PLENARY LECTURE

ADPD5-1412

STRESS AND TELOMERE MAINTENANCE MECHANISMS IN HUMAN LIFE TRAJECTORIES

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Telomeres are the protective, specialized DNA-protein complexes at the tips of chromosomes that help stabilize the genetic information. Their integrity is necessary for normal cell functioning as well as cellular replenishment. The enzyme telomerase can add DNA to telomeres, counteracting the processes that shorten them. Telomere length in humans is thus dynamic, continuously determined by systemic and cell-specific regulation of shortening and lengthening processes. However, throughout human lives, in many cells these chromosomal tips erode, with the potential eventually to cause some cells to malfunction or die. Shortening of telomeres has been shown to precede major chronic diseases linked to human aging such as cardiovascular diseases, diabetes and certain cancers, and from genetic evidence appears to contribute to some diseases. Recent emerging data suggest that telomere shortening, as typically measured in white cells from blood draws, is linked to dementias and brain functions. Telomere shortening has also been linked to chronic psychological distress, including effects coming from external influences. Conversely, exercise and other lifestyle factors have been associated with longer telomeres and their slower attrition over time. These findings raise the possibility of finding ways to slow or even alleviate the telomere shortening occurring throughout aging. An important challenge is to apply knowledge of the effects of telomere maintenance on the biological processes in aging and co-morbid diseases to improve human health.

ADPD5-2307

THE CONCEPT OF SUBCONSCIOUSNESS IN ALZHEIMER AND PARKINSON DISEASES

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Patients with Alzheimer disease progressively lose non-automatic cognitive abilities as a result of the loss of neurones in the cerebral cortex, whereas the basal ganglia, known to be involved in automatic behaviours, are essentially intact. This explains why automatic activities (ex: gait) are usually preserved in the disease. In contrast, patients with Parkinson disease have lost automatic behaviours as the result of the dysfunction of the basal ganglia, whereas the cerebral cortex is essentially preserved, thus explaining the absence of severe cognitive disorders in most patients. This suggests that the basal ganglia are playing a major role in the processing of automatic motor behaviours, and the cerebral cortex in non-automatic behaviours. The basal ganglia are composed of motor, associative and limbic territories, thus suggesting that the basal ganglia are involved in the processing of motor, but also of intellectual and emotional functions. This is what we call subconsciousness. Several examples using neuroimaging and deep brain stimulation will be provided to demonstrate that phylogenetically ancient and small structures such as the basal ganglia are directly involved in the subconscious processing of emotions in normal subjects or of emotional disturbances in patients with neuropsychiatric disorders.

ADPD5-1887

THE PRION MODEL AS A GUIDE TO DIAGNOSIS AND THERAPY OF TAUOPATHIES

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Evidence supports the idea that neurodegenerative diseases linked to protein amyloids could be caused by trans-cellular propagation of protein aggregates—a fundamental mechanism of prion diseases. We study this process in the tauopathies, which are characterized by intraneuronal accumulation of tau amyloids. We have defined heparan sulfate proteoglycans as the cell surface receptors to which tau and synuclein aggregates bind to trigger uptake and seeding into vulnerable neurons. We have also described essential strain characteristics of tau prions, in which unique conformations are propagated indefinitely in cells and in animals. Further, the conformation of the tau aggregate that enters a cell defines subsequent the structural and biochemical properties of the amplified aggregates, intracellular inclusion morphology, and subsequent neuronal pathology. Human tauopathies can now be linked to distinct groups of tau prion strains. We additionally define the minimal tau aggregate size in AD samples that is sufficient to trigger spontaneous intracellular uptake and seeding. To quantify tau seeding activity, and to test the role of proteopathic seeds in the development of pathology, we have developed a FRET-based biosensor cell assay based on induction of tau reporter aggregation. This identifies tau seeding activity far in advance of pathology detected by any other means. Our results suggest a very proximal role for seed development in pathogenesis. Finally, we have developed a new microfluidic method that we term the Multiplex Avidity Profile to rapidly define aggregate composition in patient material. We hypothesize this will enable syndromic classification of tauopathies based entirely on aggregate structure.

ADPD5-1894

HOW DO WE TREAT ALZHEIMER'S DISEASE A DECADE BEFORE DEMENTIA?

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Converging data from PET amyloid imaging, cerebrospinal fluid studies and large autopsy series suggest that one-third of clinically normal older individuals harbor a substantial burden of cerebral amyloid-ß. It remains unknown whether these individuals are in the preclinical stages of Alzheimer's disease (AD) and what proportion will progress to dementia over time. Our multi-modality imaging studies in normal older individuals have demonstrated that Aß deposition is associated with aberrant default network fMRI activity and cortical thinning in amyloid-laden regions, in a pattern similar to that observed in AD dementia. Recent studies have also reported an association between Aß burden and memory performance, and increased subjective cognitive concerns. The most compelling data come from longitudinal studies suggesting that elevated Aß burden confers a significantly increased risk of cognitive decline, particularly among older individuals with markers of both amyloid accumulation and neurodegeneration. Several secondary prevention trials in both genetic at-risk and age at-risk individuals are now ongoing, testing anti-amyloid mechanisms. The Anti-Amyloid Treatment in Asymptomatic AD (A4) trial will enroll over 1000 older individuals with evidence of amyloid accumulation on screening PET scans to determine if treatment with an anti-amyloid antibody, initiated prior to cognitive impairment, will slow neurodegeneration and the progression of memory decline towards AD dementia. A similar trial design with a BACE inhibitor in an asymptomatic AD population is being planned for a 2015 launch. One of the continued dilemmas in the field is how best to identify individuals who are clearly on the trajectory of late onset AD but at an earlier enough stage of pathology to be maximally responsive to therapeutic intervention.

ADPD5-1878

NOVEL NEUROPROTECTIVE APPROACHES IN PARKINSON´DISEASE EXPLORED IN ALPHA-SYNUCLEIN OVEREXPRESSION MODELS

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The transcription factor Nurr 1 is a key regulator of the survival of dopamine neurons during embryonic development. Recently, it has been recognized that this gene regulator may play an important role also in adult dopamine neurons, and as a mediator of the degenerative changes seen in Parkinson´s disease (PD). Rare cases of familiar PD have been associated point mutations in the Nurr1 gene, and recent observations in brains from PD patients show that the expression of Nurr 1 is reduced in affected dopamine neurons. These data suggest that reduced cellular levels of Nurr1 may be associated with increased vulnerability and impaired function in the dopaminergic system. Studies in rodents have provided further support for this idea, showing that overexpression of the disease-causing protein alpha-synuclein causes down-regulation of Nurr1, which in turn leads to impaired dopamine neurotransmission and loss of responsiveness to the neurotrophic factor GDNF. Conversely, GDNF signaling is effectively restored by overexpression of Nurr1, providing near-complete protection of nigral dopamine neurons against alpha-synuclein toxicity also in the absence of exogenous GDNF. These observations suggest that reduced Nurr1 expression, induced by increased cellular levels of alpha-synuclein, is a key element in the induction of dopamine neuron dysfunction seen in early stages of the disease, and that loss of Nurr1 contributes to the progression of the disease. Together, the clinical and experimental data point to Nurr 1 as key player in the pathogenesis of PD and identifies Nurr 1 an interesting novel therapeutic target for disease intervention in PD.

ADPD5-1634

ALZHEIMER'S DISEASE: FROM PRECLINICAL STATES TO DEMENTIA

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How should we define Alzheimer disease today? Should it be defined as a clinical disease, which starts with a dementia (classical definition)? Should it be defined as a clinical disease, which starts with the first clinical symptoms (IWG definition)? Should it be defined biologically by the presence of a positive biomarker (BM+) even in the absence of clinical symptoms (NIA-AA definition)?

If in vivo evidence of Alzheimer pathology is a fundamental feature for the further conversion to a clinical disease, it is not definitely established that all healthy subjects BM+ will develop the disease during their lifetime. At present, the risk of conversion to a clinical AD has been estimated to be around 25% after a 3-year long follow-up. Longer follow-ups are needed to demonstrate that all subjects will convert to AD. In parallel, several factors may have a negative/positive influence on the conversion: age, presence of co-morbidity, vascular risk factors, genetic predisposition, or in the opposite, significant cognitive reserve, other compensatory mechanisms of the brain, preventive genetic/episodic and life-style factors. For these reasons, normal BM+ individuals should only be considered as « asymptomatic at risk for AD (AR-AD)». Therefore, the next steps will be to identify the factors that activate or delay the dynamic process of conversion and the subtle brain changes that anticipate the clinical onset. The main clinical issue will be to identify AR-AD having the highest likelihood to convert to definite clinical AD in the subsequent months. This is especially important, as the later will be the main target population for treatment with disease-modifying drugs. When such drugs become available, the important step will be to treat AR-AD subjects to delay the onset of clinical symptoms.

Symposia – Basic Science

Symposium 02: APP PROCESSING, ABETA AND SECRETASES

ADPD5-0659

THE ADIPOCYTE DIFFERENTIATION PROTEIN APMAP IS AN ENDOGENOUS SUPPRESSOR OF A-BETA PRODUCTION IN THE BRAIN

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OBJECTIVES: Cerebral amyloid-beta (Abeta) deposition is a major pathological hallmark of Alzheimer's disease (AD). Abeta is generated by the proteolytic processing of the amyloid precursor protein (APP-C99) by gamma-secretase, an intramembrane-cleaving protease with multiple substrates. Because clinical inhibition of gamma-secretase resulted in severe side effects attributed to impaired Notch cleavage, we aimed at identifying new endogenous gamma-secretase modulating proteins that specifically affect Abeta production.

METHODS: The proteome of highly purified active gamma-secretase was analyzed by LC-MS/MS mass spectrometry. Next, the physical interactions between the newly identified endogenous gamma-secretase interacting proteins (GSPs), gamma-secretase and APP were studied in cell-based systems. Finally, the effects of the genetic inhibition of the GSPs on Abeta production were analyzed *in vitro* and *in vivo,* both in wild type and AD mice.

RESULTS: We identified the adipocyte plasma membrane associated protein **(**APMAP), a key player of adipocyte differentiation, to interact physically both with gamma-secretase and its substrate APP. In cells, partial depletion of APMAP was associated with drastic increases in APP-CTFs levels and Abeta production. In wild type and APP/PS1 transgenic mice, adeno-associated virus-mediated partial APMAP knockdown in the hippocampus increased Abeta production by ~20% and ~55%, respectively.

CONCLUSION: We demonstrate that APMAP is a negative regulator of Abeta production, through a mechanism involving APP-C99 degradation via the lysosomal-autophagic system.

Symposium 02: APP PROCESSING, ABETA AND SECRETASES

ADPD5-0665

MEMBRANE TRAFFICKING OF BETA- AND GAMMA-SECRETASES REGULATED BY GENETIC RISK FACTOR PROTEINS FOR ALZHEIMER DISEASE IMPACTS ON THE PRODUCTION OF AMYLOID-BETA PROTEINS

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Recent GWAS studies revealed that variants at *PICALM* as well as *BIN1* locus are associated with the onset of Alzheimer disease (AD) that is characterized by deposition of amyloid-β proteins (Aβ). *PICALM* gene encodes a protein called CALM which has a PtdIns(4,5)P_2-binding AP180 N-terminal homology domain at its N terminus, along with several AP2/clathrin binding motifs in the C-terminal region. BIN1 is a membrane trafficking-related adapter protein that contain N-terminal BAR and C-terminal SH3 domains. BAR domains recognize specific phospholipids and sculpt membranes to generate protrusions or invaginations. In the previous study, we showed that γ-secretase is constitutively endocytosed via clathrin-mediated pathway in a CALM dependent manner. And partial loss of CALM function decreased the rate of clathrin-mediated endocytosis of γ-secretase as well as production of the pathogenic Aβ species, Aβ42 (Kanatsu et al., *Nat Comm* 2014). Furthermore, we found that the amyloid plaque burden was significantly decreased in the piriform cortex of congenic *Picalm*$^{+/-}$ mice crossed with APP transgenic A7 mice at 12 months old, supporting our notion that partial loss-of-function of CALM is protective against AD. In contrast, we have found that BIN1 promotes BACE1 degradation by lysosomal targeting from endosome. Ablation of Bin1 expression in mouse primary neurons resulted in the increased production of Aβ and sAPPβ by augmentation of BACE1 proteins. These data suggest that Aβ production is modulated by the genetic risk factor proteins and impacts on the onset of AD.

Symposium 02: APP PROCESSING, ABETA AND SECRETASES

ADPD5-1226

SYNAPTIC LOCALIZATION OF APP PROCESSING ENZYMES

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Objectives

The objectives of this study were to investigate the synaptic localization of the Amyloid Precursor Protein (APP) processing enzymes alpha-, beta- and gamma-secretase, in order to more specifically and effectively target the production of the amyloid beta-peptide (Abeta) and thereby reduce the synaptic dysfunction in AD.

Methods

Subcellular fractionation was used to prepare crude synaptic vesicle and synaptic membrane fractions. Ultra-pure synaptic vesicles were purified by a controlled-pore glass chromatography. In situ localization of the secretases was assessed by immunocytochemistry or proximity ligation assay in hippocampal primary neurons using antibodies or a probe for active gamma-secretase. Abeta production was measured by an in vitro gamma-secretase assay followed by ELISA and beta-secretase (BACE1) activity was measured by a commercial kit.

Results

Gamma-secretase activity was found to be enriched in crude synaptic fractions but not in ultra-pure synaptic vesicles. On the contrary, BACE1 and the intermediate APP fragment APP-CTF were highly enriched in pure synaptic vesicles. In accordance with this finding, BACE1 co-localized with the synaptic vesicle marker synaptophysin in primary neurons whereas BACE1 and gamma-secretase did not co-localize.

Conclusions

We conclude that the APP-processing enzymes are present at different synaptic locations. The high enrichment of BACE1 in synaptic vesicles can enable specific targeting of synaptic BACE1.

Symposium 02: APP PROCESSING, ABETA AND SECRETASES

ADPD5-1749
SYSTEMS BIOLOGY OF BETA-AMYLOID PRODUCTION: IMPLICATIONS IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a memory disorder for which the exact cause is unknown. A defining feature of AD is the presence of amyloid-b (Ab) which is causally linked to neurodegeneration. The key molecular events leading to the generation of the amyloid b (Ab) peptide are well characterized. In principle, Ab can be produced employing only three primary components i.e., APP, b-secretase and g-secretase complex. But this entire process requires the involvement of several gene products in the cellular context. Identification of genes that regulate APP processing and Ab levels would give a clear picture of AD and would also be suitable candidates for drug development. To elucidate the cellular mechanisms that regulate APP processing we used gene-silencing strategies using small interfering RNAs (siRNA) and performed genome wide screen of all kinases and complemented it with an activity-inhibiting small molecule kinase inhibitor screen to identify key regulators of APP processing. We identified a large set of kinases as regulators of APP processing. We also found that signaling networks regulate Ab metabolism. Our results reveal that many genes regulate APP processing and many signaling networks and pathways regulate Ab metabolism. We provide experimental validation of AD being a multifactorial syndrome rather than a single disease. Moreover, we believe that systems biology approaches will play a crucial role in our future understanding of networks involved in AD pathogenesis.

Symposium 02: APP PROCESSING, ABETA AND SECRETASES

ADPD5-2071
THE T-BOX FACTOR TBX2: ITS ROLE IN REGULATING THE ALZHEIMER-RELATED PROTEINASE ADAM10

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Increased synthesis of A-beta peptides by a disturbed homeostasis of the proteinases competing for their substrate APP (amyloid precursor protein) represents a pivotal event in Alzheimer's disease pathogenesis: ADAM10 (a disintegrin and metalloproteinase 10), the physiological alpha-secretase, is decreased in favor of the A-beta generating enzyme BACE-1 (beta site APP cleaving enzyme-1). Therefore, identifying transcription factors (TFs), which contribute to the disturbed homeostasis of APP-processing by regulating the gene expression of either proteinase, might elucidate molecular mechanisms underlying AD-pathology.

Here we demonstrate that the senescence-associated TF T-box2 is a repressor of ADAM10 transcription in neuronal cells. The decrease of ADAM10 gene expression is mediated via a Tbx2 binding site within the basal promoter region as demonstrated by shift assay analysis. Coexpression and inhibition experiments revealed that the decrease of ADAM10 transcriptional activity by Tbx2 is partly conducted by its binding partner HDAC1. Analyses of cortical samples of AD patients hint at a potential contribution of Tbx2 in AD-pathogenesis: mRNA level of Tbx2 but not of the T-box gene family members Tbx3 and 21 was significantly increased in post-mortem frontal cortex tissue of AD-patients as compared to healthy age matched controls. This was paralleled by reduced ADAM10 mRNA as demonstrated earlier.

In summary, our results indicate that Tbx2 might contribute to the disturbed homeostasis of APP-processing in AD-pathogenesis.

Symposium 04: METABOLISM 1

ADPD5-0712
MODELING OF BRAIN INSULIN RESISTANCE AS A TRIGGER FOR SPORADIC ALZHEIMER'S DISEASE

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Growing evidence indicates that dysfunctionalbrain insulin and alterations downstream the insulin receptor (IR)signalling pathways in the brain precede cognitive impairment in sporadic Alzheimer's disease (sAD). Attempts to create experimental sAD condition have revealed that intracerebroventricular administration of streptozotocin (STZ-icv), a compound selectively toxic to IR and insulin producing/secreting cells, generatesan animal model which develops brain insulin resistance as a starting point and eventually demonstrates cognitive deficits and some other sAD-like features.Considering the long developmental phase in human sAD pathology, duration of post-STZ-icv treatment time might be important in validation of STZ-icv model in modelling of sAD. Characterization of STZ-icv model usually refers to the pathology developed ≤3 months post STZ-treatment. Our 9-month follow-up study of STZ-icv rat model indicates a biphasic pattern (partly reversible changes at ≤1 month,AD-like ones at ≥6 months post treatment) in onset and progression of changes in brain insulin and IR signalling accompanied by STZ dose-dependent biphasic time-pattern of memory decline. However, structural changes, particularly intracellular amyloid-β_{1-42} accumulation,become apparent not earlier than 3 months post treatment and follow a slow linear mode of progression up to 9 months. STZ-icv model provides a strong evidence of the relevance of brain insulin resistance in development of sAD-like condition.Given the resemblance of medium/late post-STZ-icv treatment stage to human sAD condition,attention should be paid to planning of preclinical drug trials in this modelwhich might contribute to better translational AD research leading to more successful clinical AD trials.

Symposium 04: METABOLISM 1

ADPD5-0882

AMYLOID-BETA-INDUCED, TAU-DEPENDENT ACTIVATION OF MTOR AT THE PLASMA MEMBRANE LEADS TO NEURONAL CELL CYCLE RE-ENTRY: A SEMINAL STEP IN ALZHEIMER'S DISEASE PATHOGENESIS

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We recently reported that neuronal cell cycle re-entry (CCR), which precedes neuron death in AD, results from Aβ oligomers (AβOs) stimulating tau phosphorylation at Y18, S409 and S416 (*J Cell Sci* 126:1278). We now describe how mTOR cooperates with AβOs, tau and Rac1 to drive neuronal CCR. In cultured neurons, CCR was prevented by reducing the abundance or activity of NCAM, Gαs, Rac1, mTORC1 or mTORC2, and required mTORC1-dependent tau phosphorylation at S262. FRET biosensors for mTORC1 revealed AβO-induced activation at plasma membrane (PM), but not lysosomes, where mTOR is activated instead by insulin, which blocks CCR. Reducing Rac1-dependent targetting of mTOR to PM or forcing mTORC1 onto lysosomes also prevented CCR. Reduction of mTor in Tg2576 mice strongly suppressed CCR and tau phosphorylation at S262, the latter of which was also reduced in 3xTg AD model mice treated with rapamycin. Finally, preliminary studies of rapidly fixed brain biopsy samples from human normal pressure hydrocephalus patients with coincident AD pathology revealed that plaque and tangle density strongly correlate with CCR markers, and tau phosphorylation at S262 and S409. AβOs thus initiate two pathways that synergistically lead to mTOR dysregulation and are obligatory for CCR: mistargetting of mTOR to PM, and tau phosphorylation at Y18, S409 and S416. The requirement for tau phosphorylation at S262 establishes that bidirectional regulation of tau and mTor integrates the pathways. Moreover, the insulin insensitivity of AD neurons, which has prompted AD's classification as "type 3 diabetes", unleashes AβOs' tau-dependent ability to induce CCR.

Symposium 04: METABOLISM 1

ADPD5-0890
THE MTOR/P70S6K PATHWAY PLAYS A KEY ROLE IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

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Objectives. Aging is the major risk factor for Alzheimer' disease (AD); however, little is known as to how the aging process facilitates the development of AD. Changes that occur in the brain as a function of age may facilitate the development of AD. Reducing the activity of the mammalian target of rapamycin (mTOR), and its downstream target p706SK, increases lifespan and health-span in genetically different species. mTOR is a protein kinase that plays a key role in regulating protein translation (via p70S6K) and degradation. Therefore, mTOR is key in controlling protein homeostasis, a process that is altered in AD and other proteinopathies. The goal of this work is to assess the role of the mTOR/p70S6K pathways in the pathogenesis of AD.
Methods: Using several mouse models, we employed multidisciplinary approaches to dissect the role of the mTOR/p70S6K signaling in AD.
Results: We will show that genetic and pharmacologic reduction of mTOR and p70S6K signaling reduced amyloid-β and tau pathology and rescued memory deficits. Mechanistically, the reduction in mTOR signaling led to an increase in autophagy induction and restored the hippocampal gene expression signature of the Tg2576 mice to wild type levels.
Conclusions: Given that mTOR and p70S6K regulate lifespan and health span, the data presented here have profound clinical implications for aging and Alzheimer's disease and provide the molecular basis for how aging may contribute to AD pathology. Our results implicate hyperactive mTOR/p70S6K signaling as a previous unidentified signaling pathway underlying gene-expression dysregulation and cognitive deficits in Alzheimer's disease.

Symposium 04: METABOLISM 1

ADPD5-0914
A NOVEL METABOLIC GENE THERAPY-BASED STRATEGY FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Objectives: To develop a therapeutic intervention targeting neuronal metabolic integrity in Alzheimer's Disease (AD). Recompartmentalization of neuronal N-acetyl-L-aspartic acid (NAA) catabolism is proposed to provide resistance to Aß-induced energetic crisis. Reductions in NAA in AD parallel compromised energetic status, and reflect a threshold effect with respect to the available metabolic resources. NAA synthesis is energy-intensive, taking place in neurons, while NAA catabolism uncouples fatty acid synthesis from the ATP-generating tricarboxylic acid (TCA) cycle in myelinating oligodendrocytes (Francis et al. 2012). We hypothesized that providing neurons with the ability to catabolize NAA in a model of familial AD would result in heightened resistance to metabolic stress via the promotion of TCA cycle integrity and overall mitochondrial function. Methods: gene therapy using recombinant adeno-associated viral vectors (AAV) was employed to recompartmentalize ASPA activity to neurons. HPLC, unbiased stereology, and cognitive testing was employed to assess the ability of this strategy to promote phenotypic rescue in the 5XFAD model of AD. Results: AAV-mediated expression of NAA-deacetylating ASPA in neurons of 5xFAD mice resulted in improved energetic status, reduced amyloid burden, the promotion of cell survival, and long-term cognitive improvement. Conclusions: The genetic recompartmentalization of NAA catabolism is a novel therapeutic strategy for AD that appears to rescue phenotype in 5xFAD mice. The utilization of a gene therapy platform, with proven clinical safety and efficacy, to target energetic integrity has relevance for the clinical development of an AD therapeutic, and for a broad spectrum of neurodegenerative diseases that manifest energetic crisis

Symposium 04: METABOLISM 1

ADPD5-1410
DECREASED HIPPOCAMPAL NEPRILYSIN IN A TYPE 1 DIABETES PRIMATE MODEL LEADS TO AN INCREASE IN ABETA LEVELS

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Objectives: Given that epidemiologic studies have shown that diabetes mellitus increases the risk of Alzheimer's disease (AD), our objective was to examine the mechanistic links between the two diseases in a non-human primate.
Methods: Tissue from multiple brain regions of a vervet monkey model of streptozotocin-induced type 1 diabetes (n=10 control; n=7 diabetic) was examined by Western blot analysis, sandwich ELISA, and qPCR for biochemical changes in tau protein and Abeta peptide, as well as changes in key enzymes that contribute to their processing and posttranslational modification.
Results: Regional brain analyses showed a global increase in tau phosphorylation in areas vulnerable to AD pathology as well as in spared structures such as the cerebellum. An examination of tau phosphatases and kinases showed a brain-wide increase in active ERK1/2. A diabetes-induced increase in Abeta levels, however, was specific to brain regions affected during the early stages of AD pathogenesis, with the greatest increase observed in the hippocampus. Examination of the amyloid precursor protein, its metabolites, and proteins involved in the clearance and degradation of brain Abeta indicated that a hippocampal-specific decrease in the Abeta-degrading enzyme neprilysin is a major contributor to this localized Abeta increase.
Conclusions: Our study suggests protein changes in the brain that link diabetes to AD risk: decreased neprilysin expression leads to an increase in Abeta in the temporal lobe structures that are at the earliest risk in AD while increased ERK1/2 activity appears to contribute to a brain-wide increase in tau phosphorylation.

Symposium 04: METABOLISM 1

ADPD5-1718
DUAL EFFECTS OF METFORMIN ON TAUOPATHY IN THE P301S MUTANT HUMAN TAU TRANSGENIC MOUSE

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Objectives:
In Alzheimer disease (AD), the progression of cerebral tau pathology correlates with cognitive impairment and appears to be influenced by several risk factors, including brain insulin resistance and the use of antidiabetic drugs such as metformin. Indeed, there is evidence that metformin enhances protein phosphatase 2A (PP2A) activity and dephosphorylates tau in cultured mouse primary cortical neurons, suggesting a potential effect on tau pathology. Here, we investigated the potential use of metformin as a therapy for tauopathy in a relevant transgenic mouse model.

Methods:
Metformin was administered in the drinking water (2mg/ml) to P301S mutant human tau (P301S-tau) transgenic and C57BL6/J wild type (WT) mice starting from 4 weeks of age for 4 months. Weight, glycemia, food and water intake were monitored weekly. Cerebral tau phosphorylation and tau inclusions were evaluated by biochemical and immunohistochemical analyses.

Results:
Chronic administration of metformin did not alter weight, glycemia, water and food intake in both WT and P301S-tau mice. In P301S-tau mice, chronic treatment with metformin affected the expression levels of PP2A and significantly decreased the levels of phosphorylated tau in the cortex and hippocampus. We also found that metformin promoted the aggregation of recombinant P301S mutant human tau in vitro. Moreover, when administered chronically, metformin significantly increased the number of β-sheet filamentous tau inclusions in the P301S-tau brain.

Conclusions:
These results indicate that chronic use metformin may favor the development of tau filamentous inclusions and suggest that metformin should be used with caution in elderly patients with dementia.

Symposium 05: NEURODEGENERATIVE DISEASES - CAN STANDARDIZATION IMPROVE THE PREDICATIVE VALUE OF ANIMAL MODELS? (PODIUM DISCUSSION)

ADPD5-0347

RECONSTRUCTING THE COMPLEXITY OF ALZHEIMER DISEASES BY USING QUANTITATIVE SYSTEMS PHARMACOLOGY AS A POSSIBLE ALTERNATIVE FOR PRECLINICAL ANIMAL MODELS

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Objectives. Succesful clinical trial development is extremely challenging in CNS neurodegenerative diseases. Possible reasons include limited predictability of preclinical animal models, and clinical trial flaws or insufficient target engagement. Most importantly is the lack of recognition of the many complex processes in the aged Alzheimer patient, driven by comorbidities, genotypes, comedications and different life histories.

Methods. We present an advanced computer-based Quantitative Systems Pharmacology (QSP) platform, a mechanism-based computer model of relevant humanized cortical networks for clinical readouts in psychiatry and neurology, calibrated with group average clinical data and has been able to blindly and correctly predict unexpected clinical outcome for a new candidate AD drug. This method differs from the traditional preclinical animal models in that it is focused primarily on clinical neurology, human neuropathology and patient outcome.

Results. We discuss a high-level QSP approach integrating many isolated preclinical experiments. A key part is the integration of this information in a web-based database, called NESTOR (NErvous SysTems Organized References). The interaction between neurotoxic and compensatory processes is described as dynamic state changes in a multi-parameter state space. These state changes are likely different from patient to patient; we explore how to possibly identify biomarkers for the individual disease trajectories. Transition rates between states are derived from preclinical and clinical data and the system output is constrained by clinical data on functional clinical scales, imaging or biochemical biomarkers.

Discussion. A novel innovative strategy for generating actionable knowledge for pharma R&D is outlined based on a humanized mechanism-based computer platform.

Symposium 05: NEURODEGENERATIVE DISEASES - CAN STANDARDIZATION IMPROVE THE PREDICATIVE VALUE OF ANIMAL MODELS? (PODIUM DISCUSSION)

ADPD5-0867
ANIMAL MODELS OF NEURODEGENERATIVE DISORDERS AND APPROACHES TO IMPROVE TRANSLATION IN DRUG DISCOVERY

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The major problem facing CNS drug discovery is that drugs that show good efficacy in preclinical models often fail efficacy endpoints in humans, with an impressive failure rate of about 99,6 % in clinical trial of AD. As a result, pharmaceutical companies may be taking the wrong compounds into clinical trials and abandoning potentially good ones. This raises the question of whether the models of neurodegeneration recapitulate the deficits seen in patients. While no mouse model is likely to capture all aspects of a human condition we can greatly improve the outcome of preclinical testing by improving the design, standardization, data collection, and interpretation of research results. Blinded studies, transparent protocols and reporting negative data are some of the ways we can improve translation in drug discovery. More thorough phenotyping rather of relying on single endpoints can also improve the predictive value of animal models. We have developed highly sensitive, automated behavioral testing systems that capture hundreds to thousands of behavioral features in a single test session, and thus can detect and track those behaviors that best define a disease model and treatment profile. The application of computer vision, robotics and bioinformatics avoids any investigator bias. Employing these technologies in early preclinical testing could have a dramatic impact on the efficiency of drug development.

Symposium 05: NEURODEGENERATIVE DISEASES - CAN STANDARDIZATION IMPROVE THE PREDICATIVE VALUE OF ANIMAL MODELS? (PODIUM DISCUSSION)

ADPD5-1906
HUMANIZATION OF ENTIRE MURINE TAU GENE FOR A BETTER MODEL OF AD

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None of the mouse models of Aβ amyloidosis reconstitute tauopathy, or NFT, without using tau mutation(s), which are causes of FTDP-17, not of AD. We consider there to exist two reasons. One is that the time necessary for Aβ amyloidosis to induce tauopathy, approximately a decade in human cases, is not enough for mice. If this happens to be true, we need to identify pathway(s) that link the two major pathologies and find measures to accelerate the processes. The other is that the physiochemical properties including the splicing profile of murine *tau* (gene) differ from those of human *tau* (gene) and thus could result in a failure of tauopathy reconstitution. To address the second question, we generated mice, in which the whole *tau* gene has been humanized. The mice appear to live normally. We have crossed these mice with single *App* knock-in mice (Saito *et al.*, ***Nat Neurosci***, 2014) and will present the latest data obtained using these mutant mice.

Symposium 05: NEURODEGENERATIVE DISEASES - CAN STANDARDIZATION IMPROVE THE PREDICATIVE VALUE OF ANIMAL MODELS? (PODIUM DISCUSSION)

ADPD5-1930
APPLICATION OF HUMAN INDUCED PLURIPOTENT STEM CELL TECHNOLOGY IN THE CONTEXT OF DRUG DISCOVERY FOR NEURODEGENERATIVE DISEASES

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Application of human induced pluripotent stem cell technology for drug discovery of neurodegenerative diseases

The use of innovative human induced pluripotent stem cell (hiPSC)-derived model systems in pharmaceutical research is expected to lead to increased disease understanding and the development of highly disease-relevant assay systems for drug screening. IPSC technology also promises to be impactful in other related areas of drug discovery & development including more predictive safety/toxicity testing, accelerated drug repositioning and patient stratification for clinical trials. Thus, IPSC technology has the potential to become a key 'translational' tool.
StemBANCC is a large-scale public-private partnership funded by the Innovative Medicines Initiative (IMI) which brings together a consortium of 35 partners from academia and industry who share their experience and work together in 12 work packages. The overall aim of this collaborative research project is the generation and characterization of high-quality human induced pluripotent stem cell lines for the investigation of a range of chronic diseases and drug safety evaluation.
In this presentation I will highlight StemBANCC activities from the work package investigating neurodegenerative diseases with a focus on Alzheimer's and Parkinson's disease which is jointly led by the University of Oxford and AbbVie.

Symposium 06: ALPHA-SYNUCLEIN: PATHOLOGICAL MECHANISMS

ADPD5-0435
PROTEOLYTIC CLEARANCE OF ALPHA-SYNUCLEIN IN VIVO: NOVEL TARGETS IN PARKINSON'S DISEASE TRANSMISSION

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Recent evidence suggests that specific extracellular ALPHA-synuclein (AS) strains are implicated in the progression of Parkinson's disease (PD) pathology. It is plausible that deregulation in the normal processing of secreted AS may be a causative risk factor for PD. Thus, elucidation of the mechanisms that regulate the protein levels of extracellular AS becomes essential. Our recent work has suggested that kallikrein-related peptidase 6 (KLK6), an extracellular enzyme physiologically present in the CSF known to cleave recombinant AS is also implicated in the regulation of naturally secreted α-syn turnover (Ximerakis et al., FASEB J, 2014). Importantly, this processing appears to be inhibited by the association of secreted α-syn with lipids. Here, we sought to investigate factors and mechanisms that regulate α-syn extracellular levels in vivo. Using KLK6 knockout mice crossed with a transgenic AS- PD mouse model we show for the first time that secreted AS oligomeric species are regulated through a proteolytic cascade involving KLK6. The effect of the protease on the in vivo propagation capacity of specific protofibrilar AS species has also been examined. Our findings clearly suggest that physiologic modifications affect the biochemical behavior of secreted AS and provide novel insights into transmission mechanisms and potential targets for therapeutic interventions.

Symposium 06: ALPHA-SYNUCLEIN: PATHOLOGICAL MECHANISMS

ADPD5-0541

ALPHA-SYNUCLEIN ACCUMULATES IN THE ENTERIC NERVOUS SYSTEM FOLLOWING INFLAMMATION IN TRANSGENIC MOUSE MODELS OF PD AND IN COLITIS ULCEROSA PATIENTS

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Objectives: Intraneuronal accumulation of alpha-synuclein (alpha-syn) is a major neuropathological hallmark of PD. Increasing evidence suggests that this also occurs in the enteric nervous system (ENS), perhaps as prodromal event leading to clinical PD. Here we explore the role of inflammation in the accumulation of alpha-syn in enteric nerves.

Methods: A dextran sodium sulfate (DSS) colitis paradigm was applied in human alpha-syn transgenic mouse lines and compared to LPS induced inflammation. The macrophage phenotype was altered by crossing one alpha-syn transgenic line with CX3CR1-eGFP transgenic mice which lack fractalkine signaling. Accumulated alpha-syn in the ENS was stereologically quantified and immune mediators were assessed based on their mRNA expression levels. Colon samples of patients with colitis ulcerosa or Crohn's disease were analyzed by immunohistochemistry.

Results: Systemic administration of DSS and LPS resulted in different types of colitis as demonstrated histologically and by cytokine/chemokine expression pattern. Whereas LPS did not induce further accumulation of human alpha-syn, DSS colitis did most prominently in submucosal plexi, which was further aggravated by the lack of CX3CR1. Also, alpha-syn aggregates persisted over 2 months after the mice had recovered from colitis. Accumulation of alpha-syn was also observed in the colon of colitis ulcerosa patients, whereas patients with Crohn's disease were devoid of it.

Conclusions: Our results in mice and humans support the hypothesis that certain types of systemic inflammation can promote alpha-syn accumulation in the ENS and that macrophages may be in involved. The relevance of this observation in PD needs to be further explored.

Symposium 06: ALPHA-SYNUCLEIN: PATHOLOGICAL MECHANISMS

ADPD5-0945

AAV-MEDIATED KNOCKDOWN OF ALPHA-SYNUCLEIN IN THE SUBSTANTIA NIGRA OF THE AFRICAN GREEN MONKEY RESULTS IN NIGRAL NEURODEGENERATION

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1.Objectives

Alpha-synuclein (a-syn) has been implicated to confer a toxic gain-of function in PD and other synucleinopathies. However, we hypothesize that a-syn is not the primary toxic species in disease; rather, a-syn aggregation produces pathology by decreasing levels of functional α-syn. Here we test our hypothesis by reducing a-syn expression in the non-human primate.

2.Methods

African Green monkeys (n=1/vector) were injected unilaterally in the substantia nigra (SN) with low ($5x10^{12}$ vector genomes (vg)/ml) or high ($2x10^{13}$vg/ml) titer AAV2/5 (including GFP as a transduction marker) expressing a shRNA designed against a-syn or scrambled shRNA as control. Animals were monitored for behavioral deficits indicative of nigrostriatal denervation for three months until sacrifice. Brains were analyzed for catecholamine content and neuropathology of the SN.

3.Results

One animal treated with a-syn shRNA exhibited a progressive deficit in a summary score of healthy behaviors. Analysis of tissue indicated that a-syn shRNA treated animals contained reduced striatal dopamine (DA). GFP+ neurons were observed throughout the SN of scrambled-shRNA treated subjects. In contrast, GFP was only seen in dorsomedial neurons of a-syn shRNA treated animals. Degeneration of TH+ neurons, preferably in the ventral tier of the a-syn shRNA treated SN, was observed. In addition, several TH- neuromelanin+ neurons were observed with a-syn shRNA treatment.

4.Conclusions

Our findings suggest that non-human primate nigral (particularly ventral tier) neurons are sensitive to the loss of a-syn. This sensitivity is not due to non-specific shRNA toxicity. Our results suggest that sufficient levels of a-syn is crucial to neuronal survival.

Symposium 06: ALPHA-SYNUCLEIN: PATHOLOGICAL MECHANISMS

ADPD5-0958
PROBING THE CONFORMATIONAL STATES OF ALPHA-SYNUCLEIN IN COMPLEX BIOLOGICAL SAMPLES

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Objectives
Alpha-synuclein (α-Syn), displays remarkable structural diversity. In physiological buffers α-Syn adopts an intrinsically disordered conformation, which can be readily switched by prolonged incubation to an amyloid structure, thought to resemble material found in Lewy bodies. Pore-like oligomers of α-Syn have also been reported and α-Syn adopts at least two different alpha-helical structures upon lipid binding. Most of our current knowledge of the structural states of α-Syn derives however from *in vitro* experiments. It would be of paramount importance to evaluate whether and which of the structures characterized so far *in vitro* resemble those formed by α-Syn *in vivo*, under physiological and pathological conditions.
Methods
To probe the conformational states of α-Syn directly in complex samples, we developed a novel approach that couples proteolytic probes and selected-reaction-monitoring mass spectrometry (Feng *et al.*, *Nat. Biotechnology*, 2014). It relies on the generation of proteolytic patterns that depend on the conformational states of a protein and can be quantified directly in complex biological backgrounds.
Results
Our approach probed the structural conversions of α-Syn directly in complex cell extracts, with a structural resolution of ~10 amino acids. It enabled the extraction of conformational markers (conformotypic peptides) for the quantification of different α-Syn conformers in biological specimens. Using a library of conformotypic peptides for different α-Syn states, we evaluated the conformational properties of α-Syn in various biological samples.
Conclusions
Our approach opens up exciting new possibilities in the analysis of α-Syn amyloidogenesis and the exploration of the novel concept of “conformational biomarkers” of PD.

Symposium 06: ALPHA-SYNUCLEIN: PATHOLOGICAL MECHANISMS

ADPD5-1192
TRUNCATED HUMAN ALPHA-SYNUCLEIN EXPRESSION RESULTS IN DOPAMINERGIC CELL DEATH THAT CAN BE RESCUED BY TARGETING AGGREGATES

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OBJECTIVES: The pathogenesis of PD and other alpha-synucleinopathies is associated with misfolding and aggregation of alpha-synuclein into oligomers and filaments with the former considered the toxic species. We have previously produced a transgenic mouse expressing 1-120 truncated alpha-synuclein with striatal synaptic alpha-synuclein aggregation and altered dopamine release but no dopaminergic cell death. We have now generated a new transgenic mouse line (called MI2) expressing higher amounts of the same 1-120 alpha-synuclein transgene under the TH promoter. Here we investigated its characteristics and the effect of the oligomer modifier anle138b on pathological markers in the nigrostriatal dopaminergic system.
RESULTS: MI2 mice showed alpha-synuclein synaptic aggregation and SNARE proteins redistribution, which were associated with a reduction in striatal dopamine release. Unlike the previous model, these changes appeared at 6 months rather than 12 months of age. Moreover, 12 month-old MI2 mice showed significant dopaminergic cell death in the substantia nigra. We also found that treatment with the oligomer modifier compound anle138b, which restores FM1-43 dye release in alpha-synuclein overexpressing PC12 cells, reduced alpha-synuclein synaptic accumulation and dopaminergic cell death. Importantly, the protective effect of anle138b on dopaminergic cells was present when treatment was started after the detection of reduced dopamine release in the striatum.
CONCLUSIONS: Our results support the hypothesis that presynaptic aggregation of alpha-synuclein and impaired striatal dopamine release precede the loss of nigral dopaminergic neurons. Importantly, we show that these deficits can be rescued by treatment with the oligomer modifier anle138b.

Symposium 06: ALPHA-SYNUCLEIN: PATHOLOGICAL MECHANISMS

ADPD5-1918
TOWARDS CLARITY FROM CONTROVERSY: A NEW MODEL FOR THE INITIATION OF PARKINSON'S DISEASE

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Misfolding and accumulation of α-synuclein (αS) occurs at low levels during brain aging and far more abundantly in 'synucleinopathies', including PD. αS missense mutations, copy number variants, and upregulated expression have each been associated with familial PD, and all 'sporadic' cases also have αS inclusions. For two decades, αS has been defined as a 'natively unfolded monomer'. In contrast, we observed in 2011 that a substantial portion of αS in healthy cells occurs as α-helical multimers sizing principally as a ~58 kDa tetramer of four N-acetylated monomers. This finding has been controversial, but our 2013 report of a protocol for crosslinking αS in intact neurons can readily reveal the tetramers and related multimers in the cytosol. We now show that levels of these multimers are relevant to PD pathogenesis by examining 5 PD-causing αS missense mutations in intact neuronal cells. By two independent methods (cell-penetrant crosslinking; fluorescent protein complementation), all 5 mutations significantly decreased the multimer:monomer ratio. Inserting the one fPD mutation (E46K) that occurs in a 'repeat motif' into two additional such motifs caused a stepwise decrease in multimerization. An engineered repeat-motif mutation (KTKEGV à KLKEGV) placed into 6 repeats abolished tetramers, raised monomers, and induced marked neurotoxicity by three different assays, accompanied by cytoplasmic aggregates. These data indicate that tetramers are a principal physiological form of αS in neurons and that destabilizing tetramers leads to neurotoxic accumulation of monomer-derived aggregates, analogous to transthyretin amyloidosis. Compounds which stabilize normal tetramers/multimers could prevent a very early event in PD pathogenesis.

Symposium 09: PROPAGATION OF TAU-PATHOLOGY

ADPD5-0718
THE ROLE OF LYSOSOMES IN TAU MISFOLDED STATE PROPAGATION
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Objectives: Tau levels are increased in the CSF of patients suffering from Alzheimer's disease but also from traumatic brain injuries and related tauopathies. Previous results have indicated that the presence of excess monomeric extracellular Tau may form a greater pathological risk to patients than hitherto suspected. We thus investigated the cellular pathway leading to exogenous Tau-derived aggregation inside cells with the aim to understand this pathway at a molecular level.
Methods: We apply super-resolution microscopy and multi-parametric imaging, both of which have been developed for the study of amyloid aggregation by our group. In particular, *d*STORM (*direct* stochastic optical reconstruction microscopy) permits imaging of amyloid species in two colours with a resolution of 20 nm, and FLIM (fluorescence lifetime imaging microscopy) permits the monitoring of amyloid aggregation in live cells with the help of a FRET sensor.
Results: We show that Tau first localises to endosomes and later to lysosomes with super-resolution microscopy. In lysosomes, exogenous Tau encounters endogenous Tau, the latter of which is naturally present due to normal physiological degradation of proteins. We further show that the low pH encountered in lysosomes is responsible for the formation of heterogeneous aggregates, the process of which can be inhibited by alkalising lysosomes.
Conclusions: Our results clearly point at lysosomes as key players in triggering Tau aggregation and thus propagation. Therefore, reducing the amount of Tau in the extracellular space could be a promising strategy to prevent disease progression.

Symposium 09: PROPAGATION OF TAU-PATHOLOGY

ADPD5-0750

ECTOSOMES: A NEW MECHANISM FOR NON-EXOSOMAL SECRETION OF TAU PROTEIN

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1. Objective

Tau, a microtubule-associated protein, aggregates in neurodegenerative disorders known as tauopathies but its secretion in the extracellular fluids may also play a role in neural network signalling. Once deregulated, secreted Tau probably participates in the spreading of Tau pathology in hierarchical pathways of neurodegeneration. The mechanisms underlying neuron-to neuron Tau transfer are still unknown. Given the place of extra-cellular vesicles (EVs) in cell-to-cell communication, we wondered whether they could involve secreted Tau. We then investigated in cell cultures, cell media and body fluids (rat and primate) if Tau might be present into 1) plasma membrane originating vesicles; the ectosomes and/or 2) in multi-vesicular bodies deriving vesicles; the exosomes.

2. Methods

EVs were purified by differential centrifugations from culture media of cell lines, neuronal primary cultures and brain interstitial fluid coming from *in vivo* models of Tauopathie. The presence of Tau was analysed using electron microscopy and biochemical assays.

3. Results

Among EVs and under basal conditions, Tau is mainly found in ectosomes, which supports the concept of a new physiological function for Tau. These specific vesicles enabled cytosolic Tau to be shuttled to the extracellular media. Moreover, the presence of Tau in vesicles coming from our pathological *in vivo* models also suggest that the over-accumulation of intra-cellular Tau results in targeting to MVBs, leading to release in exosomes.

3. Conclusions

This study brings new direct evidences that Tau transfers between cells at least via vesicular systems. This process may be involved in Tau pathology spreading.

Symposium 09: PROPAGATION OF TAU-PATHOLOGY

ADPD5-0952

PROPAGATION OF TAU PATHOLOGY BY ALZHEIMER PHF: SEEDING OF TAU ISOFORMS IN WILD-TYPE MOUSE AND IN CELLS

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Objectives: In Alzheimer's disease, the propagation of neurofibrillary tangles during progression of the disease follows neuroanatomical pathways and can reflect transysnaptic passage of abnormal tau proteins that recruit normal tau proteins in connected cells to transmit from cell to cell tau misfolding leading to aggregation. Internalisation in cells and experimental propagation in mice has been shown with synthetic tau fibrils and tau oligomers but has been less documented with bona fide "Paired helical filaments" (PHF) made of all tau isoforms extracted from human brain. This study was aimed at analyzing the propagation of tau pathology in mice after injection of human PHF, their toxic properties, and the internalisation of human PHF into cultured cells expressing different tau isoforms.

Methods : Sarkosyl-insoluble PHF were extracted from the frontal cortex of AD patients. PHF were stereotaxically injected into the dentate gyrus of wild-type mice, or added to the culture medium of cultured cells.

Results : After 3 months of incubation, mice injected with human PHF developed tau positive aggregates into neurons of the dentate gyrus that extended into Mossy fibres and away from the injection side. These tau aggregates were made only of mouse tau, and were Gallyas positive and immunoreactive with phosphotau antibodies and conformational tau antibodies. Human PHF internalized into cultured cells recruited all types of endogenous tau isoforms and tau bound to microtubules.

Conclusion : Human PHF have the ability to seed aggregation of normal tau isoforms in wild-type mice and in cells, forcing phosphorylation and conformational changes characteristic of human PHF-tau.

Symposium 09: PROPAGATION OF TAU-PATHOLOGY

ADPD5-1844
CELL TO CELL PROPAGATION OF TAU THROUGH THE EXTRACELLULAR SPACE AND THE IMPACT OF NEURONAL EXCITABILITY

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Objectives: To determine the mechanism by which tau can propagate between neurons in vitro and in vivo, and to determine the impact of neuronal hyperexcitability on tau propagation.

Methods: We have examined the transfer of endogenously generated tau between cells in physiologically relevant cell systems (primary cultures, microfluidics, induced neurons) and compared data to what is seen in affected circuits in a transgenic mouse model of tau propagation. Neuronal hyperexcitability has been induced by optogenetic stimulation and the propagation of tauopathy has been assessed in vitro, and through brian circuits.

Results: Endogenously generated full length tau can propagate between primary cell populations in culture. Tau is released into the conditioned medium and can be transferred to recipient cells suggesting that one mechanism by which it passes between cells is through the extracellular space. Uptake is via the endosomal pathways, and clearance mechanisms are induced to remove tau as it accumulates in recipient cells which identifies possible therapeutic targets to prevent tauopathy progression. Neuronal hyperexcitability leads to increased release of tau into the extracellular space, and altered distribution of tau in vivo.

Conclusions: Tau can spread between neurons via the extracellular space and propagation is accelerated by neuronal excitability. Amyloid induced hyperactivity seen in the AD brain may exacerbate the propagation of tauopathy and provide a link between the two pathologies.

Symposium 09: PROPAGATION OF TAU-PATHOLOGY

ADPD5-1928
TRANSMISSION OF CORTICAL BASAL DEGENERATION-LIKE GLIAL TAU PATHOLOGY: A NEW MODEL FOR THE PROGRESSION OF GLIA TAUOPATHIES

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Intracellular inclusions of filamentous tau proteins are the hallmark lesions of several neurodegenerative tauopathies such as Alzheimer's disease (AD) and corticobasal degeneration (CBD) which show cell type-specific and topographically distinct tau inclusions. Growing evidence supports the concept that templated transmission of tauopathies occurs through functionally interconnected neuroanatomical pathways suggesting that different self-propagating strains of pathological tau could account for the diverse manifestations of neurodegenerative tauopathies. Here, we describe the rapid and distinct cell type-specific spread of pathological tau following intracerebral injections of CBD or AD brain extracts enriched in pathological tau (designated CBD-Tau and AD-Tau, respectively) in young human mutant P301S tau transgenic (Tg) mice (line PS19) 6-9 mo before they show onset of the mutant tau transgene induced tau pathology. At 1 mo post-injection of CBD-Tau, tau inclusions developed predominantly in oligodendrocytes in the fimbria and external capsule with infrequent intraneuronal tau aggregates. In contrast, injections of AD-Tau in young PS19 mice induced tau pathology 1 mo post-injection that was predominantly in neuronal perikarya with little or no oligodendrocyte involvement. With longer post-injection survival intervals of up to 6 mo, CBD-Tau and AD-Tau induced tau pathology spread in different patterns to brain regions distant from the injection sites while maintaining the cell type specific pattern noted above, and only AD-Tau resulted in neuron degeneration. Thus, AD-Tau and CBD-Tau represent specific pathological tau strains that may underlie the distinct clinical and pathological features of these two tauopathies and these strains could become targets to develop disease-modifying therapies for CBD and AD.

Symposium 09: PROPAGATION OF TAU-PATHOLOGY

ADPD5-1941
TAU SPREADING AND PRION-LIKE PROPAGATION
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Tau pathology is characterized by intracellular aggregates of tau proteins. It is encountered in many neurodegenerative disorders, referred to as tauopathies. In the human brain, there are three tau isoforms (10-) with 3 (3R) repeats and three (10+) with 4 repeats (4R). All six Tau isoforms are aggregated in some diseases, such as AD, whereas the preferential aggregation of 3R or 4R isoforms occurs in other diseases, such as Pick's disease and progressive supranuclear palsy, respectively. In contrast to AD, where mutations have not been identified on the Tau gene (MAPT), patients presenting FTDP-17, exhibit Tau mutations. Mutant Tau proteins show a higher nucleation and fibrillogenesis than WT Tau, often leading to rapid neuronal death.
We took advantage of a new lentiviral rat model of tauopathy recently developed in our team to mimic tauopathies. Injection of lentiviral vectors encoding human wild-type (WT) or P332S Tau (3R&4R) in hippocampus resulted in NFD. However, with 3R tau WT and 3R and 4R P332S tau mutants, tau pathology was restricted to the injection site. Conversely, 4R WT human Tau protein was transferred from ventral hippocampus neurons to connected secondary neurons even at distant brain areas indicating a trans-synaptic protein transfer. Analysis of tau secretion in this rat model has been performed showing both secretion of extracellular vesicles and free forms of tau isoforms. Similar viral vector approach has been also developed in non-human primates.
Altogether, these animal models highlight differences in the molecular and cellular mechanisms underlying the pathological processes induced by tau isoforms, WT and mutant. Such observations allow for a better understanding of tau pathology propagation and the development of new approaches in diagnosis and therapeutic strategies for Tauopathies.

Symposium 10: INFLAMMATION 1

ADPD5-0328
INFLAMMATORY RESOLUTION IN ALZHEIMER'S DISEASE
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The initiation of an inflammatory response is critical to the survival of an organism. However, when inflammation fails to reach resolution (*i.e.*, repair/remodeling), a chronic inflammatory state may occur, and it becomes a major cofactor of many diseases, including Alzheimer's disease (AD). Comprehending the biological basis for altered innate immunity and inflammation in AD is a challenge that has substantial clinical importance, as restoration or preservation of immunological responses is likely to have a great importance to the lengthen of healthier lifespan. The discoveries that resolution of inflammation is a highly coordinated and active process controlled by endogenous pro-resolving and anti-inflammatory mediators, and that inflammatory cells undergo classical and alternative activation, highlight new potential molecular targets to regulate inflammation and treat chronic inflammatory diseases. Here, we will discuss novel findings from studies in human samples that demonstrate a severe impairment in signaling pathways associated with the regulation of inflammatory resolution. In addition, pre-clinical data will be presented to support the idea that restoring the activity of regulatory anti-inflammatory interleukins or pro-resolving lipid pathways can elicit protective immunity and mitigate AD-like pathology. In the future, it may be possible to generate therapies to regenerate and/or replace the endogenous inflammatory resolution pathways to prevent and/or treat AD.

Symposium 10: INFLAMMATION 1

ADPD5-0566
ACTIVATION OF THE BRAIN'S CHOROID PLEXUS FOR MONOCYTE TRAFFICKING TO THE CNS MITIGATES PATHOLOGY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder and the leading cause of senile dementia worldwide; its pathophysiology is associated with unresolved chronic neuroinflammation. In this disease, blood-borne monocyte-derived macrophages (mo-MΦ) have an role in mitigating the neuroinflammatory response, though their spontaneous entry to the CNS appears to be insufficient, and the signals which regulate their trafficking are poorly understood. Our group recently pointed to the brain's choroid plexus (CP), which forms the blood-cerebrospinal fluid-barrier (BCSFB), as a selective gateway through which mo-MΦ are recruited to the CNS following acute injury. Here we show, in 5XFAD transgenic mouse model of AD (AD-Tg), that mo-MΦ trafficking to the CNS is suppressed due to CP dysregulation of IFN-γ signaling, needed for transepithelial migration of leukocytes across the CP. Pharmacological as well as genetic manipulations in AD-Tg mice, which led to increased levels of IFN-γ at the CP, resulted in CP epithelial upregulation of leukocyte trafficking determinants, which was followed by mo-MΦ recruitment via the CP-CSF migratory pathway to cerebral sites of amyloid-beta (Aβ) accumulation, plaque removal in the hippocampus and the cortex, and reversal of cognitive decline. Collectively, our results suggest that lacking mo-MΦ infiltration to the CNS, due to CP dysfunction, takes part in AD pathophysiology, and thus point to the BCSFB as a target amenable for immunomodulation as a potential therapy for AD.
K.B. and N.R. contributed equally to this work.

Symposium 10: INFLAMMATION 1

ADPD5-0789

A COMMON PATHWAY LINKING NEURONAL INFLAMMATION TO CASPASE-6-MEDIATED AXONAL DEGENERATION IN THE NEURONS OF ALZHEIMER DISEASE.

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OBJECTIVES

Our overall goal is to determine the upstream activators of Caspase-6, an enzyme associated with Alzheimer disease (AD) pathology, age-dependent cognitive impairment and axonal degeneration. Inflammatory Caspase-1 activates Caspase-6 in human primary neurons and Caspase-1 is activated by inflammasomes. Inflammasomes have been well characterized in macrophages, but not in neurons. Here, our objective was to identify which neuronal inflammasome leads to Caspase-1-mediated Caspase-6 activation.

METHODS

The inflammasome receptors of human primary neurons, astrocytes and microglia were assessed by qRT-PCR and western blot analyses. A cell free inflammasome assay, neutralizing antibodies, and inflammasome and Caspase-1 siRNAs determined the functional neuronal inflammasome. Caspase-1 and Caspase-6 activities were measured by fluorogenic assays, and assessment of Caspase-1 or Caspase-6 cleaved proteins by western blotting or ELISA assays. Null mice were used to assess inflammasome-mediated Caspase-1 and Caspase-6 activation in brains.

RESULTS

Human CNS neurons expressed functional Nod-like receptor protein 1 (NLRP1), absent in melanoma 2 (AIM2), and ICE protease activating factor (IPAF-1), but not the NLRP3, inflammasome receptor components. NLRP1 antibodies and siRNAs impeded stress-induced neuronal Caspase-1 and Caspase-6 activation in neurons. Lipopolysaccharide induced Caspase-1 and Caspase-6 in wild-type, but not in *Nlrp1*$^{-/-}$ and *Casp1*$^{-/-}$ mice cortex. NLRP1 expression increased 25 to 30 fold and co-localized with Caspase-6 activity in AD cortical neurons.

CONCLUSIONS

CNS human neurons express functional NLRP1 inflammasomes, which activate Caspase-1 and subsequently Caspase-6. These results reveal a fundamental mechanism linking intraneuronal inflammasome activation to Caspase-1-generated interleukin-1-b-mediated neuroinflammation and Caspase-6-mediated axonal degeneration.

Symposium 10: INFLAMMATION 1

ADPD5-0819

INTRANEURONAL ABETA BURDEN AND PRECLINICAL CNS INFLAMMATION

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The efficacy of anti-inflammatories in delaying the onset of Alzheimer's disease (AD) is well documented, however, these drugs lack efficacy after clinical diagnosis. These observations would support the occurrence of a disease-aggravating inflammatory process during the preclinical stages of AD.

This presentation will illustrate the occurrence of a pro-inflammatory process in rat and mouse transgenic models of AD. We have observed inflammatory changes before the appearance of amyloid plaques and coincidental with the pathological accumulation of intracellular Aβ-immunoreactive material in hippocampal and cortical pyramidal neurons. The presence and identity of this intraneuronal material as Aβ peptides was documented by confocal and super resolution microscopy. The accompanying inflammatory process was characterized by the intermediate activation of microglia and their mobilization towards Ab-burdened neurons of the hippocampus and cerebral cortex.

At early pathological stages, in the McGill-R-Thy1-APP rat transgenic model, there is also overt astroglia activation. Both, the McGill mice and rat transgenic models show up-regulation of classical inflammatory markers such as Il-1b, COX, TNF-a and fractalkine (CX3CL1), months before the formation of extracellular amyloid plaques. This early pro-inflammatory process differs from the overt, late inflammation surrounding amyloid plaques with fully activated microglia and monocyte infiltration, and thus represents a singular pro-inflammatory modality.

These observations should have a new significance for understanding the AD pathology "ab initio", i.e. decades prior clinical diagnosis. They might also offer opportunities to find new biomarkers signalling an ongoing AD pathology as well as novel therapeutic targets for delaying or arresting disease progression.

Symposium 10: INFLAMMATION 1

ADPD5-1586
THE CELL-AUTONOMOUS ROLE OF ASTROCYTIC DOPAMINE D2 RECEPTOR IN THE SUPPRESSION OF NEUROINFLAMMATION

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Chronic neuroinflammation is a common feature of aging brain and some neurodegenerative disorders, including PD. However, the molecular and cellular mechanism underlying the regulation of innate immunity in the central nervous system remains elusive. In the present study, we investigate the role of astrocytic dopamine D2 receptor (Drd2) in the modulation of innate immunity. We demonstrate that global knockout mice lacking *Drd2*, but not *Drd1* or *Drd3*, showed remarkable inflammatory response in multiple CNS regions and increased vulnerability of nigral dopaminergic neurons to neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity. Astrocytes null for *Drd2* became hyper-responsive to immune stimuli. We found that αB-crystallin (Cryab) was one of downstream effectors of astrocytic Drd2 that might be responsible for regulating inflammatory response. Reduction of CRYAB, that is known to suppress neuroinflammation, was prominently detected in *Drd2*-null mice compared to wild-type counterparts. Interestingly, it was revealed that Cryab was markedly upregulated in the SN of PD brain. Cryab expression was also upregulated in reactive astrocytes in a neurotoxin-induced mouse PD model. Moreover, we showed increased expression of Cryab in cytoplasmic inclusions in a subset of glial cells in Parkinsonian brain. Gain- or loss-of-function studies showed that Cryab is critical for *Drd2*-mediated modulation of innate immune response in astrocytes. Furthermore, treatment of wild-type mice with a selective Drd2 agonist increased resistance of the nigral dopaminergic neurons to MPTP via partial suppression of inflammation. Our study suggests that astrocytic Drd2 activation is normally required for suppression of neuroinflammation via a Cryab-dependent manner and provides new strategy for targeting glia-mediated pathogenesis in PD.

Symposium 10: INFLAMMATION 1

ADPD5-1829

18F-GE180 PET IMAGING OF NEUROINFLAMMATION IN MICE DURING AGING AND ALZHEIMER'S DISEASE

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Objective: Chronic neuroinflammation plays an important role in aging and Alzheimer's disease (AD). *In vivo* imaging of microglial activation in patients may allow for selective monitoring of the progression of neuroinflammation and assessment of efficacy in therapeutic trials. The 18 KDa translocator protein (TSPO), a marker for activated microglia, has been used as a positron emission tomography (PET) tracer target to visualize cerebral inflammation *in vivo* in human and transgenic (Tg) mouse models.
Methods: We used the new ^{18}F-labeled GE180 TSPO PET tracer to investigate the differences in neuroinflammation between young wildtype (4 mo-old, n=4), old wildtype (26 mo-old, n=4), and old AD transgenic mice (26 mo-old, n=4).
Results: *In vivo* PET scans revealed an age-dependent elevation in whole brain uptake of ^{18}F-GE180 (peak-uptake and retention) in wildtype mice, with a further significant increase in old AD transgenic mice. A similar result was observed in hippocampal-specific uptake of ^{18}F-GE180 using co-registration of PET images with mouse brain MRI images. *Ex vivo* PET and autoradiagraphy confirmed these results and demonstrated an $SUV_{75\%}$ value reflecting enhanced uptake and specific binding of ^{18}F-GE180 in hippocampus and cortex in old AD transgenic > old wildtype > young wildtype mice. Specificity was confirmed by a cold tracer competition study. A metabolite study indicated that while total radioactivity declined over 2 hours, of the remaining radioactivity, ~90% was parent GE180.
Conclusions: We conclude that PET imaging with ^{18}F-GE180 may be useful for assessing brain inflammation in neurodegenerative diseases, and may be helpful for monitoring treatment.

Symposium 12: MITOCHONDRIA

ADPD5-0602
MITOCHONDRIA-TARGETED THERAPEUTICS FOR ALZHEIMER'S DISEASE
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We have previously demonstrated that alterations in mitochondrial dynamics precede the onset of memory phenotype and the development of amyloid plaques in three transgenic animal models of familial Alzheimer's Disease (FAD). The objective of the study was to develop a treatment to restore mitochondrial dynamics and function. Here we provide evidence that treatment with CP2, a member of a family of tricyclic pyrone compounds, restores axonal trafficking *in vivo*, and averts cognitive and motor deficit in multiple animal models of AD (APP, PS1 and APP/PS1) *in vivo*. Animals were administered CP2 via drinking water. Cognitive and behavior tests were applied at the end of a lifetime treatment (13 months) or short-term treatments (2 and 4 months). Changes in mitochondrial dynamics and function *in vivo* and *in vitro* were evaluated using real-time imaging of mitochondrial motility; an XF24 Seahorse Extracellular Flux Analyzer; the activity of OXFOS Complexes I-V was done using enzymatic reactions; and metabolic changes were measured using LC- and GC-MS-based metabolomics. Behavior and memory functions were detected with a battery of tests. Alleviation of the motor and memory phenotype was accompanied with partial reduction in amyloid burden. Investigation of the molecular mechanism revealed that CP2 modulates mitochondria energetics and activates a cascade of events protecting mitochondrial dynamics. Our findings, for the first time, demonstrate that restoration of mitochondrial trafficking protects against cognitive dysfunction in AD. Our data validate mitochondrial motility as an early therapeutic target for AD and poise CP2 as a promising therapeutic compound.

Symposium 12: MITOCHONDRIA

ADPD5-0771
MITOCHONDRIAL FUNCTION AND PARKINSON'S DISEASE: PINK1 REGULATES COMPLEX I ACTIVITY VIA NDUFA10 UBIQUINONE UNCOUPLING

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Objectives: PINK1, a mitochondrial kinase causes Parkinson's Disease (PD), has further strengthened the involvement of mitochondrial dysfunction in PD. The fact that PINK1 regulates Complex I enzymatic activity resulting in loss of mitochondrial membrane potential and compromised synaptic activity suggests a role for this gene in mitochondrial maintenance. However, the underlying molecular mechanisms remain to be elucidated.
Methods: Phosphoproteomics was performed on PINK1 mouse brain and liver to identify putative PINK1 substrates. Mouse and Drosophila PINK1 deficient model, human PINK1 patient fibroblasts and iPSC derived neuron were used to assess mitochondrial membrane potential, ATP content and Complex I enzymatic activity.
Results: Under resting conditions *Pink1* knockout cells and cells derived from patients with *PINK1* mutations display a loss of Complex I activity causing a decrease in the mitochondrial membrane potential. Analyzing the phosphoproteome of Complex I in *Pink1*$^{-/-}$ mice we found specific loss of phosphorylation of Ser^{250} in Complex I subunit NdufA10. Phosphorylation of Ser^{250} was needed for ubiquinone reduction by Complex I. Phosphomimetic NdufA10 reverse Pink1 deficits in mouse knockout cells and rescue mitochondrial depolarization and synaptic transmission defects in *pink*^{B9} null *Drosophila* mutant. Complex I deficits and ATP synthesis were also rescued in cells derived from PINK1 patients.
Conclusions: We show in three different PINK1 models that NdufA10 is phosphorylated in a PINK1-dependent fashion, and this modification is required for Complex I mediated ubiquinone uncoupling. This evolutionary conserved pathway contributes to the pathogenic cascade that eventually leads to Parkinson's Disease in patients with PINK1 mutations.

Symposium 12: MITOCHONDRIA

ADPD5-1374

REDOX-SENSITIVE RYR2-MEDIATED CALCIUM RELEASE HAS A KEY ROLE IN HIPPOCAMPAL LEARNING AND MEMORY DEFECTS PRODUCED BY SOLUBLE ABETA OLIGOMERS

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Objectives: We have reported that persistent RyR-mediated calcium signals invoked by soluble Aβ oligomers (AβOs) prevent the spine remodeling invoked by brain-derived neurotrophic factor, decrease RyR2 mRNA and protein contents and provoke mitochondrial fragmentation. Pre-incubation with N-acetylcysteine (NAC), an effective antioxidant precursor of glutathione, abolishes the cytoplasmic calcium increases and mitochondrial fragmentation induced by AβOs. Here, we investigated whether reactive oxygen species (ROS) modulate the RyR2 expression changes induced by AβOs and if decreased RyR2 expression affects hippocampal-dependent spatial memory. **Methods**: Primary hippocampal neurons were transfected with mito-Pericam or HyPerMito to detect mitochondrial calcium and hydrogen peroxide production, respectively, or incubated with MitoSox to sense mitochondrial superoxide generation. Mitochondrial fragmentation was detected in neurons loaded with MitoTracker. Male rats, bilaterally injected intra-hippocampus with AβOs or antisense oligonucleotide anti-RyR2 (O-RyR2), were trained in the Oasis Maze task to evaluate hippocampal-dependent spatial learning and memory. Fluorescence images acquired by confocal or spinning disk microscopy were analyzed with ImageJ software; RyR2 mRNA expression was evaluated by q-PCR and protein content by WB analysis. **Results:** Intra-hippocampal injections of AβOs or O-RyR2 decreased RyR2 protein content, without alterations in RyR3 protein levels, and impaired spatial learning. The decrease in RyR2 mRNA levels induced by AβOs required mitochondrial ROS generation, but not cytoplasmic ROS production by NOS or NOX. **Conclusions:** Our results suggest that redox-sensitive RyR2-mediated calcium release is crucial for spatial memory processes, and suggest that deficient RyR2-mediated calcium signaling contributes to AβOs-induced learning and memory deficits. **Support:** BNI (P-09-015F); FONDECYT (11110322, 1100052); CENEM-ICM-P10-001-F.

Symposium 12: MITOCHONDRIA

ADPD5-1453

P66SHC ADAPTOR PROTEIN – A PROMISING NOVEL TARGET IN ALZHEIMER'S DISEASE THERAPY?

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Objectives

The mammalian ShcA adaptor protein p66^{shc} has previously emerged as a key regulator of mitochondrial reactive oxygen species (ROS) production and an important mediator of Aβ-induced ROS generation and cytotoxicity *in vitro*. The involvement of p66^{shc} in mammalian longevity and life span determination has been demonstrated in the p66^{Shc} knockout mice which are characterized by a 30% prolonged lifespan, lower ROS levels, and protection from age-related impairment of physical and cognitive performance. These protective effects have partly been explained by a decrease in cellular ROS production and higher resistance to oxidative stress.

Methods

Building on the hypothesis that p66^{Shc} would also be protective against Aβ-induced mitochondrial dysfunction and ROS production *in vivo*, we investigated mitochondrial function in freshly isolated brain mitochondria of a newly generated p66^{Shc}-ablated APP transgenic mouse model of beta-amyloidosis.

Results

p66^{Shc}-ablated APP transgenic mice were characterized by improved brain mitochondrial respiration, increased ATP production and reduced ROS levels along with a reversal of Aβ-related cognitive deficits. These beneficial metabolic and cognitive effects were independent of Aβ levels and amyloid plaque deposition.

Conclusion

The results of our study suggest that p66^{Shc} inhibition may be an effective novel therapeutic approach against Aβ-induced mitochondrial and synaptic dysfunction *in vivo*.

Symposium 12: MITOCHONDRIA

ADPD5-1584
USE OF ISOGENIC HUMAN IPSCS TO ELUCIDATE REDOX-MEDIATED PATHWAYS TO AD/PD, AND OTHER NEUROLOGIC DISORDERS

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PD is initially characterized by loss of A9-type dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). An association has been reported between PD and exposure to mitochondrial toxins, including environmental pesticides paraquat, maneb, and rotenone. Here, using a robust, patient-derived stem cell model of PD allowing comparison of A53T α-synuclein (α-syn) mutant cells and isogenic mutation-corrected controls, we identify mitochondrial toxin-induced perturbations in A53T α-syn A9 DA neurons (hNs). We report a novel pathway whereby basal and toxin-induced nitrosative/oxidative stress results in S-nitrosylation of transcription factor MEF2C (to form SNO-MEF2C) in A53T hNs compared to corrected controls. This redox reaction inhibits the MEF2C-PGC1α transcriptional network, contributing to mitochondrial dysfunction and apoptotic cell death. Our data provide mechanistic insight into gene-environmental interaction (GxE) in the pathogenesis of PD. Furthermore, using small molecule high-throughput screening, we identify the MEF2C-PGC1α pathway as a therapeutic target to combat PD (Ryan et al., Cell 2013). Recently, we have found similar reaction mechanisms in other neurological diseases, forming SNO-MEF2A or SNO-MEF2C that enhances neuronal apoptosis in stroke and inhibits neurogenesis in AD (Okamoto et al. Cell Rep, 2014).

Symposium 12: MITOCHONDRIA

ADPD5-1815
NICOTINAMIDE RIBOSIDE INCREASES SIRT3 ACTIVITY AND PROTECTS AGAINST MPTP TOXICITY

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An optimal neuroprotective therapy for Parkinson's disease (PD) would prevent both the loss of dopaminergic neurons in the substantia nigra as well as their axonal projections. A promising approach is to increase intracellular nicotinamide adenine dinucleotide (NAD+), which activates sirtuins, exerts neuroprotective effects, and prevents axonal degeneration. NAD+ induces PGC-1alpha, a transcriptional coactivator which increases expression of antioxidant enzyme and mitochondrial biogenesis, and whose deficiency is linked to PD. SIRT1 protects against α-synuclein aggregation and increase life span in mice with the A53T α-synuclein. Nicotinamide riboside (NR) is a precursor to NAD+. NR increases NAD+ levels in several cell lines. We showed that NR increases NAD+ levels in brain tissue and in brain mitochondria. The magnitude of the increases exceeds two-fold in brain mitochondria an effect unknown for any other precursor to NAD+ We found that administration of NR to a neuronal cell line increased gene expression of SIRT1, SIRT3, PGC-1α, MnSOD, and downstream mitochondrial genes. NR treatment also protected cells dose dependently against MPP+ toxicity, restored ATP levels and protected against depletion of GSH. NR in vivo increased SIRT1 mRNA, and SIRT1, SIRT3, PGC-1α and mitochondrial electron transport chain complex protein levels. Nicotinamide riboside treatment attenuated MPTP induced dopamine depletion in the striatum, and loss of dopaminergic neurons in the SNpc. Lastly we showed that NR treatment of Q111 striatal cells produced dose-dependent increases in PGC1-alpha protein levels. These studies show that NR is a effective neuroprotective agent with great promise as a disease modifying therapy for PD.

Symposium 13: PROTEIN CREATION, FOLDING, MISFOLDING AND DEGRADATION

ADPD5-0946
AMYLOID BETA CATABOLISM GENERATES N-TERMINAL TRUNCATIONS THAT ARE LARGELY ASSOCIATED WITH THE PROCESS OF AMYLOIDOGENESIS AND PERPETUATION OF FIBRILLAR DEPOSITS
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Objective: A heterogeneous group of amyloid beta (Abeta) species constitute the parenchymal and cerebrovascular amyloid deposits in Alzheimer's disease (AD). Besides the classic full-length peptides, biochemical and proteomic analysis of AD deposits revealed high degree of Abeta heterogeneity at both N- and C-terminal ends likely resulting from the local action of multiple proteolytic enzymes. Interestingly, many of these fragments are also normal components of cerebrospinal fluid, suggesting their active participation in clearance mechanisms. Increasing evidence indicates that deficient brain clearance largely contributes to Abeta accumulation; thus, we compared the biophysical properties and lesion distribution of various N- and C-terminally degraded Abeta fragments to better understand their biological importance.
Methods: Synthetic homologues of *in vivo* identified truncated peptides were used to compare solubility properties, formation of beta-sheet-rich structures, binding to thioflavin T, self-oligomerization and formation of amyloid-like fibrils. Novel antibodies recognizing specific N- and C-terminal truncations were generated and employed to immunolabel amyloid deposits and conduct biochemical analysis in transgenic models and AD brains.
Results: N- and C-terminally truncated peptides exhibited completely different biophysical properties and brain tissue distribution. C-terminally degraded Abeta fragments were extremely soluble, did not convert to beta-sheet-rich structures, failed to aggregate or form fibrils and did not co-localize with plaque deposits. Contrastingly, those degraded at the N-terminus were poorly soluble, with high tendency to aggregate and fibrillize and specifically co-localize with Congo-red-positive plaque cores.
Conclusions: Degradation at the C-terminal-end of Abeta generates fragments likely associated to catabolic/clearance mechanisms while truncations at the N-terminus favor the process of amyloidogenesis.

Symposium 13: PROTEIN CREATION, FOLDING, MISFOLDING AND DEGRADATION

ADPD5-1206

IDENTIFICATION OF THE DEUBIQUITINASE USP8 AS A CRITICAL REGULATOR OF K63-LINKED UBIQUITIN CHAINS ON ALPHA-SYNUCLEIN IN DOPAMINERGIC NEURONS WITH LEWY BODIES.

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Objectives

A major neuropathological hallmark of PD is the accumulation of alpha-synuclein in ubiquitinated neuronal inclusions termed Lewy bodies. An important outstanding question is whether ubiquitination in Lewy bodies is catalysed by enzymes that are directly relevant to alpha-synuclein degradation and toxicity or represent a non-specific modification.

Methods

Fifteen Lewy body disease brains were used to study the composition of ubiquitin conjugates in inclusions and potential protein interactors, iPSc-derived dopaminergic neurons for localization studies and cell lines for biochemical assays of alpha-synuclein ubiquitination. Toxicity studies were performed by dopaminergic neuron- or eye-specific knockdown of relevant de-ubiquitinases in the alpha-synuclein *Drosophila* model.

Results

Using comparative analysis in human brains, we found that ubiquitin immunoreactivity in Lewy bodies is largely comprised of K63-linked ubiquitin chains, significantly reduced in the substantia nigra compared to the neocortex and inversely correlated with the up-regulation and pathological localisation of the deubiquitinase Usp8. Usp8 recognised alpha-synuclein and directly regulated its degradation by de-ubiquitinating preferentially K63-linked chains on alpha-synuclein. In human iPSc-derived neurons, Usp8 partly co-localised with alpha-synuclein and knockdown of Usp8 in dopaminergic neurons *in vivo* protected *Drosophila* from alpha-synuclein-induced locomotor deficits. Accordingly eye-specific Usp8 knockdown rescued the eye toxicity by reducing specifically the soluble pool of alpha-synuclein.

Conclusions

We present multiple lines of evidence suggesting that enhanced recycling of K63-linked ubiquitin chains by Usp8 is a pathogenic step in Lewy body biogenesis and a potential mechanism by which K63-linked ubiquitin-mediated trafficking of alpha-synuclein fails in dopaminergic neurons in Parkinson's disease.

Symposium 13: PROTEIN CREATION, FOLDING, MISFOLDING AND DEGRADATION

ADPD5-1216
THE ROLE OF TETRASPANIN 6 IN THE REGULATION OF THE AMYLOIDOGENIC PATHWAY

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Objective. Despite the fact that mutations on presenilins and APP genes trigger the Aβ deposition in patients with familiar AD, little is known about the biological processes behind the sporadic AD cases leading to Aβ deposition. Our laboratory demonstrated that specific proteins of the tetraspanins family modulate Aβ production. Based on these findings, we searched for tetraspanins associated to sporadic AD and affecting Aβ production. We centered on tetraspanin 6 (Tspan6) because its levels in AD brains are elevated. **Methods**. We used human brain samples to study Tspan6 levels in healthy people and AD patients. The biological function of Tspan6 and its impact on the amyloidogenic pathway was studied *in vitro*. Finally we created a Tspan6 knockout mouse in order to corroborate our findings *in vivo*. **Results**. Interestingly our findings suggest that Tspan6 enhances the amyloidogenic pathway by interacting and stabilizing the substrate for Aβ production, APP-CTF. We provide evidences of the role played by the autophagosomal/lysosomal pathway. **Conclusions**. We conclude that tspan6 levels are increased in sporadic AD brains and enhance Aβ production by stabilizing the APP-CTF levels. We determined that the impaired degradation of APP-CTF by the autophagosomal/lysosomal pathway is the mechanism driving this effect. Our study corroborates the importance of the lysosomal/autophagosomal pathway in sporadic AD and presents Tspan6 as a key player linking lysosomal-dependent degradation of APP-CTF and Aβ generation.

Symposium 13: PROTEIN CREATION, FOLDING, MISFOLDING AND DEGRADATION

ADPD5-1590
RIBOSOME FUNCTION UNDER PRESSURE AND IN DISEASES

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Ribosomes are essential for life of all organisms, as they generate all of the proteins required for cells existence and growth. They are macromolecular assemblies, built of long rRNA chains and many rProteins in stoichiometric ratios. These universal cellular machines translate the genetic code into proteins and perform their basic functions, namely decoding and creation of nascent chains in an almost identical mode in all living cells. However, recent evidence correlate stress and variations in the copy number of part of mRNA chains that code for ribosomal proteins. The likelihoods of actual translation of these mRNA chains, thus creating non stoichiometric ribosomes and their probable functional aspects will be discussed.

Symposium 13: PROTEIN CREATION, FOLDING, MISFOLDING AND DEGRADATION

ADPD5-1663
PROTEOSTASIS AND THE EVOLUTION PATHOLOGY IN ALZHEIMER'S DISEASE

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The extracellular accumulation of beta-amyloid peptide is a key trigger in the pathogenesis of Alzheimer's disease (AD). As the disease progresses, "secondary" intracellular protein aggregate pathology is formed by proteins such as Tau, α-synuclein, and TDP-43. How the deposition of amyloid triggers the "secondary" pathologies of AD is a topic of intense study. Over the past few years, we have pursued the hypothesis that diminished function of protein homeostatic machinery, trigged by amyloid deposition, may be a primary mechanism in the progression of AD. Although mice with Alzheimer-type amyloidosis do not develop the expected secondary pathology of human AD, we asked whether the accumulation of amyloid may produce abnormalities that would be indicative of altered proteostasis. We developed a sequential detergent extraction method, followed by SDS-PAGE separation of the various fractions, in-gel trypsin digestions, and LC-MS/MS proteomic approaches to identify proteins that lose solubility in the brains of APPswe/PS1dE9 (line 85) mice with high amyloid burden. By this approach, numerous cytosolic proteins were identified as becoming aberrantly insoluble (PMC3690965). These data provided in vivo evidence that the accumulation of extracellular amyloid can lead to diminished function of the intracellular protein homeostasis network. To investigate the mechanisms by which amyloid may interfere with proteostasis, we have begun working in cultured cell models to assess whether exposure to amyloid fibrils, oligomeric Abeta, or monomeric Abeta induces changes in cytosolic protein solubility as was seen in the mice. The outcomes of these studies will be presented at the meeting.

Symposium 13: PROTEIN CREATION, FOLDING, MISFOLDING AND DEGRADATION

ADPD5-1943

A NOVEL APPROACH TO AMELIORATE ALZHEIMER'S DISEASE PHENOTYPES USING A SMALL PEPTIDE DERIVED FROM A PHYSIOLOGICAL ACTIVATOR, P35, OF CYCLIN- DEPENDENT KINASE (CDK5).

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Besides the hallmark pathology of amyloid plaques and neurofibrillary tangles (NFTs), it has been reported that cyclin-dependent kinase 5 (Cdk5), a critical neuronal kinase in nervous system development, function and survival, is deregulated and hyperactivated in AD brains and is, in part, responsible for the AD and other neurodegenerative disease pathology. Under physiological conditions, Cdk5 activity is tightly regulated. The deregulation and hyperactivation of Cdk5/p25 induces neuropathology e.g.AD. Thus Cdk5/p25 becomes prime therapeutic target for AD and neurodegenerative diseases associated with the hyperactivation of Cdk5. In order to prevent hyperactivation of Cdk5/p25, we have designed several small peptides of p25 on the basis of Cdk5/p25 crystal structure and checked for competition with p25 and there by inhibiting the hyperactivity of Cdk5. We discovered a small peptide (p5) comprising of 24 amino acids, inhibited Cdk5 hyperactivation selectively in ***vivo.*** The modification of p5 to TFP5 crosses BBB, was tested in a transgenic AD model mouse. The p25 transgenic AD model (p25Tg) mouse was chosen since these mice show similar phenotypes to other AD model mice as well as in AD patients. Post TFP5 injections in p25Tg mice displayed significant reduction in Cdk5/p25 hyperactivity, A- beta plaque formation along with AD behavioral rescue. TFP5 does not inhibit normal Cdk5/p35 activity, and therefore has no toxic side effects. In addition, treated mice rescued synaptic dysfunction, neuroinflammation and a reduction in phospho-neurofilaments / tau and cell death. These results indicate that TFP5 has a potential to be a therapeutic target for AD.

Symposium 15: AMYLOID PRODUCTION AND ABETA TOXICITY

ADPD5-0488
AMYLOID BETA PROCESSING AND TOXIC MECHANISMS RELATED TO NEURONAL LIPID RAFTS.

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Lipid rafts are considered functional platforms where several proteins interact and initiate signal transduction. They also appear to be responsible for different aggregating processes, including amyloid beta oligomerization. Structural raft lipids are altered early during neuropathological development.
In this study, we have investigated whether amyloid beta formation and toxicity may be related to neuronal raft impairment.
Using lipid raft fractions isolated from human cortical brain areas as well as immortalized human neuroblastoma cells, we have analyzed by inmunoprecipitation, inmunochemistry and immunoblotting the potential differences in the presence of amyloid beta processing machinery (amyloid precursor protein, APP, and β/γ secretases) in Alzheimer's disease patients of early and late stages, as compared to healthy controls.
Our results demonstrated that b-secretase accumulates in lipid rafts of AD subjects even at the earliest stages, enhancing its interaction with APP. Interestingly, APP and the amyloid were shown to be associated with a voltage dependent anion channel (VDAC) involved in extrinsic apoptosis. This interaction induced VDAC dephosphorylation and neuronal death.
Overall, these results suggest that lipid raft impairments in AD are connected to protein rearrangements that promote amyloid beta formation and activation of toxic signaling pathways.
Supported by SAF2010-22114-C02-01/02

Symposium 15: AMYLOID PRODUCTION AND ABETA TOXICITY

ADPD5-0492

ALZHEIMER'S DISEASE-LIKE PATHOLOGY INDUCED BY ABETA OLIGOMERS IN NON- HUMAN PRIMATES

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder and a major medical problem. Here, we have investigated the impact of amyloid-β (Aβ) oligomers, AD-related neurotoxins, in the brains of rats and adult non-human primates (cynomolgus macaques). Soluble Aβ oligomers are known to accumulate in the brains of AD patients and correlate with disease-associated cognitive dysfunction. When injected into the lateral ventricle of rats and macaques, Aβ oligomers diffused into the brain and accumulated in several regions associated with memory and cognitive functions. Cardinal features of AD pathology, including synapse loss, tau hyperphosphorylation, astrocyte and microglial activation were observed in regions of the macaque brain where Aβ oligomers were abundantly detected. Tangles were be detected with different specific antibodies (PHF-1, Alz50, CP13 and MC1) and electron microscopy. Most importantly, oligomer injections induced AD–type neurofibrillary tangle formation in the macaque brain. These outcomes were specifically associated with Ab oligomers, as fibrillar amyloid deposits were not detected in oligomer-injected brains. Human and macaque brains share significant similarities in terms of overall architecture and functional networks. Thus, generation of a macaque model of AD that links Ab oligomers to tau and synaptic pathology has the potential to greatly advance our understanding of mechanisms centrally implicated in AD pathogenesis. Furthermore, development of disease-modifying therapeutics for AD has been hampered by the difficulty in translating therapies that work in rodents to humans. This new approach may be a highly relevant non-human primate model for testing therapeutic interventions for AD.

Symposium 15: AMYLOID PRODUCTION AND ABETA TOXICITY

ADPD5-0950
IN VITRO AND IN VIVO EFFECTS OF LENTIVIRAL BETA-SYNUCLEIN OVEREXPRESSION ON APP AND TAU PROTEIN

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Objectives:
Beta-Synuclein (Beta-Syn) belongs to the highly conserved Synuclein protein family. Alpha-Synuclein is the most prominent member as it aggregates in the so called Lewy Bodies, one major hallmark of Parkinson's disease. Remarkably, parts of its hydrophobic peptide backbone (NAC) have been also detected in amyloid plaques of Alzheimer's disease (AD) patients. Beta-Syn lacks the NAC region and is able to prevent Alpha-Synuclein and amyloid aggregation. In addition, Beta-Syn interferes with several intracellular regulatory and signaling pathways. Aim of this study was to clarify whether Beta-Syn overexpression can alter amyloid pathology in an *in vitro* and *in vivo* model of AD.
Methods:
In vitro, human neuroglioma cells expressing human Amyloid Precursor Protein (APP) infected with Beta-Syn expressing lentiviral particles were investigated. *In vivo*, mice overexpressing human APP with Swedish and London mutations ($hAPP_{SL}$) were injected with lentiviral particles encoding human Beta-Syn into the hippocampus. An empty lentiviral vector was used as negative control. Afterwards behavioral, biochemical and histological analyses were performed.
Results:
Exogenous Beta-Syn expression revealed differential effects on APP expression and amyloid aggregation depending on time and concentration. In addition, the influence of Beta-Syn on phosphorylation of Glycogen synthase 3beta (GSK3-beta), a known tau kinase, was shown in cells and the tg-mouse model. As a consequence tau phosphorylation was affected after overexpression of Beta-Syn *in vitro*.
Conclusion:
Depending on the concentration Beta-Syn seems to be able to ameliorate or deteriorate amyloid expression and aggregation. Furthermore, the results indicate an influence on tau phosphorylation by Beta-Syn via regulation of GSK3-beta.

Symposium 15: AMYLOID PRODUCTION AND ABETA TOXICITY

ADPD5-1566

THE ROLE OF THE AMYLOID PRECURSOR PROTEIN IN NEURAL STEM CELL PROLIFERATION AND DIFFERENTIATION

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The amyloid-β precursor protein (APP) has been very well studied for its role in Alzheimer's disease. However, the normal function of APP remains uncertain. A large number of studies have suggested that APP has a trophic role in stimulating neuronal cell growth. Our own studies have shown that APP can stimulate the proliferation and neuronal differentiation of neural stem or progenitor cells (NSPCs). The effect of APP on NSPC proliferation is mediated by secreted cystatin C. In the present study, we examined the role of APP in NSPC differentiation into neurons. Cystatin C did not induce NSPC differentiation. Therefore, to identify proteins that may mediate the effect of APP on NSPC differentiation, we used a gene array approach to identify genes whose expression correlated with APP-induced neurogenesis. The expression of neurogenin 2 (Ngn2), which is a basic helix-loop-helix transcription factor, was significantly downregulated in NSPCs from APP knockout mice (APPKO). The expression of Ngn2 was increased in NSPCs from APP overexpressing (Tg2576) mice. Ngn2 overexpression in APPKO NSPCs promoted neuronal differentiation, whereas siRNA knockdown of Ngn2 expression in wild-type NSPCs decreased neuronal differentiation. The results demonstrate that APP-stimulated neuronal differentiation of NSPCs is mediated by Ngn2. These experiments are likely to have implications for understanding APP's trophic function in the adult or aging brain.

Symposium 15: AMYLOID PRODUCTION AND ABETA TOXICITY

ADPD5-1624
ADAM10 ENDOCYTOSIS: LOOKING FOR NEW STRATEGIES FOR ALZHEIMER'S DISEASE THERAPY

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Objectives: ADAM10 is the enzyme responsible for preventing Abeta production, thus fostering its activity represents a suitable strategy for Alzheimer's disease (AD) therapy. ADAM10 is active towards its substrates when properly inserted in the postsynaptic membranes. We have recently identified a binding motif for the clathrin adaptor AP2 in ADAM10 tail, which is relevant to ADAM10 endocytosis and to its synaptic localization/activity. Moreover ADAM10/AP2 interaction is significantly increased in AD patients compared to control subjects. We aim at developing cell permeable peptides (CPPs) able to interfere with ADAM10/AP2 complex and, thereby, to reduce ADAM10 endocytosis in order to increase ADAM10 membrane levels/synaptic activity.
Methods: We designed four CPPs. Taking advantage of several approaches we validated CPPs efficacy and specificity both different systems.
Results: We observed that two CPPs were able to interfere with ADAM10/AP2 association. In neuronal cultures the CPPs disrupt ADAM10/AP2 interaction, decrease ADAM10 internalization rate and increase the levels of ADAM10 at synaptic membranes. Moreover they are specific because they do not affect AP2 binding to other partners. We performed acute in vivo treatment and we verified that CPPs are able to cross the blood-brain barrier and are effective in vivo. Indeed, CPPs treatment reduces ADAM10/AP2 binding and increases ADAM10 synaptic levels/activity.
Conclusions: A finely balanced membrane level of ADAM10 is an essential prerequisite to control enzyme activity. We designed a powerful tool able to interfere with mechanisms regulating ADAM10 intracellular trafficking and to modulate its membrane availability, and thereby to shift APP metabolism towards the non-amyloidogenic pathway.

Symposium 15: AMYLOID PRODUCTION AND ABETA TOXICITY

ADPD5-1744
ACAT INHIBITION AND APP PROCESSING

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Alzheimer's disease brains exhibit large deposits of aggregated amyloid β-protein (Aβ). We have previously found that inhibition of the cholesterol-modifying enzyme acyl-coenzyme A: cholesterol acyltransferase (ACAT) results in decreased amyloid pathology. ACAT inhibitors used in cells or AD mouse models and knockdown or knockout of ACAT1 in cells all reduced Aβ production. An independent group has also confirmed our findings in ACAT1 knockout animals. Our recent studies have focused on the mechanism by which ACAT1 inhibition decreases Aβ levels. First, we found that the amyloid precursor protein (APP) is palmitoylated in the ER and palmitoylated APP (*pal*APP) is preferentially targeted to lipid rafts. *Pal*APP appears to be a better β- than α-secretase substrate, especially in lipid raft fractions. APP processing and Aβ levels directly correlate with the amount of *pal*APP in experiments using palmitoyl acyltransferases or palmitoylation inhibitors. Palmitoylation promotes APP dimerization as *pal*APP is three times more likely to form dimers than total APP. Interestingly, we found that ACAT activity regulates palmitoylation of APP. Cells lacking ACAT activity almost entirely lack *pal*APP and do not produce detectable levels of Aβ. Two different ACAT inhibitors decrease total *pal*APP by about 50% and severely reduce lipid raft-associated *pal*APP in cells, while also inhibiting APP processing. Co-immunoprecipitation and FLIM/FRET assays showed that APP dimerization is reduced by ~50% in cells treated with the ACAT inhibitors. Taken together, our data suggest that ACAT inhibitors reduce *pal*APP-dimers to lower Aβ generation and further support development of ACAT inhibitors as a therapeutic strategy for AD.

Symposium 17: NEURONAL NETWORKS AND SYNAPTIC FUNCTION

ADPD5-1063
INCREASED IN VIVO APP INTRACELLULAR DOMAIN (AICD) PRODUCTION IN CA1 HIPPOCAMPAL NEURONS AFFECTS GLUTAMATERGIC SYNATIC FUNCTION.

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Alzheimer's disease (AD) is a neurodegenerative disease that begins as mild short-term memory deficits and culminates in total loss of cognition and executive functions. Until recently, a main culprit of the disease, resulting from Amyloid-Precursor Protein (APP) processing, has been thought to be amyloid-b peptide (Ab). However, despite the genetic and cell biological evidence that supports the amyloid cascade hypothesis, it is becoming clear that AD etiology is complex and that Ab alone is unable to account for all aspects of AD [Pimplikar *et al.* J Neurosci.30: 14946. 2010]. It is, therefore, currently urgent to identify other possible culprits of the disease in the hope to develop alternative therapeutic strategies. Gamma-secretase not only liberates Ab, but also its C-terminal intracellular counterpart called APP intracellular domain (AICD) [Passer. et al. JAlzheimers Dis.2: 289-301. 2000], which is known to also accumulate in AD patient's brain [Ghosal et al. PNAS.106:18367. 2009], but surprisingly little is known about its functions in hippocampus.

To address this crucial issue, we increased AICD production *in vivo* in adult CA1 pyramidal neurons, mimicking the human pathological condition. Different ex-vivo electrophysiological and pharmacological approaches, including double- patch of neighbor neurons were used.

We clearly demonstrate that AICD strongly affects glutamatergic synaptic transmission. We further show that this effect impairs synaptic plasticity, which we were able to rescue by different pharmacological approaches.

Our results provide convincing and entirely novel evidence that increased *in vivo* production of AICD is enough, *per se*, to cause synaptic dysfunction in CA1 hippocampal neurons.

Symposium 17: NEURONAL NETWORKS AND SYNAPTIC FUNCTION

ADPD5-1573
DYSREGULATION OF EEF1A EXPRESSION IS ASSOCIATED WITH SYNAPTIC PLASTICITY IMPAIRMENTS IN ALZHEIMER MODEL MICE

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Objective: Synaptic dysfunction has been linked to pathogenesis of Alzheimer's disease (AD). Understanding molecular mechanisms underlying AD-associated synaptic failure may help identify novel therapeutic targets for the disease. Previous studies implicated a critical role of eukaryotic translation elongation factor 1A (eEF1A) in maintaining long-term synaptic plasticity and memory. Our objective is to investigate whether eEF1A expression associated with neuronal plasticity is compromised in AD, and if so, can restoring eEF1A levels help mitigate impairments of synaptic plasticity in AD model mice. **Methods:** Transgenic mice including AD model mice (APP/PS1 and Tg2576), and brain-specific *Tuberin* (TSC2) knockdown mice were used. Electrophysiology experiments were performed on hippocampal slices to measure long-term potentiation (LTP), induced at CA3-CA1 synapses. Western blotting and immunofluorescence combined with confocal microscopy were performed on brain tissue samples, including human AD. **Results:** Levels of eEF1A (CA1 regions of hippocampus) in AD model mice were reduced, compared to those of wild type littermates. Further, treatment of slices with mTORC1 inhibitor rapamycin caused down-regulation of eEF1A levels in wild type, but no effect on AD model mice. Moreover, forskolin (PKA agonist)-induced eEF1A expression was blunted in AD model mice. Finally, amyloid beta-induced LTP failure was alleviated in slices prepared from TSC2 knockdown mice, in which the mTORC1 signaling (upstream regulator of eEF1A expression) is elevated. **Conclusions:** Together, our data indicate that eEF1A dysregulation may contribute to the synaptic plasticity deficits displayed in AD, thus provide insights into molecular mechanisms and accordingly novel therapeutic targets for the disease.

Symposium 17: NEURONAL NETWORKS AND SYNAPTIC FUNCTION

ADPD5-1765
MECHANISMS AND TREATMENT OF NEURAL NETWORK DYSFUNCTION IN DEMENTING DISORDERS

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Dementing disorders are associated with profound alterations in the activity and connectivity of neural networks, including shifts in spectral power of network oscillations, abnormalities in behavior-associated modulations of brain rhythms, and hypersynchrony that can escalate into epileptic activity. Studies in rodent models suggest that such network dysfunctions is caused by co-pathogenic interactions between proteins that characteristically accumulate in the brains of patients with dementing disorders, particularly amyloid-beta, tau and alpha-synuclein. Potential underlying mechanisms include depletion of specific sodium channels from inhibitory interneurons, imbalances in the activation of intra- versus extra-synaptic NMDA receptors, and changes in intrinsic neuronal excitability. These abnormalities and the network dysfunction they cause likely contribute to cognitive decline. Over time, they may also promote detrimental changes in gene expression and neurodegenerative processes. For example, aberrant network activity can lead to an accumulation of neuronal activity-induced DNA double strand breaks, possibly by interfering with effective DNA repair. Interventions that prevent or reverse network dysfunction and related pathogenic cascades in animal models include reduction of tau, overexpression of the anti-aging factor klotho, treatment with specific anti-epileptic drugs, and enhancement of interneuronal functions. The potential relevance of these models and therapeutic strategies to dementing disorders will be discussed.

References: *Ann. Neurol.* 76: 443 (2014), *Cell* 148: 1204 (2012), *Cell* 149: 1 (2012), *Cell Rep.* 22: 1065 (2014), *JAMA Neurology* 70: 1158 (2013), *Nat. Neurosci.* 16: 616 (2013), *Neuron* 70: 410 (2011), *PNAS* 109: E2895 (2012)

Supported by US National Institutes of Health, S.D. Bechtel, Jr. Foundation, MetLife Foundation, and AFAR

Symposium 17: NEURONAL NETWORKS AND SYNAPTIC FUNCTION

ADPD5-1889

COGNITIVE IMPAIRMENT CAUSED BY NON-AMYLOID-RELATED PROTEINS IN THE BRAIN

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Objective

Amyloid fibrils containing in-register β-sheets accumulate as Aβ-containing amyloid plaques and tau-containing neurofibrillary tangles in Alzheimer's disease (AD), but they do not appear to be directly involved in the pathogenesis of AD. The goal of this talk is to report on the results of recent studies to determine whether non-amyloid-related Aβ and tau proteins, which lack in-register β-sheets, may be involved in the pathogenesis of AD, by evaluating their effects on cognition in mouse models.

Methods

Fibrillation assays or polyclonal OC antibodies were used to identify amyloid-related tau and Aβ proteins; Aβ and tau proteins that were non-reactive or non-fibrillar were defined as non-amyloid-related. Tests of spatial reference memory were used to assess the effects of these proteins on cognition in various lines of transgenic mice expressing tau and APP variants.

Results

Two non-amyloid-related proteins associated with impaired cognition were identified; one tau and one Aβ protein. In rTg4510 mice, a 35 kDa non-amyloid-related tau cleavage product (TCP35), formed by proteolysis by caspase-2, correlated with impaired memory, and reducing caspase-2 levels lowered TCP35 and restored memory. In J20, Tg2576 and APP/TTA mice, there were non-amyloid-related Aβ oligomers, including Aβ*56, which correlated with impaired learning and memory. In APP/TTA mice, reducing APP selectively reduced non-amyloid related Aβ oligomers and improved learning and memory.

Conclusion

Two non-amyloid-related tau and Aβ proteins have been shown to disrupt cognitive function in mice, suggesting they may be directly involved in the pathogenesis of AD and therefore useful targets for diagnosing and treating AD.

Symposium 17: NEURONAL NETWORKS AND SYNAPTIC FUNCTION

ADPD5-2048
NOT ONLY PRESYNAPTIC TERMINALS: ALPHA-SYNUCLEIN EXCESS MODIFIES THE EXCITABILITY OF POST-SYNAPTIC NEURONS AT RISK IN PARKINSON'S DISEASE

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The pathological impact of alpha-synuclein (aSyn) overexpression (ASOX) is typically identified with presynaptic terminals where aSyn interacts with phosphatidylinositol 4,5-bisphosphate (PIP2). However, PIP2 is also a potent modulator of voltage-activated calcium (Cav) channels that are major determinants of post-synaptic neuronal excitability. Calcium influx through Cav channels gives rise to excessive mitochondrial oxidative stress (mOS) in neurons vulnerable in Parkinson's disease (PD). Here, we report that ASOX, which is also associated with excessive mOS, alters calcium influx while maintaining normal autonomous pacemaking in vulnerable neurons.

We found that Cav currents in vagal motoneurons of the A53T-haSyn mouse that overexpresses mutant (A53T) human aSyn were down-regulated and that the voltage half-activation of Cav channels was depolarized relative to wild-type animals. Together, these modifications reduced the calcium influx associated with individual action potentials, while maintaining normal pacemaking rates. Furthermore, we identified an upstream control of these dramatic Cav channel phenotypes, such that up-regulation of microRNA silencers of Cav1.2 channels was accompanied by a down-regulation of neuronal Cav1.2 and Cav2.3 channel transcripts. This is likely to represent an adaptive response of DMV motoneurons to the aSyn insult. Preliminary results indicate that Cav channel down-regulation reflects modified binding of aSyn to PIP2. Taken together, our findings point to a novel pathophysiological role of aSyn and a novel neuroprotective strategy that identifies PIP2 as a viable therapeutic target to regulate mOS in pacemaking neurons, such as mesencephalic dopamine neurons, whose degeneration and Lewy body accumulation more directly impacts PD symptoms.

Support: Michael J. Fox Foundation.

Symposium 17: NEURONAL NETWORKS AND SYNAPTIC FUNCTION

ADPD5-2317
ABETA INDUCED SYNAPTIC TOXICITY
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Increasing evidence favors the synapse as one of the initial sites of neuronal damage by amyloid b-protein (Aβ) and this synaptic injury is thought to underlie the cognitive deficits seen in Alzhiemer's disease. An interesting picture has emerged whereby neuronal activity augments processing of the amyloid precursor protein (APP) to enhance Aβ production and release, which in turn depresses synaptic activity and synaptic plasticity as well as causing synapse loss. However, while a number of plausible pathways have been proposed, the molecular mechanisms of Aβ-induced synaptic injury remain to be clearly elucidated. Of these, one centers on the involvement of APP in Aβ-initiated synaptic damage. Specifically, previous studies have indicated that caspase cleavage of APP in the cytosolic domain after the aspartate residue at position 664 leads to neuronal injury, possibly through release of a peptide containing the last 31 amino acids the C-terminus of APP, coined C31. However, transgenic mice overexpressing human APP with the D664A mutation, engineered to abrogate this cleavage event, produced mixed results. Consequently, it is unclear whether cleavage of APP at position 664 is synaptotoxic in brain. In this presentation, I will present data on expression of the D664A construct in organotypic slice cultures as well as from an APP knockin mouse line. Both sets of experiments do show that substituting alanine for aspartate at this position attenuates Aβ-induced synaptic injury. It remains unclear, however, whether it is specifically the loss of C31 that is responsible for this protective effect.

Symposium 20: DISEASE MECHANISMS

ADPD5-0383
SHARED MOLECULAR MECHANISMS IN ALZHEIMER'S DISEASE AND ALS: NEUROFILAMENT-DEPENDENT TRANSPORT OF SAPP, FUS, AND TDP-43 WITH ENDOPLASMIC RETICULUM TUBULES

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Objectives. Amyotrophic lateral sclerosis (ALS), a debilitating neurodegenerative disorder of brainstem and motor neurons, involves the deterioration of the neuromuscular junctions by poorly understood mechanisms. The proper function of the neuromuscular junction requires sAPP, a proteolytic fragment of amyloid-beta precursor protein (APP) - a transmembrane protein implicated in the pathology of Alzheimer's disease (AD) - and also the RNA binding proteins TDP-43 and FUS; these proteins cause ALS when mutated. A general trait of ALS-afflicted neurons is the disorganization of neurofilaments (NFs). Now, we show that the anterograde transport of sAPP, TDP-43, and FUS occurs together with endoplasmic reticulum (ER) tubules, and requires peripherin NFs. **Methods.** The transport of sAPP, TDP-43, FUS, and the translocation of the ER protein Reticulon 4 (Rtn4) into neurites was studied in CAD cells, a brainstem-derived neuronal cell line relevant to AD and ALS. **Results.** We show that sAPP is generated in the soma, at a juxtanuclear ER subdomain colocalized with, and stabilized by, NFs. Along neurites, sAPP localizes to Rtn4-positive ER tubules that extend from the soma into the growth cone, and colocalize with peripherin NFs. Knocking down peripherin with siRNA disrupts the NF network, and diminishes the accumulation of sAPP, TDP-43, FUS, and Rtn4 at terminals. **Conclusions.** We propose that deficient ER translocation into neurites prevents the transport of proteins with essential function at the synapse, including sAPP, TDP-43, and FUS, and could be part of the mechanisms leading to AD and ALS. Support: NIH AG039668 (Z.L.M.), New Jersey Health Foundation (Z.L.M., V.M.).

Symposium 20: DISEASE MECHANISMS

ADPD5-1067
MICROGLIA INVOLVED IN A COMMON CELLULAR MECHANISM OF NEURODEGNERATION

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Genetic variants in the triggering receptor expressed on myeloid cells 2 (TREM2) have been linked to Nasu-Hakola disease, Alzheimer's disease (AD), PD, amyotrophic lateral sclerosis, frontotemporal dementia (FTD) and FTD-like syndrome without bone involvement. TREM2 is an innate immune receptor preferentially expressed in microglia and involved in inflammation and phagocytosis. Whether and how TREM2 missense mutations affect TREM2 function is elusive. Here we report that missense mutations associated with FTD and FTD-like syndrome reduce TREM2 maturation, abolish shedding by ADAM proteases and impair phagocytosis. As a consequence of reduced shedding TREM2 is virtually absent in the cerebrospinal fluid (CSF) and plasma of a patient with FTD-like syndrome. Lower levels of TREM2 were also observed in CSF of AD and FTD patients further supporting that reduced TREM2 function may contribute to the risk for two prominent neurodegenerative disorders.

Symposium 20: DISEASE MECHANISMS

ADPD5-1481

INCREASE OF CSF AMYLOID-BETA DURING THE INITIAL STAGE OF CEREBRAL AMYLOID-BETA DEPOSITION IN MOUSE MODELS

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Background and objectives: Alzheimer's Disease (AD) is thought to start decades before the first clinical symptoms emerge. The diagnosis of patients at this 'Pre-clinical AD' stage relies exclusively in fluid (CSF) or imaging (MRI or amyloid-PET scans) biomarkers and is limited by the lack of long-term biomarker profile changes and lack of pathological confirmation of the disease in such patients. To this end APP transgenic (APP-tg) mouse models can be consistently used for translational biomarker research, as AD pathology is easily assessable, followed over time and can be correlated directly to the fluid biomarker changes of the same animals.

Methods: We assessed CSF Aβ40 and 42 levels in 3 different APP-tg mouse models (APP23, APP24 and APP51) and compared the observed biomarker changes to Aβ pathological and biochemical changes in the brain.

Results: We observed a temporary 20-30% increase in CSF Aβ 40 and 42 in all the models before the CSF Aβ42 decline associated to Aβ-pathology progression. Remarkably, such initial CSF Aβ peak coincided with the first plaques appearing throughout the brain cortex, which corresponded to the initial increase in brain Aβ levels in every model. Increased brain APP processing and insufficient brain to blood Aβ clearance, probably underlie this finding in the early stages of Aβ deposition in mice.

Conclusion: If confirmed in humans, our observation may constitute the first detectable biomarker changes in AD pathological process, opening new perspectives in patient selection and stratification for preventive treatment strategies and in the discovery additional early 'Pre-clinical AD' biomarkers.

Symposium 20: DISEASE MECHANISMS

ADPD5-1685
MOLECULAR PATHOLOGY AND TRANSLATIONAL APPROACHES ON ALZHEIMER'S DISEASE

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Deposition of amyloid beta peptides (Abeta) as senile plaques and that of tau as neurofibrillary changes are the hallmark neuropathological lesions of Alzheimer's disease (AD), which are implicated in its pathogenesis and deemed as the prime target for the disease-modifying therapies (DMT). Abeta is produced by sequential proteolytic cleavages by beta- and gamma-secretases. gamma-Secretase, harboring presenilins (PS) as the catalytic center, forms the C terminus of Abeta that determines its propensity to aggregate: missense mutations in PS genes cause familial AD by altering the preferred gamma-secretase cleavage sites to increase production of pathogenic Abeta42 species. Recently, the relationship between neural activity and secretion or extracellular release of Abeta and tau is highlighted as the key events underlying the progression of the AD pathology, which may be novel targets for DMT of AD. Efforts to clinically develop the DMTs for AD, including establishment of imaging and fluid biomarkers that surrogate the AD pathology, through longitudinal multi-center clinical studies like AD Neuroimaging Initiative (ADNI), are underway worldwide, towards the goal of very early treatment hopefully at the preclinical AD stage. The current status of Japanese ADNI will also be presented.

Symposium 20: DISEASE MECHANISMS

ADPD5-1885
IMPACT OF CO-MORBIDITIES ON ALZHEIMER'S PATHOGENESIS AND COGNITIVE FUNCTION IN MOUSE MODELS

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"Impact of co-morbidities on Alzheimer's pathogenesis and cognitive function in mouse models."

Introduction: The etiology of Alzheimer disease (AD) is complex, as a combination of genetic and epigenetic causes along with lifestyle factors contribute to disease progression. Additionally, elderly subjects with AD suffer from a variety of co-morbidities including stroke (ischemia), stress, diabetes, seizures, osteoporosis, cancer, and renal disease. Understanding the molecular interactions between co-morbid disorders is of critical significance, and to date, largely remains an unexplored area of investigation. Here, we seek to determine the effect of three clinically relevant co-morbid conditions (including, stress, cerebral ischemia and diabetes) on AD pathology and cognition.

Methods: To properly address this question, we used a combination of newly developed transgenic mouse models along with biochemical, histological and behavioral approaches to elucidate the underlying molecular mechanism by which several comorbid conditions impact AD pathogenesis and cognition.

Results: Our studies show that stress, cerebral ischemia and diabetes can contribute to AD pathogenesis by modulating both Ab and tau pathology and profoundly affecting synaptic and cognitive function.

Conclusions: Our studies reveal that comorbid conditions (i.e. stress, cerebral ischemia and diabetes) are key factors that trigger AD pathogenesis and severely affect synaptic and cognitive function. Therefore, therapies aimed to reduce these comorbid conditions (i.e. stress, cerebral ischemia, and diabetes) might be a promising approach to mitigate AD pathology and alleviate cognitive impairments.

Symposium 20: DISEASE MECHANISMS

ADPD5-2226
USING INDUCED PLURIPOTENT STEM CELLS TO PROBE MECHANISMS OF ALZHEIMER'S DISEASE

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Although animal models of Alzheimer's disease have been very useful, they have not yet led to an effective treatment or a complete understanding of what goes wrong in the human brain during AD. Human in vitro models derived from human induced pluripotent stem cells (hIPSC) may prove to be of substantial utility given that they are euploid and can differentiate to bona fide cell types. In addition, purified cells with controlled genetic background and content can be cultured and differentiated together to develop functional cell structures composed of different cell types and genomes. In my talk, I will discuss our recent progress including a description of our drug screening efforts with FAD APP duplication neurons, which have turned up some very interesting potential leads. I will also summarize our efforts studying and comparing early phenotypes of FAD mutations in isogenic backgrounds. Finally, I will discuss how we are using naturally occurring variants that increase or decrease risk of developing AD in the human population using hIPSC to study complex human haplotypes.

Symposium 22: SEEDING, SPREADING, AND PRION-LIKE MECHANISMS

ADPD5-0528
PEAKS AND CLIFFS IN THE PATHOGENESIS OF PRECLINICAL PRION DISEASE

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Objectives: Prion diseases exhibit a preclinical phase that can last for decades followed by rapid progression after clinical onset. We hypothesized that down-regulation noted for the PrP-like shadoo protein during prion infections[1] might also apply to PrP^{C}, the precursor to PrP^{Sc}. We set out to measure PrP^{C} reductions and their relationship to a two-step model of disease pathogenesis[2].

Methods: Sucrose gradient fractionations of prion-infected brains in conjunction with conformation dependent immunoassay (CDI), scrapie cell assay (SCA) and *in vitro* misfolding reactions.

Results: PrP^{C} is quantitatively reduced at endpoint in rodent models of scrapie, CJD and CWD and altered qualitatively in glycotype profile[3]. We now show that reduction occurs at or before the midpoint of the preclinical phase, with the kinetics of PrP^{C} down-regulation in two paradigms matching plateau effects for infectivity. Wt and *Prnp*$^{+/o}$ mice at disease end-stage had different profiles of infectivity and PrP isoforms.

Conclusions: As PrP^{C} is the precursor for the generation of misfolded forms of the prion protein and is also required for pathogenic signaling from misfolded PrP, down-regulation must impact disease pathogenesis; this effect may represent a generalized host defence mechanism and is the likely cause of a plateau in infectivity[1]. However, the concept of a lethal PrP isoform (PrP^{L}) common to wt and *Prnp*$^{+/o}$ mice at disease end-stage[1] was not supported by CDI and SCA analyses.

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Symposium 22: SEEDING, SPREADING, AND PRION-LIKE MECHANISMS

ADPD5-0962
EXPERIMENTAL TRANSMISSIBILITY OF ALZHEIMER PATHOLOGY IN A NON-HUMAN PRIMATE

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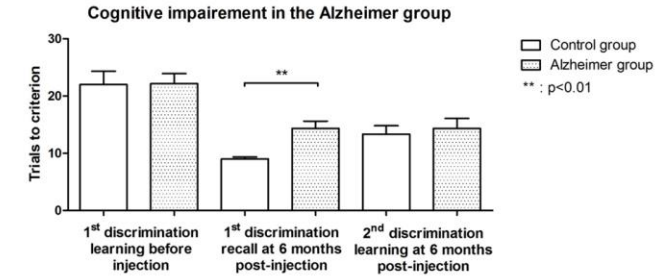
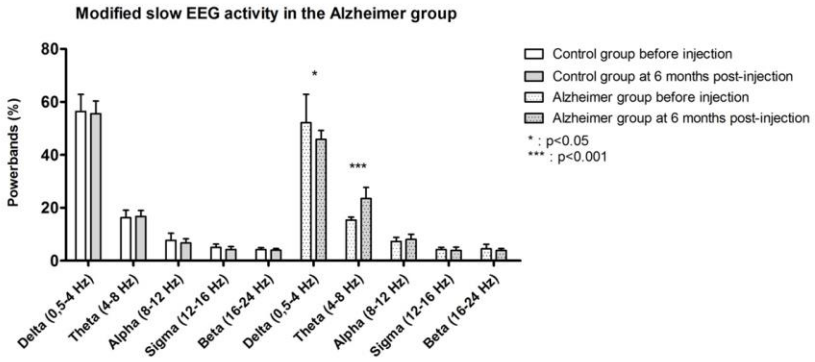
Objectives: Alzheimer's disease (AD) is a fatal chronic neurodegenerative disorder characterized by the intracerebral accumulation of abnormal beta-amyloid peptides and tau proteins. In transgenic mice overexpressing mutated forms of these proteins, the intracerebral injection of brain extracts containing these lesions accelerates the occurrence of amyloid and tau pathologies [1, 2]. We aim to determine if administration of human Alzheimer brain homogenates can also induce spontaneous amyloid and tau pathologies in the mouse lemur primate.

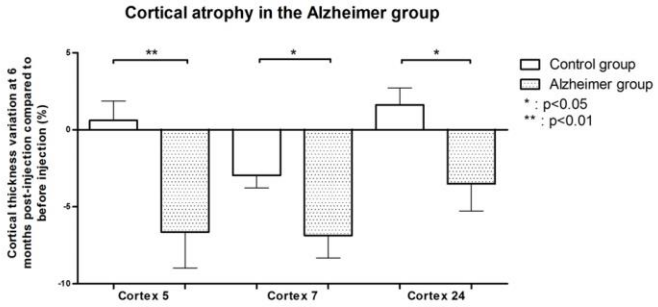
Methods: Brain extracts from two patients with AD and one control subject were injected to 12 mouse lemur primates (n=6 in the control group and n=6 in the “Alzheimer” group; 4x6.25µl, hippocampus and subjacent cortex (Brodmann area 5)). We present here 6 months of follow-up with behavioral, electroencephalography and Magnetic Resonance Imaging studies.

Results: Before injection, all the animals were able to memorize a discrimination task. However, 6 months post-injection, the Alzheimer group lost the ability to remember this task although they were still able to learn a new discrimination paradigm. As soon as 6 months post-injection, electroencephalogram slow activity was modified with decreased delta and increased theta frequency bands and cerebral atrophy was detected in the cingulate and parietal cortices in the Alzheimer group as

compared to the control group.







Conclusion: Our observations suggest that administration of Alzheimer brain homogenates could rapidly induce pathologic events leading to cognitive impairment, functional alterations and cortical atrophy in primates.

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Acknowledgements: France-Alzheimer association.

Symposium 22: SEEDING, SPREADING, AND PRION-LIKE MECHANISMS

ADPD5-1389
NEUROPROTECTIVE REGULATION OF EXOSOME SECRETION BY CYSTATIN C

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Objectives: The extracellular secretion of exosomes, generated within endosomal multivesicular bodies, can serve as an alternative pathway for the release of endocytosed material that does not reach the lysosome. We have demonstrated the presence in exosomes of cystatin C (CysC), which is implicated in neuroprotection and repair in the nervous system. In this study, we have examined the role that CysC plays in modulating exosome secretion.
Methods: Using a novel method developed in our laboratory, the levels of brain exosomes were compared between transgenic mice with increased CysC expression and littermate wild-type mice. Exosomal content in the extracellular space was quantified by measuring exosomal total protein as well as levels of the exosomal markers, standardized relative to protein content in brain homogenates.
Results: Higher exosome levels were observed in the brain extracellular space of CysC overexpressing mice as compared to littermate controls, demonstrating that increased CysC expression enhances exosome secretion *in vivo*.
Conclusions: Our studies of exosome levels in the brain have identified CysC as a previously unappreciated regulator of exosome secretion. Our finding that CysC expression positively regulates exosome secretion lays the foundation for the development of CysC-loaded exosomes as a treatment for neurodegenerative disorders where endosomal disruption occurs, such as Alzheimer's disease, Down's syndrome, amyotrophic lateral sclerosis, CHMP2B-frontotemporal dementia, and Niemann-Pick type C. We hypothesize that dysfunctions in the endocytic system that disrupt the efficient transport of membrane lipids and cargo for degradation in lysosomes can be alleviated by clearance of accumulated vesicular content through exosome secretion.

Symposium 22: SEEDING, SPREADING, AND PRION-LIKE MECHANISMS

ADPD5-1516

EVIDENCE OF SELF-PROPAGATION OF AMYLOID-BETA MISFOLDING IN VIVO BY LIMITING DILUTIONS AND DIFFERENT ROUTES OF ADMINISTRATION

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Objectives: We and others have shown that amyloid-beta (Abeta) pathology can be induced in animals in a similar way as described for prion diseases. These findings call for additional experiments directed to understand how Abeta misfolding spreads in Alzheimer's disease.

Methods: In this study, two prion-like properties were tested *in vivo* for misfolded Abeta: titration of the inducible agent by limiting dilutions and induction of pathological changes by different routes of exposure. Experiments were done in Tg2576 mice. Induced pathological changes were compared to amyloid deposition naturally developed in transgenic animals at different ages.

Results: Our results show that induction of Abeta was titrable and the minimum amount of Abeta seeds able to accelerate pathological changes was equivalent to a million-fold dilution of the brain, which is in the range of what is found for *bona fide* prions. In addition, several routes of administration of Abeta seeds were tested, including oral gavages, eye drops, and intra-muscular and intra-peritoneal injections. Many of them were able to induce pathological changes in the brain, although in different degrees.

Conclusions: Our findings, together with previously published reports, suggest that some aspects of AD pathology might be transmissible. These results may contribute to understand the mechanisms implicated in the initiation of Abeta pathology and therefore be useful to develop new therapeutic strategies for the prevention and treatment of this devastating disease.

Symposium 22: SEEDING, SPREADING, AND PRION-LIKE MECHANISMS

ADPD5-1870
PRIONIC LOOPS, ANTI-PRIONS, AND DEPENDENCE RECEPTORS IN NEURODEGENERATION

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Abstract - AD/PD 2015
Dale E. Bredesen, MD
UCLA and Buck Institute for Research on Aging

Title: Prionic Loops, Dependence Receptors, and Systems Therapeutics for Alzheimer's Disease

Alzheimer's disease (AD) represents a major healthcare problem, affecting over 5 million Americans and approximately 30 million globally, without truly effective therapy.

In studies of neural cell death, we identified dependence receptors which induce pcd when their trophic ligands are withdrawn. Over 20 such receptors have been identified to date, including the beta-amyloid precursor protein, APP, which functions as a molecular switch: interaction with netrin-1 enhances cleavage at the alpha-cleavage site, producing sAPPalpha and alphaCTF, which mediate neurite extension, caspase inhibition, and the inhibition of Abeta peptide formation. Conversely, interaction of APP with Abeta itself leads to cleavage of APP at three sites, producing four peptides—sAPPbeta, Abeta, Jcasp, and C31—that mediate neurite retraction, caspase activation, and synaptic reorganization. Thus Abeta may function as an anti-trophin, creating a prionic loop with APP.

These alternative APP-mediated signals can be manipulated genetically or pharmacologically to inhibit the Alzheimer's phenotype. Furthermore, ApoE4 alters this critical balance in favor of the pro-AD signaling, at least in part via a reduction in SirT1.

These results suggest a model in which AD results from a chronic imbalance between synaptoblastic signaling and synaptoclastic signaling, amplified by prionic loops such as APP-Abeta. Since this balance is affected by many different factors in the environment—the same supportive factors whose reduction is associated with increased risk of AD—we have created an extensive therapeutic program that alters the balance to favor synaptoblastic signaling, instead of attempting to treat AD with a monotherapy that fails to address the many members of the underlying network.

Symposium 22: SEEDING, SPREADING, AND PRION-LIKE MECHANISMS

ADPD5-2263
TRANSMISSION OF PATHOLOGY IN NEURODEGENERATIVE DISEASES
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The accumulation of misfolded proteins is a fundamental pathogenic process in neurodegenerative diseases. However, the factors that trigger aggregation and spreading of these disease proteins in brain are poorly understood. Recent studies demonstrate that misfolded disease proteins including alpha-synuclein in Parkinson's disease and tau in Alzheimer's disease and frontotemporal degeneration can be propagated from cell-to-cell through the recruitment of their endogenous normal counterparts. Moreover, pathologic misfolded aggregates propagated along major central nervous system (CNS) pathways to regions far beyond injection sites and appear to follow neuroanatomical interconnectomes. This spreading of pathology is progressive and leads to behavior impairments and eventually compromises neuronal survival but immune therapy reduces the spread of pathology, rescue behavior phenotypes and neuron loss. Our findings open up new avenues for understanding the mechanisms of disease progression and for developing novel therapeutics.

Symposium 24: ANIMAL MODELS - NEW WAYS TO GO?

ADPD5-0200
LOW PREDICTIVE VALIDITY OF ANIMAL MODEL IN AD: DID WE ASK THE RIGHT QUESTIONS?

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Over the last 20 years a wide range of genetically modified animal models to study Alzheimer´s disease has been developed, some of them specific for different pathogenically important targets of the disease, others combining different features of the human disorders in a complex way, like combining up to 5 transgenes. Drugs designed for different targets have been successfully tested in these models, what is contrasted by the fact that during the last 10 years about 99.6 % of all clinical AD trials failed! First we have to ask was defined a success in the animals? In most of the cases it was a pronounced influence on brain pathology and brain biochemistry, functional readouts were sometimes missing or weak. Reviewing the quality of papers concerning behavioral results raises doubts that this was done in the best possible way. There was the optimistic assumption that prevention or reduction of pathology will automatically result in an improvement of cognitive performance in patients! What do we call failure in the clinical trial? Many of the drugs were also able to shift biomarkers in human subjects very similar as they did animal models, the failure is they did not succeed to improve or stabilize the cognitive performance and the functional status. This translational problem advocates for more refined studies on behavioral effects of drugs for AD. As an alternative to the traditional behavioral test systems, the use of high-tech methods for phenotypic screening should be considered. The application of state of the art robotics, computer vision and bioinformatics can produce complex datasets like acquisition and analysis of more than2000 different behavioral features in an automatic, high-throughput way. These “big data” provide outstanding statistical power and allow precise conclusions about the influence of compounds on distinct behavioral patterns. An advantage is that experiments are performed without influence of a human investigator in a highly standardized way. Steadily growing databases allow direct comparison to effects of standard compound, which is integrated in the overall data analysis process. Based on experience from other CNS disorders this high-tech approach in combination with robust and already well-characterized and phenotyped animal models could bring a breakthrough in predictive preclinical in vivo drug testing.

Symposium 24: ANIMAL MODELS - NEW WAYS TO GO?

ADPD5-0723

EPILEPTOGENICITY AND PRECOCIOUS MORTALITY OF BIGENIC BIAT MICE THAT EXPRESS AMYLOID AND TAU.

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Bigenic biAT mice express APP.V717I and Tau.P301L and develop combined Alzheimer pathology when ageing (>10-12 months) (Terwel et al, 2005, 2008, Crespo-Biel et al, 2014).

Results

Important precocious mortality of young biAT mice (<6 months) with epileptic symptoms was totally absent in the parental monogenic mice. No authentic amyloid or tauopathy was evident yet in brain of young biAT mice. Amyloid accumulation and pathology in biAT and parental APP.V717I mice was similar, while tau phosphorylation and oligomerization was more early and more extensive than in age-matched Tau.P301L mice. Surprisingly, tangles were less in brainstem of old biAT mice that escaped early epileptic death. To explain survival beyond 12 months, the age-limit of Tau.P301L mice, we demonstrated activation of GSK3 by amyloid in biAT mice, resorting the same effects as co-expression of GSK3β with Tau.P301L in biGT mice. Despite different timing the biochemical pathway to tauopathy was similar: progressive tau-phosphorylation, soluble oligomers, insoluble oligomers, aggregates, tangles and threads.

Conclusions

The death of young biAT mice is caused by epileptogenic activity, demonstrated by clinical symptoms in the homecage and during tests: seizures, convulsions, freezing, salivation, tongue biting, posture of corpses. Moreover, high spontaneous and induced epileptogenic activity was evident during electrophysiological recordings from brain sections of young biAT mice. Additionally, young biAT mice already suffer functional and structural defects in synapses and dendritic spines in cortex and hippocampus that underlie or contribute to electric hyperactivity.

The reported high incidence of epilepsy in AD patients deserves further attention by retrospective and prospective studies.

Symposium 24: ANIMAL MODELS - NEW WAYS TO GO?

ADPD5-1060

A NOVEL AAV BASED MODEL MIMICS EARLY FEATURES OF ALZHEIMER'S DISEASE HUMAN PATHOLOGY

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Evaluation of biomarkers and therapies for Alzheimer's disease (AD) suffers from lack of models close to disease progression in human. Most of transgenic models express supraphysiological levels of APP metabolites to mimic AD lesions such as amyloid plaques and neurofibrillary tangles.

We describe here the development of an innovative AAV-based mouse model with two major objectives: create a relevant model closer to human physiopathology and mimic early stages of AD. This model was obtained by co-injection of two AAV vectors coding the human Amyloid Protein Precursor (APPsl) and the human Presinilin 1 (PS1M146L). Our strategy allows a stable and low expression of transgenes without overexpression of APP. This leads to βCTF and Aβ42 production as soon as 1 month post-injection without late symptoms appearance like senile plaque.

Interestingly, only co-injection of both viruses increased the ratio Aβ42/Aβ40 and triggered hyperphosphorylation of the murine Tau protein which was correlated with increased levels of GSK-3β. Otherwise, we measured physiopathological amounts of APP, βCTF and Aβ peptides compared to human samples and transgenic mice. We therefore measured a decrease of PSD95 associated with synaptic defects such as extrasynaptic NMDAR activity and an alteration of the GABA metabolism pathway. Finally significant behavior impairments appeared from 3 months post-injection.

This strategy overcame major pitfalls of transgenic models such as continuous expression of transgenes from *in utero* and limitations to the transfer to other species. Moreover, results highlighted here are strong evidences that a lot of various mechanisms appear long before the hallmarks of AD.

Symposium 24: ANIMAL MODELS - NEW WAYS TO GO?

ADPD5-1285
STRUCTURAL AND FUNCTIONAL MRI COMPARISON OF APP TRANSGENIC LINES

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[2]Division of Psychiatry Research, University of Zurich, Zurich, Switzerland

Alterations in brain structure or in functional connectivity (FC) as detected with MRI occur early in Alzheimer's disease, already affecting asymptomatic and pre-clinical cases. MRI studies in transgenic mice may enable understanding the mechanisms underlying these changes. We applied a comparative approach to identify structural and functional deficits in three mouse lines expressing different aspects of amyloid pathology.
ArcAbeta (Swe/Arc), Osaka (E693Δ), and PSAPP (Swe + PSEN1 L166P) transgenic mice and wild-type littermates were imaged at 13-15 months (n = 10-15) using an animal MRI scanner operating at 9.4T. Resting-state fMRI in mice anesthetized with medetomidine/isoflurane yielded information on functional connectivity (FC) diffusion tensor imaging on white matter integrity (fractional anisotropy, FA), and high resolution 3D images on volume changes of brain structures (voxel-based morphometric analysis, VBM).
ArcAbeta mice presented significant alterations in cortical FC in contrast to PSAPP and Osaka mice. Cortical volume was reduced in ArcAbeta and increased volume in cholinergic nuclei in pons and basal forebrain, while FA values were reduced in the anterior external capsule and corpus callosum. PSAPP mice presented increased cortical volume and increased FA in most white matter tracts while no significant structural alteration were observed in Osaka mice neither with FA nor VBM.
We observed high variability in structural and functional phenotype among different APP mouse lines, which has to be taken into consideration when comparing such models. Specific structure-function relationship may yield important insight into the role of amyloid in determining these pathological changes.

Symposium 24: ANIMAL MODELS - NEW WAYS TO GO?

ADPD5-1572
DEVELOPING ANIMAL MODELS TO STUDY THE CO-MORBIDITY OF ALZHEIMER'S DISEASE AND VASCULAR DEMENTIA

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Vascular contributions to cognitive impairment and dementia (VCID) is the second most common cause of dementia after Alzheimer's disease (AD). In addition, it is estimated that almost half of all AD patients have significant cerebrovascular disease co-morbid with their AD pathology. We hypothesize that cerebrovascular disease alters the neuroinflammatory response of the brain to Alzheimer's disease pathology. We have developed the hyperhomocysteinemia (HHCy) model of VCID that results in hemorrhagic cerebrovascular pathology. We have induced this model in both wild type mice as a model of VCID and APP/PS1 transgenic mice as as model of AD/VCID co-morbidity. We have examined amyloid pathology, learning and memory and neuroinflammation.

In wild type mice, HHCy results in significant cortical micro hemorrhage, M1 type neuroinflammation and spatial memory impairment in the radial arm water maze. Under normal conditions, the APP/PS1 mice with established amyloid pathology show high expression of alternative, M2a inflammatory markers. When cerebrovascular disease is present, there is a distinct switch in the inflammatory state toward an M1, pro-inflammatory bias. Furthermore, the presence of cerebrovascular disease with amyloid pathology results in an additive effect on memory impairment. Despite unchanging Abeta load, the distribution of amyloid is altered, favoring cerebrovascular deposition.

Overall, our data suggests that cerebrovascular disease alters amyloid distribution and results in further cognitive impairment. This is in association with a dramatic shift in the neuroinflammatory state of the brain. We believe this data has significant implications to the treatment of dementia patients who have Alzheimer's and cerebrovascular pathologies present.

Symposium 24: ANIMAL MODELS - NEW WAYS TO GO?

ADPD5-1834
WNT SIGNALING IS INVOLVED IN THE OCTODDON DEGUS MODEL OF ALZHEIMER DISEASE

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Alzheimer's disease (AD) is a common neurodegenerative disorder and the leading cause of age-related dementia worldwide. Some AD models for AD have been described, however, these models do not recapitulate the entire spectrum of lesions present in human AD brains. Thus, the identification and validation of a natural, wild-type AD model that mimic the pathological hallmarks observed in AD would be highly useful to unravel the mechanisms of AD. The Chilean rodent *Octodon degus* (*O. degus*) naturally develops extracellular amyloid plaques, neurofibrillary tangles and synaptic dysfunction in an age-related manner, all pathological hallmarks described in the beginning and progression of the AD. Wnt signaling has been widely implicated in the pathogenesis of AD, and plays a crucial role in maintaining the structure and function of neuronal circuits. Hence, elucidating the molecular mechanisms of Wnt dysfunction and synaptic disassembly in a 'natural' model that recapitulates AD pathology has the potential to identify targets to slow down AD-like neuropathology. We have explored age-related changes in the expression of Wnt components in *O. degus* brain. Down-regulation of Wnt ligands and receptors with an increase in GSK-3b activity plus an increment in the sFRPs and Dickkopf-1 antagonists was observed. These molecular changes indicate that Wnt signaling is deregulated during 'physiological' ageing, and may contribute to progressive neuronal degeneration, memory loss and cognitive deficits such as those observed in AD. Our results support the idea that Wnt signaling might be a novel therapeutic target for AD.

Symposium 30: APP BIOLOGY 1

ADPD5-0449

TYROSINE-682 OF THE AMYLOID PRECURSOR PROTEIN AS NEW TARGET TO EXPLORE MECHANISMS OF AGE-RELATED NEURONAL DEGENERATION.

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Recently emerging evidence supports a pivotal role for the intracellular domain of amyloid precursor protein (APP) for the function of APP. In particular, we are interested in studying the highly conserved 682-YENPTY-687 motif, which is recognized by proteins containing phosphotyrosine interaction domains (PIDs) (Tamayev et al., 2009). The APP intracellular domain contains eight potential phosphorylation sites; seven of these (Y653, S655, T668, S675, Y682, T686 and Y687) were phosphorylated in APP from brains of AD patients (Lee *et al*.2003). Often located in protein interaction sites, phosphorylatable residues may interfere with binding to cytoplasmic adaptor proteins, and thus we predict with APP function. To address this hypothesis we have recently characterized a knock-in (KI) mouse model of dementia and premature aging in which the Y682 of the 682-YENPTY-687 motif is replaced by glycine (Y682G). We unexpectedly found a key role for Y682 in development and aging, with age-dependent cognitive and locomotor decline, loss of synaptic connections, decreased cholinergic tone, and defects in nerve growth factor signaling in mice (Matrone *et al*., 2011; 2012). In addition, Y682G neurons show an anomalous compartmentalization of APP, a defect in the autophagic machinery, and an alteration in APP binding to several adaptor proteins, clearly suggesting that Y682 plays a crucial role in all the events described above (La Rosa *et al*., submitted). Taken together, our studies provide novel information on the function of the 682-YENPTY-687 motif in the physiological function of APP paving the way for potential therapeutic strategies to address AD in humans.

Symposium 30: APP BIOLOGY 1

ADPD5-0603
THE ROLE OF AMYLOID ABETA IN LIPID-MEDIATED TOXICITY
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Objectives:
The transmembrane sequence (TMS) of APP (Aβ residues 29 to 49) is a key determinant of Aβ42 oligomerization and toxicity, with G33 being the critical residue (Harmeier et al., 2009). Our systematic analysis of Aβ-lipid interactions revealed that the neurotoxic Aβ42 binds significantly to sphingomyelin (SM), GM1, phosphatidylcholine (PC), and brain total lipid extract (BTLE) as compared to its non-toxic counterparts Aβ40 and Aβ42 G33I. Developing molecules that interfere with Aβ-lipid interactions may enable the design of amyloid selective therapeutics to combat Alzheimer disease (AD).
Methods:
Surface plasmon resonance (SPR), Size-exclusion chromatography (SEC), organotypic slice cultures, electrophysiology, microscopy techniques, MALDI-MS, and *Drosophila melanogaster* as an *in vivo* model for AD.
Results:
To date, we have found that an eight amino acid peptide with alternating hydrophobic and hydrophilic amino acids ('aggregation inhibitory peptide' - AIP) neutralizes Aβ42-induced neurotoxicity by preferentially binding to the C-terminal region of highly toxic Aβ tetra-/hexamers. Our data indicate (i) that co-administration of AIP with Aβ42 prevents the loss of excitatory synapses and rescues long-term potentiation (LTP) in CA1 organotypic hippocampal slice cultures, and (ii) that the AIP exhibits protective effects in our *in vivo* AD model.
Conclusions:
Membrane regions with a high percentage of SM, (e.g., 'lipid rafts') could be of particular importance for the interaction with Aβ42 and may play a crucial role in mediating amyloid toxicity *in vivo*. The development of compounds that interfere with toxic Aβ-lipid interactions may have a preventive and therapeutic potential.

Symposium 30: APP BIOLOGY 1

ADPD5-0933

AMYLOID PRECURSOR PROTEIN SYNAPTIC FUNCTION INVOLVES TRANS-DIMERIZATION AND FE65/FE65L1 SIGNALING

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Objectives
Accumulating evidence suggests that the Amyloid Precursor Protein (APP) has an essential synaptic function. The current data suggest that sAPP as well as trans-directed dimerization of membrane bound APP are involved in this function.
Methods
We performed a cell-clustering assay with semi-adherent cells and a mixed co-culture assay of HEK293 cells and primary neurons, previously used to show that APP can promote presynaptic differentiation of contacting axons. Further we used for analysis of Fe65/Fe65L1 knockout mice different immunohistochemical and electrophysiological methods as well as behavioral studies.
Results
Here we report that inhibition of APP shedding increases its cell adhesion features and increases its synaptogenic function in a mixed co-culture assay of HEK293 cells and primary neurons. This suggests that trans-interaction of APP is required to induce presynaptic differentiation of contacting axons. Further, we show that mice lacking the Fe65 and Fe65L1 APP adaptor protein exhibit severe deficits in formation of the peripheral synapse, and deficits in pre-and postsynaptic function of the central synapse associated with deficits in LTP and learning. These phenotypes resemble some key characteristics of genetically modified APP/APLP2 knockout mice, lacking the intracellular domain that mediates binding to Fe65/Fe65L1. This indicates that Fe65/Fe65L1 might mediate APP signaling at the synapse.
Conclusions
Together our data corroborate the view that APP function depends on trans-dimerization at the synapse and suggest that Fe65/Fe65L1 is involved in APP synaptic signaling.

Symposium 30: APP BIOLOGY 1

ADPD5-1603

SECRETED APP AND MEMBRANE-ANCHORED APP COOPERATE TO INDUCE G PROTEIN-MEDIATED ACTIVATION OF THE AKT SURVIVAL PATHWAY

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Here we investigated the neuroprotective function of the soluble APP ectodomain sAPPalpha which is generated by cleavage of APP by alpha-secretase along the non-amyloidogenic pathway. Recombinant sAPPalpha protected primary hippocampal neurons and SH-SY5Y neuroblastoma cells from cell death induced by trophic factor deprivation. We show that this protective effect is abrogated in neurons from APP-knockout animals and APP-depleted SH-SY5Y cells, but not in APP-like protein 1- and 2- (APLP1 and APLP2) depleted cells, indicating that expression of membrane-bound holo-APP is required for sAPPα-dependent neuroprotection. Trophic factor deprivation diminished the activity of the Akt survival pathway. Strikingly, both recombinant sAPPα and the APP-E1 domain were able to stimulate Akt activity in wild-type (wt) fibroblasts, SH-SY5Y cells and neurons, but failed to rescue in APP-deficient neurons or fibroblasts. The ADAM10 (a disintegrin and metalloproteinase domain-containing protein 10) inhibitor GI254023X exacerbated neuron death in organotypic (hippocampal) slice cultures of wt mice subjected to trophic factor and glucose deprivation. This cell death-enhancing effect of GI254023X could be completely rescued by applying exogenous sAPPalpha. Interestingly, sAPPalpha-dependent Akt induction was unaffected in neurons of APP-ΔCT15 mice that lack the C-terminal YENPTY motif of the APP intracellular region. In contrast, sAPPalpha-dependent rescue of Akt activation was completely abolished in cells expressing an APP mutant lacking the G-protein interaction motif located in the APP C-terminus. Collectively, our data provide new mechanistic insights into the physiologic role of APP in antagonizing neurotoxic stress: they suggest that cell surface APP mediates sAPPα-induced neuroprotection via G-protein-coupled activation of the Akt pathway.

Symposium 30: APP BIOLOGY 1

ADPD5-1793
DECIPHERING THE MECHANISMS BY WHICH INHIBITING APP DIMERIZATION REDUCES ABETA PRODUCTION

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Objectives: Increased amyloid precursor protein (APP) dimerization has been shown to enhance Abeta peptide formation. Our goal is to identify small molecules that inhibit APP dimerization and lower Abeta levels.
Methods: To define the mechanism by which APP dimerization affects the production of Abeta, a high throughput screen (HTS) for small molecule modulators of APP dimerization was conducted using APP-*Firefly* luciferase enzyme complementation to detect APP dimerization. Selected modulators identified from a library of 77,440 compounds were tested for their effects on Abeta and other APP fragments using ELISA and western blotting, respectively. APP phosphorylation was examined by IP-western blotting using anti Abeta/APP antibody for IP, and anti-phospho-tyrosine for western blot.
Results: One APP dimerization inhibitor significantly lowered Abeta and sAPPbeta levels without affecting sAPPalpha or gamma-CTF levels, suggesting that blocking the dimerization is preventing the cleavage by beta-secretase in the amyloidogenic processing pathway of APP. Interestingly, this inhibitor and its analog increased APP phosphorylation on tyrosine.
Conclusions: Inhibition of APP dimerization has previously been suggested as a therapeutic target for AD. To our knowledge, this is the first HTS effort to identify small molecule modulators of APP dimerization. The findings reported here further support the notion that modulation of APP dimerization could be a viable means of reducing the production of Abeta. The precise mechanism of action of the identified inhibitors is currently being investigated.

Symposium 33: PRESENILIN BIOLOGY

ADPD5-0833
FUNCTION AND MECHANISM OF GAMMA-SECRETASE: A SIGNALING RECEPTOR INVOLVED IN NEUROINFLAMMATION IS AN UNUSUAL SUBSTRATE

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Objectives: The use of gamma-secretase as a drug target in Alzheimer's disease (AD) is hampered by the fact that it cleaves many different membrane protein substrates. A better understanding of the molecular mechanisms of substrate cleavage and substrate specificity may allow to selectively inhibit amyloid beta production without interfering with other gamma-secretase functions.
Methods: Pharmacologic and genetic inhibition of gamma-secretase in cell lines and primary human B cells.
Results: The dogma holds that gamma-secretase does not directly cleave a membrane protein, but requires the previous truncation of the membrane protein-ectodomain by another protease, e.g. alpha-secretase. Here, we discovered a novel function for gamma-secretase, namely the direct shedding of a full-length membrane protein-receptor of the TNF receptor-superfamily, the B cell maturation antigen (BCMA). BCMA did not require prior ectodomain truncation. Gamma-secretase cleavage was found to control both BCMA protein levels at the cell surface and BCMA signaling, which is required for the survival of immunoglobulin-secreting plasma-cells. BCMA has an unusually short ectodomain of 54 amino acids. Increasing the length of the ectodomain reduced gamma-cleavage efficiency, suggesting that the short length of the ectodomain allows direct shedding by gamma-secretase. However, other natural membrane proteins with short ectodomains were not cleaved by gamma-secretase demonstrating that short ectodomain length is not sufficient for gamma-cleavage.
Conclusions: Together, BCMA is identified as the first membrane protein directly shed by gamma-secretase, suggesting that gamma-secretase cleaves a whole new class of transmembrane proteins. Moreover, BCMA gamma-cleavage constitutes a novel immunoregulatory mechanism which may be affected by AD drugs targeting gamma-secretase.

Symposium 33: PRESENILIN BIOLOGY

ADPD5-0889
THE ROLE OF PRESENILIN IN THE PRESYNAPTIC MECHANISMS AT HIPPOCAMPAL MOSSY FIBERS REVEALED BY A GRANULE CELL-TARGETED OPTOGENETIC APPROACH

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Presenilin (PS), the catalytic subunit of the intramembrane protease gamma-secretase, cleaves various synaptic proteins including APP, N-cadherin, neurexin, ephrin, nectin and alcadein but the role of these processings in synaptic function is not known. Importantly, more than 150 missense mutations have been found on PS1 gene which cause the early-onset form of familial Alzheimer's disease (FAD) and decrease the proteolytic activity of PS.

PS has been recently found to regulate presynaptic plasticity by an unknown mechanism. The aim of this project is to understand the role of presenilin in presynaptic plasticity. We examine the role of PS at the synapse between the dentate gyrus granule cells and the CA3 pyramidal neurons of the hippocampus which is characterized by a large presynaptic plasticity essential to memory encoding.

This project involves new optogenetic tools specifically targeted to the dentate gyrus which combine cell-specific activation to cell-specific genetic manipulation. We developed a bicistronic lentiviral tool to co-express the variant of channelrhodopsin ChIEF together with a transgene of interest separated by a 2A-peptide. This construct is specifically targeted to dentate gyrus granule cells via the use of a promoter specific of hippocampal granule cells cloned for the first time. Stereotaxic injection of a virus which allows to co-express channelrodopsin with Cre recombinase permitted to knock-down PS1 gene in PS1-floxed mouse and helped to reveal the role of PS1 in presynaptic facilitation. This regulation also depends on the fragment CTFbeta of APP, a main substrate of gamma-secretase, which accumulates in absence of PS1.

Symposium 33: PRESENILIN BIOLOGY

ADPD5-1565
FUNCTION AND DYSFUNCTION OF PRESENILIN IN ALZHEIMER'S DISEASE
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Mutations in *presenilin-1* (*PSEN1*) are the major cause of familial Alzheimer's disease (FAD). How *PSEN1* mutations perturb Presenilin-1 (PS1) function in the brain to produce FAD is unresolved. To determine the impact of pathogenic mutations on PS1 function *in vivo*, we generated two independent *Psen1* knock-in (KI) mice in which the FAD mutation L435F or C410Y was introduced into the genomic *Psen1* locus. Surprisingly, homozygous KI/KI mice display perinatal lethality and developmental defects indistinguishable from those of *Psen1*-null mice. *Psen1* mRNA levels are unchanged in KI/KI mice, but PS1 endoproteolysis is impaired. In *vitro* γ-secretase assay revealed that γ-secretase-mediated cleavage of APP, Notch and N-Cadherin is abolished in KI/KI embryonic brains and reduced (~50%) in KI/+ brains. Levels of mouse endogenous Aβ40 and Aβ42 are reduced in the adult cerebral cortex of KI/+ mice, and the Aβ42/40 ratio is increased at ~15% due to the greater reduction of Aβ40. Similarly, levels of human Aβ derived from transgenic mice are also reduced in the cerebral cortex of KI/+ mice, and the Aβ42/40 ratio is increased, leading to accelerated amyloid deposition. Using three behavioral paradigms assessing hippocampal memory, we found that spatial memory and pattern completion are impaired. Electrophysiological analysis of the hippocampal Schaeffer collateral and commissural/associational synapses showed reduced short-term and long-term synaptic plasticity. Stereological analysis revealed that the *PSEN1* mutation causes age-dependent neurodegeneration, increased apoptosis and gliosis. Collectively, our results demonstrate that pathogenic *PSEN1* mutations produce a full spectrum of AD related phenotypes through a loss of Presenilin function mechanism.

Symposium 33: PRESENILIN BIOLOGY

ADPD5-1606
MODULATION OF GAMMA-SECRETASE ACTIVITY BY NICASTRIN
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The g-secretase complex, composed of PS, PEN-2, Aph1 and nicastrin (NCT), is essential for intramembranous processing of APP, Notch and several type I membrane proteins. In view of evidence that NCT plays a critical role in substrate recognition, we and others have examined the feasibility of modulating NCT function using antibody-based approaches, with the notion that NCT-specific antibodies could bind to, and modulate the binding of NCT to individual substrates. In this regard, we have reported that a synthetic antibody fragment that targets the NCT ectodomain (ECD) impairs g-secretase-mediated processing of both Notch and APP in *in vitro* assays. We generated additional NCT-specific synthetic antibodies using phage display technology, then reformatted the cDNAs encoding these antibodies to corresponding cDNAs encoding single-chain variable fragments (scFv) that were then stably expressed in HEK293 cells expressing the APP "Swedish" (APPswe) variant. We have analyzed two anti-NCT-specific antibodies, which following conversion to scFvs, bind to the NCT ECD *in vitro*. In HEK293 cells that stably express one of these antibodies, NCT maturation is impaired and leads to reduced levels on the cell surface, where the Notch derivative, NEXT, is subject to g-secretase processing. On the other hand, g-secretase that is present in the Golgi apparatus and endosomal/recycling compartments in cells expressing scFv G9 is fully capable of processing APPswe to generate Ab peptides. Thus, we suggest that scFvG9 affects both the maturation of NCT and subcellular distribution of g-secretase that leads to differential processing of APP versus Notch.
Supported by NIH AG027854 (SSS), Cure Alzheimer's Fund (CAF), Alzheimer's Association, AHAF, the Edward H. Levi Fund and the Adler Foundation. SSS is a paid Consultant of Eisai Research Labs Inc., AZTherapies Inc. and Jannsen Pharmaceutica NV.

Symposium 33: PRESENILIN BIOLOGY

ADPD5-1891

PRESENILIN1 FUNCTIONS AS A POSITIVE REGULATOR OF NEURONAL, BUT NOT GLIAL, RECEPTORS AND LIGAND-DEPENDENT NEUROPROTECTION

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Epidermal growth factor receptor (EGFR) plays pivotal roles in cell growth, differentiation and tissue function acting as integrator where extracellular growth and survival signals converge and transform into intracellular outputs. In the brain, binding of EGF ligands to EGFR regulates neuronal function and stimulates survival kinases providing neuroprotection against toxic insults. Functions of EGFR are implicated in aging and neurometabolic disorders such as diabetes and Alzheimer's disease (AD). Genome-wide and protein-protein interaction studies identified EGFR as a risk factor for AD while transcriptional profiling indicates that ApoE4 affects the brain expression of EGFR. Furthermore, EGFR functions may mediate Aβ42-induced memory loss in animal models. Additional work indicates that EGFR interacts with presenilin (PS) and that PS1 regulates differentiation of brain neural progenitor cells through EGFR. We found that PS1 null cortical neurons contain little EGFR and show no EGF-dependent phosphorylation of survival kinases or neuroprotection. In contrast, absence of PS1 has no effect on the EGFR of primary glial cells. Acute downregulation of PS1 suppresses neuronal EGFR while exogenous PS1 stimulates expression of EGFR. Absence of PS1 results in dramatic decrease (>95%) of neuronal EGFR mRNA although PS1 affects the stability of neither EGFR nor its mRNA. Furthermore, expression of neuronal EGFR is independent of both γ-secretase and PS2 suggesting that PS1-dependent downregulation of this receptor may contribute to developmental abnormalities and lethal phenotypes specifically found in PS1, but not in PS2, null mice. In summary, the data shows that PS1 is necessary for the expression of neuronal, but not glial, EGFR thus controlling its biological functions in a cell-specific manner. This lecture will also discuss effects of PS FAD mutants on PS-dependent transcriptional mechanisms regulating expression of neuronal receptors.

Symposium 39: PROTEIN MODIFICATION AND DEGRADATION

ADPD5-0299
N-TRUNCATED ABETA 4-42 A TARGET FOR ALZHEIMER'S DISEASE
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Objectives: N-truncated $Abeta_{4-42}$ is highly abundant in AD brain. We have recently generated a novel transgenic mouse model expressing $A\beta_{4-42}$ (named Tg4-42), which develops a massive CA1 pyramidal neuron loss without plaque formation. The hippocampus-specific neurodegeneration correlates well with age-dependent spatial reference memory deficits assessed by the Morris water maze test. In addition we have generated a novel monoclonal antibody NT4X-167 specific for the N-terminus of $Abeta_{4-42}$. One objective was to identify the level of neuron loss correlating with behavioural deficits in an age-dependent manner. The second objective was whether NT4X-167 can be used to treat two AD mouse models (Tg4-42 and 5XFAD).
Methods: Morris water maze, stereology for neuron count, passive immunization, mouse transgenic model, neuropathology, plaque load.
Results: 4-month-old Tg4-42 mice show a loss of 45% CA1 neurons and are still able to learn, whereas 5-month-old mice have a 55% neuron death and are impaired in the reference memory task of the Morris water maze. We have treated Tg4-42 mice by passive immunization with NT4X-167 between 3 and 6 months of age. The treatment results in rescue of spatial memory deficits. Moreover by passive immunization of the conventional amyloid mouse model 5XFAD we demonstrate that the antibody NT4X-167 significantly reduces plaque load.
Conclusions: $A\beta_{4-42}$ induces neuron loss without plaque formation. Passive immunization with NT4X-167 rescues behavioural deficits associated with neuron loss and reduces plaque load. These observations demonstrate that $A\beta_{4-42}$ is a relevant disease target and that the antibody NT4X-167 has potential as a therapeutic tool.

Symposium 39: PROTEIN MODIFICATION AND DEGRADATION

ADPD5-1047

MEPRIN BETA, BUT NOT ITS ISOENZYME MEPRIN ALPHA, CATALYZES THE FORMATION OF N-TRUNCATED ABETA PEPTIDES IN VITRO

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Objective: A significant proportion of amyloid-beta (Aβ) peptides in Alzheimer's disease (AD) is truncated at the N-terminus. Among these N-truncated peptides, pGlu-modified amyloid Aβ3pE-40/42 and Aβ11pE-40/42, have been shown to correlate with disease progression and being overrepresented in early-onset forms of inherited AD. After truncation, these peptides are modified by the enzyme glutaminyl cyclase (QC), which has been shown to be upregulated in AD.
Methods: We expressed different APP proteins (wt or familial Alzheimer's mutations) in the cell lines HEK293 and CHO. The APP processing and production was assessed using Western Blot analysis and ELISAs detecting N-truncated or full length Aβ. Maldi-TOF mass spectrometry was used to analyze cleavage of APP-derived peptides.
Results: We could show that APP carrying the Swedish (APP^{695} [$KM^{595,596}NL$]) mutation leads to generation of full-length Aβ (Aβ(1-40/42)), whereas processing of APP wt results in significant formation of N-truncated peptides suggesting alternative processing. Co-expression of Meprin β, but not Meprin α, resulted in production of N-truncated Aβ peptides Aβ(2-40/42). These results were corroborated by cleavage of APP-related peptides by Meprin *in vitro*. Interestingly, Meprin β cleaved preferably APPwt and not APPswedish, which contrasts to the β-secretase BACE.
Conclusion: The data support a BACE-independent processing of APPwt that may contribute to formation of N-truncated Aβ. These truncated forms, in turn, might be prone to further post-translational modification. Thus, Meprin β might represent a potential upstream target to suppress formation of pGlu-Abeta.

Symposium 39: PROTEIN MODIFICATION AND DEGRADATION

ADPD5-1079

AT LEAST 2 INDEPENDENT PROTEOLYTIC PATHWAYS CONTRIBUTE TO THE FORMATION OF BETA-AMYLOID IN PRIMARY NEURONS AND MAMMALIAN CELLS

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Objectives:
To date no BACE I inhibitor passed clinical development for the treatment of AD. Mounting evidence shows that a heterogeneous mixture of Aβ peptides is deposited in AD and it is unclear what molecular pathways lead to their generation. Since truncated forms of Aβ, e.g. pGlu-3 Aβ generated by catalysis of Glutaminyl Cyclase (QC), show an overt aggregation propensity and neurotoxicity *in vitro* and *in vivo*, we analyzed pathways of N-truncated Aβ liberation.

Methods:
Co-secretion of Aβ with QC and neurotransmitters was studied in primary bovine chromaffin cells and neuroblastoma cells. Liberation of N-truncated Aβ was studied in cells lines transfected with APP (wt) or APP “Swedish” (sw), in primary neurons from APP (wt) and APP (sw) expressing mice and in primary human neurons derived from iPS cells. Analysis comprised immunofluorescence, ELISA, Western Blot and mass spectrometry.

Results:
Aβ and QC show an activity-dependent, regulated co-secretion from dense-core secretory vesicles together with neurotransmitters. Furthermore, the secretion of N-truncated Aβ peptides from different cell types was only partially dependent on BACE I as demonstrated by application of a BACE I inhibitor and BACE I siRNA nucleotides or in experiments using cells derived from BACE ko mice. This suggests the presence of alternative cleavage by yet unknown proteases generating truncated Aβ species.

Conclusions:
We discovered at least 2 independent pathways leading to the generation of the total Abeta pool secreted from cellular models. This might have implications for the development of additional treatment strategies for Alzheimer’s disease.

Symposium 39: PROTEIN MODIFICATION AND DEGRADATION

ADPD5-1097
ISOGLUTAMINYL CYCLASE CONTRIBUTES TO CCL2-DRIVEN NEUROINFLAMMATION IN ALZHEIMER'S DISEASE

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Objectives: The brains of Alzheimer's disease (AD) patients are characterized by deposits of Abeta peptides and by accompanying chronic inflammation. Here, we provide evidence that the enzyme isoglutaminyl cyclase (isoQC) is a novel factor contributing to both aspects of AD pathology.
Methods: We used APP-transgenic mouse and human control and AD brains for enzymatic activity assays, ELISAs, qRT-PCR and double immunoflourescent labellings to reveal the expression of isoQC, pGlu-CCL2 and pGlu-Abeta.
Results: Two putative substrates of isoQC, N-truncated Abeta peptides and the monocyte chemoattractant chemokine CCL2, undergo isoQC-catalyzed pyroglutamate (pGlu) modification. This triggers Abeta aggregation and facilitates the biological activity of CCL2, which collectively results in the formation of high molecular weight Abeta aggregates, glial cell activation, neuroinflammation and neuronal cell death. In mouse brain we found isoQC to be neuron-specifically expressed and co-localized with its substrate CCL2. During aging of wild type and Tg2576 mice, isoQC and CCL2 proteins are up-regulated and were found to be co-induced in Abeta plaque-associated reactive astrocytes. Also, in mouse primary astrocyte culture, a simultaneous up-regulation of isoQC and CCL2 expression was revealed upon pro-inflammatory LPS/IFN-g stimulation. In brains of AD patients, the expression of isoQC and CCL2 mRNA and protein is up-regulated compared to controls and correlates with pGlu-Abeta load and with the decline in mini mental state examination.
Conclusions: Our observations provide evidence for a dual involvement of isoQC in AD pathogenesis by catalysis of pGlu-Abeta and pGlu-CCL2 formation which mutually stimulate inflammatory events and affect cognition.

Symposium 39: PROTEIN MODIFICATION AND DEGRADATION

ADPD5-1132

TWO SOMATOSTATIN RECEPTOR SUBTYPES REGULATE THE MAJOR ABETA DEGRADING ENZYME NEPRILYSIN

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Objectives

Neprilysin is a major Aβ degrading enzyme, the expression of which declines upon aging. This increases Aβ levels which may lead to Alzheimer's disease (AD). In a previous search for activators of neprilysin, we identified the neuropeptide somatostatin (Saito et al., Nat Med 2005). Somatostatin binds to G protein-coupled somatostatin receptors (SSTR), four of which are expressed in hippocampus and cortex, and induces translocation of neprilysin to cell surface (Kakiya et al., JBC 2012). Here we identified the SSTR subtypes which regulate neprilysin.

Methods

SSTR single knockout mice were investigated but no changes in neprilysin occurred. We therefore generated knockout mice lacking two of the four SSTR subtypes in a combinatorial manner. Neprilysin levels and localization and Aβ levels were investigated. Primary neurons were used to investigate in vitro the effects on neprilysin of SSTR knockout.

Results

We found that the simultaneous knockout of two of the SSTR subtypes significantly decrease the levels of neprilysin in lacunosum molecular layer of dendate gyrus. Importantly, the lowered neprilysin levels lead to markedly increased Aβ levels in hippocampus. Consistently, stimulation of primary neurons derived from wildtype mice with receptor agonists specific for these two receptors significantly increased neprilysin activity while neurons from double SSTR knockout mice did not respond to somatostatin.

Conclusions

Our resul**ts sho**w unambiguously that two subtypes of the SSTRs regulate in parallel neprilysin in hippocampus and therefore directly control the Aβ levels. This opens up for a potential AD treatment using specific agonists targeting these two receptor subtypes to activate neprilysin.

Symposium 39: PROTEIN MODIFICATION AND DEGRADATION

ADPD5-1243
PHOSPHORYLATED ABETA PEPTIDES - MAKER OR MARKER IN ALZHEIMER'S DISEASE

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Objective
Aβ is generated by proteolytic processing involving sequential cleavages of the amyloid precursor protein (APP) by β- and γ-secretase. Several familial AD (FAD) mutations that cause early onset AD are found within the Aβ and result in alterations of peptide conformation and promote its aggregation. However, such mutations are very rare and account for only a very small number of cases. We recently demonstrated that extracellular Aβ undergoes phosphorylation by secreted variants of protein kinase A and that the phosphorylation of Aβ at serine residue 8 promotes its aggregation into oligomeric and fibrillar assemblies.
Methods
We applied cell biological, biochemical, biophysical and neuropathological methods to characterize the phosphorylation of Aβ in cell culture models as well as in brains of human AD cases and transgenic mice.
Results
Phosphorylated Aβ shows increased propensity to form oligomeric and fibrillar aggregates and adopt β-sheet conformation. By using highly specific phosphorylation state specific antibodies, we demonstrate abundant presence of phosphorylated Aβ in human AD and APP transgenic mouse brain. Phosphorylated Aβ species were detected in extracellular plaques, inside of neurons, and in cerebral vessels, thereby indicating a contribution to all characteristic Aβ associated lesions in AD brains. Phosphorylated Aβ species appear enriched in clinically manifested AD as compared to pathologically preclinical stages.
Conclusions
Phosphorylated Aβ peptides are common and abundant species in human AD brains and transgenic mouse models. The detection of these variants could be further explored as targets for AD therapy and prevention as well as markers for AD pathogenesis.

Symposium 40: DEMENTIA WITH LEWY BODIES: UPDATES FROM PRE-CLINICAL AND CLINICAL STUDIES, PART I

ADPD5-1021
PATHOLOGICAL PHENOTYPING: WHAT IS THE BEST CORRELATE FOR DLB?
G.M. Halliday[1]
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Objectives: Describe the pathology and its progression in different phenotypes of patients with alpha-synuclein-immunoreactive Lewy bodies
Methods: Examination of immunohistochemically stained brain tissue sections with antibodies to alpha-synuclein in a large longitudinally studied cohort.
Results: Lewy bodies are intraneuronal cytoplasmic inclusions made of many proteins, but the core fibrils are made from the abnormal aggregation of alpha-synuclein. Lewy bodies form from a build up of punctate membrane aggregates of phosphorylated alpha-synuclein that coalesce into loosely packed filaments that undergo ubiquitination but not degradation, rather “maturing” by truncation and compaction. Lewy bodies occur to a small degree in the elderly (now considered preclinical) and in patients with diverse neurological and psychiatric disorders. At autopsy, the largest numbers of patients with Lewy bodies are 1) those with PD, 2) those with dementia with Lewy bodies (DLB), and 3) those with Alzheimer’s disease (AD). These patient types are separated by the early onset of dementia (DLB and AD) and by the regional distribution and severity of Lewy body and AD-type pathologies. Assessment of these different pathological cohorts suggests that the timing and tempo of the different pathologies relates to the clinical phenotype and that such pathophysiological differences are likely to involve different molecular interactions.
Conclusion: A slow pace of relatively restricted regional Lewy body involvement occurs in PD, while the most rapid and spatially expansive molecular involvement occurs in patients with DLB.

Symposium 40: DEMENTIA WITH LEWY BODIES: UPDATES FROM PRE-CLINICAL AND CLINICAL STUDIES, PART I

ADPD5-1126

MECHANISM OF PROGRESSIVE SPREADING IN SYNUCLEINOPATHIES

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Deposition of alpha-synuclein aggregates occurs widely in the central and peripheral nervous systems in Parkinson's disease (PD). Although recent evidence has suggested that cell-to-cell transmission of alpha-synuclein aggregates is associated with the progression of PD, the mechanism by which alpha-synuclein aggregates spread remains undefined. In this talk, I will show that alpha-synuclein aggregates are transmitted from cell to cell through a cycle involving uptake of external aggregates, co-aggregation with endogenous alpha-synuclein, and exocytosis of the co-aggregates. Moreover, my colleague and I found that glucocerebrosidase depletion, which has previously been strongly associated with PD and increased cognitive impairment, promoted propagation of alpha-synuclein aggregates. Depletion of other genes such as ctsd (cathepsin D) and ATP13A2, resulted in mixed outcomes in lysosomal functions. The cell lines with these gene depletions further confirmed that lysosomal dysfunction is the key modulator of spreading of synucleinopathy. These studies define how alpha-synuclein aggregates spread among neuronal cells and may provide an explanation for how glucocerebrosidase mutations increase the risk of developing PD and other synucleinopathies.

Symposium 40: DEMENTIA WITH LEWY BODIES: UPDATES FROM PRE-CLINICAL AND CLINICAL STUDIES, PART I

ADPD5-1588

GBA1-LINKED PARKINSONISM: FOCUS ON COGNITION AMONG ASHKENAZI JEWS IN ISRAEL

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Background: Subjects who are heterozygotes with severe mutations in the *GBA* gene have a higher risk to develop PD. The aim of this study was to establish a well characterized group of Ashkenazi Jewish PD patients and asymptomatic subjects, carriers of severe mutations in the *GBA* gene.
Methods: Genotype was assessed in 1050 Ashkenazi PD cohorts, 220 of them were GBA mutation carriers (20.9%) and 54 were carriers of severe GBA mutations (24.5%). 83 asymptomatic relatives were also assessed, 39 of them were carriers of severe GBA mutations (47%). All participants underwent a thorough neurological assessment.
Results: Patients with PD carriers of severe GBA mutations tended to have an earlier age of motor symptoms onset (56.9±8.8 vs. 59.55±10.3). PD patients, carriers of severe GBA mutations, demonstrated lower performance on cognitive tests compared to carriers of mild mutations and patients with PD who were non-carriers . Patients with PD who are carriers of severe GBA mutations also reported more REM Sleep Behavior Disorder symptoms than carriers of mild mutations and patients with PD who were non-carriers ($p<0.030$). No differences were observed in age, gender, years of education, autonomic function, behavioral profile or smell identification, between asymptomatic carriers of severe GBA mutations and carriers of mild mutations or non-carriers. However, asymptomatic carriers of severe GBA mutations performed poorer on executive function tasks (TMT).
Conclusions: PD patients and asymptomatic carriers with severe GBA mutations have specific cognitive profile among PD patients and healthy heterozygotes.

Symposium 40: DEMENTIA WITH LEWY BODIES: UPDATES FROM PRE-CLINICAL AND CLINICAL STUDIES, PART I

ADPD5-1645
RADIOLOGICAL PHENOTYPING OF DLB: HOW COULD IMAGING HELP DIAGNOSTIC ACCURACY?
S. Gomperts[1]
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Background: The sensitivity and specificity of clinical criteria of the Third Consortium on Dementia with Lewy bodies (DLB) for neuropathologically proven DLB approach 85% and 75%, respectively, at tertiary care centers.
Goals: To assess imaging biomarkers that differentiate DLB from related Lewy body diseases, AD and HCS.
Methods: Cross-sectional and longitudinal prospective studies in subjects with DLB, PD with normal cognition (PD-nl), PD with mild cognitive impairment (PD-MCI), and PD with dementia (PDD), contrasted with Alzheimer's disease (AD) and healthy control subjects (HCS). Subjects underwent formal neurologic examination, detailed neuropsychological assessments, MRI, and PET scans with radioligands altropane (DAT: dopamine transporter), PiB (Aβ amyloid) and FDG (glucose metabolism).
Results: Putamen DAT concentrations were similar in DLB and PD and differentiated DLB from HCS and AD. Decreased caudate DAT concentration related to cognitive impairment in DLB. PiB uptake was greatest in DLB and AD. However, cortical PiB retention was common in PD and predicted cognitive decline. In contrast to DAT and PiB, FDG distinguished PD-MCI from PD-nl groups on the basis of cortical hypometabolism. PET imaging of paired helical filament tau has potential for differentiating DLB and AD from the parkinsonian tauopathies.
Conclusion:
Multimodal PET imaging differentiates DLB from other disease groups and may increase diagnostic accuracy in the clinic, improve cohort uniformity for clinical trials, and serve as biomarkers for targeted molecular therapies.

Symposium 40: DEMENTIA WITH LEWY BODIES: UPDATES FROM PRE-CLINICAL AND CLINICAL STUDIES, PART I

ADPD5-1755
CLINICAL CHARACTERIZATION OF PRODROMAL DLB
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Prodromal DLB can be defined by a relevant clinical deficit which is combined with a biomarker suggestive of an underlying alpha-synucleinopathy. Cases presenting with prodromal cognitive symptoms are most likely to be characterised as non-amnestic MCI with prominent attentional, executive and visuo-perceptual dysfunction. Conversion to DLB is reported to occur in two-thirds of such cases within a seven year follow up with baseline presence of REM sleep behaviour disorder, fluctuations, daytime sleepiness and mild parkinsonism increasing risk. Non-cognitive prodromal presentations include psychiatric (unexplained delirium, stupor, mood disorders and psychosis), hyposmia and autonomic dysfunction (constipation, orthostatic dizziness and urinary incontinence). Since most of these symptoms lack disease specificity, it is the order of their appearance and the total number of symptoms experienced that may be most important in identifying early stage Lewy body disease.
Biomarkers available for current use include structural MRI for medial temporal lobe preservation, perfusion and metabolic SPECT/PET for occipital hypofunction, dopamine transporter SPECT for nigro-striatal dysfunction and cardiac scintigraphy. Although these techniques may in combination be useful for confirmation of moderate or advanced LB disease cases, it is as yet unclear how they can contribute to prodromal diagnosis. Histological confirmation by autonomic ganglia or cutaneous nerve biopsy may ultimately provide the best evidence of an underlying alpha-synucleinopathy in early stage cases and might be considered necessary before starting long-term, disease-specific preventative treatment.

Symposium 40: DEMENTIA WITH LEWY BODIES: UPDATES FROM PRE-CLINICAL AND CLINICAL STUDIES, PART I

ADPD5-1972
TECHNOLOGIES FOR STUDYING AD AND PD GENE FUNCTIONS IN HUMAN NEURONS: POTENTIAL AND LIMITATIONS OF iN Cells

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Over the last five years, my laboratory has developed approaches to study human neurons in collaboration with the Wernig laboratory (also at Stanford). We have described protocols for the rapid trans-differentiation of fibroblasts and iPS/ES cells into neurons (referred to as iN cells), and for the efficient introduction of conditional mutations into these neurons using AAV- or CRISPR-mediated homologous recombination. Our present goal, as described in my lecture, is twofold: First, to optimize and standardize this method, to clarify the biological basis for its success, and to apply it to study synaptic function both in healthy and diseased human neurons. Second, to generate mutations in AD- and PD-linked genes in these human neurons, and to analyze the effects of such mutations on neuronal function, in particular synaptic transmission. I will discuss the iN cell technology and outline present results towards these goals with the hope of convincing the audience of the utility of the approach.

Symposium 42: TDP 43, C9ORF72 AND TTR

ADPD5-0340
A NOVEL THERAPEUTIC APPROACH TO ALS: INHIBITION OF TDP-43 AGGREGATION AND FORMATION OF TDP-43 POSITIVE PATHOLOGICAL STRESS GRANULES

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We have identified compounds that inhibit cellular aggregation of TDP-43, which is genetically linked to amyotrophic lateral sclerosis (ALS), and accumulates as pathology in ALS and frontotemporal dementia (FTLD-TDP). Pathological TDP-43 forms insoluble protein aggregates that accumulate predominantly in the cytoplasm, but can also appear in the nucleus. Extensive research suggests that these insoluble protein aggregates form via the stress granules (SG), which are a type of RNA granule that forms in response to many different types of cellular stress. Accumulating data suggests that TDP-43 aggregates lead to disease by sequestering TDP-43 in the insoluble aggregates, perhaps because of reduced availability of soluble, functional TDP-43, or by directly stimulating RNA degradation and cell death pathways through the SG pathway. In both scenarios, inhibiting formation of SG containing insoluble TDP-43 could inhibit the pathophysiology of ALS and FTLD-TDP and delay disease.

Using a cell based screening approach, we identified a series of compounds that inhibit TDP-43 inclusion formation in a reproducible and dose-dependent manner, while showing little-to-no cytotoxicity. Inhibition of TPD-43 aggregation is observed in cell lines, primary cortical neurons and in induced pluripotent stem cells from controls and ALS subjects. Biochemical studies indicate that our lead compound preferentially reduces TDP-43 aggregates and cleavage products, while only slightly reducing levels of TDP-43 monomer. The compounds appear to act through a mechanism independent of TDP-43 ubiquitination. The compounds also reduce the deleterious effects of TDP-43 expression in *C. elegans*. This work potentially identifies a novel therapeutic approach for treating ALS and FTLD-TDP.

Symposium 42: TDP 43, C9ORF72 AND TTR

ADPD5-1442

TRANSTHYRETIN EXPRESSION IS INCREASED IN HUMAN AD AND BEHAVES AS A NEURONAL STRESS PROTEIN IN APP23 AD MODEL MICE DETOXIFYING A BETA AND MODULATING ITS PRODUCTION

J. Buxbaum[1], *X. Li*[1]
[1]*MEM, The Scripps Research Institute, La Jolla, USA*

Background: We have shown that neurons from human AD brains stain with an anti-transthyretin (TTR) antibody, that over-expression of a wild type human *TTR* gene suppresses the AD phenotype in APP23 mice and that TTR inhibits Aβ aggregation, fibril formation and cytotoxicity *in vitro* and in tissue culture by binding to monomers, small oligomers and fibrils. In cultured neurons and hippocampi of APP23 mice *TTR* transcription is increased through the action of the stress response transcription factor HSF1. In analyses of brains from APP23 mice genetically programmed to over-express human TTR we noted that the production of Aβ fragments appeared to be reduced, raising the question of whether TTR could bind to Aβ precursors perhaps interfering with processing.

Methods and Results: Collaborative NMR studies with Y. Song in the Sanders laboratory at Vanderbilt University showed that TTR interacted with C99 at residues G659, A665, T668 (APP 695 numbering). Transfection of Aβ-producing 7PA2 CHO cells with a human TTR construct resulted in diminished Aβ in the culture medium while immunoprecipitation of DSP cross-linked lysates of the cells with an anti-TTR antibody pulled down both C99 and APP.

Conclusions: human TTR appears to behave as a neuronal chaperone regulated by HSF1 and interferes with Aβ aggregation *in vivo* and *in vitro*. We now find that it interacts with the Aβ precursors APP and C99 and appears to interfere with their processing and release of Aβ into the media. The effect is likely to be related to interference with phosphorylation of threonine 668.

Symposium 42: TDP 43, C9ORF72 AND TTR

ADPD5-1495

TDP-43 MEDIATED SYNAPTIC ALTERATIONS IN THE PATHOGENESIS OF TDP-43 PROTEINOPATHIES

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Background: TPD-43 mediated neurodegeneration results from the pathological miss-processing and/or functional change of this RND-binding-protein. Recent research has indicated that TDP-43 alterations may have an early, underappreciated, pathological role at the synapse.
Objectives: Characterise the pre- and post-synaptic pathology occurring in the cortex of the TDP-43^{A315T} mouse model of ALS with regional immunohistochemistry and spine density analysis. Spine analysis was investigated in TDP-43^{A315T} YFP-H fluorescent mice.
Methods: Pre-synaptic pathology was investigated using immunohistochemistry (Synaptophysin, VGluT and VGAT1). Dendrite spines in the TDP-43^{A315T}YFP-H mice were investigated on the Zeiss LSM-510-Meta confocal microscope using Neurolucida™ software.
Results: Whilst there was no significant difference in Synaptophysin labelled puncta**,** Glutamatergic and GABAergic pre-synaptic vesicle transporters were significantly reduced at day 90 (symptom onset) in the TDP-43^{A315T} mice compared to wild-type controls (Figure 1). These changes were specific to motor cortex and not present in somatosensory cortex. However, there was significant total reduction in spine densities in somatodendritic and apical TDP-43^{A315T} x YFP-H dendrites at this symptom onset, day 90, time-point. There was no significant difference in spine densities during the development of the TDP-43^{A315T} x YFP-H mouse, investigated at day 30.
Conclusion: Our investigations highlight potential pathogenic roles for mutated TDP-43 at the synapse. Understanding the role that TDP-43 plays in synaptic dysfunction may reveal new therapeutic windows for intervention in TDP-43 proteinopathies.

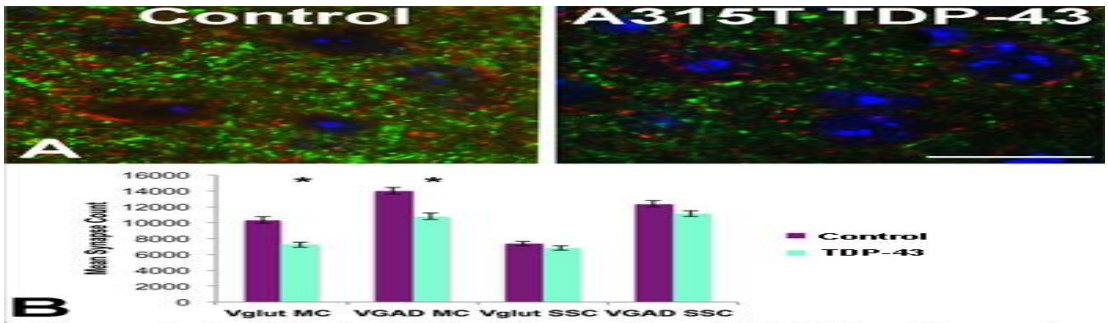

Figure 1. A. IHC for VGlut (green) and VGAD (red) puncta in the motor cortex (MC) in control and A315T TDP-43 mice at day 90 (blue = DAPI) B. Synapse counts for both VGlut and VGAD were significantly reduced in the motor cortex with no change in the somatosensory cortex (SSC) at day 90, in comparison to control (*p<0.05). Scale bar = 50µm

Symposium 42: TDP 43, C9ORF72 AND TTR

ADPD5-1567
FULL-LENGTH TDP-43 FORMS TOXIC AMYLOID OLIGOMERS THAT ARE PRESENT IN FRONTOTEMPORAL LOBAR DEMENTIA-TDP PATIENTS
Y.R. Chen[1]
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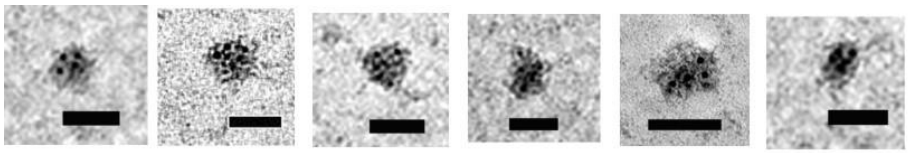
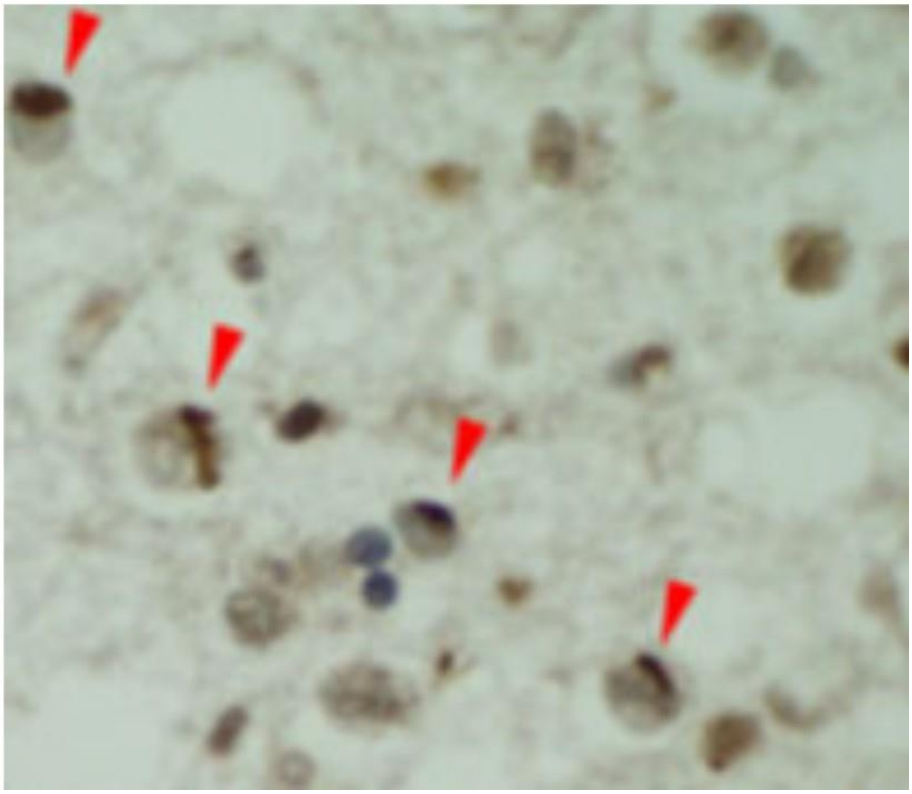
Proteinaceous inclusions are common hallmarks of many neurodegenerative diseases. TDP-43 proteinopathies, consisting of several neurodegenerative diseases including frontotemporal lobar dementia (FTLD) and amyotrophic lateral sclerosis (ALS), are characterized by inclusion bodies formed by polyubiquitinated and hyperphosphorylated full-length and truncated TDP-43. The structural properties of TDP-43 aggregates and their relationship to the pathogenesis are still ambiguous. Here, we demonstrated that the recombinant full-length human TDP-43 forms structurally stable, spherical oligomers that share common epitopes with an anti-amyloid oligomer-specific antibody. The TDP-43 oligomers are stable, have exposed hydrophobic surfaces, exhibit reduced DNA binding capability, and are neurotoxic *in vitro* and *in vivo*. Moreover, TDP-43 oligomers are capable of cross-seeding Alzheimer's amyloid-β to form amyloid oligomers, showing the inter-convertibility between the amyloid species. Such oligomers are present in the forebrain of transgenic TDP-43 mice and FTLD-TDP patients. Our results suggest aside from filamentous aggregates TDP-43 oligomers may play a role in TDP-43 pathogenesis.





Reference:

Yu-Sheng Fang, Kuen-Jer Tsai, Yu-Jen Chang, Patricia Kao, Rima Woods, Pan-Hsien Kuo, Cheng-Chun Wu, Jhih-Ying Liao, Shih-Chieh Chou, Vinson Lin, Lee-Way Jin, Hanna S. Yuan, Irene H Cheng, Pang-Hsien Tu, and **Yun-Ru Chen***. "Full-Length TDP-43 Forms Toxic Amyloid Oligomers that are Present in Frontotemporal Lobar Dementia-TDP Patients." (*corresponding author) (**2014**) (**Nature Communications,** 5:4824, DOI: 10.1038/ncomms5824, 1-13).

Symposium 42: TDP 43, C9ORF72 AND TTR

ADPD5-1840

THERAPY DEVELOPMENT FOR AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA WITH C9ORF72 EXPANSION

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Objectives:

Expanded hexanucleotide repeats in a non-coding region of the *C9orf72* gene were recently identified as the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The pathogenic mechanisms of this expansion are not understood, but initial observations point to either a loss of function of the endogenous *C9orf72* gene, and/or a toxic gain of function of the expanded RNA. The latter may be mediated either by sequestration of RNA binding proteins into RNA foci or by production of aberrant polypeptide(s) through repeat-associated non-ATG-dependent (RAN) translation. Our objectives are to develop cellular and animal models to dissect disease mechanisms and develop a therapeutic strategy lowering *C9orf72* RNAs.

Methods:

To test the efficacy and tolerability of antisense oligonucleotides (ASOs) targeting *C9orf72* expanded RNAs in transgenic mice and neurons directly converted from patient fibroblasts.

Results:

We have generated mice modeling either a loss of C9orf72 function or a toxic gain of function to unravel the relative contributions of each mechanism. We established multiple lines of BAC transgenic mice expressing different repeat lengths of a repeat-containing human *C9orf72* gene. Pathologic RNA foci containing both sense and antisense repeat RNAs and RAN dipeptides are identified in brains and spinal cords of these *C9orf72* mouse models and in neurons derived from *C9orf72* patient fibroblasts.

Conclusions:

A therapeutic strategy using antisense oligonucleotides (ASOs) that mediate degradation of RNAs carrying the *C9orf72* hexanucleotide expansion is developed to target a gain of toxic function from expanded *C9orf72* in ALS and FTD patients.

Symposium 42: TDP 43, C9ORF72 AND TTR

ADPD5-1861
NEUROPROTECTION OF CK-1 INHIBITORS AGAINST TDP-43 AND LPS TOXICITY

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1. Objectives: To show that protein kinase CK-1 inhibitors are useful innovative drug candidates for TDP-43 mediated neurodegenerative diseases and also Parkinson disease [1,2]
2. Methods: Chemical genetics approach to identify new CK-1 inhibitors using an in-house chemical library of heterocyclic small molecules. Hit to lead optimization trough medicinal chemistry programs. Lead to candidate selection based on ADME-Tox properties. Cell based and animal studies with leads and candidates to confirm the efficacy of CK-1 inhibitors.
3. Results: Identification of two new families of CK-1 inhibitors. A reduction of TDP-43 phosphorylation is observed after CK-1 inhibitors treatment in cell cultures. Neuronal toxicity induced by hTDP-43 protein on transgenic flies is decreased after the treatment with our CK-1 inhibitors. CK-1 inhibitors also shown neuroprotection in cell based and in vivo models of Parkinson disease
4. Conclusions: CK-1 inhibitors, and specially our small molecule candidate IGS2.7, are promising drug candidates for the future treatment of neurodegenerative diseases such as Alzheimer disease, amyotrophic lateral sclerosis, frontotemporal dementia and Parkinson disease

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[2] Kametani F, Nonaka T, Suzuki T, Arai T, Dohmae N, Akiyama H, Hasegawa M. Identification of casein kinase-1 phosphorylation sites on TDP-43. Biochem Biophys Res Commun. 2009 May 1;382(2):405-9.

Symposium 43: FUNCTIONS OF ETA SECRETASE AND BACE1

ADPD5-0940
BETA-SECRETASE AND INFLAMMATION MARKERS IN PRECLINICAL ALZHEIMER'S DISEASE

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Objectives: To investigate biomarkers in CSF of relevant pathophysiological pathways in a large multicentre cohort of cognitively normal subjects.
Methods: We measured markers of amyloid precursor protein processing (A[beta]42, sAPP-[beta], [beta]-secretase activity), neuronal damage (t-Tau, p-Tau) and neuroinflammation (YKL-40) in the cerebrospinal fluid (CSF) of 266 cognitively normal subjects recruited as part of a large multicentre study. We used NIA-AA research criteria for classification into different stages of preclinical AD. We analyzed the relationship between CSF biomarkers, clinical variables and *APOE* genotype, and we compared CSF biomarkers across diagnostic groups.
Results: Age showed a positive correlation with CSF t-Tau, p-Tau and YKL-40. Age also correlated with CSF A[beta]42, but only in APOE[epsilon]4 carriers. CSF A[beta]42 correlated positively with Tau, sAPP[beta] and YKL-40 in subjects with normal A[beta]42 levels. These correlations were negative or non-significant in participants with low A[beta]42 levels. Subjects labelled as preclinical AD (stage 2 and 3), and 'suspected non-amyloid pathology' (SNAP) had higher levels of YKL-40 than participants in Stage 0 and Stage 1. No differences in sAPP[beta]levels of [beta]-secretase activity in CSF were detected across groups.
Conclusions: CSF [beta]-secretase is not altered in preclinical AD. CSF A[beta]42 correlates with tau but the directionality of the correlations differs depending on the A[beta]42 status. Moreover, subjects in preclinical stages 2-3 of AD and with SNAP showed high levels of YKL-40. This suggests that inflammation is a very early process in neurodegenerative diseases.

Symposium 43: FUNCTIONS OF ETA SECRETASE AND BACE1

ADPD5-1023
A PHYSIOLOGICAL ROLE FOR THE ALZHEIMER'S DISEASE-RELATED BACE1 IN REGENERATING NEURONS

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Objectives: To identify a physiological role for BACE1 in the central nervous system.
Methods: Animals were anaesthetized and the right facial nerve was transected at its exit from the stylomastoid foramen. The contralateral side was left intact and served as a paired internal control. Survival times studied were 3, 7, 14 and 21 days. Sixteen-micrometer-thick coronal cryosections of the brain stem were collected onto glass slides, fixed in buffered formaldehyde solution, washed in 0.01 M PBS and incubated with rabbit polyclonal anti-BACE1 antibody (00/6) for two hours at room temperature. Slides were subsequently incubated with Alexa 488-conjugated goat anti-rabbit secondary antibody for 1h at room temperature and viewed under confocal microscopy.
Results: We observed a rapid and sustained up regulation of BACE1 protein expression in activated microglia and astrocytes. In addition, we also observed an increase in BACE1 protein expression in injured neurons in the facial nucleus up to 21 days (longest time-point evaluated) following axotomy of the facial nerve.
Conclusions: Up regulation of BACE1 expression in astrocyte cultures has been previously reported. However, its expression *in vivo* in both activated astrocytes and microglia is novel. Its up regulation in neurons might point to an association with repair mechanisms whereas its presence in activated glia may constitute a signaling mechanism whereby its expression in proliferating glial cells surrounding the damaged site may aid in the clean up and repair processes. The high level of BACE1 expression in regenerating neurons highlights a novel physiological role for BACE1 in the CNS.

Symposium 43: FUNCTIONS OF ETA SECRETASE AND BACE1

ADPD5-1068
ETA-SECRETASE GENERATES APP FRAGMENTS THAT MODULATE LTP AND ACCUMULATE UPON BACE1 INHIBITION

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Alzheimer's disease (AD) pathology is characterized by the accumulation of amyloid plaques, which are predominantly composed of amyloid b-peptide (Aβ). Two principal physiological pathways either prevent or promote Aβ generation from its precursor (amyloid precursor protein; APP) in a competitive manner. Modulation of the amyloidogenic pathway is currently exploited by anti-Aβ therapeutic strategies. Although APP processing has been studied in great detail, unknown proteolytic events appear to hinder stoichiometric analyses of APP metabolism in vivo. We now identified a higher molecular weight C-terminal fragment of APP (CTF-η) in addition to the long-known fragments CTF-α and CTF-β, which are generated by α- and β-secretase (a disintegrin and metalloproteinase; ADAM10 and β-site APP cleaving enzyme 1; BACE1). CTF-η is generated by MT5-MMP, a membrane bound matrix-metalloproteinase referred to as η-secretase, which co-localizes with senile plaques in AD. MT5-MMP mediated cleavage results in the release of a truncated, soluble APP ectodomain (sAPP-η). Upon shedding of sAPP-η, CTF-η is further processed by ADAM10 and BACE1 to release long and short Aβ peptides (Aη-α and Aη-β). η-Secretase activity is enriched in dystrophic neurites in an AD mouse model and human AD brains. Genetic and pharmacological inhibition of BACE1 activity results in a robust accumulation of CTF-η and Aη-α. Strikingly, Aη-α lowers hippocampal long-term potentiation similar to synaptotoxic Aβ oligomers. These findings not only demonstrate a novel physiologically and pathologically relevant APP processing pathway but are also of immediate translational relevance.

Symposium 43: FUNCTIONS OF ETA SECRETASE AND BACE1

ADPD5-1385

LOSS OF FUNCTION OF SPINOCEREBELLAR ATAXIA TYPE-1 PROTEIN, ATXN1, ENHANCES BACE1 LEVELS AND BETA-AMYLOID PATHOLOGY IN THE CEREBRUM

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1. Objectives

Abnormal expansion of CAG repeat in the ataxin-1 gene (*ATXN1*) causes the degeneration of Purkinje cells and cerebellar ataxia type 1 (SCA1), a neurodegenerative movement disease. Previously our laboratory has reported *ATXN1* is associated with Alzheimer's disease (AD) and knockdown of ATXN1 increases Abeta generation in cultured mammalian cells. Here, we investigate whether ATXN1 expression affects the processing of amyloid precursor protein (APP) and the pathogenesis of AD.

2. Methods

We examined the levels of APP cleavage products and the secretases in the brains of ATXN1 knockout (KO) and wild-type mice. APP processing and Abeta pathology were further analyzed after crossing the ATXN1 mice with APPswe/PS1deltaE9 AD mice.

3. Results

We found ATXN1 KO increases BACE1 levels in cortex and hippocampus, but not in cerebellum and brain stem. This increase of BACE1 is concordant with the shift of APP processing into the beta-secretase cleavage pathway along with an increase in Abeta levels and plaque load in the brains of APPswe/PS1deltaE9 mice. In addition, the dendritic development of immature neurons, a neurogenic marker in adult brain, was impaired in the hippocampus of ATXN1 KO mice.

4. Conclusions

Together, these findings suggest that the loss of ATXN1 function potentiates beta-amyloid pathology by increasing BACE1 levels and the subsequent beta-secretase cleavage of APP.

Symposium 43: FUNCTIONS OF ETA SECRETASE AND BACE1

ADPD5-1672
SCHWANN CELL BACE1 IS REQUIRED FOR REMYELINATION OF PERIPHERAL NERVES

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Objectives: Inhibition of BACE1 is being pursued as a therapeutic target for treating patients suffering from Alzheimer's disease because BACE1 is a critical β-secretase for generating β-amyloid peptide. Knowledge regarding the other cellular functions of BACE1 is therefore critical for the safe use of BACE1 inhibitors in human patients. BACE1 deficiency in mice causes hypomyelination during development and impairs remyelination in injured sciatic nerves. Since BACE1 is expressed by both neurons and myelinating Schwann cells, we asked whether axonal or Schwann cell BACE1 is required for optimal remyelination. **Methods:** By swapping sciatic nerve segments from BACE1-null mice with the corresponding wild-type nerve segments or vice versa, we tested how deficiency of BACE1 in Schwann cells or axons affected remyelination. **Results:** Our results show that BACE1 in axons and Schwann cells is similarly important for remyelination of regenerated axons. We found that neuregulin 1 (Nrg1) is expressed by Schwann cells and that abolished Nrg1 cleavage in BACE1-null Schwann cells contributes to decreased remyelination of regenerated axons. Although the myelin sheath was thinned in BACE1-null mice, the number of Schwann cells in BACE1-null mice was higher than wild-type. **Conclusions:** This study is the first to provide evidence that remyelination requires non-neuronal sources of BACE1.

Symposium 43: FUNCTIONS OF ETA SECRETASE AND BACE1

ADPD5-1747
BACE1 IN HEALTH AND ALZHEIMER'S DISEASE
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The β-secretase, β-site amyloid precursor protein (APP) cleaving enzyme 1 (BACE1), is the first of two proteases (the second being γ-secretase) to cut APP to generate the β-amyloid peptide (Aβ) implicated in Alzheimer's disease (AD) pathogenesis. As such, BACE1 is a prime therapeutic target for lowering cerebral Aβ levels as a treatment strategy for AD. The highest levels of BACE1 in the body are found in neurons of the brain, where it is localized within endosomal compartments of cell bodies and presynaptic terminals under non-pathologic conditions. However, in AD brain, BACE1 levels increase several fold, and vesicles containing the enzyme accumulate within swollen, distended axons that pass near amyloid plaques. These peri-plaque dystrophic axons also accumulate APP, suggesting the possibility that increased enzyme and substrate might accelerate Aβ production near amyloid deposits, thus exacerbating plaque growth and AD progression. Indeed, we will present evidence that BACE1 accumulation in dystrophic axons is associated with increased levels of BACE1-cleaved fragments of APP and Aβ in our 5XFAD mouse model of AD amyloidosis. Possible mechanisms of Aβ-induced BACE1 accumulation will be discussed. We conclude that Aβ causes increased levels of BACE1 that in turn lead to further Aβ production, instigating a vicious cycle of AD pathogenesis.

Symposium 45: PINK1, LRRK AND PARKIN

ADPD5-0496
AGE-DEPENDENT, SELECTIVE DOPAMINERGIC NEURODEGENERATION, PROTEIN ACCUMULATION AND AUTOPHAGY IMPAIRMENT IN LRRK-DEFICIENT MICE

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Mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) gene are the most common genetic cause of Parkinson's disease (PD), but the pathogenic mechanism is unclear. We previously generated *LRRK2*-/- mice and found no detectable phenotypes in the brain, but the kidney develops striking age-dependent PD-like changes, including accumulation of alpha-synuclein and ubiquitinated proteins, impairment of the autophagy-lysosomal pathway, increases of apoptosis, inflammatory responses, and oxidative damage. To determine whether the absence of the phenotypes in the *LRRK2*-/- brain might be due to the relative high expression of LRRK1 in the brain, we developed double knockout mice lacking both LRRK1 and LRRK2. *LRRK1/2*-/- mice are viable and fertile, but show reduced weight gain in adulthood and early mortality at ~15 months of age. Interestingly, stereological neuron counting revealed significant loss of dopaminergic neurons in the *substantia nigra pars compacta* and noradrenergic neurons in the *locus coeruleus* of *LRRK1/2*-/- mice at 15 months of age, whereas the number of dopaminergic neurons is normal in early ages. Furthermore, the volume of the cerebral cortex and the number of cortical neurons are normal in *LRRK1/2*-/- old mice, suggesting that the loss of dopaminergic and noradrenergic neurons is specific. In addition, at 15 months we observed increased levels of α-synuclein, impairment of autophagy function, increases of apoptosis, astrogliosis and microgliosis in *LRRK1/2*-/- brains. These findings show that loss of LRRK function results in age-dependent autophagy impairment and selective loss of dopaminergic neurons, indicating essential role of LRRK in autophagy regulation and survival of dopaminergic neurons.

Symposium 45: PINK1, LRRK AND PARKIN

ADPD5-0895
XBP-1S LINKS PARKIN TO DJ-1
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- Unfolded Protein Response that has been linked to the physiopathology of Parkinson's disease (PD) is a cellular response to Endoplasmic Reticulum (ER) stress induced by the accumulation of protein aggregates. Interestingly the E3 ubiquitin ligase Parkin and the chaperone protein DJ-1, two proteins related to autosomal recessive early-onset PD, are regulated by ER stress. Moreover, Parkin was reported to interact in stress conditions with DJ-1monomeres. We have unraveled that Parkin is also a transcription factor that modulates p53, presenilin 1 and 2 transcription. In this study we have investigated whether Parkin could control, in basal or ER stress conditions, DJ-1 expression through its transcription factor activity.
- We have overexpressed in different cell types (SH-SY5Y, TSM1, HEK293) wild-type Parkin or its ubiquintin ligase active or inactive mutants. We have used MEF cells invalidated or not for the gene coding for Parkin, p53 and also for the ER stress induced transcription factor: XBP-1S. mRNAs and proteins expression were recorded using Q-PCR and Western-blot techniques, while promoter activity and XBP-1S responsive element were analysed and identified by reporter gene assay, mutagenesis, gel shift and ChIP experiments.
- PK ubiquitine ligase activity is not involved in DJ-1 regulation. In contrast, in ER stress conditions, PK transcriptional repression of p53, activates XBP-1S that directly interacts with *DJ-1* promoter ultimately leading to the up regulation of DJ-1.
- Our study unravels an ER stress signalling pathway by which Parkin and the chaperone protein DJ-1 could control part of the UPR response in PD.

Symposium 45: PINK1, LRRK AND PARKIN

ADPD5-1165
LRRK2 BAC TRANSGENIC RATS DEVELOP PROGRESSIVE DEFICITS IN DOPAMINERGIC TRANSMISSION AND MOTOR DYSFUNCTION IN A NEW MODEL OF PARKINSON'S DISEASE

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Objectives: Mutations in leucine rich repeat kinase-II (LRRK2) cause autosomal dominant Parkinson's disease (PD). Transgenic animals have been crucial in modelling the mechanisms underlying PD progression. Bacterial artificial chromosome (BAC) transgenics generate physiologically relevant disease models encompassing large genomic DNA sequences using endogenous promoters and regulatory sequences. We have therefore developed BAC transgenic rats expressing human LRRK2. These lines express either G2019S mutant, R1441C mutant or wild-type LRRK2. Utilising rats allows for more advanced behavioural, neuroanatomical and neurophysiological analyses.
Methods: Young and old animals were assessed. Behaviour was assessed using a battery of motor and non-motor behavioural tests. Levodopa was administered in an attempt to rescue motor phenotypes. Striatal dopamine release and content were examined using fast-scan cyclic voltammetry and high-performance liquid chromatography. Stereological counts of neurons in the substantia nigra pars compacta (SNpc) were performed along with molecular assays to assess alterations related proteins. *In vivo* electrophysiological analysis was performed on dopaminergic neurons in the SNpc.
Results: Aged *LRRK2* mutant transgenic rats display impaired dorsal striatal dopamine release and corresponding motor and cognitive deficits. Motor deficits were shown to be levodopa-responsive. R1441C rats show alterations in SNpc neuronal firing patterns. Behavioural and dopamine release deficits occur in the absence of any overt degeneration of the SNpc neurons. *LRRK2* mutant transgenic animals show early changes in LRRK2 phosphorylation at key residues, although no accumulation of α-synuclein was seen in aged animals.
Conclusions: These rats recapitulate many of the key features of PD and inform on the sequence of disease progression.

Symposium 45: PINK1, LRRK AND PARKIN

ADPD5-1784
LRRK2 PHOSPHORYLATES TAU AT LITTLE EXPLORED EPITOPES AND ENHANCES TAU PATHOLOGY AND NEURONAL LOSS IN TRANSGENIC MICE.
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We recently discovered that LRRK2 phosphorylates the T149 and T153 epitopes in tau in vitro, cell culture and in a transgenic mouse model of tauopathy. Few previous studies have been performed on either T149 or T153, and our finding that LRRK2 preferentially targeted these sites and in mouse models enhanced tauopathy was entirely novel. In unpublished work, we have now extended these studies to demonstrate that the presence of tubulin significantly promotes the phosphorylation of tau by LRRK2 at these epitopes. We can also manipulate the degree by which LRRK2 can promote tau phosphorylation with physiologically relevant factors. Additionally, we have now identified a third epitope which appears to be more strongly targeted by LRRK2 than the previously described epitopes. Finally, we now show data that the modest overexpression of wild-type LRRK2 in a tau transgenic model enhances tau-directed neurodegeneration. The data will expand our currently poor understanding of the relationship between LRRK2 and tau pathology.

Symposium 45: PINK1, LRRK AND PARKIN

ADPD5-1892
PARKIN-P53 INTERPLAY IN PARKINSON AND OTHER BRAIN DISEASES
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Parkin has been described as an ubiquitin-ligase responsible for most of early-recessive cases of Parkinson's disease (PD). Interestingly, we 2 demonstrated that parkin also behaves as a transcriptional repressor of the tumor suppressor p53[1] and Presenilins 1 and 2, allowing to delineate a consensus parkin responsive element[2]. It is noteworthy that there exists an inverse relationship between the risk of developing PD and brain cancer. We showed that parkin levels are inversely correlated to p53 levels but also to the grade of either, oligodendroglioma, mixed gliomas and glioblastomas. We demonstrated that p53 exerts a positive control on parkin transcription i cellq as well as in vivo and that a lack of function of mutant p53 likely accounts for reduced levels of parkin in pathological biopsies[3]. Therefore, parkin is clearly a pleiotropic protein involved in various brain pathologies.
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3-VIOTTI, J., et al. (2014) Loss of function of p53 transcriptional activity results in parkin depletion that correlates with glioma tumor grade. *Oncogene.* **33**, 1764-1775

Symposium 45: PINK1, LRRK AND PARKIN

ADPD5-1965
ROLE OF THE PINK1-PARL-PGAM5 AXIS IN PARKINSON DISEASE
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Mutations in Pink1 cause a recessive form of Parkinson's disease. These mutations affect mitophagy in cell culture after CCCP depolarization. Interestingly in untreated cells these mutations lead to complex I deficiency which causes a mild depolarization of the mitochondria and a reduced ATP production. Similar observations in mouse brain, Drosophila and iPS cells from patients have led us to postulate that this role of PINK1 in complex I regulation is an early effect in patients. We have shown that Pink1 regulates NdufA10, a complex I component involved in ubiquinone reduction function. Intriguingly Pink1 is cleaved by the rhomboid PARL which resides at the inner membrane of the mitochondrium. PGAM5, a phosphatase, is a second substrate of PARL. PARL knock out mice show a massive neurodegeneration in their brain. We will present experiments that investigate the role of PARL processing of these kinase and phosphatase in the quality control of mitochondria.

Symposium 48: MICROGLIA AND INNATE IMMUNITY

ADPD5-0458
INTERLEUKIN-10 DEFICIENCY RE-BALANCES INNATE IMMUNITY TO MITIGATE ALZHEIMER-LIKE PATHOLOGY

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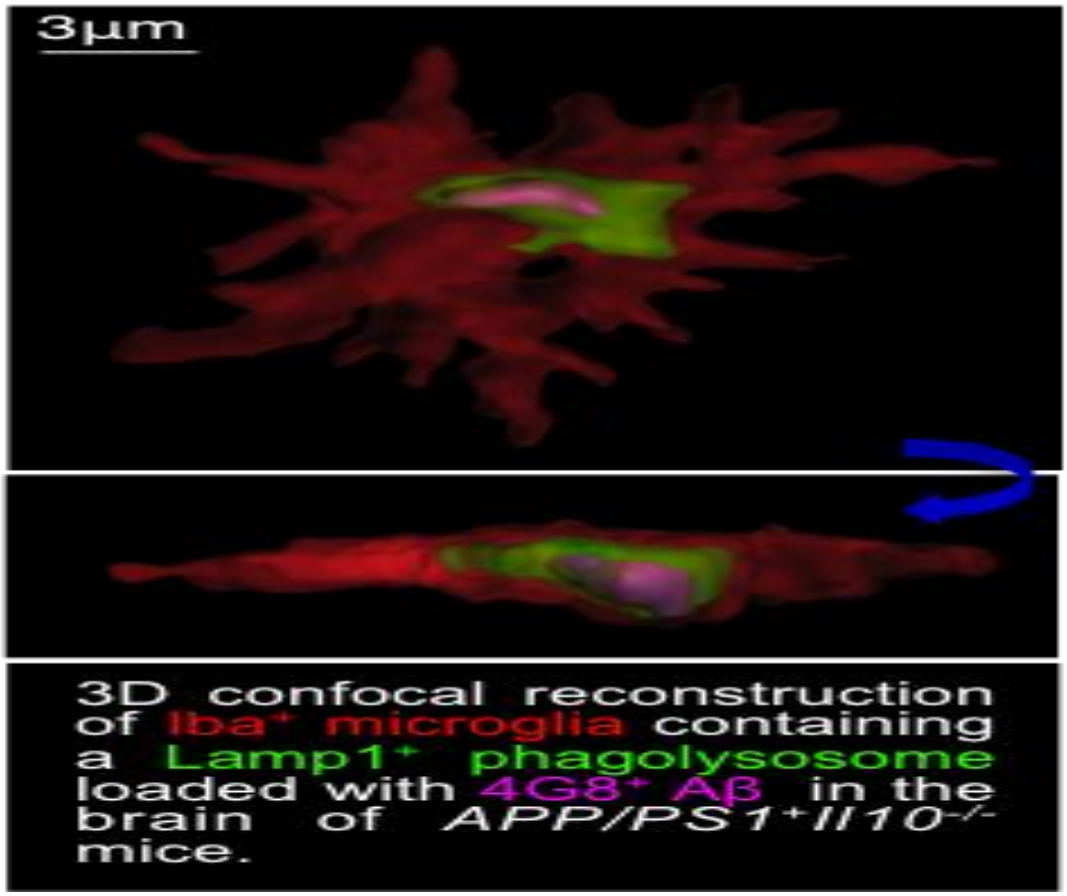
Objectives: Neuroinflammation and failure in Abeta clearance are accepted as essential participants in sporadic Alzheimer's disease (AD) evolution. Human evidence suggests that the anti-inflammatory cytokine, interleukin-10 (*Il10*), is etiologically involved in AD. Our goal is to delineate the contribution of IL-10 pathway in AD-like pathology.
Methods: We analyzed IL-10 signaling in AD patient brains *post-mortem* using immunostaining and protein analyses. To determine the impact of *Il10* deficiency on AD, we crossed the APP/PS1 mouse model of cerebral amyloidosis with a mouse deficient for *Il10.* We used 3D confocal reconstruction *in silico* to quantify Abeta phagocytosis in *APP/PS1^{+}Il10$^{-/-}$* mouse brains, and genome-wide RNA sequencing to identify immune genes modulation. We analyzed mice cognitive alteration and measured synaptic damage by synaptophysin labeling. IL-10 effect on phagocytic capacity was examined *in vitro* by live imaging of *Il10$^{-/-}$* versus *Il10$^{+/+}$* primary microglia.
Results: We observed elevated IL-10 signaling in AD patient brains. *Il10* deficiency in APP/PS1 mice activated innate immune cells to restrict cerebral amyloidosis and modulated innate immune genes driving neuroinflammation. Synaptic integrity and cognitive deficits were partially restored. *In vitro*, IL-10 ablation endorsed microglial Abeta uptake into phagolysosomes, whereas IL-10 addition reduced Abeta phagocytosis. Conclusion: Our results suggest that elevated IL-10 signaling in AD patient brains inhibits microglial Abeta phagocytosis and blocking anti-inflammatory

pathways can stimulate microglia to clear Abeta without inducing neurotoxicity.



Symposium 48: MICROGLIA AND INNATE IMMUNITY

ADPD5-1205
ABCA7 MEDIATES MICROGLIAL CLEARANCE OF AMYLOID-BETA OLIGOMERS

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Objectives: Genome-wide association studies indicate that ATP-binding cassette transporter A7 (ABCA7) is a strong risk factor for late-onset Alzheimer's disease (AD). We have previously demonstrated that deletion of ABCA7 in the J20 amyloidogenic mouse caused significant increases in insoluble amyloid-beta levels with concomitant increases in the number of amyloid-beta plaques in the hippocampus. ABCA7 has also been implicated in the role of phagocytosis. However, the mechanism by which ABCA7 reduces amyloid-beta load in the brain is unknown. In this study we investigated whether ABCA7 mediates microglial clearance of amyloid-beta oligomers.

Methods: We isolated the brain from ABCA7 knockout mice (n=6) and wild type littermates (n=6) following thorough perfusion with Hank's buffer. The whole brains were homogenized, filtered through cell strainer and cultured in flasks using L929 media. Following shaking the floating microglia were harvested and seeded onto chamber slides. They were then treated with FITC-labelled amyloid-beta40 (Ab40) and amyloid-beta42 (Ab42) oligomers, separately, and the uptake of Ab oligomers were analysed using fluorescence microscopy.

Results: The clearance of both Ab40 and Ab42 oligomers was significantly reduced (~50%) in ABCA7 null microglia compared to wild type microglia.

Conclusion: ABCA7 mediates microglial clearance of amyloid-beta oligomers, providing a pathogenic mechanism by which ABCA7 is implicated in AD neuropathology.

Symposium 48: MICROGLIA AND INNATE IMMUNITY

ADPD5-1340
A ROLE FOR MIROGLIA IN AGEING AND DEMENTIA
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Genetic risk factors for Alzheimer's disease (AD) recently identified imply that inflammation plays a causal role in AD. Using the MRC Cognitive Function and Ageing Study (CFAS), we investigated the role of microglia in human brain ageing and dementia.
Frontal cortex from 298 cases were analysed for CD68 (phagocytic activity); macrophage scavenger receptor (MSR)-A (plaque related), CD64 (Fcγ receptor I), Iba1 (resting and activated microglia), TREM2 (phagocytic vs proinflammatory microglial activity) and HLA-DR (antigen presenting function). All analyses were adjusted for age of death and sex.
Overall, MMSE was associated negatively with CD68 and HLA-DR and positively with CD64 and Iba1. Among the cases without dementia, associations were observed for: diffuse plaques with all markers except MSR-A which was strongly associated with neuritic plaques. HLA-DR and Iba1 were negatively associated with tangles. In the cases with dementia and AD pathology, CD64 was strongly associated with all neurodegenerative pathologies except tangles, CD68 with plaques and tangles, and MSR-A with neuritic plaques and tangles. HLA-DR was significantly related to plaques and tangles and Iba1 with increase in all neuropathological features. TREM2 recognized only monocytes/macrophages. With regard to the *APOE* polymorphism, ε2 was associated with expression of Iba1 and MSR-A and ε4 with CD68, CD64 and HLA-DR. These data suggest that microglia may respond differently to Aβ and tau in subjects with and without dementia so that the microglial response may influence the likelihood of developing dementia. Interestingly the findings also suggest that *APOE* polymorphism may influence the microglial profile.

Symposium 48: MICROGLIA AND INNATE IMMUNITY

ADPD5-1407

IMPAIRMENT IN EXPRESSION OF PARKINSON'S DISEASE RELATED GENES IN MICROGLIA TRIGGERS NEUROTOXICITY TO DOPAMINERGIC NEURONS

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Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects more than 1% of individuals over the age of 55. A pathological hallmark of PD is intercellular inclusions, which the main component is α-Synuclein (αSyn) protein. To date, 15 genes have been identified as related to PD. These gene mutations can cause both loss and gain of function in their respective proteins, and can result in autosomal recessive and autosomal dominant PD. Microglia activation in PD may result in neuronal cell death. DJ-1 is an oxidative stress sensor that localizes to the mitochondria. We discovered that down-regulation of DJ-1, as found in PD, in microglia, increased cell sensitivity to dopamine, as measured by secreted pro-inflammatory cytokines such as IL-1β and IL-6, and by the elevation of reactive oxygen species (ROS), which resulted in dopaminergic neuronal death. PTEN induced kinase 1(PINK1) has been shown to protect cells against oxidative stress-induced apoptosis. We discovered that down-regulation of PINK1 in microglia results in increased sensitivity to αSyn stimulation resulting in increased secretion of pro-inflammatory cytokines and neurotoxicity**.** Furthermore, we showed that DJ-1 and PINK-1 deficient microglia exhibited reduced autophagy, and impaired degradation of αSyn. Our results imply that DJ-1 and PINK1 deficiency mediates microglia dopaminergic neurotoxicity and affects their ability to phagocytose and to degrade αSyn. Further studies of PD gene-mediated cellular pathways in microglia may provide useful insights into the etiology of PD.

Symposium 48: MICROGLIA AND INNATE IMMUNITY

ADPD5-1609
HARNESSING INNATE IMMUNITY TO TREAT NEURODEGENERATIVE DISEASES

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Altered central nervous system (CNS) proteostasis, characterized by accumulation of extracellular or intracellular proteinaceous deposits, is thought to be a key trigger of many neurodegenerative disorders. There is considerable evidence that various assemblies of the aggregated proteins that form these inclusions can activate the innate immune system which, in turn, can contribute to the degenerative cascade. There is also growing evidence that alterations in innate immune signaling can play a key role in regulating proteostasis of key pathogenic proteins linked to neurodegenerative disorders. We term this complex interplay between the innate immune system and proteinopathy, immunoproteostasis. In a contextually dependent fashion, immunoproteostasis can have positive or negative effects on the proteinopathy and degenerative phenotype. Using our rAAV-based somatic brain and spinal cord transgenic technology, we have explored a number of paradigms to attempt to harness innate immunity for therapeutic benefit. We will present several examples of our preclinical studies showing both the potential and the challenges of our attempts to harness innate immunity for therapeutic benefit. Studies on Il-10 reveal that it is protective in SOD1 familial ALS mouse model, but harmful in APP and a-synuclein models. More recent studies using soluble Toll-like receptor Fc fusion proteins reveal the remarkable potential of these for therapeutic benefit in APP mouse models. In addition, we will discuss how novel theragnostic imaging methods might enable us to evaluate the effects of targeting innate immunity in the living brain.

Symposium 48: MICROGLIA AND INNATE IMMUNITY

ADPD5-2297
TREM2 REGULATES MICROGLIAL CELL ACTIVATION IN RESPONSE TO OLIGODENDROCYTE DAMAGE IN VIVO

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Microglia are phagocytic cells that survey the brain and perform neuroprotective functions in response to tissue damage, but their activating receptors are largely unknown. Triggering receptor expressed on myeloid cells 2 (TREM2) is a microglial immunoreceptor whose loss-of-function mutations in humans cause presenile dementia, while genetic variants are associated to increased risk of neurodegenerative diseases. In myeloid cells, TREM2 has been involved in the regulation of phagocytosis, cell proliferation and inflammatory responses *in vitro*. However, it is unknown how TREM2 contributes to microglia function *in vivo*. Here, we identify an essential role for TREM2 in the activation and function of microglia during cuprizone (CPZ)-induced demyelination.
TREM2-deficient (TREM2-/-) mice had defective clearance of myelin debris and more axonal pathology, resulting in impaired clinical performances compared to wild-type (WT) mice. TREM2-/- microglia proliferated less in areas of demyelination and were less activated, displaying a more resting morphology and decreased expression of the activation markers MHC II and iNOS as compared to WT. Mechanistically gene expression and ultrastructural analysis of microglia suggested a defect in myelin degradation and phagosome processing during CPZ intoxication in TREM2-/- microglia. These findings place TREM2 as a key regulator of microglia activation *in vivo* in response to tissue damage.

Symposium 50: RETROMERS IN AD AND PD

ADPD5-1073
MECHANISMS BY WHICH BY RETROMER TRANSPORTS APP VIA SORL1
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Processing of the Amyloid precursor protein (APP) into the Amyloid beta-peptide is considered a major pathological event in the cascade that leads to onset of Alzheimer's disease (AD).
SorLA is a cargo receptor for the retromer complex, genetically and functional linked to Alzheimer's disease (AD). The gene encoding the sorLA protein (*SORL1*) is associated with early- as well as late-onset AD, and sorLA protein levels are decreased in brains from many AD patients.
We have initially identified sorLA as a sorting receptor for APP, where sorLA activity protects against the amyloidogenic cleavage of APP in cell cultures and neurons. A physiological role of sorLA in amyloidogenesis was later demonstrated, as sorLA deficiency accelerates and aggravates pathology in AD mouse models.
We have also shown that sorLA regulates the maturation and exit of APP from the Golgi in the secretory pathway, and that sorLA levels in these compartments are determined by the efficient retrieval from endosomes to the Golgi/TGN by interaction with the retromer complex.
Our recent studies have identified novel ways to regulate the intracellular levels of functional sorLA that is available to act in APP transport. SorLA carries complex glycosylations that actively determine shedding efficiency of the luminal receptor domain, thus having direct impact on functional sorLA activity in the cell. We have also identified a strong regulation of sorLA at the transcriptional level, and determined *cis*- and *trans*-regulatory elements that control the temporal and spatial expression profile of sorLA.

Symposium 50: RETROMERS IN AD AND PD

ADPD5-1884
GENETICALLY LINKING THE RETROMER CORE MOLECULE VPS35 TO PD
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A *de novo* mutation in vacuolar protein sorting 35 (VPS35 p.D620N), a core component of the retromer complex, was recently identified by exome analysis and linked to familial late-onset PD. More recently, in Canadian Mennonite families with late onset PD, a mutation was described in receptor-mediated endocytosis 8 (DNAJC13 p.N855S) by similar methods. The consequences of mutation to specific protein cargos in terms of altered intracellular trafficking to trans-Golgi network, lysosomes or the plasma membrane have yet to be elucidated. However, both knock-down and overexpression of VPS35 alters surface delivery of AMPA-type glutamate receptors and synaptic transmission. Retromer also plays an important role in governing the traffic and processing of proteins important to dementia, including SorLA, amyloid precursor protein and progranulin. Thus an overlapping pathway has emerged in some forms of parkinsonism and dementia, supported by unbiased genetic discovery. While most cargos and their fate have yet to be elucidated in neurons (let alone in the context of pathogenic mutations linked to disease) a model for retromer subunit interactions with the WASH-complex, receptor-mediated endocytosis 8 and sorting nexins has recently been described. Genetic analysis of these protein components will be reviewed.

Symposium 50: RETROMERS IN AD AND PD

ADPD5-1888
GENETICALLY LINKING SORL1 AND OTHER RETROMER RECEPTORS TO AD
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Common single nucleotide polymorphisms in the *SORL1* gene have been associated with late onset Alzheimer's disease (LOAD) but causal variants have not been fully characterized nor has the mechanism been established. We conducted a combined family- and cohort-based genetic association study. Caribbean Hispanics with familial and sporadic LOAD and similarly aged controls recruited from the United States and the Dominican Republic, and patients with sporadic disease of Northern European origin recruited from Canada.
Prioritized coding variants in *SORL1* detected by targeted re-sequencing were validated by genotyping in additional family members and unrelated healthy controls. Variants were then transfected into human embryonic kidney 293 (HEK) cell lines and tested for Aβ40 and Aβ42 secretion and the amount of the amyloid precursor protein (APP) secreted at the cell surface.
We identified 17 coding exonic variants that were significantly associated with LOAD. Two rare variants (rs117260922-E270K and rs143571823-T947M) with MAF<1% and one common variant (rs2298813-A528T) with MAF=14.9% segregated within families and were deemed deleterious to the coding protein. Transfected cell lines showed increased Aβ40 and Aβ42 secretion for the rare variants (E270K and T947M) and increased Aβ42 secretion for the common variant (A528T). All mutants increased the amount of APP at the cell surface, though in slightly different ways, thereby failing to direct full-length APP into the retromer-recycling endosome pathway.
Common and rare variants in *SORL1* elevate the risk of LOAD by directly affecting APP processing which in turn can result in increased Aβ40 and Aβ42 secretion.

Symposium 50: RETROMERS IN AD AND PD

ADPD5-1890
THE CELL BIOLOGY OF RETROMER GENES IMPLICATED IN AD AND PD
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The Retromer complex is a conserved protein assembly that operates in endosomal protein sorting. Retromer is responsible for sorting membrane proteins into two distinct pathways: endosome-to-Golgi retrieval and endosome-to-cell surface recycling and its activity has been linked to the pathogenesis of Alzheimer disease (AD) through the role that retromer plays in controlling the localisation of the SorL1 protein that interacts with amyloid precursor protein (APP). Additionally retromer serves as a hub for recruiting additional endosomal sorting machinery to the membrane including the F-actin-promoting WASH complex.
A key element of the retromer complex is the VPS35 protein that, together with VPS26 and VPS29 forms the cargo-selective subcomplex. A point mutation in VPS35 has been shown to cause an inherited form of PD. I will report experiments from my lab that have investigated the effect of the PD-causing VPS35 mutation on the assembly of retromer and its association with accessory factors such as the WASH complex. Additionally I will report the results of a genome-wide RNAi screen for novel components of the endosome-to-Golgi retrieval pathway that has revealed an unexpected role for multi-pass membrane proteins in this pathway.

Symposium 50: RETROMERS IN AD AND PD

ADPD5-1902
THE RETROMER TRANSPORT PATHWAY IN THE PATHOGENESIS OF PD
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Human genetic studies have linked mutations in over a dozen genes with rare familial forms of Parkinson's disease (PD). Furthermore, large-scale genome-wide association studies have implicated additional genetic loci in PD risk in the general population. We have pursued potential common mechanisms of action of such disease-associated mutations and common variants. Using a variety of strategies -- including the analysis of human brain transcriptome datasets, primary rodent neuron in vitro models, and model organism in vivo approaches -- our studies support a role for multiple PD-associated genetic loci within an essential cellular pathway that regulates intraneuronal protein trafficking through endosomes. Disease-associated forms of the LRRK2, RAB7L1, and VPS35 genetic loci appear to be associated with endolysosomal and retromer complex sorting defects. Taken together, these studies further implicate retromer and lysosomal pathway alterations in PD risk. We will present additional components of this putative disease pathway, based on cell and in vivo analyses.

Symposium 50: RETROMERS IN AD AND PD

ADPD5-1924
RETROMER MEDIATES AMYLOID, TAU, AND MICROGLIA PATHOLOGY THROUGH SEPARATE MECHANISMS

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Retromer is an assembly of proteins that traffics and transports cargo out of endosomes. A range genetic and expression studies have implicated retromer in the pathogenesis of Alzheimer's disease (AD). Retromer dysfunction in the brain can lead to different but well-defined pathophysiological consequences. I will review these pathophysiological consequences and how each might be separately linked to core features of AD—amyloid accumulation, glia abnormalities, and tau toxicity.

Symposium 51: TAU BIOLOGY AND DISEASE MECHANISMS 2

ADPD5-0284
CAN THE MUTANT HUNTINGTIN GENE PRODUCT SPREAD FROM CELL TO CELL: EVIDENCE FROM NEURONAL ALLOGRAFTS IN HUNTINGTON'S DISEASE PATIENTS

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Huntington's disease (HD) is caused by a genetically encoded pathological protein (mutant huntingtin (mHtt)), which is thought to exert its effects in a cell-autonomous manner where degeneration occurs within individual cells that carry the aberrant gene. Here, we investigated the hypothesis that mHtt is capable of spreading within cerebral tissue.
The brains of four patients with HD who received genetically unrelated fetal neural allografts at least a decade earlier were examined post-mortem. The presence of mHtt aggregates within the grafted tissue was confirmed using an array of techniques including microscopy, western immunoblotting and infrared spectroscopy, and a number of different antibodies targeting different epitopes of mHtt aggregates.
A number of mHtt protein aggregates were located within intracerebral allografts of striatal tissue in three of these HD patients. The mHtt+ aggregates were observed in the extracellular matrix of the genetically unrelated transplanted tissue while in the host brain they were localized in neurons, neuropil, extracellular matrix and blood vessels. In addition, peripheral immune cells in separate HD patients contained mHtt. There are a thus a number of non cell-autonomous mechanisms which could explain these observations including transynaptic propagation as well as hematogenous transport of mHtt, among others.
This is the first demonstration for the presence of mHtt in genetically normal and unrelated allografted neural tissue transplanted into the brains of HD patients. These observations raise questions on the importance of non-cell autonomous mechanisms of protein spread in genetic disorders of the CNS, and further provide new targets for the development of therapeutic strategies.

Symposium 51: TAU BIOLOGY AND DISEASE MECHANISMS 2

ADPD5-1351

EXTRACELLULAR AND INTRACELLULAR EFFECTS OF LOW-N OLIGOMERS OF PRO-AGGREGANT TAU REPEAT DOMAIN (TAURD-DELTA K280)

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Objectives:

The repeat domain of Tau protein with the pro-aggregant mutation (TauRD-Delta-K280) induces toxicity in transgenic mice and organotypic hippocampal slice culture models (Sydow et al., JN 2011, Messing et al., NBA 2013). One current concept of Tau-mediated toxicity is that it is based on low-n oligomeric species, rather than higher aggregated forms. Therefore, we investigated structural and functional aspects of low-n oligomers of TauRD-DeltaK280.

Methods:

Recombinant TauRD-DeltaK280 oligomers were purified by hydrophobic interaction chromatography. Biophysical and structural characterization was performed by ThS, ANS, CD, AFM and DLS. Functional aspects of oligomers were investigated by MTT, LDH, ROS production and calcium assays. Protein transfection, FACS and western blotting were employed to investigate the role of low-n oligomers at intracellular space.

Results:

The TauRD-DeltaK280 oligomers have predominantly disordered structure (by ThS, CD), but reveal conformational changes compared with monomers (by ANS). AFM reveals globular shapes with sizes from 1.6-5.4 nm. The hydrodynamic radius of oligomers (~5.2 nm) is dominated by that of tetramers (DLS). In neurons, TauRD-DeltaK280 oligomers cause an increase in ROS production, elevation of intracellular calcium and synapse loss without affecting cell viability (MTT, LDH). Intracellular transduction of oligomers induces the aggregation of endogenous tau in SH-SY5Y cells.

Conclusions:

Extracellular applications of TauRD-DeltaK280 oligomers are toxic to the synapse. TauRD-DeltaK280 oligomers induce the aggregation of endogenous tau when present inside the neurons.

Symposium 51: TAU BIOLOGY AND DISEASE MECHANISMS 2

ADPD5-1436

SPPL2B IS DRASTICALLY INCREASED IN EARLY STAGES OF ALZHEIMER'S DISEASE AND ASSOCIATES WITH TAU PATHOLOGY.

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Introduction

Alzheimer's disease (AD) is characterized by the presence of amyloid beta (Aβ) plaques and neurofibrillary tangles (NFT). SPPL2b is a novel enzyme that processes TNFα and BRI2, substrates involved in Aβ plaque and NFT formation. Since changes in BRI2 and TNFα were previously identified in early stages of AD, we have extensively characterized SPPL2b in human brain tissue from controls and AD patients at different stages and its relationship with plaques and NFT.

Methods

SPPL2b expression levels were quantified in post-mortem human hippocampus from AD patients (n=14) and age-matched controls (n=13) by western blot and immunohistochemestry. The relationship of SPPL2b with tau was evaluated by double immunohistochemistry and immunoprecipitation (IP) experiments.

Results

SPPL2b levels were 10-fold increased ($p < 0.0001$) in AD hippocampus compared to non-demented controls. SPPL2b increase strongly correlated to Braak stages ($r = 0.785$, $p < 0.0001$), started in early AD stages (Braak II-III) and showed a drastic increase from Braak III to IV, stages in which cognitive impairment starts. SPPL2b immunoreactivity was associated with both plaques and NFT and immunoprecipitation experiments revealed that SPPL2b is a novel tau binding protein.

Conclusions

We found a dramatic increase of SPPL2b in early stages of AD associated with tau pathology. SPPL2b processes BRI2 and TNFα, proteins involved not only in Aβ homeostasis but also in chronic inflammation and NFT formation. These data reveal a novel potential AD etiological factor that links Aβ plaques and NFT and thus forms a new promising target for disease modifying therapies.

Symposium 51: TAU BIOLOGY AND DISEASE MECHANISMS 2

ADPD5-1447
ALPHA-SYNUCLEIN AND TAU OLIGOMERS INTERACT IN SYNUCLEINOPATHIES: TAU OLIGOMERS AS A THERAPEUTIC TARGET

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Objectives:
As life expectancy and therefore, the amount of people living with neurodegenerative synucleinopathies rise, it is increasingly important that we find an effective prevention and treatment strategy. While Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are characterized by the deposition of alpha-synuclein, multiple disease-causing proteins frequently overlap within a single disorder. Additionally, studies suggest that intermediate aggregates known as oligomers which form prior to fibrils are the true toxic species in disease. Previous work from our lab and others have shown that oligomeric tau and α-synuclein accumulate in disease and in mouse models. Here we directly investigate the relationship of the oligomeric species of both proteins with each other.
Methods:
We have evaluated brain tissue and isolated oligomers from PD and DLB patients and the A53T synucleinopathy mouse model using biochemical and immunohistochemical analysis with our novel antibodies for alpha-synuclein oligomers—F8H7 and Syn33—and for tau oligomers—T22. Immunotherapy studies using anti-tau oligomer monoclonal antibody (TOMA) in A53T mice are ongoing.
Results:
We found that both alpha-synuclein and tau oligomers are elevated in disease and in A53T mice compared to age-matched controls. Moreover, oligomers of tau and alpha-synuclein form "hybrid oligomers" in these patient brains and immunoprecipitated tau oligomer complexes contained α-synuclein oligomers and vice versa.
Conclusions:
Our results suggest that tau and alpha-synuclein oligomers may have a synergistic relationship, implicating tau oligomers in the progression of synucleinopathies. These studies suggest that TOMA may be a viable immunotherapeutic agent against pathological tau and alpha-synuclein in PD and LBD.

Symposium 51: TAU BIOLOGY AND DISEASE MECHANISMS 2

ADPD5-1519
TAU MISSORTING AS PART OF GLUCOCORTICOID-TRIGGERED SYNAPTIC PATHOLOGY

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Objectives: Despite mainly axonal protein, Tau is recently found in dendritic tree and spines where may interfere with synaptic structure and function. While sustained elevations in stress hormones, glucocorticoids (GCs), have been implicated in neurodegenerative mechanisms and we previously showed that stress and GC trigger Tau hyperphosphorylation, the exact cellular cascades and mechanisms underlying the GC-evoked dendritic remodeling and spine atrophy are poorly investigated. *Methods*: With the emerging idea that Tau may penetrate into the synaptic compartment, we monitored Tau dynamics and localization in hippocampus of GC-treated, 3-4 months old rats using both WB subcellular fractionation and electron microscope approaches followed by detailed 3-dimensional morphometric analysis of dendritic branches and spines. *Results*: We demonstrated that prolong GC administration resulted in dendritic remodeling and loss of specific spine categories in rats demonstrating Tau missorting to hippocampal synapses with the participation of specific phosphorylated Tau isoforms whereas GC impact on Tau phosphorylation state exhibit a clear subcellularly distinct pattern pointing to differential trafficking of Tau isoforms. *Conclusions:* These *in vivo* findings add to our limited knowledge about the underlying mechanisms of GC-evoked dendritic plasticity and spine atrophy implicating Tau missorting in mechanism(s) of synaptic damage, beyond Alzheimer's disease pathology.

Symposium 51: TAU BIOLOGY AND DISEASE MECHANISMS 2

ADPD5-1871

HUNTINGTON'S DISEASE IS A TAUOPATHY: LESSONS FROM MICE MODELS

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Aims: Huntington disease (HD) is an autosomal dominant neurodegenerative disorder caused by a polyglutamine expansion in the N-terminal region of the huntingtin protein. At the cellular level, HD is characterized by proteolytic cleavage, misfolding and aggregation of huntingtin. Aggregates of hyperphosphorylated tau proteins define a class of neurodegenerative disease called tauopathies. HD is not thought to be a tauopathy, but there are several articles reporting limited tau pathology in HD patients. These observations prompted us to hypothesized that HD pathology might promote tau hyperphosphorylation.

Methods: To test this hypothesis, we used two mice models of HD (R6/2, Q175), and analyzed tau phosphorylation before and after the onset of HD symptoms. We also used various cell models.

Results: We found that before the onset of HD symptoms, R6/2 mice presented a slight elevation of tau phosphorylation, while symptomatic mice displayed tau hyperphosphorylation at multiple tau phospho-epitopes. There was no activation of major tau kinases that could explain this observation, but we found that calcineurin/PP2B was downregulated in R6/2 mice. We observed similar changes in tau phosphorylation and calcineurin expression in Q175 mice. Calcineurin was also reduced in Q111 compared to Q7 cells. Finally, pharmacological or genetic inhibition of endogenous calcineurin was sufficient to promote tau hyperphosphorylation in neuronal cells.

Conclusion: Taken together, our data suggest that, in R6/2 and Q175 mice, mutant huntingtin lead to deregulation of calcineurin and consequent tau hyperphosphorylation, and that the mild tau pathology seen in HD might, to some extent, stem from impaired calcineurin regulation.

Symposium 54: TAU BIOLOGY AND DISEASE MECHANISMS 3

ADPD5-0593

FUNCTIONAL CHARACTERIZATION OF NEW N-TERMINALLY TRUNCATED TAU FRAGMENTS UNDERLINES THE ROLE OF N-TERMINAL REGION OF TAU IN MICROTUBULE STABILIZATION

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Objectives: Tau is a microtubule-associated protein involved in the regulation of microtubule dynamics and its related functions in neurons. Alzheimer's disease (AD) is characterized by the occurrence of abnormal post-translational modifications of Tau proteins, including the truncation, which can result in either a loss of function or a gain of toxic function. A major step forward in understanding of the role of Tau truncation would be to identify the precise cleavage sites of the several truncated Tau fragments that are observed in AD brains, especially those at N-terminus which are less characterized than those truncated at C-terminus.

Methods: Tau proteins were immunoprecipitated from human brain samples and subjected to primary amines labeling using a covalently linked biotin prior to enzymatic digestion. Peptides were then purified on streptavidin column and identified using LC-MS/MS. Expression vectors containing coding sequences of truncated-Tau were generated and used in cell-based assays. Effects on microtubules were mainly analyzed by biochemical studies.

Results: We have identified several new N-terminally truncated Tau species in the human brain, with N-terminal residues located throughout the Tau sequence. Our cell-based assays showed that the ability of Tau to bind and stabilize microtubules was greater when N-terminal region of Tau is truncated.

Conclusions: Our cell-based assays suggest that N-terminal region of Tau could directly affect microtubule dynamics. Future studies based on our new N-terminally truncated-Tau species will provide new knowledge on the role of truncation in Tau biology as well as in AD pathological process.

Symposium 54: TAU BIOLOGY AND DISEASE MECHANISMS 3

ADPD5-0722
ROLE OF GSK3 AT EACH STEP OF THE DENTATE GYRUS NEUROGENESIS. POSSIBLE CONSEQUENCES IN ALZHEIMER DISEASE

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Alzheimer disease (AD) is characterized by the presence of two aberrant structures: tau and beta amyloid (Aβ) aggregates. Both components (Aβ and tau) play a role in the disease and one connection between both is through GSK3, a kinase that is activated by Aβ and modifies tau.
Adult neurogenesis at the dentate gyrus is related to the appearance of new episodic memories, memories that are impaired in Alzheimer disease.
In this work, we have analyzed, using different models of genetically modify mice, the effect of a GSK3β overexpression, at the dentate gyrus, at different steps of that adult neurogenesis. It seems that tau may play a role in the GSK3 overexpression effect on dentate gyrus adult neurogenesis. Changes in GSK3 activity taken place in AD patients could result in an impairment of adult neurogenesis.

Symposium 54: TAU BIOLOGY AND DISEASE MECHANISMS 3

ADPD5-0785

INVESTIGATING THE FUNCTION OF MICROTUBULE-ASSOCIATED PROTEIN TAU (MAPT) AND ITS GENETIC ASSOCIATION WITH PARKINSON'S USING HUMAN IPSC-DERIVED DOPAMINE NEURONS

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OBJECTIVES

The microtubule associated protein tau (*MAPT*) locus is highly associated with Parkinson's (PD); however, the mechanisms underlying susceptibility remain unclear. We propose that polymorphisms within the *MAPT* haplotype sequence have functional consequences on *MAPT* expression and tau protein function in the dopamine neurons notably lost in PD. To examine this we chose the following objectives:

- To investigate allele-specific regulation of tau expression in iPSC-derived dopamine neurons.
- To investigate the physiological role of tau in axonal transport by perturbing tau expression.

METHODS

Induced pluripotent stem cells derived from healthy heterozygous (H1/H2) donors were differentiated into midbrain-type neuronal cultures. Dopamine neurons were isolated using rapid fixation immunostaining for tyrosine hydroxylase (TH) followed by fluorescence-activated cell sorting and RNA extraction. For tau perturbation, RNAi targeting specific isoforms or total tau was designed, with initial knockdown performed in neuroblastoma cells.

RESULTS

Transcripts of *TH* were >7-fold enriched in FACS-isolated neurons and mature *MAPT* isoforms (exon 3+ or 10+) were detected with extended culture. siRNA-mediated specific knockdown of exon 3+ (~60%) or exon 10+ (~90%) *MAPT* isoforms was achieved. Isoform-specific and total *MAPT* shRNA sequences were incorporated into lentivirus plasmids for delivery to neuronal cultures, in addition to those expressing fluorescent amyloid precursor protein for imaging of live axonal transport.

CONCLUSIONS

Examining haplotype-specific tau expression and function for the first time in dopamine cultures allows us to understand the effect of haplotype on tau protein in the neurons that degenerate in PD, and to identify therapeutic targets to reduce progression beyond disease's earliest signs.

Symposium 54: TAU BIOLOGY AND DISEASE MECHANISMS 3

ADPD5-0809
CELL TYPE-SPECIFIC TAU PATHOLOGY IN PS19 MICE FOLLOWING INTRACEREBRAL INJECTIONS OF PATHOLOGICAL TAU FROM ALZHEIMER'S DISEASE OR CORTICOBASAL DEGENERATION BRAINS

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Objective:
Intracellular tau inclusions are the hallmark lesions of tauopathies, a group of diseases that include Alzheimer's disease and corticobasal degeneration, which show distinct topographic and cell type-specific distribution of tau. Recent studies suggest that tau transmits through synaptically connected pathways and that different strains of tau could account for the diverse manifestations of tauopathies. Our aim was to investigate the differential induction and spread of tau pathology in PS19 transgenic mice after intracerebral injection of human brain derived extracts from Alzheimer's disease (AD) and corticobasal degeneration (CBD).
Methods:
We injected enriched pathological tau human extracts from AD and CBD brains into cortex and hippocampus of young PS19 tau transgenic mice (n=24) and analyzed the resulting tau pathology at 1mo, 3mo and 6mo after injection.
Results:
At 1 mo postinjection CBD injected mice showed tau inclusions in oligodendrocytes of fimbria and external capsule near the injection site with infrequent intraneuronal inclusions. In contrast, AD injected mice showed abundant intracellular tau pathology in neurons of the hippocampus without involvement of oligodendrocytes. With prolonged time postinjection pathology increased in intensity and spread to distant regions of the brain. Additionally, AD injected mice showed neuronal loss in CA3 at 3 mo after injection.
Conclusion:
We present mouse models of CBD-like and AD-like tauopathies that rapidly develop distinct glial and intraneuronal tau inclusions, respectively, recapitulating the human counterpart that will provide systems for studies on transmission of tau pathology, tau mediated neurodegeneration and development of disease-modifying therapies for CBD and AD.

Symposium 54: TAU BIOLOGY AND DISEASE MECHANISMS 3

ADPD5-0938

DIFFERENTIAL SPREADING OF 3 AND 4R TAU ISOFORMS IN THE RAT BRAIN

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1. Objectives

Six tau isoforms with either 3 or 4 microtubule-binding domains (3R or 4R) are expressed in the human brain. They are generated by alternative splicing. Such Tau splicing is defective in fronto-temporal dementia exhibiting some MAPT (tau gene) mutations (FTDP-17). Moreover, these 3R and 4R Tau isoforms differentially aggregate among Tauopathies (3R in Pick's disease, 4R in progressive supranuclear palsy and 3R+4R in Alzheimer's disease). Finally, there is a progressive and hierarchical propagation of Tau pathology in some sporadic tauopathies. In the present work, we analyzed how Tau isoforms and/or FTDP-17mutations act on the propagation of tau pathology.

2. Methods

We took advantage of a lentiviral rat model of tauopathy developed in our team to overexpress either 3R or 4R isoforms in the hippocampus. The propagation of tau pathology was analysed by immunohistochemistry and tau secretion by ELISA tau quantification in cerebro-spinal and interstitial fluids.

3. Results

We demonstrated that Tau isoforms are differentially transferred from the hippocampus to trans-synaptically connected regions. In fact, 4R-Tau is more prone to propagate than 3R-Tau. Such observations are correlated to a dramatic decrease of 3R-Tau secretion in biological fluids compared to 4R-Tau. Interestingly, these observations are also seen when FTDP-17 mutations are present either in 3R or 4R-Tau.

4. Conclusions

Tau isoforms with or without FTDP-17 mutations display differential spreading properties that may participate to various phenotypes associated to specific tauopathies.

Symposium 54: TAU BIOLOGY AND DISEASE MECHANISMS 3

ADPD5-1352

PRO-AGGREGANT TAU IMPAIRS MOSSY FIBER TRANSMISSION AND PLASTICITY DUE TO STRUCTURAL CHANGES, VESICLE DEPLETION AND CA++ DYSREGULATION

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Objectives:

An inducible mouse model expressing the Tau repeat domain (Tau_{RD}) with the pro-aggregant mutation delta-K280 was used to analyze consequences of Tau aggregation on presynaptic function in the hippocampus.

Methods:

We used aged mice either expressing a pro-or anti-aggregant variant of Tau_{RD} and Tau knockout animals. Additionally we used organotypic hippocampal cultures, transiently transfected hippocampal cell-cultures and N2a cells.

Results:

Expression of pro-aggregant Tau_{RD} leads to phosphorylation, aggregation and missorting of Tau in area CA3 and the dentate gyrus. To test presynaptic pathophysiology we used electrophysiology in the mossy fiber tract. Synaptic transmission was severely decreased in pro-aggregant Tau_{RD} and knockout mice. Long-term depression of the mossy fiber tract failed in 12 month old pro-aggregant Tau_{RD} mice, but not at an age of 2 month. In 12 month old mice we observed an increase in bouton size, but a decline in numbers and presynaptic markers. Both pre- and postsynaptic structural deficits are preventable by inhibition of Tau_{RD} aggregation. Calcium imaging revealed progressive, pathological calcium dysregulation in boutons of pro-aggregant Tau_{RD} slice cultures. In N2a cells we observed the same effect even in cells without tangle load. In primary hippocampal cells transient Tau_{RD} expression had a similar effect. By ultrastructural analysis we observed a depletion of the presynaptic vesicle pool leading to impairment of synaptic function together with calcium dysregulation and structural changes.

Conclusions:

We conclude that oligomer -formation during Tau_{RD} aggregation causes pre- and postsynaptic deterioration and plasticity deficits by dysregulation of activity-dependent calcium dynamics.

Symposium 55: INFLAMMATION 2

ADPD5-0269

M2 MICROGLIA ARE NECESSARY AND SUFFICIENT FOR ABETA PLAQUE REDUCTION DURING NEUROINFLAMMATION

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Objectives
Neuroinflammation has long been considered a driver of Alzheimer's disease progression. However, experiments developed to explore the interaction between neuroinflammation and AD pathology showed a surprising reduction in Abeta plaque deposition. We sought to understand this unexpected outcome by examining microglial phenotypes using multiple mouse models of chronic neuroinflammation.
Methods
Mice in the first chronic inflammation model harbored a cre-activated human IL-1beta transgene driven by the mouse GFAP promoter. The second model used an adeno associated virus vector carrying a human IL-1beta cDNA to transduce mice. Inflammation was induced in one hippocampus of 8-month-old APP/PS1 mice for 4 weeks, while the other hemisphere received a control injection. The alternatively activated M2 marker Arginase 1 was used to observe the presence of M2 cells and cellular uptake of Abeta was demonstrated by confocal microscopy. Injections of interleukin-4 were used to specifically induce M2 cells and a mini-pump and intra-hippocampal cannula was used to deliver an IL-4Ralpha antibody to block M2 induction.
Results
We observed a robust activation of Arginase 1+ M2 microglia in areas of chronic inflammation and interestingly these cells were shown to contain Abeta. When IL-4 was used to specifically induce M2 microglia, there was significant plaque reduction. Conversely blocking M2 microglia during chronic inflammation impaired plaque clearance.
Conclusions
Together these findings demonstrate that M2 microglia are necessary and sufficient for Abeta plaque reduction during neuroinflammation, opening up possible avenues for immunomodulatory therapy of AD.
This research was supported by NIH/NIA grant RO1AG030149

Symposium 55: INFLAMMATION 2

ADPD5-0616
CLASSICAL COMPLEMENT CASCADE MEDIATES EARLY SYNAPSE LOSS IN AD MOUSE MODELS

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1. Objectives: Region-specific synapse loss is an early hallmark of Alzheimer's disease (AD); however, mechanisms behind synapse dysfunction remain unclear. Recent work has identified unexpected roles for an immune pathway—proteins of the classical complement cascade, C1q and C3, and microglia—for elimination and refinement of synaptic connections in the healthy developing mouse brain. Interestingly, AD brains have highly increased levels of complement and certain complement cascade interactors have emerged as AD susceptibility genes. We hypothesized that similar developmental synapse pruning mechanism may drive synapse loss in early stages of AD.
2. Methods: Using *in vivo* high and super resolution imaging, we determined levels of complement and their co-localization at synapses in J20 and APP/PS1 transgenic brains and in an acute *in* vivo model of Aβ oligomer synaptotoxicity. To test whether complement is necessary for Aβ-induced synapse loss, we utilized mice lacking C1qA or C3 and also generated APP/PS1 mice lacking C3. Finally, we developed an assay to measure microglial engulfment of hippocampal synapses *in vivo*.
3. Results: We found a region-specific upregulation and deposition of complement onto synapses well before pathological phenotypes in J20 and APP/PS1 mice and in wildtype mice injected with soluble Aβ oligomers. Microglia in oligomer-injected mice engulfed more synapses vs. monomer-injected. Importantly, genetic deletion of complement protected against synapse loss in these models.
4. Conclusions: Our results suggest that aberrant reactivation of a normal developmental pruning pathway helps mediate early synapse loss in pre-plaque brains, marking an important step in the development of AD synaptic pathology.

Symposium 55: INFLAMMATION 2

ADPD5-0937

T CELL EXTRAVASATION IN ALZHEIMER'S DISEASE: EVIDENCE FROM TRANSGENIC MOUSE MODELS AND POST-MORTEM HUMAN MATERIAL

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Objectives. A mounting body of evidence indicates a key role for inflammation in Alzheimer's disease (AD); however, little is known on the involvement of the adaptive immune system. Here, we have performed a systematic study of T cell occurrence in brains from APP transgenic mouse models and post-mortem human AD material.
Methods. CD3-positive T cells were studied via bright field and confocal microscopy in brains from APP-overexpressing mice. Total, intra- and extravascular T cells were quantified from confocal stacks of 100 mm-thick sections. The expression of the adhesion molecules VCAM1 and ICAM1 was investigated using specific monoclonal antibodies. Furthermore, T cell presence was studied in post-mortem human AD and control brains.
Results. Clusters of CD3-positive cells were observed in the brains of all APP-overexpressing models examined. While intravascular counts were not different between transgenic and control animals, extravascular cells were significantly increased in SweArc transgenic mice. No correlation was observed with parenchymal plaques, but several amyloid-laden vessels were accompanied by CD3 cells. Supporting a role for vascular permeability in T cell extravasation, both ICAM1 and VCAM1 were found to be upregulated in brains from APP transgenic mice. T cells were also increased in the hippocampus of AD post-mortem brains, but not in the mid frontal gyrus.
Conclusions. Our results suggest that T cells infiltrate brains affected by AD pathology, likely due to increased vascular permeability. Extravasated T cells could cross-talk with and modulate cells of the CNS. The role of T cells in AD remains to be elucidated.

Symposium 55: INFLAMMATION 2

ADPD5-0955

GENERATION OF AN IMMUNE-DEFICIENT MODEL OF ALZHEIMER'S REVEALS A CRITICAL ROLE FOR THE ADAPTIVE IMMUNE SYSTEM IN DISEASE PATHOGENESIS

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Objectives:

The innate immune system is strongly implicated in Alzheimer disease (AD) pathogenesis. In contrast, very few studies have examined the potential role of the adaptive immune response in this disorder. The extensive testing and clinical assessment of beta-amyloid vaccination strategies has only further heightened the need to better understand the relationships and interplay between the adaptive immune system and AD neuropathology.

Methods:

To address this issue, we generated a novel immune-deficient transgenic AD model by backcrossing 5xfAD mice onto a Rag2-/- il2r-gamma-/- double knockout background. The resulting line, termed Rag-5xfAD mice, lacks B-cells, T-cells, and Natural Killer (NK) cells but overexpress mutant forms of both amyloid precursor protein and presenilin-1.

Results:

While Rag-5xfAD mice replicate many of the salient neuropathological features observed in immune-competent 5xfAD mice, disease pathogenesis is significantly accelerated by the loss of these peripheral immune populations. Levels of both soluble and insoluble beta-amyloid, are almost doubled in Rag-5xfAD mice versus strain-matched immune-competent transgenics. Likewise, AD-associated increases in several pro-inflammatory cytokines are also exacerbated by the deletion of T-, B-, and NK-cell populations. Interestingly, changes in microglial number and reactivity are also detected, suggesting that the adaptive immune system could influence AD at least in part by modulating the innate immune response.

Conclusions:

Taken together, our data reveal that T, B, and NK-cells significantly impact the progression of AD pathogenesis and neuroinflammation. These studies also suggest that peripheral immune health and interactions between adaptive and innate immune systems could dramatically influence the development and progression of AD.

Symposium 55: INFLAMMATION 2

ADPD5-1537
TREM2 DEFICIENCY BLOCKS ACCUMULATION OF INFILTRATING MONOCYTES AROUND AMYLOID PATHOLOGY

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Variants in the *Triggering Receptor Expressed on Myeloid cells 2* (*TREM2*) gene confer risk for Alzheimer's disease (AD), as well as Nasu-Hakola disease, frontrotemporal dementia, Parkinson's disease and amytrophic lateral sclerosis. Therefore, because TREM2 appears to play a central role in numerous age-related neurodegenerative conditions, understanding more about TREM2 biology promises to provide novel avenues for targeting neuroinflammation as a basis for novel neurodegenerative disease therapeutics. The current study examines the role of TREM2 in AD, and specifically demonstrates that loss of function for TREM2 in mouse models of AD alters amyloid-assoicated plaque pathology, with reduced β-amyloid (Aβ) deposition, diminished reactive astrocytosis, reduced phosphorylation of microtubule-associated protein tau (MAPT), lower expression of pro-inflammatory mediators and enhanced expression of some genes associated with alternative macrophage activation. To address mechanisms linking TREM2 loss of function to altered Aβ-plaque pathology, we characterized TREM2 expression. TREM2 is strongly expressed on myeloid cells surrounding Aβ deposits in both AD-autopsy brain sections and mouse models of amyloid deposition. Based on cell surface marker expression, TREM2 positive plaque-associated macrophages are largely derived from peripheral monocytes rather than brain-resident microglia. TREM2 loss of function eliminates association of these macrophages with Aβ deposits. Taken together, these surprising findings suggest that TREM2 is critical for accumulation of infiltrating peripheral monocytes around Aβ deposits and that TREM2 positive cells may promote Aβ-related inflammatory pathologies. These findings document a unique and largely unexpected role of TREM2 in AD and provides an important basis for development of TREM2-directed therapies.

Symposium 55: INFLAMMATION 2

ADPD5-1557
IMAGING INFLAMMATION AND ITS ROLE IN NEURODEGENERATION
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Microglia comprise around 15% of white cells in the brain and are normally in a resting state monitoring the brain milieu. Any injury to the brain results in microglial activation which provides a marker of disease activity. The function of activated microglia can be both detrimental and beneficial depending on the phenotype. Microglia with the M1 phenotype release cytokines which may drive disease progression. M2 microglia become intrinsic macrophages, express restorative growth factors, help clear cellular debris and abnormal protein aggregations, and re-model connections as an adaptive response to brain damage. In practice the two phenotypes are inter-convertible. All activated microglia express translocator protein (TSPO) on their mitochondrial membrane and M2 type express surface cannabinoid CB2 sites which allows their presence be imaged in vivo with positron emission tomography (PET) radioligands. In this talk the different approaches to microglial imaging will be detailed snd the different patterns of microglial imaging in Parkinsonian disorders, dementias, and other neurodegenerative and inflammatory brain diseases will be presented. The value of suppressing microglial activation and possible strategies to achieve this will be debated.

Symposia - Clinical

Symposium 01: ADVANCED CHARACTERIZATION OF EARLY DISEASE STAGE

ADPD5-0685
USING 18-MONTH COST AND CAREGIVER OUTCOMES TO EVALUATE MEANINGFUL DEFINITIONS OF DISEASE PROGRESSION IN ALZHEIMER'S DISEASE

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Objectives: Disease progression definitions of a meaningful magnitude related to cognition and functioning in Alzheimer's disease (AD) were evaluated using costs and caregiver outcomes.
Methods: GERAS is an 18-month European observational study of costs associated with AD which enrolled patients with Mini-Mental State Examination (MMSE) ≤26. Data collected at baseline and 6-month intervals included MMSE, Alzheimer's Disease Cooperative Study – Activities of Daily Living (ADCS-ADL) (baseline and 18-months only), Resource Use in Dementia and Zarit Burden Inventory (ZBI). Health and social care resource use and caregiver time spent on informal care were used for cost estimation. The literature suggests the following meaningful changes in disease progression: (1) cognitive progression - a worsening of ≥3 MMSE points and (2) functional progression - decline in either ≥1 basic ADL item or >20% instrumental ADL items. Generalized linear models adjusting for disease severity, sex, country and baseline score provided estimates of the difference in total societal costs, total caregiver hours, caregiver supervision hours and ZBI total score associated with progression.
Results: N=1495 patients were included in these analyses. Cognitive progression/non-progression was found in 44%/45% (11% missing) and functional progression/non-progression in 48%/17% (35% missing) patients. Functional progression had the largest association with total societal cost (40% increase) and total caregiver hours (45% higher), as well as caregiver burden (greater total mean 5.4). Greater caregiver supervision time was associated with cognitive progression (30% increase).
Conclusion: The present analyses suggest that these literature-derived thresholds of disease progression are associated with meaningful increased costs and care needs.
This study is sponsored and funded by Eli Lilly and Company Limited.

Symposium 01: ADVANCED CHARACTERIZATION OF EARLY DISEASE STAGE

ADPD5-1001
IMPROVING DIAGNOSTIC ACCURACY IN ALZHEIMER'S DISEASE CLINICAL TRIALS

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Objectives: Many reasons have been suggested for the recent failures of confirmatory randomized clinical trials (RCT) of drugs for Alzheimer's disease (AD), the most salient being variable diagnostic accuracy. For clinical trials, the diagnosis of AD should be made based on published clinical criteria, documented progression of cognitive decline, and neuroimaging data congruent with a diagnosis of AD. Unfortunately, evidence of progressive cognitive decline in clinical studies is typically neglected and neuroimaging confirmation of AD is often lacking. Poor diagnostic accuracy may produce less than expected cognitive decline with placebo treatment, thereby reducing the perceived efficacy of an active comparator compound. Our objective was to review the accuracy of AD diagnoses in completed clinical trials from external sources and suggest areas for improvement.
Methods: We have provided examples from a series of controlled AD clinical trials from external sources, identifying the most common pitfalls in study enrollment and execution, and presenting techniques to increase diagnostic accuracy and study validity.
Results: The lack of evidence of progressive cognitive decline appears to be the most frequent challenge in current clinical studies. Only a few protocols specifically requested evidence of cognitive deterioration prior to baseline as an inclusion criterion.
Conclusions: Clinical diagnosis of AD among screening subjects in RCTs needs to be carefully evaluated before study participation. We propose integrating centralized eligibility randomization services, focused on diagnoses congruent with protocol-specific criteria, and driven by medical experts, as one potential solution to this problem.

Symposium 01: ADVANCED CHARACTERIZATION OF EARLY DISEASE STAGE

ADPD5-1315
A MULTIDOMAIN TWO-YEAR RANDOMIZED CONTROLLED TRIAL TO PREVENT COGNITIVE IMPAIRMENT – THE FINGER STUDY RESULTS

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Background: Observational studies indicate that vascular and lifestyle-related risk factors are associated with risk of cognitive impairment and Alzheimer's disease. The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) is a randomized controlled trial (RCT) investigating whether a multidomain intervention will prevent cognitive impairment. Here we report the main results on cognition after 2 years of intervention.
Methods: FINGER study is a 2-year multicentre randomized controlled trial with 1260 participants aged 60-77 years recruited from previous population-based non-intervention studies. The inclusion criteria were CAIDE dementia risk score $\geq$6 indicating presence of modifiable risk factors, and cognitive performance at the mean level or slightly lower than expected for age. The participants were randomized (1:1) into multidomain intervention consisting of nutritional guidance, exercise, cognitive training, and monitoring of vascular risk factors and control groups. Primary outcome is cognitive performance measured by a comprehensive neuropsychological test battery (NTB) Z score. Linear mixed-model analyses were used.
Results: In the intention-to-treat analyses we found a significant beneficial intervention effect on cognitive performance. The modelled difference between groups (intervention minus control group) in the change of NTB total score per year was 0.022 (95 % confidence interval [CI] 0.002 to 0.042). Drop our rate was only 12.1 %, and adverse events were very few and mild.
Conclusions: This is the first large RCT showing that it is possible to prevent cognitive decline among older at-risk individuals. The results highlight the value of the feasible and effective novel multidomain approach for several cognitive domains.

Symposium 01: ADVANCED CHARACTERIZATION OF EARLY DISEASE STAGE

ADPD5-1761

RELEVANCE OF RHYTHMIC AUDITORY STIMULATION IN FREEZING GAIT FOR PARKINSON'S DISEASE PATIENTS

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Objective

The aim of this word was to compare several auditory stimulation modalities during freezing gait episodes in Parkinson's disease.

Methods

43 patients (22 women and 21 men), mean age 77.3 years with idiopathic PD during the fluctuating stage were included in the study. Mean UPDRS score was 33.3. The study was conducted in patients during their OFF period. The LTeM system was used to detect freezing gait, which allowed to apply the auditory stimulation to the patient right from the detection moment.

Four conditions were studied:

Condition A: right from freezing detection: vocal command "stop" followed 10 seconds later by the vocal command "walk".

Condition B: right from freezing detection: vocal command "walk"

Condition C: condition A followed by 19 rhythmic auditory stimuli based on the patient's spontaneous gait pattern during the ON period.

Condition D: Condition B followed by 19 rhythmic auditory stimuli based on the patient's spontaneous gait pattern during the ON period.

The effects of the four conditions on the 3 gait parameters: mean Inter Step Interval (ISI), variation coefficient of mean ISI and number of steps performed without freezing were studied.

Results

Condition C was significantly different from the other three for all 3 gait parameters: greater number of steps, increased mean ISI and lower variation coefficient of the mean ISI.

Conclusion

Using a specific system to detect gait freezing and applying auditory stimulation of 19 stimuli after 10 seconds after having stopped walking could improve gait conditions in patients in advanced stages of PD.

Symposium 01: ADVANCED CHARACTERIZATION OF EARLY DISEASE STAGE

ADPD5-1852

THE RELEVANCE OF SUBJECTIVE MEMORY AND SUBJECTIVE SPATIAL NAVIGATION COMPLAINTS IN SUBJECTS AT RISK OF ALZHEIMER DISEASE

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Alzheimer disease (AD) is considered to be a continuum ranging from preclinical stages, over mild cognitive impairment (MCI) to dementia syndrome. Biomarker-based diagnostic approach may have a limited worldwide utility beyond specialized memory clinics, therefore a growing research interest is focused to identify the first patients' subjective complaints or objective clinical symptoms. Subjective memory complaints (SMC) may occur independently of objective cognitive impairment and precede MCI syndrome. SMC comprise heterogenic group of symptoms, and SMC subjects have various risk of AD. The effort is to investigate predictive value of SMC for AD by analyzing specific complaints and questionnaires. Previously, we have reported that spatial navigation (SN) is similarly impaired in MCI and in early AD subjects, and that SN has been associated with hippocampal atrophy and certain ApoE4 and TOMM40 genotypes. We investigate the association of subjective SN complaints with objective SN performance, neuropsychology and brain volumetry in SMC and MCI subjects. Our data suggest that a specific question may be a useful screening tool for exploring difficulties with SN, the skill, which is impaired very early in the course of AD. This question may help to differentiate SMC subjects with anxiety and depression from those at risk of AD. Screening and timely diagnosis of AD outside of specialized memory clinics at the level of family or primary care physicians would require reliable, inexpensive and less time consuming screening tool. Subjectively reported symptoms, including SN complaints, may guide further decision making on patients at risk of AD.

Symposium 01: ADVANCED CHARACTERIZATION OF EARLY DISEASE STAGE

ADPD5-2311
INFORMATION AND COMMUNICATION TECHNOLOGIES FOR THE ASSESSMENT AND THE TREATMENTS OF NEURODEGENERATIVE DISEASES

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Alzheimer disease (AD) and other related dementia represent a major challenge for health care systems within the aging population. It is therefore important to develop better instruments for assessing disease severity and disease progression to optimize patient's care and support to care providers, and also provide better tools for clinical research. In this area, Information and Communication Technologies (ICT) are of particular interest. Such techniques enable accurate and standardized assessments of patients' performance and actions in real time and real life situations. ICT may also help to improve non-pharmacological approaches, stimulation and rehabilitation. The aims of this presentation are to systematically analyze the strengths, weaknesses, opportunities, and threats of employing ICT for assessment, stimulation in AD and other related disorders. This is also the occasion to provide recommendations for the use of ICT in clinical research. This will be illustrated by results coming from EU FP7 program (Dem@Care, VERVE, InMindd) and the from the French Azgame program.

Symposium 03: PROFILING DLB, PD AND FTD

ADPD5-0300
PROSPECTIVE ANALYSIS OF CLINICAL SYMPTOMS IN PRODROMAL DLB: THE NEWCASTLE LEWYPRO STUDY

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Objectives
In DLB the underlying synucleinopathy develops over years before clinical symptoms and involves not only the brain but extracranial elements of the nervous system. Thus, unlike Alzheimer's disease, DLB as a synucleinopathy characteristically has a wide range of non-cognitive (including motor, neuropsychiatric, autonomic, sleep) symptoms and these frequently begin before the dementia phase of the illness. Previous studies examining symptoms in people who progressed to DLB have been retrospective and prospective analyses are needed to develop very early diagnostic criteria to identify prodromal disease.
Methods
The LewyPro study is a clinical cohort study conducted in the North East of England to prospectively assess a broad range of symptoms in the prodromal phase of the illness to determine the pattern and timing of symptoms in this pre-dementia stage to facilitate the development of future diagnostic criteria. All subjects receive a medical assessment by a fully qualified psychiatrist, neuropsychological assessment, FP-CIT SPECT imaging and at baseline have a consensus panel clinical diagnosis of mild cognitive impairment (NIA-AA).
Results
These will be of the first 50 subjects comparing those who are biomarker positive (by FP-CIT) versus biomarker negative in six symptom domains: cognitive; neurological; neuropsychiatric; sleep; autonomic; visual. Currently 40% are FP-CIT positive with these having a higher prevalence of symptoms related to sleep and Parkinsonism. Preliminary data on those converting to dementia, comparing DLB with non-LB dementia, will also be presented.
Conclusions
In people presenting to memory and dementia services sleep and parkinsonian symptoms may be early features of prodromal DLB.

Symposium 03: PROFILING DLB, PD AND FTD

ADPD5-0384
DOPAMINE D1, D2, D3 RECEPTORS, VESICULAR MONOAMINE TRANSPORTER TYPE-2 (VMAT2) AND DOPAMINE TRANSPORTER (DAT) DENSITIES IN AGED AND DIFFUSE LEWY BODY DISEASE (DLBD) HUMAN BRAINS

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The dopamine D_1, D_2, D_3 receptors, vesicular monoamine transporter type-2 (VMAT2), and dopamine transporter (DAT) densities were measured in aged and diffuse Lewy body disease (DLBD) human brains by quantitative autoradiography. The density of D_1 receptors, VMAT2, and DAT was measured using [^{3}H]SCH23390, [^{3}H]dihydrotetrabenazine, and [^{3}H]Win35428, respectively. The density of D_2 and D_3 receptors was calculated using the D_3 selective radioligand, [^{3}H]**WC-10** and the D_2-preferring radioligand [^{3}H]raclopride using a mathematical model described previously. Dopamine D_1, D_2, and D_3 receptors are extensively distributed throughout striatum; the highest density of D_3 receptors occurred in the nucleus accumbens (NAc). Dopamine D_3 receptor density exceeded D_1 and D_2 receptor densities in extrastriatal regionse, and thalamus contained a high level of D_3 receptors with negligible D_2 receptors. The density of the DAT was negligible in the extrastriatal regions whereas the VMAT2 was expressed in moderate density. There were no significant changes in the dopamine D_1 and D_2 receptor densities in any brain regions measured. VMAT2 and DAT densities were reduced in all the brain regions measured in DLB/PDD, however the significant reduction was found in putamen for DAT and in the NAc and SN for VMAT2. The decrease of dopamine pre-synaptic markers implies neuronal loss in the substantia nigra pars compacta (SNpc) in these DLB/PDD cases, while the increase of D_3 receptors in striatal regions could be attributed to dopaminergic medication history and psychiatric state such as hallucinations. Whether it also reflects compensatory regulation upon dopaminergic denervation warrants further confirmations on larger populations.

Symposium 03: PROFILING DLB, PD AND FTD

ADPD5-0865
BRAIN GBA1 & GBA2 ACTIVITIES; A FACTOR TO CONSIDER IN PD
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Gaucher disease is a lysosomal storage disorder caused by a deficiency of the lysosomal b-glucosidase (GBA1). An association between Gaucher disease (GD) and Parkinson disease (PD) was recognised in 1996. Previously, we described a patient with very mild GD (N370S/L444P) but a severe early onset PD who had undetectable levels of not just the dopamine metabolite (HVA) but also the serotonin metabolite (5-HIAA) in CSF. Subsequently, we have found that this patient had undetectable leucocyte levels of the non-lysosomal b-glucosidase (GBA2). Recently, we demonstrated that GBA2 is the predominant glucosidase in brain. We have also shown that some GD patients have elevated leucocyte GBA2 while others do not. Furthermore, we have observed undetectable levels of GBA 2 in 5% of GD patients, GD carriers and unaffected persons. This raises the possibility that GBA2 is disease modifying in GD with perhaps both negative and positive effects. GBA1 activity is also decreased in the substantia nigra of not just GD Parkinson's patients but also those with idiopathic PD. Whether this loss of activity directly contributes to PD pathogenesis in such patients is not currently known. Using a neuronal cell culture model (SHSY5Y) we have shown that inhibition of GBA1 and/or GBA2 increases cellular susceptibility to oxidative stress. Loss of activity of either or both glucosidases may therefore be a factor to consider with regards to our understanding of the pathogenesis of GD/PD and idiopathic PD.

Symposium 03: PROFILING DLB, PD AND FTD

ADPD5-1320

THE NEUROPSYCHOLOGICAL DEFICIT PROFILE OF DEMENTIA WITH LEWY BODIES (DLB) IS INFLUENCED BY DEGREE OF ALZHEIMER'S DISEASE (AD) PATHOLOGY

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Objective: DLB is characterized by cell loss and Lewy body deposition in brain stem nuclei, limbic regions, and neocortex. Concomitant AD pathology is present to varying degrees in its typical limbic/neocortical distribution. Our objective was to determine if degree of concomitant AD pathology observed at autopsy is related to the pattern of neuropsychological deficits associated with DLB at presentation.

Method: We retrospectively examined the performance of mildly-demented patients with autopsy-confirmed DLB (n=88) or "pure" AD (n=239) on cognitive domain scores. We then reexamined domain scores after subdividing DLB patients into those with high (Braak stage V-VI; n=38) or low (Braak stage II-IV; n=49) degrees of concomitant AD pathology.

Results: Despite similar mental status scores, DLB were more impaired than AD in Visuospatial and Attention/Executive Function, but less impaired in Memory ($F(4,322)=4.09$;$p=.003$;Wilk'sλ=.952). Pattern analysis showed that AD had greatest impairment in Memory and least impairment in Visuospatial, whereas DLB showed the opposite pattern. This difference in deficit patterns was exaggerated when AD and DLB with Low-Braak were compared ($F(4,283)=7.15$;$p<.001$;Wilk'sλ=.908). However, the patterns converged and became indistinguishable when AD and High-Braak DLB were compared ($F(4,273)=0.30$;$p=.878$;Wilk'sλ =.996).

Conclusions: The initial cognitive deficit profile of DLB is influenced by degree of concomitant AD pathology to the extent that DLB with a high degree of AD pathology has a pattern of deficits almost identical to that of pure AD. Thus, a DLB deficit profile may indicate the presence of DLB pathology, but lack of a DLB deficit profile does not rule out DLB pathology.

Symposium 03: PROFILING DLB, PD AND FTD

ADPD5-1452
PREDICTION OF PARKINSON'S DISEASE PROGRESSION USING DIFFUSION TENSOR IMAGING
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Objectives: 1) To determine the degree to which rates of declining motor control in Parkinson's disease (PD) are associated with diffusion tensor imaging (DTI) detected abnormal white matter microstructures at baseline; 2) To determine the value of baseline DTI for predicting fast versus slow PD progression.
Methods: 126 patients with early PD (age=60.3±9) enrolled in the Parkinson's Progression Marker Initiative (PPMI) had DTI scans at baseline and clinical follow-up after 12.6±1 months. DTI processing included estimations of fractional anisotropy (FA), an index of white matter integrity, in 72 brain regions. Linear mixed-effect models were used to examine relations between baseline FA values and rate of motor decline based on UPDRS scores. Machine learning together with a receiver operator characteristic analysis were used to identify the anatomical pattern of FA values that predicted fast versus slow PD progression.
Results: High rates of motor control decline were associated with low baseline FA values primarily of the posterior limb of the internal capsule, the medial lemniscus, and temporal lobe white matter regions (all $p < 0.02$). A moderate accuracy of 63 ± 0.8 % was achieved with baseline DTI in predicting fast versus slow decline in PD motor symptoms. The anatomical pattern of abnormal FA values that contributed to the prediction involved primarily the superior corona radiata, the cingulum as well as superior parietal, superior frontal, superior cerebellar peduncle regions ($p<0.01$).
Conclusions: The findings suggest that an abnormal baseline FA pattern from DTI is a potential predictor of PD progression.

Symposium 03: PROFILING DLB, PD AND FTD

ADPD5-1709

LONGITUDINAL STUDIES OF FAMILIAL FRONTOTEMPORAL DEMENTIA - A WINDOW ON DISEASE PROGRESSION AND POTENTIAL INTERVENTION

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A family history is common in patients with frontotemporal dementia (FTD) and in approximately 20% the disease is inherited on an autosomal dominant basis. Mutations in three genes, namely, *MAPT, GRN and C9orf,* account for the majority of cases, although an expanding number of rarer genetic causes are being identified. Of the three prototypic syndromes found in association with FTD, behavioural variant, progressive non-fluent aphasia and semantic dementia, the former two are most commonly found in autosomal dominant disease but with considerable phenotypic overlap and neuropathological overlap with sporadic disease. Natural history studies of autosomal dominant neurodegenerative disorders can provide major insights into the development and progression of disease as exemplified by Huntington's Disease and familial Alzheimer's disease (the DIAN study). A number of small studies of familial FTD have indicated a significant premanifest stage of neurodegeneration and changes in biomarkers such as serum progranulin. Recently a multinational consortium (the GENetic Frontotemporal dementia Initiative – GENFI), comprising 11 sites across Europe and Canada, has analysed data from 220 participants consisting of 118 mutation carriers (40 symptomatic, 78 asymptomatic) and 102 non carriers. This has revealed significant changes in neuropsychological measures around five years prior to onset and atrophy on MRI occurring up to 10 years before onset. These studies allow elucidation of the premanifest stage and the identification of biomarkers of proximity to symptomatic onset. Such studies are essential for assessing potential therapeutic interventions prior to irreversible neurodegeneration.

Symposium 07: LIPIDIDIET - NUTRITIONAL INTERVENTION IN PRODROMAL AD

ADPD5-0519

ABCA1 AGONIST PEPTIDES: A NEW THERAPEUTIC AVENUE FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Objectives: ApoE4 is the most prevalent genetic risk factor for Alzheimer's disease (AD). We have previously shown that the pathological effects of apoE4 can be reversed using the RXR agonist, bexarotene, and that this is achieved via upregulation of ABCA1, resulting in increased lipidation of apoE4. Since the RXR system has numerous other targets, it is important to devise the means of activating ABCA1 directly. Accordingly, we presently utilized peptides shown to directly activate ABCA1 *in vitro*, and examined the extent to which they can affect the degree of lipidation of apoE4 *in vivo* and counteract the brain and behavioral pathological effect of apoE4.
Methods: The ABCA1 agonist peptide CS6253 was injected i.p. in 2 dosages (20mg and 60mg/kg/48h) for 8 weeks to 2.5 months old apoE3 and apoE4 targeted replacement mice (n=6/group) after which the resulting effects of the treatment on apoE4-driven brain and behavioral deficits were assessed.
Results: I.p. injection of the ABCA1 agonist CS6253 reversed the hypolipidation of apoE4 relative to apoE3 in a dose-dependent manner, without affecting the levels of apoE. Furthermore, the ABCA1-agonist abolished the apoE4-driven accumulation of Abeta42 and hyperphosphorylated tau in hippocampal neurons and the associated behavioral deficits in an object recognition test.
Conclusion: These findings support the hypothesis that selective ABCA1 agonist treatment can reverse AD phenotype in apoE4 mice suggesting a novel therapeutic approach for the treatment of AD in apoE4 carriers.

Symposium 07: LIPIDIDIET - NUTRITIONAL INTERVENTION IN PRODROMAL AD

ADPD5-0620

IMPACT OF A SPECIFIC NUTRIENT COMBINATION DIET ON CEREBRAL CIRCULATION, NEURONAL INTEGRITY AND COGNITION IN MOUSE MODELS FOR ALZHEIMER'S DISEASE

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Recently, a combination of omega-3 with precursors and cofactors in membrane synthesis (Fortasyn) was developed for dietary management of AD. This diet enhances formation of neuronal membranes, and support synaptogenesis in rodents. Studies in mild AD patients supplemented with this diet indicate improvements in verbal recall task and better cognitive performance. **Objectives**. Within the EU 7th Framework project Lipididiet (nº 202167) we evaluated impact of a Fortasyn-containing diet on cerebral hemodynamics in relation to cognition and cerebral network integrity in APP/PS1, and ApoE4 mice. **Methods**. We examined 12 and 18 months old $APP_{swe}/PS1_{dE9}$, apoE4, and C57BL6/J mice. From 2 months of age, mice were fed a standard control diet or a specific nutrient-combination diet containing fish oil, phospholipids, UMP, choline, B-vitamins and antioxidants (Fortasyn). All MR measurements (CBF, rs fMRI, MRS, DTI) were performed on a 11.7T scanner. Cognition was assessed in MWM and open field. Neurogenesis and synaptic plasticity was quantified post mortem via IHC. **Results**. The diet restored neurogenesis and decreased anxiety-related behavior in open field and preserves white and gray matter integrity and CBF in AβPP-PS1 mice. The combination diet restores reduced CBF, impaired functional connectivity and white and grey matter integrity in aging ApoE4 mice as well. **Conclusions**. A specific combination diet restores reduced CBF and concomitant impaired functional connectivity and white and grey matter integrity in aging mouse models for AD. Diet may provide in preventive strategies in very early phases of AD.

The research received funding from EU Seventh Framework Programme (FP7/2007-2013) nº 202167

Symposium 07: LIPIDIDIET - NUTRITIONAL INTERVENTION IN PRODROMAL AD

ADPD5-1025

LIPID-BASED DIETS HAVE POSITIVE EFFECT ON MUSCARINIC RECEPTORS-MEDIATED G-PROTEIN ACTIVATION IN FRONTAL CORTEX AND HIPPOCAMPUS OF TRANSGENIC APPSWE/PS1DE9 FEMALE MICE.

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Objectives: Transgenic APPswe/PS1dE9 mice overproduce human amyloid-beta (Abeta) and mimic amyloid plaque formation characteristic for human AD. We have previously detected early impairment of presynaptic cholinergic parameters and functionality of muscarinic receptor signal transduction in the course of amyloid accumulation (Machova et al., Neurobiol.Aging 2008 and Neurobiol.Dis. 2010). Now we investigated whether fish oil-based experimental diets (LipiDiDiet endothelial, mitochondrial and combinational) modify markers of cholinergic muscarinic transmission in the hippocampus and frontal cortex.

Methods: Transgenic 3-month-old female mice were fed either control or endothelial, mitochondrial or combinational diet for 10 months. They were killed under deep anesthesia, brain regions dissected, and stored at -80 °C until used for biochemical determinations.

Results: In hippocampus, all experimental diets increased the potency of muscarinic agonist in activation of G-proteins and decreased the concentration of soluble Abeta40. Endothelial diet decreased cholineacetyltransferase activity but increased its gene expression. Mitochondrial diet had no influence on cholineacetyltransferase activity but increased its gene expression. Endothelial and mitochondrial diets increased gene expression of the high-affinty choline transporter (ChT1). In frontal cortex, all tested diets increased the sensitivity of muscarinic receptors-mediated G-protein activation to agonist but had no influence on soluble Abeta40/42. They did not influence cholineacetyltransferase activity but decreased its gene expression. Combinational diet, in addition, decreased gene expression of ChT1.

Conclusions: Compared to control diet, fish oil-based diets differentially influence parameters of muscarinic cholinergic neurotransmission in different brain regions. The common profitable effect of all diets is the potentiation of muscarinic receptor-mediated G-protein activation in middle-aged transgenic mice.

Symposium 07: LIPIDIDIET - NUTRITIONAL INTERVENTION IN PRODROMAL AD

ADPD5-1478
UNDERSTANDING THE MECHANISMS HOW LIPIDS AFFECT AMYLOIDOGENIC PROCESSING

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Objectives:
Lipids, including cholesterol, phytosterols, phospholipids, sphingolipids, glycosphingolipids, ceramide, are major factors determining the balance between amyloidogenic versus non-amyloidogenic processing and the total amount of Abeta. One major goal of the LipiDiDiet study is to identify the mechanisms by which lipids are able to change APP processing.
Methods:
We systematically studied the influence of major lipids and their effects on gene transcription, protein levels, secretase activities, interaction with APP and Abeta, subcellular trafficking, lipid microdomain composition, lipid enzymes, protein modification, combinatory effects with other nutritional factors like vitamins, protein stability and degradation, cellular energy, lipid composition, feed-back regulation, lipid homeostatic proteins and validated results in human brains samples.
Results:
Pro- and non-amyloidogenic lipids act by multiple but overlapping and common mechanism. Amyloidogenic processing preferentially takes place in lipid microdomains and is targeted by several lipids. E.g. cholesterol increases raft associated amyloidogenic processing, others, including polyunsaturated fatty acids and the plant sterol stigmasterol cause a displacement of cholesterol to the non-lipid microdomain fraction, shift in gamma-secretase localization, increased alpha-secretase mediated processing and reduced Abeta levels. Other mechanisms include e.g. targeting secretase levels and subcellular trafficking. Biogenesis of several lipids is influenced by Abeta, AICD and APPs, an increase in these lipids triggers release of APP processing products which decrease respective lipid anabolic enzymes.
Conclusions:
Lipid alter APP processing is due to largely similar or overlapping mechanisms. This knowledge can be used for the design of nutrition based AD prevention strategies.
[1]Funded by the EU FP7 project LipiDiDiet, Grant Agreement N°211696

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ADPD5-1770

STATUS OF A CLINCIAL STUDY INVESTIGATING THE EFFECTS OF A SPECIFIC NUTRITION COMBINATION IN PRODROMAL ALZHEIMER'S DISEASE: THE LIPIDIDIET STUDY

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Objectives The specific nutrition combination Fortasyn Connect is designed to support synapse formation and function in patients with Alzheimer's disease (AD) by delivering specific nutritional precursors and cofactors for the formation of neuronal membranes. Two randomised controlled trials (RCTs) have shown that the nutrition combination improves memory performance in drug-naïve mild AD patients (MMSE 20-26 and MMSE≥20), indicating that it may have promising effects in (very)early AD as well. The LipiDiDiet study[1] is one of the first RCTs in prodromal AD, investigating the effects of the nutrition combination on cognitive functioning.
Methods The LipiDiDiet study is a 24-month, double-blind, parallel-group, multi-centre, multi-country RCT in subjects with prodromal AD (criteria Dubois 2007), receiving the nutritional intervention or an iso-caloric control product once daily. Primary outcome measure is cognitive functioning as assessed by a neuropsychological test battery.
Results In total, 312 subjects have been randomised, 197(63%) of whom completed the study, 55(18%) are ongoing and 60(19%) dropped-out (status September 2014). Three optional 12-month double-blind extension studies are ongoing, including respectively 110, 28 and 6 subjects at this moment. Baseline characteristics of the study population conform to the criteria for prodromal AD, with evidence for underlying AD pathology. A planned blinded interim analysis on safety and efficacy was completed in July 2014 and reviewed by an independent Data Monitoring Committee, which advised to continue the study as planned.
Conclusions Further details on the progress of the study will be presented at the conference.
[1]Funded by the EU FP7 project LipiDiDiet, Grant Agreement N°211696.

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ADPD5-2329
Dementia risk estimation tools and their use in clinical trials

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Background: Many risk factors have been associated with dementia and Alzheimer's disease (AD) in epidemiological studies. Such factors are often studied individually, but it is essential to investigate their combined effects and formulate risk estimation tools for identifying at-risk individuals who may benefit most from preventive interventions.
Methods: During the LipiDiDiet project, several dietary and vascular factors were investigated in relation to AD in epidemiological studies. Based on data from the Cardiovascular Risk Factors, Aging and Dementia (CAIDE) study, a Healthy Diet Index and a Dementia Risk Score were developed. In addition, a novel supervised learning method (Disease State Index, DSI) and accompanying visualisation method (Disease State Fingerprint, DSF) are being tested for dementia risk profiling.
Results: Persons with healthy diet (high score in the Healthy Diet Index) at midlife had significantly decreased risk for AD later in life compared to persons with unhealthy diet. The midlife CAIDE Dementia Risk Score (including several modifiable risk factors) has been validated and it was shown to relate to brain MRI changes up to 30 years later. It was also used to select participants in the FINGER 2-year multidomain randomised trial. DSI performed well in predicting dementia 7 years later in the CAIDE population based on a profile of late-life risk factors.
Conclusions: Several modifiable dietary and vascular risk factors already at midlife are linked with AD risk. Risk scores and profiling tools could be useful for selecting participants in prevention trials and identifying individuals who are most likely to respond to preventive interventions

Symposium 08: CSF AND IMAGING BIOMARKERS OF AD

ADPD5-0242

CEREBRAL MICROBLEEDS AND THE IMPACT ON CEREBROSPINAL FLUID BIOMARKERS IN DEMENTIA

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Objective: Cerebral microbleeds (CMBs) are hypothesised to have an important, yet unknown role, in the dementia disease pathology. In this study we analysed the impact of CMBs on routine cerebrospinal fluid (CSF) biomarkers in a broad range of dementia diagnoses, in order to determine the importance of CMBs in various dementia diagnoses.
Method: 1039patients undergoing dementia investigation (mean age 62 (±10), 53% female; 10 different dementia diagnoses) were analysed. All patients underwent lumbar puncture, and an MRI scan. CSF samples were analysed for amyloid β (Aβ)42, total tau (T-tau), tau phosphorylated at threonine 18(P-tau) and CSF/serum albumin ratios. Univariate and multivariate linear regression models were used to determine the impact of number/topography of CMBs on CSF biomarkers.
Results: Patients with CMBs had lower levels of Aβ42, and higher CSF/serum albumin ratios, when compared to patients without CMBs, in the whole cohort. In the subgroups Aβ42 decreased with increasing number of CMBs in alcohol related dementia (standardized beta: -1.71, P=0.026), Alzheimer's disease (standardized beta: -0.20, P=0.001), mild cognitive impairment (standardized beta: -0.18, P=0.002) and vascular dementia (standardized beta: -0.50, P=0.013). There was no impact on T-tau and P-tau with number of CMBs. CSF biomarkers varied with CMB topography, demonstrating lower Aβ42 with lobar topographies, and decreased T-tau and P-tau with infratentorial topographies.
Conclusion: Aβ42 is the routine CSF biomarker mostly affected by CMBs in dementia suggesting a direct relationship between CMBs and Alzheimer's pathology. CMBs impact on CSF-biomarkers varies with topography, demonstrating different pathology behind lobar and deep and infratentorial CMBs.

Symposium 08: CSF AND IMAGING BIOMARKERS OF AD

ADPD5-0792

BENCHMARKING THE UPDATED CRITERIA ON ALZHEIMER'S DISEASE - DATA FROM THE SWEDISH DEMENTIA REGISTRY

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Background:
The diagnosis of Alzheimer's disease (AD) has traditionally been made by clinical criteria, but novel research criteria have incorporated biomarkers for diagnosis. In the newly updated guidelines from the International Working Group (IWG-2), a cerebrospinal fluid (CSF) profile with low levels of amyloid beta 1-42 (Abeta42) together with high levels of either total tau (T-tau) or phosphorylated tau (P-tau) has been included as a research diagnostic criterion for AD. The aim of this study was to test how implementation of the new IWG-2 diagnostic criteria would influence AD prevalence using data from the Swedish Dementia Registry (SveDem).
Methods:
By cross-referencing a laboratory database containing measurements of CSF Abeta42, T-tau and P-tau with the SveDem registry, 2375 patients with a clinical diagnosis of AD were acquired. The proportion of patients with pathological CSF biomarkers was evaluated.
Findings:
A pathological biomarker profile was found in 76·5% of patients with a clinical diagnosis of AD, based on levels of Abeta42 and T-tau, and in about 60·4%, based on levels of Abeta42 and P-tau. Altogether, 76·8% fulfilled the IWG-2 CSF biomarker criteria.
Interpretation:
This is the first study to evaluate the new IWG-2 CSF biomarker criteria on patients with diagnosed AD. In a large cohort of AD patients diagnosed in clinical practice about a quarter did not fullfil the IWG-2 biomarker based criteria for AD. This discrepancy may partly reflect false positive clinical diagnosis. Implementation of biomarker informed criteria, such as IWG-2, would reduce the number of patients diagnosed with AD.

Symposium 08: CSF AND IMAGING BIOMARKERS OF AD

ADPD5-0841
COMPARISON OF THREE AMYLOID BIOMARKERS FOR PRECLINICAL ALZHEIMER'S DISEASE

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Objectives: Biomarkers for amyloid play a central role in the NIA-AA research definition of preclinical AD. In a prospective community-recruited cohort of cognitively intact older adults, we compared three amyloid markers within-subjects: ^{11}C-PIB, ^{18}F-flutemetamol, and CSF $A\beta_{42}$.
Methods: 34 cognitively intact healthy subjects (65-80 years) participated (22 underwent all 3 procedures, 12 underwent ^{18}F-flutemetamol together with one of the other tests). CSF $A\beta_{42}$ was measured with ELISA (Fujirebio Europe). The primary outcome measures were the concordance between binary classification based on ^{18}F-flutemetamol versus ^{11}C-PIB according to visual reads or semiquantitative assessment (standardized uptake value ratio in composite cortical volume, $SUVR_{comp}$). Our secondary outcome measures were the concordance between binary classification based on ^{18}F-flutemetamol $SUVR_{comp}$ versus CSF $A\beta_{42}$ cut-off, and the correlation between ^{18}F-flutemetamol and ^{11}C-PIB $SUVR_{comp}$.
Results: Binary classification based on semiquantitative cut-offs was concordant between ^{11}C-PIB and ^{18}F-flutemetamol in 94%. Concordance of blinded binary visual reads between tracers was 84%. Classification based on $A\beta_{42}$ was concordant with ^{18}F-flutemetamol semiquantitative classification in 80% (7 discordant cases were positive according to ^{18}F-flutemetamol but negative according to $A\beta_{42}$). ^{18}F-flutemetamol and ^{11}C-PIB $SUVR_{comp}$ were highly correlated ($\rho=0.84$, $p<0.0001$).
Conclusions: For the definition of preclinical AD based on amyloid imaging semiquantitative cut-offs produce more consistent results than visual reads. Our data provide no indication that CSF $A\beta_{42}$ would be more sensitive than amyloid imaging in this population.

Symposium 08: CSF AND IMAGING BIOMARKERS OF AD

ADPD5-0888
DETECTION OF PHF-TAU PATHOLOGY WITH [18F]T807 IN BRAIN SECTIONS FROM CHRONIC TRAUMATIC ENCEPHALOPATHY (CTE) PATIENTS

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Objective: To establish the ability of the radiotracer [18F]T807, also known as [18F]AV-1451, to detect Tau aggregates in postmortem human CTE brain tissue.
Methods: Frozen sections from CTE, Alzheimer's disease (positive control) and age matched controls subjects were exposed to [18F]T807 and examined by autoradiography. Adjacent slides from the same tissue samples were stained with PHF Tau antibodies (AT8, AT100, 3R and 4R) in order to establish the presence of pathological Tau aggregation in the [18F]T807 positive regions. In addition, several fluorescent reporter compounds that compete with [18F]T807 binding were used to double stain these tissues to identify any colocalization between the tracer and PHF Tau aggregates revealed by the antibodies.
Results: The positive signals obtained by autoradiography from [18F]T807 in CTE brain tissue were located in areas that contained PHF tau as determined by Tau antibodies. All [18F]T807 surrogate fluorescent compounds colocalized with immunofluorescence signal obtained with antibodies that stain pathological Tau aggregations but also with those that stain the 3R and 4R variants of the protein.
Conclusions: Autoradiography results suggest that the radiotracer [18F]T807 is capable of binding Tau in CTE tissue. There was good colocalization of fluorescent signals from T807 structurally related reporter compounds and tau antibodies AT8, AT100, 3R and 4R in CTE.

Symposium 08: CSF AND IMAGING BIOMARKERS OF AD

ADPD5-1202

COMPARING CONTROL GROUPS FROM SEVEN LARGE-SCALE COHORTS: DIFFERENCES IN DEMOGRAPHIC-CLINICAL FACTORS AND ASSOCIATIONS WITH IMAGING MARKERS OF ALZHEIMER'S DISEASE

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[6]INSERM U 558, University of Toulouse, Toulouse, France
[7]Third Department of Neurology, Aristoteleion Panepistimeion Thessalonikis, Thessaloniki, Greece
[8]Medical University of Lodz, Medical University of Lodz, Lodz, Poland
[9]University Hospital of Kuopio, University of Eastern Finland, Kuopio, Finland
[10]Psychiatry Warneford Hospital, University of Oxford, London, United Kingdom
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[12]Institute of Psychiatry, King's College London, London, United Kingdom

Objectives. Strict criteria for recruiting participants normally lead to collection of highly selected samples (e.g. ADNI). It is unclear to what extent control groups recruited through these mechanisms are representative of the general population and hence whether results are generalizable. We compared the control groups from three reference large-scale studies (ADNI, AIBL, and AddNeuroMed) and four large population-based cohorts in terms of demographic and clinical characteristics, and studied associations with imaging markers of Alzheimer's disease.

Methods. 1464 healthy controls were included (228 ADNI, 100 AddNeuroMed, 155 AIBL, 59 BRC, 237 GENIC, 279 NOMAS, 406 PIVUS). Differences among cohorts were analysed with ANOVA, ANCOVA, and Chi-square tests. Multiple lineal regression was used to study associations between demographic-clinical variables and imaging markers.

Results. ADNI, BRC and PIVUS recruited the oldest groups and GENIC the youngest one. Subjects in ADNI had higher MMSE scores while subjects in GENIC and PIVUS had the lowest scores. ADNI, BRC and AIBL were the groups with less global brain atrophy and NOMAS the one with greatest global brain atrophy. Differences among cohorts in demographic-clinical variables significantly influenced hippocampal volumes. Moreover, between-cohorts differences were still significant after adjusting for demographic-clinical factors showing that BCR and PIVUS had greater hippocampal atrophy.

Conclusions. This study together with recent research show that highly selected samples are not totally representative of the general population. These findings may

have important implications for research in Alzheimer's disease since most of results from previous studies directly depend on the characteristics of the control groups.

Symposium 08: CSF AND IMAGING BIOMARKERS OF AD

ADPD5-1241

BRAIN AREAS WITH NORMATIVELY INCREASED BLOOD FLOW ARE MORE SUSCEPTIBLE TO AMYLOID DEPOSITION

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Recent studies suggest a relationship between brain perfusion abnormalities and the deposition of beta amyloid. Interpretation of this relationship is limited by the inability to disambiguate cause from effect. It is possible that amyloid pathology causes perfusion abnormalities. Alternatively, areas with normatively increased or decreased perfusion may be susceptible to amyloid deposition. We examined whether PET-derived amyloid uptake values in older adults varied regionally as a function of normative blood flow.

One hundred twenty-eight older adults (mean age=69.8 years) received amyloid PET scans (Pittsburgh Compound B). Mean amyloid uptake values were calculated among these subjects in 31 regions-of-interest (ROIs) defined by a neuroanatomical atlas. A normative blood flow atlas was created by averaging blood flow values from SPECT scans of 47 healthy younger adults (mean age=34.3 years) in the same ROIs. The 31 ROIs were rank ordered from lowest to highest blood flow value and divided into quartiles. We compared the mean amyloid uptake values across the four quartiles.

Areas with higher mean normative blood flow had higher amyloid uptake values [$F(3,27)=3.19$, $p=0.036$]; there was a linear trend ($p=0.029$) showing a monotonic increase in amyloid values across the four quartiles.

Brain areas that have relatively higher blood flow in young, healthy adults are more likely to have higher levels of amyloid deposition in older individuals. Regional increased blood flow throughout life may render the brain parenchyma susceptible to amyloid deposition through mechanisms yet to be determined.

Symposium 11: UPDATE ON TREATMENT OF PD

ADPD5-0357
ASSESSMENT OF MOTOR CONTROL FLUCTUATIONS IN PD PATIENTS UNDER DOPAMINERGIC TREATMENT WITH NEUROSKILL DEVICE: OFFICE AND HOME MONITORING.

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1. Objectives

To monitor motor fluctuations in PD patients using both Neuroskill and UPDRS-III evaluations in clinic setting and establish correlation between them.
To assess Sensitivity and Specificity of Neuroskill in discriminating PD subjects from healthy subjects.
To home monitor a subset of PD patients.

2. Methods

Neuroskill measures accelerations and pressures during cursive writing tasks and derives Criteria of Stability, Smoothness and Synchronization of handwriting movements.
A clinical study with 45 PD subjects (H&Y Stage II and III; mean age 65±9 years) and 33 healthy, age and gender matched controls was conducted at the Colorado Neurological Institute under IRB approved Protocol. PD subjects continued on their regular dopaminergic medications. In clinic, all subjects were assessed with Neuroskill and UPDRS-III five times at hourly intervals. In addition, 22 PD patients used the device at home for self-monitoring during 24 hours.

3. Results

The study demonstrated significant fluctuations in motor control of PD patients (Cr.I 64.4±8.7) vs. remarkable stability in controls (Cr.I 84.9±4.6). Pearson's correlation between Neuroskill Criterion-I (Stability) and UPDRS-III for 225 concurrent assessments demonstrated an inverse correlation –0.48 ($p<0.00001$).
Using standard ROC curve, it was established that Criterion 1 Signature OFF does extremely well in discriminating PD from non-PD individuals. It exhibited high values of both Sensitivity and Specificity for cutpoints in the range of 75 - 84.

4. Conclusions

Neuroskill appears to provide accurate assessment of motor status in PD subjects and can be utilized in home setting for remote monitoring, which may permit optimization of individual therapy.

Symposium 11: UPDATE ON TREATMENT OF PD

ADPD5-0401
INHIBITION OF RHO KINASE AS A DISEASE-MODIFYING STRATEGY FOR NEURODEGENERATIVE DISORDERS

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Disease-modifying therapies for neurodegenerative diseases should attenuate the pathology and foster regeneration. As we could show in cell culture and animal models of Parkinson's disease and Amyotrophic lateral sclerosis, pharmacological inhibition of Rho kinase (ROCK) has protective properties on neuronal survival and acts in a pro-regenerative manner on axonal outgrowth. In the SOD1-G93A mouse model of ALS, oral application of the ROCK inhibitor Fasudil increased survival and improved motor behavior. This was associated with the activation of intracellular pro-survival signaling cascades, e.g. Akt/PKB, and in addition implicated immune-modulatory effects on microglia. In toxin-based models of Parkinson's disease (MPTP and 6-OHDA), Fasudil increased the number of nigral dopaminergic neurons, regenerative sprouting to the striatum and striatal dopamine metabolites. Interestingly, we could also observe that Fasudil affected alpha-synuclein aggregation in cell free aggregation assays and cell cultures in vitro. Since Fasudil is already licensed for human use and has a favorable safety profile, it should be further evaluated in clinical trials for its properties as a disease-modifying drug in human neurodegenerative disorders.

Symposium 11: UPDATE ON TREATMENT OF PD

ADPD5-1883
PERSONALIZED MEDICINE AND DISEASE COURSE MODIFICATION IN PD
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The diagnosis of Parkinson disease (PD) is based on clinical criteria, and is needed for useful symptomatic therapy. However, it became quite clear in recent years that the same features can result from different etiopathogenic mechanisms. Thus, it is accepted now that what is called PD is the result of phenotypic convergence. Even pathological diagnosis of PD, based on the demonstration of typical distribution of alpha-synuclein deposits, is a manifestation of phenotypic convergence at the tissue level.

Since the clinical manifestations of PD can be the result of quite heterogeneous mechanisms, it is unlikely that an intervention can be developed which will be able to influence the development of the disease in all patients. Such disease-modifying therapy should be based not on clinical but rather on understanding the underling pathogenetic processes which differ among cases.

Individualized therapy to interrupt, or at least slow, disease progression must be based on elucidation of the metabolic processes. Some patients may develop PD as a result of mitochondrial damage. Correction of these abnormalities will not affect the progression of the disease among other PD patients, in whom an identical syndrome derives from defects in the proteasome system, etc.

Precision medicine can be used now to identify the underlying pathogenic mechanisms in individual patients, paving the way to the development of real disease modification.

Symposium 11: UPDATE ON TREATMENT OF PD

ADPD5-1893
NEW CONCEPT IN THE TREATMENT OF PD
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The approach to Parkinson's disease (PD) imply important decision such as when and how start treatment. The rate of disease progression vary in the early stage being slower in the very early patients. Thus the early period after diagnosis is critical in term of rate of progression but is also in the early stage of the disease that pharmacological intervention may modify the course of the disease. Levodopa remain the gold standard in the treatment of PD but the STRIDE-PD study reinforce the concept that risk of developing dyskinesia and wearing-off each increased with higher levodopa doses. Thus physicians should use the lowest dose of levodopa that provides satisfactory clinical control to minimize the risk of dyskinesia and wearing off. Recently a pragmatic, open label randomized trial to evaluate which of three classes of drug (levodopa, dopamine agonists, or MAOBI), as initial treatment, provides the most effective long-term control of symptoms and best quality of life for people with early PD has been published. The authors found a very small but persistent benefits for patient-rated mobility scores when treatment is initiated with levodopa compared with levodopa-sparing therapy. Today we may also consider a combination of drugs rather than using a single drug at high dose. MAOBI can be successfully combined with DA-agonists and L-dopa and L-dopa can be combined with a DA and MAOBI. Combination of drugs may be more or equally effective but may reduce the dose of each of them minimizing the risks of side effects.

Symposium 11: UPDATE ON TREATMENT OF PD

ADPD5-1956
IMPACT OF DOPAMINERGIC TREATMENT ON BRAIN CONNECTIVITY IN PARKINSON'S DISEASE

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Patients with Parkinson's disease (PD) are characterized by motor impairment but also by executive dysfunctions, characterized by deficits in internal control of attention, set shifting, planning, inhibition, conflict resolution, impairment in dual task performance, and on a range of decision-making and social cognition tasks, due to degeneration of dopaminergic neurons in basal ganglia. It has been well demonstrated that levodopa, and dopamine agonists impact not only on parkinsonism but also on executive control. Executive tasks may become easier with L-dopa, suggesting a phenomenon of functional restitution and reallocation of the cortical activity under dopamine.

The lecture will touch upon brain connectivity changes as assessed by functional MRI (fMRI) both at rest and during a specific task performance. Different techniques have been used so far for MRI data analysis including the hypothesis- driven methods (e.g. seed-based functional/ effective connectivity), and data-drive methods (e.g. independent component analysis, graph theory based methods). As compared to age-matched controls, studies in PD and PD-dementia (PDD) have shown early changes particularly in the motor basal ganglia networks while rather variable results have been reported in cognition-related networks including the default mode network (DMN), extrastriate visual network, fronto-parietal and salience networks. The impact of dopaminergic therapy on connectivity of these networks will be discussed, with a special focus on the resting state connectivity of the DMN and the basal ganglia network.

Supported by the grant NT/13499 of the Czech Ministry of Health

Symposium 11: UPDATE ON TREATMENT OF PD

ADPD5-2303
APATHY AND IMPULSIVITY IN PARKINSON'S DISEASE
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From the wide range of neuropsychiatric complications usually seen in Parkinson's disease (PD), disorders of motivation are common manifestations. They range from the decreased emotional reactivity and goal-directed behavior found in apathetic patients, to impulsivity, disinhibition and addictive behaviors as found in those developing impulse control disorders (ICDs). Apathy and ICDs have a major impact on the quality of life of the patients and caregivers. However, proper understanding and management of these entities still represents a challenge.
The meso-cortico-limbic dopaminergic system plays a critical role in reward sensitivity, motivation and reinforcement-learning since it derives the pleasure or pain that results from our actions and guides future decisions and behavior. Dysregulation of this system has been suggested to sub-serve the clinical expression of these alterations of motivation in PD.
Here we review current topics and data supporting the implication of the meso-cortico-limbic dopaminergic system on the development of apathy and impulsivity in PD and how it explains the co-existence of entities that represent opposite sides of the same behavioral and dopamine-dependent continuum. We provide arguments showing that both entities relate to different patterns of dopaminergic dysregulation mainly mediated by a dissimilar course of neurodegeneration and effects of dopaminergic stimulation. We shall also discuss how apathy and ICDs are associated to different clinical characteristics, cognitive profiles and prognosis. We will finally provide insights on future directions to guide research on apathy and motivation in PD.

Symposium 14: GENETICS 1

ADPD5-0774
EXOME SEQUENCING IN NEURODEGENERATIVE DISEASES - MORE THAN A FAMILY BUSINESS

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The development and application of next-generation sequencing technologies has allowed for an exponential increase in the number of findings of genetic causes associated with disease.
These technologies are now commonly used to study Mendelian forms of neurodegenerative diseases, usually by sequencing the exomes of affected and unaffected family members, and by comparing the resulting variants. This approach has not only identified several new genes, but has also revealed unexpected presentations for mutations in previously known disease genes.
Although extremely well suited to the study of families, these techniques can also be used to study cohorts of deeply phenotyped cases and large case/control samples.
In this talk, I will summarize the major contributions of exome sequencing to the study of neurodegenerative diseases and I will discuss the interface between Mendelian and complex neurological diseases with particular focus on pleiotropic events and the role of rare variants.

Symposium 14: GENETICS 1

ADPD5-1604
DISSECTING THE MOLECULAR MECHANISMS OF THE GENETIC CAUSES OF PD REVEALS NOVEL MECHANISMS OF NEURODEGENERATION

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Parkinson's disease (PD) is characterized by the major clinical features of including bradykinesia, rigidity, rest tremor and postural instability, which are caused by the progressive loss of dopamine neurons in the substantia nigra pars compacta. Autonomic dysfunction, anxiety, depression, abnormalities of sleep, cognitive impairment, among other clinical features are due to the degeneration of other neuronal populations and/or neuronal dysfunction. A major contributor to these pathologic derangements is the accumulation and aggregation of α-synuclein, the major protein constituent of Lewy Bodies and Lewy Neurites. Many fresh insights into the pathogenesis of PD have come from advances in the genetics of PD. Mutations in leucine-rich repeat kinase 2 (LRRK2) and α-synuclein cause autosomal dominant PD. Mutations in parkin, PINK1 and DJ-1 cause autosomal recessive PD. Other genes identified from genome wide association and linkage studies determine one's relative risk of developing PD. Oxidative and nitrosative stress as well as tyrosine phosphorylation of parkin via activation of the non-tyrosine receptor kinase, c-Abl inactivates Parkin in idiopathic PD. PARIS and AIMP2, parkin substrates, accumulate in models of α-synuclein induced degeneration paralleling what occurs in PD due to deletion or inactivation of parkin. α-Synuclein induced degeneration is reversed by knockout of c-Abl via prevention of the inactivation of parkin and the accumulation of the parkin substrates, AIMP2 and PARIS. α-Synuclein induced degeneration is also reversed by deletion of PARIS. Thus, the parkin substrates, AIMP2 and PARIS, are downstream of α-synuclein pathology and highlight the intersection of recessive PD with idiopathic PD.

Symposium 14: GENETICS 1

ADPD5-1877
GENETICS OF EARLY-ONSET ALZHEIMER DEMENTIA: WHAT IS MISSING?
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Molecular genetic studies have contributed greatly to our current knowledge of the pathogenesis of neurodegenerative dementia. Most progress was made in Alzheimer disease (AD) which, at young age, has strong a familial predisposition though onset ages can vary considerably among mutation carriers. In autosomal dominant families, frequent high-penetrant mutations were identified for AD in APP, PSEN1 and PSEN2. The proteins involved are being studied in detail and have resulted in important biological hypotheses that are currently being pursued for the development of suitable and more effective treatments. Nonetheless, a considerable fraction of the genetic etiology has not yet been resolved. Mutations in the known genes cannot explain disease in all early-onset families, suggesting that there have to be other causal genes to be found. In cohorts, mutations are observed in only a small percentage of patients. Furthermore, genetic factors underlying the wide spread in onset ages are largely unmapped.
We systematically screen AD patients using a multiple amplicon, exon-targeted sequencing-based MASTR assay (www.multiplicom.com), for genetic variation in set of 30 genes associated with neurodegenerative brain diseases. In large pedigrees and patient cohorts, we use next-generation sequencing (NGS) to identify new causal and/or genetic modifier genes. For these studies, distant related patients are selected of extended families while of cohorts patients with extreme phenotypes (familial, early-onset age, pathology confirmed, etc.) are included. In mutation carriers, we use different NGS approaches to identify genetic modifiers. To identify putative modifying genetic variants, we link the genetic data to biological data obtained by brain transcriptomics and serum proteomics of mutation carriers.

Symposium 14: GENETICS 1

ADPD5-1881
DECODING ALZHEIMER'S DISEASE IN THE WHOLE GENOME ERA
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Mass. General Hospital/Harvard Medical School, Boston, USA
Alzheimer's disease (AD) is strongly influenced by genetic factors such as the APP, PSEN1, and PSEN2, and APOE. Since 2005, we have led the Alzheimer's Genome Project, which in 2008, reported novel AD genes including the spinocerebellar ataxia 1 gene, ATXN1, CD33, and ADAM10. ATXN1 knockout increases BACE1 levels in cortex and hippocampus, but not in cerebellum and brain stem. This increase of BACE1 is concordant with the shift of APP processing into the beta-secretase cleavage pathway along with an increase in Abeta levels and plaque load in the brains of APPswe/PS1deltaE9 mice. CD33 is one of several genes involved in the innate immune system of the brain that have been associated with AD risk. CD33 knock-out led to 1. decreased Abeta load owing to enhanced phagocytosis by microglia and 2. reduced numbers of M1-activated microglia in *APP/PS1* and 5XFAD tg mouse brains. Re-sequencing of ADAM10 revealed two rare mutations that tightly co-segregated with AD and impair ADAM10 non-amyloidogenic cleavage of APP in transgenic mice. We also carried out whole genome sequencing on 440 subjects from 452 multiplex NIMH AD families. We then employed a multi-pronged approach to identify highly penetrant functional variants underlying the association of previously reported AD genes that emerged from genome-wide association studies. Our analyses revealed several novel pathogenic variants in the known and GWAS-confirmed AD genes. The elucidation of the genes and functional variants influencing risk for AD should continue to enhance our understanding of AD etiology and pathogenesis. Ultimately, these genes will be used to predict risk for AD and guide novel the development of therapies for the effective treatment and prevention of this terrible disease.

Symposium 14: GENETICS 1

ADPD5-1900
DEFINING THE GENETIC ARCHITECTURE OF ALZHEIMER'S DISEASE. WHERE NEXT?

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For seventeen years, the *APOE* gene was the only known susceptibility gene for late-onset AD. In 2009, we identified three new genome-wide significant susceptibility genes(1,2). Recently, in the IGAP collaboration, we have published fifteen additional susceptibility loci(3–5). IGAP utilised 25,580 AD cases and 48,958 controls to identify eleven new loci reaching genome-wide significance(6), and further new loci through gene-based burden analysis(7). The susceptibility loci we have identified are not randomly distributed with respect to their functions; pathway analysis shows significant evidence for clusters of genes implicating ubiquitination, endocytosis, cholesterol transport, and immunity.

A significant proportion of genetic variation in disease pathology is yet to be detected. Thus, we are investigating rare variants in disease through exome chip and next generation sequencing experiments, which have already identified new AD-protective(8) and AD-risk variants(9). Combining common and rare variant data will provide us with the most comprehensive risk estimates of AD. Using a polygenic score approach we can identify population groups with the greatest and least biological susceptibility to AD. This method has proved more effective in predicting disease status, than individual, genome-wide significant variants of small/moderate effect(10). Future studies will establish the specific functional changes that contribute to disease by piloting novel cellular modelling techniques using reprogrammed induced pluripotent stem cells (iPSc) cells from individuals with selected genetic risk profiles. This will allow a variety of cell and animal models to be produced to help understand disease mechanisms, test new drug therapies and produce iPSc as a research resource.

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Symposium 14: GENETICS 1

ADPD5-1923
GENETIC ANALYSIS OF ALZHEIMER AND PARKINSON DISEASE RISK
J. Hardy[1]
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Genetic analysis of AD/PD and other neurodegenerative diseases
John Hardy UCL Institute of Neurology, London, UK. J.hardy@ucl.ac.uk
In my talk I will describe the Mendelian and non-mendelian causes of AD and PD related diseases. I will then discuss more generally how genetic analysis is beginning to give us insights into the underlying causes of selective vulnerability with different neuronal types having specific weaknesses based on their underlying function… the catastrophic cliff theory of selective vulnerability

Symposium 18: EPIGENETICS AND GENETIC MECHANISMS

ADPD5-0615

ACCURATE, NON-INVASIVE AND LOW COST PREDICTIVE MODEL FOR PD

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Figure 1: Area under the curve.

Objectives: To identify non-invasive and cost-effective factors accurately predicting if participants in longitudinal studies were PD cases. **Methods**: We mined baseline biomarker, clinical and genetic data in the Michael J. Fox foundation's Parkinson's Progression Markers Initiative (PPMI). Stepwise regression models were used to identify candidate factors for modeling based on Akaike information criterion. Factors remaining in the model included a genetic risk score incorporating 28 common risk loci as well as G2019S (LRRK2) and N370S (GBA1), University of Pennsylvania Smell Identification Test score (UPSIT), family history, gender and age. We developed the model using the PPMI cohort and then fit it to the Parkinson Associated Risk Study (PARS) cohort. We are pursuing further replication in the NINDS Parkinson's Disease Biomarker Program (PDBP) cohort. **Results**: Both PPMI and PARS showed > 93% predictive accuracy (area under the curve). Logistic regression examining predictive model fit showed significance at p-value < 2E-16. In the prospective PDBP cohort, we expect further replication and a retrospective estimate of accuracy relative to time from diagnosis. This second replication phase will also address possible recruitment bias in PARS. **Conclusions**: This is an important model as we show we can accurately predict Parkinson's disease in populations using non-invasive and relatively inexpensive information costing no more than $150 USD per sample.

Figure 1: Area under the curve.

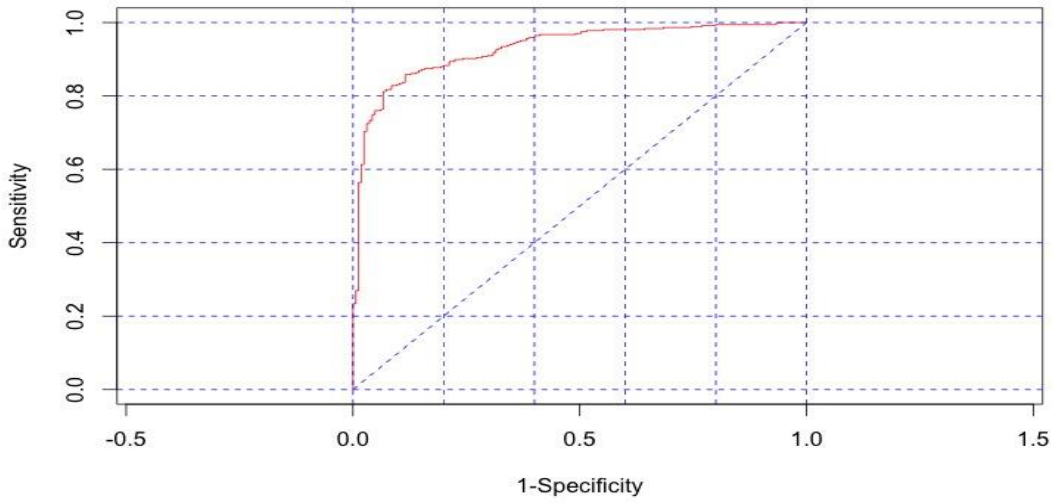
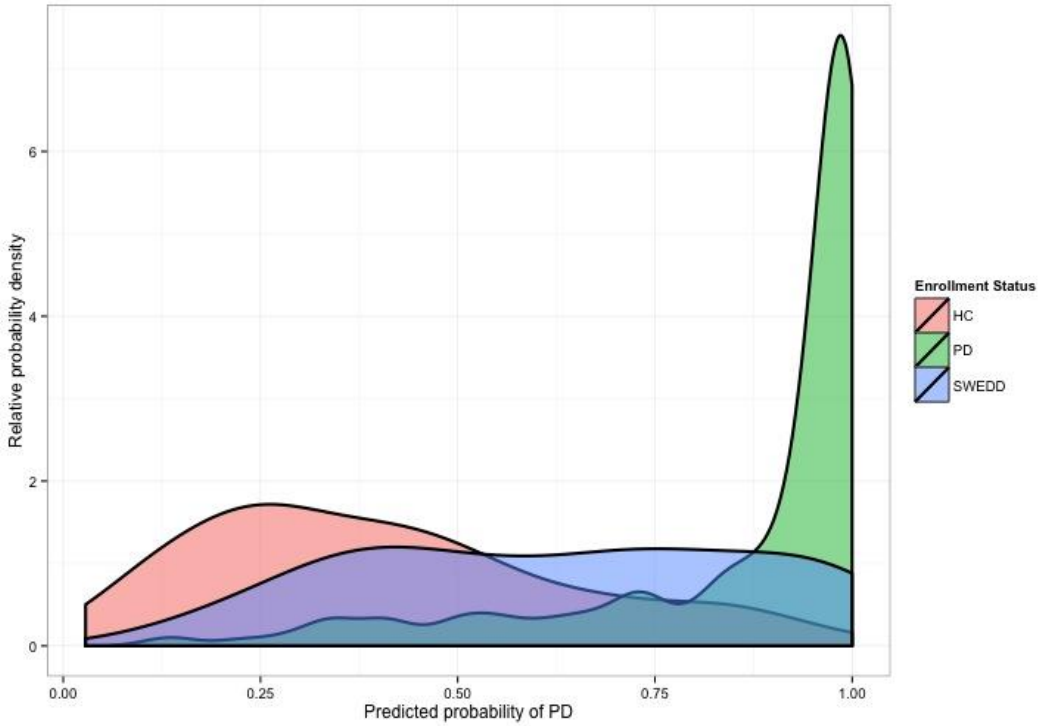


Figure 2: Predicted probabilities in PPMI.


Relative probability density
0
2
4
6
0.00
0.25
0.50
0.75
1.00
Predicted probability of PD
Enrollment Status
HC
PD
SWEDD

Symposium 18: EPIGENETICS AND GENETIC MECHANISMS

ADPD5-1197
PATHOLOGICAL PHENOTYPE OF THE FAMILIAL PD USING INDUCED PLURIPOTENT STEM CELLS

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Leucine-rich repeat kinase 2 (LRRK2) is the causative molecule of the autosomal dominant hereditary form of PD, PARK8, which was originally defined in a study of a Japanese family (the Sagamihara family) harboring the I2020T mutation in the kinase domain. Although a number of reported studies have focused on cell death mediated by mutant LRRK2, details of the pathogenetic effect of LRRK2 remain completely unknown. In the present study, to elucidate the mechanism of neurodegeneration in PD caused by LRRK2, we generated induced pluripotent stem cells (iPSC) derived from dermal fibroblasts isolated from two PARK8 patients (LA and LB) in the Sagamihara family using retroviruses carrying Oct4, Sox2, Klf4, and c-Myc genes, as previously described. To evaluate the iPSC lines established from the patient, we characterized their properties. All of the patient iPSC lines demonstrated differentiation of all three germ layers spontaneously in vivo, and maintained a normal karyotype. We found that more than 80% of the differentiated cells were positive for βIII-tubulin (a neuron-specific marker). We also found that I2020T mutant LRRK2 iPSC-derived neurons released less dopamine than control iPSC-derived neurons. Furthermore, we demonstrated that patient iPSC-derived neurons had a lower phospho-AKT level than control iPSCs-derived neurons, and that the former showed an increased incidence of apoptosis relative to the controls. These results suggest that I2020T LRRK2-iPSC could be a promising new tool for reproducing the pathology of PD in the brain caused by the I2020T mutation, and applicable as a model in studies of targeted therapeutics.

Symposium 18: EPIGENETICS AND GENETIC MECHANISMS

ADPD5-1550

LONG NON-CODING RNAS IN NEURODEGENERATIVE BRAIN AND PATIENTS' BLOOD CELLS: FROM STRUCTURE TO FUNCTION

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Objectives: Parkinson's disease (PD) is the most prevalent motor disorder, but specific disease biomarkers are still sought and new targets for future therapeutic interference are urgently needed. Long non-coding RNAs (lncRNAs) comprise a novel, important class of RNAs with largely unknown biological functions.
Methods: Using RNA sequencing technology and a computational workflow for in-depth analysis of whole-transcriptome RNA-seq datasets, we detected and analyzed lncRNAs in rRNA-depleted sequenced libraries from PD patients' post-mortem substantia nigra and controls.
Results: We identified a total of 7244 lncRNAs that were detected at or above 1 normalized reads (FPKM) at least in one group of the obtained datasets, with several of those changed significantly in PD substantia nigra. Disease-specific changes in known and novel lncRNAs and corresponding changes in alternative splicing, protein domains and miRNA binding sites demonstrated widespread transcript variations, including a decline in the PD-risk gene antisense lncRNA PINK1-AS as well as decreases in microRNA host genes (e.g. miR124-2HG, miR22-HG) in the substantia nigra from PD patients compared to controls.
Conclusions: Our results suggest lncRNA regulatory involvement in PD; and provide a workflow that will be of use to the increasing number of laboratories producing RNA-Seq data in a wide range of biomedical studies.

Symposium 18: EPIGENETICS AND GENETIC MECHANISMS

ADPD5-1886
IMMUNE AND NEURAL EPIGENOMICS OF ALZHEIMER'S DISEASEIMMUNE AND NEURAL EPIGENOMICS OF ALZHEIMER'S DISEASE

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Transcriptomic analyses of human postmortem Alzheimer's disease (AD) brains have revealed global changes in gene expression patterns that are characterized by the downregulation of genes associated with synaptic function, learning, memory, and the upregulation of adaptive as well as innate immune response genes. However, the mechanisms underlying these changes are still poorly understood. Here, we conducted transcriptomic analysis of brains from the CK-p25 mouse model of neurodegeneration, which recapitulates various cardinal hallmark AD phenotypes, found that these mice display gene expression changes that are highly reminiscent of those that are characteristic of AD. In an attempt to understand the chromatin landscape associated with these gene expression changes, we utilized chromatin immunoprecipitation combined with next-generation sequencing (ChIP-seq) in CK-p25 mice and controls. This allows us to assess the distribution of various histone modifications that specify genomic elements, such as promoters and active/inactive enhancers. These studies revealed that neuron-specific enhancers in CK-p25 brains show reduced activity, whereas microglia/monocyte-specific enhancers show increased activity. These increased activity enhancers are enriched for binding sites of the transcription factors ETS1 and PU.1. Furthermore, mouse orthologs of human AD-associated genetic variants map predominantly to higher-activity, immune-related enhancers, and not to lower-activity neuronal enhancers. These results suggest that genetic predisposition to AD is related to immune function, whereas non-genetic factors affect neuronal pathways. We have also conducted global chromatin profiling and gene expression profiling in human induced pluripotent stem cell (iPSC)-derived neural and immune cells from familial and sporadic AD patients as well as healthy individuals. Results from these studies provide further insight into the roles of the brain's neural and immune cells in the pathogenesis of AD, and how AD-related genetic variants impact these distinct cell types.

Symposium 18: EPIGENETICS AND GENETIC MECHANISMS

ADPD5-1896
UNRAVELLING THE GENETICS OF A LARGE FAMILIAL AD KINDRED IN ANTIOQUIA COLOMBIA

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Genomic studies in PSEN1 E280A kindred: A Protective Variant Delays Onset

We have sequenced the complete genomes of 72 individuals affected with early onset familial Alzheimer's disease (EOFAD) caused by the E280E autosomal dominant, highly penetrant mutation in the presenilin-1 gene (*PSEN1*). We performed a genome-wide association test to identify variants that modify age at onset (AAO) of AD. Our analysis revealed a haplotype of single nucleotide polymorphisms (SNPs) on chromosome 17 associated with a delayed AAO for mild cognitive impairment and dementia. Individuals carrying this haplotype had an average age at onset of mild cognitive impairment at 51.0 ± 5.2 years (mean ± std. dev.) compared to 41.1 ± 7.4 for those without these SNPs. These striking results suggest a genomic locus modifying Alzheimer's AAO conferring a large (~10 year) protective effect.

Symposium 18: EPIGENETICS AND GENETIC MECHANISMS

ADPD5-2309
THE ROLE OF MICRO RNAS IN AD AND PD: A GENETIC PERSPECTIVE
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A substantial portion of the susceptibility underlying both Alzheimer's disease (AD) and Parkinson's disease (PD) is genetically determined. Most of the currently known risk genes were identified in genome-wide association studies (GWAS) correlating disease risk with genotypes at millions of polymorphic DNA sequence variants, e.g. single-nucleotide polymorphisms (SNPs). While the genetic evidence underlying these findings is very compelling for most recently identified loci, the mechanisms by which they exert their pathogenic effects remain less well understood. One such mechanism could involve interference with micro-RNA (miRNA) mediated expressional regulation. MiRNAs are a class of small, non-coding RNAs that modulate protein expression by binding to mRNA thereby decreasing translation. Binding between miRNAs to their mRNA targets is largely determined by nucleotide sequence complementarity. Our group has recently begun to investigate the potential role of disease-associated SNPs in interfering with miRNA-to-mRNA binding by affecting this sequence complementarity. To this end, we have developed an *in silico* pipeline systematically modelling the effects of SNPs on miRNA-to-mRNA binding. Application of this algorithm to recent AD or PD GWAS findings highlight a number of variants predicted to exert strong effects on protein expression. In my presentation, I will present a summary of these *in silico* findings and discuss how they correlate with ongoing experiments assessing these predicted effects *in vitro*. In addition, I will discuss how the findings from our genetics-centered approach correspond to studies aimed at identifying profiles of disease-associated miRNAs in blood or cerebrospinal fluid as potential biomarkers for AD or PD.

Symposium 19: TAUOPATHIES 1

ADPD5-0747
A NEURONAL DNA DAMAGE RESPONSE IS DETECTED AT LOW BRAAK STAGES AND CORRELATES WITH COGNITIVE IMPAIRMENT IN THE AGEING BRAIN

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Objective: We have previously demonstrated a wide variation in oxidative stress and a DNA damage response (DDR) in the ageing brain, which shows an inverse relationship to Braak stage [1]. We hypothesise that neuronal dysfunction as a result of oxidative DNA damage accounts for some of the cognitive impairment that is not fully explained by Alzheimer-type pathology.
Methods: Frontal cortex frozen tissue (Braak stage 0-II, 39 cases) was obtained from the Medical Research Council Cognitive Function and Ageing Study cohort. Oxidative stress (malondialdehyde, MDA) and the neuronal DDR (γH2AX and DNA-dependent protein kinase catalytic subunit, DNA-PKcs) were assessed, and the relationship to cognitive impairment determined. Neurones were isolated from 10 cases (5 high and 5 low neuronal DDR) by laser capture microdissection and changes in the neuronal transcriptome identified by microarray analysis.
Results: Both DNA-PKcs+ (r_s=-0.43, p=0.006) and γH2AX+ (r_s=-0.41, p=0.009) neuronal counts correlated inversely with the patients' last MMSE score. 1005 genes were significantly differentially expressed (639 up-regulated, 366 down-regulated genes, p<0.001) in cases with a high neuronal DDR. Functional grouping analysis identified dysregulation of genes associated with insulin signalling and wnt signalling, and upregulation of GSK3B.
Conclusion: We present the gene expression signature of neurones in response to DNA damage at the earliest Braak stages, and propose that down-regulation of insulin and wnt signalling results in the up-regulation of GSK3B which contributes to neuronal dysfunction and cognitive impairment independent of Alzheimer pathology in the ageing brain.
[1] Simpson et al. NAN 2010; 36: 25-40.

Symposium 19: TAUOPATHIES 1

ADPD5-1361
EARLY ONSET FAMILIAL ALZHEIMER-TYPE DEMENTIA ASSOCIATED WITH TAUOPATHY AND TDP-43 PROTEINOPATHY

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Objective: To describe the genetic findings of a kindred with progressive Alzheimer-type dementia in which the proband had autopsy-confirmed tauopathy with TDP-43 proteinopathy.

Methods: We first analysed neurodegeneration-related genes and then performed whole exome sequencing on two affected siblings and one unaffected aunt. By identifying identical by descent regions in both siblings, we uncovered a large number of candidate genes potentially involved in the etiology of the phenotype under study.
Results: Analysis of the *GRN, TARDBP, APP, PSEN 1, 2,* and *MAPT* genes excluded pathogenic mutations. Genes that were found to harbor more variants shared identical by descent among the siblings, included *LRRK2, CSMD1, AHNAK2, PCNT*, and *TMEM176B*. According to our analysis of variants shared among the siblings there is an enrichment in genes involved in nervous system development, synaptic transmission, neurogenesis, muscle structure development, brain and forebrain development, lipid transport and localization, behavior, learning or memory. In addition, pathway analysis revealed pathways related to Alzheimer disease.
Conclusion: This condition does not fit into any previously characterized tauopathy or TDP-43 proteinopathy, and the clinical picture deviated from more frequent presentations like frontotemporal dementia with parkinsonism. Although further genetic studies may eventually disclose the etiology, our results highlight the possibility that there is no single causative gene in this case study but a set of genes working together in different pathways contributing to the etiology of a complex phenotype.
Supported by European Commission's 7th Framework Programme under GA No 278486, 'DEVELAGE'.

Symposium 19: TAUOPATHIES 1

ADPD5-1455
AMYLOIDOSIS AND FORMATION OF OLIGOMERIC TAU STRAINS IN ALZHEIMER'S AND Pd
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Objectives
Tau pathology is implicated in a number of neurodegenerative diseases including Alzheimer's (AD) and Parkinson's disease (PD). Studies repeatedly suggest that oligomeric tau is the most toxic in disease, and spreads in a prion-like mechanism. We found that oligomeric tau forms in both AD and PD. The exact mechanism and the role of these tau species in disease progression are still unknown. We hypothesize that these tau structures represent different oligomeric strains and that their conformational diversity depends upon other amyloid proteins, namely amyloid beta oligomers in AD and alpha-synuclein oligomers in PD. Therefore, it is of great importance to establish immunological and biochemical characteristics of these entities.
Methods
We generated and characterized multiple anti-tau oligomer-specific mouse monoclonal antibodies (TOMAs) and performed preliminary immunological analyses of brain tissue from AD and PD for the presence of tau oligomeric strains. In addition, we are investigating the biochemical characteristics and stability of these strains.
Results
Tau forms multiple oligomeric strains which can be distinguished biochemically and immunohistochemically with different TOMA clones. Different tau strains are capable of seeding in vitro. Strain-specific properties were also analyzed by Proteinase K digestion.
Conclusion
The ability of tau to form different oligomeric conformers may play a critical role in disease phenotype and progression. As TOMA clones display distinct preferences for different subsets of tau oligomers, they have great potential for early diagnostics, the design of strain-specific therapeutic approaches and distinguishing polymorphisms and complexity of tau aggregation in different neurodegenerative tauopathies and for personalized medicine.

Symposium 19: TAUOPATHIES 1

ADPD5-1651
A NOVEL MECHANISM UNDERLYING THE PATHOGENESIS OF PROGRESSIVE SUPRANUCLEAR PALSY

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Progressive Supranuclear Palsy (PSP) is a movement disorder characterized by the presence of neurofibrillary tangles (NFTs) comprised of abnormally hyperphosporylated/aggregated tau protein, extensive gliosis and neuronal loss within specific brain regions. A clear mechanism and treatment strategy for PSP has been lacking so far. Appoptosin (SLC25A38) is a pro-apoptotic protein that mediates neuronal cell death through intrinsic caspase activation. A single nucleotide polymorphism (rs1768208 C/T) near *SLC25A38* may be a risk factor for PSP. Our study identified a much higher T-allele occurrence as well as increased levels of appoptosin, activated caspase-3 and caspase-cleaved tau in PSP patients. Increased appoptosin associates with T-allele occurrence and correlates with activated caspase-3 and caspase-cleaved tau. Appoptosin overexpression increased caspase-mediated tau cleavage and concomitant tau aggregation and synaptic dysfunction, whereas appoptosin deficiency exhibited reduced tau cleavage and aggregation. Appoptosin transduction in the globus pallidus impaired multiple motor functions and exacerbated neuropathology in tau-transgenic mice, with little effect in tau knockout mice. Our findings reveal a novel role for appoptosin in PSP pathogenesis, linking caspase-mediated tau cleavage to synaptic dysfunction and behavioral/motor defects in tauopathies. In addition, our findings may have implications in other disorders involving caspase-3 dysregulation. Indeed, appoptosin is a component genetically-linked to sideroblastic anemia (*Nat Genet* 2009, 41(6):651-3). We also found that appoptosin knock-out embryos are prenatally inviable due to anemic complications (including defects in heme synthesis) and observed an aberrant upregulation of appoptosin in leukemic B-cells. Hence, we anticipate future work to determine the association of appoptosin variants with many non-neurodegenerative disorders.

Symposium 19: TAUOPATHIES 1

ADPD5-1919
COGNITIVE ASPECTS OF PROGRESSIVE SUPRANUCLEAR PALSY
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Cognitive difficulties are a prevalent clinical feature in progressive supranuclear palsy (PSP). We evaluated a cross-sectional sample of 350 patients who met the NINDS-SPSP Criteria for PSP with a variety of commonly used neuropsychological tests. We found that approximately 1 in 4 patients had a Dementia Rating Scale Total score at or below the 1st percentile and had at least two tests at or below the 5th percentile. More than half of the sample had a primary executive dysfunction (e.g., 63% impaired on the Frontal Assessment Battery), with milder difficulties in memory, construction, and naming. These results have important clinical implications for clinicians following patients with PSP.

Symposium 19: TAUOPATHIES 1

ADPD5-2314

TAUOPATHIES AND THE MULTIPLE FACETS OF TAU DYSFUNCTION

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Objective

Aggregates of microtubule-associated protein tau constitute the characteristic neuropathological features of several neurodegenerative diseases known as tauopathies such as progressive supranuclear palsy and Alzheimer's disease. The mechanisms of tau toxicity are not yet certain. While studies in human neurons will contribute to clarify tau toxic process, investigations are now performed in mouse models where tau aggregation and toxicity are reproduced. In human P301S tau transgenic mice, we have shown that transplantation of newly differentiated control astrocytes is able to prevent tau induced neuronal death. This effect was surprising because neuronal death occurs in the P301S tau mice despite a clear increase in the number of endogenous astrocytes. The aim of the present study was to determine and compare the neuroprotective effect of endogenous P301S tau and transplanted control astrocytes.

Methods

The characteristics of astrocytes in P301S tau and control mice were investigated using immunohistochemistry, immunoblotting and proteomics. Similar studies were performed in primary cultures of postnatal P301S and control mouse astrocytes in co-cultures of neurons and astrocytes as well as of neurons and astrocyte conditioned medium.

Results

We found that astrocytes grown from human P301S tau transgenic mice are not protective for P301S tau or control neurones. This characteristic is maintained in the supernatant from P301S tau astrocyte cultures.

Conclusions

We have determined that endogenous astrocytes in human P301S tau transgenic mice, differently from transplanted control astrocytes are not neuroprotective. The toxic effect is present also in astrocyte-cultured medium. The identification of the toxic element will help to understand tau pathological mechanisms.

Symposium 25: BIOMARKERS 2: PD and LBD

ADPD5-0725
CORRELATION BETWEEN CLINICAL, CEREBROSPINAL AND NEUROPATHOLOGICAL OBSERVATIONS IN SUBJECTS WITH THE CLINICAL DIAGNOSIS OF IDIOPATHIC NORMAL PRESSURE HYDROCEPHALUS

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Objective: For diagnosis of Alzheimer's Disease (AD) three approaches are implemented: clinical assessment, assessment of cerebrospinal fluid (CSF) biomarkers and imaging applying Pittsburgh Compound B (PiB-PET). Assessment of a brain biopsy is not carried out in AD. In subjects with idiopathic normal pressure hydrocephalus (iNPH) a brain biopsy can be obtained at the ventriculo-peritoneal shunt operation. Here we assessed correlation between AD related clinical, CSF and biopsy findings in subjects with the clinical diagnosis of iNPH.
Methods: 164 brain biopsies measured approximately 20 mm^2 in size were obtained during a shunt operation in 2010 to 2013. A pre-operative clinical assessment of cognitive status and a lumbar puncture was performed on most but the data was available for this study in 111 subjects. For assessment of cognitive function Mini Mental state examination (MMSE) was carried out. Levels of CSF β-amyloid (Aβ42), total tau (T-tau) and hyperphosphorylated tau (HPtau) were measured applying commercial ELISA. The tissue samples were assessed applying Aperio image analysis positive pixel count (PPC) and the area of grey matter assessed for the load of Aβ42 and HPtau measured 9.5 mm^2 ± SE 1,2 mm^2.
Result: Regarding Aβ the correlation was -0.32 ($p<0.001$) biopsy/CSF; -0.23 ($p<0.012$) biopsy/MMSE and 0.3 ($p<0,001$) MMSE/CSF. Regarding HPtau the correlation was 0.32 ($p<0.001$) biopsy/CSF, -0.26 ($p<0.006$) biopsy/MMSE and -0.17 ($p<0,063$) MMSE/CSF
Conclusion: Our results indicate that there is a significant correlation between AD related clinical, CSF and biopsy "markers" but the correlations even if being significant included a considerable number of "outliers"

Symposium 25: BIOMARKERS 2: PD and LBD

ADPD5-0879
CSF ALPHA-SYNUCLEIN SPECIES AS PROGRESSION BIOMARKERS FOR PD
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Objectives: To evaluate the potential use of alpha-synuclein (α-syn) species namely total (t-α-syn), oligomeric (o-α-syn) and phosphorylated at serine 129 (p-S129-α-syn) in cerebrospinal fluid (CSF) as progression biomarkers for PD.
Methods: Following the development and validation of novel enzyme-linked immunoadsorbent assays (ELISAs) using our novel specific monoclonal antibodies (mAbs) for α-syn oligomers, phosphorylated Ser129 α-syn or total-α-syn, we examined the levels of α-syn species in CSF from PD patients and age-matched healthy controls. Then, we assessed the potential use of α-syn species as progression markers in CSF samples that were taken at baseline and endpoint from a large cohort of patients that were early diagnosed with PD who participated in the DATATOP study.
Results: Combining the measurements of different CSF α-syn species, we found a clear differential CSF pattern between PD and control subjects. Interestingly, the levels of CSF α-syn species in DATATOP cohort were significantly altered at the end point compared to the baseline.
Conclusion: Our study highlights the usefulness of combining CSF α-syn species in improving PD diagnostic accuracy and prognostic evaluation. However, our findings need to be further confirmed in prospectively planned, independent cohort, particularly in samples where PD has been longitudinally assessed such as the Parkinson's Progression Markers Initiative (PPMI) cohort.

Symposium 25: BIOMARKERS 2: PD and LBD

ADPD5-1230

IDENTIFCATION OF CANDIDATE CEREBROSPINAL FLUID BIOMARKERS IN PARKINSONISM USING PROTEOMICS

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Background: Neurodegenerative parkinsonian syndromes have significant clinical and pathological overlap, making their early diagnosis difficult. They include Parkinson's disease (PD) and atypical parksinsonian syndromes (APS): progressive supranuclear palsy (PSP), multiple system atrophy (MSA) and corticobasal syndrome (CBS).

Objectives: To identify candidate cerebrospinal fluid (CSF) biomarkers, which can discriminate parkinsonian syndromes from healthy controls using proteomics.

Methods: CSF was consecutively collected from 134 participants; patients with PSP (n=36), PD (n=26), MSA (n=28), CBS (n=14), and elderly healthy controls (n=30). Participants were divided into an experimental and a validation set. Proteomic analysis with multiplex isobaric labeling was used for protein identification and quantification. The identified proteins were analyzed using multivariate discriminant analysis. Defining healthy controls and APS patients as two classes, an OPLS model was created using the discovery set. The model was then evaluated on the validation set. Proteins responsible for the separation were further evaluated by performing t-tests on the individual proteins, with multiple testing correction.

Results: There was 100% sensitivity and 70% specificity differentiating APS from healthy controls when the OPLS model created was applied to the validation set. PD data were positioned in between APS and healthy controls. 20 proteins were both significantly discriminant in the multivariate model and in the univariate t-tests. They consisted of acute phase/inflammatory markers and neuronal/synaptic markers, which were respectively increased and decreased in APS compared to healthy controls.

Conclusion Using an unbiased approach, proteins differentiating parkinsonian patients from healthy controls were identified, which may be useful as candidate biomarkers.

Symposium 25: BIOMARKERS 2: PD and LBD

ADPD5-1249

GLUCOCEREBROSIDASE (GBA) LEVELS AND ACTIVITY ARE REDUCED IN SPORADIC PARKINSON'S DISEASE

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Background: Mutations in glucocerebrosidase (GBA) represent the most common genetic risk factor for Parkinson's disease (PD) and other synucleinopathies. Many of these mutations lead to decreased enzymatic activity/stability and have been shown to impair lysosomal functionality, including clearance of α-synuclein.
Objective: To determine whether there is a decrease in GBA activity/levels in sporadic PD.
Methods: We sequenced all 11 exons of the GBA gene from 179 PD and 62 controls. GBA enzymatic activity was measured using a fluorescent artificial substrate, 4-methylumbelliferyl-β-d-glucoside. Protein levels were assessed by Western blotting (GBA and α-synuclein) and with a fluorescent GBA-probe, MDW941, which binds irreversibly to GBA.
Results: 18 GBA mutation carriers were identified, all in the PD group. We see a significant (P = 0.0127) 16.2% decrease in GBA enzymatic activity in superior temporal gyrus (STG) tissues from sporadic PD (n=38) compared to controls (*n*=34). GBA mutation carriers (*n*=8) had a further reduction in GBA activity, 55.9% decrease (P < 0.0001), compared to controls. In a subset of the STG samples, we measured a significant (P < 0.0001) increase in α-synuclein which correlates with the decreased GBA activity. Using the fluorescent GBA-probe we show a significant (P < 0.0001) decrease in lysosomal GBA in sporadic PD (*n*=18) compared to control (*n*=18).
Conclusions: These data demonstrate a significant reduction in the levels and activity of GBA in PD cases, both with and without GBA mutations. Additional human data, from other brain regions, together with mechanistic insights will provide a foundation for drug discovery programs.

Symposium 25: BIOMARKERS 2: PD and LBD

ADPD5-1401
CSF BIOMARKERS IN DLB: RESULTS FROM A LARGE EUROPEAN MULTICENTRE COHORT

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Objective. Most biomarker studies in DLB have included small cohorts from single centres. Building on a new pan-European consortium, which aims at collecting large multicentre cohorts of DLB and PD patients to describe longitudinal course and detect diagnostic and prognostic biomarkers, as well as providing guidelines for longitudinal cohort studies, we here describe the proportion with pathological CSF AD biomarkers in DLB and PD.
Methods. Out of a cohort of 1616 patients (DLB 581, PD 887, and PDD 148), CSF levels of abeta42 (Aβ42), total tau (t-tau), and phosphorylated tau (p-tau), as measured at the different centres, were collected. The locally available cut-off values for each marker were used to distinguish between normal and abnormal values
Results. CSF biomarker results were available for 474 patients from nine centres (DLB 367, PDD 46, PD 61) (Table 1 & Table 2). Low Aβ42 was more common in DLB than in PD, and high t-tau and p-tau were more common in DLB than in PDD and PD (Table 2).
Conclusion: In the largest DLB cohort studied for CSF, 49% had pathological Aβ42, and nearly a third had increased t-tau and p-tau values. However, there were large differences in Aβ42-values between centres (Table 1), which are likely due to pre-analytic procedures and the use of different ELISA tests. Future studies should explore the association of CSF markers with clinical phenotype, disease progression, imaging and other biomarkers in DLB.

Table 1| Characteristics of the largest DLB cohorts

	Karolinska Institute (n=24)	Lund University (n=91)	University Hospital Strasbourg (n=71)	VU University Medical Center (n=101)	Paracelsus-Elena-Klinik (n=62)

Age, years§ Mean ± SD	72.75±6.10	74.51±6.24	68.63±9.96	67.73±8.21	71.15±6.02
Gender Male Female	17 (70.8%) 7 (39.2%)	53 (59.6%) 36 (40.4%)	38 (53.5%) 33 (46.5%)	84 (83.2%) 17 (16.8%)	37 (59.7%) 25 (40.3%)
Duration,years‡ Mean ± SD	2.23±1.95	5.44±3.12	5.96±4.93	N/A	2.18±1.92
MMSE score† Mean ± SD	23.79±3.97	20.94±5.09	24.35±8.29	22.22±4.51	18.85±6.87
Aβ42 pathological (%) normal (%)	6 (25%) 18 (75%)	74 (81.3%) 17 (18.7%)	20 (28.2%) 51 (71.8%)	38 (37.6%) 61 (62.4%)	27 (44.3%) 34 (55.7%)
t-tau pathological (%) normal (%)	7 (29.2%) 17 (70.8%)	32 (35.6%) 58 (64.4%)	14 (20.0%) 56 (80.0%)	33 (32.7%) 68 (67.3%)	9 (14.5%) 53 (85.5%)
p-tau pathological (%) normal (%)	1 (4.2%) 23 (95.8%)	12 (14.8%) 69 (85.2%)	24 (33.8%) 47 (66.2%)	46 (45.5%) 55 (54.5%)	17 (40.5%) 25 (59.5%)

Data are expressed as Mean ± SD) for continuous variables, and as n (%) for categorical variables.
Abbreviations: MMSE, Mini-Mental State Examination; Aβ42, Amyloid-β_{42}; t-tau, total tau; p-tau, tau phosphorylated at threonine 181.
§ Karolinska Institute: n=24; Lund University: n=91; University Hospital of Strasbourg: n=71; VU University Medical Center: n=101; Paracelsus-Elena-Klinik: n=60
‡ Karolinska Institute: n=24; Lund University: n=16 ; University Hospital of Strasbourg: n=49; VU University Medical Center: n=0; Paracelsus-Elena-Klinik: n=49
† Karolinska Institute: n=21; Lund University: n=17 ; University Hospital of Strasbourg: n=71; VU University Medical Center: n=97; Paracelsus-Elena-Klinik: n=59
Data from University of Stavanger's Centre for Age-Related Medicine, University of Chieti, and University Medical Centre Ljubljana are not expressed.

Table 2 | CSF markers by diagnostic group

	DLB (n=376)	PDD (n=46)	PD (n=61)
Age, years§ Mean ± SD	71.02±8.39[b]	70.04±8.12	68.33±6.33
Gender Male Female	240 (65.8%) 125 (34.2%)	33 (71.7%) 13 (28.3%)	38 (62.3%) 23 (37.7%)
MMSE score† Mean ± SD	22.09±6.41[b]	22.50±3.92[b]	28.54±1.76
Aβ42 pathological (%) normal (%)	179 (48.8%)[b] 187 (51.1%)	19 (41.3%)[b] 27 (58.7%)	1 (1.6%) 60 (98.4%)
t-tau			

pathological (%) normal (%)	105 (28.8%)[b] 260 (71.2%)	7 (15.6%) 38 (84.4%)	4 (6.6%) 57 (93.4%)
p-tau pathological (%) normal (%)	 109 (32.3%)[a,b] 228 (67.7%)	 3 (7.5%) 37 (92.5%)	 2 (3.3%) 59 (96.7%)

Data are expressed as Mean ± SD for continuous variables, and as n (%) for categorical variables.
Differences between groups were assessed with ANOVA followed by Bonferroni post-hoc test for continuous variables, or with Pearson Chi square test for categorical variables.
Abbreviations: MMSE, Mini-Mental State Examination; Aβ42, Amyloid-β_{42}; t-tau, total tau; p-tau, tau phosphorylated at threonine 181.
§ DLB: n=365; PDD: n=27; PD: n=61
†DLB: n=266; PDD: n=26; PD: n=56
[a]p<0.05 compared to PDD
[b]p<0.001 compared to PD

Symposium 25: BIOMARKERS 2: PD and LBD

ADPD5-2312
THE PARKINSON'S PROGRESSION MARKER INITIATIVE – DEVELOPING TRANSLATIONAL TOOLS TO ACCELERATE PD THERAPIES

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The Parkinson Progression Marker Initiative (PPMI) is designed to identify clinical, imaging and biospecimen biomarkers of PD progression to establish objective outcomes for PD therapeutic studies. The goal of PPMI is to define biomarker subsets throughout the clinical course of PD from prodromal PD to manifest disease that may reflect clinical progression and clinical response to therapeutic intervention. PPMI utilizes standardized methods of acquisition and analysis of biomarker data. Rapid ongoing public access to PPMI data and biospecimen is available at www.ppmi-info.org.

PPMI has completed enrollment of early untreated PD (n=423), healthy (HV) (n=196) and SWEDD (n=64) subjects and is currently enrolling prodromal subjects (n=70) defined by RBD or olfactory loss and DAT deficit, and genetic PD subjects (n=600) defined as subjects with a LRRK2, GBA, and/or SNCA mutation who either are asymptomatic or have manifest PD, at 32 clinical sites worldwide. All subjects are and will be comprehensively followed longitudinally.

Key initial findings include -

· Assessments of non-motor symptoms including cognition, depression, anxiety and autonomic function show abnormalities in PD subjects compared to HS
· DAT striatal binding ratios in putamen are reduced by about 60% in PD compared to HS
· CSF alpha synuclein, p-Tau and T-tau are reduced by about 20% in PD compared to HS
· SWEDD subjects show DAT and CSF finding similar to HS
· About 50% of PD subjects were treated with dopaminergic meds within 12 months.

The PD, HV, and SWEDD subjects have all reached the one-year PPMI follow-up assessment. There have been more than 300,000 data downloads and 45 request for study samples from the PPMI website. The prodromal and all genetic cohorts are expected to complete enrollment in 2015.

Symposium 27: BIOMARKERS AND MRI 1

ADPD5-0245

CEREBROSPINAL FLUID BIOMARKERS AND CLINICAL PROGRESSION OF PD

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Objective
To investigate if certain cerebrospinal fluid (CSF) biomarkers at baseline can predict future progression of motor symptoms and cognitive decline in patients with Parkinson's disease (PD).
Methods
Study participants were recruited from hospitals in southern Sweden as part of the prospective, longitudinal Swedish BioFinder study. In the present study, we included 42 patients with PD and 69 controls with clinical assessment and lumbar puncture at baseline. Baseline CSF were analysed for alpha-synuclein (alpha-syn), $Abeta_{42}$, tau, phosphorylated tau (P-tau) and neurofilament light (NFL). Associations between CSF markers at baseline and change in clinical characteristics after two years were investigated using multivariate models adjusting for age, gender, disease duration, and levodopa-equivalent daily dose.
Results
Higher levels of alpha-syn within the PD group were associated with progression of motor symptoms and cognitive decline over two years, indicated by significant relationships between alpha-syn and change in Hoehn & Yahr (beta=0.394, p=0.043), Unified Parkinson's Disease Rating Scale-3 (UPDRS-3) (beta=0.449, p=0.013), Timed Up and Go (beta=0.406, p=0.023) and A Quick Test of cognitive speed (AQT, beta=0.423, p=0.018). Lower levels of $Abeta_{42}$ were associated with worsening on delayed memory recall (F=5.834, p=0.022). High levels of P-tau were associated with worsening in motor symptoms (UPDRS-3, beta=0.350, p=0.045; Hoehn & Yahr, beta=0.366, p=0.038).
Conclusion
Higher levels of alpha-syn at baseline seem to be associated with worsening of motor symptoms and cognitive speed over two years in PD. Increased alpha-syn might be a marker of more intense synaptic degeneration in PD. Amyloid pathology (low CSF $Abeta_{42}$) is associated with memory decline.

Symposium 27: BIOMARKERS AND MRI 1

ADPD5-0600

CORTICAL THICKNESS IN PRODROMAL DEMENTIA WITH LEWY BODIES AND PRODROMAL ALZHEIMER'S DISEASE

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Aims

To assess and compare cortical thickness (CTh) of patients with prodromal Dementia with Lewy bodies (pro-DLB), prodromal Alzheimer's disease (pro-AD), DLB dementia (DLB-d), AD dementia (AD-d) and normal ageing.

Methods

Study participants (N=173) underwent 3 Tesla T1 3D MRI and detailed clinical and cognitive assessments. We used FreeSurfer analysis package to measure CTh and investigated the patterns of cortical thinning across groups.

Results

Comparison of CTh between pro-DLB and pro-AD ($p<0.05$, FDR corrected) showed more right anterior insula thinning in pro-DLB, and more bilateral parietal lobe thinning in pro-AD. Comparison of prodromal patients to healthy elderly controls showed the involvement of the same regions. In DLB-d cortical thinning was found predominantly in the temporo-parietal junctions, insula and cingulate cortices. In AD-d, the most significant areas affected included the temporal and parietal lobes.

Conclusion

Right anterior insula involvement may be a key region at the prodromal stage of DLB and needs further investigation.

Symposium 27: BIOMARKERS AND MRI 1

ADPD5-1162

PRESUBICULUM ATROPHY IN MILD COGNITIVE IMPAIRMENT PATIENTS WITH AD PATHOLOGY

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Objectives. Hippocampal atrophy is a useful biomarker to identify MCI who progress to dementia from those who remain stable.
This study is aimed to compare subfields hippocampal volumetry between Abeta-negative (Abeta-) and Abeta-positive (Abeta+) aMCI patients in order to identify specific patterns of atrophy in Abeta+ aMCI and correlations with neuropsychological tests.
Methods. 145 MCI patients were enrolled in WP5 of PharmaCOG (E-ADNI) and underwent neuropsychological evaluation (memory, language and executive functions), CSF collection and high resolution 3T MRI. Hippocampus and hippocampal subfields volumes were computed using Freesurfer. Comparisons between Abeta+ and Abeta- were performed using an analysis of covariance model with age and gender as covariates. Correlations were computed using the Pearson's R test.
Results. Right hippocampal atrophy was reported in Abeta+ relative to Abeta- patients (-7.7%, p=.019) and left hippocampus presented a similar trend (-6.0%, p=.052). Greater volume reduction was detected bilaterally in the presubiculum (right: -10.7%, p=.003; left: -8.4, p=.018) of Abeta+ compared to Abeta- MCI and was significantly correlated with long term memory and naming, domains typically impaired in prodromal AD patients (Pearson R ranging from 0.20 to 0.43, all p<.001).
Conclusions. These preliminary data highlight the validity of presubiculum atrophy assessment to increase the accuracy and the sensitivity of total hippocampal atrophy in diagnosing MCI who progress to dementia. Longitudinal analysis is ongoing.
Pharmacog is funded by the EU-FP7 for the Innovative Medicine Initiative (grant n°115009).

Symposium 27: BIOMARKERS AND MRI 1

ADPD5-1258
DIFFUSION KURTOSIS IMAGING CHANGES IN PD WITH AND WITHOUT COGNITIVE IMPAIRMENT

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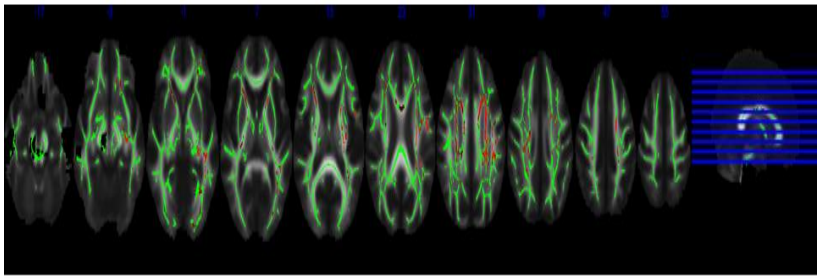
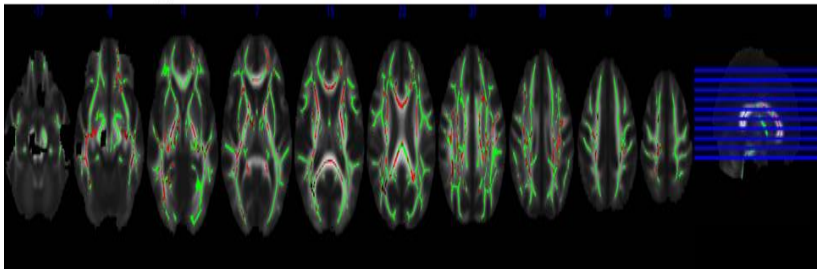
PURPOSE: Neuroanatomical correlates of cognitive changes in Parkinson's disease (PD) are of great interest to understand the underlying pathophysiology and monitor response to treatments. Along with well-characterized atrophy in cortical/subcortical regions, white matter (WM) microstructural changes have been demonstrated. Diffusional kurtosis imaging (DKI) is an extension of diffusion tensor imaging (DTI) that characterizes non-Gaussian water diffusion providing more comprehensive evaluation of WM microstructural integrity.
METHODS: We investigated DKI changes in 20 PD (65.5±5.0y), 17 PD with mild cognitive impairment (PD-MCI, 69.3±8.9y), and 26 normal controls (NC, 70.7±7.2y). The study cohort underwent detailed clinical and neuropsychological evaluations and a 3T DKI-MRI to provide parametric maps of mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD), fractional anisotropy (FA), mean kurtosis (MK), axial kurtosis (AK), and radial kurtosis (RK). Using track-based spatial statistics (TBSS), skeletonized voxel-wise analysis identified group differences on the WM skeleton. Also, 44 regions were drawn based on the JHU WM atlas to test for group differences using ANCOVA with age as a covariate.
RESULTS: Both TBSS and ROI-analysis showed widespread differences including decreased AK values in PD vs. NC and decreased FA and increased MD, AD and RD in PD-MCI vs. both NC and PD. ROI with significant differences included cingulum, hippocampus, fornix, and corpus callosum correlating with changes in Visuospatial, Executive, and Memory domains.
CONCLUSION: Disruption of WM connectivity may be a useful biomarker for exploring pathologic mechanisms in PD, predicting cognitive change in PD and as an outcome for PD clinical trials.

DKI Changes NC vs. PD

Mean kurtosis MK



Axial kurtosis $K_{||}$



Symposium 27: BIOMARKERS AND MRI 1

ADPD5-1363
PARKINSON'S PATIENTS SHOW DISTINCT PATTERNS OF ATROPHY IN THE FIRST AND SECOND DECADE OF DISEASE

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OBJECTIVE:PD is a neurodegenerative disorder that is characterized by alpha-synuclein deposition that follows a nonrandom cortical distribution. Our goal was to model in vivo cortical progression in PD based on the change in cortical thickness with disease duration. We compared cortical atrophy between PD patients with short duration (2-10 years) and long duration (>10 years) of disease. We hypothesized that the rates of cortical atrophy would be regionally distinct, and driven by disease duration.
METHODS: All 205 PD patients underwent clinical evaluation and anatomical MRI scan at 3T. Cortical thickness was evaluated with FreeSurfer in brain regions identified by the Destrieux Atlas. A bootstrapped linear regression was performed to estimate rate of decrease in cortical thickness with disease duration (mm/year). Thickness values and rate of thinning were compared between groups.
RESULTS: Thickness decreased bilaterally (p
CONCLUSIONS: In PD, distinct brain regions atrophy at different rates in a manner dependent on duration of disease.

Symposium 27: BIOMARKERS AND MRI 1

ADPD5-1830
LONGITUDINAL CORTICAL THINNING IN PARKINSON'S DISEASE
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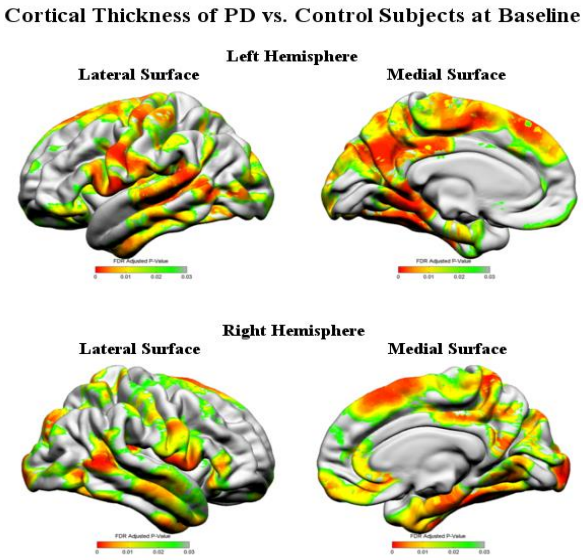
Objective: Parkinson's disease (PD) is characterized by dopaminergic cell death in the substantia nigra. Post-mortem studies reveal the presence of Lewy pathology in cortical areas, especially in later stages. This study aimed to gain insight into PD-related "macroscopic" cortical changes *in vivo* using longitudinal structural MRI.
Methods: Structural MRI images were obtained at baseline, 18, and 36 months in 78 non-demented PD subjects and 79 controls matched for age, gender, and number of visits. Cortical thickness was compared by region between PD and controls, and between PD subgroups based on disease duration [<1yr (PD-early, n=19), 1-5yr (PD-middle, n=34), >5yr (PD-later, n=25)]. Linear mixed modelling and F-tests were used to compare the amount and rate of cortical thinning between groups and subgroups. Region-level p-values were adjusted using a false discovery rate (FDR) of 0.05.
Results: Compared to controls, PD subjects at all visits had widespread cortical thinning that was particularly prominent in temporal, pre-central, precuneus, para-central, and post-central cortical regions. PD-early and PD-middle subjects had accelerated rates of overall cortical atrophy (p=0.049, p=0.004), whereas PD-later subjects showed no significant difference in atrophy rate (p=0.143).
Conclusions: Widespread cortical atrophy occurs in PD, even in the early stages. Accelerated cortical thinning occurs mainly in early- and middle-stage PD. The plateau of cortical atrophy in later stage is intriguing and further investigation is

warranted.


Cortical Thickness of PD vs. Control Subjects at Baseline
Left Hemisphere
Lateral Surface
Medial Surface
Right Hemisphere
Lateral Surface
Medial Surface

Symposium 28: POTENTIAL NOVEL BIOMARKERS

ADPD5-0342
ELEVATED LEVELS OF THE ALZHEIMER'S DISEASE-RISK FACTOR SORL1 TRIGGER ANXIETY

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Objectives
SORL1 is a well-studied risk factor in Alzheimer's disease and is known to inhibit amyloidogenic processing of amyloid precursor protein (APP) and mediate lysosomal sorting of the neurotrophic factor GDNF. Both APP and GDNF are involved in neurodegenerative disease but also play important roles in the developing brain. In the present study, we investigated if SORL1 might also be implicated in neurodevelopmental disorders.
Methods and Results
We find highly increased levels of SORL1 among individuals suffering from anxiety disorder compared to controls ($P=1.8x10^{-14}$), and that its encoding gene *SORL1* shows a gene-wise significant association with this disorder. Interestingly, SORL1 is moderately expressed in the embryonic brain cortex but rises steeply after birth until it plateaus during puberty, suggesting that SORL1 expression is strictly controlled in early postnatal development when neuronal circuitries controlling anxiety are established. Indeed, postnatal hippocampal neurons from Sorl1 deficient mice displayed increased survival and dendritic complexity when stimulated with GDNF compared to wildtype controls. Furthermore, lack of Sorl1 rescued the anxiety phenotype observed in mice with reduced GDNF expression while conditional overexpression of human SORL1 triggered anxiety-related behaviour.
Conclusions
Anxiety is an emotional state characterized by extreme nervousness and even panic in the absence of reason. Anxiety-related behavior is conserved through evolution from invertebrates to humans but its biology remains poorly understood. Importantly, our present findings establish SORL1 as a critical regulator of anxiety-related behaviour in both mice and humans.

Symposium 28: POTENTIAL NOVEL BIOMARKERS

ADPD5-0568
ANALYSIS OF AUTOANTIBODIES FROM CSF AND SERUM OF AD AND AMCI SUBJECTS: POTENTIAL ROLE IN PATHOLOGY PROGRESSION AND PREDICTION

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Objectives: Mounting evidence support the involvement of immune system in AD progression including the participation of inflammatory and autoimmune components in the neurodegenerative process. Immune system components have been detected in the brain, cerebrospinal fluid (CSF) and serum of AD subjects and their trend of variation correlates with disease progression. Nevertheless, AD patients present significantly lower levels of antibodies against Aß in serum and CSF in respect to healthy controls. Within this scenario, incomplete and often controversial results are reported about CNS immune/autoimmune responses during AD and a better comprehension is needed. Our research aims to shed light on the nature and potential role of autoantibodies in CSF and serum from AD and aMCI patients compared to healthy subjects.
Method: Our method employ immune-proteomics approach in order to discern natural occurring antibodies in human biofluids by the identification of brain antigen targeted by self-IgGs.
Results: Overall our data reveal that the alterations of autoantibodies profiling both in CSF and serum follow disease staging and progression. Furthermore, we demonstrate a fair overlap between CSF and serum auto-IgGs suggesting the existence of different immunogenic events between CNS and periphery. Interestingly, CSF autoantibodies recognized, among others, key players of energy metabolic pathways, including glycolysis and the TCA cycle, found oxidatively modified in previous studies on AD brain.
Conclusions: Our results show the manifestation of autoantibodies targeting brain proteins in AD and aMCI and suggest/propose the occurrence of a chain of events among brain oxidative damage, CSF autoantibodies production and reduced energy metabolism.

Proteins recognized by autoantibodies from AD and aMCI subjects:

CSF	Serum
Dihydropteridine reductase	**Dihydropteridine reductase**
Triosephosphate Isomerase	**Triosephosphate isomerase**
Protein-L-isoaspartate(D-aspartate) O-methyltransferase	Heat shock cognate 71 kDa protein
Glyceraldehyde-3-phosphate dehydrogenase	Dihydropyrimidinase-related protein 2
Carbonic Anydrase 2	Glial fibrillary acidic protein
Glutamate Dehydrogenase	Tubulin alpha (varie isoforme)
Aconitate hydratase	L-lactate dehydrogenase B chain
2',3'-cyclic-nucleotie 3'-phosphodiesterase	Phosphatidylethanolamine-binding protein 1
Tripartite motif-containing protein 73	
ATP synthase α sub	
Pyruvate kinase isozymes M1/M2	
Cullin-3	
Syntaxin-binding protein 1	

Symposium 28: POTENTIAL NOVEL BIOMARKERS

ADPD5-0582

THE SYNAPTIC MARKER CSF NEUROGRANIN IN ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE SUBJECTS

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Objective
Alzheimer's disease (AD) is characterized by extracellular deposits of aggregated amyloid-β (Aβ) peptides, intracellular tangles, and loss of synapses. Up to recently, no biomarkers reflecting the third hallmark, progressive dysfunction and degeneration of synapses, have been available. The objective of the present study was therefore to evaluate cerebrospinal fluid (CSF) levels of the post-synaptic protein neurogranin (Ng) in the ADNI-1 cohort.

Methods
CSF samples from the ADNI-1 cohort including AD dementia (N=95), mild cognitive impairment (MCI, N=186) and controls (N=105) were analyzed by an in-house developed immunoassay. The immunoassay employs the in-house generated monoclonal antibody Ng7 as capturing antibody.

Results
Higher CSF Ng levels were found in AD dementia and progressive MCI (pMCI, progression to AD dementia during follow-up), but also in stable MCI (sMCI, no progression to AD dementia during at least 2 year follow up) as compared to controls ($p<0.001$). These differences were confined to Aβ positive MCI subjects (CSF Aβ42<192 pg/mL), while Aβ negative cases had CSF Ng levels in the same range as Aβ negative controls. Further, high CSF Ng levels predicted future hippocampal atrophy in controls and sMCI ($P<0.05$), and weakly in pMCI ($P=0.08$), but not in AD dementia cases.

Conclusion
The CSF concentration of Ng is increased in MCI and AD dementia compared to controls. The increase in CSF Ng was dependent on Aβ positivity, and predicted progression of hippocampal atrophy in predementia cases. These findings suggest that CSF Ng is an early biomarker to monitor synaptic function or degeneration.

Symposium 28: POTENTIAL NOVEL BIOMARKERS

ADPD5-1040

CSF JKN3 KINASE IS A PROGNOSTIC BIOMARKER IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is clinically marked by memory troubles, followed by aphasia, apraxia and agnosia. Senile plaques made of β-amyloid (Aβ) peptides, neurofibrillary tangles formed by hyperphosphorylated tau proteins, and neuronal losses are the typical features of the disease. Cerebrospinal fluid (CSF) Aβ, T-tau and p-tau are now widely used as biomarkers. C-Jun N-terminal kinases (JNKs) are kinases activated by phosphorylation. JNK3 is mainly detected in the brain and JNKs are implicated in neuronal death. The levels of brain JNK isoforms and CSF JNK3 were assessed in AD and neurological disease control patients. In 10 AD and 10 control brains, JNKs levels were evaluated by western blots and $A\beta_{42}$ was measured by ELISA. In 8 AD and 9 control brains JNK immunostainings were also carried out. In addition to classic biomarkers, JNK3 levels were determined by immunoblot in 30 AD patients and 26 neurological disease controls. Patients were followed from two to three years using MMSE scores. Significant increased JNK3 and phosphorylated JNK levels were detected in AD brains and JNK3 levels correlated with $A\beta_{42}$ levels. Confocal microscopy revealed that JNK3 was associated with Aβ in senile plaques. CSF JNK3 levels were significantly enhanced in AD patients and were statistically correlated with the rate of cognitive decline. A moderate increase of CSF JNK3 level was associated with an exacerbated cognitive impairment In conclusion. CSF JNK3 levels are increased in AD are linked to the rate of cognitive decline. CSF JNK3 could be a new biomarker in AD.

Symposium 28: POTENTIAL NOVEL BIOMARKERS

ADPD5-1262
DETECTION OF ABETA DEGRADATION INTERMEDIATES IN THE CSF OF PATIENTS WITH MILD COGNITIVE IMPAIRMENT AND ALZHEIMER DISEASE
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An imbalance between production and clearance of the amyloid-beta peptide (Abeta) might be key to start the pathological cascade of sporadic Alzheimer disease (AD). While Abeta is a heterogeneous mixture of peptides with different solubilities, stabilities, and toxic properties, Abeta42 in particular is believed to be one of the chief culprits in AD pathogenesis. A variety of enzymes (including neprilysin 1/2, insulin-degrading enzyme, endothelin-converting enzyme, BACE1/2 and cathepsin B/D) degrade Abeta to different less-toxic intermediates. The intermediate, Abeta34, can either be generated by gamma-secretase, beta-secretase, BACE1 or its close homolog BACE2. In particular, BACE1/2-derived Abeta34 is generated from soluble Abeta40 and 42 substrates.
By studying Abeta catabolism in patients with mild cognitive impairment (MCI) and AD, we strive to understand the enzymatic amyloid clearance in early stages of AD. We have recently generated a monoclonal, neoepitope antibody that specifically recognizes Abeta34 with picomolar affinity. We found that Abeta34 was significantly elevated in cerebrospinal fluid (CSF) samples of patients with MCI compared to those from non-AD and AD individuals. These results suggest that during MCI, there is an elevated enzymatic Abeta-clearance activity, evident by an increase of the intermediate Abeta34, and that this process may become impaired with the progress of AD pathogenesis.
In summary, we hypothesize that at the MCI stage, Abeta40 and 42 can be degraded via stable and non-toxic intermediates (e.g. Abeta34), which can easily be detected in human CSF samples.

Symposium 28: POTENTIAL NOVEL BIOMARKERS

ADPD5-1851
HOW TO INTERROGATE LIPID DYSREGULATION IN LATE-ONSET DEMENTIAS?

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The emerging field of neurolipidomics seeks to understand how dynamic changes in membrane composition regulate brain cell function and how these changes can be used a biomarkers to predict disease outcome and track disease fate. Commonly conceptualized as undulating fields of identical molecules, neuronal membranes are, in fact, made up of hundreds of chemically and molecularly diverse lipid species. For the first time, significant technological advances in high performance liquid chromatography (LC), electrospray ionization (ESI), and matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) are enabling membrane composition to be profiled comprehensively at the molecular level. Coupled with subcellular fractionation and careful consideration of extraction protocols that enrich for different phospholipid families, species that vary by only one double bond, a single methylene group, or carbon chain linkage can now be quantified directly in synaptic preparations. These advances are allowing for discovery of novel biomarkers of disease transition, progression, and fate and new mechanistic insight into the determinative roles of lipid metabolism in neurodegenerative disease. Yet, as with imaging biomarkers, accuracy and reproducibility are fundamentally dependent on how lipid biomarkers are measured. Here, we ask whether changes in phosphocholine (PC) membrane predict transition from a pre-symptomatic to symptomatic state distinguish normal elderly from mild cognitive impairment (MCI) and AD and we describe challenges in harmonization of protocols, analyses, and lipid identification required for replication of biomarker results obtained in through neurolipidomic investigations.

Symposium 31: PET 1: TAU IMAGING

ADPD5-0741
TAU PET IMAGING ([18F]THK-5117) IN A MULTI-TRACER COHORT
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Objectives: The recent advances in PET imaging enabled the development of novel tau tracers that detect neurofibrillary tangles pathology. However, the *in vivo* regional progression of neurofibrillary tangles in relation to the other pathological hallmarks of Alzheimer's Disease (AD) pathology, neurodegeneration and amyloid plaques, remains largely unknown.
Methods: Patients with Mild Cognitive Impairment (MCI), dementia due to AD and Healthy Controls (HC) underwent a dynamic PET scan (0-90 minutes) with the novel tau tracer [18F]THK5117. The age-matched HC, MCI and AD patients were also scanned with [11C]PIB. Additional [18F]FDG scans were performed in the MCI and AD patients. All participating individuals underwent a high-resolution structural MRI (T1). Standard Uptake Value Ratio images were created for all tracers in respect to the grey matter of the cerebellum, and regional sampling was performed based on the Hammer's atlas.
Results: Comparisons were conducted between the [18F]THK5117 retention in cortical and subcortical regions across diagnostic groups. Moreover, the regional distribution of [18F]THK5117 retention was compared with those of [11C]PIB and [18F]FDG.
Conclusions: This study provides further insight into imaging *in vivo* the regional distribution of tau deposition with the novel PET tau tracer [18F]THK5117 at different stages of AD in relation to amyloid plaque pathology and neuronal degeneration.

Symposium 31: PET 1: TAU IMAGING

ADPD5-0994
[11C]PBB3-PET DETECTS TAU-PATHOLOGY IN PROGRESSIVE SUPRANUCLEAR PALSY AND CORTICOBASAL DEGENERATION

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Objectives:[^{11}C]PBB3 has been recently introduced as a tau imaging PET ligand that has high affinity and selectivity for tau deposits. The aim of the present study is to investigate distribution of tau-pathology in progressive supranuclar palsy (PSP) and corticobasal degeneration (CBD) by [^{11}C]PBB3 PET.
Methods: Twelve patients with PSP, 8 patients with corticobasal syndrome (CBS), and 25 age-matched healthy controls (HC) participated in this study. Seventeen patients with Alzheimer's disease (AD) also took part in the study as disease control. Sequential PET scans were performed for 70 min following intravenous injection of [^{11}C]PBB3. Standardized uptake value ratio (SUVR) at 30-50 min was calculated using the cerebellar cortex as reference region. Cerebral beta-amyloid depositions were estimated using [^{11}C]Pittsburgh compound B (PIB) PET.
Results: All patients and HCs were PIB-negative except one patient with CBS and 4 HCs. SPM analysis showed high [^{11}C]PBB3 binding in globus pallidus, putamen, thalamus, midbrain, pons, and peri-rolandic areas in PSP patients compared with 21 HCs. Seven PIB-negative CBS patients showed high [^{11}C]PBB3 binding in peri-rolandic areas, supplementary motor area, and midbrain compared with 21 HCs. One PIB-positive patient with CBS showed high [^{11}C]PBB3 binding in the whole cerebral cortex including limbic cortex like AD patients.
Conclusions: The distribution of [^{11}C]PBB3 binding in the patients was mostly in agreement with the known distribution of tau pathology in PSP and CBD, suggesting that [^{11}C]PBB3-PET may be useful for the diagnosis of these disorders and therapeutic monitoring of anti-tau therapy.

Symposium 31: PET 1: TAU IMAGING

ADPD5-1650
PET RADIOLIGANDS REVEAL THE BASES OF DEMENTIA IN PARKINSON DISEASE

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Background: Cognitive impairments and dementia are among the most feared consequences of PD; the lifetime risk of dementia approaches 80%.
Goals: To identify in life the molecular signature of pathologic changes that have been identified in postmortem brain examinations and that have been linked to PD dementia.
Methods: Cross sectional and longitudinal prospective studies in PD subjects with normal cognition(PD-nl), PD with mild cognitive impairment (PD-MCI), PD with dementia (PDD) and subjects with dementia Lewy body (DLB) contrasted with normal subjects (HCS). All underwent formal neurologic examination, extensive neuropsychological assessments and PET scans with the radioligands altropane (DAT), PiB (β-amyloid), and FDG (synapses).
Results: DAT concentrations in putamen and caudate were similar in PD and DLB and were significantly lower than in HCS; the caudate decrease was related to indices of cognitive impairment in DLB. PiB uptake was greatest in DLB and significantly linked to impaired cognition. There was substantial PiB retention in ~40% of PDs as well; it did not distinguish PD-nl from PD-MCI, but was a strong risk factor for developing dementia over time. In contrast to DAT and PiB, FDG did distinguish PD-MCI from PD-nl, based on multiple cortical areas of hypometabolism in PD-MCI. PET scans with the radioligand T807 that detects tau shows promise in assessing the contribution of neurofibrillary tangles to PDD and even PD-MCI.
Conclusion: PET scans with the appropriate radioligands are powerful ways to assess the biochemical and molecular bases of cognitive impairment and dementia in PD during life.

Symposium 31: PET 1: TAU IMAGING

ADPD5-1831

CLINICAL PET STUDY OF A NOVEL TAU PET TRACER [18F]THK-5351 IN PATIENTS WITH ALZHEIMER'S DISEASE

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Objectives: For early detection of disease-related tau pathology in the human brain, we developed a novel tau PET tracer [^{18}F]THK-5351, that displayed high binding affinity and selectivity for PHF-tau. In this study, we evaluated the clinical utility of [^{18}F]THK-5351 in humans with PET.
Methods: Twenty-two participants including 4 young normal, 7 aged normal subjects and 11 Alzheimer's disease (AD) patients underwent [^{18}F]THK-5351 and [^{11}C]PiB PET. Two AD patients additionally performed [^{18}F]THK-5117 PET for comparison with [^{18}F]THK-5351. Standardized uptake value ratios at 50-60 min and 40-70 min post injection were calculated for [^{18}F]THK-5351 and [^{11}C]PiB, respectively, using the cerebellar cortex as the reference region.
Results: AD patients showed significantly higher [^{18}F]THK-5351 retention in the temporal, parietal, posterior cingulate, ventrolateral prefrontal and hippocampus than young and aged normal subjects. In addition, higher [^{18}F]THK-5351 retention was observed in the hippocampus of aged normal subjects when compared to young normal subjects. [^{18}F]THK-5351 showed faster kinetics and lower white matter retention than [^{18}F]THK-5117. The pattern of [^{18}F]THK-5351 retention followed the known distribution of PHF-tau in AD brain and did not correlate with the cortical retention of [^{11}C]PiB.
Conclusion: High signal-to-background ratio of [^{18}F]THK-5351 allows clear visual inspection of PET images and sensitive detection of PHF-tau. Further studies are needed to study longitudinal changes in tau pathology.

Symposium 31: PET 1: TAU IMAGING

ADPD5-1880
TAU PET IMAGING IN ALZHEIMER´S DISEASE
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Combination of different biomarkers will not only improve the understanding of AD disease mechanisms but will also probably be necessary for reliable clinical diagnosis.We therefore explore the possibility to visualize the pathological deposition of tau in different stages of AD as well as in other forms of dementia using the PET Tau tracer 18F-(S)THK5117, in order to better understand the temporal evolution of AD pathophysiology and the possible role of tau imaging as a new and early biomarker for AD progression and for evaluation of new drug targets. Our multi-tracer PET system also include 11C-PIB for measurement of fibrillar amyloid plaques and 18F-FDG for cerebral glucose metabolism. Patients with clinical diagnosis of AD, mild cognitive impairment (MCI), different non-AD dementia are included as healthy young and old controls . All patients are undergoing neuropsychology testing and CSF biomarker assays. The introduction of a novel tau PET tracer will provide a crucial non-invasive tool for the investigation of neurofibrillary tangle pathology, in vivo, as they constitute an attractive target for early and accurate AD diagnosis. Moreover, by shedding light onto the underlying interrelationship of tau pathology with other aspects of the disease, such as cognitive decline and amyloid burden. In the future tau imaging will play an important role in the evaluation of new treatment strategies with target on tau pathology such tau immunization .

Symposium 31: PET 1: TAU IMAGING

ADPD5-2318
Tau PET in aging and dementia: initial experience with [18F]T807
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Detection of focal brain tau deposition during life could greatly facilitate accurate diagnosis of Alzheimer's disease (AD), staging and monitoring of disease progression, and development of disease modifying therapies. We have tested a recently developed PET method for selective imaging of tau pathology in patients with AD dementia, mild impairment with amyloidosis, non-AD dementia, and clinically normal controls. Preliminary results comparing tau PET measures to clinical, cognitive, brain structural, CSF, and amyloid-beta imaging variables will be presented. Our findings support the concept of tau PET as an imaging biomarker for AD pathologic change that may be useful for diagnosis and therapy development.

Symposium 32: BIOMARKERS 3

ADPD5-0316

LONGITUDINAL CEREBROSPINAL FLUID BIOMARKER MEASUREMENTS AND PRECLINICAL SPORADIC ALZHEIMER'S DISEASE: A PROSPECTIVE 9-YEARS COHORT STUDY

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Importance: Ascertainment of the pattern and temporal change of biomarkers in preclinical sporadic Alzheimer's disease (AD) will increase knowledge about early pathogenesis and facilitate therapeutic trials.
Methods: In this prospective and longitudinal study 54 cognitively healthy individuals (60-87 years) underwent cognitive assessments and CSF sampling at baseline. Follow-ups were performed after 5 and 9 years, including repeated CSF measurements. Forty-four individuals were followed for at least 9 years and this subgroup was included in the main analyses.
Results: Twelve subjects (27 %) had low CSF Aβ42 levels (<192 ng/L) at baseline and out of these five converted to AD and one to dementia with Lewy Bodies (DLB). In the remaining cases with low baseline Aβ42 levels, four cases were cognitively normal over nine years and two cases developed MCI. CSF Aβ42 predicted development of AD/DLB with a sensitivity of 100 %, specificity 84 %, positive predictive value 50 %, and negative predictive value 100 %.
When analyzing repeated CSF samples no additional decrease in Ab_{1-42} levels was observed in the participants with low baseline levels. In the subjects with normal baseline Aβ42 levels, 19% developed pathologic levels during follow-up, of which none developed AD/DLB/MCI over nine years.
Conclusions: Low CSF Aβ42 levels predict sporadic AD and DLB at least nine years before dementia onset with high sensitivity and Aβ42 levels have already reached a plateau at this time. However, quite many individuals can harbor brain amyloid accumulation over a decade without any signs of cognitive dysfunction.

Symposium 32: BIOMARKERS 3

ADPD5-1055
THE UNBEARABLE LIGHTNESS OF BEING OLIGOMERS
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Immunotherapies targeting a-beta monomers or insoluble aggregates have succeeded in lowering deposits of beta amyloid in brain but not in producing clinically significant results. Shifting from a-beta aggregates , a soluble a-beta hypothesis considers oligomers rather than plaques central toxic species being responsible for synaptic dysfunction . Many candidates of soluble oligomeric species have been proposed to have high affinity to synapses of subsets of hippocampal and cortical neurons causing neurotoxicity via cell surface receptors The exact structure and localization of these toxicologically relevant oligomers and receptors have not been characterized in human brain and a relationship between specific oligomers and the initiation of the disease has not been established. Up to present oligomers secreted from in vitro preparations or extracted from post-mortem brain tissue have been mostly studied , hence, it is not clear if these oligomers are present in the original brain tissue or are products of artifactual oligomerization. We report about the localization of oligomers in neuropathologically confirmed Alzheimer disease (AD), and the relation between oligomeric densities, neuritic plaques , neurofibrllary tangles and synaptic integrity . This study of human autopsy material combines neuropathological assessment and localization of a-beta oligomers, AD lesions and Western blot analyses in cognitively intact and AD diagnosed very old individuals. Our results reveal that mid-range molecular weight a-beta oligomers are present in both normal aging and AD brain areas and are in strict topographic association with fibrillar amyloid deposits. In the cerebellum , despite the absence of fibrillary deposits we demonstrate the presence of oligomers in both normal aging and AD.

Symposium 32: BIOMARKERS 3

ADPD5-1821
LONGITUDINAL IMAGING AND PHENOCONVERSION IN THE PARS PRODROMAL COHORT

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The PARS cohort involves 203 hyposmic and 100 normosmic subjects. Data from the baseline imaging indicates 11% of subjects have DAT deficit (
Methods: Subjects completed baseline, 2-yr and 4-yr clinical and ^{123}I-ß-CIT/SPECT evaluations. Clinical evaluations (UPDRS, cognitive testing, diagnosis assignment) were performed blind to imaging and olfactory data. Change in striatal binding ratio (SBR) between baseline, 2-yr and 4-yr imaging was compared based on olfactory and DAT imaging status. Phenoconversion to PD at baseline, 2-yr and 4-yr imaging was examined.
Results: 203 hyposmics and 100 normosmics completed baseline assessments, 262 completed 2-yr, 173 completed 4-yr visits. Hyposmics with DAT deficit at any scan, 35% (10/31) phenoconverted by year 2 and 51% (21/41) phenoconverted by year 4. SBR mean percent change in hyposmic phenoconverters was -13.4 (±9.4) at yr 2 and -23.2 (±14.7) at yr 4 compared to -2.0 (±16.8) at yr 2 and 4.8 (±16.6) at yr 4 for hyposmic non-converters.
Conclusion: Longitudinal follow-up of PARS cohort demonstrates 51% phenoconversion among hyposmics with DAT deficit within the 4-year follow-up period. Longitudinal DAT imaging indicates that phenoconverters have higher loss in DAT compared to hyposmic non-converters. Additional follow-up will allow for more precise estimation of the phenoconversion rate and identification of markers that may predict the rate of progression in the pre-diagnostic phase of PD.

Symposium 32: BIOMARKERS 3

ADPD5-1856
IDENTIFICATION OF PRE-SYMPTOMATIC ALZHEIMER'S DISEASE IN OLDER ADULTS AT HIGH-RISK: HIGH RESOLUTION RETINAL IMAGING IN PRECLINICAL DISEASE

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Alzheimers disease (AD) is associated with volume reductions in retinal nerve cell layers, as well as Aβ deposits within the retina and lens. Cross-sectional studies of changes for individual cell layers (MCI/AD vs. cognitively normal [CN] controls) suggest a loss of tissue with increasing disease severity. We sought to characterize structural differences in retinal layers and to determine whether these are associated with visual field impairments in CN older adults at risk for AD.
CN older adults (n=63) underwent ^{18}F-florbetapir PET neuroimaging to determine cortical Aβ burden. All participants also underwent spectral-domain optical coherence tomography (SD-OCT) and visual field testing. Compared to CN older adults with low Aβ (n=53), CN older adults with high Aβ (n=10) showed increased thickness of a moderate magnitude in the parafoveal peripheral measures of the retinal nerve fiber layer (d=0.61, p=.07), inner nuclear layer (d=0.68, p=.18) and inner plexiform layer (d=0.73, p=.04). Increased Aβ burden was significantly associated with worse visual perimetry performance using the SAP stimuli (d=0.55, p=.04).
These results suggest that, in the likely preclinical stage of AD (i.e., CN older adults with high cortical Aβ), several of the retinal nerve cell layers show measurable increases in thickness possibly reflecting an inflammatory process. We believe that this may precede eventual decreases with disease progression. Numerous studies have suggested that Aβ-induced inflammation may play a critical and early role in AD pathogenesis, and one study found similar evidence of inflammation in the rat retina following intravitreal administration of Aβ (Howlett et al., 2011). Impairments in visual fields were also identified with increasing Aβ levels. Taken together, the results provide preliminary but important insights into the early structural and functional retinal changes in preclinical AD.

Symposium 32: BIOMARKERS 3

ADPD5-1869

THE TRAJECTORY OF CSF AMYLOID BETA LEVELS IN PRECLINICAL LOAD

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Objective

Reduced CSF Aβ1-42 concentrations are found in the preclinical phase of Alzheimer's disease (AD), but infrequently elevations are reported. Our objective was to test the hypothesis that in the dementia trajectory of late onset AD (LOAD), Aβ1-42 elevations precede reductions.

Methods

Community residing normal subjects were recruited by random population sampling. We conducted a cross sectional study of subjects aged 21-88y (n=270) and a longitudinal study with 2y and 6y follow-ups (n=92 and 39 respectively). All subjects received standardized clinical, LP and imaging exams. Outcome measures included cognition, ventricular enlargement, PIB-PET, and CSF P-tau levels.

Results

Across the adult lifespan, increasing numbers of subjects show both elevations and reductions in CSF Aβ1-42 ($p<.05$). Quadratic relationships between the Aβ1-42 and both P-tau and cognition were observed indicating pathology with both high and low Aβ1-42 levels. Elevated PIB uptake and increased ventricle size also showed a quadratic association with Aβ1-42 levels, providing P-tau levels were elevated. Elevated Aβ1-42 predicted longitudinal reductions in Aβ1-42 levels suggesting direction. Longitudinally, cognitive decline was best predicted by P-tau/Aβ1-42 ratio, which in turn was predominantly driven by reductions in Aβ1-42.

Conclusions

Both elevated and reduced Aβ1-42 levels are found after age 60, and both are related to cognitive decline and elevated P-tau levels. In the presence of elevated P-tau, both elevated and reduced Aβ1-42 levels are further associated with brain atrophy and amyloid load. While still not possible to conclude that in LOAD CSF Aβ1-42 elevations precede the reductions, this trajectory remains a possibility.

Symposium 32: BIOMARKERS 3

ADPD5-1925
WHAT DOES CSF TELLS US ABOUT ALZHEIMER'S AND RELATED DISORDERS?

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Past decade witnessed significant effort to translate basic findings about Alzheimer's and related disorders into clinical practice with the goal of improving diagnostics and exploring therapeutics. As a result, today, several hallmark lesions and proteins intimately linked to the pathogenesis of Alzheimer's and related disorders, can be visualized in the brain and measured in body fluids.

As part of these efforts, amyloid, tau and other proteins can be reliably measured in the cerebrospinal fluid. Since specific changes in the cerebrospinal fluid levels of these proteins have been shown to correlate with Alzheimer's and related disease phenotypes, specific combinations of changes in these proteins, in particular, have been widely used as diagnostic biomarkers of Alzheimer's and related disorders. These biomarkers are unique, compared to many others, considering cerebrospinal fluid assessment offers opportunity to critically test for a wide spectrum of pathologies that often need to be excluded in the process of differential diagnostics. To date, despite inclusion of cerebrospinal fluid biomarkers into diagnostic criteria of Alzheimer's and related disorders, the rationale for using cerebrospinal fluid biomarkers per se as well as compared to other biomarkers remains unsettled. Importantly, despite the wealth of data reporting protein changes in the cerebrospinal fluid in Alzheimer's and related disorders few studies and even fewer interpretations elucidate on the value of cerebrospinal fluid findings in understanding the pathogenesis of Alzheimer's and related disorders.

Symposium 35: PET 2: AMYLOID AND RECEPTORS

ADPD5-0460
ALZHEIMER'S DISEASE RISK GENES MODULATE THE RELATIONSHIP BETWEEN PLASMA APOE AND PIB BINDING IN THE BRAIN

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[3]N/A, Oakland University, Rochester, USA
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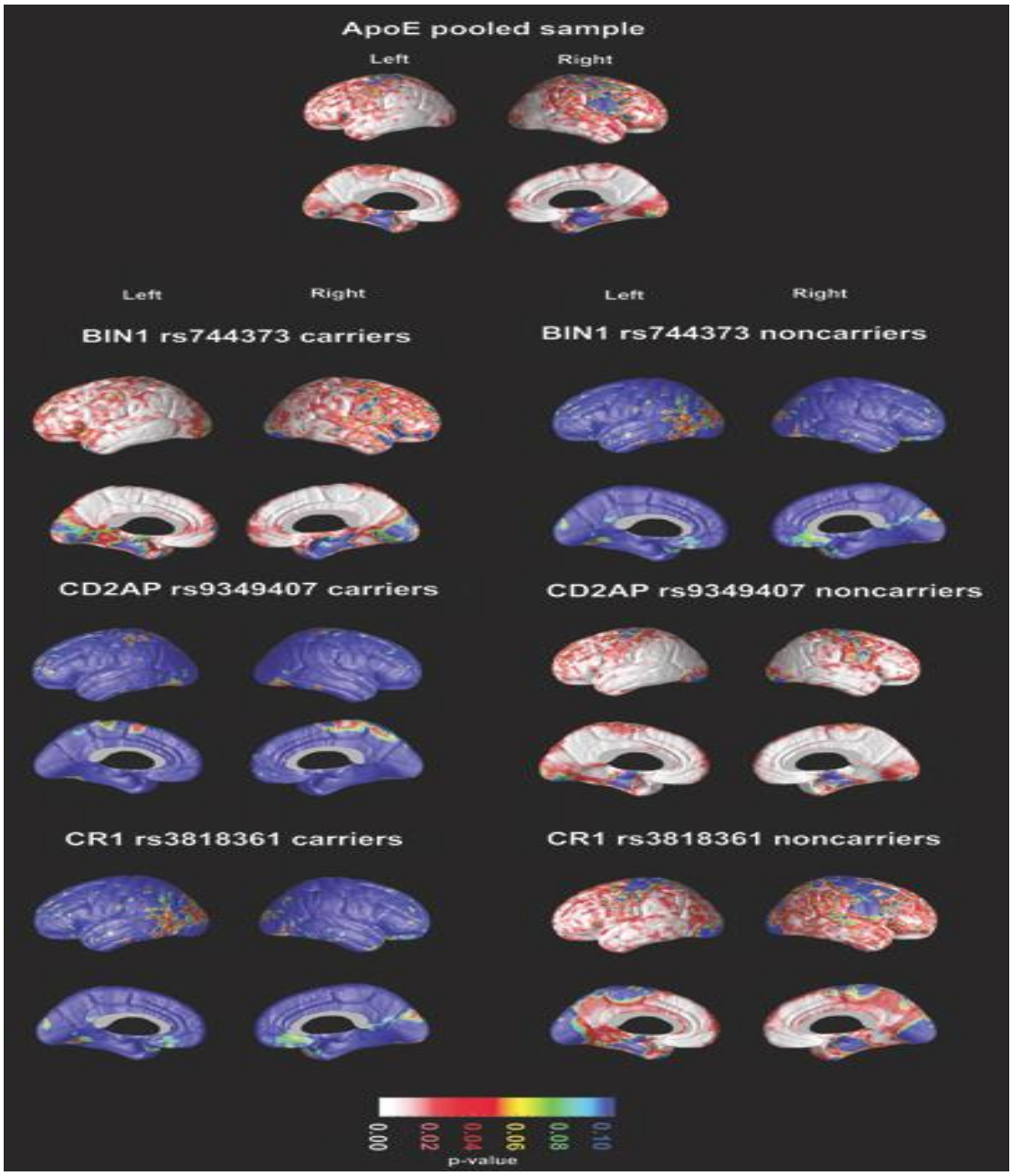
Objective: Genome-wide association studies have identified many risk genes for Alzheimer's disease (AD). The mechanism through which many of these genes exert their effect remains unknown. Here we investigated the association between plasma apoE protein and brain amyloidosis and the effect of the top AD risk genes on that association.
Methods: Our dataset consisted of 18 AD, 52 mild cognitive impairment and 3 cognitively normal Alzheimer's Disease Neuroimaging Initiative 1 (ADNI1) subjects with available [11C]-PIB and peripheral blood protein data. We applied the cortical pattern matching methodology to study the associations between plasma ApoE protein and cortical PIB binding in 3D. Next, we studied the effect of carrier/noncarrier status for the remaining top 10 AD risk genes variants available in ADNI1 GWAS on this association. Correction for multiple comparisons was done with permutation testing using the stringent threshold of p
Results: Plasma apoE protein showed a significant negative association with PIB SUVR throughout except for the sensorimotor and entorhinal cortex. Effect on this association was seen for *BIN1* rs744373 where the association was only observed in minor allele carriers, and *CD2AP* rs9349407 and *CR1* rs3818361 where the association was only preserved in minor alleles noncarriers. We did not find evidence for modulation of the ApoE protein-PIB association by the available *CLU, PICALM, ABCA7, BIN1* and *MS4A6A* genotypes.
Conclusion: Our data show that *BIN1* rs744373, *CD2AP* rs9349407, and *CR1* rs3818361 modulate the association between plasma ApoE protein and brain amyloidosis. These findings imply a potential epigenetic or downstream interaction.


ApoE pooled sample
Left
Right
Left
Right
BIN1 rs744373 carriers
Left
Right
BIN1 rs744373 noncarriers
CD2AP rs9349407 carriers
CD2AP rs9349407 noncarriers
CR1 rs3818361 carriers
CR1 rs3818361 noncarriers
0.00
0.02
0.04
0.06
0.08
0.10
p-value

Symposium 35: PET 2: AMYLOID AND RECEPTORS

ADPD5-0681

DIAGNOSTIC COMPARISON OF REGIONAL AMYLOID PET MEASURES AND DIFFERENT CSF ASSAYS FOR PREDICTING ALZHEIMER'S DISEASE

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Objectives

Biomarkers of cerebral amyloid β (Aβ) are important for diagnosing Alzheimer's disease (AD) early. We performed a head-to-head comparison of two cerebrospinal fluid (CSF) assays and PET measures of eight regional and global amyloid depositions.

Methods

34 consecutive patients with mild cognitive symptoms who developed AD within 4 years were compared with 122 controls. The amyloid PET tracer [18F]Flutememetamol was used. CSF Aβ42 and P-tau were analysed with ELISA INNOTEST and Aβ42, Aβ42/Aβ40 and Tau with ADx assays.

Results

The best CSF measures were Aβ42/Tau and Aβ42/P-tau (AUC 0.93–0.94, INNOTEST and ADx), which performed equally to the best PET measures (AUC of anterior cingulate, posterior cingulate/precuneus, global/composite score, prefrontal and parietal: 0.91–0.92; $P>0.35$). No PET measure was significantly better than Aβ42 INNOTEST (AUC 0.90) or Aβ42/Aβ40 ADx (AUC 0.86). Using a priori cutoffs, the best sensitivities and specificities for CSF were produced by Aβ42/Tau INNOTEST (97%, 83%) and for PET the prefrontal region (88%, 86%). The combination of composite PET and Aβ42/P-tau or Aβ42/Tau (AUC 0.95–0.96) was not significantly better than either variable alone, but composite PET and Aβ42 INNOTEST (AUC 0.95) were slightly better than Aβ42 INNOTEST alone (AUC 0.90; $P<0.01$).

Conclusions

There was no significant difference between the best CSF and amyloid PET measures and no significant improvement when combining them. Regional PET measures were not better than a composite PET score. The choice between CSF analysis and amyloid PET can be based on availability, costs and doctor/patient preferences since both have equally high diagnostic accuracy.

Symposium 35: PET 2: AMYLOID AND RECEPTORS

ADPD5-0734
PET IMAGING OF SYNAPTIC NORADRENALINE WITH [11C]ORM-13070
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Objectives: The PET tracer [^{11}C]ORM-13070 was recently validated for receptor occupancy analysis of brain alpha2C-adrenoceptors, and PET experiments in monkeys and humans indicated that tracer uptake into the caudate and putamen was reduced by interventions that increased synaptic noradrenaline concentrations in the brain. This study aimed to confirm the sensitivity of [^{11}C]ORM-13070 binding to increased levels of synaptic noradrenaline.
Methods: PET imaging of the brain was performed with a 3D High Resolution Research Tomograph. Eight subjects underwent a control [^{11}C]ORM-13070 PET scan and two PET scans after two different noradrenaline challenges, i.e. a sub-anaesthetic infusion of ketamine and oral intake of atomoxetine combined with cold stimulation. Tracer uptake in the caudate nucleus and putamen was described with AUC values in scan time windows of 10-20 min and 5-30 min post injection, and quantified with the ratio method. Voxel-based analysis was performed with average B/F images.
Results: Both challenges caused small but statistically significant (10-20%, $p<0.05$) reductions in tracer uptake in both target regions. Voxel-based analysis revealed significant clusters in the dorsal putamen with both challenges. Ketamine was associated with significant elevations in circulating noradrenaline and adrenaline levels, while the atomoxetine + cold treatment was not.
Conclusions: Strong experimental support was gained for the feasibility of [^{11}C]ORM-13070 PET imaging of brain noradrenergic neurotransmission.

Symposium 35: PET 2: AMYLOID AND RECEPTORS

ADPD5-0857
DIFFERENTIAL DIAGNOSTIC ACCURACY OF [11C]PIB PET AND CSF BIOMARKERS IN PRODROMAL ALZHEIMER'S DISEASE

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Objectives: While showing good convergence across the spectrum of Alzheimer's disease (AD), some studies highlight imperfect agreement between positron emission tomography (PET) and cerebrospinal fluid (CSF) measures of beta-amyloid (AB_{42}), and differential performance with respect to the identification of prodromal AD. Using a sample of patients with mild cognitive impairment (MCI) and AD, we sought to determine 1) concordance between CSF-AB_{42} (alone, and with tau) and Pittsburgh Compound-B (PIB) PET and 2) classification accuracy of PIB PET and CSF-AB_{42} (alone, and with tau) with respect to stable (sMCI) versus MCI who progressed to AD dementia (pMCI). **Methods:** Diagnosis of MCI (n=34) and AD (n=35) were issued using the 2004 Petersen and the 1984 McKhann criteria, respectively. PIB PET standard uptake value ratio maps were calculated using the cerebellar cortex as a reference region and subsequently registered nonlinearly to a population-based PIB template. CSF levels of $A\beta_{42}$ and phosphorylated tau were determined using sandwich enzyme-linked immunosorbent assay. **Results:** Concordance within groups was highest for sMCI (76%), followed by AD (60%) and pMCI (33%). Using $A\beta_{42}$/p-tau, concordance was highest for pMCI (100%), followed by AD (96%) and sMCI (62%). Logistic regression showed that classification accuracy of PIB-PET was superior to AB_{42} (79% vs. 63%) or AB_{42}/p-tau (65%). **Conclusions:** Relative to CSF measures of AB_{42} or AB_{42}/p-tau, PIB PET may prove a better predictor of progression to AD in patients with MCI. Discordance between PET and CSF markers for AB_{42} suggests they cannot be used interchangeably, as is currently the case.

Symposium 35: PET 2: AMYLOID AND RECEPTORS

ADPD5-1684
BETWEEN SNAP AND A HARD ABETA ROCK: CHARACTERIZING THE FATE OF PRECLINICAL ALZHEIMER'S DISEASE

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Objectives. The objective of the study was to characterize a two-imaging marker construct in the preclinical stages of Alzheimer disease.
Methods. 493 cognitively unimpaired individuals (HC, 73.0±6.2years; 56%female) were included in the study. Aβ status (**A**) was determined with either PiB, flutemetamol or florbetapir, while neurodegeneration (**N**) was established using hippocampal volume. Following Jack et al, individuals were categorized as either A-N-, A+N-, A+N+, or suspected non–Alzheimer disease pathophysiology (SNAP, A-N+). Clinical progression, cognitive domain-specific trajectories and global composite scores for 461 HC were assessed.
Results: 65% of HC were classified as A-N-, 18% as A+N-, 5% as A+N+, and 12% as A-N+. Participants in the A-N- group were significantly younger, and while males were more prevalent among A+N+ and A-N+, females were more prevalent among A+N- and A-N-. ApoE4 carriage was more frequent in A+N-(48%) and A+N+(58%) than in A-N-(19%) and A-N+(23%). While no significant differences were observed in baseline scores significantly faster cognitive decline was observed in A+N+(-0.30SD/yr) and A+N-(-0.08SD/yr) when compared to A-N-(+0.02SD/yr). The A-N- and A-N+ groups did not show significant decline over time, although A-N+ was associated with a lower baseline performance. While A+N+ and A+N- were more impaired in memory domains, A-N+ were more impaired in language and executive domains. Within 4.5 years, 22.2% of A+N+ progressed to amnestic MCI/AD, compared to only 7.3% of A-N+(2MCI, 1AD, 1VaD)
Conclusions: Increasing marker abnormality was reflected in faster cognitive decline. Distinct cognitive domains were affected in those with AD and non-AD pathology, likely suggesting different underlying pathophysiological mechanisms.

Symposium 35: PET 2: AMYLOID AND RECEPTORS

ADPD5-1733

CEREBRAL GLUCOSE ANALYSIS SERVES AS AN EXCELLENT BIOMARKER FOR NEURODEGENERATIVE BRAIN DISEASES

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Objectives

Reliable and objective diagnosis of parkinsonian disorders on the basis of cerebral glucose imaging (FDG-PET) by applying a multivariate and data-driven technique.

Methods

We studied FDG PET brain data of patients who underwent FDG PET scanning in the context of their clinical workup. Data were analyzed using scaled subprofile model and principal component analysis (SSM PCA). Disease-specific patterns were identified in patients with PD, MSA, PSP and AD. A disease-related pattern can be prospectively applied to the FDG PET data of new patients. For each patient, a score on each pattern can be calculated denoting the extent of its expression in that patient. Pattern expression at baseline was compared with the final clinical diagnosis after follow-up visits.

Results

A multicenter and international website data entry database was developed recently (www.glimpsproject.com) and contains at present more than 200 FDG PET scans. Only on the basis of glucose consumption the multivariate methods (blind for the classes) were able to differentiate in over 90% of selected cases the conditions PD, MSA and PSP. Also new patterns were developed in the domain of dementia.

Conclusion

Glucose metabolism with the application of multivariate analysis techniques can be used as an objective screening tool to achieve high accuracy in diagnosing parkinsonian and dementing disorders in routine clinical practice. In addition, it may be used to identify ideal candidates in clinical trials.

Symposium 37: MECHANISMS AND THERAPEUTIC STATEGIES IN ALS/FTD SPECTRUM DISEASES

ADPD5-1299

THERAPEUTIC STRATEGIES TO COMBAT MOLECULAR MECHANISMS OF TOXICITY IN FTD/ALS

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Objectives: Given hexanucleotide (G_4C_2) repeat expansion in *C9orf72* is now known to be the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), we seek to identify therapeutic strategies to combat *C9orf72*-linked disorders (c9FTD/ALS). We and others have demonstrated that the RNA structure of *C9orf72* repeat expansion may cause neurodegeneration via their accumulation into discrete structures in the nucleus, termed RNA foci, and by serving as a template for the synthesis of aggregation-prone dipeptide repeat (DPR) “c9RAN proteins” by repeat-associated non-ATG (RAN) translation. We have also shown that *C9orf72* mRNA expression is reduced in c9FTD/ALS as a result of epigenetic changes.

Methods: To further elucidate the molecular mechanisms underlying c9FTD/ALS and sporadic ALS (sALS), we used a variety of approaches, including the generation of novel antibodies, *in vitro* and *in vivo* assays as well as analyses of human tissue.

Results: We discover a biomarker and lead small molecules to target r(G_4C_2)-associated defects in c9FTD/ALS. In addition, we show poly(GA) DPRs mediate toxicity in cell culture and animal model systems through endoplasmic-reticulum-associated mechanisms. We also identify an abundance of transcriptome defects and epigenetic modifications in c9ALS and sALS cases.

Conclusions: We demonstrate the viability of therapeutic strategies targeting the RNA structures necessary for RAN translation and foci formation for the treatment of c9FTD/ALS as well as the use of DPRs in patient cerebrospinal fluid as a potential biomarker. Taken together, our findings may be applicable for other neurodegenerative diseases.

Symposium 37: MECHANISMS AND THERAPEUTIC STATEGIES IN ALS/FTD SPECTRUM DISEASES

ADPD5-1553

INCREASED SURVIVAL AND IMPROVEMENT OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) PATHOLOGY BY INHIBITION THE CXCR4/CXCL12 SIGNALING

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Chemokine receptors, including the G-protein-coupled receptor CXCR4, are expressed widely in neurons and glial cell. The ligand of CXCR4, the chemokine stromal-derived factor 1 (SDF-1), also known as CXCL12, evokes glutamate release and thereby modulates neuronal function or apoptosis. Inhibition of the CXCR4/CXCL12 signaling might result in preventing the toxic cascade of glutamate release from astrocytes and the eventual neuronal apoptosis in ALS
.AMD3100 a bicyclam molecule that specifically and reversibly blocks SDF-1 binding to CXCR4 was used in this study as a proof of concept of the new approach
Experimental
The CXCR4/CXCL12 signaling pathway was inhibited by subcutaneous administration of AMD3100. ALS Tg G93A mice were treated with AMD3100 for once and twice a week, and compared with mice treated with PBS. The effect of AMD3100 on ALS pathology was examined regarding survival, weight loss , motor function and pathological markers
Results
AMD3100 treatment extended the survival about 27 days, compared with mice treated with PBS. In addition, the treatment resulted in less weight loss as disease retrogrades and performance in Rotarod test, which resembles motor function, was better **Bi**ochemical analysis showed reduction in inflammatory markers levels and proinflammatory cytokines, increase in integrity of blood-spinal cord-barrier (BSCB) as well as of EEAT2 levels
Conclusion
The multi-faceted action of AMD3100which Inhibits the CXCR4/CXCL12 signaling provide a novel and pleiotropic option for ALS therapy with an increased margin of safety compared to other drugs which improve only one of these aspects of the diseases.

Symposium 37: MECHANISMS AND THERAPEUTIC STATEGIES IN ALS/FTD SPECTRUM DISEASES

ADPD5-1591
ADVANCES IN THE MOLECULAR NEUROPATHOLOGY OF FRONTOTEMPORAL DEMENTIA AND AMYOTROPHIC LATERAL SCLEROSIS

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The most common genetic defect in frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) is the abnormal expansion of a GGGGCC hexanucleotide repeat in a non-coding region of the chromosome 9 open reading frame 72 gene (*C9ORF72*). The neuropathology of *C9ORF72* mutation cases is consistently characterized by TDP-43 pathology in neurons and glia. Cases with clinical FTD show FTLD-TDP which most often fits the type B pattern and cases with clinical ALS show TDP-43 pathology indistinguishable from classical sporadic ALS. However, compared to other TDP-43 proteinopathies, *C9ORF72* mutation cases are associated with additional pathologies which might play pathogenic roles. Aggregates of RNA, composed of the massively expanded repeat, can be frequently demonstrated in neuronal nuclei using fluorescent in situ hybridization. These RNA foci are thought to bind and sequester specific RNA binding proteins, leading to the abnormal splicing of other genes. Another absolutely sensitive and specific pathological change is the presence of TDP-43 negative inclusions in the cerebellum, hippocampus and other neuroanatomical sites being composed of dipeptide repeat (DPR) proteins that result from the unconventional translation of the expanded repeats in both sense and antisense direction (poly-GA, -GP, -GR and poly-PA, -PG, -PR respectively). Clinicopathological correlative studies have shown that the anatomical distribution of TDP-43 pathology correlates closely with the pattern of neurodegeneration and clinical phenotype. In contrast, the distribution of DPR pathology (based on anti-GA and GP) is highly consistent among cases, with no clinical correlation, suggesting that DPR inclusions are a valuable pathological marker to predict the *C9ORF72* mutation but are of uncertain pathogenic significance.

Symposium 37: MECHANISMS AND THERAPEUTIC STATEGIES IN ALS/FTD SPECTRUM DISEASES

ADPD5-1862
NATURAL HISTORIES IN HEREDITARY DEMENTIAS: ANATOMICAL VULNERABILITIES AND PROTEIN SPREAD

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Neuropathologic analysis of multiple individuals from a single kindred may provide a powerful way to investigate the natural history of hereditary diseases, characterized by cognitive decline and dementia. The study can be even more fruitful if pre-mortem imaging studies are combined with post-mortem neuropathology.
Correlating imaging with neuropathology data provides essential information for defining which areas of the brain are affected in the course of degeneration. If neuropathologic analyses can be carried out in the early stages of disease, even before onset of symptoms, the combination of imaging and neuropathology may give insights about pathogenesis.
We have studied hereditary dementias associated with prionopathies and tauopathies. Studied individuals, affected by Gerstmann Sträussler Scheinker disease (GSS) or by hereditary tauopathies, belong to multiple generations of kindreds followed for decades. We illustrate the salient points of disease spread and abnormal protein deposition in patients at different stages of illness. In GSS, the first foci of prion protein amyloid are found in the cerebellum; in hereditary tauopathies, the frontal cortex shows the earliest presence of tau in nerve cells.
Identifying earliest vulnerability in anatomical areas facilitates the formulation of hypotheses related to pathways of protein spread. Spreading of pathologic proteins may be inferred on the basis of anatomical protein distribution and synaptic connectivity in the involved brain regions. The analysis of protein spread becomes complex when more than one protein aggregates in brain. One protein may be central in determining aggregation of the second one as in the case of GSS.

Symposium 37: MECHANISMS AND THERAPEUTIC STATEGIES IN ALS/FTD SPECTRUM DISEASES

ADPD5-1879
MECHANISM AND THERAPY IN ALS/FTD AND BEYOND

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The genes whose expression cause human neurodegenerative disease are widely expressed, producing damage not only within the most vulnerable neurons but also within their partner neurons and glia. This is certainly true for Amyotrophic Lateral Sclerosis (ALS), some instances of which are caused by mutation in the ubiquitously expressed superoxide dismutase (SOD1). Despite absence of consensus on the mechanism(s) of toxicity, slowed disease progression has been achieved by a clinically feasible infusion into the nervous system of antisense oligonucleotides (ASOs) that direct RNase H-dependent destruction of SOD1 mRNA within the nervous system.

The most frequent genetic cause of ALS and the second most common dementia (frontal temporal dementia) is hexanucleotide expansion in a non-coding region of the *C9orf72* gene. Sense and antisense strand repeat-containing RNAs have been found to accumulate in nuclear RNA foci, the hallmark feature of repeat expansion RNA-mediated toxicity. ASOs have been developed that selectively target sense strand repeat-containing RNAs and reduce sense-oriented foci without affecting overall *C9orf72* expression. These findings establish ASO-mediated degradation of repeat-containing RNAs as an attractive therapeutic approach.

Huntington's disease is caused by toxicity from CAG-repeat expansions in the widely expressed Huntington gene. ASO infusion to catalyze rapid degradation of huntingtin mRNA mediates partial, sustained reversal of disease that persists much longer than the mRNA knockdown. These findings establish transient ASO-mediated silencing as a feasible therapy for Huntington's disease.

Overall, ASO infusion into the nervous system is widely applicable for therapy in neurodegenerative diseases, with trials ongoing in spinal muscular atrophy and myotonic dystrophy and expected to initiate shortly in ALS, ALS/FTD and Huntington's disease. Alpha-synuclein, APP, presenilin, and tau are attractive targets for extending this approach to Parkinson's and Alzheimer's diseases.

Symposium 38: MRI 2

ADPD5-0728
FUNCTIONAL CONNECTIVITY AND IMPAIRED RECOGNITION OF FAMILIAR FACES IN ALZHEIMER'S DISEASE

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1.Objectives: Studies have reported that patients with Alzheimer's disease (AD) experience difficulties recognizing their own faces in recent photographs. Two case reports of late-stage AD reported that this loss of self-face recognition was temporally graded: photographs from the remote past were recognized more easily than recent photographs. Neuroimaging studies in healthy adults have related own face recognition abilities to a bilateral fronto-temporo-parieto-occipital network, involved in core, extended face perception and self-systems.
2.Methods: In this study, behavioral experiments and fMRI experiment were conducted to compare moderate AD patients with healthy older participants in a recognition task involving self and familiar faces from different decades of the participants' life. Variable performance allowed us to examine correlations between scores and resting-state fMRI in order to link behavioral data to cerebral connectivity. Critical regions of interest involved in familiar face perception and self-system were selected for seed to voxel analyses.
3.Results: Moderate AD patients had difficulties recognizing themselves in photographs taken at recent periods of their life (65 years and over), suggesting a temporally graded loss of self-face recognition. fMRI results showed that the higher the connectivity between the dorsomedial-prefrontal cortex (DMPFC) and the right superior-frontal gyrus, the lower the self- and familiar-face recognition scores in moderate AD patients.
4.Conclusions: DMPFC is part of face perception and self-system while previous studies have related the superior frontal region to control processes rather than face recognition processes. Impaired face recognition in AD appears to be related to functional dedifferentiation between brain networks.

Symposium 38: MRI 2

ADPD5-0927
AUTOMATED SEGMENTATION OF AMYLOID PLAQUES ON IN VIVO MRI AFTER GD-STAINING

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Objectives: Magnetic resonance imaging (MRI) combined with intra-cerebro-ventricular administration of Gadolinium (Gd-DOTA) contrast agents can be used to detect individual amyloid plaques in transgenic live mice (ICV-Gd-staining protocol [1]). Our objective was to implement an automated protocol to segment amyloid plaques from MRI in order to facilitate the estimation of amyloid load during preclinical therapeutic evaluation.

Methods: Three-D Gradient-echo MR images (resolution: 29x29x117µm^3) were recorded on 14 APP/PS1 transgenic mice after ICV-Gd-staining, a protocol based on the use of a clinically approved MR contrast agent [1]. SPM mouse [2] and "amyloid mouse" templates were used to automatically segment amyloid plaques. After imaging, mice were sacrificed and amyloid plaques were detected from histological sections.

Results: Gd-staining allowed plaque detection by MRI (Figure-top). SPM mouse was able to create probability maps with segmented amyloid plaques and can be used to quantify amyloid plaques on a longitudinal way from MRI (Figure-bottom). Plaques from segmented MR images were registered to amyloid plaques detected by histology.

Conclusion: Here, we show that amyloid plaques can be detected by in vivo MRI with a very high in-plane resolution (29µm) and segmented automatically. The protocol presented here can be used as a rapid and reliable reference method for anti-amyloid drug development trials.

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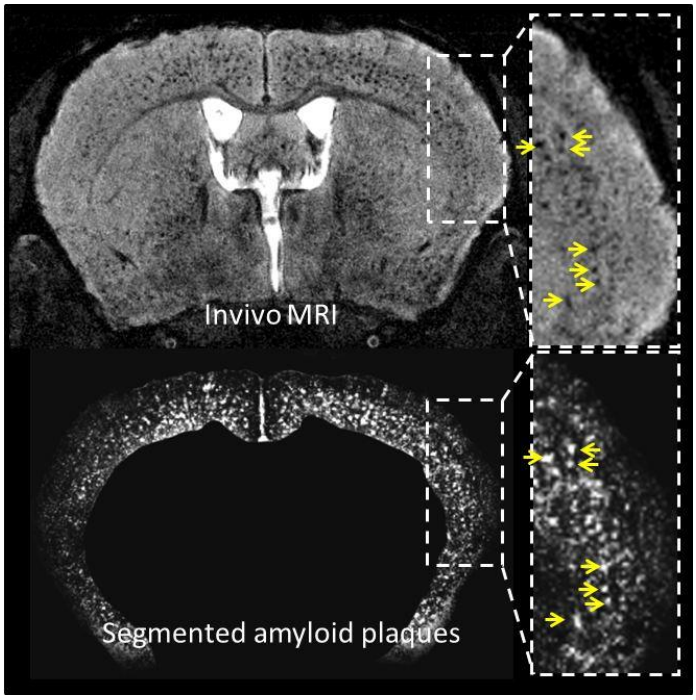
Acknowledgements: Medicen (Pôle_de_compétitivité Île-de-France, TransAl_program), France-Alzheimer association, Alliance Biosecure.


Invivo MRI
Segmented amyloid plaques

Symposium 38: MRI 2

ADPD5-0947

HIPPOCAMPAL TEXTURE PREDICTS AD CONVERSION IN AMYLOID POSITIVE MILD COGNITIVELY IMPAIRED SUBJECTS

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Objectives

We investigate whether hippocampal texture predicts conversion from mild cognitive impairment (MCI) to Alzheimer's disease (AD) in amyloid positive subjects.

Methods

The study dataset consisted of 98 MCI subjects (23 12-month converters, 46 24-month converters) from the Alzheimer's Disease Neuroimaging Initiative with a cerebrospinal fluid amyloid beta 1 to 42 peptide concentration below 192 pg/ml [1]. Hippocampal volume and hippocampal texture [2] were computed from each subject's structural magnetic resonance imaging (MRI) scan. Both MRI biomarkers were based on a segmentation of the hippocampi obtained using cross-sectional FreeSurfer (v.5.1.0, default parameters). Hippocampal volume was divided by the intra-cranial volume. Each biomarker was entered into a separate logistic regression model with age as covariate.

Results

MCI-to-AD conversion performance was quantified using the area under the receiver operating characteristic curve, and p-values were obtained using a DeLong test (see the table).

	12 month conversion	24 month conversion
Texture	0.701 ($p < 0.001$)	0.654 ($p = 0.005$)
Volume	0.648 ($p = 0.033$)	0.595 ($p = 0.099$)

Conclusions

Hippocampal texture predicts conversion to AD in amyloid positive MCI subjects. Interestingly, the commonly applied hippocampal volume does not predict a two year conversion. Hippocampal texture is a promising structural MRI biomarker for enrichment of AD trials using early AD and amyloid positive patients.

References

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[2] Sørensen L, *et al*. (2013) Hippocampal Texture Predicts Conversion from MCI to AD. Alzheimer's & Dementia, suppl.

Symposium 38: MRI 2

ADPD5-0949
PROGRESSION OF WHITE MATTER DEGENERATION IN EARLY PD: A MULTICENTER EVALUATION USING DIFFUSION TENSOR IMAGING
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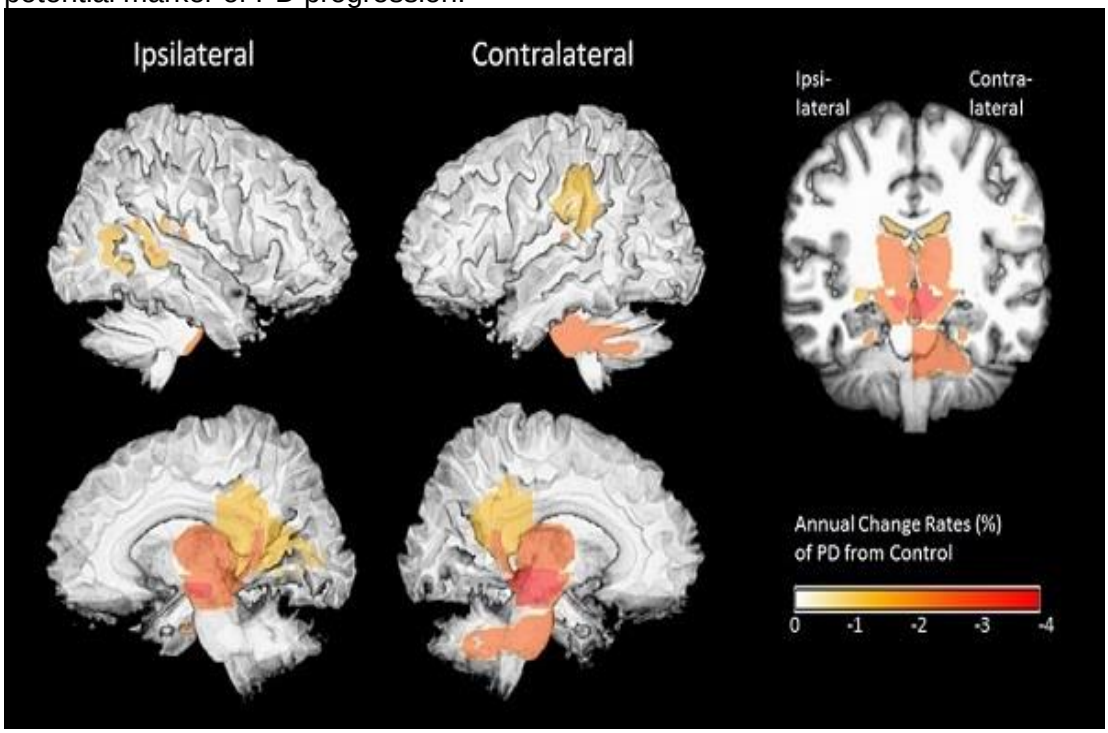
Objectives: This study aimed to identify the utility of MRI diffusion tensor imaging (DTI) in measuring abnormal white matter (WM) progression in patients enrolled in the Parkinson's progression marker initiative (PPMI). PPMI is an international, multicenter, longitudinal study assessing biochemical, clinical, and imaging biomarkers of PD progression.
Methods: 48 healthy controls (age=59.9±11) and 110 de-novo PD patients (age=60.3±9) had MRI-DTI scans at baseline and follow-up over 12.6±1 months. Image processing included an intra-subject registration of all time points and an inter-subjects registration to a brain atlas using DARTEL. Fractional anisotropy (FA) and WM probabilistic index were measured in 108 WM and 18 basal ganglia ROIs of each subject at each time point. Linear mixed-effect models between-group within-subject analyses were conducted to examine group differences in rates of FA.
Results: The Figure indicates regional pattern of significantly increased annual rates of FA reduction (FDR-adjusted $p<0.05$) in patients compared to controls. PD was associated with greater rates of FA reduction predominantly in the basal ganglia and the medial temproparietal regions, without significant differences between ipsilateral and contralateral hemispheres (corresponding to the body side of symptom at onset). Among these regions, substantia nigra yielded the highest rate of FA reduction (contralateral=3.8%/year, ipsilateral=3.7%/year) from baseline.
Conclusions: The results suggest that increased rates of WM degeneration are a

potential marker of PD progression.



Symposium 38: MRI 2

ADPD5-1273
WHITE MATTER DEGENERATION IN ATYPICAL ALZHEIMER'S DISEASE
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Objective. To assess white matter (WM) tract damage in patients with atypical Alzheimer's disease, including early age-of-onset AD, logopenic variant of primary progressive aphasia (lvPPA) and posterior cortical atrophy (PCA) using diffusion tensor (DT) MRI, and to identify similarities and differences across the AD spectrum.
Methods. WM tract damage and cortical atrophy were assessed in 28 EOAD, 12 lvPPA and 13 PCA patients relative to age- and sex-matched healthy subjects. Conjunction and interaction analyses were used to define overlapping and syndrome-specific patterns of brain damage.
Results. EOAD, lvPPA and PCA patients shared a common pattern of WM damage involving the body of the corpus callosum, fornix, and main anterior-posterior pathways, and cortical atrophy of the left temporo-parietal regions and precuneus. EOAD patients had also a specific damage to the genu and splenium of the corpus callosum, and parahippocampal tract bilaterally. In all AD patients, particularly in the two focal forms (lvPPA and PCA), WM damage was more severe and widely distributed than expected on the basis of cortical atrophy.
Discussion. In atypical AD clinical phenotypes, the distribution of WM damage exceeds cortical atrophy and may reflect the dissemination of pathology through structural connections from atrophic to unaffected cortical regions. WM degeneration may be an early marker of AD pathology in EOAD and focal AD forms.
Funding. Italian Ministry of Health (Grant #GR-2010-2303035).

Symposium 38: MRI 2

ADPD5-1636
DECREASED DEFAULT AND VENTRAL ATTENTION NETWORK CONNECTIVITY MEDIATE THE AMNESTIC EFFECTS OF SCOPOLAMINE ADMINISTRATION

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Objective: Disrupted cholinergic neurotransmission plays a key role in AD-related mnemonic deficits. Here we use functional connectivity MRI (fcMRI) to study cholinergic modulation of distributed neural networks following scopolamine administration. We determine the pattern of changing connectivity following anticholinergic challenge, and assess whether particular networks may mediate scopolamine-induced amnestic effects.
Methods: A double-blind, crossover design was used to assess cognitive function and connectivity across 10 cortical networks in 34 cognitively-normal elderly participants receiving placebo or scopolamine (0.2mg IV) on two separate visits.
Results: Scopolamine induced significant deficits in several cognitive tasks, most notably the delayed recall portion of the Selective Reminding Task (SRT-DR). Drug-induced changes in connectivity were observed in 4 of 10 cortical networks, with the largest decreases seen in two cognitive networks: the Default (DN) and Ventral Attention Networks (VAN; both $p < 0.001$, cohen's $d > 1$). Alterations in DN and VAN connectivity were highly predictive of subsequent memory performance, and mediation analysis demonstrated DN and VAN connectivity fully mediated the effect of scopolamine on SRT-DR performance.
Interpretation: Scopolamine administration led to significantly altered connectivity in a subset of cognitive networks, without changing connectivity in visual and motor networks. In particular, the DN and VAN were highly sensitive to cholinergic modulation, and decreased connectivity in these networks demonstrated full statistical mediation of scopolamine-induced deficits in the SRT-DR. These results highlight the importance of DN and VAN connectivity to memory, as well as the potential of fcMRI as a tool to better understand drug induced changes in cognition.

Symposium 41: TAU BIOLOGY AND DISEASE MECHANISMS 1

ADPD5-0252
INTRACEREBRAL INJECTION OF PREFORMED SYNTHETIC TAU FIBRILS INITIATES WIDESPREAD TAUOPATHY AS WELL AS NEURONAL LOSS IN THE BRAINS OF TAU TRANSGENIC MICE

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Neurofibrillary tangles (NFTs) composed of hyperphosphorylated fibrillized tau are found in numerous tauopathies including Alzheimer's Disease (AD). Increasing evidence suggests that tau pathology can be transmitted from cell-to-cell; however, the mechanisms involved in the initiation of tau fibrillization and spreading of disease linked to progression of tau pathology are poorly understood.
Intracerebral injections of preformed synthetic tau fibrils (PFFs) into the hippocampus or frontal cortex of young tau transgenic (Tg) mice expressing mutant human P301L tau induces tau hyperphosphorylation and aggregation around the site of injection, as well as a time-dependent propagation of tau pathology to interconnected brain areas distant from the injection site. Both the injection site and the nature of the seed affect the distribution and characteristics of the resulting tau pathology. Furthermore, we show that injection of tau PFFs into the hippocampus induces selective loss of CA1 neurons.
Together, our data confirm previous studies on the seeded induction and the spreading of tau pathology in a different tau Tg mouse model and reveals for the first time neuronal loss after intracerebral injection of tau PFFs in tau Tg mouse brain. These results further validate the utility of the tau seeding model in studying disease transmission, and provide a more complete in vivo tauopathy model with associated neurodegeneration which can be used to investigate the mechanisms involved in tau aggregation and spreading, as well as aid in the search for disease modifying treatments for AD and related tauopathies.
Eve Peeraer et al. accepted for publication in Neurobiology of Disease

Symposium 41: TAU BIOLOGY AND DISEASE MECHANISMS 1

ADPD5-0581
THE UNFOLDED PROTEIN RESPONSE: AN EARLY FACTOR IN TAU PATHOLOGY

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1. Objectives

The unfolded protein response (UPR) is a homeostatic stress response that is strategically positioned in the endoplasmic reticulum to sense and transduce signals from metabolic and inflammatory pathways. Our previous work shows activation of the UPR in the brain of tauopathy patients in neurons containing early stage tau pathology. In this study we investigate the mechanistic connection between the UPR and tau.

2. Methods

Post-mortem brain tissue, animal and cell models to modulate and analyse UPR signaling and tau phosphorylation and aggregation.

3. Results

UPR activation leads to tau phosphorylation, which is initially a reversible event involving the PERK pathway of the UPR. This is illustrated by our data that demonstrate that the UPR is also connected with physiological and reversible tau phosphorylation during metabolic stress in cell and animal models. Our data show that dysfunction of the autophagy/lysosomal system contributes to the persistence of tau phosphorylation and the initiation of tau aggregation.

4. Conclusions

We propose that phosphorylation of tau is part of the adaptive UPR. Our data are in accordance with a model where early in AD pathogenesis the adaptive UPR is activated in response to cellular stress, with tau phosphorylation as part of this response. Inability to restore homeostasis, for example by dysfunction of the autophagy/lysosomal system, ultimately leads to the formation of neurofibrillary tangles and neuronal loss. The functional involvement of the UPR in the earliest phases of tau pathology may provide an interesting window of opportunity by intervention in specific UPR signaling pathways.

Symposium 41: TAU BIOLOGY AND DISEASE MECHANISMS 1

ADPD5-1376

CRITICAL ROLE OF TAU ACETYLATION IN MEDIATING TAU AGGREGATION AND SYNAPTIC TOXICITY

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Background

Neurodegenerative tauopathies are characterized by pathogenic accumulation of tau. How pathogenic tau accumulates and induces neurodegeneration remains largely unknown. Tau is extensively modified post-translationally. Emerging evidence from our lab and others demonstrated that tau is acetylated by acetyltransferases p300/CBP at various lysine residues and that aberrant tau acetylation is elevated in all tauopathies. We hypothesize that tau acetylation plays a critical role in tau accumulation and tau-mediated neurodegeneration.

Methods

High performance liquid chromatography–electrospray tandem mass spectrometry was used to identify the acetyllysines in the soluble lysates from AD brains. We also developed monoclonal and polyclonal antibodies against specific acetyllysines on tau. The effects of acetylated-tau on tau accumulation and neuronal circuitry in vivo were examined in transgenic mice or in hippocampus using AAV-mediated transduction, followed by behavioral, electrophysiological, biochemical and pathological analyses.

Results and Conclusions

Several acetyllysine sites on tau were identified in AD brains, including ac-K174 and ac-K274. Compared with mice expressing wildtype human tau, mice expressing tau mutants that mimic tau acetylation exhibited elevated tau accumulation, most likely via blocking tau clearance mechanisms. Acetylation of tau at specific lysine residues also led to enhanced tau oligomerization. Moreover, acetyl-mimic tau is mis-sorted to somatodendritic compartments, leading to impaired long-term potentiation and cognitive deficits. Notably, reducing tau acetylation with small molecule inhibitors protected against tau-mediated deficits in tauopathy mouse models, providing proof-of-principle that inhibitors of tau acetylation represent promising new therapeutic strategies.

Symposium 41: TAU BIOLOGY AND DISEASE MECHANISMS 1

ADPD5-1629
FAMILIAL TAUOPATHIES
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Tauopathies are characterized by the pathologic accumulation of hyperphosphorylated, insoluble tau protein (Tau), which forms neurofibrillary tangles in the brain. Tau binds axonal microtubules, promotes their assembly, and stabilizes the cytoskeleton. Microtubule-associated protein tau gene complex (*MAPT*) located on chromosome 17 encodes Tau. *MAPT* consists of a non-coding exon followed by 14 coding exons. Alternative splicing leads to six major transcripts observed in the human brain and tau isoforms that contain either three or four 18-amino acid imperfect repeats (3R-tau and 4R-tau). In normal adult human brains, 3R-tau and 4R-tau are equally expressed. The 3R-tau/4R-tau ratio is altered in taupathies. Some taupathies such as Pick's disease contain predominantly hyperphosphorylated 3R-tau. 4R tauopathies include progressive supranuclear palsy, corticobasal degeneration, age-related medial temporal lobe tauopathy, globular glial tauopathies, and argyrophilic grain disease. In Alzheimer's disease, the neurofibrillary pathology is composed of approximately equal amounts of hyperphosphorylated 3R-tau and 4R-tau,
Most tauopathies are sporadic. However, more than 40 different mutations have been identified in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) due to *MAPT* mutations. In FTDP-17 due to *MAPT* mutations, tau pathology can be of 3R or 4R type. Clinical presentation and pathological characteristics differ widely.
Mutations in the leucine-rich repeat kinase 2 gene (*LRRK2*) on chromosome 12 usually presents as a synucleinopathy; however, *LRRK2* G2019S and *LRRK2* R1441C mutation carriers can present with taupathies and PSP-like symptoms. Currently, our Mayo Udall Center concentrates on the identification of other genetic forms of tauopathies, and their clinical, pathological, and genetic characterizations.

Symposium 41: TAU BIOLOGY AND DISEASE MECHANISMS 1

ADPD5-2321

MISSORTING OF TAU IN DEGENERATING NEURONS: Abeta, TAU, SPASTIN, AND MICROTUBULE BREAKDOWN

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Objectives

We are interested in mechanisms by which exposure of neurons to Abeta causes pathological effects on the level of tau and microtubules observed in AD, such as missorting of tau into the somadendritic compartment, destabilization of microtubules, and synapse decay.

Methods

Primary neurons from mature wildtype and Tau knockout mice were exposed to A-beta oligomers. Changes in the Tau/microtubule system were observed by confocal microscopy and staining with antibodies against components of the microtubule cytoskeleton.

Results

Microtubule breakdown occurs in Abeta-exposed dendrites invaded by Tau. This process is mediated by spastin, a microtubule-severing enzyme. Spastin is recruited to microtubules modified by polyglutamylation through TTLL6 (Tubulin-Tyrosine-Ligase-Like-6 enzyme). Photoconversion of Dendra2-labeled Tau reveals that missorted Tau is newly synthesized and not derived from axons. The toxic effects of A-beta are reversible and involve the activitation of the kinase MARK. In absence of Tau (TauKO neurons), microtubules and synapses are resistant to A-beta induced toxicity because mislocalization of TTLL6 and polyglutamylation of microtubules are prevented, end there is no recruitment of spastin nor microtubule breakdown. Reintroduction of Tau re-establishes A-beta induced toxicity in TauKO neurons.

Conclusions

Abeta oligomers can activate a cascade leading to breakdown of microtubules in dendrites and thus impairing intraneuronal traffic. The results point to the importance of posttranslational modifications of microtubules and the role of microtubule-severing enzymes such as spastin. These Abeta-induced effects depend on the presence of Tau and thus can be suppressed by reducing tau.

Acknowledgement: Funding by DZNE, MPG, Tau Consortium

Symposium 41: TAU BIOLOGY AND DISEASE MECHANISMS 1

ADPD5-2322

STRUCTURE AND TOXICITY OF TAU OLIGOMERS

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Objectives

To compare effects of Tau mutation A152T (related to PSP, with mutation outside the MT-binding domain) with pro-aggregant mutation DeltaK280 (related to FTDP, mutation within MT-binding domain) in vitro and in transgenic mouse models in order to understand different pathological features.

Methods

Analysis of Tau mutants by biochemical and biophysical methods (microtubule binding and Tau aggregation assays, electron and AFM microscopy). Generation of transgenic mice, analysis of histopathology and behavior, electrophysiology of acute and organotypic slices.

Results

Comparison of MT binding and Tau aggregation shows that interaction of Tau-A152T with MT is weakened, aggregation into filaments is decreased, but there is pronounced increase of soluble Tau oligomers in Tau-A152T. This is remarkable considering that residue152 is far from the repeat domain, the basis of MT binding and Tau aggregation.

Transgenic mice overexpressing Tau-A152T under the Thy-1 promoter show characteristic features of tau pathology (hyperphosphorylation, aggregation, missorting, neuronal loss). Signs of inflammation are pronounced (microgliosis, astrocytosis), cognitive decline sets in comparatively late (see abstract Sydow et al.).

Organotypic slices of TauA152T expressing mice reveal presence of Tau both in pre- and postsynaptic compartments, in contrast to normal Tau. There is increased epileptiform activity and excitotoxicity, correlating with an increase of extracellular glutamate. Calcium levels are strongly increased after membrane depolarization with KCl. All of these are unique features not observed in slices from proaggregant mice (see abstract Krüger et al.).

Conclusions

Tau-A152T reduces Tau-MT interaction and filamentous Tau aggregation but shifts the balance towards oligomers. Even though the mutation is far from the domain regulating microtubule interactions and aggregation, there is pronounced Alzheimer-like pathology, coupled with excitotoxicity, and a remarkable degree of inflammation.

Acknowledgement: Funding by DZNE, MPG, Tau Consortium

Symposium 46: VASCULAR ASPECTS OF NEURODEGENERATION

ADPD5-0970

CEREBROVASCULAR DISEASE IS ASSOCIATED WITH DECREASED AMYLOID-BETA SPECIES BUT NOT WITH THE AMYLOID-BETA42/AMYLOID-BETA40 RATIO OR 18-F FLUTAMETAMOL UPTAKE IN MCI

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Objectives:
To investigate the relationship between cerebrovascular disease and amyloid deposition including multiple forms of β-amyloid (Aβ) in MCI and AD.
Methods:
CSF-Aβ38, CSF-Aβ40 and CSF-Aβ42 were determined and the Aβ42/Aβ40-ratio calculated in 267 healthy elderly controls (M/F:102/162; mean age 72.9 years), 359 MCI subjects (M/F:179/178; mean age 70.7 years) and 20 AD subjects (M/F:5/15; mean age 72.9) were analyzed including. A composite score of [18F]flutametamol uptake (PET) was available for a subpopulation (n=350).White Matter Hyperintensities (WMH) on MRI FLAIR-images, graded using Fazekas and AWMRC-scales, were used as proxy for cerebrovascular disease.
Results:
In MCI patients, the total Fazekas score, continuous and dichotomized using cut-off 4, and the total ARWMC score were negatively associated with CSF-Aβ38, CSF-Aβ40, and CSF-Aβ42, but not with the Aβ42/Aβ40-ratio. In healthy controls, there was no association between WMH and CSF-biomarkers or the Abeta42/Abeta40 ratio (Table 1). The composite score from [18F]flutametamol PET was not associated with WMH in any of the groups.
Conclusions:
We found that in MCI, cerebrovascular disease present as white matter hyperintensities was associated only with lower levels of CSF-Aβ38, CSF-Aβ40, and CSF-Aβ42, while the Aβ42/Aβ40 ratio was unaffected. This suggests that subcortical cerebrovascular disease is related to a general decrease in the production of Aβ, rather than AD specific aggregation of Aβ42, which was supported by the amyloid PET imaging data.

Table 1. Beta- and p-values from linear regression models that tested associations between white matter lesions on MRI as a measure of cerebrovascular disease as independent variable, and CSF-biomarkers and PET composite scores as dependent variables. Significant ($p<0.05$) associations are marked with an asterix (*).

		CSF-Aβ38	CSF-Aβ40	CSF-Aβ42	Aβ42/Aβ40 ratio
MCI	Fazekas (continuous)	beta = -0.21	beta = -0.22	beta = -0.16	beta = 0.01
		$p<0.001$*	$p<0.001$*	$p=0.004$*	$p=0.87$
	Fazekas (dichotomized)	beta=-0.12	beta=-0.14	beta = -0.11	beta=-0.01
		$p=0.036$*	$p=0.015$*	$p=0.05$*	$p=0.90$
	AWMRC	beta=-0.20	beta=-0.19	beta=-0.16	beta=-0.02
		$p<0.001$*	$p<0.001$*	$p=0.003$*	$p=0.77$
AD	Fazekas (continuous)	beta=-0.58	beta=-0.33	beta=-0.52	beta=-0.18
		$p=0.052$	$p=0.23$	$p=0.039$*	$p=0.45$
Controls	Fazekas (continuous)	NA	NA	NA	NA
		$p>0.17$	$p>0.17$	$p>0.17$	$p>0.17$

Symposium 46: VASCULAR ASPECTS OF NEURODEGENERATION

ADPD5-1231
EARLY- VERSUS LATE- ONSET SUBCORTICAL VASCULAR COGNITIVE IMPAIRMENT
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Background: Early onset Alzheimer's disease and early onset frontotemporal dementia have been largely studied, while little attention was paid to early onset subcortical vascular cognitive impairment (EOSVCI). The aim of this study was to evaluate the differences between EOSVCI and late onset SVCI (LOSVCI) in terms of small vessel disease burden, amyloid burden, brain atrophy pattern, and cognitive dysfunction.
Methods: We prospectively recruited 137 patients from a single referral center. Patients were divided into EOSVCI (n=30, onset age <65 years) and LOSVCI (n=107, onset age ≥ 65 years). All patients underwent brain MRI, PiB-PET and detailed neuropsychological testing. Cortical thickness and volume of subcortical structures were analyzed.
Results: The educational level or severity of cognitive impairment did not differ between EOSVCI and LOSVCI patients. History of stroke and obesity were more prevalent in EOSVCI patients. EOSVCI patients tend to have higher number of lacune and had lower PiB-retention-ratio than LOSVCI patients. Atrophy in temporal and occipital cortex, amygdala, and hippocampus were more severe in LOSVCI, while pallidal atrophy was more severe in EOSVCI. The neuropsychological test showed that frontal-executive dysfunction was more prominant in EOSVCI patients while memory dyfunction was more prominent in LOSVCI patients.
Conclusions: EOSVCI patients had more vascular related factors while LOSVCI patients exhibited more Alzheimer's disease related characteristics. This suggests that cognitive dysfunction in LOSVCI is partially driven by aging related factors such as amyloid burden, whereas in EOSVCI, more extensive amount of vascular factors are necessary to reach the same stage of cognitive impairment.

Symposium 46: VASCULAR ASPECTS OF NEURODEGENERATION

ADPD5-1402
CEREBRAL VASCULAR AMYLOID SEEDS STRUCTURALLY DISTINCT FIBRILLAR ASSEMBLY

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Cerebrovascular accumulation of the amyloid ß-protein (Aß), a condition known as cerebral amyloid angiopathy (CAA), is an important driver of vascular cognitive impairment and dementia (VCID) and is a common comorbidity of patients with Alzheimer's disease (AD). In addition to its prevalence in AD, several related familial CAA disorders result from specific mutations that reside within the Aß peptide sequence of the Aß precursor protein including the Dutch-type (E22Q) and Iowa-type (D23N) mutations. Presently, there are no reliable biomarkers or effective therapies specifically for CAA and VCID. These deficiencies are complicated by a poor understanding of the unique structural attributes of cerebral vascular amyloid and their distinctive features and processes compared to parenchymal plaque amyloid. Previous studies suggest that differences exist between cerebrovascular amyloid and parenchymal plaque amyloid. Here we show that CAA mutant forms of Aß more readily adopt a anti-parallel fibril structure in contrast to wild-type Aß42 that largely assembles into parallel fibril structures commonly found in parenchymal plaques. In vitro, CAA mutant amyloid fibril seeds promote wild-type Aß peptide assembly. Similarly, in transgenic mice CAA mutant vascular amyloid deposits dramatically promote the assembly and co-deposition of wild-type Aß on cerebral vessels. Further, isolated CAA mutant amyloid seeds were found to drive anti-parallel fibrillar assembly of wild-type Aß. These findings suggest that CAA deposits exhibit an anti-parallel fibril structure that can serve as a template to enhance wild-type Aß assembly into similar anti-parallel structures.

Symposium 46: VASCULAR ASPECTS OF NEURODEGENERATION

ADPD5-1560
VASCULAR COGNITIVE IMPAIRMENT
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Vascular brain abnormalities influence the evolution of clinical Alzheimer´s disease (AD). The existence of pure vascular dementia is considered to be exceptional and there has been a shift in nomenclature to vascular cognitive impairment. New guidelines for the diagnosis of vascular cognitive impairment (VCI) represent an important step in the definition of this clinical entity. However, these guidelines still remain vague in the definition of "vascular" brain lesions causing cognitive decline, because longitudinal correlative imaging studies are still scarce. I will explore which abnormalities are likely to contribute to VCI based on a proven vascular etiology, fast progression and their incidence or progression being related to cognitive decline. Among focal changes visible on standard MRI these features apply for coalescent white matter changes. The evidence for lacunes and microbleeds is much less convincing. Microstructural alterations in normal appearing brain tissue which can be detected by new MRI techniques such as magnetization transfer imaging (MTI), diffusion tensor imaging (DTI) and high resolution MR appear to better correlate with cognitive decline, but the etiology of these changes and their histopathological correlates is still incompletely understood as is their evolution over time. New multimodal image processing such as voxel-based lesion-symptom mapping (VLSM) or combinations of DTI and voxel-based analysis will allow to allocate the lesion patterns that show the greatest covariance with clinical outcome. Such data and more longitudinal correlative data on lacunes and microbleeds will increase our pathophysiologic understanding of the vascular component in dementia including the interplay with primary degenerative processes and will lead to refinement of current VCI criteria

Symposium 46: VASCULAR ASPECTS OF NEURODEGENERATION

ADPD5-1836
APOE AND CEREBROVASCULATURE IN ALZHEIMER'S DISEASE
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While amyloid-β (Aβ) is a key pathogenic molecule in AD, epidemiological studies have shown that several well-established risk factors for AD including diabetes, atherosclerosis, stroke, and hypertension all have a vascular component that reduces cerebral perfusion. Therefore, disturbance of cerebrovascular system is likely a major contributor to AD pathogenesis. Among the three human apolipoprotein E (apoE) isoforms (E2, E3 and E4), *APOE4* is the strongest genetic risk factor for late-onset AD and cerebral amyloid angiopathy (CAA). The most consistent finding that differentiates apoE4 from apoE3 is their respective roles in brain Aβ clearance, where apoE4 is either less efficient or inhibitory compared to apoE3. To address the differential functions of apoE isoforms, we compared cerebral blood flow, behaviors and Aβ metabolism among human *APOE* isoform (E2, E3 and E4)-targeted replacement (TR) mice at young and old ages. We found that cerebral blood flow and memory performance are reduced, while endogenous Aβ levels are elevated, in aged *APOE4*-TR mice compared to *APOE3*-TR mice. Brain glucose metabolism was also compromised in aged *APOE4*-TR mice. To specifically address the function of a major apoE and Aβ receptor LRP1 in cerebrovasculature, we generated conditional knockout mice deleting *Lrp1* in vascular mural cells (smLRP1-KO), which include smooth muscle cells and pericytes. We found that cerebral blood flow and vessel functions are significantly impaired in smLRP1-KO mice. Our results demonstrate that the presence of *APOE4* or an absence of apoE receptor LRP1 leads to cerebrovascular defects, which compromise Aβ clearance machinery resulting in Aβ accumulation in the brain. The resulting Aβ aggregates further exacerbate cerebrovascular dysfunction in AD.

Symposium 46: VASCULAR ASPECTS OF NEURODEGENERATION

ADPD5-2280
PROGRESSION OF VASCULAR RISK FACTORS PREVALENCE IN ALZHEIMER'S DISEASE, MIXED DEMENTIA AND VASCULAR DEMENTIA IN A MEMORY CENTER

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Background
Some studies show a decrease in the prevalence of dementia. This could be due to a decrease in cerebrovascular pathology that may magnify the effect of Alzheimer pathology. The aim of this study was to evaluate the progression of the prevalence of vascular risk factors(VRF) in patients admitted in a memory center diagnosed with Alzheimer's disease(AD), vascular dementia(VD) or both (mixed dementia[MD]).
Methods
All consecutive patients were prospectively included. We compared the prevalence of VRF (hypertension, diabetes mellitus and hypercholesterolemia) and the diagnostic distribution across three 5-year periods: 1995-1999(P1), 2000-2004(P2), 2005-2009(P3). We used statistical tests such as anova and X^2 for simple comparison, and Poisson regression for multivariate analysis.
Results
We included 3428 patients (P1=1067, P2=1275, P3=1086). The prevalence of VRF increased in each period (hypertension: P1=33.4%, P2=50.5%, P3=63.0%; diabetes: P1=10.1%, P2=15.8%, P3=19.1%; hypercholesterolemia: P1=unreliable, P2=22.4%, P3=41%). The mean dementia onset age significantly increased (P1=69.6, P2=71.6, P3=71.9 years). The diagnostic distribution evolved with a larger number of MD at the expense of AD (AD: P1=59.9%, P2=41.9%, P3=29.1%; MD: P1=25.1%, P2=43.0%, P3=53.9%). Mean MMSE score at inclusion did not change significantly (P1=19.4, P2=20.1, P3=19.9).
Conclusion
In patients with dementia, the prevalence of VRF increased with time, contrary to what is observed in the general population except for diabetes mellitus, as well as the proportion of mixed dementia, while the age at onset was delayed. These findings support the role of cerebrovascular pathology in dementia and highlight the necessity for better VRF management to prevent or delay dementia.

Symposium 49: UPDATE ON DLB: CLINICAL AND PRE-CLINICAL STUDIES (PART 2)

ADPD5-0526

WHERE AND WHEN TO TARGET PATHOGENIC SYNUCLEIN VARIANTS FOR THERAPEUTIC PURPOSES IN PRECLINICAL MODELS?

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Background: A variety of precilinal models based on overexpression or transmission of alpha-synuclein (AS) in the nervous system are now available, although their relevance to human synucleinopathies remains questionable. In parallel, therapeutic strategies to combat the generation of aberrant AS species or their detrimental effects are being developed. A case in point is the enhancement of Chaperone-Mediated Autophagy (CMA) through overexpression of its rate-limiting step Lamp-2a, which achieves the double effect of minimizing aberrant AS species and mitigating their detrimental effects on lysosomes (Xilouri et al., 2013). A major challenge lies in the selection of the timing and site of application of such strategies in a progressive synucleinopathy model, so as to most closely mimic a possible intervention in a clinical trial.

Objective: To evaluate the timing and site of intervention of enhancement of CMA in a progressive synucleinopathy model.

Methods: We are using human AS BAC Tg rats, which develop widespread alpha-synucleinopathy and dopaminergic neurodegeneration (Nuber et al., 2013). We are injecting them at various time points and at various sites with Adeno Associated Virus (AAV) expressing Lamp-2a (Xilouri et al., 2013). We are evaluating Lamp-2a expression and effects on AS species and indices of neurodegeneration.

Results: Fractionated Western immunoblotting shows widespread accumulation of oligomeric alpha-synuclein in various brain areas of hAS BAC Tg rats, including hippocampus and cortex. Injections of Lamp-2a AAV have been performed in striatum and nigra and effects on AS species and dopaminergic neurodegeneration will be evaluated.

Conclusions: Our study represents an example of a therepautic intervention in a synucleinopathy animal model that addresses the issues of timing and site of intervention. Similar strategies undertaken by others will be discussed.

This work was supported by ARISTEIA (GSRT), SINERGASIA (GSRT) and MULTISYN (EU FP7) grants, as well as the NEURASYNC ITN (EU FP7) to LS.

Symposium 49: UPDATE ON DLB: CLINICAL AND PRE-CLINICAL STUDIES (PART 2)

ADPD5-1344
ENHANCING GBA1 ACTIVITY AS A THERAPEUTIC STRATEGY IN LEWY BODY DISEASES

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Gaucher disease, the most common lysosomal storage disease, is caused by a recessively inherited deficiency in glucocerebrosidase and subsequent accumulation of toxic lipid substrates. Heterozygous mutations in the lysosomal glucocerebrosidase gene (GBA1) have recently been recognized as the highest genetic risk factor for the development of ?-synuclein aggregation disorders (“synucleinopathies”), including Parkinson’s disease (PD) and dementia with Lewy bodies (DLB). Despite the wealth of experimental, clinical and genetic evidence that supports the association between mutant genotypes and synucleinopathy risk, the precise mechanisms by which GBA1 mutations lead to PD and DLB remain unclear. Decreased glucocerebrosidase activity has been demonstrated to promote ?-synuclein misprocessing. Furthermore, aberrant ?-synuclein species have been reported to downregulate glucocerebrosidase activity, which further contributes to disease progression. We will summarize the recent findings that highlight the complexity of this pathogenetic link and how several pathways that connect glucocerebrosidase insufficiency with ?-synuclein misprocessing have emerged as potential therapeutic targets. From a translational perspective, we will discuss how glucocerebrosidase augmentation has been explored for the treatment of GBA1-related synucleinopathies, and potentially, for non-GBA1-associated neurodegenerative diseases. In summary, the link between GBA1 and synucleinopathies has become the paradigm of how the study of a rare lysosomal disease can transform the understanding of the etiopathology, and hopefully the treatment, of a more prevalent and multifactorial disorder.

Symposium 49: UPDATE ON DLB: CLINICAL AND PRE-CLINICAL STUDIES (PART 2)

ADPD5-1475

MODELING DLB IN MICE: A BI-GENIC APPROACH COMBINING HUMAN SNCA MULTIPLICATION WITH MUTANT GBA1 GENES

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Objectives: We have created a new animal model of complex genetic susceptibility informed by dementia, parkinsonism and other alpha-synuclein-associated diseases of the human brain: the SYNERGY mouse (Synucleinopathy related to Gaucher's and Lewy body dementia). These animals carry three distinct susceptibility traits at two loci (SNCA, GBA1); each trait is strongly linked to dementia and parkinsonism.

Methods: The SYNERGY mouse was generated by crossing a PAC1-hSNCA^{A53T} line, which carries 148Kb of the human SNCA locus [Kuo et al., 2010], with Gba1^{D409V} knock-in mice [Xu et al., 2003]. SYNERGY mice thus combine: (1) SNCA gene multiplication (i.e., four copies); (2) A53T point mutations in all SNCA alleles; (3) absence of murine Snca; and (4) two Gba1^{D409V} knock-in mutations.

Results: We observe significant motor, cognitive and olfactory deficits in SYNERGY mice compared to wild-type control animals at 3 to 4 months of age; biochemically, we detect altered glycolipid profiles and aggregates of alpha-synuclein in select brain areas of 10 week-old mice. Assessment of age-dependent progression for these early proteomic, lipidomic and microscopic changes as well as of behavioural deficits is currently ongoing.

Conclusions: We posit that SYNERGY mice represent the etiologically most relevant model for Lewy body dementia created to date. We are hopeful that our bi-genic animals will advance preclinical research by permitting the study of multi-systems changes that underlie SNCA-associated neurodegeneration in humans, and by serving as a platform for target validation and drug screening in the future.

Symposium 49: UPDATE ON DLB: CLINICAL AND PRE-CLINICAL STUDIES (PART 2)

ADPD5-1587
RIPK3 AS A POSSIBLE THERAPEUTIC TARGET FOR GAUCHER DISEASE AND OTHER NEURODEGENERATIVE DISEASES
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Gaucher disease (GD), an inherited metabolic disorder caused by mutations in the glucocerebrosidase (*GBA*) gene, is the most common lysosomal storage disease (LSD). Heterozygous mutations in *GBA* are a major risk factor for Parkinson's disease (PD). GD is divided into three clinical sub–types based on the absence (type 1) or presence (types 2 and 3) of neurological signs. Type 1 GD was the first lysosomal disease for which enzyme therapy became available, and although infusions of recombinant glucocerebrosidase (GCase) ameliorate the systemic effects of GD, lack of efficacy for the neurological manifestations, along with the significant expense and inconvenience of enzyme therapy for patients, renders the search for alternative or complementary therapies paramount. Glucosylceramide (GlcCer) and glucosylsphingosine accumulation in the brain leads to massive neuronal loss in neuronopathic GD (nGD) patients and in nGD mouse models. However, the mode of neuronal death is not known. Here, I will present data showing that modulating the receptor–interacting protein kinase 3 (Ripk3) pathway markedly improves neurological and visceral disease in a mouse model of GD. Importantly, *Ripk3* deficiency dramatically improved the clinical course of GD mice with increased survival, motor coordination and salutary effects on cerebral as well as hepatic injury. The relevance of these findings to PD will be discussed.

Symposium 49: UPDATE ON DLB: CLINICAL AND PRE-CLINICAL STUDIES (PART 2)

ADPD5-1882
LONGITUDINAL PD BIOMARKER STUDIES: DENOPA AND PPMI
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Objectives: To demonstrate first longitudinal data on one single- [De No Parkinson's disease (PD): DeNoPa] and one multicenter biomarker study (Parkinson Progression Marker Initiative of the Michael J Fox Foundation; PPMI) for progression biomarkers as outcome measures for clinical trials
Methods: clinical measures (motor scales, non-motor symptoms, cognitive tests, sleep),volumetric imaging [magnetic resonance imaging (MRI)] and blood/cerebrospinal fluid (CSF) biomarkers have been investigated in two longitudinal cohorts on de novo PD and healthy controls.
Results: 159 patients and 110 healthy controls have been recruited in the DeNoPa study and followed after 24 months. A multimodal panel of clinical, functional and imaging measures significantly reflect the progression of the disease in DeNoPa.While non-motor symptoms progress (MDS-UPDRS 1 and Scopa AUT), sleep deteriorates (ESS) and volumetric imaging of grey-matter and hippocampal area decrease significantly, the CSF parameters remain relatively stable (α-synuclein) or increase slighlty (β-amyloid 1-42, total tau protein, neurofilaments) from baseline to the first follow-up period in comparison of patients and controls. This propoed progression panel needs validation by continuing follow-up and in independent, larger cohorts, such as PPMI. For PPMI 24 sites in the USA and Europe recruited 232 de novo PD patients and 196 healthy controls. Follow-up assessments including clinical data, MRI, dopamine transporter imaging, CSF and blood sampling are done every 6 months.
Conclusion: A multimodal panel of progression marker can serve as outcome measure for clinical trials in de novo PD. More accurate biomarkers in biological fluids for the progression of PD need to be established.

Symposium 49: UPDATE ON DLB: CLINICAL AND PRE-CLINICAL STUDIES (PART 2)

ADPD5-1994
VARIANTS IN SYNUCLEIN GENES UNDERLYING A DLB PHENOTYPE

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The relationship between Parkinson disease (PD), PD with dementia (PDD) and dementia with Lewy bodies (clinically DLB or diffuse Lewy body disease (DLBD) at autopsy) is long debated. Although PD is primarily considered a motor disorder cognitive impairment is often present at diagnosis, and only ~15% of patients remain cognitively intact in the long term. Alpha-synuclein is a major constituent of Lewy bodies and Lewy neuritic pathology, and although the regional and quantitative burden differs, PD, PDD, and DLB may represent a disease continuum. Alpha-synuclein was first implicated in pathogenesis when *SNCA* point mutations and locus multiplications were identified in familial parkinsonism with dementia. In world-wide populations *SNCA* genetic variability remains the most reproducible risk factor for idiopathic PD, and the synuclein gene family has been implicated in DLB/DLBD. However, the precise variant(s) driving those associations have yet to be elucidated. The relationship between genetic variability, gene and protein expression is contentious, while the physiologic and pathologic roles of alpha-synuclein, and their relationship, remain enigmatic. Hence, comprehensive analysis of the *SNCA* locus is ongoing in four cohorts: 1) families with *SNCA* and *SNCB* mutations; 2) the Progressive Parkinson's Markers Initiative *de novo* cohort, focused on biomarker measurement of protein in plasma and cerebrospinal fluid; 3) the Movement Disorders Society PD-Mild Cognitive Impairment cohort, focused on the classification and progression of cognitive decline in advancing PD; 4) studies in brain bank series focusing on Lewy neuropathology, protein and gene expression. A brief summary and synthesis of interim findings will be presented.

Symposium 52: CLINICAL TRIALS PODIUM DISCUSSION - IS THERE A WAY TO INCREASE THE CHANCE OF SUCCESS IN PHASE 3?

ADPD5-2313
DESIGNING CLINICAL TRIALS IN AD

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Recent failures of large size Phase III randomized clinical trials (RCT) in mild to moderate stages of Alzheimer's disease (AD), or even prodromal AD, comparing placebo to drugs interfering with the amyloid cascade are raising serious concerns about the likelihood of success for the ongoing RCTs in similar populations. Patients currently under study have either dementia or MCI, using the modified NINCDS criteria or the Dubois criteria with ages usually ranging from 55 to 90. The relative weight of the underlying pathology is different in younger vs older patients: younger patients will show predominantly amyloid pathology, have a different disease course and atrophy pattern, whereas the older patients will have variable levels of tau hyperphosphorylation, small vessel disease and Lewy Body pathology. The plan of analysis should compare the clinical efficacy in younger vs older subjects, with the hypothesis that the effect size will be higher in younger patients using therapy directed against amyloid. In future studies, proof-of-concept could be attempted in Phase II by enrolling younger (age 50-70 year) subjects with predominantly familial early onset AD, like in the API trial in PS1 carriers, whereas Phase III should require proof of amyloid pathology with low CSF Aβ42 levels, correcting for APOE status,using the best available laboratories to process the samples or using a single reference laboratory, or use a positive amyloid PET scan judged centrally as entry criterion. . However, studies show that having amyloid per se does not predict progression. This may jeopardize the power of the study to detect a difference. An enrichment strategy to increase the likelihood clinical progression, as well as decrease heterogeneity, could consist of including APOE4 carriers only, or include patients with relatively high tau values in CSF, or requiring the presence of a certain amount of bilateral hippocampal atrophy at baseline, or using a randomised start design by documenting clinical or atrophy progression in a run-in period of 6 months and randomizing those with progression only. Coexisting pathology may decrease the chance of success of a therapy directed at amyloid pathology. Presence of concomitant Lewy Body pathology could be ruled out by requiring DAT-SPECT scans at entry, as a proxy for the yet absent reliable alpha-synuclein marker in CSF or serum, or by ruling out clinical features such as visual hallucinations, motor rigidity, or fluctuations of cognition. Since structural MRIs are now done on all patients at screening, the relative amount of white matter lesions could be quantified using a structured method, allowing sub-analysis for low vs relatively high amount of vascular co-morbidity. Patients with the lowest levels of vascular and Lewy body pathology and the highest levels of amyloid pathology would be expected to show the highest clinical response to anti-amyloid therapy. The current designs and primary endpoints are modeled on the ChEI symptomatic trials: fixed time end-points (18 months), dual cognitive and global or functional outcomes. Statistically significant differences between treatment arms for these outcomes at 18 months determine the fate of the

drugs being tested. We argue that this will be not be easily achieved in an analysis putting together all the patients diagnosed as “probable AD” ages 55 to 90 considering the heterogeneity of the underlying age-associated pathology. Futhermore, even if such a difference was demonstrated, the clinical relevance of such a finding will be questioned by regulators, third part payers and future users. Clinically meaningful endpoints such as delay of progression from very mild to moderate dementia, operationally defined as CDR 0.5, progressing to CDR 1 or 2, should be added on the primary plan of analysis. In addition, I argue for the development of more meaningful IADL scales that tap into the activities of the younger patients as defined above, and that are more sensitive to change than the current available scales (Sikkes et al 2009). Considering involving patient panels in the design of future trials and asking them what they consider is important, may beneficial as well. Delaying progression will be even more meaningful in future studies enrolling patients with “prodromal AD”, based on the revised NINCDS-ADRDA criteria (Dubois et al 2007), where (1) placebo can be used until dementia is diagnosed, (2) the underlying pathology may be more amenable to arrest of progression, (3) delaying further cognitive decline, emergence of functional decline or dementia will offer high face validity. We cannot use the RCT design of short-term symptomatic drugs when dealing with longer term disease modification. Innovative plans of analysis are required for the ongoing studies, and the shift to prodromal AD for current and future RCTs. In short we argue to rethink the design of RCT in AD and put more focus on including as homogeneous as possible populations in Phase II, use the prodromal AD criteria, and shift the current emphasis on cognitive outcomes in short-term studies to prevention of global and functional decline in longer Phase III studies. A constant dialogue is necessary between patients, clinicians, pharmaceutical sponsors and regulators in order to move this field forward.

Symposium 52: CLINICAL TRIALS PODIUM DISCUSSION - IS THERE A WAY TO INCREASE THE CHANCE OF SUCCESS IN PHASE 3?

ADPD5-2319
THE FUTURE FOR ALZHEIMER CLINICAL TRIALS

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Despite marked advances in knowledge, biomarkers, and drug discovery, phase 2 and 3 Alzheimer-related clinical trials methods have remained relatively stagnant, although some advances have been made. We review trials methods that mostly showed lack of efficacy, at best, modest efficacy; discuss ongoing trials' inclusion criteria, targets, focus, designs, and outcomes. Then we estimate the likelihood for success based on prior evidence.

Current trials include mild/moderate AD, prodromal or MCI due to AD, preclinical AD, secondary prevention, at-risk, and primary prevention trials. Many include features such as biomarkers for sample enrichment intended to increase likelihood for Alzheimer pathology. Outcomes, however, continue to be basic cognitive and functional assessments; although some modifications include executive function tests, computerized administration, and 'composite' batteries derived from scales used in observation cohort studies; biomarkers are exploratory. Compared to earlier prevention trials, current prevention trials use evolved selection criteria, potentially interesting outcomes and no doubt will offer new lessons.

Predicting expectations or designing trials that may increase likelihood for success is an uncertain endeavor as it requires predicting or exploiting advances in knowledge, targets and drugs. A few changes in methods are probable that involve changing management, infrastructure, preparation, recruitment methods, medication/therapy delivery, and regulatory oversight. There will be very few clinic visits, little direct fact-to-face testing, and physicians may not need to be involved. Assessments will be brief, frequent, active and passive, rely on sensors, biosensors, holograms, and virtual systems to assess pharmacokinetics, cognitive and functional behaviors, and health effectiveness. Conducting trials will become faster, cheaper, and ecologically valid. Scenarios for Alzheimer clinical trials of the future utilizing these approaches will be described. Whether these changes increase the chances for success remains to be determined.

Symposia – Treatment

Symposium 16: TRANSLATIONAL STRATEGIES 1: ABETA TARGET

ADPD5-2112
THERAPY DEVELOPMENT FOR ALZHEIMER'S DISEASE BASED ON ABETA OLIGOMER ELIMINATION

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1. Objectives:
Strong evidence exists for a central role of amyloid-beta (Abeta) oligomers in the pathogenesis of Alzheimer's disease (AD). The potency to eliminate Abeta oligomers is one of the most desirable criteria for the selection of agents for drug development. Starting from the lead compound 'D3", we set out to identify derivatives with increased efficacy in Abeta oligomer elimination.
2. Methods
We have designed an assay for the quantitative determination of interference with Abeta aggregate size distribution (QIAD). It is a fast, reliable and robust in vitro assay that is able to quantify the Abeta oligomer eliminating potential of any compound. We characterized D3 derivatives with QIAD and with other in vitro assays for their Abeta oligomer eliminating efficacy and binding affinities to Abeta species. Moreover, the most promising derivatives were also characterized in various animal models.
3. Results
D3 and its derivatives specifically eliminated Abeta oligomers and converted them into non-amyloidogenic, non-fibrilar and non-toxic species. We show that next to plaque load and inflammation reduction, oral application of the compounds slowed down neurodegeneration and improved cognitive performance in several transgenic AD mouse models. We demonstrate the predictive power of the QIAD assay for in vivo efficacy by comparing QIAD outcomes of several therapeutically interesting compounds with their treatment effects in animal models.
4. Conclusions
Some D3 derivatives show superior properties in vitro und in vivo. The relation between Abeta oligomer elimination efficacy and in vivo outcome underlines the role of Abeta oligomers in AD pathogenesis.

Symposium 16: TRANSLATIONAL STRATEGIES 1: ABETA TARGET

ADPD5-2021

PRECLINICAL PROFILE OF MK-8931, A STRUCTURALLY NOVEL, CENTRALLY-ACTIVE, BETA-SECRETASE (BACE1) INHIBITOR FOR THE TREATMENT OF ALZHEIMER'S DISEASE.

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Alzheimer's disease (AD) patients need disease modifying therapies that slow progression or delay onset of AD. The amyloid hypothesis proposes a critical pathological role for toxic β-amyloid (Aβ)-containing oligomers and plaques. Inhibition of the aspartyl protease β-secretase (BACE1) may be a robust strategy for testing the amyloid hypothesis because BACE1 is required for Aβ synthesis. MK-8931 is a structurally novel, non-peptidic, BACE inhibitor that was discovered through the intensive use of structure-based drug design and in vivo screening for CSF Aβ lowering in rodents. Here we describe the pharmacological and pharmacokinetic characterization of MK-8931 across multiple preclinical species. MK-8931 is a potent inhibitor of the human BACE1 enzyme with excellent selectivity over other human aspartyl proteases. MK-8931 potently inhibits amyloid precursor protein processing in cells. Acute oral administration of MK-8931 to rats dose- and time-dependently reduces plasma, CSF and brain Aβ40. Acute oral administration to cynomolgus monkeys robust lowers CSF and cortical Aβ40. MK-8931 has moderate to high bioavailability, a moderate $T_{1/2}$ in all species, and good brain penetration in rats and monkeys. The compound does not inhibit or induce the major human CYP450 enzymes and is a weak inhibitor of the hERG channel. Pharmacodynamic and pharmacokinetic modeling based on preclinical data was used to estimate the exposure required for 75% reduction of CSF Aβ in humans. Phase 1 studies have shown that MK-8931 potently and effectively lowers CSF Aβ peptides in humans and MK-8931 has progressed to Phase 3 clinical trials in mild-moderate and prodromal Alzheimer’s disease.

Symposium 16: TRANSLATIONAL STRATEGIES 1: ABETA TARGET

ADPD5-1486

SOLUBLE GAMA-SECRETASE MODULATORS INHIBIT PRODUCTION OF ABETA42 AND ABETA40 AND AUGMENT PRODUCTION OF MULTIPLE CARBOXY-TRUNCATED ABETA SPECIES

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Alzheimer's disease is characterized pathologically by an abundance of extracellular neuritic plaques composed primarily of the amyloid β peptide variant (Abeta42). In the majority of familial AD (FAD) cases, e.g. those harboring mutations in presenilin 1 (PS1), there is a relative increase in the levels Abeta42 compared to Abeta40. We previously reported the characterization of a series of aminothiazole-bridged aromates referred to as aryl aminothiazole gamma-secretase modulators or AGSMs (Kounnas et al. 2010 Neuron 67:769-780) and showed their potential for use in the treatment of FAD (Wagner et al. 2012 Arch. Neurol . 69:1255-1258). Here we describe a series of GSMs with improved physicochemical properties compared to AGSMs. Specific heterocycle replacements of the phenyl rings in AGSMs provided potent molecules with improved aqueous solubilities. A number of these soluble gamma-secretase modulators (SGSMs) potently lowered Abeta42 levels without inhibiting proteolysis of Notch or causing accumulation of amyloid precursor protein carboxy-terminal fragments (APP-CTFs), even at concentrations approximately 1000-fold over their IC_{50}'s for reducing Abeta42 levels. The effects of one potent SGSM on Abeta peptide production were verified by MALDI-TOF mass spectrometry, showing enhanced production of a number of carboxy-truncated Abeta species. This SGSM also inhibited Abeta42 peptide production in a highly purified reconstituted gamma-secretase *in vitro* assay system and retained the ability to modulate gamma-secretase-mediated proteolysis in a stably-transfected cell culture model over-expressing a human PS1 mutation validating the potential for use in FAD.

Symposium 16: TRANSLATIONAL STRATEGIES 1: ABETA TARGET

ADPD5-0821
TOWARDS TREATMENT OF AMYLOID TOXICITY IN DEMENTIA
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The correlation between the total amount of fibrillar aggregates and cognitive decline depends not only on the quantity of the amyloid protein deposited but also on the number of synapses to be destroyed. Indeed, patient with Alzheimer's disease (AD) exhibiting similar level of clinical severity, higher levels of education are associated with more severe disease related changes of ß-amyloid PET or CSF Aß42 levels suggesting that amyloid toxicity may be the same in patients with matched clinical severity but gross differences in AD pathology. Pre-fibrillary Aß species rather than elongated amyloid fibrils are likely to represent the primary pathogenic agents simply because the former provide on their surfaces more chemical groups than the latter, such as hydrophobic side chains and unbound hydrogen bonds that would not be accessible within amyloid. The origine of the toxicity of the oligomers may arise from inappropriate interaction in trans with the folding of cellular and extracellular structures including proteins, lipid membranes and nucleic acids. Therefore, prevention and treatment of protein misfolding disorders needs to address aggregation and misfolding in cis and trans by decreasing the concentration and disrupting the formation of these toxic species. This can be achieved, as we have shown as first, with Aß-analogues that selectively bind to the native state of the peptide that suppress nucleation and proliferation of toxic pre-amyloid species, by antibodies to reduce the level of highly trans-aggregation prone species (such as Aß oligomers), and by stimulating clearance by proteolytic degradation.

Symposium 16: TRANSLATIONAL STRATEGIES 1: ABETA TARGET

ADPD5-0594

NATURALLY OCCURRING AUTOANTIBODIES AGAINST BETA-AMYLOID OLIGOMERS RESCUES MEMORY DEFICITS IN TRANSGENIC MICE WITH ALZHEIMER'S DISEASE

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Objectives: Previous reports showed that naturally occurring autoantibodies, intravenous immunoglobulin (IVIG), had the benefits in patients with moderate-stage AD who carried an APOE ε4 allele. However, the mechanism by which IVIG played a role in patients remained unclear. Here, we investigated the properties of the antibodies in IVIG in vitro and in vivo.

Methods: Naturally occurring autoantibodies against amyloid-β oligomers (nAbs-Aβo) were purified from IVIG by Aβ oligomer or Cibacron blue affinity chromatography. The effect of nAbs-Aβo on Aβ aggregation and cytotoxicity were analyzed by thioflavin T fluorescence and MTT assay, respectively. The antibodies were injected intraperitoneally to the APPswe/PS1dE9 transgenic mice and the spatial memory and cognition were assessed by morris water maze test and novel object recognition.

Results: nAbs-Aβo purified by both affinity chromatography specifically recognized the Aβ oligomers, inhibited Aβ42 aggregation and attenuated Aβ42-induced cytotoxicity in SH-SY5Y neuroblastoma cells. nAbs-Aβo significantly improved the spatial memory and cognition, reduced the soluble Aβ and oligomers levels. Moreover, nAbs-Aβo reduced the production of pro-inflammatory cytokines, such as TNF-α and IL-6 in AD mice.

Conclusions: Our findings suggested that nAbs-Aβo may have therapeutic potential for AD mice and patients by inhibiting neurotoxicity of Aβ oligomers, and decreasing Aβ levels and pro-inflammatory cytokine production.

Symposium 16: TRANSLATIONAL STRATEGIES 1: ABETA TARGET

ADPD5-0255

NPT088: A DRUG CANDIDATE THAT TARGETS AMYLOID-BETA, TAU, AND ALPHA-SYNUCLEIN AGGREGATES

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Protein misfolding that produces assemblies of toxic and transmissible aggregates is a central feature of the pathobiology of neurodegenerative diseases. A serendipitous discovery that direct exposure to filamentous bacteriophage mediates reductions of both amyloid-beta and tau deposits in brains of transgenic mouse models of Alzheimer's disease led to a search for the mechanism. We have isolated and characterized a fragment of the capsid protein responsible for the amyloid targeting activities of the phage, and we show that an immunoglobulin fusion of this General Amyloid Interaction Motif (GAIM), called NPT088, recapitulates the efficacy of the phage both in vitro and in transgenic models of Alzheimer's disease, tauopathy, and Parkinson's disease following chronic systemic administration. Assays for amyloid fiber remodeling, fiber assembly inhibition, and neuroprotection from cytotoxic oligomers together suggest that this GAIM mediates these activities by potently preventing edge-to-edge beta strand aggregation. NPT088 represents a novel, potent, and specific therapeutic candidate for reducing pathologic misfolded protein assemblies that are central players in neurodegenerative diseases.

Symposium 21: TRANSLATIONAL STRATEGIES 2: NUTRITION, TAU TARGETS

ADPD5-2326

DETECTING EFFICACY OF SOUVENAID IN PATIENTS WITH ALZHEIMER'S DISEASE USING NETWORK ANALYSIS

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Alzheimer's disease (AD) clinical trials typically employ an array of outcome measures in order to capture the high dimensionality of dementia. These outcomes are traditionally analysed separately, resulting in much useful clinical information being lost. For this reason, new analytical methods to recognize treatment responses, using techniques that better embrace the high dimensionality of the dementia syndrome, might prove useful.

This study aimed to ascertain the viability of detecting treatment signals using network analysis, a well-established set of techniques employed in a wide range of applications (e.g. ecology to stock markets) but to date little used in dementia clinical trials. Our specific objectives were to evaluate the feasibility and performance of analysing changes in the degree of network connectivity in recent clinical trials of Souvenaid®, a medical nutritional drink for the dietary management of mild AD. Souvenir I and Souvenir II trials both showed significantly more connectivity in the treatment group compared to the placebo, with the difference being evident as early as 12 weeks. The increased connectivity in the Souvenir I and II studies suggest a more widespread treatment effect than when the measures are analysed individually. In this way, the network analysis approach allows overall treatment change to be demonstrated. By considering each treatment arm in a clinical trial as a network, the information from all outcome measures employed in the trial can be evaluated. Further work on translating this into clinically recognizable treatment effects is ongoing.

Symposium 21: TRANSLATIONAL STRATEGIES 2: NUTRITION, TAU TARGETS

ADPD5-2325
SOUVENAID: A NUTRITIONAL INTERVENTION DESIGNED TO ENHANCE SYNAPSE FORMATION IN EARLY ALZHEIMER'S DISEASE
D. Wilkinson
Synapse loss and synaptic dysfunction are established features of the neuropathological processes involved in the early stages of Alzheimer's disease (AD). This early involvement of synapses is very likely the neuropathological substrate for the early symptoms. Indeed, synapse loss has been shown to be the closest pathological correlate of memory dysfunction. Consequently, synapse loss and membrane-related pathology provide viable targets for intervention in AD. Studies have shown that blood levels of key nutrients, essential for the formation and turnover of synapses are lower in patients with AD. This includes uridine, docosahexaenoic acid, eicosapentaenoic acid, choline, phospholipids, in combinations with certain vitamins and cofactors such as folic acid, vitamins B12, B6, C, E, and selenium. Furthermore lower brain and CSF levels of these nutrients have been demonstrated in subjects with AD.
The specific nutrient combination Souvenaid, was developed to provide the key nutrients required for synapse formation and membrane-related pathology in order to ameliorate synapse dysfunction and loss in AD.
This talk will focus on the rationale and preclinical evidence behind the hypothesis, that providing nutritional precursors and cofactors will act together to support membrane formation, and the broad clinical study program investigating the potential of this nutrient combination in AD.

Symposium 21: TRANSLATIONAL STRATEGIES 2: NUTRITION, TAU TARGETS

ADPD5-1901
TRANSLATING THE BIOLOGY OF AGING TO NOVEL THERAPEUTICS FOR NEURODEGENERATIVE DISEASE

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Aging is clearly the leading risk factor for sporadic Alzheimers disease. While research on the biology of aging has progressed rapidly in the past decades, translating this knowledge to develop a better understanding of how aging and age-related diseases might ultimately cause neuronal dysfunction and death in Alzheimers has not been extensively explored. Age-related pathobiological mechanisms can also link lifestyle and other risk factors to cognitive aging and Alzheimers with implications for prevention. Applying knowledge from the biology of aging to neurodegeneration creates opportunities for new drugs to prevent and treat Alzheimers disease. Here, we will provide an overview of drug discovery and development for Alzheimers based on our understanding of the biology of aging as it relates to neurodegeneration.

Symposium 21: TRANSLATIONAL STRATEGIES 2: NUTRITION, TAU TARGETS

ADPD5-1866
GENE THERAPY APPROACHES TO TAUOPATHY
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Arginase-1 converts arginine to ornithine and subsequently polyamines, such as spermidine and spermine. It also can deplete arginine, reducing the production of nitric oxide. In cultured C3H cells overexpressing human tau, increased arginase1 expression led to decreased tau, decreased phosphotau, decreased nitrated tau and reduced mTOR, implying increased autophagy. Adding excess arginine to these cells increased tau, phospho-tau and elevated mTOR, implying reduced autophagy. When added to in vitro tau aggregation assays, spermidine and spermine inhibited tau aggregation at physiological concentrations. These results suggest that increased arginase1 expression may benefit the tau phenotype. 4 mo old Tg4510 mice were injected bilaterally with arginase1-AAV9 into the hippocampi and tissues were collected at 8 mo of age. Histological analysis of one hemisphere indicated successful increases in arginase expression. They also revealed significant 40% reductions in phospho-tau, Gallyas silver staining and reduced atrophy of the hippocampus. Western analysis of SDS soluble fractions found decreased levels of 50-60kD phospho-tau isoforms and nitrated tau plus 130-180kD multimers of total tau, phospho-tau and nitrated tau. Kinases GSK-3B, p38MAPK, CDK-5 (but not casein kinase) were reduced by the treatment. Inflammatory cytokines IL-1B, IL-12, and INF-gamma were reduced to nontransgenic levels. Proteins known to inhibit autophagy were also reduced, suggesting increased autophagy. These results suggest that gene therapy using arginase1 may have therapeutic potential for tauopathies.

Symposium 21: TRANSLATIONAL STRATEGIES 2: NUTRITION, TAU TARGETS

ADPD5-1562
THE LEUKOTRIENES PATHWAY IN TAUOPATHY
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Tauopathies are diseases characterized by an accumulation of pathological tau protein leading to progressive neurodegeneration. Current drug interventions for these diseases are limited to the treatment of symptoms without directly affecting tau pathology or the resultant neuronal dysfunction, underscoring the need for development of tau-targeted therapeutics.
The 5-lipoxygenase is an enzyme widely expressed in the central nervous system and the source of pro-inflammatory lipids called leukotrienes (LTs). Recent evidence suggests that pharmacological or genetic modulation of their levels directly influences neurodegenerative processes and improve cognition.
However, no data are available as to whether leukotriene pathway is elevated in human tauopathy, or if it directly influences tau pathology in a relevant model of the disease. In this study we provide the first evidence that the leukotrienes pathway is up-regulated in human tauopathy (i.e, Progressive supranuclear palsy) and in transgenic tau mice. Furthermore, we demonstrate that leukotrienes play a functional role in the development of tau pathologic phenotype since their pharmacological suppression in transgenic tau mice results in memory improvement, rescue of synaptic integrity and dysfunction and reduction of tau pathology via a cdk5-dependent mechanism.
Our results establish leukotrienes as a key player in the development of the entire spectrum of tau pathology phenotype, and a novel viable therapeutic target for the pharmacological treatment of human tauopathy.

Symposium 21: TRANSLATIONAL STRATEGIES 2: NUTRITION, TAU TARGETS

ADPD5-1499
TAU ANTIBODY EFFECTOR FUNCTION STATUS DETERMINES INFLAMMATORY RESPONSES IN TARGETING PROPAGATION OF TAU PATHOLOGY

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Immunotherapy to neurodegenerative diseases has been demonstrated to hold promise in preclinical models and in clinical trials. However, despite exquisite target specificity, antibodies harbor the risk of aggravating neuroinflammation in an already chronically inflamed brain. We hypothesized that antibody effector function might be both beneficial in slowing the spreading of tau, yet have the potential to cause neural toxicity by stimulating gliosis. Anti-Tau with full effector function may promote microglia engulfment and clearance of extracellular toxic protein, while also inducing FcgR-mediated microglial activation that could have deleterious effects on neurons. We therefore tested, in a tauopathy mouse model, whether effector function is required for efficacy. Tau-P301L transgenic mice were dosed with full-effector or effectorless antibodies targeting S409 phospho-tau for 3 months. Both reduced tau pathology relative to control-treated mice, measured by IHC and WB. In primary cultures, both antibodies rescued neurons from extracellular tau-mediated toxicity independent of effector function. In cultured microglia effector function enhanced tau uptake, however also induced release of proinflammatory cytokines. Effectorless antibodies prevented both tau uptake and activation by microglia. In neuron-microglia co-cultures only the effectorless antibody rescued neurons. We show that effector function is not required for efficacy in vivo, and propose that effector function status may determine antibody mechanism-of-action. Furthermore, we suggest that effectorless antibodies are potentially safer. We conclude that engineering Tau antibodies to have attenuated effector function may mitigate potential deleterious inflammatory consequences of antibody treatment, while still preserving the ability to limit the spread of toxic proteins in vivo.

Symposium 23: TRANSLATIONAL STRATEGIES 3: CHOLINERGIC TARGETS, STEM CELLS AND GROWTH FACTORS

ADPD5-1903
TARGETING THE P75 NEUROTROPHIN RECEPTOR FOR ALZHEIMER'S THERAPEUTICS: BASIC MECHANISMS THROUGH PHASE 1
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The p75 neurotrophin receptor ($p75^{NTR}$) is linked to signaling networks promoting synaptic dysfunction and degeneration in AD. Its expression occurs in neuronal populations undergoing degeneration, including cortical, hippocampal, basal forebrain, raphe, and locus coeruleus neurons and is increased in AD and mouse AD models. In its predominant signaling state, $p75^{NTR}$ promotes degenerative signaling and knockout studies demonstrate that it enables Aβ-induced degeneration *in vitro* and *in vivo*. We have developed small molecule $p75^{NTR}$ ligands that inhibit its degenerative signaling and prevent Aβ-oligomer induced neurite and synaptic degeneration. *In vitro* studies demonstrate inhibition of Aβ-induced: GSK3β, cdk5, p38 and c-Jun activation; tau phosphorylation and missorting; compromised AKT and CREB function; RhoA/cofilin activation; FYN activation/NR2B phosphorylation; and inhibition of LTP. *In vivo* studies employing oral administration in $APP^{Lond/Swe}$ mice demonstrate $p75^{NTR}$ target engagement, inhibition of tau phosphorylation and misfolding, inhibition of neurite and spine degeneration (including late-stage reversal of degeneration), inhibition of microglial activation; and improved performance in multiple behavioral tests, with no effects on Aβ levels. Inhibition of neuronal degeneration in Tg2576 and Ts65Dn mice, along with reversal in wild type mice of age-related cholinergic neurite atrophy, indicate that mechanisms extend beyond a single transgenic mouse model. $APP^{Lond/Swe}$ mouse micro-PET and autoradiography studies with a TSPO PET ligand revealed $p75^{NTR}$ ligand-mediated inhibition of elevated signaling consistent with inhibition of microglial activation. Phase 1 safety and PK studies with a derivative of the prototype $p75^{NTR}$ ligand have been successfully completed and Phase 2a-enabling studies are underway. Funding: ADDF, Alzheimer’s Association, NIA

Symposium 23: TRANSLATIONAL STRATEGIES 3: CHOLINERGIC TARGETS, STEM CELLS AND GROWTH FACTORS

ADPD5-1526
ALPHA7 RECEPTOR AGONISTS AS PRO-COGNITIVE AGENTS
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While anti-amyloid therapies in Alzheimer's disease (AD) hold great promise, such treatments may only slow functional decline. Therefore additional therapies are needed that will help to restore lost function. We discuss the current understanding of the mechanism of action of alpha7 nicotinic acetylcholine receptor (alpha 7) agonists, therapies that may restore cognitive function. We will compare clinical efficacy data for encenicline, an alpha 7 partial agonist currently in phase 3 development for AD and cognitive impairment in schizophrenia, with preclinical data. We will propose a model of how alpha 7 agonists may affect neuronal networks and enhance cognition. Encenicline activates alpha 7 receptors at concentrations below the Ki in the presence of acetylcholine, a phenomenon we term priming. alpha 7 agonists affect hippocampal neuronal networks involved in cognition at priming concentrations through enhancing GABAergic inhibitory post-synaptic currents, long-term potentiation (LTP), and theta synchrony. Independent pharmacokinetic (PK) studies and pharmacodynamics-pharmacokinetic modeling confirmed that plasma concentrations capable of increasing theta rhythm power *in vivo* corresponded to priming concentrations *in vitro* and to active concentrations in preclinical models of cognition. These data extend the concept of priming activity at alpha 7 receptors into neuronal systems at the circuit level. At priming concentrations, these data with encenicline analogs are consistent with the important role for increased GABAergic tone, mediated by interneurons, in the generation of theta synchrony and its concomitant role in memory.

Symposium 23: TRANSLATIONAL STRATEGIES 3: CHOLINERGIC TARGETS, STEM CELLS AND GROWTH FACTORS

ADPD5-1434

THE GALANTAMINE PRODRUG, MEMOGAIN®, REVERSES DEFICITS IN HIPPOCAMPAL NEUROGENESIS AND MIGRATION FOLLOWING CHOLINERGIC DENERVATION.

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Loss of basal forebrain cholinergic innervation of the hippocampus and severe neuronal loss within the hippocampal CA1 region are early hallmarks of Alzheimer's disease (AD), and are strongly correlated with cognitive status. This loss of cholinergic innervation is a key factor underlying alterations in hippocampal neurogenesis, which are also characteristic of AD. We have previously reported the effects of various cholinergic compounds on hippocampal neurogenesis indicating that acetylcholine serves as a potent neurogenic regulator.

Memogain® (GLN 1062) is an inactive galantamine pro-drug with 15 fold higher brain availability than galantamine. It is designed to provide improved blood brain barrier penetration, greater potency, and fewer side effects than the cholinesterase inhibitors currently used for the treatment of AD. Galantamine is unique among the cholinesterase inhibitors in that it also has allosteric actions at α-7 nicotinic receptors, linked to both disease-modifying and cognitive enhancing effects.

The immunotoxin, 192IgG saporin (SAP), used to induce selective basal forebrain cholinergic cell loss, resulted in a pronounced loss of basal forebrain cholinergic neurons and hippocampal ChAT fiber density. SAP-lesioned animals also displayed significant reductions in hippocampal neuroprogenitor populations, reduced cell proliferation, and disrupted neuronal migration, when compared to sham-operated control animals, as well as significant impairments in spatial working memory. By contrast, animals treated with Memogain® displayed a restoration of hippocampal cell proliferation, increased neuronal cell counts, normalized neuronal migration, and improvements in cognitive function. Thus, the beneficial effects of Memogain® may extend beyond acute cognitive enhancement, to include disease modification through support of hippocampal neurogenesis.

Symposium 23: TRANSLATIONAL STRATEGIES 3: CHOLINERGIC TARGETS, STEM CELLS AND GROWTH FACTORS

ADPD5-1002

ROLE OF CREB IN AMYLOID-BETA-INDUCED BDNF DOWN-REGULATION

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Objectives: Accumulation of amyloid-β (Aβ) peptide results in loss of brain-derived neurotrophic factor (BDNF), loss of functional synapses and subsequent neurodegeneration and memory loss. Previous work has demonstrated that Aβ decreases activity-induced BDNF expression by regulating CREB phosphorylation, which occurs via both activation of GSK3β and inactivation of PKA. However, the mechanism by which Aβ reduces basal levels of BDNF remains unclear.

Methods: Differentiated, unstimulated human neuroblastoma (SH-SY5Y) cells were treated with 5μM oligomeric Aβ prior to quantification of BDNF and CREB mRNA via qRT-PCR. Phosphorylated and total CREB protein levels were analyzed in both the cytoplasmic and nuclear fractions. Lastly, the GSK3β inhibitor CT99021 or the PKA activator forskolin were added to SH-SY5Y cells with or without Aβ treatment to determine if activating CREB by either mechanism could protect against Aβ-induced loss of basal BDNF expression.

Results: CREB mRNA was reduced in Aβ-treated cells compared to controls. Phosphorylated and total CREB proteins were decreased in both the cytoplasm and nucleus of Aβ-treated cells. However, neither pCREB129 nor pCREB133 levels were altered relative to total CREB levels. Although CT99021 did not protect cells against Aβ-induced BDNF down-regulation, forskolin increased pCREB133 levels and prevented Aβ-induced BDNF down-regulation.

Conclusions: Aβ down-regulates basal levels of BDNF in the absence of cell stimulation. This is a consequence of Aβ-induced CREB transcriptional down-regulation rather than changes in CREB phosphorylation. Aβ reduces basal BDNF expression and activity-induced BDNF expression by different mechanisms. However, pCREB133 protects against both basal and activity-induced BDNF down-regulation following Aβ treatment.

Symposium 23: TRANSLATIONAL STRATEGIES 3: CHOLINERGIC TARGETS, STEM CELLS AND GROWTH FACTORS

ADPD5-0993
POTENTIAL STEM CELL THERAPY FOR AD AND PD
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Recent studies of stem cell show its therapeutic potential for neurodegenerative disorders. The aim of the present study was to elucidate the preventive and therapeutic potential of stem cells for AD and PD. Among stem cells, autologous human adipose-derived stem cells (hASCs) elicit no immune rejection responses, tumorigenesis, or ethical problems.
Here, we used hASCs and examined whether intravenously or intracerebrally transplanted hASCs could have therapeutic and preventive effects in AD mouse model (Tg2576). We first report that intravenously or intracerebrally transplanted hASCs significantly rescues memory deficit and neuropathology in the brains of Tg mice by up-regulating IL-10 and VEGF and by elevating synaptic and dendritic stability. More importantly, our findings that transplanted hASCs prevent or delay the onset and progression of the disease strongly suggest that the simple, convenient and safe intravenous injection of hASCs can be very useful in both the prevention and treatment of AD.
Parkinson's disease (PD) is caused by the progressive degeneration of dopaminergic neurons and is characterized by cytoplasmic inclusions known as lewy bodies in the substantia nigra. Recently, treatment of PD using stem cells has been in the spotlight. In many studies, it has been described that the structural and functional alteration of mitochondria were associated with neurodegenerative diseases including PD. Therefore, we investigated the effects of intra-venous injection of hASCs on mitochondrial functions in PD mouse model induced by 6-hydroxydopamine (6-OHDA). Intravenous injection of hASCs greatly improved behavior and mitochondrial dysfunction in PD mice model.

Symposium 23: TRANSLATIONAL STRATEGIES 3: CHOLINERGIC TARGETS, STEM CELLS AND GROWTH FACTORS

ADPD5-0266

DEFICIENT NEUROGENESIS PLAYS A ROLE IN COGNITIVE IMPAIRMENTS IN ALZHEIMER'S DISEASE

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Objectives (1) Examine whether adult neurogenesis plays a role in learning and memory impairments and cognitive deficits. (2) Determine whether deficient neurogenesis can induce cognitive deficits in Alzheimer's disease (3) Determine whether loss of gamma-secretase function in neural progenitor cells induces cognitive deficits.

Methods For objective 1,2 we temporally depleted neurogenesis by conditional nestin-regulated expression of δ-HSV-TK in neural progenitor cells in the brain of mice harboring FAD-linked APPswe/PS1-delta-E9. For objective 3 expression of presenilin-1 in hippocampal neural progenitor cells was downregulated by lentiviral vectors expressing shRNA for the targeting of presenilin-1.

Results (1) Depletion of neurogenesis was manifested by a dramatic decrease in the number of new neurons in the subgranular layer of the dentate gyrus of the hippocampus. (2) Depletion of neurogenesis induced and enhanced deficits in contextual memory, pattern separation, novelty recognition and spatial memory in the FAD mice. (3) Downregulation of presenilin-1 in hippocampal neural progenitor cells induces learning and memory deficits in adult mice. New neurons expressing downregulated presenilin-1 levels exhibit reduced dendritic branching and reduced number of dendritic spines. (4) Presenilin-1 regulates adult neurogenesis via beta-catenin and notch signaling.

Conclusions These experiments provide, for the first time, (1) Evidence that impairments in neurogenesis promote cognitive deficits in Alzheimer's disease (2) A novel mechanism underlying learning and memory deficits in Alzheimer's disease. Enhancing neurogenesis may attenuate or prevent learning and memory impairments characterizing the disease.

Symposium 26: AMYLOID-REDUCING THERAPIES IN ALZHEIMER'S DISEASE

ADPD5-1927
TREATMENT OF ALZHEIMER DISEASE; THE PATHWAY TO 2025

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In 2012, the World Health Organization presented a report where Alzheimer Disease (AD) and other dementias should be regarded as a global public health priority. A similar policy declaration has been presented by the European Union and USA. The main reason for these policy declarations is the awareness of trends that sometimes are described as a "time-bomb". In 2010, the number of people affected by dementia worldwide was estimated to 36 million, with an estimated cost of approx 600 bUSD. The prevalence of dementia is expected to reach 115 million in 2050, with an equivalent cost increase.

The progressive nature of dementia influences the whole life situation for families during several years-decades and so far, no cure or highly significant symptom relieving treatment is available. Increased understanding of the pathophysiology of AD has given us new therapeutic targets, and by using new biomarkers possibilities to diagnose patients earlier.

The last drug to enter the market place was in 2002. Since then, many products in different development phases have failed. Why? Wrong molecules, inappropriate animal models, inappropriate proof-of-concept studies, heterogeneous patient groups, too advanced disease, non-relevant outcome measures, intercenter variability in increasingly globalised multi-centre trials?

Our hope for the future is not only to give the patient an early symptomatic relief but that new therapies could potentially slow or even halt the progression of the disease. Increased global collaboration between academia, industry and regulatory authorities is a vital step for a successful drug development.

We have good hope that until 2025, ongoing studies directed towards beta-amyloid and/or tau metabolism have proven effective and are on the market.

Symposium 26: AMYLOID-REDUCING THERAPIES IN ALZHEIMER'S DISEASE

ADPD5-1683

A 52-WEEK PILOT STUDY TARGETING ABETA WITH PBT2: NEUROIMAGING RESULTS

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Objectives The trial aimed to explore putative imaging markers to help inform future clinical trial design of PBT2 in Alzheimer's disease (AD).

Methods We conducted a 52-week, double-blind, randomized, placebo-controlled trial involving 42 patients with mild AD (71.1±9.2 years, MMSE 24.2±2.5) with a baseline ^{11}C-PiB-SUVR ≥1.7. Participants received either PBT2 (250mg/day) or placebo for 52 weeks. Primary outcome measure was Aβ burden as measured by ^{11}C-PiB-PET at baseline and 12 months. Participants also underwent periodic neuropsychological evaluation, FDG-PET and MRI scans.

Results PBT2 was well tolerated. At baseline, there were no significant between-group differences in age, gender, ApoE, hippocampal volume, PiB or FDG SUVR. Baseline ^{11}C-PiB-SUVR ranged between 1.73 and 3.31 (median 2.5). Despite PBT2-treated patients showing a significant decrease in Aβ burden at 12 months (-2.5%, p=0.048) post-hoc, this was accompanied by a similar decrease in the placebo group (-3.1%, p=0.06), yielding no significant between-group differences. Similarly, no significant between-group differences were found on cognition, FDG or MRI volumetrics, though a trend towards a slower rate of hippocampal atrophy on PBT2-treated patients (p=0.09) was observed. When analyzed separately, PBT2-treated participants with baseline >SUVR 2.5 (n=11) showed significant reductions in PiB-SUVR (p=0.002) while those with <SUVR 2.5 (n=14) did not (p=0.6), and while the between-groups differences became more pronounced, they did not reach statistical significance.

Conclusions There were no between-group differences in Aβ burden in PBT2 treated and untreated mild AD patients, despite a significant reduction in Aβ in the PBT2-treated patients, especially in those with a baseline PiB-SUVR ≥2.5.

Symposium 26: AMYLOID-REDUCING THERAPIES IN ALZHEIMER'S DISEASE

ADPD5-1525

BIOMARKER PATTERN OF ARIA-E SUBJECTS IN 2 PHASE 3 CLINICAL TRIALS OF BAPINEUZUMAB

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Objective: Amyloid-Related Imaging Abnormalities with edema/effusion (ARIA-E) are observed with anti-amyloid therapies in Alzheimer's disease (AD). This study evaluated whether ARIA-E was associated with specific biomarker patterns.
Methods: Bapineuzumab, an anti-amyloid-β therapy, was evaluated in separate trials for *APOE*4 carriers and non-carriers with mild-moderate AD. Subsets underwent PET, CSF, or volumetric MRI assessments.
Results: 223, 558, and 1398 subjects underwent PET, CSF, and vMRI assessments, and 22, 64, and 134, respectively, developed ARIA-E. No differences in baseline brain PET amyloid signal (GCA SUVr) or vMRI measures (WBV, VV, HCV) were observed between ARIA-E and non-ARIA-E subjects. No differences in baseline CSF Aβ42, Aβ40, t-tau, or p-tau were observed, except Aβ42 in *APOE*4 non-carrier ARIA-E vs non-ARIA-E (bapineuzumab/placebo p=0.026/0.012). Bapineuzumab-treated subjects with ARIA-E showed greater reduction at week 71 in GCA SUVr and greater VBSI and HBSI vs non-ARIA-E (all comparisons PET: p<0.001-0.059; VBSI/HBSI: p<0.001). Greater CSF p-tau reduction at week 71 was observed in ARIA-E subjects (all comparisons p<0.001-0.096). Greater t-tau reduction was seen for ARIA-E vs non-ARIA-E (*APOE*4 carriers: bapineuzumab/placebo p=0.022/0.018; non-carriers placebo: p=0.054). No differences in changes at week 71 were observed in CSF Aβ42 or Aβ40, except for decreased Aβ40 in ARIA-E *APOE*4 carriers vs non-ARIA-E (bapineuzumab/placebo p= 0.042/0.089). **Conclusion:** Except for PET amyloid signal and CSF Aβ42 in *APOE*4 non-carriers, baseline biomarkers do not predict risk for developing ARIA-E. Longitudinal changes in several biomarkers were significantly

associated with ARIA-E. These data support the hypothesis that ARIA-E may be related to Aβ efflux from the brain.

Symposium 26: AMYLOID-REDUCING THERAPIES IN ALZHEIMER'S DISEASE

ADPD5-1471
PROFILING THE DYNAMICS OF CSF AND PLASMA ABETA REDUCTION WITH JNJ-54861911, AN ORAL BACE INHIBITOR

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Objectives
Reduction of Aβ production by inhibition of BACE1 has been proposed as a promising treatment in Alzheimer's Disease. JNJ-54861911 is a novel oral BACE inhibitor. We report plasma and CSF Aβ reductions in phase 1 clinical trial, investigating target engagement.
Methods
Healthy participants, aged 55 to 85, were randomized (JNJ-54861911/placebo) in a single dose (1-150mg) and subsequent multiple ascending 14-day dose (5-90mg) trial, to assess safety, tolerability, plasma and CSF (36h catheterization) pharmacokinetics (PK) and pharmacodynamics (PD) including Aβ (Aβ1-37, 1-38, 1-40 and 1-42), sAPPα and sAPPβ. To account for inter-subject variability of baseline Aβ and sAPP levels, reductions were expressed as % of predose.
Results
After single dose, plasma Aβ reached maximal reduction after 3-4 hours, while CSF Abeta started declining only after 6-8 hours post-dose. Aβ reductions persisted long after compound levels declined. This resulted in sustained Aβ reductions, up to 95%, after 14 days of dosing. The changes observed for all 4 Aβ peptides were dose-dependent and similar, but Aβ1-40 (most prevalent in plasma and CSF) had the best assay characteristics. sAPPβ peptides reduced in parallel, while sAPPα increased up to 2-3 fold. These effects appear to be independent of baseline factors (e.g. Aβ or APOE genotype).
Conclusions
JNJ-54861911 is a potent, brain-penetrant BACE inhibitor, achieving up to 95% Aβ reduction with once daily oral dosing. Aβ reduction outlasted plasma/CSF PK, leading to sustained PD activity. It is plausible that plasma and CSF Aβ reductions are driven by peripheral and central activity, respectively.

Symposium 26: AMYLOID-REDUCING THERAPIES IN ALZHEIMER'S DISEASE

ADPD5-0966

RANDOMIZED, DOUBLE-BLIND, PHASE 1B STUDY OF BIIB037, AN ANTI-AMYLOID BETA MONOCLONAL ANTIBODY, IN PATIENTS WITH PRODROMAL OR MILD ALZHEIMER'S DISEASE

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Objectives: BIIB037 is a fully human monoclonal antibody selective for aggregated forms of amyloid beta-peptide being investigated as a disease-modifying treatment for Alzheimer's disease (AD). Results of an interim analysis of a Phase 1b study evaluating safety, tolerability, pharmacokinetics, and pharmacodynamics of BIIB037 in patients with prodromal or mild AD will be presented.
Methods: This is a multicenter, randomized, double-blind, placebo-controlled, multiple-dose study of BIIB037. Patients were 50–90 years of age, positive for amyloid beta deposition as assessed by florbetapir (^{18}F-AV-45) positron emission tomography (PET) scan, and met clinical criteria for prodromal AD or mild AD. During the double-blind, placebo-controlled phase, patients received BIIB037 or placebo by intravenous infusion once every 4 weeks for 52 weeks. In a staggered, parallel-group design, patients were randomized to 1 of 5 treatment arms and stratified by APOE epsilon 4 status (carrier or non-carrier). The primary endpoints were safety and tolerability. Secondary endpoints included BIIB037 pharmacokinetics and change from baseline to Week 26 in brain amyloid plaque burden as measured by PET imaging.
Results: A total of 126 patients were randomized. Incidence of adverse events, serious adverse events, and amyloid-related imaging abnormalities (ARIA) will be presented. Quantitative analysis of ^{18}F-AV-45 PET imaging will assess the effect of BIIB037 on cerebral amyloid plaque content.
Conclusions: These data will provide information on the safety and tolerability of BIIB037. The effectiveness of BIIB037 in reducing amyloid plaque burden will be addressed.

Symposium 29: ER STRESS, SIGMA-1 RECEPTORS AND GPCRS AS THERAPEUTIC TARGETS IN CNS DISEASE

ADPD5-1916
ABETA 42 OLIGOMER BINDING TO NEURONAL SIGMA-2/PGRMC1 RECEPTORS IS DISPLACED BY DRUG CANDIDATES THAT IMPROVE COGNITIVE DEFICITS

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Objectives: We have discovered novel therapeutic antagonists of a receptor not previously associated with AD (sigma-2/PGRMC1) capable of blocking Abeta oligomer toxic effects on synapses in vitro and cognitive deficits in vivo. We sought to define the role of this receptor in mediating Abeta oligomer-induced deficits.
Methods: Knockdown of sigma-2/PGRMC1 (progesterone receptor membrane component 1) protein expression in vitro using siRNA results in a highly correlated reduction in binding of exogenous Abeta oligomers to neurons of more than 90%. Expression of sigma-2/PGRMC1 protein is upregulated in vitro by treatment with Abeta oligomers, and is dysregulated in Alzheimer's disease patients' brain compared to age-matched, normal individuals. Specific, high affinity small molecule receptor antagonists can displace synthetic Abeta oligomer binding to synaptic puncta in vitro and displace endogenous human AD patient oligomers from brain tissue sections in a dose-dependent manner. These receptor antagonists prevent and reverse the effects of Abeta oligomers on membrane trafficking and synapse loss in vitro and cognitive deficits in AD mouse models.
Results: These findings suggest sigma-2/PGRMC1 receptors mediate saturable oligomer binding to synaptic puncta on neurons and that brain penetrant, small molecules can displace endogenous and synthetic oligomers and improve cognitive deficits in AD models.
Conclusions: We propose that sigma-2/PGRMC1 is a key mediator of the pathological effects of Abeta oligomers in AD and is a tractable target for small molecule disease-modifying therapeutics. These receptor antagonists represent promising disease modifying drug candidates that will be studied in planned clinical trials.

Symposium 29: ER STRESS, SIGMA-1 RECEPTORS AND GPCRS AS THERAPEUTIC TARGETS IN CNS DISEASE

ADPD5-1598
TARGETING THE SIGMA-1 PROTEIN FOR NEUROPROTECTION IN ALZHEIMER'S DISEASE

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The sigma-1 receptor (S1R) is a ligand-operated molecular chaperone expressed in the brain and localized on endoplasmic reticulum (ER), mitochondria and plasma membranes. Its activation modulates IP_3 receptor-dependent Ca^{2+} mobilizations, facilitates the activation of ER stress sensor proteins and kinase pathways. Under chronic activation, it is also involved in recomposition of lipid domains on the plasma membrane, which are highly functionalized domains. Interestingly, the chaperone can be directly activated (or inactivated) by several classes of natural and synthetic ligands, explaining its historic misunderstanding as a classical membrane receptor. Ligands modulating the S1R ligands have been shown to be potent neuromodulatory and protective drugs in different neurodegenerative insults and apthologies (stroke, Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis…). We examine the involement of the S1R in Ad pathology and validate selective or non-selective S1R agonists as neuroprotective agents. First, we analyzed the impact of S1R invalidation (using S1R KO mice) on the vulnerability to AD pathology. Two main AD models were used, a nontransgenic model by direct icv injection of oligomeric amyloid-b (Ab) protein fragments [25-35] (Ab_{25-35}) in mice and transgenic animals overexpressing $hAPP_{Swe}$ or $hAPP_{SweInd}$. We observed that AD toxicity is significantly amplified in S1R KO mice injected with Ab_{25-35} and in S1R KO x $hAPP_m$ lines. Second, we showed the protective potency of S1R agonists and mixed muscarinic/S1R ligands in AD models. The pathology was analyzed in terms of ER and oxidative stress, inflammation, mitochondrial damage, cell loss, memory deficits, increased APP processing and Tau hyperphosphorylation. We therefore confirmed the role of endogenous neuroprotection system in neurodegenerative processes and identified S1R agonists as potent neuroprotective and putatively disease-modifying agents.

Symposium 29: ER STRESS, SIGMA-1 RECEPTORS AND GPCRS AS THERAPEUTIC TARGETS IN CNS DISEASE

ADPD5-0875
CELLULAR STRESS SIGNALING AT THE MITOCHONDRION-ER-NUCLEUS AXIS: ROLES OF THE SIGMA-1 RECEPTOR CHAPERONE

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The sigma-1 receptor (Sig-1R) is ligand-operated endoplasmic reticulum (ER) chaperone that resides specifically at the ER-mitochondrion interface referred to as the MAM (**M**itochondrion **A**ssociated ER **M**embrane). At the MAM, Sig-1Rs serve to (1) chaperone the IP3 receptor type 3 to maintain proper Ca2+ signaling from the ER into mitochondria to ensure sustenance of sufficient bioenergetics; (2) attenuate free radical generation at the ER for proper ER-mitochondrion-plasma membrane signaling that is essential for dendritic spine formation; (3) chaperone the ER stress sensor IRE1 to ensure proper transmission of stress signaling in the mitochondrion-ER-nucleus axis to enhance cellular survival at the genomic level. Interestingly, in addition to those actions at the MAM, Sig-1R agonists like psycho-stimulant cocaine or certain neurosteroids can cause the Sig-1R to dissociate from their cognate co-chaperone BiP at the MAM and translocate the Sig-1R thereby to the plasma membrane to interact with other receptors, ion channels, or kinases to trigger a plethora of pharmacological responses. The Sig-1R agonists can also cause Sig-1Rs to translocate to the nucleus and extracellular space. We are in the process of understanding the complete underpinning mechanism of the dynamic action of this molecular chaperone Sig-1R as it has been implicated in diseases like Alzheimer's, Parkinson's, ALS/FTLD, HIV-associated neuro-dementia, and addiction, and may thus provide new therapeutic avenues for those diseases.

Symposium 29: ER STRESS, SIGMA-1 RECEPTORS AND GPCRS AS THERAPEUTIC TARGETS IN CNS DISEASE

ADPD5-0828
POSSIBLE ROLES OF CALCIUM STORES IN THE INITIATION OF AD
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Objectives: Early life stress has been shown to affect adult brain and behavior, and to accelerate the development of mental disorders. In our earlier studies we found that release of calcium from stores is altered by stress, to affect the ability to undergo long term potentiation (LTP) of reactivity to stimulation. We have now tested the hypothesis that juvenile stress (JS) contributes to the development of deficits in neuronal plasticity in the triple-transgenic mouse model for Alzheimer's disease (3xTgAD) by interacting with regulation of calcium stores. Methods: We studied LTP in 3, 6 and 8 month-old 3xTgAD and wt mice. Juvenile animals were exposed to three stressors; restrain, forced swim and elevated platform. EPSPs were recorded in stratum radiatum of CA1 region of hippocampal slices. Results: Adult 3xTgAD mice exhibited significant deficits in LTP compared with wt mice. LTP in 3xTgAD control and stressed mice was rescued by pre-exposure to 0.2 μM ryanodine, in an age-dependent manner. Acting at a beta adrenergic receptor, isoproterenol (Iso) converted STP to LTP. This was mediated by activation of calcium stores, and was suppressed by JS, especially in the 3xTgAD slices. Biochemically, 3xTgAD express a reduced level of Sirtuin-1, a deacetylase involved in synaptic plasticity. Furthermore, stressed mice have altered ratio of Aβ42/40. Conclusions: These and more recent results highlight the intricate relations among calcium stores, ryanodine receptors, stress and catecholamines in the regulation of cognitive decline associated with AD, adding new dimensions to the plethora of factors that lead to the disease.

Symposium 29: ER STRESS, SIGMA-1 RECEPTORS AND GPCRS AS THERAPEUTIC TARGETS IN CNS DISEASE

ADPD5-0215
PRESENILINS, NEURONAL STORE OPERATED CALCIUM ENTRY AND SYNAPTIC LOSS IN ALZHEIMER´S DISEASE

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Background. Mutations in presenilins result in familial Alzheimer's disease (FAD). These mutations affect gamma-secretase function of presenilins and influence Ab42/Ab40 ratio. Our previous studies suggested that FAD mutations also affect endoplasmic reticulum (ER) calcium (Ca^{2+}) function of presenilins and result in ER Ca^{2+} overload (Tu at al, 2006. Cell 126, 981-993; Zhang at al. 2010. J Neurosci 30, 8566-8580). However, mechanistic connection between ER Ca^{2+} signaling dysregulation and synaptic loss in AD has not been previously established.
Methods. Perform studies of Ca^{2+} signaling and synaptic loss in hippocampal neurons from presenilin 1 (PS1) M146V knockin (KI) model of FAD. Our approach is centered on measurements of synaptic Ca^{2+} signals and analysis of postsynaptic spine shapes in wild type and PS1-M146V KI neurons.
Results: We discovered that PS1-M146V KI neurons compensate for ER calcium overload by downregulating STIM2 protein, a master regulator of neuronal store operated calcium entry pathway (nSOC). We further demonstrated that similar downregulation of STIM2 occurs as a result of normal aging process. In experiments with STIM2 conditional knockout mice we demonstrate that knockout of STIM2 results in destabilization of mushroom spines in hippocampal neurons. We further demonstrate that overexpression of STIM2 results in rescue of mushroom spine deficiency in PS1-M146V KI hippocampal neurons. We determined that synaptic CaMKII acts downstream of nSOC in spines.
Conclusions. We concluded that (1) STIM2-nSOC-CaMKII pathway is essential for long-term maintenance of mushroom spines; (2) function of this pathway is compromised in aging and AD neurons due to downregulation of STIM2.

Symposium 34: TRANSLATIONAL STRATEGIES 4: Gsk3BETA , Tau Targets (2)

ADPD5-2245
POTENTIAL FOR TAU ISOFORM-SPECIFIC ANTIBODIES FOR PASSIVE IMMUNOTHERAPY IN TAUOPATHIES

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Introduction: From Braak staging in Alzheimer's disease, it is now recognised that tau pathology spreads along anatomically connected pathways with progression of disease. The agent of this "prion-like" aggregation and trans-synaptic spread are pathological conformers of tau that are released by cells, endocytosed by neighbouring healthy neurons and in turn convert normal tau in a cascade-like fashion. Studies have shown the potential therapeutic benefit of targeting this extracellular tau with specific antibodies.
Objective: RD3 and RD4 antibodies that target regions in the microtubule-binding repeat domain of tau that are crucial for aggregation were investigated to demonstrate their efficacy in preventing pathological tau uptake and aggregation in a cell-based model.
Methods: Sequences encoding the wild-type, deltaK280 (Δ) and P301L/V337M (LM) double mutant tau repeat region were cloned into pEGFP-N1 and pDsRed-monomer-N1. SH-SY5Y neuroblastoma cells were co-transfected with mutant and WT tau constructs. After 48 hours, fixed cells were analysed by fluorescence resonance energy transfer (FRET) using both acceptor photo bleaching microscopy and a fluorescence plate reader with fixed excitation/emission windows.
Results: Strongest FRET signals in cells co-expressing the Δ and LM mutants were observed. Pre-incubation of the cells with AD brain homogenate further increased the signal. This was progressively abolished with pre-incubation of the brain homogenate with increasing concentrations of RD3 antibody with a more modest efficacy of the RD4 antibody.
Conclusions: The well-characterized RD3 antibody appears to be effective in preventing the cellular uptake and seeding of tau aggregation. These findings suggest that RD3 could be developed for passive immunotherapy.

Symposium 34: TRANSLATIONAL STRATEGIES 4: Gsk3BETA , Tau Targets (2)

ADPD5-2111

TAU VACCINE AADVAC1 - THE ROAD TO PHASE II CLINICAL TRIALS

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The microscopic changes that underlie the syndrome of Alzheimer's disease (AD) dementia are accumulation of pathological amyloid-β and tau proteins, accompanied by synaptic and neuronal loss and neuroinflammation. The progress and spreading of neurofibrillary tau pathology through the brain correlates with the cognitive symptoms, neuronal loss and atrophy observed in AD. We have analyzed the building blocks of tau pathology and identified the most toxic species, which were used to develop transgenic animal models that faithfully replicated human neurofibrillary pathology. We have developed an active and passive vaccine against neurofibrillary pathology. These vaccines are phosphorylation-independent, instead targeting a conformational epitope found in all stages of human Alzheimer's disease, and throughout the AD tau proteome, both on early and late pathological tau species.

The vaccines reduced the neurobehavioral impairment of transgenic animals, and reduced the number of neurofibrillary tangles and the total amount of insoluble tau protein in their brains, also eliminating tau hyperphosphorylation at multiple AD-related tau epitopes.

The active vaccine AADvac1 was tested in GLP toxicology and safety pharmacology studies, displaying an excellent safety profile.

AADvac1 was applied in a clinical phase 1 trial (EudraCT 2012-003916-29) to 30 patients with mild to moderate AD. The safety and immunogenicity results are presented.

An 18-month follow-up study (EudraCT 2013-004499-36) studies these patients further.

ADAMANT, a phase II 24-month randomized, blinded, placebo-controlled safety/efficacy study of AADvac1, will assess long-term safety of AADvac1 and its impact on patient cognition, function and biomarkers. The study begins in Q2 2015.

Symposium 34: TRANSLATIONAL STRATEGIES 4: Gsk3BETA , Tau Targets (2)

ADPD5-2044

TARGETING THE SHARED PATHOLOGICAL CONFORMERS OF BOTH ABETA AND HYPERPHOSPHYLATED TAU WITH CONFORMATION SELECTIVE MONOCLONAL ANTIBODIES

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Immunomodulation holds promise as a treatment for AD but current attempts only address one aspect of the pathology: either amyloid β (Aβ) or the hyperphosphorylated tau (ptau) protein. We developed a novel immunomodulatory approach using a pBri peptide. We tested this approach in APP/PS1, 3xTg and TgSwDI AD models and have documented pBri as an immunogen reduces: amyloid plaques, vascular amyloid deposits and neurofibrillary tangles. Using pBri we have developed monoclonal antibodies (mAbs).

MAbs were obtained from pBri inoculations of BALBc mice. Positive hybridomas were selected by their shared reactivity against Aβ, PHF and PrP^{Res}. The best mAbs were characterized by blots, surface plasmon resonance (SPR) and histology in AD tissue. One such mAb is being tested in 3xTg mice with both tau and Aβ related pathology. These mAbs on tissue sections specifically immunolabel AD tissue. On Western blots these mAbs detect purified paired helical filament preparations, aggregated/oligomeric Aβ and PrP^{Res}, extracted from CJD brain tissue. SPR shows high affinity binding to oligomeric/aggregated Aβ, with no binding to monomeric Aβ. One of these mAbs is being tested therapeutically in 3xTg mice.

We have developed a novel immunization procedure which we have used to produce monoclonal antibodies (mAbs) that recognize multiple pathological proteins. We are characterizing these mAbs which give selective immunolabeling in AD tissue and on Western blots to pathological conformers. We believe that immunotherapy that specifically targets the most toxic, oligomeric forms of Aβ and ptau, has a greater chance of success clinically with much less risk of toxicity.

Symposium 34: TRANSLATIONAL STRATEGIES 4: Gsk3BETA , Tau Targets (2)

ADPD5-2040
TAU REDUCTION PREVENTS ABETA-INDUCED AXONAL TRANSPORT DEFICITS BY BLOCKING ACTIVATION OF GLYCOGEN SYNTHASE KINASE 3 BETA

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Objectives: Determine mechanisms by which tau enables Aβ-induced deficits of axonal transport.

Methods: We prepared primary hippocampal neuronal cultures from *Tau*$^{+/+}$, *Tau*$^{+/-}$, and *Tau*$^{-/-}$ mice, with or without expression of familial Alzheimer's disease-linked forms of human amyloid precursor protein (hAPP). We treated cultures with a γ-secretase modulator and used tau ablation, tau knockdown, and tau reconstitution to investigate mechanisms of Aβ/tau-dependent deficits in axonal transport of mitochondria. Axonal mitochondrial motility was quantified by transfecting cultures with a mito-RFP plasmid, fluorescence microscopy, and kymograph analysis in ImageJ.

Results: We demonstrate deficits in anterograde axonal transport of mitochondria in primary neurons from transgenic mice expressing hAPP. We show that these deficits depend on $A\beta_{1-42}$ production and are prevented by partial tau reduction or tau ablation. Expression of mutant tau constructs in *Tau*$^{-/-}$ neurons and knockdown of endogenous tau revealed that the copathogenic effects of tau do not depend on its microtubule binding, interactions with Fyn, or potential roles in neuronal development. Inhibition of neuronal activity, NMDA receptor function, or GSK3β activity or expression abolished Aβ-induced transport deficits. Tau ablation prevented Aβ-induced GSK3β activation.

Conclusions: Tau allows Aβ oligomers to inhibit axonal transport of mitochondria through activation of GSK3β, possibly by facilitating aberrant neuronal activity. Our findings suggest a role for tau in Aβ-induced neuronal dysfunction that is independent of microtubule binding and upstream of GSK3β activation. Our tau-reconstitution assay provides a new approach to dissecting mechanisms underlying Aβ- and tau-dependent neuronal deficits, including the intriguing mediator role that GSK3β has in this process.

Symposium 34: TRANSLATIONAL STRATEGIES 4: Gsk3BETA , Tau Targets (2)

ADPD5-2022

DP-C016, AN INNOVATIVE MULTIVALENT VACCINE TARGETING HYPER-PHOSPHORYLATED TAU

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Objectives: Effective vaccines to treat tauopathies will need to specifically eliminate abnormally hyper-phosphorylated tau proteins while causing neither encephalopathy nor microhemorrhage. Declensional peptides, defined as amino acid copolymers [glatiramer acetate (Copaxone™; Teva) with antigen specificity, constitute a new class of immunomodulators which can achieve this goal.
Methods: We design cationic peptides with high alanine content and incorporating more than one amino acid at a given position, capable of inducing an immune response against multiple antigenic variants, while driving antigen-presenting cells into an anti-inflammatory phenotype. DP-C016 declensional peptides target multiple phosphorylation sites in the C-terminal region of tau. We have tested these compounds in monocyte activation and human T-cell proliferation assays, and immunogenicity studies in mice.
Results: We have identified DP-C016.11 as a prototype compound: it activates a monocyte cell line and induces the acute release of the anti-inflammatory T_H2 chemokine CCL22; it induces the proliferation of a significant proportion of naïve CD4+, but very little CD8+, T-lymphocytes in a 6-day assay using CFSE-labeled PBMCs isolated from healthy donors; it is a very strong T_H2 immunogen in mice even without adjuvant. Antibodies induced by DP-C016.11 cross-react against PHFs isolated from autopsy brain tissue from AD patients and stain, by immunohistology, neuritic elements and NFTs in the hippocampus CA1 field of the same patients.
Conclusions: DP-C016 peptides combine a broad immunogenicity against multiple phosphorylation sites of tau, in the absence of strong adjuvant, with anti-inflammatory, neuroprotective properties. We are currently testing DP-C016 peptides in tau-transgenic, JNPL3 mice.

Symposium 34: TRANSLATIONAL STRATEGIES 4: Gsk3BETA , Tau Targets (2)

ADPD5-2002
GSK-3 INTERACTS WITH LYSOSOME NETWORKING AND CONTRIBUTES TO NEURODEGENERATION

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Glycogen synthase kinase-3 (GSK-3) has been recently implicated in the etiological mechanisms of neurodegenerative disorders including Alzheimer's disease (AD). Inhibition of GSK-3 produces multiple therapeutic benefits in AD animal models including the ability to reduce amyloid-β deposition (Aβ), a major pathological hallmark of AD. This raises the possibility that GSK-3 interacts with pathways responsible for cellular clearance.

Lysosomes are acidic organelles responsible for clearance of damaged and obsolete proteins and organelles. Deficiency in lysosomal function is found in human pathogenesis and has been recently implicated in enhance build-up of Ab deposits and reduced cognitive performance in the context of AD. Agents that repair defects in lysosomal function are thus considered a potential therapeutic intervention.

In a recent work we identified the lysosome as a GSK-3 target. We showed that hyperactive GSK-3 impairs lysosomal acidification and that inhibition of GSK-3 restores lysosomal acidification, reduces Ab loads, and improves cognitive performance in an AD mouse model, the 5XFAD mice. Further research using AD-like cell cultures or primary hippocampal neurons indicated that GSK-3 suppresses autophagic flux by impairing lysosomal acidification. In addition, GSK-3 interplay with mammalian target of rapamycin (mTOR) inhibits autophagy and regulates de-novo lysosome biogenesis. We suggest that the therapeutic activity achieved with GSK-3 inhibitors is mediated via enhanced lysosomal acidification thus accelerating degradation of Aβ deposits. GSK-3 inhibitors may be used as a potential therapy targeting lysosomes.

Symposium 36: APOE: MECHANISMS AND APOE-BASED TREATMENT STRATEGIES

ADPD5-1283

APOE INCREASES RISK FOR DEMENTIA WITH LEWY BODIES: INDEPENDENT EFFECT OR CONTAMINATION OF ALZHEIMER-RELATED PATHOLOGY?

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Objectives: ApoE was recently found to be the strongest genetic risk factor for dementia with Lewy bodies (DLB) in a large multi-national study but it remains unclear whether this association was merely detected due to contamination of DLB patients with frequent concomitant Alzheimer disease (AD)–type pathology. Here, we examine whether the ApoE epsilon (e) 4 risk allele influences the burden of cortical Lewy bodies in DLB brains and whether the ApoE protein and mRNA levels are increased independent of AD-type pathology.
Methods: The ApoE allele frequencies and Lewy and Alzheimer-related pathologies were examined in 290 autopsied subjects; part of the largest prospective study of dementia and aging in UK (OPTIMA).These subjects were further stratified into four groups: pure DLB (n=30); DLB with AD pathology (n=39); pure AD (n=139) and controls (n=82). In addition, we examined ApoE protein and mRNA levels in cingulate, entorhinal and occipital cortex in 20 subjects of each group (10 e4 carriers vs. 10 non-e4 carriers).
Results: The ApoE genotype distribution was significantly different between study groups (Chi-square, $p<0.001$), highest e4 allele frequency seen in DLB-AD group (51%). Apo e4 carriers also showed higher cortical Lewy body densities in compared to non-ε4 carriers in a model adjusted for age, gender and AD-type pathology. The protein and mRNA levels of ApoE were increased in brain regions with prominent Lewy body pathology.
Conclusions: Our results suggest that ApoE e4 allele may contribute to dementing synucleinopathies via increased cortical Lewy body burden and not mechanisms related to amyloid processing.

Symposium 36: APOE: MECHANISMS AND APOE-BASED TREATMENT STRATEGIES

ADPD5-1242
APOE4 TARGETED PHARMACOLOGICAL AND IMMUNOLOGICAL THERAPY OF ALZHEIMER'S DISEASE

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Brain pathology of Alzheimer's diseases (AD) and the genetics of autosomal dominant familial AD have been the "lamp posts" for looking for AD therapeutic targets. Although this approach still remains valid, none of the compounds tested to date have produced clinically meaningful results. This calls for developing complementary therapeutic approaches and AD targets. The allele ε4 of apolipoprotein E4 (APOE-ε4), which is the most prevalent genetic risk factor for sporadic AD and is expressed in more than half of the patients, presents such an AD therapeutic target. .

The pathological effects of the protein apoE4 which is coded by the allele APOE-ε4 can be due to loss of a structural feature which the "good", AD benign allele, apoE3 possesses and/or to gain of structural features and functions specific to apoE4. The contribution of the gain of structural effects of apoE4 will be presently assessed by investigation of the extent to which the pathological effects of apoE4 can be counteracted by i.c.v. injection of anti-apoE4 mAbs whereas the possible role of loss of structure related mechanisms will be assessed by measurements of the extent to which correction of the impaired lipidation of apoE4 utilizing either the RXR-agonist Bexarotene or by direct activators of the apoE lipidating protein ABCA1, can reverse its pathological effects. The results obtained utilizing naïve apoE4 and apoE3 targeted replacement mice suggest that the pathological effects of apoE4 are mediated by both loss and gain of structural features and that apoE4 directed therapy should thus target both mechanisms.

Symposium 36: APOE: MECHANISMS AND APOE-BASED TREATMENT STRATEGIES

ADPD5-1190

ANTI-APOE4 IMMUNOTHERAPY FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Introduction: Recent findings suggest that key pathological effects of apoE4, the most prevalent Alzheimer's disease (AD) genetic risk factor, are associated with gain-of-toxic function mechanisms.

Objective: This study examined the extent to which anti-apoE4 mAbs can counteract the cognitive and pathological effects apoE4 in targeted-replacement (TR) mice.

Results: The anti-apoE4 mAbs were injected i.p to TR mice, which express either apoE4 or its AD benign isoform apoE3, via a preventive paradigm (10 weekly injections, starting at the age of 4 weeks) and a treatment paradigm (3 weekly injections, at the age of 4 months).

Examination of naïve 4 months old apoE4 mice revealed distinct hippocampal pathologies. Of these, the apoE4-driven accumulation of hyperphosphorylated tau and the decreased apoER2 levels were abolished by the injection of anti-apoE4 mAbs in both the preventive and treatment paradigms. In contrast, the apoE4 accumulation of Aβ was not affected by either treatment. Behavioral studies revealed that the anti-apoE4 mAbs counteract the cognitive deficits of the apoE4 mice. These effects were associated with the accumulation of IgG in the brain and its co-localization with apoE4.

Conclusion: Peripherally injected Anti-apoE4 mAbs penetrate the brain and counteract key pathological effects of apoE4 in TR mice. This suggests a novel therapeutic approach for treatment of apoE4 carriers in AD and in other acute and chronic diseases.

Symposium 36: APOE: MECHANISMS AND APOE-BASED TREATMENT STRATEGIES

ADPD5-1095

NEUROANATOMIC EFFECT OF APOE POLYMORPHISMS ON HIPPOCAMPAL VOLUME: A MULTI-COHORT STUDY IN ALZHEIMER'S DISEASE, MILD COGNITIVE IMPAIRMENT AND HEALTHY AGEING

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Objectives: To examine, using a cross-sectional design, the genetic influence of ApoE gene polymorphisms on hippocampal volume in a large AD, Mild Cognitive Impairment (MCI) and normal ageing dataset (n=1781), as well as a multicentre study of 14-year old healthy adolescents (n=1387).

Methods: A linear mixed model regression was used to compare automated Freesurfer derived hippocampal volumes between ApoE groups in AD patients, MCI, cognitively normal (CN), and non-demented individuals. Further ApoE comparisons were also made in MCI ε4 carriers that converted to a future AD diagnosis (MCI-converters) compared to those that remained clinically stable over follow-up, and CN individuals stratified by amyloid positivity.

Results: Hippocampal volumes were significantly smaller in ε4 carrier AD patients and MCI subjects. A similar effect of the ε4 allele was found in MCI subjects that progressed to AD at follow up and cognitively normal (CN) individuals who met the criteria for amyloid positivity as assessed by positron emission tomography (PET). In

contrast, volumes of ε2 carriers from healthy and non-demented groups were found to be comparatively larger than the AD group. No evidence of an ApoE genotype effect on hippocampal volume was established in non-demented and CN individuals and young healthy adolescents.
Conclusions: Our results show that hippocampal volumes are most affected in AD patients and subjects with either prodromal or pre-clinical stages of disease possessing the ε4 allele, whereas no such effect is present in older and young healthy groups of individuals.

Figure 1

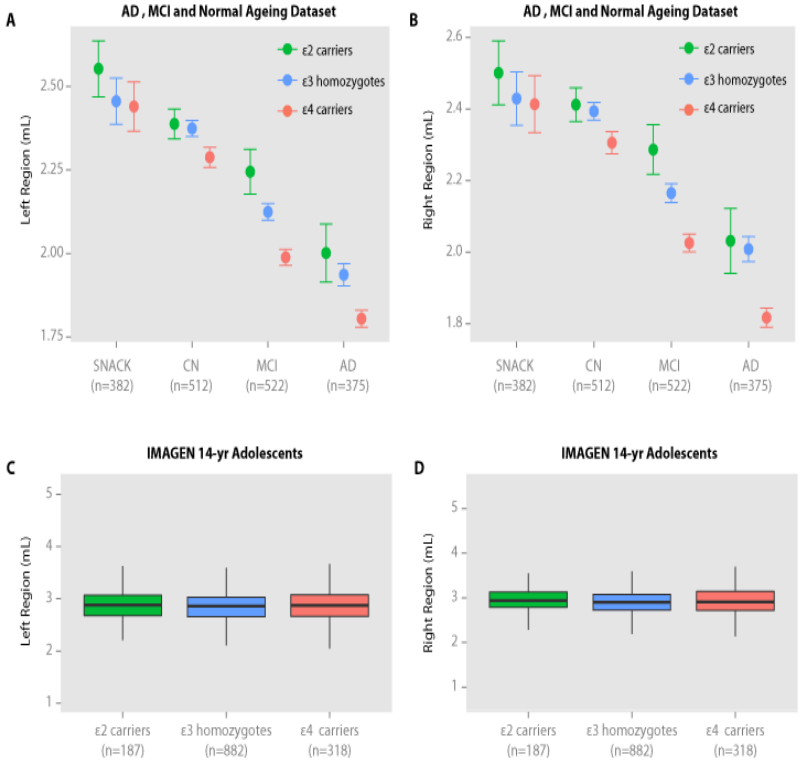
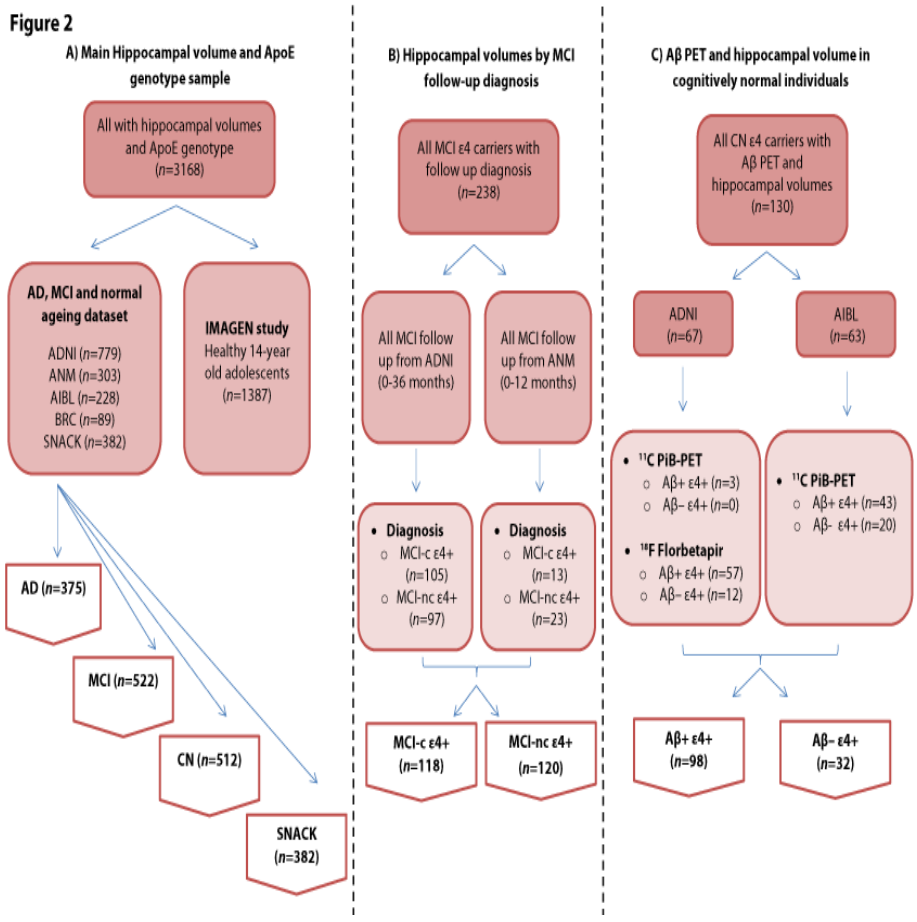

A
AD , MCI and Normal Ageing Dataset
ε2 carriers
ε3 homozygotes
ε4 carriers
Left Region (mL)
2.50
2.25
2.00
1.75
SNACK (n=382)
CN (n=512)
MCI (n=522)
AD (n=375)
B
AD , MCI and Normal Ageing Dataset
ε2 carriers
ε3 homozygotes
ε4 carriers
Right Region (mL)
2.6
2.4
2.2
2.0
1.8
SNACK (n=382)
CN (n=512)
MCI (n=522)
AD (n=375)
C
IMAGEN 14-yr Adolescents
Left Region (mL)
5
4
3
2
1
ε2 carriers (n=187)
ε3 homozygotes (n=882)
ε4 carriers (n=318)
D
IMAGEN 14-yr Adolescents
Right Region (mL)
5
4
3
2
1
ε2 carriers (n=187)
ε3 homozygotes (n=882)
ε4 carriers (n=318)

Figure 2


A) Main Hippocampal volume and ApoE genotype sample
All with hippocampal volumes and ApoE genotype (n=3168)
AD, MCI and normal ageing dataset
ADNI (n=779)
ANM (n=303)
AIBL (n=228)
BRC (n=89)
SNACK (n=382)
IMAGEN study
Healthy 14-year old adolescents (n=1387)
AD (n=375)
MCI (n=522)
CN (n=512)
SNACK (n=382)
B) Hippocampal volumes by MCI follow-up diagnosis
All MCI ε4 carriers with follow up diagnosis (n=238)
All MCI follow up from ADNI (0-36 months)
All MCI follow up from ANM (0-12 months)
Diagnosis
MCI-c ε4+ (n=105)
MCI-nc ε4+ (n=97)
Diagnosis
MCI-c ε4+ (n=13)
MCI-nc ε4+ (n=23)
MCI-c ε4+ (n=118)
MCI-nc ε4+ (n=120)
C) Aβ PET and hippocampal volume in cognitively normal individuals
All CN ε4 carriers with Aβ PET and hippocampal volumes (n=130)
ADNI (n=67)
AIBL (n=63)
11C PiB-PET
Aβ+ ε4+ (n=3)
Aβ− ε4+ (n=0)
18F Florbetapir
Aβ+ ε4+ (n=57)
Aβ− ε4+ (n=12)
11C PiB-PET
Aβ+ ε4+ (n=43)
Aβ- ε4+ (n=20)
Aβ+ ε4+ (n=98)
Aβ− ε4+ (n=32)

Symposium 36: APOE: MECHANISMS AND APOE-BASED TREATMENT STRATEGIES

ADPD5-0871

DIFFERENTIAL BINDING OF ABETA43 TO APOLIPOPROTEIN E ISOFORMS MAY AFFECT ITS CLEARANCE ACROSS THE BLOOD-BRAIN BARRIER

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Background: A range of Abeta peptides that vary in length have been found in the human brain with Abeta40 and Abeta42 being the most studied. However, recently the Abeta43 peptide was found to be more abundant than previously thought and might play a role in AD development. Interestingly, it has been described that Abeta43 does not accumulate in CAA. We hypothesize this is due to an altered blood-brain barrier clearance compared to shorter Abeta peptides. Several studies indicate that interaction with apolipoprotein E (ApoE) is important for clearance of Abeta.
Objectives: To gain more insight in the mechanism of cerebral Abeta clearance, by studying the interaction of Abeta peptides with ApoE isoforms.
Methods: Interaction of ApoE isoforms with Abeta peptides is analyzed using SDS-PAGE and native PAGE plus Western Blotting. The interaction between peptide (complexes) and cerebrovascular cells is determined using viability assays.
Results: Preliminary results indicate that Abeta43 is more toxic towards cerebrovascular cells than other Abeta peptides. Results based on immunoblot quantification show that lipidation of ApoE strongly affects its interaction with Abeta. Furthermore, the binding affinity of this interaction is dependent on the Abeta isoform, with Abeta40 and Abeta42 showing much stronger interaction with ApoE2/E3 than Abeta43.
Conclusions: Our results indicate that the Abeta43 peptide shows increased toxicity towards cerebrovascular cells and decreased binding affinity for ApoE compared to other Abeta peptides. Since Abeta43 is not associated with CAA our results suggest that its decreased interaction with ApoE may affect its clearance efficacy across the blood-brain barrier.

Symposium 36: APOE: MECHANISMS AND APOE-BASED TREATMENT STRATEGIES

ADPD5-0271
PHOSPHOLIPID DYSREGULATION CONTRIBUTES TO APOE4-INDUCED COGNITIVE DEFICITS IN ALZHEIMER'S DISEASE PATHOGENESIS

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[2]Pathology, Westchester Medical Center New York Medical College, Vahalla, USA
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[6]Psychiatry, Icahn School of Medicine at Mount Sinai, New York City, USA

Objectives: The apolipoprotein Eε4 (ApoEε4) allele is the strongest genetic risk factor for developing sporadic Alzheimer's Disease (AD). However, the mechanisms underlying the pathogenic nature of ApoE4 are not well understood. In this study, we test the hypothesis that ApoE is essential for maintenance of brain phospholipid homeostasis and that the ApoE4 isoform is dysfunctional in this process. **Methods:** We performed phospholipid analysis using high pressure liquid chromatogram (HPLC) with suppressed conductivity in human and mouse brain samples, as well as primary neurons expressing ApoE4 alleles. We also developed a haploinsufficiency mouse model of PIP_2 degrading enzyme, the phosphoinositol phosphatase synaptojanin 1 (synj1) with ApoE4 background for further studies. **Results:** We have found that the levels of phosphoinositol biphosphate (PIP_2) are reduced in postmortem human brain tissues of ApoE4 carriers, as well as in ApoE4 knock-in (KI) mouse brains. We found similar changes in primary neurons expressing ApoE4 alleles compared to neurons expressing the ApoE3 allele. These changes are secondary to increased expression of synaptojanin 1 (synj1) in ApoE4 carriers. Further studies indicate that ApoE4 which behaves like ApoE null conditions, fails to degrade synj1 mRNA efficiently unlike ApoE3 does, partially due to differential expression levels of miR155. These data suggest a loss-of-function of ApoE4 genotype in regulating PIP_2 homeostasis. Most importantly, genetic reduction of synj1 in ApoE4 KI mouse models restores PIP_2 levels, and rescues AD-related cognitive deficits. **Conclusions:** Together, our findings uncover a novel mechanism that links ApoE4-related lipid changes to the pathogenic nature of ApoE4 in AD.

Symposium 44: TAU IMUNOTHERAPY

ADPD5-1781
TAU IMMUNOTHERAPY
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In recent years, tau immunotherapy has advanced from proof-of-concept studies (Sigurdsson, EM, NIH R01AG020197, 2001; Asuni AA et al, J Neurosci, 27, 2007), which have now been confirmed and extended by us and others. Phase 1 clinical trials on active tau immunizations are being conducted, with several passive tau antibody trials likely to be initiated in the near future for Alzheimer's disease and other tauopathies. Because tau pathology correlates better with the degree of dementia than amyloid-β (Aβ) pathology, greater clinical efficacy may be achieved by clearing tau- than Aβ aggregates in the later stages of the disease, when cognitive impairments become evident.
Substantial insight has now been obtained regarding which epitopes to target, mechanism of action, and potential toxicity but much remains to be clarified. All of these factors likely depend on the model/disease or stage of pathology and the immunogen/antibody. Interestingly, tau antibodies interact with the protein both extra and intracellularly but the importance of each site for tau clearance is not well defined. Some antibodies are readily taken up into neurons whereas others are not. It can be argued that extracellular clearance may be safer but less efficacious than intraneuronal clearance and/or sequestration to prevent secretion and further spread of tau pathology.
Development of therapeutic tau antibodies has led to antibody-derived imaging probes, which are more specific than the dye-based compounds that are already in clinical trials. Such specificity may give valuable information on pathological tau epitope profile, which could then guide the selection of therapeutic antibodies for maximal efficacy and safety.
Hopefully, tau immunotherapy will be effective in clinical trials, and further advanced by mechanistic clarification in experimental models with insights from biomarkers and postmortem analyses of clinical subjects.

Symposium 44: TAU IMUNOTHERAPY

ADPD5-1686

EFFECTS AND MECHANISMS OF ANTI-TAU ANTIBODIES IN TAUOPATHIES

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[2]Psychiatry, Washington University, St. Louis, USA
[3]Neurology and Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, USA

Objective
We previously found a strong reduction in tau pathology, insoluble tau, and improved cognitive performance in P301S tau transgenic mice following intracerebroventricular infusion of the anti-tau antibody HJ8.5. We evaluated the effects of HJ8.5 in the same model of tauopathy following intraperitoneal administration.
Methods
Six-month old P301S mice received one of two dose levels of HJ8.5 through intraperitoneal injection for 3 months. Following treatment, we performed immunostaining for p-tau, anatomical and biochemical analysis, and behavioral tests.
Results
Both 10 mg/kg and 50 mg/kg treatment with HJ8.5 significantly reduced the loss of cortical and hippocampal tissue volumes compared to control treated mice. Mice treated with HJ8.5 showed a very strong decrease in detergent-insoluble human tau as well as reduced phospho-tau staining in the hippocampal CA1 cellular layer. Both doses of HJ8.5 reduced thio-S positive tau aggregates in the piriform cortex and amygdala, and mice treated with HJ8.5 at 50 mg/kg showed significantly improved sensorimotor performance on both the inverted screen and ledge tests compared to vehicle treated mice. In BV2-microglial cells, we observed significantly higher uptake of P301S tau aggregates in the presence of HJ8.5. In addition, HJ8.5 treatment resulted in a large dose dependent increase of tau in the plasma.
Interpretation
Systemically administered anti-tau antibody HJ8.5 significantly decreased insoluble tau and brain atrophy. It also improved motor function in a mouse model of tauopathy. These data indicate that passive immunization targeting tau should be strongly considered as a therapeutic strategy for tauopathies.

Symposium 44: TAU IMUNOTHERAPY

ADPD5-1615
ANTIBODY AGAINST EARLY PATHOGENIC TAU PRION FOR TREATING ALZHEIMER'S DISEASE AND OTHER TAUOPATHIES
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[2]Micheli Center for Sports Injury Prevention,
Children's Hospital Boston Harvard Medical School, Boston, USA
[3]Neurology, Beth Israel Deaconess Medical Center Harvard Medical School, Boston, USA
[4]Center for the Study of Traumatic Encephalopathy,
Boston University School of Medicine, Boston, USA
Tau pathology (tauopathy) is a neuropathological hallmark in Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE) associated with traumatic brain injury (TBI). Since tau pathologies, as detected by commonly known antibodies or methods, are not obvious acutely after TBI, whether tauopathy is an end result or early driver of TBI pathology is unclear, although TBI is a potential risk factor for AD. We have previously shown that Pin1-catalyzed *cis-trans* isomerization of tau and APP after proline-directed phosphorylation is a unique signaling mechanism protecting against tau and Abeta pathologies in Alzheimer's disease (AD). To visualize Pin1-catalyzed post-phopshorylation conformational regulation, we have developed novel peptide chemistry that allow the generation of *cis* and *trans*-specific polyclonal antibodies, and use them to raise antibodies specific for isomers of phosphorylated tau (p-tau) (Cell 149: 232-244). Using these cis and trans conformation-specific antibodies, we have shown that *trans* p-tau is the physiological form that promotes microtubule assembly, whereas the *cis* form is the previously unrecognized early pathogenic pretangle tau conformation that not only loses its normal function, but also gains toxic function, leading to tauopathy in AD. We have now further generated *cis* and *trans*-specific p-tau monoclonal antibodies and identified *cis* p-tau as an early central mediator of brain injury after TBI that is effectively stopped by antibody in vitro and in vivo. These insights uncover a novel disease mechanism in TBI and its connection with AD, and suggest a potentially exciting new immunotherapy for treating TBI, CTE and AD.

Symposium 44: TAU IMUNOTHERAPY

ADPD5-0928
TAU SPREAD AND IMMUNOTHERAPY TARGETING ITS AMINO-TERMINAL DOMAIN

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New York State Institute for Basic Research in Developmental Disabilities,
Staten Island NY, USA

Objectives: A key lesion of Alzheimer disease (AD) and related tauopathies is the accumulation of abnormally hyperphosphorylated tau (ptau) which forms neurofibrillary tangles. Unlike normal tau which interacts with tubulin and promotes its assembly into microtubules, the AD P-tau sequesters normal tau, disrupts microtubules and serves as a template for its prion-like aggregation and spread.
Methods: These previous studies of ours led us to attempt tau immunotherapy employing antibodies to N-terminal projection domain of tau which we would expect to be accessible in the AD P-tau/N-tau aggregates. For immunotherapy we employed mouse mAb 43D to tau_{6-18}, and 77E9 to $tau_{184-195}$, and as control, mouse IgG.
Results: Intraperitoneal administration of 100 micrograms 43D IgG/injection/week for 4 weeks in 14-17-month-old 3xTg-AD mice at moderate to severe stage of tau and Abeta pathologies showed a significant decrease in tau hyperphosphorylated at Ser199, Ser202/Thr205, Ser262/356 and Ser396/404, and a trend for reduction in Abeta pathology, and significant improvement in reference memory by Morris Water Maze task. Identical treatment with mAb 77E9 to $tau_{184-195}$ also significantly reduced the levels of hyperphosphorylated taus and showed a trend to reduce Abeta pathology as above found with mAb 43D, but could not improve the reference memory of the treated animals.
Conclusions: These studies show (1) that passive immunization targeting N-terminal projection domain of tau can effectively clear the hyperphosphorylated protein and possibly reduce Abeta pathology and (2) that targeting tau_{6-18} is potentially an especially promising strategy for the treatment of AD and other tauopathies.

Symposium 44: TAU IMUNOTHERAPY

ADPD5-0537
PASSIVE IMMUNIZATION WITH A NEW MONOCLONAL ANTIBODY TO PHOSPHO-SER413-TAU ATTENUATES TAUOPATHY PHENOTYPES IN MICE
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[3]Faculty of Pharmaceutical Sciences, Himeji Dokkyo University, Himeji, Japan
Objectives: Cellular inclusions of hyperphosphorylated tau are a hallmark of tauopathies, which are neurodegenerative disorders that include Alzheimer's disease (AD). Active and passive immunization against hyperphosphorylated tau has been shown to attenuate phenotypes in model mice. We developed new monoclonal antibodies to hyperphosphorylated tau and sought high therapeutic efficacy for future clinical use.
Methods: Using more than 20 antibodies, we investigated which sites on tau are phosphorylated early and highly in the tauopathy mouse models tau609 and tau784. These mice display tau hyperphosphorylation, synapse loss, memory impairment at 6 months, and tangle formation and neuronal loss at 15 months. We generated mouse monoclonal antibodies to selected epitopes and examined their effects on memory and tau pathology in aged tau609 and tau784 mice by the Morris water maze and by histological and biochemical analyses.
Results: Immunohistochemical screening revealed that pSer413 is expressed early and highly. Monoclonal antibodies to pSer413 and to pSer396 (control) were generated. These antibodies specifically recognized pathological tau in AD brains but not normal tau in control brains according to Western blots. Representative anti-pSer413 and anti-pSer396 antibodies were injected intraperitoneally into 10-11- or 14-month-old mice once a week at 0.1 or 1 mg/shot 5 times. The anti-pSer413 antibody significantly improved memory, while the anti-pSer396 antibodies showed less effect. The cognitive improvement paralleled a reduction in the levels of tau hyperphosphorylation, tau oligomer accumulation, synapse loss, tangle formation, and neuronal loss.
Conclusions: These results indicate that pSer413 is a promising target in the treatment of tauopathy.

Symposium 44: TAU IMUNOTHERAPY

ADPD5-0270
TARGETING INTRA VS EXTRACELLULAR TAU BY RECOMBINANT ANTIBODIES
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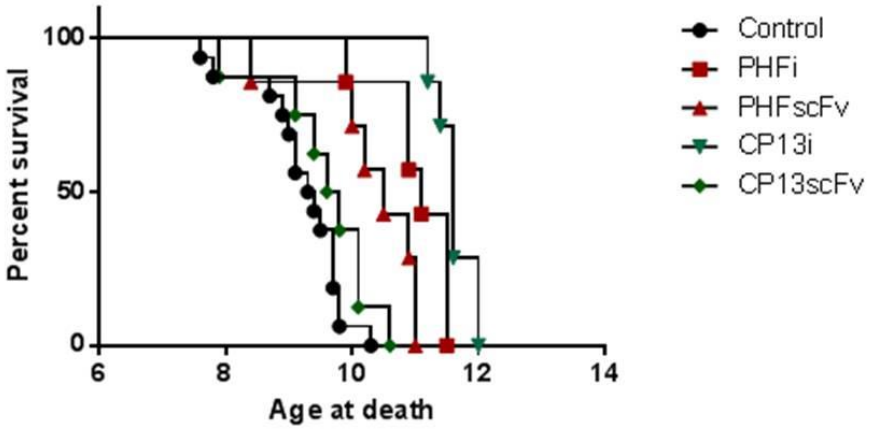
Objectives
The mechanisms underlying the abnormal phosphorylation and accumulation of tau in AD remain unclear, but one of the possibilities is that it might be due to conformational changes in tau in the diseased brain. Anti-tau immunotherapy has recently emerged as a promising approach to target tau, but many mechanistic questions regarding the optimal form of anti-tau immunotherapy remain. We hypothesize that anti-tau immunotherapy may be optimized by targeting both intracellular and extracellular pools of tau and that specific binding of hyperphosphorylated tau by single chain variable fragments (scFv) or by intracellularly expressed intrabodies will prevent its toxicity and formation of neurofibrillary tangles.
Methods
We cloned scFvs from two monoclonal anti-phospho-Tau antibodies (PHF and CP13) and generated stable intracellular anti-Ptau scFvs (intrabodies), as well as scFvs, directed into the secretory pathway. Then we expressed these intrabodies and scFvs in the brains of newborn transgenic rTg4510 mice and in spinal cords of homozygous JNPL p301L mice. Effects on pathology and life expectancy were assessed at three and 12 month of age, respectively
Results
Analysis of three month old rTg4510 brains revealed that both intrabodies and scFvs attenuated tau pathology relative to PBS-injected controls with CP13i being the most effective. Similarly, CP13i as well as PHFi significantly prolonged life expectancy of homozygous JNPL P301L mice (Fig).
Conclusions
These studies suggest that targeting phosphorylated tau in the intracellular vs. extracellular compartment is feasible therapeutic approach against tauopathy in animal models, whereas antibody effector functions are not required.



Expression of intracellular single chain fragments against phosphorylated Tau prolongs life expectancy in homozygous JNPL P301Lmice following intraspinal delivery of AAV expressing PHF scFv, PHF intrabody, CP13 scFv, and CP13 intrabody.

Symposium 47: SYNUCLEIONOPATHIES AND PRION DISEASES

ADPD5-2286
PD-61-W3 (SYNUCLERE™) IS A NOVEL SMALL MOLECULE AND CLINICAL CANDIDATE FOR TREATMENT OF ALPHA-SYNUCLEIN AGGREGATE ACCUMULATION IN PARKINSON'S DISEASE AND OTHER SYNUCLEINOPATHIES

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PD-61-W3 (Synuclere™) is a novel small molecule that prevents alpha-synuclein aggregation at substiochiometric proportions and rapidly remodels, reduces and detoxifies pre-formed alpha-synuclein aggregates, a central pathogenic component in Parkinson's disease (PD) and other synucleinopathies. PD-61-W3 efficacy was tested in independent studies using PD-relevant Thy-1 human wild-type alpha-synuclein transgenic mice (Line 61). These animal model studies demonstrated that PD-61-W3 is well-tolerated in vivo and that PD-61-W3 targets brain alpha-synuclein accumulation, as demonstrated by marked reduction in alpha-synuclein aggregate load in substangia nigra, cortex and hippocampus following 3-months of subcutaneous (s.c.) treatment in younger mice (45-90% reduction) or 6-months of i.p. treatment in older mice (79-91% reduction). Western blot analysis of brain extracts showed that PD-61-W3 significantly reduced alpha-synuclein oligomers by ~72%. Reduced alpha-synuclein aggregates in PD-61-W3-treated transgenic mice were accompanied by improved motor performance on the challenging beam traversal and pole tests. Importantly, PD-61-W3 exhibited good CNS drug properties including: micromolar exposure in brain, plasma and CSF following a single s.c. injection in mice at a therapeutic dose level; no significant off-target binding to a panel of brain receptors, transporters or ion channels; no significant CYP450 inhibition; and good chemical stability. Furthermore, PD-61-W3 was non-mutagenic in the Ames test. These studies support the advancement of PD-61-W3 into human clinical trials as a disease-modifying small molecule drug for the treatment of alpha-synuclein aggregation in Parkinson's disease and other synucleinopathies.
Funded by ProteoTech Inc. and the MJFox Foundation for Parkinson's Disease Research

Symposium 47: SYNUCLEIONOPATHIES AND PRION DISEASES

ADPD5-1321
NATIVE ALPHA-SYNUCLEIN MULTIMERS ARE DESTABILIZED BY PD-LINKED POINT MUTATIONS; THEIR COMPLETE ABOLITION BY MUTATING THE REPEAT MOTIFS CAUSES ACUTE NEUROTOXICITY

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There is growing evidence that alpha-synuclein (aS) can occur normally in alpha-helix-rich tetramers and related physiological low-n assemblies (collectively, aS multimers).
Combining data from cultured cells, mouse brains and patient-derived iPSC neurons and using two independent assays, in vivo crosslinking and fluorescent protein complementation, we have obtained highly consistent evidence that all known familial PD-linked aS missense mutations destabilize native aS multimers.
In particular, mutations G51D and E46K strongly reduced tetramer:monomer ratios, and this effect was amplified by stepwise introduction of E46K-like mutations (KTKEGV becomes KTKKGV) into additional aS repeat motifs. When we sought to abolish the physiological propensity of aS to multimerize by introducing deletion mutations, we unexpectedly found that aS multimerization still occurred in each of fourteen sequential 10-amino acid deletion-mutants, suggesting compensatory effects among the 6 highly conserved (KTKEGV) and up to 3 additional aS repeats. We therefore introduced selected mutations into all relevant repeats and found that certain mutations repeated in-register across these motifs completely abolished aS multimerization. Thus, the altered repeat motifs KLKEGV, KTKKGV, KTKEIV or KTKEGW did not support multimer formation, suggesting the importance of certain charged and small residues for normal self-interaction. Expression in human neuroblastoma cells showed that all multimer-abolishing (but no neutral) mutants induced neurotoxic effects (see abstract by von Saucken *et al.*). Moreover, all multimer-abolishing mutants were enriched in PBS-insoluble fractions of the cells and showed pathological aggregation in biochemical and confocal microscopy assays. We hypothesize that the neurotoxicity from multimer-to-monomer conversion recapitulates early events in the pathogenesis of synucleinopathies.

Symposium 47: SYNUCLEIONOPATHIES AND PRION DISEASES

ADPD5-0374
ENHANCED ALPHA-SYNUCLEIN INDUCED DOPAMINERGIC NEURODEGENERATION IN G2019S-BAC TRANSGENIC RATS
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Objectives: The leucine-rich repeat kinase 2 (LRRK2) gene is associated with a form of parkinsonism that is indistinguishable from idiopathic Parkinson's disease (PD). We previously reported LRRK2-knockout rats as resistant to dopaminergic neurodegeneration elicited by overexpression of human α-synuclein, and such resistance correlated with reduced pro-inflammatory myeloid cells recruited to the midbrain. Recently, we characterized novel G2019S-BAC transgenic rats and found robust expression of LRRK2 in the substantia nigra. Here, we aim to test whether G2019S-LRRK2 expression in rats enhances α-synuclein induced dopaminergic neurodegeneration and inflammatory responses.
Methods: 10-12 weeks-old G2019S-LRRK2 rats and littermate controls negative for the BAC transgene were injected with recombinant adeno-associated virus 2/1 (rAAV2/1)-α-synuclein vector into the right substantia nigra pars compacta (SNpc). Unbiased stereological analysis was subsequently performed to estimate the total number of dopaminergic neurons and proinflammatory myeloid cells in these animals.
Results: We observed that G2019S-LRRK2 expression resulted in enhanced dopaminergic neurodegeneration elicited by rAAV2- α-synuclein mediated neurodegeneration. Pro-inflammatory cells recruited to the substantia nigra were enhanced in G2019S-LRRK2 rats and correlated well with neurodegeneration.
Conclusions: These data show that, similar to some mouse transgenic models of PD, G20129S-LRRK2 exacerbates α-synuclein-linked neurodegeneration. The relatively short timeline of the model (4 weeks) makes this model ideal to test novel neuroprotective therapies.

Symposium 47: SYNUCLEIONOPATHIES AND PRION DISEASES

ADPD5-0363
AGED CATTLE BRAIN DISPLAYS ALZHEIMER'S DISEASE–LIKE PATHOLOGY
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[2]-, Institute for Research in Biomedicine, Barcelona, Spain
Amyloid beta (Aβ) and hyper-phosphorylated tau (ptau) are the proteins undergoing misfolding in Alzheimer's disease (AD). Recent studies have shown that brain homogenates rich in amyloid aggregates are able to seed the misfolding and aggregation of amyloidogenic proteins inducing an earlier onset of the disease in mouse models of AD. Prion diseases are able to be transmitted by the inoculation of the misfolded prion protein noted in cattle affected by bovine spongiform encephalopathy. The infectious agent can be propagated from cattle to human beings. In the case of AD, it has been reported that Aβ aggregates and neurofibrillary tangles (NFTs) are present in the brain of several non-human mammals, including **a**ged monkeys, bears, dogs, and cheetahs. We have analyzed the hippocampus, temporal cortex, and thalamus in more than 60 cows ranging from 13 to 23 years old. After a complete histopathological analysis, we could observe many of the typical hallmarks detected in human AD brains, including Aβ and tau aggregates. Cow amyloid deposits are reactive against human anti-Aβ antibody and thioflavin-S. The morphological characteristics of these deposits are remarkably similar to human aggregates. When the cattle brains were stained with anti-ptau antibody and silver-stained, they displayed NFT-like structures in the cortical area. These results could have a huge repercussion in public health since AD has been shown to be transmissible under certain circumstances. We are currently investigating whether cattle tissue containing Aβ aggregates and/or NFTs are able to seed amyloid misfolding and aggregation**,** similarly delineated in prion diseases**.**

Symposium 47: SYNUCLEIONOPATHIES AND PRION DISEASES

ADPD5-0244
GLYCATION DISRUPTS PROTEOSTASIS AND PROMOTES NEURODEGENERATIVE ALTERATIONS IN SYNUCLEINOPATHIES

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α-synuclein (aSyn) aggregation in Lewy bodies is a pathological hallmark of Parkinson's disease (PD) and other synucleinopathies. Glycation, an age-dependent protein modification, is present in Lewy bodies. Here, we investigated the effect of the natural glycating agent methylglyoxal on aSyn biology and found that glycation increased aSyn aggregation and toxicity. Notably, striatal injection of methylglyoxal in mice caused neuronal loss. Genetic and pharmacological manipulation of methylglyoxal increased aSyn-dependent toxicity in human LUHMES cells and in PD patient-derived iPSCs, and decreased motor performance and survival in aSyn transgenic flies. Furthermore, glycated aSyn impaired synaptic transmission in rat hippocampal slices. Methylglyoxal promoted aSyn oligomerization by affecting its N-terminal structure and impairing lipid-binding ability. Glycation disrupted proteostasis, reducing aSyn turnover, aggregation, and release, likely the mechanistic link underlying the phenotypes observed. In total, our study uncovers glycation as a novel player in synucleinopathies, opening novel avenues for the design of therapeutic strategies.

Symposium 47: SYNUCLEIONOPATHIES AND PRION DISEASES

ADPD5-0222
ALDEHYDE DEHYDROGENASE 1 DEFINES AND PROTECTS A SUBPOPULATION OF NIGROSTRIATAL DOPAMINERGIC NEURONS IN PD
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There exist subpopulations of dopaminergic (DA) neurons in *substantia nigra pars compacta* (SNpc) that display differential vulnerabilities in Parkinson's disease (PD). The underlying molecular mechanism is unknown. Rodent SNpc DA neurons can be divided into two subpopulations based on the expression of aldehyde dehydrogenase 1 (ALDH1A1). Here we show in PD-related alpha-synuclein transgenic mice that DA neurodegeneration mainly occurred in the dorsomedial ALDH1A1-negative subpopulation, which was also prone to cytotoxic alpha-synuclein aggregation. Notably, ALDH1A1 also exhibited a conserved topographic expression pattern in human SNpc. Studies with postmortem PD brains revealed a severe reduction of ALDH1A1 expression and neurodegeneration in the ventral ALDH1A1-positive subpopulations. ALDH1A1 expression was also suppressed in alpha-synuclein transgenic mice. Genetic inhibition of *Aldh1a1* exacerbated alpha-synuclein-induced DA neurodegeneration and alpha-synuclein aggregation, whereas overexpression of *ALDH1A1* was protective. Furthermore, ALDH1A1 appeared to specifically protect against alpha-synuclein-mediated DA neurodegeneration. *Aldh1a1*-null and control DA neurons showed comparable sensitivity to 1-methyl-4-phenylpyridinium (MPP+), glutamate, or camptothecin-induced cell death, while overexpression of *ALDH1A1* did not rescue alpha-synuclein-induced loss of cortical neurons. Together, our findings suggest ALDH1A1 plays an important role in protecting subpopulations of SNpc DA neurons through preventing the accumulation of dopamine aldehyde intermediates and formation of cytotoxic alpha-synuclein oligomers.

Symposium 53: OTHER TREATMENT STRATEGIES

ADPD5-1600

DEEP BRAIN STIMULATION AS A SYMPTOMATIC TREATMENT OF MEMORY IMPAIRMENT IN ALZHEIMER DISEASE : FEASIBILITY STUDY AND LONG TERM OUTCOME

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Recent studies have suggested that neuronal circuits involved in memory can be modulated by deep brain stimulation (DBS) and that this propriety might be used to slow the cognitive decline of Alzheimer Disease (AD) patients.
We conducted a prospective study whose objective was to evaluate the feasibility and safety of DBS in AD patients. Inclusion criteria were: adult patients under 70 years old, with AD diagnosed for less than 2 years, with Mini Mental Status (MMSE) between 20 and 24 and predominant impairment of episodic memory. The fornix was stimulated bilaterally by electrodes implanted stereotactically in the hypothalamus (3V, 130 Hz). Clinical, biological, neuropsychological and imaging evaluation was conducted 3 months before and 6, 12, 24 and 36 months after surgery.
During the one year- inclusion period, 108 patients with recently diagnosed AD and episodic memory impairment were screened in a specialized consultation. Only 8 patients fulfilled all the inclusion criteria, 4 accepted to be included but only one was finally operated and followed for 3.5 years. The stimulation was perfectly tolerated. The memory scores (MMSE, ADAS-Cog, Grober & Buschke) were stabilized compared to baseline during 2 years of continuous stimulation, and slightly worsened after 3 years. 18FDG PET scan showed an increase of the mesial temporal lobes metabolism compared to baseline, sustained at long term.
This pilot study brings additional data about the safety of fornix DBS in the hypothalamus in AD patients. Clinical and functional imaging data suggest that the fornix DBS effect might be maintained over time.

Symposium 53: OTHER TREATMENT STRATEGIES

ADPD5-1544
PERK INHIBITION REVERSES STRUCTURAL AND FUNCTIONAL ABNORMALITIES IN TAU TRANSGENIC MICE.
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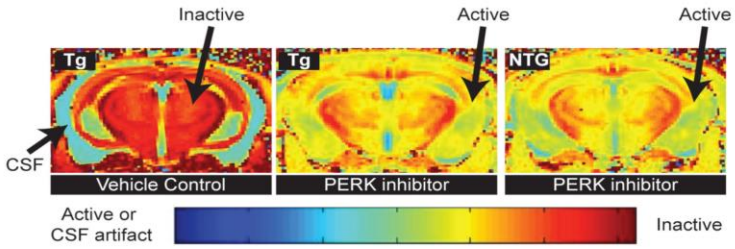
A major challenge in tauopathy research is the lack of effective therapeutic strategies. We recently established that the most toxic form of tau chronically activates the endoplasmic reticulum (ER) stress sensor PERK. Under conditions of ER stress, PERK inhibits RNA translation. If sustained, as it occurs in tauopathies, extended shut down of protein synthesis weakens and kills neurons and other tau-bearing cells. We inhibited PERK with a novel compound, GSK2606414, in the rTg4510 tau transgenic mouse model. Mice were treated from 6 to 9 months of age, which is after abundant tau pathology, extensive brain atrophy, and significant neuronal dysfunction appear (5.5 months). Every month during the treatment course, we measured neuronal function and brain volume by adapting an innovative imaging technique called MEMRI (manganese-enhanced MRI). At the end of treatment, we performed cognitive testing and quantified changes in tau pathology.
The drug effectively inhibited PERK in the tau transgenic mice. In addition, drug-treated tau mice showed virtually complete recovery of brain 1) structure and 2) function, 3) significant cognitive improvements, and 4) dramatically reduced soluble tau levels. Meanwhile, pathological tau deposits remained the same as vehicle-treated transgenic controls.
These data suggest that PERK is a potent therapeutic target for tauopathies. A major advantage of our strategy is rooted on the **therapeutic** paradigm of our study. Therefore, PERK inhibition could positively impact early and late stage tauopathic patients. Future efforts aim to develop safe and effective PERK inhibitors for the clinic.



Representative MEMRI images after 3 months of treatment with PERK inhibitor. Warm colors indicate no or reduced activity, while cold colors (blue and green) represent abundant activity; due to compromise of the blood-brain barrier and dramatic brain atrophy, manganese leaks into the CSF and shows positive in the MEMRI images of tau transgenic mice treated with vehicle. PERK inhibitor-treated transgenic mice showed virtually complete recovery of MEMRI signal compared to non-transgenic controls (NTG).

Symposium 53: OTHER TREATMENT STRATEGIES

ADPD5-0919
TRICYCLIC ANTIDEPRESSANTS REDUCE ALPHA-SYNUCLEIN ACCUMULATION IN TWO ANIMAL MODELS OF SYNUCLEINOPATHY

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Objectives. We previously demonstrated that tricyclic antidepressants, including amitriptyline (AMI), delay need for dopaminergic therapy in an early cohort of Parkinson's disease (PD) patients (Paumier et al., 2012), suggesting they may have disease-modifying properties. Recently, the tricyclic drug nortriptyline (NOR) was found to reduce alpha-synuclein (alpha-syn) aggregation in *in vitro* aggregation assays (unpublished data). These findings prompted us to examine the effects of tricyclics on alpha-syn accumulation in two distinct animal models of synucleinopathy.
Methods. 1) We conducted a dose response study in a human wild-type alpha-syn overexpression mouse model driven by the PDGF promoter. Four-month-old mice were injected (i.p.) with 0.5, 5.0 or 25 mg/kg NOR daily for 30 days. 2) We investigated whether AMI or NOR reduced accumulation of alpha-syn in a rat model produced by injections of pre-formed fibrillar (PFF) alpha-syn (Luk et al., 2012). PFF alpha-syn (8µg/4µl) was injected unilaterally into striatum. Daily injections with saline/tricyclics began 2 weeks prior to and continued for 8 weeks following PFF injections (AMI 5 or 15mg/kg; NOR 5 or 20mg/kg).
Results. NOR significantly reduces human alpha-syn accumulation in a dose-dependent manner within both the cortex and hippocampus of transgenic mice. In the rat PFF model, both AMI and NOR reduce the accumulation of alpha-syn in a dose dependent manner; however, NOR was significantly more potent than both doses of AMI.
Conclusions. Reduction of alpha-syn accumulation by tricyclic drugs supports the view that in addition to their antidepressant properties these drugs hold potential as a disease-modifying therapeutic for PD.

Symposium 53: OTHER TREATMENT STRATEGIES

ADPD5-0775

COMBINED GENETIC TREATMENT IN A TOXIN-INDUCED MOUSE MODEL OF MULTIPLE SYSTEM ATROPHY IMPROVED MOTOR FUNCTIONS

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Background: Multiple System Atrophy (MSA) is a sporadic neurodegenerative disorder found in 4 people per 100,000 individuals with a mean survival of 7-9 years after the diagnosis. The clinical features of MSA include autonomic failures combined with Parkinsonism (MSA-P subtype) in 80% of cases, or cerebellar ataxia (MSA-C subtype) in 20% of cases. Pathologically, MSA is characterized by glial cytoplasmic inclusions (GCIs), gliosis, and striatonigral and olivo-pontocerebellar degeneration. Studies in animal models of MSA and MSA patients have identified several factors that may contribute to the neurodegeneration, such as inflammation, oxidative stress, and mitochondrial dysfunction. These factors are assumed to be linked to alterations in glutamate homeostasis, as observed in the CSF of MSA patients. To examine whether changes in glutamate homeostasis impacts the disease, we used 3-nitropropionic acid (3-NP) to generate a mouse model of striatonigral degeneration in order to evaluate the therapeutic effect of a mixture of three genes (*Nrf2, EAAT2, GDH2*) involved in glutamate homeostasis and oxidative stress.

Results: Following intrastriatal injection of 3-NP in C57 black mice we observed amphetamine and apomorphine induced ipsilateral rotations as well as contralateral motor deficiency. Mice that were injected intrastrataly with a mixture of *Nrf2, EAAT2* and *GDH2* prior to 3-NP injections, showed improvement in motor function and in apomorphine induced ipsilateral rotations, but not in amphetamine induced ipsilateral rotations.

Conclusions: Our findings suggest that the our novel genetic treatment combining *Nrf2, EAAT2* and *GDH2* genes, protects striatal cells from excitotoxicity. These results may provide a novel potential therapeutic approach for MSA.

Symposium 53: OTHER TREATMENT STRATEGIES

ADPD5-0372
SEXUAL DICHOTOMY ALZHEIMER'S DISEASE: NOVEL INSIGHTS AND FUTURE INTERVENTIONS BASED ON ADNP

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Activity-dependent neuroprotective protein (ADNP), essential for brain formation, mutated in autism in men and the only protein reduced in Alzheimer's disease (AD) patient serum, is regulating tauopathy, which is shared by autistic and AD patients. Thus, $ADNP^{+/-}$ mice exhibit tauopathy, age-driven neurodegeneration and behavioral deficits.

Objectives: 1] Is there a sexual difference in ADNP expression, associated with differential AD prevalence?

2] Are the sex-specific ADNP gene targets?

Methods: $ADNP^{+/+}$ mice were compared to $ADNP^{+/-}$ mice behaviorally and gene expression was analyzed by quantitative real time PCR.

Results: Our most recent findings identified sexual dichotomy in hippocampal ADNP expression in men and mice (with a significantly higher ADNP expression in males compared to females) and different behavioral impairments in ADNP-deficient male compared to female mice. Furthermore, the hippocampal transcript content for apolipoprotein E (the major risk gene for AD) was doubled in female compared to male mice, and further doubled in the $ADNP^{+/-}$ females, suggesting differential association with AD in females, also taking into consideration that AD is more prevalent in women.

Conclusions: The identification of sexual dichotomy in the hippocampal expression of ADNP and downstream regulation of apolipoprotein E, coupled with our recent identification of a precise target for ADNP/NAP (davunetide) neuroprotection[1] provides for rational drug development.

1. Oz S, Kapitansky O, Ivashco-Pachima Y, Malishkevich A, Giladi E, Skalka N, Rosin-Arbesfeld R, Mittelman L, Segev O, Hirsch JA, Gozes I. The NAP motif of activity-dependent neuroprotective protein (ADNP) regulates dendritic spines through microtubule end binding proteins. Mol Psychiatry 2014.

Symposium 53: OTHER TREATMENT STRATEGIES

ADPD5-0319
ULTRASOUND AS A THERAPEUTIC AVENUE IN ALZHEIMER'S DISEASE
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BACKGROUND. Alzheimer's disease (AD) and related disorders are characterized by the presence of oligomeric forms of peptides and proteins that eventually aggregate into intra- and extracellular deposits (Ittner & Götz, Nature Rev Neurosci 2011). Levels of these molecules are elevated because of their increased production and/or impaired removal, with recent therapeutic strategies targeting both processes.
METHODS. In our study we aimed to determine whether a transient opening of the blood-brain barrier (BBB) together with the delivery of therapeutic antibodies would assist in the clearance of these molecules. Only one method has been demonstrated to open the BBB non-invasively and repeatedly: non-thermal focused ultrasound coupled with the intravenous injection of microbubbles, widely used ultrasound contrast agents. This is the method we used because it allows for a transient opening of the BBB in the absence of tissue damage, as demonstrated in many experimental species, including rhesus macaques (McDonnald et al., Cancer Res 2012).
RESULTS. We applied focused ultrasound to transgenic mouse models with a pronounced AD pathology and found that this resulted in a significant improvement biochemically, histologically and behaviourally, without causing any tissue damage. We will present mechanistic insight that has been generated in different cohorts of mice.
CONCLUSIONS. Our study highlights the potential of focused ultrasound as a viable therapeutic approach for AD, and possibly other diseases involving protein aggregation, such as frontotemporal dementia and motor neuron disease.

POSTERS

ADPD5-1392

EFFECTS OF ANANDAMIDE ON NEURONAL DEATH AND TAU PHOSPHORYLATION LEVELS

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Objectives: Cannabinoids have demonstrated in recent studies a important role in the physiopathological development of neurodegenerative diseases such as Alzheimer's Disease. However, it is still very controversial the influence of an endogenous cannabinoid, anandamide, in Alzheimer's Disease as well as it is unknown its influence on tau protein phosphorylation . Thus. the aim of this study was to evaluate the effect of anandamide on cell death and tau phosphorylation in differentiated and non-differentiated neuronal-cells.
Methods: Neuroblastoma cells SH-SY5Y were cultivated for seven days in **24 wells plates with** Dulbecco's Modified Eagle Medium **with bovine fetal serum (SFB 15%**) and differentiated cells have retinoic acid 100uM added to **the medium (SFB 1%**) in the days 1, 4 and 7. **Also in day 7,** anandamide was added at the concentrations: 0,1 uM, 1uM e 10uM **and cell viability test or protein extraction was performed 24 hours after the administration**. Cell viability was evaluated by Tripan blue exclusion test. Protein quantification was performed using Western Blotting technique
Results: Anandamide at concentrations 1,0 uM and 3,0 uM promoted significative cell death, but only on non-differentiated cells, differentiated cells showed no alterations. Quantitative values were analyzed by one-way ANOVA (Duncan's post-test, $p<0.05$). Preliminary data of Western blotting analysis revealed a tendency of increasing strongly tau phosphorylation levels in both cells types, although different concentrations have promoted distinct effects in differentiated cells compared to non-differentiated.
Conclusions: Anandamide promoted cell death and probably increases phosphorylation.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0274

MOROCCAN CASES WITH ALZHEIMER DISEASE: CLINICAL, GENETIC AND PROTEOMIC ASPECTS

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Abstract

In Morocco, genes predisposing individuals to Alzheimer disease AD and predicting disease incidence remain elusive and prevent health care professionals from identifying AD in its early stages, with the goal of slowing down the progression. The purpose of the present study is to:

- Evaluate the genetic contribution of mutations in in the presenilin-1 (PS1) and presenilin-2 (PS2) genes to familial early-onset AD cases (FAD) and sporadic late-onset AD cases;
- Elucidate the critical role of GAPDH in the blood of FAD cases carrying presenilin mutations and its interaction with Aβ amyloid.

A higher level of atrophy reflects a decrease in neuropsychological performance. We identified 1 novel frameshift mutation in the PS1 gene and 2 novel frameshift mutations in the PS2 gene. The activity of GAPDH in FAD cases, was significantly decreased as compared to sporadic cases and healthy controls. The expression of GAPDH in blood samples from Mutant tau transgenic mice and FAD cases was decreased as compared to sporadic cases and healthy controls. The Dot blotting examination showed an incresead Aβ accumulation in the blood samples from FAD cases.EM examination showed an increase in amyloid fibrils both in the blood and brain samples. Our mutational analysis report a correlation between clinical symptoms and genetic factors in our cases and suggesting that these mutations increase the risk for developing AD. Our proteomic analysis, report the involvement of GAPDH in AD that may influence the pathogenesis of AD.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0349
NOSOLOGICAL DIFFERENTIATION OF MIXED DEMENTIA (PET-CSF STUDY)
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“Mixeddementia” (MD) is defined as a combination of Alzheimer's disease (AD) andvascular dementia (VaD). According to various studies it’s frequency ranges in15-25%. But pathological, clinical and instrumental studies recently showeddata of much more higher frequency of MD: about 80-95%.
We examined257 patients aged 64,8±8,2 years with various cognitive impairment. In 85patients were diagnosed both vascular and neurodegenerative cognitiveimpairment at the stage of mild cognitive impairment or dementia. In 148patients were investigated levels of Aβ-42 amyloid and total tau-protein in cerebrospinalfluid. 38 patients underwent positron emission imaging with 18-FDG to evaluatecerebral metabolic changes.
In MD Aβ-42level was significantly reduced (195,7±105,5 pg/ml) as compared to patientswith VaD and AD. The concentration of tau was higher (1239,3±582,0 pg/ml)compared with AD patients (677,6±444,0 pg/ml) and VaD (460,9±151,4 pg/ml). Suchdifferences can be due to mutual potentiation of neurodegeneration andcerebrovascular damage. There was significant decrease in glucose metabolismin the temporo-parietal regions and hyppocampus in MD and in AD. In 16 of 18patients with VaD (not at the stage of MCI) specific AD hypometabolic pattern wasobserved, but in a less extent.
Cerebrovascular damage is a significant riskfactor for the progression of neurodegeneration. Pathophysiological mechanismsof AD development, such as amyloidogenesis and tauopathy determine progressionof ischemic brain damage. It can be assumed that the majority of vascular andneurodegenerative cognitive disorders are inherently mixed and this probabilityis even more higher at the stage of dementia.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0360

ALZHEIMER'S DISEASE PATHOLOGY AND OXIDATIVE STRESS WORSENS IN HYPERTENSION WITH DIABETES. NEUROPROETCTION BY TIO2 NANODELIVERY OF ANTIOXIDANT H-290/51

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Diabetes and hypertension are the key co-morbidity factors in several neurodegenerative diseases and could precipitate Alzheimer's Disease (AD) pathophysiology in human populations. Thus, a possibility exists that amyloid beta peptide (AbP) infusion in diabetic and hypertensive rats may induce greater AD pathology than in normal animals. Since these co-morbidity factors are associated with severe oxidative stress, we investigated effects of a potent anti-oxidant H-290/51 in AD pathology associated with hypertensive diabetic (HYDB) rats.

Rats were made hypertensive by 1 kidney 1 Clip (1K1C) method and after 4 weeks administered streptozotocine (60 mg/kg, i.p. for 3 days) to develop clinical symptoms of HYDB. AD like pathology was produced by AbP infusion (50 ng/10 µl, i.c.v. for 4 weeks) in healthy and HYDB rats. Blood-brain barrier (BBB) breakdown, brain edema and neuronal injuries and oxidative stress parameters were investigated. We found that HYDB rats after AbP infusion showed 6 to 12 fold increase in oxidative parameters and exacerbated BBB breakdown to proteins, edema formation and neuronal damages as compared to AbP infusion in healthy animals. TiO2-naowired H-290/51 administered (50 mg/kg, i.v. daily for 2 weeks after AbP infusion) resulted in marked neuroprotection and reducing in oxidative stress parameters. These observations are the first to point out that AD pathology worsens in HYDB cases and nanodelivery of antioxidants may have profound therapeutic value in clinics, not reported earlier.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0393
PROTEIN AGGREGATION VERSUS TOXICITY: DEVELOPING DROSOPHILA MODELS TO STUDY AMYLOID-BETA PATHOLOGY

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A major hallmark of Alzheimer's disease (AD) is the aggregation of the Amyloid-beta (A-beta) peptide. Until now the exact role of these aggregates in AD pathology is not understood, as the amount of aggregated A-beta poorly correlates with disease severity. This raises the question how aggregation and toxicity are linked. Interestingly, A-beta aggregates do not appear randomly during disease progression but spread throughout the brain in a stereotypical manner. How exactly this spreading occurs is still under debate but recent data suggest a prion-like seeding mechanism. In our lab, we are developing *Drosophila in vivo* models that mimic this spreading phenotype. We expressed several A-beta constructs in the *Drosophila* brain and observed differences in their toxicity that did not necessarily correlate with the amount of aggregated A-beta. Hence, our constructs are suitable to examine the relationship between aggregation and toxicity. By simultaneously expressing a slow- and a fast-aggregating A-beta species in the same fly brain, we are analyzing whether there is a crosstalk between the two variants. We hypothesize that A-beta variants with distinct aggregation capacities will influence each other's aggregation kinetics, which might affect the speed of A-beta deposition and thereby alter toxicity levels. This would speak in favor of a "seeding-mechanism". As a read-out we apply various methods to detect parallel changes in toxicity and aggregate formation. Using these novel in vivo models, we can gain new insights into how A-beta pathology spreads throughout the brain and how A-beta aggregation and toxicity are linked to each other.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0400
MUTATIONS AND MODIFICATIONS IN THE METAL-BINDING DOMAIN OF ABETA AFFECT ITS ZINC-INDUCED INTERACTION WITH DNA
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Objectives: Post-translational modifications and/or point mutations in the metal-binding domain (MBD) alter the ability of Abeta to form neurotoxic oligomers. The objective of this work was to evaluate the role of mutations and modifications in zinc-induced interaction of MBD with naturally occurring polyanions, using synthetic single- and double-stranded DNA as a model.
Methods: Surface plasmon resonance biosensing; set of synthetics peptides representing MBD of Abeta natural variants associated with Alzheimer's disease.
Results: (1) Acetylation of the N-terminus amino group and the H6R substitution ("English" mutation) results in a decrease of Zn-induced binding of MBD to DNA. This agrees with the known involvement of the N-terminal amino group and histidine 6 in the formation of Zn-induced dimers of the metal-binding domain. (2) Phosphorylation of serine 8, known to enhance the ability of the metal-binding domain to oligomerize in the presence of zinc ions, and the D7N substitution ("Tottory" mutation) increase the ability of MBD to bind to DNA. (3) In contrast to other bivalent metal ions (Cu, Mg, Ca, Ni, Mn) zinc is a necessary molecular factor for the interaction of MBD with DNA for all peptide variants studied.
Conclusions: (1) MBD is the Zn-dependent DNA binding site of Abeta. (2) The ability of MBD to bind to DNA depends on the peptide oligomerization state. (3) The D7N substitution presumably enhances zinc-induced oligomerization of MBD. (4) Natural polyanions presented in the cerebral spinal fluid can modulate Zn-induced aggregation of Abeta.
Supported by the Russian Scientific Foundation (grant #14-24-00100)

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0451

THE METAL-BINDING DOMAIN 1-16 OF ABETA COMPRISING ISOMERIZED ASPARTATE 7 IS THE MINIMAL AGGREGATION SEED

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Objectives: to determine influence of the isomerization of Aspartate 7, the most abundant spontaneous aging-related chemical modification of Abeta, on amyloidogenic, neurotoxic, structural and aggregation properties of the soluble Abeta species corresponding to the metal-binding domain 1-16.

Methods: transgenic mice model of Alzheimer's disease, histology, neuronal cell cultures, solution NMR spectroscopy, molecular modelling, surface plasmon resonance biosensing.

Resuts: (1) intracerebral injections of synthetic metal-binding domain of Abeta comprising the isomerized Asp7 (isoAbeta16) increase the amyloid burden in transgenic mice; (2) isoAbeta16 is not toxic for neuronal cells; (3) isoAbeta16 forms zinc-induced heterodimers with the intact Abeta16; (4) the primary zinc recognition site 11-14 controls formation of the zinc-bound homo- and hetero-dimers involving isoAbeta16; (5) zinc ions trigger aggregation of isoAbeta16; (6) NMR data reveal structural similarity between zinc-bound dimers of isoAbeta16 and the H6R mutant of Abeta16 (H6RAbeta16); (7) 3D model of the isoAbeta16 zinc-bound dimer structure has been built using solution structure of the zinc-bound dimer of H6RAbeta16 as a template.

Conclusions: (1) soluble isoAbeta16 appears to be the minimal molecular agent triggering aggregation of the endogenous Abeta in vivo; (2) possible molecular basis of the isoAbeta16 amyloidogenicity is related to its ability to form zinc-induced aggregation seeds; (3) blocking the 11-14 site of Abeta responsible for the formation of seeds is a potential anti-aggregation strategy for the therapy of Alzheimer's disease.

Supported by the Russian Scientific Foundation grant No.14-24-00100.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0476
AGGREGATION IN ALZHEIMER'S DISEASE AND PD IS CONTROLLED BY MONOMER RECONFIGURATION
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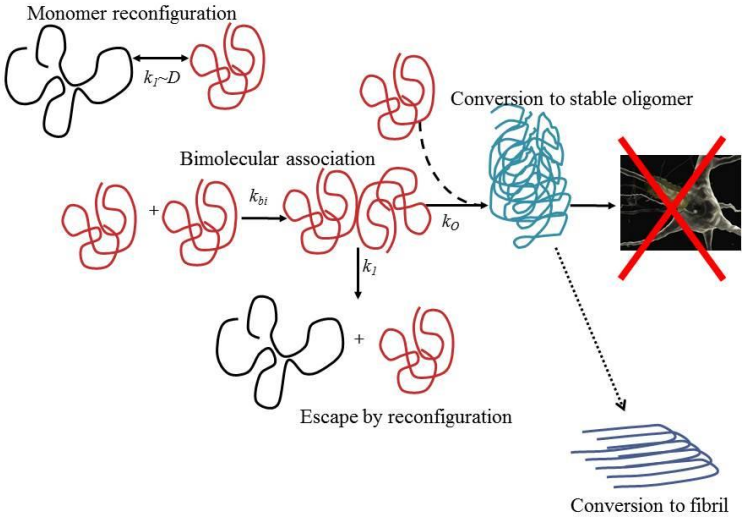
Objectives: We have developed a model of the first step of aggregation, the formation of a dimer, that is kinetically controlled by the reconfiguration of the monomer. When reconfiguration is rapid there is not enough time for two monomers to make stabilizing interactions and form an oligomer. When reconfiguration slows down to the rate of bimolecular diffusion, then stabilizing interactions can be made. We have investigated this hypothesis with measurements of alpha-synuclein and the Alzheimer's peptide.
Methods: To measure reconfiguration we use the method of Trp-Cys contact quenching. One tryptophan and one cysteine are mutated into the sequence. The lifetime of the tryptophan triplet state depends on the likelihood of making a close contact with the cysteine on the same chain and the intramolecular diffusion coefficient, which then yield the rate of reconfiguration.
Results: We find that the reconfiguration rate is highly correlated with conditions that accelerate aggregation (e.g. temperature, pH, mutation). We also find that various small molecule aggregation inhibitors increase the rate of reconfiguration to keep the proteins from forming oligomers.
Conclusions: These experiments give a new view on why these proteins are so prone to aggregation and how therapeutics may be assayed.



01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0539
QIAD ASSAY FOR QUANTITATING A COMPOUND'S EFFICACY IN A-BETA OLIGOMER REMOVAL

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1. Objectives:
Strong evidence exists for a central role of amyloid-beta oligomers in the pathogenesis of Alzheimer's disease (AD). The potency to eliminate A-beta oligomers is, from the current point of view, one of the most desirable criteria for the selection of agents as lead compounds for drug development towards AD treatment. Any screening for oligomer eliminating compounds requires a well characterized target. Therefore, new methods for the preparation, purification and quantification of specific A-beta oligomers, which are representative for the toxic oligomers involved in AD pathogenesis, are urgently needed in AD drug development.
2. Methods
We have designed an assay for the quantitative determination of interference with A-beta aggregate size distribution (QIAD). It is a fast, reliable and robust *in vitro* assay that is able to quantify the A-beta oligomer eliminating potential of any chemical compound.
3. Results
The predictive power of the assay for *in vivo* efficacy is demonstrated by comparing QIAD outcomes of several therapeutically interesting compounds with their treatment effects in animal models and clinical studies.
4. Conclusions
The hereby described A-beta-QIAD assay for analysis of agent-induced A-beta oligomer elimination allows comprehensive and reliable *in vitro* screening of drug candidates for maximum efficacy in the elimination of cytotoxic A-beta oligomers. The quantitative nature of the assay allows comparison between various drug candidates. Furthermore, the observed relation between A-beta oligomer elimination and *in vivo* results strengthens the role of Aβ oligomers in AD pathology.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0555
THE ROLE OF ABETA INTERMEDIATES DURING INDUCED ABETA AGGREGATION
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Objectives
The accumulation of Amyloid-beta leading to the formation of plaque deposits in Alzheimer's disease (AD) is a nucleation-dependent polymerization process. The fibrillogenesis from Abeta monomers occurs through the stepwise formation of oligomers to protofibrils and to finally fibrils. Previous studies have shown that intracerebral injection of Abeta-containing brain homogenate induced Abeta plaque deposition in APP transgenic mice. However, the role of Abeta intermediates during this induced amyloid plaque formation remains elusive.
Methods:
To identify critical factors that are required for the induction of Abeta plaque formation in vivo, we injected APP transgenic mice with 1) either Abeta-containing brain homogenate from APP transgenic mice or with brain homogenate immunodepleted for 2) Abeta and 3) Abeta intermediates. Furthermore, we immunized those intracerebrally injected APP transgenic mice with an antibody against Abeta intermediates. The $Abeta^{+}$ area was defined as seeding area and quantified by immunohistochemical staining.

Results:
Postmortem analysis at different time points revealed that mice injected with brain homogenate depleted for Abeta intermediates showed less seeded plaques when compared to control mice. Passive immunization with an antibody against Abeta intermediates showed similar results, although induced Abeta deposition was still evident.
Conclusion:
Our results suggest that intermediate forms of Abeta are important for the early and rapid plaque formation, but the lack of intermediates in the brain homogenate *per se* doesn't completely prevent the formation of plaques. We conclude that monomeric species of Abeta are sufficient for the initiation of plaque formation and postulate that Abeta intermediates are not the real culprit.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0574

PROTECTIVE EFFECT OF NMDA RECEPTOR ANTAGONIST MK-801 AGAINST SMALL BETA-AMYLOID(1-42) OLIGOMER-INDUCED NEURONAL DEATH

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Soluble beta amyloid (Aβ) forms – one of the most important and critical factors in the pathogenesis of Alzheimer's disease, particularly $A\beta_{1-42}$ oligomers. However, the molecular mechanism how $A\beta_{1-42}$ oligomers trigger neurotoxic cascades are not fully elucidated.

The aim of this study was to investigate the toxic effects of small $A\beta_{1-42}$ oligomers on neurons and glia cells in primary neuronal/glial cerebellar granule cell cultures (CGC) and pure microglia cultures, evaluating whether these neurotoxic effects caused by $A\beta_{1-42}$ oligomer are sensitive to NMDA receptor antagonist MK-801.

Membrane potential of cells was monitored using fluorescent dye DiBAC4(3). The viability of cells was assessed using a fluorescent microscopy, changes in glutamate concentration were measured by the fluorimetric method.

We found that during 0.5-1h incubation small $A\beta_{1-42}$ oligomers caused depolarization of neurons and microglia membranes and MK-801 protected only the microglia cells from $A\beta_{1-42}$-induced membrane depolarization. The effect of MK-801 was also seen in pure microglia cultures. Measuring glutamate level in CGC culture medium we found that during 0,5-4h incubation $A\beta_{1-42}$ oligomers increased glutamate level and this was prevented by MK-801. In pure microglial cultures, $A\beta_{1-42}$ oligomers did not cause release of glutamate into medium. We found that MK-801 preserved neuronal viability and protected CGC from $A\beta_{1-42}$-induced neurotoxicity during 24h incubation.

In conclusion, $A\beta_{1-42}$ oligomers induced rapid and NMDA receptor–dependent microglial plasma membrane depolarization **and rapid** NMDA receptor–independent neuronal depolarization and NMDA receptor–dependent release of glutamate in CGC cultures leading to excitotoxic neuronal death.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0673

FIBRILLIZATION OF THE MIXTURES OF AMYLOID BETA 1-40 AND 1-42

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Objectives
Ratio of Amyloid-beta (Abeta) 42 and 40 in the peptide mixture and agitation conditions are important determinants of the fibrillization kinetics *in vitro*. Our aim was the quantitative determination of the effects of these factors.
Methods
Abeta aggregation was monitored by Thioflavin T fluorescence intensity in a fluorimetric cell equipped with a magnetic stirrer.
Results
Under quiescent conditions carefully defibrillized Abeta solutions were stable for several days, whereas in agitated solutions the fibrillization was completed in less than 2 hours. Agitation was crucial in the initial exponential phase of the process, fibrillization rate of Abeta40 and Abeta 40/42 mixtures, but not that of Abeta 42 were enhanced by agitation also in the elongation phase. Kinetic parameters of the fibrillization depend on the ratio of Abeta 40/42 ratio in the mixture. Adding 10% of Abeta42 to Abeta40 shortened the lag period almost to the level observed with pure Abeta42. Importantly, 10 and 50% Abeta42 did not increased the fibrillization rate that started to increase only when Abeta42 content was 90%.
Conclusions
Fibrillization of Abeta 40 and 42 peptides and their mixtures is triggered by agitation most likely by secondary nucleation mechanism. In the agitated solutions both peptides demonstrated similar fibrillization kinetics, lag period for Abeta 42 was twofold shorter and fibril growth rate twofold faster than that of Abeta 40. The different amyloidogenity of the peptides most likely arise from different ability to form fibrillar seeds and grow under “quiescent” conditions and different stability of their aggregates *in vivo*.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0677

ANALYSIS OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) CONTRIBUTION TO ALZHEIMER NEUROPATHOLOGY USING 2ND GENERATION MODEL MICE

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Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been known to serve as a key enzyme in the glycolytic pathway. Besides, recent evidence suggests that accumulation of s-nitrosylated GAPDH in the nucleus initiates induction of apoptosis. Aggregates consisting of GAPDH have been detected in Alzheimer's disease (AD) brains although the mechanism(s) by which GAPDH affects the progression of AD pathology have remained elusive. In order to examine the contribution of GAPDH to AD pathology, we inspected the expression levels and patterns of GAPDH in a newly established AD model mice. In this novel mouse model, the Ab sequence of the murine APP gene has been humanized, and the Swedish (KM670/671NL), the Arctic (E693G) and the Beyreuther/Iberian (I716F) mutations have been introduced by knock-in strategy (APP^{NL-GF} mice) (Saito et.al., Nat. Neurosci., 2014). In the present study, we investigated the expression profile of GAPDH in APP^{NL-GF} mice by immunohistochemistry. 3 month old mice carrying premature amyloid plaques in hippocampus and cortex showed a modest GAPDH expression compared to age-matched wild-type controls. 7 month old mice carrying a larger number of cored plaques exhibited apparent plaques-like staining of GAPDH near some of the amyloid plaques in hippocampus and cortex. These results suggest that GAPDH plaques appear near mature amyloid plaques. Consistently, we observed similar GAPDH plaques in the hippocampus of AD brains. Further investigation of the mechanism(s) by which GAPDH accumulates in AD model mice may lead to elucidation of the link between GAPDH accumulation and subsequent pathological events such as tauopathy and neurodegeneration.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0684

ROLE OF TRANS EPSILON-VINIFERIN IN CELLULAR AND ANIMAL MODELS OF ALZHEIMER'S DISEASE

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Objectives: Alzheimer's disease (AD) is notably characterized by senile plaques, oxidative stress and inflammation. Polyphenols, such as stilbenoïds, have antioxidant effects and interact with amyloid peptides. These properties could rescue biological impairments observed in experimental AD models. The objectives were to determinate the potential neuroprotective role of *trans*-epsilon-viniferin, isolated from grape cane extracts using new technology (Patent WO2010063980), by studying anti-inflammatory action and anti-aggregating effects on $Abeta_{1-42}$ *in vitro* and *in vivo* AD models.

Methodology: Cellular model of AD was murine primary cultures of neurons and astrocytes treated by aggregated $Abeta_{1-42}$ and IL-1beta. Anti-inflammatory action was studied by quantifying cytokine levels with X-MAP® Luminex assay. Anti-aggregating and disaggregating effects on $Abeta_{1-42}$ were studied *in vitro* by scanning electron microscopy. Animal model of AD was APPswePS1dE9 mice, which received an intraperitoneal injection of viniferin every week between 3 and 6 months or 6 and 12 months to study respectively putative its preventive or curative effects. Anti-inflammatory action and effects on amyloid load were respectively studied by quantifying levels of cytokines by ELISA and immunofluorescence.

Results: We demonstrated that viniferin inhibited inflammation in cellular model of AD and $A\beta_{1-42}$ aggregation process with a superior efficacy than resveratrol and even induced disaggregation of aggregated peptide. In APPswePS1dE9 mice, we confirmed that viniferin go through the blood barrier. Analysis of neuroprotective effects are in progress.

Conclusion: These results promote us to investigate other viniferin's analogs, in large study.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0708
THE N-TERMINAL REGION OF AMYLOID? CONTROLS THE AGGREGATION RATE AND FIBRIL STABILITY AT LOW PH THROUGH A GAIN OF FUNCTION MECHANISM

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Alzheimer's disease is linked to a pathological polymerization of the endogenous amyloid β-peptide (Aβ) that ultimately forms amyloid plaques within the human brain. We used surface plasmon resonance (SPR) to measure the kinetic properties of Aβ fibril formation under different conditions during the polymerization process. For all polymerization processes, a critical concentration of free monomers, as defined by the dissociation equilibrium constant (K(D)), is required for the buildup of the polymer, for example, amyloid fibrils. At concentrations below the K(D), polymerization cannot occur. However, the K(D) for Aβ has previously been shown to be several orders of magnitude higher than the concentrations found in the cerebrospinal and interstitial fluids of the human brain, and the mechanism by which Aβ amyloid forms in vivo has been a matter of debate. Using SPR, we found that the K(D) of Aβ dramatically decreases as a result of lowering the pH. Importantly, this effect enables Aβ to polymerize within a picomolar concentration range that is close to the physiological Aβ concentration within the human brain. The stabilizing effect is dynamic, fully reversible, and notably pronounced within the pH range found within the endosomal and lysosomal pathways. Through sequential truncation, we show that the N-terminal region of Aβ contributes to the enhanced fibrillar stability due to a gain of function mechanism at low pH. Our results present a possible route for amyloid formation at very low Aβ concentrations and raise the question of whether amyloid formation in vivo is restricted to a low pH environment.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0724

A YEAST MODEL SHOWING SYNERGISTIC TOXICITY BETWEEN ABETA42 AND TAU.

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The molecular basis behind Alzheimer's disease is inadequately understood. We have developed a humanized yeast model that shows a clear synergistic toxicity between Abeta42 and Tau. In this model, coexpression of Abeta42 and Tau leads to increased oxidative stress and cellular necrosis. Furthermore, Tau becomes hyperphosphorylated on pathologically relevant epitopes when coexpressed with Abeta42. We identified *MDS1*, the yeast orthologue of human GSK-3beta as a major regulator of this process. Not only is this hyperphosphorylation reduced in a *mds1* deletion strain, the synergistic toxicity observed in wild type strains is significantly decreased, providing evidence for the importance of Tau hyperphosphorylation in the disease progression. However, additional toxicity is still observed in *mds1* deletion strains, meaning at least one other factor is left to be discovered. Furthermore, the growth defect was even more pronounced when Abeta42 was coexpressed with FTDP-17 Tau mutants known to be more aggregation-prone. Finally, we studied a number of deletion strains in which the toxicity of Abeta42 is strongly increased. Upon coexpression with Tau, cellular growth of these mutants fell to nearly zero, showing that our model provides a screenable phenotype that can be a valuable tool in the search for disease modulators. In addition, we performed a synthetic genetic array (SGA) with the genome wide collection of yeast deletion strains and a collection of compromised alleles of essential genes (DAmP library). This revealed a significantly reduced toxicity when *TUB4*, encoding gamma-tubulin essential for microtubule nucleation in both yeast and mammalian cells, was knocked-down (DAmP library).

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0807

ASSESSMENT OF A NEW THREALOSE-CONJUGATED PEPTIDE AS POTENTIAL DRUG FOR ALZHEIMER'S DISEASE THERAPEUTICS

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[2]CNRS UMR 5293 -, Institut des Maladies Neurodégénératives, Universitè de Bordeaux, France

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Objectives: Amyloid-beta oligomers (Abeta-o) are key intermediates in Alzeimer's disease-related synaptic dysfunction. Our aim is to assess the therapeutic potential of a new trehalose-conjugated peptide by probing its ability to 1) hinder Abeta aggregation 2) hold back the synaptic targeting of Abeta-o 3) decrease the neuro-toxicity of Abeta-o, and 4) improve the cognitive deficit due to the accumulation of Abeta-o.

Methods: We have conjugated a trehalose moiety to the LPFFD pentapeptide, to give the Ac-LPFFD-Th. The ability of Ac-LPFFD-Th to bind monomeric Abeta has been investigated by means of NMR, ESI-MS analyses of proteolysis experiments, and Thioflavine-T. The impact of Ac-LPFFD-Th on the assembly of Aβo has been assessed by western-blot analysis. The ability of Ac-LPFFD-Th to neutralize the binding of toxic Abeta-o to synapses and to reduce Abeta-o-induced toxicity has been investigated using cultured rat cortical neurons and subcellular fractionation. We are checking *in vivo* the impact of Ac-LPFFD-Th on the Abeta-o-induced cognitive decline by using the Y-maze spontaneous discrimination task in mice. Abeta-o or Abeta-o+Ac-LPFFD-th will be injected intracerebroventricularly 14 days before the cognitive performance evaluation.

Results: We have characterized the Abeta sequence involved in the molecular recognition with Ac-LPFFD-Th. We have obtained evidences that Ac-LPFFD-Th modify the aggregative features of Abeta-o, reduces the Abeta-o binding to synapse and protects neurons from toxic insults. We also expect that the Ac-LPFFD-Th treatment in rodents will reduce the cognitive impairment induced by Abeta-o.

Conclusions: Based on our findings Ac-LPFFD-Th is a promising candidate for the treatment and prevention of AD.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0894

USING YEAST TO UNDERSTAND INTRACELLULAR PATHWAYS THAT REDUCE ACCUMULATION AND TOXICITY OF ALZHEIMER'S BETA AMYLOID

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The aggregation and accumulation of the beta amyloid (Aβ) protein in the brain is known to be one of the major contributors to neurodegeneration and cognitive decline in AD. Enhancing the removal of Aβ aggregates has been suggested as one approach to preventing neuronal toxicity. Intracellular clearance pathways such as autophagy play a vital role in maintaining cellular homeostasis and survival by removing damaged organelles or aggregated proteins, including Aβ aggregates. However, the cellular mechanisms underlying autophagy mediated Aβ clearance and protection against toxicity is poorly understood. I have developed a yeast cell model expressing Aβ tagged with green fluorescent protein (GFP-Aβ) to help provide insight into its aggregation, toxicity and intracellular clearance pathways that regulate Aβ accumulation.

In yeast, I showed that aggregated Aβ can be selectively targeted for degradation in the cell and enhancing the autophagy-lysosomal activity reduced Aβ accumulation and toxicity. To understand the mechanisms underlying autophagy mediated protection against Aβ, I recently undertook a screening analysis of Aβ expression and toxicity in a gene knock-out autophagy mutant library in yeast. Findings from the study indicated an important role for Hsp70/40 chaperone mediated pathways in the clearance of Aβ aggregates and protection against toxicity. We are currently investigating the protective role of these chaperones in modulating aggregation, Aβ42 clearance and alleviating toxicity using overexpression systems. In addition to its use as a biological screen to dissect disease-relevant pathways, these yeast cell based systems are also ideally suited for high-throughput combinatorial screening techniques for novel drug discovery in AD.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0900
TARGETING THE EARLY MOLECULAR EVENTS OF BETA-AMYLOID PEPTIDE AGGREGATION USING NEO-EPITOPE ANTIBODIES.
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Soluble beta-amyloid (Abeta) oligomers account for the decline in synaptic plasticity and loss of memory associated with dementia and therefore represent an early diagnostic and therapeutic target for the treatment of Alzheimer's disease (AD). In order to elucidate the mechanisms of peptide misfolding in the early stages of AD pathogenesis, it is important to develop sensitive and specific assays to identify and monitor key molecular triggers and biological markers before the development of clinical symptoms. In this work we have investigated the early events of Abeta peptide aggregation using high resolution mass spectrometry and have identified key molecular changes associated with Abeta peptide oligomerisation. Analytical dissection of the molecular events associated with peptide aggregation has enabled us to design and develop a new type of anti-Abeta antibodies (neo-epitope), which specifically target molecular triggers appearing during the initial, early stages of peptide oligomerisation. Screening of AD transgenic mice and human post-mortem AD brain samples, using a combination of antibody targeted and quantitative mass spectrometry revealed that these molecular changes in Abeta peptide are highly relevant *in vivo*. Our findings show that the use of neo-epitope antibodies offers a novel analytical tool for the identification of early molecular seeds of Abeta oligomers. Here we reveal crucial information not only for understanding and targeting Abeta peptide aggregation, but also for monitoring the pathological associated changes in levels of Abeta found in human biofluids.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0908
INHIBITION OF AMYLOID BETA AGGREGATION BY FUNCTIONALIZED METAL NANOPARTICLES

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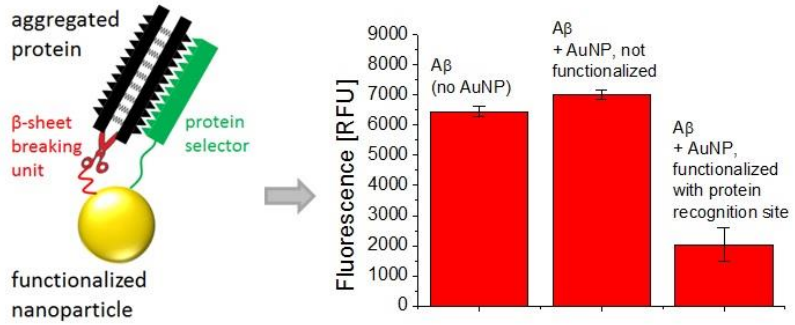
1. Objectives: Neurodegenerative disorders affect an ever increasing number of people in aging societies. In many of these diseases, protein misfolding and aggregation is involved. Therefore, the development of new methods to detect, understand and prevent pathological protein aggregation processes is essential. In our approach, tailored nanoparticles are used as organizational platform and transport vehicle to combine different functional units. These are intended to cooperate synergistically, so that they perform peptide recognition, beta-sheet breakage and peptide cleavage.
2. Methods: Surfactant-free, monodisperse nanoparticles are fabricated by laser ablation in saline solution and conjugated to different self-synthesized ligands. Biophysical assays such as ThioflavinT-staining (beta-sheet content), circular dichroism (secondary structure), density gradient centrifugation and ELISA (oligomerization) reveal the influence of the new materials on Abeta-aggregation.
3. Results: Immobilization of various functional units on gold has a profound influence on colloidal stability kinetics. It was found that different ligand modifications require specific ligand to nanoparticle ratios to secure an overall positive or negative net charge. Most stable conjugates were tested in biophysical experiments, showing that nanoparticles functionalized with an Abeta-selector interact with the model protein and inhibit its aggregation, whereas non-functionalized nanoparticles do not affect protein aggregation.
4. Conclusions: The inhibitory influence of stable, mono-functionalized gold nanoparticle conjugates on Abeta-aggregation was shown. Next, we will synthesize bi-functional nanoparticles carrying an additional beta-sheet breaking unit.



01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0917
ROLE OF REDOX SIGNALING EVENTS IN PROTEIN MISFOLDING DISEASES
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There is accumulating evidence that Alzheimer´s disease (AD) pathogenesis correlates with mitochondrial damage and increased oxidative stress due to the overproduction of reactive oxygen species (ROS) and an imbalance in anti-oxidative defenses. AD pathology is firmly associated with oxidative stress endproducts which can be found at the endpoint of AD pathology, in brains of AD patients. However, it is still not well characterized whether oxidative stress is the cause or consequence of AD pathology or whether ROS act as signaling molecules in the onset and progression of AD.
We are using novel genetically encoded fluorescence-labeled redox sensors which are fused to redox proteins specific for real-time-imaging of glutathione redox potential or H_2O_2. This powerful tool allows the quantitative measurement of redox changes in specific cellular compartments *in vivo.* We have introduced them into our *in vivo Drosophila* model of Amyloid-beta (Abeta) aggregation to gain novel insights into the role of oxidative stress in the onset and progression of AD. With the tools we can follow redox changes in real-time and in parallel to Abeta aggregation and neurodegeneration. The establishment of dual-expression *Drosophila* models, further allows the expression of different Abeta variants (non-toxic/toxic) and the redox sensors in neurons or glia cells, to explore neuron-glia communication. Interestingly, preliminary data show that changes in oxidized glutathione levels in neurons parallel, but not in glia cells, correlate with the deposition of toxic Abeta42 species. The major challenge will be to unravel the direct molecular mechanisms of ROS signaling in neurodegeneration and AD pathogenesis.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0924

APP-INDUCED NEURODEGENERATION IS ABETA-INDEPENDENT AND MEDIATED BY THE UNFOLDED PROTEIN RESPONSE

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Objective:
Accumulation of amyloid-β (Aβ) peptide is a hallmark of Alzheimer's disease (AD) and thought to be the cause of neurodegeneration. However, some animal models produce widespread Aβ deposits but no clear neuronal loss questioning this Aβ hypothesis. We sought to determine the underlying mechanism for olfactory loss associated with AD.

Methods:
We utilized a previously developed an in vivo mouse model that reveals amyloid precursor protein (APP) induced apoptosis is cell-autonomous in olfactory sensory neurons (OSNs). Together with additional transgenic lines and a combination of molecular and biochemical assays we analyzed the basis of OSN neurodegeneration.

Results:
Here we show, in 2 distinct transgenic models that APP-induced apoptosis of OSNs occurs early and is independent of Aβ, highlighting the presence of other pathogenic mechanisms. We further demonstrate that APP-induced apoptosis is dependent on the Unfolded Protein Response (UPR) and that blocking the activity of protein kinase R-like endoplasmic reticulum kinase (PERK) can rescue both anatomical and behavioral phenotypes, thus revealing a key regulatory function.

Conclusions:
These findings support olfactory disruption as an early indicator of AD and present a mechanistic basis for the associated olfactory loss that may prove to be an effective therapeutic target.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0982
LET-7C DECREASED ABETA PRODUCTION VIA ACTIVATION OF BACE2
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Alzheimer's disease(AD) is the most common form of dementia. Nearly all Down syndrome(DS) patients develop AD neuropathology including neuritic plaques and neurofibrillary tangles after 40 years of age. It was believed that some genes on chromosome 21 contributed to the AD pathogenesis in DS patients. miRNAs are regulatory RNAs playing vital roles in physiology and pathology conditions. To study the contribution of miRNAs in AD pathogenesis in DS, we used low density array to screen for differential expression of miRNAs between DS and normal cells. The taqman based array identified 42 differential miRNAs. Our data showed let-7c was increased in DS cells and brain tissues. Let-7c decreased Abeta production, but had no effect on APP or BACE1 expression. Nevertheless, let-7c increased the C99 cleavage within Abeta domain. Further study showed let-7c increased BACE2 expression via activation of BACE2 gene transcription. And this activation depended on Ago1/2 complex. Our study here demonstrated that let-7c, as an activating miRNA, can decrease Abeta production via activation of BACE2.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-0986

QUATERNARY STRUCTURE DEFINES A LARGE CLASS OF AMYLOID-BETA OLIGOMERS NEUTRALIZED BY SEQUESTRATION

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Objectives

Amyloid-beta (Abeta) oligomers have been identified in Alzheimer's disease (AD) brain, but no general consensus has yet been reached as to which are the most significant in AD pathogenesis. This study addresses the following questions related to brain-derived Abeta oligomers: 1) are there distinct subtypes of Abeta oligomers based on quaternary structural motifs; 2) what is the most abundant type of Abeta oligomer produced and how does it affect neurological function?

Methods

We classified brain–derived Abeta assemblies into amyloid-related Abeta oligomers (AmAbetaO's) and non-AmAbetaO's based on their spatial, temporal, and structural relationships to fibrils. We quantified the relative levels of AmAbetaO's and non-AmAbetaO's in Tg2576 and rTg9191amyloid precursor protein (APP) transgenic mice. Using rTg9191 mice, which predominantly produce AmAbetaO's, we examined the effects of AmAbetaO's on cognitive function.

Results

AmAbetaO's, immunoreactive to OC antibodies recognizing in-register parallel beta-sheet structure, appear plaque-dependently and represent the most abundant soluble assemblies in both Tg2576 and rTg9191 mice. AmAbetaO's are concentrated in the vicinity of dense-core plaques and largely overlap with plaque-associated neuropathology. Non-AmAbetaO's, recognized by A11 antibodies, emerge before plaque formation. In contrast, Abeta*56, a memory-impairing non-AmAbetaO's that is produced at low levels, is distributed throughout the cortex. AmAbetaO's do not impair cognition in rTg9191 mice.

Conclusions

Our results highlight spatial distribution as a key factor determining the role of distinct Abeta assemblies on cognition. In the APP transgenic mice studied here, AmAbetaO's, despite being the most abundant soluble Abeta assemblies, are rendered innocuous because of their effective containment within plaques.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1009

VALIDATION AND CHARACTERISATION OF A NOVEL PEPTIDE THAT BINDS BETA AMYLOID AGGREGATES.

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Objective: We have previously reported that a novel 15mer peptide, isolated via phage display screening, targeted Aβ and attenuated its neurotoxicity. Here, we aimed to generate and biochemically characterise analogues of this peptide with improved stability.

Methods: The stable peptide analogue (15MS.A.) underwent the following analysis: stability in brain homogenate, ability to inhibit Aβ aggregates and attenuate neurotoxicity, ability to bind monomeric, oligomeric and fibrillar Aβ using co-immunoprecpitation or Surface Plasmon Resonance analysis; staining for Aβ plaques on brain tissue from transgenic mice and analysis in plasma and brain homogenate following i.v. injection of tritiated (^{3}H) peptide into to mice.

Results: We found that 15M S.A. retained the activity and potency of the parent peptide and demonstrated improved proteolytic resistance *in vitro* and reduced the formation of soluble Aβ42 oligomers. The 15M S.A. candidate directly interacted with oligomeric Aβ42, with an affinity in the low μM range. Furthermore, this peptide bound fibrillar Aβ42 and also stained plaques *ex vivo* in brain tissue from AD model mice. Following i.v. administration, plasma concentrations of ^{3}H peptide decreased with time, as expected, however the peptide was detected in brain homogenate with levels remaining stable over time. Further validation, particularly the ability of the peptide to bind Aβ aggregates *in vivo* is required.

Conclusion: Given its multifaceted ability to target monomeric and aggregated Aβ42 species, this candidate holds potential for novel preclinical AD imaging and therapeutic strategies.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1010

CORRELATING MEMBRANE BINDING AND TOXICITY OF SYNTHETICALLY PREPARED AMYLOID BETA PEPTIDE MUTANTS.

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The key protein causing Alzheimer's disease (AD) is called amyloid beta (Aβ). This protein can acquire a destructive nature to brain neurons and it is unclear why this occurs in a subset of the aging population. Therefore, to better understand what makes this protein neurotoxic, we hypothesise that its toxicity is correlated with binding to the lipid components of the plasma membrane. Further, we have identified the specific amino acids, glutamine (Q), at position 15 and lysine (K) at positions 16 and 28 in Aβ that may have a critical role in mediating the binding to lipid membranes. We will present data showing the biophysical, cell binding and cell toxicity properties of the mutated Aβ peptides. **Methods:** To study Aβ binding to lipid membrane surfaces, giant unilamellar vesicles will be prepared and treated with the Aβ peptides and the extent of binding will be quantitated. ThT aggregation assays will be performed. Primary cortical neuronal cultures will be used for cell toxicity and cell binding studies. **Results:** All three mutant peptides have 1) diminished neurotoxic activity while the Q15A mutant peptide was not toxic at all 2) diminished levels of peptide binding to cells in culture 3) decreased rate of aggregation and fibril formation. **Conclusion:** By understanding the role of these key residues within the Aβ peptide sequence in mediating cell binding and toxicity, this will assist us in future design of therapeutic drugs for the treatment of AD.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1039

INHIBITION OF AMYLOID-BETA UPTAKE BY LYSOZYME AND ITS PATHOLOGICAL RELEVANCE IN ALZHEIMER'S DISEASE

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Objective: Apart from ageing; brain injury and brain inflammation are believed to be important risk factors to trigger Alzheimer disease (AD) pathology. We have studied the role of lysozyme in AD. Lysozyme is a protein of the innate immune system, and is secreted from microglia and astrocytes during inflammatory conditions.
Methods: Lysozyme levels were quantified in cerebrospinal fluid from Alzheimer patients and immunohistochemistry of lysozyme and amyloid plaques were performed in brain sections of sporadic AD patients. Aβ aggregation was investigated using ThT, cell viability was detected using XTT, cellular uptake of Aβ was studied by flow cytometer and binding of Aβ and lysozyme was examined using fluorescence resonance energy transfer.
Results: We have found increased levels of lysozyme in Alzheimer patients' cerebrospinal fluid and sizable deposits of lysozymes in Aβ plaques in the brain of sporadic Alzheimer patients. Pathological importance of lysozyme in AD was revealed by *in-vitro* toxicity studies, where lysozyme prevents Aβ aggregation and rescues neuroblastoma cells from Aβ toxicity. Lysozyme binds to monomers of $A\beta_{(1-42,1-40,1-38)}$ and this binding inhibits neuronal uptake of Aβ.
Conclusions: These results indicate that increased levels of lysozyme could be a preventive stress response during AD that eventually gets overwhelmed by the AD pathology development. Understanding of details about Aβ uptake inhibition and internalization mechanisms by lysozyme will give insights about how to stop intracellular Aβ accumulation and subsequent disease progression, a path breaking mechanism in AD. The study will further enlighten the therapeutic potential of lysozyme to halt AD pathology.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1083
MODIFIERS OF AMYLOID-BETA TOXICITY IN ALZHEIMER'S DISEASE
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Objectives
Processing of Amyloid precursor protein leads to production of two reservoirs of Aβ: a secreted pool and non-secreted cytoplasmic pool. Secreted Aβ contributes to extracellular plaques but despite various studies, the role of cytoplasmic Aβ in AD pathogenesis remains unclear. In this study, we hypothesise that cytoplasmic Aβ contributes to the toxicity of secreted Aβ through specific transmembrane interactions in our fly model of AD.
Methods
Our preliminary experiments show that expression of aggregation prone form of extracellular Aβ leads to a reduction in longevity, locomotor deficits and deposition of plaques while that of cytoplasmic Aβ, on the other hand, is non-toxic. Interestingly, the co-expression of cytoplasmic Aβ enhances the plaque deposition and toxicity of extracellular Aβ. To investigate how extracellular and cytoplasmic Aβ may interact we undertook an RNAi modifier screen of 115 candidate genes in *Drosophila*. We measured the increase in longevity caused by RNAi in flies expressing extracellular and cytoplasmic Aβ. Only those RNAi constructs that did not also cause increased longevity in control flies (those expressing only extracellular Aβ and those not expressing Aβ) were retained.
Results
Thirteen genes are found to specifically rescue the combined toxicity of cytoplasmic with extracellular Aβ. The longevity data is also supported by brain histology, gene and protein expression measurements. Presently, we are further investigating these genes in mammalian cell culture.
Conclusions
Our results suggest a synergistic interaction between two pools of Aβ and highlight the importance of transmembrane Aβ interactions in AD pathology
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01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1114

MODULATION OF ENDOPLASMATIC RETICULUM-MITOCHONDRIA CONTACT SITES DECREASES INTRACELLULAR AMYLOID BETA-PEPTIDE LEVELS

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Objectives The metabolism of glucose, calcium, lipids and cholesterol is controlled in the contact points formed between ER and mitochondria. Interestingly, all these important cellular processes are impaired in Alzheimer's disease. Here, we show that modulation of ER-mitochondria contacts by knock-down of the tethering protein Mitofusin2 (Mfn2) decreases the intracellular levels of amyloid beta-peptide (Abeta).

Methods HEK293 cells overexpressing mutated amyloid precursor protein were transfected with siRNA to knock-down Mfn2. Decreased Mfn2 expression was confirmed by Western Blot. ELISA was used to detect intra- and extracellular levels of Abeta 1-40/1-42. Alamar Blue assay was used to assess cell viability.

Results Knock-down of Mfn2 was successfully performed and did not affect cell viability during a 48 h period. In cells treated with Mfn2 siRNA decreased intracellular levels of both Abeta 1-40 and Abeta1-42 were detected. However, the levels of secreted Abeta were not affected.

Conclusions APP and gamma-secretase complexes are present in the ER-mitochondria interface and interestingly we have recently shown that Abeta is indeed generated in these contact points (Schreiner et al 2014 JAD *DOI10.3233/JAD-132543*). Here we clearly show that intracellular Abeta levels are regulated by ER-mitochondria interplay, while secreted Abeta levels are not affected. This may be interpreted as Abeta is produced at different subcellular sites and that the pool produced in ER-mitochondria contact points normally accumulates intracellularly. In our future work we will test the hypothesis that modulation of ER-mitochondria interplay affects formation of autophagosomes and intracellular production/transport/deposition of Abeta.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1128

OLIGOMER MIMICKING STANDARD PROTEIN FOR MULTIMER DETECTION SYSTEM

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Objectives
Protein misfolding-related diseases or proteinopathies are becoming significant in age-related diseases, such as Alzheimer's disease, Parkinson's disease. Several commercial kits were developed and used to detect their respective oligomer proteins in diseases which are mentioned above. Due to their nature of aggregation, these proteins tend to aggregate and oligomerize often, making the standardization of the measurement in trouble. Hence, it was difficult to quantify these aggregating proteins without expansive devices or kits in a reasonable time frame.
Methods
Standard Amyloid beta 42 (Aβ42) protein was constructed by attaching various fragment peptides of Aβ42 protein onto carrier proteins. The carrier protein would have no immunological relations with anti-Aβ protein antibodies. Three types of standard proteins were constructed for amyloid beta peptides, which was a biomarker for Alzheimer's disease. The artificial standard proteins were characterized by ELISA, SDS-PAGE and western blot.
Results
Oligomer Mimicking Standard Proteins were well detected based on the sequence of the Aβ42 peptide and specific antibodies against N- or C-termini. Oligomer Mimicking Standards Proteins can be applied to MDS as controls and references. Further studies are in progress to construct the correlations between Aβ42 Oligomer Mimicking Standard Proteins. Optimization, stabilization, and features of Artificial Standard Proteins are also in the works. Standard proteins for other protein-aggregating diseases are being developed.
Conclusions
These standard proteins could be efficiently applied as standard references in the Multimer Detection System, which was developed for differentiating oligomers in various proteins aggregating disease.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1281

NEURONAL TOXICITY OF AMYLOID-BETA OLIGOMERS

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Objectives:

Aggregation of the amyloid-beta peptide (Aβ), especially into oligomeric forms, is considered to trigger the pathology of Alzheimer‘s disease (AD) by causing synaptic loss and neurodegeneration. In order to gain a deeper insight into their neurotoxic properties, we characterized synthetic and recombinant (wild-type and E693G mutated (Arctic mutation)) Aβ oligomer species (ADDLs) and their effects on cell survival, formation of reactive oxygen species (ROS) and tau phosphorylation in neuronal cultures.

Methods:

Aβ oligomers were routinely characterized by western blot and silver staining. Binding of oligomers to hippocampal neurons was assessed by immunocytochemistry and confocal imaging. We further investigated generation of ROS after Aβ oligomer treatment using the CellRox dye. The viability of neurons was assessed by LDH assay. Changes in the phosphorylation state of virally overexpressed human tau were examined by western blot.

Results:

We show that the preparations of synthetic and recombinant Aβ oligomers lead to a distinct oligomer composition. These exhibited differential abilities to bind to the surface of neurons and displayed differences in neuronal toxicity. Further, synthetic Aβ oligomers were able to induce tau phosphorylation and ROS formation in neurons.

Conclusions / Outlook:

Aβ oligomers induce AD-typic neuronal phenotypes e.g. oxidative stress and hyperphosphorylated tau. However, although oligomer preparations were highly reproducible according to SDS-PAGE and western blot, we observed high variations within individual experiments. Therefore, we will further characterize the structural properties of our oligomeric Aβ preparations. Moreover, we will investigate the interplay of tau phosphorylation, oxidative stress and neuronal cell death.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1302
DESIGN OF OLIGOMER-SPECIFIC ANTIBODIES
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Selective targeting of oligomeric assembles using specific antibodies provides promising therapeutic and diagnostic opportunities for protein misfolding diseases. Unfortunately, the molecular properties associated with oligomer-specific antibodies are not well understood, and this limits targeted design and development. In this work we demonstrate a generic method that enables the design and optimization of oligomer-specific antibodies. The method takes a two-step approach. The first step discriminates between oligomers and fibrils and is accomplished through identification of cryptic epitopes exclusively buried within the structure of the fibrillar form. The second step discriminates between monomers and oligomers based on differences in avidity. We show inhere that a simple bivalent mode of interaction, as within e. g. the IgG isotype, can increase the binding strength of the antibody up to 1500 times compared to its monovalent counterpart. We expose how the ability to bind oligomers is affected by the monovalent affinity and the turnover rate of the binding and, importantly, also how oligomer specificity is only valid within a specific concentration range. We provide an example of the method by creating and characterizing a spectrum of different monoclonal antibodies against both the A beta peptide and alpha-synuclein that are associated with Alzheimer's and Parkinson's diseases, respectively. The approach is however generic, does not require identification of oligomer-specific architectures, and is, in essence, applicable to all polypeptides that form oligomeric and fibrillar assemblies.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1365

EFFECT OF ALOE VERA ON LIFE SPAN, MOTOR ACTIVITY, AND OXIDATIVE STRESS PARAMETERS IN THE BRAIN OF DROSOPHILA MODEL OF ALZHEIMER'S DISEASE

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Objectives- The present study was conducted to test the neuroprotective effect of *Aloe vera* extract in *Drosophila* AD model.
Methods- Genetic cross was set by using UAS/GAL4 system to get *Drosophila* model of Aβ, expressing amyloid-β ($A\beta_{42}$) specifically in eye and neuronal tissues under the control of UAS promoter. *Aloe vera* Liquid extract (5, 10, 15 and 20ml/L of *Drosophila* food) was orally administered to Aβ-driven flies. Polyphenolic and flavonoid content in Aloe vera extract was determined by HPLC.The memory-enhancing effects of the *Aloe vera* extract were studied by means of Climbing and Longevity assay. Also, the antioxidant activity in *Drosophila* head was assessed using superoxide dismutase (SOD), catalase (CAT), Malondialdehyde (MDA), and protein carbonyl assays.
Results- Eye specific expression of human $A\beta_{42}$ resulted in absolute degeneration of eye ommatidial structures that progress with age. This ommatidial neurodegeneration phenotype was rescued significantly by the treatment with Aloe vera extract. Better rescue was found at concentration of 15ml/L. Similarly *elavGal4* driven Aβ flies showing improved climbing ability and increased longevity. Significantly increase in SOD and CAT level was observed in *Aloe vera* treated flies whereas MDA and protein carbonyl content was significantly reduced.
Conclusions- Our results suggest that the *Aloe vera* extract ameliorates amyloid beta (1–42)-induced spatial memory impairment by attenuation of the oxidative stress in AD model of *Drosophila. Aloe vera* showed a dose dependent significant delay in the loss of activity pattern, reduction in the oxidative stress and apoptosis, and increase in the life span of AD flies.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1423

THE PERMANENT OXIDATION OF ABETA-MET35 MODULATES THE PEPTIDE'S AGGREGATION AND INCREMENTS ITS TOXICITY IN VIVO

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Objectives: To examine the role of Abeta-peptide Met^{35} redox state on its aggregation and toxicity *in vivo,* using a *C. elegans* model of the human amyloidogenic disease Inclusion Body Myositis.

Methods: We used transgenic strains that express the Abeta-peptide intracellularly in muscle cells and, to maintain the peptide´s Met^{35} permanently oxidized, we introduced a deletion of the methionine sulfoxide reductase A-1 gene (*msra-1*). The enzyme MSRA-1 repairs oxidized methionines in proteins.

Results: In the case of a constitutive Abeta transgenic strain, we found that in the absence of MSRA-1, the number of amyloid aggregates decreases while the oligomeric Abeta species increase. These results correlate with increased synaptic dysfunction and misslocalization of the nicotinic acetylcholine receptor ACR-16. In an inducible Abeta strain, which allows us to analyze Abeta toxicity in its early phase of aggregation, the absence of MSRA-1 causes paralysis delay indicating decreased toxicity in these initial stages.

Conclusion: Our approach aims at modulating the oxidation of Abeta Met^{35} *in vivo* and analyzing its aggregation and toxicity, which we measured as changes in locomotor behavior, and neuromuscular junction function and structure. Understanding the process of Abeta aggregation and toxicity related to Met^{35} redox state will contribute to elucidate its role in amyloidogenic diseases. Our results suggest that therapies that boost the activity of the Msr system could have a beneficial effect in managing amyloidogenic diseases at their early stages.

Financial support: FONDECYT 1120213 to RA

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1438

ASSEMBLY OF THE ALZHEIMER'S DISEASE ASSOCIATED AMYLOID BETA PEPTIDE DURING THE LAG PHASE OF FIBRIL FORMATION.

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One of the most important targets in Alzheimer's Disease (AD) therapy is still the proteolytic fragment of the amyloid precursor protein, called amyloid beta peptide. This fragment is marked by its high tendency to self-associate. The self-assembly, which finally leads to the formation of amyloid fibrils, has to proceed via an unknown number of intermediate structures, among which a more potent therapeutic target than the fibril is suspected. OBJECTIVES: The objective was to identify and characterize possible early, distinct assemblies of the amyloid beta peptide in solution regarding size, shape and fraction.
METHODS: Sedimentation velocity centrifugation has been complemented by small angle neutron scattering experiments, atomic force microscopy, and CD-spectroscopy.
RESULTS: By sedimentation velocity centrifugation a small number of s-value species could be reliably detected corresponding to monomers, dodecamers and octadecamers of the amyloid beta peptide, thus corroborating the assumption, that certain assembly states possess a higher probability than others. The observed oligomeric species possessed globular shape, increased beta sheet content in comparison to the mostly random coil structure of the monomer, were negative for thioflavin T staining, and exhibited a stronger cytotoxicity than amyloid fibrils at the same mg/ml concentrations.
CONCLUSIONS: Our study of small Abeta peptide assemblies present in solution during the lag phase of amyloid formation provided size-, as well as shape information and relative fractions of distinct oligomeric species and thus adds to the characterization of a possible new target for AD therapy. The collected quantitative data further allow designing a model for the self-assembly process.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1454
LABEL-FREE SINGLE PARTICLE CHARACTERIZATION OF A-BETA OLIGOMERS IN SOLUTION

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This work determines the size of individual amyloid-beta oligomers in solution without chemical labels by taking advantage of resistive-pulse sensing when single amyloid-beta particles move through the molecular-scale volumes in lipid bilayer-coated nanopores. By measuring the magniture of resistive pulses due to translocation of individual amyloid-beta oligomers, protofibrils, or fibers, this method makes it possible to account for the large heterogeneity of amyloid-beta aggregate sizes and shapes. We propose that this emerging single-molecule technology may help to elucidate the pathological role of various amyloid oligomers in amyloid-associated diseases by characterizing the dynamic distributions in size and shape of these oligomers with high resolution.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1476

PRE-PLAQUE AMYLOID AGGREGATION IN BRAIN WITH ALZHEIMER'S DISEASE PATHOGENESIS

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Objective: To determine the native conformation(s) of beta-amyloid (Abeta) in normal brain and to examine how these conformations change in brain early in the course of Alzheimer's disease (AD) pathogenesis. Prior studies attempting to analyse early Abeta conformations in brain have relied on methods that can alter the conformation of Abeta and/or require fixation. To visualize secondary structure of proteins in their natural state we use label free synchrotron based Fourier transform infrared microspectroscopy (FTIRM) on unprocessed brain sections and primary neurons of AD transgenic mice.

Methods: FTIRM, confocal immunofluorescence microscopy and nondenaturing Western blot are used to analyse Abeta in brains and primary neurons of wild type and AD transgenic mice (Tg19959 with Swedish & Indiana APP mutations) at different ages or time in culture, respectively.

Results: Comparisons of the FTIRM spectral intensity of the alpha and beta bands support that 1. Abeta in the brains of AD transgenic mice starts to aggregate before plaques can be found, and 2. this Abeta starts to aggregate within neurons. In contrast, beta-aggregation is not apparent in wild type mouse brains or primary neurons. Abeta in brain and cells runs as a high molecular weight complex on native gels.

Conclusions: Our data support that localized beta-aggregation precedes amyloid plaque formation in brains of AD transgenic mice and within primary neurons of AD transgenic but not wild type mice. These results further support the growing awareness that misfolded intraneuronal Abeta can be a key pathogenic species involved in causing synapse dysfunction in AD.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1730

PYROGLUTAMYLATED AMYLOID BETA AFFECTS THE STRUCTURE, MORPHOLOGY AND TOXICITY OF THE FULL LENGTH BY SMALL QUANTITIES

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Objectives: to determine which is the ratio in the mixture of AβpE3-42 and Aβ1-42, where AβpE3-42 has the major influence on the aggregation kinetics, secondary structure, morphology and toxicity of Aβ1-42.
Methods: We characterized the cell toxicity by analysis of the intracellular calcium dysregulation; aggregation kinetics by turbidity assay; conformation by Circular Dichroism; morphology by TEM and AFM; secondary structure of single oligomer by NanoIR.
Results: The different mixtures and peptides alone were differently effective in inducing free Ca^{2+} increase in the exposed cells, but the mixture with 5% of AβpE3-42 showed the higher increase. The 5% mixture showed fast aggregation kinetic, similar to that of AβpE3-42 alone, the aggregation kinetics for the other mixtures were inversely proportional to the amount of pEpeptide. The Nano IR spectroscopy revealed that the 5% mixture has an oligomeric population with a prevailing β-sheet conformation and some oligomers with unordered secondary structure, that are not found neither in full length, nor in AβpE3-42. Morphological analysis was in agreement with the previous data. The 5% mixture has a morphology characterized by globular aggregates with more segmented and unstructured fibers, so it is different from the AβpE3-42, Aβ1-42 and from the other mixtures
Conclusions: here we showed that AβpE3-42 has an active role in the formation of pathological oligomers and revealed its maximum effective when it is present at 5% in the amyloid beta mixture.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1732

ALZHEIMER'S DISEASE -CAUSING PROLINE SUBSTITUTIONS LEAD TO PRESENILIN 1 AGGREGATION, DEPOSITION AND ATTENUATED FUNCTION

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Objectives: PXXP proline substitutions in the sequence of presenilin 1 lead to familial Alzhiemer disease (AD). Cyclophilin folding chaperone recognition of presenilin 1 is affected in these mutations. We studied the cellular and biochemical consequences of presenilin 1 misfolding brought about by cyclophilin inhibition. **Methods:** cell biological and biochemical assays were utilized to observe misfolding, aggregation and accumulation of presenilin 1 as a result of PXXP mutations or cyclophilin inhibition. Gamma secretase activity assays were preformed to measure presenilin 1 function. **Results:** Similarly, to their key role in the folding of the prion protein, cyclophilin activity is critically required for the correct folding and functionality of PS1. Inhibition of cyclophilin activity results in PS1 misfolding aggregation and deposition in the endoplasmic reticulum quality control compartment- the ERQC. Correspondingly, the substitution of proline 264 or 267 abolishes a cyclophilin recognition site essential for the proper maturation of PS1 leading to its aggregation. The loss of PS1 function reduces gamma secretase activity, modulates the ratio between the aggregative prone amyloid beta 42 product to 40 and impairs mitochondrial distribution and function initiating the pathological process that underlies familial AD.
Conclusions: Our discoveries point at the cyclophilin family of folding chaperones as a novel linker between toxic protein aggregation, the aging process and neurodegenerative diseases and suggest the elevation of cyclophilin activity as a potential target for therapeutic intervention.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1763

C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSION IN PATIENTS IN THE CLINICAL SPECTRUM OF ALZHEIMER´S DISEASE

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Objectives: To investigate the presence of the pathogenic *C9orf72* repeat expansion in patients with the clinical diagnosis of Alzheimer Disease (AD) or mild cognitive impairment (MCI) not having an imaging and/or a cerebrospinal fluid biomarkers profile compatible with the disease.
Methods: Sixty two patients assisted in the Dementia outpatient clinic of Coimbra University Hospital having a brain imaging and/or CSF profile not typical of AD were included in the study. 15 patients with the clinical diagnosis of AD and 47 MCI were studied for the presence of *C9orf72* expansion.
Results: Three patients (*age* 58 to *81 years*) harboring the *C9orf72* G_4C_2 repeat expansion have been identified (4,8%), all of them with the clinical diagnosis of amnestic mild cognitive impairment based on their neuropsychological assessment. Two *cases were* initially *selected* because they presented atypical CSF levels of $A\beta_{1-42}$, T-tau, and P-tau_{181P}, whereas the remaining one had shown a negative PiB-PET imaging. The MRI scan revealed asymmetrical hippocampal atrophy in all cases, more pronounced in the left mesial temporal lobe. All patients had rapid progressive course to dementia within 12 months.
Conclusions: This study suggest that, albeit rare, *C9orf72* G_4C_2 expansion can be detected in clinical MCI patients. Therefore, our data underscore the utility of testing this particular mutation in clinical diagnosed AD or MCI patients with an asymmetrical pattern of hippocampal atrophy and in patients who presented atypical AD biomarkers profile. This procedure will correct misclassified FTLD cases with direct implications in genetic counseling of both patients and family members.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1771

USING GENETICS, BIOMARKER AND MENDELIAN RANDOMIZATION TO IDENTIFY COMMON PATHWAYS AND GENES IMPLICATED ON ALZHEIMER'S AND PARKINSON'S DISEASE

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Latest genetic studies suggest that there is some genetic overlap between Alzheimer's disease (AD) and Parkinson disease (PD). Similarly, recent studies also supports CSF Aβ and tau levels are potential biomarkers for not only AD but also PD. However it is not clear whether the difference in the CSF Aβ and tau levels is a cause or an effect of the disease. In this project we will use the ADNI, the PPMI, and Washington University genetic and biomarker data to identify common biomarkers and genetic risk factors for AD and PD.

Alzheimer's disease (AD) and Parkinson disease (PD) share common genes, pathways and biochemical biomarkers. A relative large proportion of PD cases also have memory problems. We also hypothesize that AD-related genes and pathways may explain the memory problems in those individuals.

In this project we will use the CSF Aβ, tau and α-synuclein levels together with the genetic information (used as instrumental variable) to perform mendelian randomization studies and determine whether the CSF Aβ, tau and α-synuclein levels are true endophenotypes for PD. Second, we will to use the CSF Aβ, tau and α-synuclein for genetic studies to identify additional variants associated with CSF protein levels and risk for AD and PD. Third, in this proposal, we plan to use genetic data (both rare and common variants) as well as biomarker levels to identify common genes and pathways between AD and PD.

We will present the results at the meeting.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1841

CEREBROSPINAL FLUID BETA AMYLOID1–42 LEVELS IN THE DIFFERENTIAL DIAGNOSIS OF ALZHEIMER'S DISEASE – SYSTEMATIC REVIEW AND META-ANALYSIS

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ObjectivesThe purpose of this study was to carry out systematic review of the literature and meta-analysis to evaluate the diagnostic utility of cerebrospinal fluid Ab_{1-42} as a biomarker for differentiating Alzheimer's disease (AD) from non-AD dementia.
METHODS Design Systematicliterature review was used to evaluate the effectiveness of the Ab for the diagnosis of Alzheimer's disease. The Scottish Intercollegiate Guidelines Network (SIGN) tool was used by two evaluators to evaluate independently the quality of the 17 studies.**Data sources** The literature review covered from October 27, 1946, to October 22, 2013, and searched eight domestic databases including Korea Med and international databases including Ovid-MEDLINE, EMBASE, and Cochrane Library.**Data Extraction and Synthesis**Primary criteria for inclusion were valid studies on (i) patients with mild cognitive impairmentwith confirmed or suspected AD and non-AD dementia, and (ii) assessment of Ab_{1-42} levels using appropriate comparative tests.**Results** total of 17 studies were identified in which levels of CSF Ab_{1-42} were assessed. Meta-analysis was performed on eleven robust studies that compared confirmed AD with healthy individuals, 10 studies that compared AD with non-AD dementias, and 5 studies that compared a-MCI with na-MCI subjects. Overall, the CSF $Ab_{1–42}$ levels were reduced in patients with AD patients compared to healthy controls or non-AD dementia. The effectiveness of this test was evaluated for diagnostic accuracy (pooled sensitivity, 0.80 (95% CI 0.78–0.82); pooled specificity, 0.76 (95% CI 0.74–0.78).**Conclusions** Reduced CSF $Ab_{1–42}$ levels are of potential utility in the differential diagnosis of AD versus non-AD dementias and healthy controls.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1873
EFFECT OF METAL CHELATORS ON THE AGGREGATION OF AMYLOID ? PEPTIDES IN THE PRESENCE OF METAL IONS

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Redox-active metal ions, copper and iron, can bind to β-amyloid (Aβ) peptides in the brain of patients with Alzheimer's disease, and they have been implicated in Alzheimer's disease, as their dyshomeostasis and imbalance may induce aggregation of amyloidogenic peptide. In this work, first, we examined the formation of all types of Aβ40 and Aβ42 aggregates (oligomers, fibrils, and amorphous aggregates) in the presence of the added metal ions, iron and copper, by using fluorescence spectroscopy and atomic force microscopy. Copper and iron differentially altered the morphology of amyloid aggregates. The morphology is crucial for Aβ neurotoxicity and Alzheimer's disease progression. It has been proposed that Aβ oligomers are the most toxic species of Aβ and play a pivotal role in Alzheimer's disease pathogenesis. We have found that iron ions are involved in fibril formation and copper ions induce the formation of amorphous aggregates and oligomers and prevent the formation of fibrillary aggregates. Since metal chelation has been proposed as a therapy for Alzheimer's disease on the basis that it may prevent Aβ aggregation, we also investigated the metal chelating ability of two chelators, ethylenediaminetetraacetic acid and clioquinol (5-chloro-7-iodo-8-hydroxyquinoline), to bind these metal ions and the effects of them on metal-triggered amyloid-β aggregation. Our results showed that both clioquinol and ethylenediaminetetraacetic suppressed the iron ion-induced fibrillization and the copper ion-induced oligomerization of Aβ40 and Aβ42. They also induced changes in the morphology of Aβ aggregates.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1938
MOLECULAR INTERACTIONS OF GLYCODENDRIMERS IN ALZHEIMER´S DISEASE
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Objectives:
H-bond active glycodendrimers are presented as potential Anti-Alzheimer agent in-vitro and in-vivo. To examine Anti-Alzheimer effect of glycodendrimers in vitro and in vivo focusing on the molecular interactions against various amyloidogenic peptides ($A\beta_{1-28}$, $A\beta_{1-40}$, $A\beta_{1-42}$, and human AD brain extracted Aβ).
Methods:
Behavioral evaluation of AD transgenic mice treated with glycodendrimers, Confocal immunofluorescence microscopy, cell viability assay, fluorescence measurements, TEM, Western blot are used to analyze Abeta in cultured cells, in brains and primary neurons of wild type and AD transgenic mice.
Results:
Glycodendrimers are suited to modulate fibril formation of various amyloidogenic peptides and are partly able to reduce the toxicity of Alzheimer's disease brain extracts in human neuroblastoma cells. For in-vivo study both cationic and neutral glycodendrimers are capable to cross blood-brain-barrier and to modify the aggregation state of β-amyloid burden in APP/PS1 mice (*Biomacromolecules* **2011**, *12*, 3903; *New J. Chem.* **2012**, *36*, 350; *Biomacromolecules* **2013**, *14*, 3570). Recent progress is that partial substitution of maltose with histidine significantly increased the ability of PPI maltose dendrimers to cross the BBB and improves cognitive performance in APP/PS1 transgenic mice as a model of cerebral β-amyloidosis with similarities to familial AD.
Conclusions:
Cationic glycodendrimers do not outline the promising in-vivo properties as determined by neutral glycodendrimers. Next efforts are directed to integrate promising biologically active units in neutral glycodendrimers to understand a mechanism of glycodendrimer´s action as anti-Alzheimer agent under in-vivo.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1980

MEMBRANE TOXICITY OF ABETA BY PORE FORMING MECHANISMS: INHIBITION BY A NOVEL PENTAPEPTIDE

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[2]Oxidation Biol. Lab., University of Melbourne, Melbourne, Australia

Objectives

Several studies suggest that beta amyloid (Abeta) fragments produced in the brain by enzymatic processing can generate toxic effects similar to those of $Abeta_{42}$. Here, we used fragments derived from different regions of Abeta with the aim of determining the role of each region on neurotoxicity. Moreover, we identified a neuroprotective pentapeptide having the sequence of the glycine zipper region of the C-terminal of Abeta ($G_{33}LMVG_{37}$).

Methods

In order to evaluate the neuroprotective pentapeptide and the toxicity of $Abeta_{1-28}$ (N-terminal region), $Abeta_{25-35}$ (central region) and $Abeta_{17-42}$ (C-terminal region), we performed patch clamp, immunofluorescence, western blot, FRET, electronic microscopy, calcium imaging and viability assays.

Conclusion

We conclude that Abeta fragments produced toxic effects similar to those observed with $Abeta_{42}$. The results suggest that the center region of Abeta (25-35) is important for membrane perforation and intracellular calcium increase; the N-terminal region (1-28) contributes to the increase in intracellular calcium, but not in the membrane perforation, likely by interacting with other calcium effectors; and the C-terminal region containing the fragment produced by the α-secretase (17-42) induced mitochondrial toxicity, but not membrane perforation and calcium increase, supporting the idea of less toxicity in the non-amyloidogenic pathway.

The effects of GLMVG could be explained by a direct interaction between Abeta and this pentapeptide, which prevents the synaptotoxic effects associated with Abeta that are initiated at the plasma membrane. These results provide a novel rationale for drug development against Alzheimer´s disease.

Supported by Conicyt, Fondecyt 1140473

ADPD5-2096

CONNECTIVITY ANALYSIS IN ALZHEIMER'S DISEASE (AD) BY INTERREGIONAL CORRELATION COMBINING [11C] PIB AND [18F] FDG PET

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Objectives: to determine whether [^{18}F] FDG PET brain pattern correlates with [^{11}C] PIB uptake in AD specific brain region using a SPM (Statistical Parametric Mapping) based network analysis.
Methods: Combined [^{11}C] PIB and [^{18}F] FDG PET patterns data of 15 patients: 3 controls, 8 probable Alzheimer's disease and 4 possible Alzheimer's disease. We used Frontal Cortex and Precuneus as 'seed region' defining [11C]-PIB uptake normalized to cerebellum (CBL) as covariate for a multiple regression analysis of the corresponding [^{18}F] FDG PET data.
Results: PIB uptake in Precuneus (Figure 1) and Frontal Cortex (Figure 2) showed negative correlation with FDG uptake in the parietal cortex (Significance level uncorrected P= 0.005, Extend threshold = 50 voxels).
Conclusions: For specific brain regions PET PIB biomarker for amyloid load correlates with PET FDG biomarker representing brain glucose metabolism. The area of negative correlation is in the parietal cortex. Combined [^{11}C] PIB and [^{18}F] FDG PET scanning needs to be extended to confirm this negative correlation and investigate other functional networks of neurodegeneration.

Figure 1: Area of correlation PIB and FDG PET with Precuneus as 'seed region'

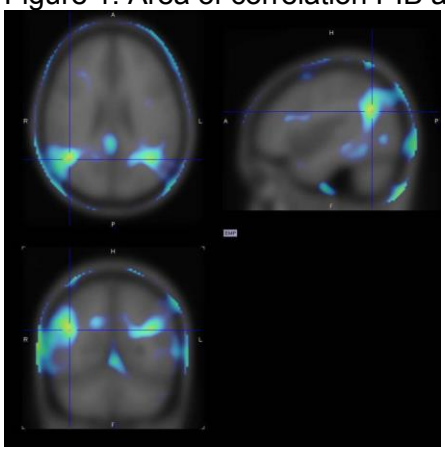
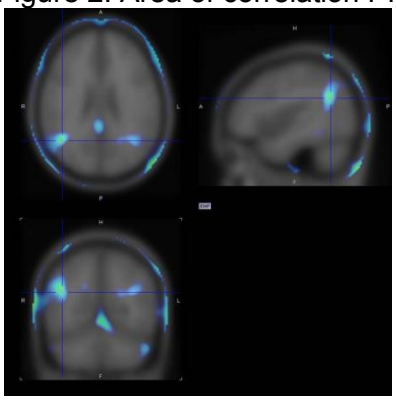


Figure 2: Area of correlation PIB and FDG PET with Frontal Cortex as 'seed region'



01b. Protein Misfolding & Aggregation: Abeta

ADPD5-2131

CORTICAL AND CEREBELLAR A-BETA OLIGOMERS AND A-BETA DEPOSITIONS IN NORMAL CONTROLS AND ALZHEIMER PATIENTS

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Objectives: presence (Western blots), the regional distribution and the co-localisation (immunohistochemistry) of ß-amyloid and oligomer-deposits were studied in cerebral and cerebellar cortex in controls and in Alzheimer's disease patients.
Methods: 69 cases (43-104 years old) were studied by immunohistochemistry using anti-Aß (4G8), anti-oligomer (NU-1 monoclonal antibody) and anti-tau (AT8) antibodies in the hippocampus and the temporal, frontal, occipital neocortex. Moreover, oligomers were examined with Western blots in the temporal cortex (TC) and in the cerebellum of 21 aged-matched cases (11 controls, 10 AD).
Results: A parallel evolution and a similar localization of oligomer deposits with Aß deposition were observed in all studied area. Co-localisation of amyloid deposition and oligomers was observed in the senile plaques. On Western blots all signals detected with the NU-1 antibody against the Aß-amyloid oligomers were present in all cases (controls and AD) in the cortex and in the cerebellum, despite the total absence of plaques in the TC of 6 controls and one «tau only AD», as well as in all cases in the cerebellum. Only the 48 kDa band, corresponding to a dodecamer in the TC and the cerebellum and was significantly increased in the AD patients and was statistically associated with the severity of plaque deposition of the TC.
Conclusions: Aß oligomers are globally present in the human brain aging and the dodecamer (48 kDa) is statistically associated with the severity of plaques. Aß-amyloid and oligomer deposition parallels in all cases in neocortical areas including hippocampal formation.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-2142

THE RELATIONSHIP BETWEEN AMYLOID ANGIOPATHY AND CEREBRAL MICROBLEEDS IN A TWO YEARS AUTOPSY COHORT

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1. Objectives

Cerebral microbleeds (CMB) are hemosiderin-containing macrophages around small vessels on histological examination. The physiopathology of CMBs is largely debated. Several authors report an association with hypertensive encephalopathy in basal ganglia CMB and more associated with amyloid angiopathy (CAA) in superficial, lobar lesions. The goal of our study was to examine the frequency of CMB in different brain regions and the relationship between CAA and CMB with histological and immunhistological methods in a non-selected autopsy population.

2. Methods

Four regions: the frontal, parietal, occipital cortex with the adjacent white matter and basal ganglia of 88 cases of consecutive autopsies with authorisation for research were examined. CMBs were identified on haematoxylin-eosin-stained histological slides, CAA using antibody anti-amyloid.

3. Results

CMBs were present in at least one region in 95.5 % of cases and CAA was observed in 46.6 % of this series. CMB were more frequent in the parietal and frontal lobe followed by the occipital region and basal ganglia. In contrast, CAA was most frequent in the occipital lobe. No statistical correlation was found between the two lesions. In lobar localisation, amyloid of the vessels was present in the cerebral cortex, in contrast to microbleeds, which were mainly in subcortical localisation and both affected different type of arteries.

4. Conclusions

CMB are frequent lesions in postmortem examination and are not associated with CAA, and affects different anatomical subregions and vessel type.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-2162

THE CO-EXISTENCE OF AN EQUAL AMOUNT OF ALZHEIMER'S AMYLOID-B 40 AND 42 FORMS STRUCTURALLY STABLE AND TOXIC OLIGOMERS THROUGH A DISTINCT PATHWAY

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Fibrillar amyloid-β (Aβ) is the major constituent of senile plaques in the brain of patients with Alzheimer's disease (AD). Aβ is a short peptide generated from amyloid precursor protein with two main isoforms, Aβ40 and Aβ42, with the latter having two additional hydrophobic residues at the C-terminus. The two isoforms have distinct characteristics, in which Aβ42 plays a more pathogenic role. Some early-onset familial AD cases possess an elevated Aβ42/Aβ40 level, and biochemical studies show the two species interact with each other. Therefore, understanding structural conversion in the aggregation of mixed Aβ isoforms is essential for elucidating AD pathogenesis. Here, we systematically examined the differences among Aβ42, Aβ40, and various Aβ42/Aβ40 mixtures by monitoring the fibrillization kinetics, epitope changes, assembly, morphology, and induced cytotoxicity. We found the minor Aβ species in different mixing ratios modulated the major aggregation pathway. Size-exclusion chromatography, circular dichroism spectroscopy, and photo-crosslinking assay showed that soluble Aβ42 oligomers were stabilized after Aβ40 addition, and the equimolar Aβ42/Aβ40 mixture rapidly formed spherical oligomers. These oligomers were the most toxic among those examined as evidenced by neurite degeneration and neuronal toxicity. However, these oligomers were not responsible for intracellular calcium elevation. Overall, our results demonstrated that differently mixed Aβ species repartitioned oligomer intermediates on the major aggregation pathway. Furthermore, the equimolar mixture rapidly formed structurally stable and the most toxic oligomers. These results provided information on the potential pathological mechanisms underlying the elevated Aβ42/Aβ40 ratio in familial AD patients and in the local environment of sporadic AD brains.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-2167
GENETIC VARIANT OF AMYLOID BETA A2T MUTANT SLOW DOWN THE FIBRIL FORMATION AND REDUCE CELL CYTOTOXICITY

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Alzheimer's disease (AD) is the most common dementia in the world. Dementia describes significant loss of certain mental functions such as memory, attention, and abstract thinking. The primary neuropathologic hallmark in AD is amyloid plaques. Plaque formation results from increased production or decreased clearance of amyloid beta peptide, which is generated by proteolytic cleavage of the amyloid precursor protein (APP). Genetic mutations reside in APP gene result in familial AD that cause early-onset. Most APP mutations are found in and around abeta region. Previous study showed that the mutations located in N-terminus of abeta, such as the English (H6R) and Tottori (D7N) mutations, would promote fibril formation and increase cell toxicity. However, A673T mutant (A2T in abeta numbering) located in abeta N-terminus close to the beta-secretase cleavage site not only affect the production of abeta but also showed low-prevalence incidence of AD compared to the normal controls. In this study, we used several aggregation and toxicity assays to elucidate the differences reside in wile type, A2T mutant, and A2V mutants. We found that fibril formation in A2T mutantsignificantly retarded and induced less neurotoxicity. The results suggest that N-terminus mutation on abeta could alter its aggregation and toxicity property even though it is not included in the current abeta oligomer and fibril structural models. Further investigation on the involving residues at the N-terminal region is undergoing.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-2185
NITRATION OF Y10 IN AB1-42 HAS SIGNIFICANT EFFECTS ON ITS AGGREGATION

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Alzheimer's diease（AD）is a neurodegenerative disease that is characterized by the deposition of A β_{1-42}.What's more， oxidative /nitrative stress is always detected in patients brain. So，in this way， A β_{1-42} is easy to be nitrated. We used synthetic nitrated peptide to study the effects of nitration of Y10 of A β_{1-42}.We used thioflavin-T（ThT）binding assay and transmission electron microscopy （TEM）to test the aggregation of nitrated A β_{1-42}. We found that nitration of Y10 of A β_{1-42} has significant effects on its aggregation. It can reduce the aggregation of A β_{1-42}. As we know，the formation of oligomers of A β_{1-42} was believed to play a crucial role in AD.These results may suggest that nitration of A β_{1-42} can reduce the toxicity of A β_{1-42} .Our founding may lead a detailed understanding of the function of A β_{1-42} and be helpful in curing AD.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-2232

HEPARAN SULFATE IN NEUROPATHOLOGY IN AMYLOID-BETA PRECURSOR PROTEIN TRANSGENIC MICE

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Objectives:
Heparan sulfate (HS) co-localize with amyloid-beta (Aβ) deposits in Alzheimer's disease (AD) brain and in Aβ precursor protein (AβPP) transgenic mice. HSPGs are widely expressed in extracellular matrices and at cell surfaces, but their pathogenic role in AD is unclear. Our aim was to test if HS participates in Aβ-pathogenesis *in vivo* relating to immune-based mechanisms.
Methods:
Heparanase degrades HS, the glycosaminoglycan side-chains of HSPGs. We compared neuropathology in 15-months-old transgenic mice overexpressing both human heparanase and human AβPP with the Swedish mutation (tgHpa*Swe, n=17) and AβPP single-transgenic mice (tgSwe, n=17). Brains were examined with Congo red and Aβ- and HS-immunohistochemistry. For AβPP-processing, Aβ and sAβPPβ levels were measured in 2.5-months-old mice by ELISAs and western blots. Finally, we investigated the effect of heparin on $Aβ_{1-42}$-aggregation in an *in vitro* Thioflavin T assay.
Results:
The double-transgenic tgHpa*Swe mice had significantly lower amyloid burden (p_{x-42}-burden (p_{1-42}-aggregation *in vitro* (p_{x-40}, and $Aβ_{x-42}$ in 2.5-months-old mice did not differ. Preliminary data of the influence of a peripheral inflammatory stimulus will also be presented.
Conclusions:
HS contributes to amyloid deposition in tgSwe mice by increasing Aβ fibril formation, since the HS-like glycosaminoglycan, heparin, increased $Aβ_{1-42}$ aggregation *in vitro* and heparanase-induced fragmentation of HS led to reduced amyloid burden *in vivo*. Heparanase overexpression did not directly alter AβPP processing in young tgHpa*Swe mice.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-2259
PORTABLE AMYLOID AGGREGATION ASSAYS
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This paper presents the design of portable amyloid aggregation assays for sorting amyloid-beta (Abeta) aggregates by size using nanochannels and then measuring the concentrations of the different Abeta aggregation stages using nanoreactors. In addition, this portable device not only will keep the sample of cerebrospinal fluid (CSF) at a controlled temperature after the extraction, but also will allow to study the correlation between the different Abeta aggregation stages and their cytotoxicity.
First, the CSF sample will be injected into the sampling section. Most of the fluid will be sent to the storage reservoir for the cytotoxicity tests, but a small fraction will be injected into the analytical system. Then, in the sample preparation section, the CSF will be processed to avoid further Abeta aggregation (e.g., by chemical cross-linking), thus ensuring the biological relevance of the analysis. After the injection of the reagents needed to avoid further aggregation, the sample will go through several loops, thus ensuring complete mixing with the reagents. Finally, in the detection/separation section, the sample will go through a series of reservoirs separated by arrays of nanochannels of different widths and heights (from 10nm to 200nm), thus sorting the Abeta aggregates of the CSF sample according to their sizes. Once the sorting is performed, the concentrations of the different Abeta aggregation states will be quantified in each reservoir by nanoreactors, which consist of antibody-functionalized vesicles containing enzymes that can specifically bind to the Abeta aggregates and then can be captured by a surface immobilized primary antibody (IgG).

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-1022

CLINICAL FEATURES OF ALZHEIMER'S DISEASE WITH LEWY BODIES

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Objectives. Among those with Alzheimer's disease (AD), we examined the clinical differences between individuals with and without Lewy body (LB) pathology.
Methods. A total of 531 participants who met neuropathologic criteria for 'high' and 'intermediate' "likelihood" of AD according to the National Institute on Aging-Ronald Reagan Institute guidelines were included. All participants underwent a clinical assessment within two years of death. Chi-square and t-test were used for unadjusted analyses and general linear models were used for adjusted analyses.
Results. Age at onset and age at death were lower among participants with LBs than without. Men more often had LBs. Participants with LBs more often had a demented parent and at least one *APOE* ε4 allele. After adjustment for age, gender, education, neuritic and diffuse plaques, and tangles, scores on the Geriatric Depression Scale (GDS), Neuropsychiatric Inventory Questionnaire (NPIQ), the Unified Parkinson's Disease Rating Scale (UPDRS) motor scale and Wechsler Adult Intelligence Scale-Revised Digit Symbol subtest were more severe for participants with LBs.
Conclusions. In AD, LBs presence related to a younger age at onset and death. Persons with LBs are more often male, have had a parent with a history of dementia and have at least one *APOE* ε4 allele. Participants with LBs have more depressive symptoms, neuropsychiatric symptoms, motor disturbances and slower visual-motor processing. Comorbid Lewy body pathology in AD results in a different clinical phenotype.
Funding acknowledgement: NACC Grant (U01 AG016976)

01g. Protein Misfolding & Aggregation: parkin

ADPD5-2151

INTERLEUKIN-1 DRIVES PROTEIN AGGREGATION IN ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE

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A growing body of evidence establishes neuroinflammation as central in the pathogenesis of both Alzheimer's (AD) and Parkinson's (PD). Here we show evidence that the pluripotent pro-inflammatory cytokine IL-1 regulates the synthesis and function of two of the major components of protein degradation and/or recycling.
Objective: To define and characterize the relationship between IL-1, Parkin, and NEDD8 in human AD and PD, an animal model, and human neuronal cell cultures.
Methods: Using immunohistochemistry and Immunofluorescence, we probed the intercellular localization of NEDD8 and Parkin in cell culture and human brain from AD, PD, and controls. We measured IL-1 and Parkin levels in an AD mouse model and measured Parkin levels in response to IL-1 in primary rat and human (NT2) neuronal cultures.
Results: In an animal model of AD, IL-1 and E3 ubiquitin ligase Parkin increased with age. Further, we showed that neuronal IL-1 treatment increases Parkin expression and mediates translocation of its activator NEDD8. Importantly, in human brain, NEDD8 immunoreactivity is absent in the nucleus and Parkin is aggregated and increased in both AD and PD compared to that in age-matched controls.
Conclusions: Our results are consistent with the idea that IL-1 is a driver of neddylation dysfunction and Parkin aggregation in AD and PD. Thus, we predict that early intervention to appropriately regulate IL-1 expression and activity would prevent or delay protein recycling dysfunctions in neurodegenerative disease, and may be generalized to all disorders characterized by glial activation and IL-1 overexpression. Supported by AG12411 and the Windgate Foundation.

01m. Protein Misfolding & Aggregation: other

ADPD5-0333

CONSUMPTION OF GREEN TEA IS ASSOCIATED WITH REDUCED RISK OF COGNITIVE DECLINE

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Objectives
We aimed to determine whether the consumption of green tea, coffee, or black tea is associated with the incidence of dementia and mild cognitive impairment (MCI) in the general population.
Methods
We conducted a population-based prospective study with Japanese residents aged >60 years from Nakajima, Japan (the Nakajima Project). Participants received an evaluation of cognitive function and blood tests. The consumption of green tea, coffee, and black tea was also evaluated at baseline.
Results
Of 723 participants with normal cognitive function at a baseline survey (2007-2008), 490 completed the follow up survey in 2011-2013. The incidence of dementia during the follow-up period (mean ± SD: 4.9 ± 0.9 years) was 5.3%, and that of MCI was 13.1%. With regard to the incidence of dementia, multiple-adjusted odds ratio was 0.26 (95% CI: 0.06–1.06) for consuming green tea every day compared with those who did not consume green tea at all. Regarding the incidence of cognitive decline (dementia or MCI), multiple-adjusted odds ratio was 0.32 (95% CI: 0.16–0.64) among individuals who consumed green tea every day and 0.47 (95% CI: 0.25–0.86) among those who consumed green tea 1–6 days per week compared with individuals who did not consume green tea at all.
No association was found between coffee or black tea consumption and the incidence of dementia or MCI.
Conclusions
Higher green tea consumption was associated with lower incidence of cognitive decline (dementia or MCI), even after adjustment for possible confounding factors.

01m. Protein Misfolding & Aggregation: other

ADPD5-0455

LOW DOSE CO-APPLICATION OF CELASTROL AND ARIMOCLOMOL INDUCES HEAT SHOCK PROTEINS IN DIFFERENTIATED HUMAN NEURONS: RELEVANCE TO NEURODEGENERATIVE DISEASE TREATMENT

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Neurodegenerative diseases such as Alzheimer's, Parkinson's, and Amyotrophic Lateral Sclerosis (ALS) have been termed 'protein misfolding disorders' that are characterized by the accumulation of aggregation-prone, misfolded proteins which trigger pathogenic cascades that lead to cell death in specific populations of neurons. Heat shock proteins (Hsps) are protein repair agents which can detect and repair misfolded proteins. Upregulation of Hsps has been suggested as a potential therapeutic strategy to combat neurodegenerative protein misfolding disorders. Celastrol induces Hsps by acting on heat shock transcription factor 1 (HSF1). Arimoclomol is a co-inducer of Hsps that potentiates their induction. *In vivo* administration of celastrol in a mouse model of Alzheimer's reduces a key neuropathological feature, namely aggregation of amyloid-beta protein, while *in vivo* administration of arimoclomol in a mouse model of ALS delays the time course of the disorder. Using differentiated SH-SY5Y human neuronal cells, we examined if co-application of celastrol and arimoclomol, at low dosages that do not affect cell viability or neuronal process morphology, triggered induction of Hsps in neurons that is greater than that obtained by their individual application. Low dosage co-application induced Hsps in the human neuronal cells whereas single application did not. Hence this may represent a promising strategy to upregulate a set of neuroprotective Hsps in the treatment of neurodegenerative diseases. Supported by grants from NSERC, Canada and a Canada Research Chair (Tier I) in Neuroscience to IRB.

01m. Protein Misfolding & Aggregation: other

ADPD5-0456

STRESS-INDUCED LOCALIZATION OF HSP70 HEAT SHOCK PROTEINS TO DISCRETE CYTOPLASMIC AND NUCLEAR STRUCTURES OF DIFFERENTIATED HUMAN NEURONS BY LIVING IMAGING AND FRAP

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Heat shock proteins (Hsps) are protein repair agents that can detect and refold misfolded, aggregation-prone proteins that accumulate during neurodegenerative diseases, such as Alzheimer's, Parkinson's and ALS, triggering pathogenic cascades that result in cell death in specific populations of neurons. The localization of Hsps to particular cytoplasmic and nuclear sites was employed as an index to identify structures in differentiated human neuronal SH-SY5Y cells that are particularly sensitive to cellular stress and require the recruitment of Hsps to refold misfolded, aggregation-prone neuronal proteins. Live imaging, using spinning disk microscopy, revealed that YFP-tagged members of the Hsp70 multigene family rapidly localized to discrete neuronal cytoplasmic structures (centrioles) after cellular stress and also to particular elements of the nucleus (nuclear speckles, rich in RNA splicing factors, and the nucleolus). The cytoplasmic and nuclear structures that were targeted by the YFP-tagged HSPA6 (Hsp70B' protein) and HSPA1A (Hsp70-1 protein) were subjected to FRAP (Fluorescence Recovery After Photobleaching). The recovery kinetics, after laser induced photobleaching, demonstrated that the stress-induced recruitment of the Hsp70 proteins into specific cytoplasmic and nuclear structures of human neuronal cells was highly dynamic. Supported by grants from NSERC, Canada and a Canada Research Chair (Tier I) in Neuroscience to IRB.

01m. Protein Misfolding & Aggregation: other

ADPD5-1174

DESIGN OF SPECIFIC DNA PRIMERS TO DETECT OF VARIABLE CLU GENOMIC LESIONS IN ALZHEIMER'S DISEASE

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Recently has been implicated polymorphisms on clusterin (CLU) gene is significantly associated with lipid metabolism, atherogenesis and Alzheimer's disease (AD). However, the influence of genetic variation has not been examined in Korean. So far, there were many techniques have been developed for genome-wide analysis of fusion genes, a more efficient method is desired. In this study, we have developed PCR primer pairs that target specific regions of previously sequenced genes from CLU gene. Primers were targeted to amplify 8 exons, and flanking region its genes which were optimized PCR analysis. This study comprised ten Alzheimer's disease (AD) patients, one Parkinson's disease (PD) patients and two transient global amnesia (TGA) patiens were selected for genotyping. We identified four nucleotide sequence polymorphisms (SNPs) in APOJ/CLU, by direct sequencing from PCR products. These include there (rs7982, rs2279590 and rs3216167) previously reported variants in the SNP database (dbSNP) established by NCBI and as well as one new (NEW1) or extremely low frequency mutations was found in more than one individual in LOAD subjects. Our study suggests that CLU variants may be an AD susceptibility factor in southern Korean population. Larger genetic studies in different ethnics and future metaanalysis are needed to clarify the relationship between the CLU gene and AD.

ADPD5-0386

THE COMMON BIOCHEMICAL PHENOTYPES OF CELLS EXPRESSING APP-MUTANTS LINKED TO FAMILIAL ALZHEIMER'S DISEASE IS INCREASE IN THE SECRETION OF ABETA-OLIGOMERS

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Objectives: Alzheimer's disease (AD) is the most common cause of dementia. Recently, many studies support the oligomer hypothesis that soluble amyloid β (Aβ) oligomers are primarily neurotoxic and affect synaptic plasticity. However, it is unclear whether production of Aβ oligomers would actually increase or not in AD. We examined the production of Aβ protein species in cultured cells expressing mutants of an amyloid precursor protein (APP) linked to Alzheimer's disease in order to determine the common biochemical abnormalities of Aβ formation in various FAD mutations. Methods: Flp-In 293 cells were transfected with wild-, Swedish-, Dutch- and London-type mutant APP, and cultured for two days. We measured the amount of Aβ1-40, Aβ1-42 and Aβ oligomer species in culture media with Western blotting and ELISA. We performed comparative analyses of the productive potencies of Aβ monomer and oligomers among cultured cells harboring wild-type and mutated APP genes. Results: The amounts of both Aβ1-42 and Aβ1-40 secreted from the cells with Swedish-type APP were about ten times more than those with wild-type APP. In London-type APP, the Aβ1-42/Aβ1-40 ratio was increased. Western blotting of culture media using 6E10 antibody showed an intense band of Aβ monomer in cells with wild-type and mutant APP. The amount of Aβ oligomers increased in all the cultured media of cells with the 3 different APP mutants, compared with that of cells with wild-type APP.
Conclusion: We propose that increase in the secretion of Aβ oligomers is the common biological abnormalities in various APP mutants related to FAD.

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-0498

ENDOCYTIC DISTURBANCE DISRUPTS ABETA CLEARANCE IN ASTROCYTES WITHOUT AFFECTING ABETA UPTAKE.

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We previously showed that aging attenuates the interaction between dynein-dynactin complexes in cynomolgus monkey brain and that dynein dysfunction reproduces age-dependent endocytic pathology such as intracellular accumulation of abnormally enlarged endosomes and endosomal APP accumulation. On the other hand, it remains unclear whether such age-dependent endocytic disturbance also occurs in glial cells. Here, we show that intracellular accumulation of enlarged endosomes occurs even in astrocytes of aged monkey brains. Moreover, we found that Abeta accumulates in these enlarged endosomes. RNA interference studies demonstrated that dynein dysfunction reproduces astroglial endocytic pathology and disrupts Abeta clearance in astrocytes via endocytic disturbances without affectin its uptake. These findings suggest that endocytic disturbance can alter astroglial functions and also may be involved in age-dependent Abeta pathology.

ADPD5-0513

INTRABODY-BASED CONFORMATIONAL-SELECTIVE INTERFERENCE WITH Aß OLIGOMERS IN LIVING CELLS TO DISCOVER NEW SUBCELLULAR MECHANISMS OF ALZHEIMER'S DISEASE

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Objectives

Aß oligomers (AßOs) are recognized as the most neurotoxic species in Alzheimer's Disease (AD) but they are still mysterious in terms of assembly, traffic and actions in living cells.

Recently, we established a new subcellularly-localized Conformational-Selective Interference (CSI) approach (Meli et al., Nat Comms 2014) by which we demonstrated that intracellular Aβ undergoes pathological oligomerization through critical conformations formed inside the endoplasmic reticulum (ER).

Here, we study the functional effects mediated by the ER-localized CSI on some AD-relevant subcellular alterations and mitochondrial disfunctions, in order to discover new subcellular mechanisms in AD pathogenesis.

Methods

The CSI approach is based on the expression of conformation-sensitive anti-AßOs antibody fragments as intracellular antibodies (intrabodies), targeted to the ER.

The ER-localized intrabody is expressed in some AD-relevant cell lines and in different primary cells derived from the AD mouse models, such as the neuronal stem cells (NSC) from adult brain subventricular zone (SVZ).

Subcellular and mitochondrial alterations are evaluated by morphological, bioenergetic and biochemical studies.

Results

Conformational-sensitive intrabodies targeted to the ER control the levels and the assembly state of intracellular and extracellular AßOs. This triggers a strong rescue of mitochondrial dysfunctions and bioenergetic deficits in CHO cell lines carrying fAD APP mutations. Ultrastructural changes on mitochondrial-ER junctions can be observed. Studies on primary cells are in progress.

Conclusions

The anti-AßOs intrabodies are unique tools to study the intracellular actions of AßOs; in this way we are deciphering new functional links ER-mitochondria and subcellular mechanisms relevant in the AD pathogenesis

ADPD5-0546

SEPT5 AND ITS POTENTIAL ROLE IN THE MOLECULAR PATHOGENESIS OF ALZHEIMER'S DISEASE

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Objectives: Septins are a highly conserved family of guanosine triphosphate-binding proteins, which play a central role in the axonal transport and vesicle trafficking in the synapses. Particularly SEPT5 has been shown to interact with syntaxin-1 of the SNARE complex and regulate synaptic vesicle (SV) localization at the presynaptic terminal. Furthermore, SEPT5 interacts with SEPT8, which in turn has been suggested to impact SV recycling. Thus, SEPT5 is a potential target for further studies in the molecular pathogenesis of Alzheimer's disease (AD).
Methods: Here, we have investigated the possible alterations in SEPT5 mRNA expression and splicing in relation to the AD-related neurofibrillary pathology in the temporal cortex of human brain. Furthermore, we investigated whether the siRNA-mediated down-regulation of SEPT5 in human SH-SY5Y neuroblastoma cells impacts amyloid precursor protein (APP) processing and amyloid-β (Aβ) production.
Results: Our data suggest that the expression of SEPT5 is moderately decreased in relation to AD-related neurofibrillary pathology in the brain and that the down-regulation of SEPT5 reduces β-secretase (BACE1), soluble APPβ and Aβ levels *in vitro*.
Conclusions: Considering the known mechanistic functions and interactions of SEPT5, our results suggest that SEPT5 plays a role in the regulation of post-translational levels and activity of BACE1. Further characterizations of the potential role of SEPT5 in the early molecular pathogenesis of AD are currently undergoing.

ADPD5-0556

APP A673T MUTATION REDUCES NOT ONLY BETA-CLEAVAGE BUT ALSO GAMMA-CLEAVAGE OF APP IN CELLS

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Objective: APP A673T mutation has been reported to protect against Alzheimer's disease and age-related cognitive disorders by reducing β-cleavage of APP (Jonsson et a., 2012). Although A673T reduces productions of sAPPβ and Aβ, it is unclear whether this mutation causes a concomitant reduction in C99 production leading to reduced Aβ level. The aim of this study is to examine main effects of A673T mutation on Aβ production.

Methods: (1) We expressed A673T APP in CHO cells to examine intracellular level of β-cleaved fragment of APP, C99. (2) We expressed A2T C99 (equivalent to A673T APP) in the cells to evaluate Aβ production. (3) We performed sucrose gradient centrifugation to visualize subcellular distribution of A2T C99 in the cells.

Results: (1) Although the level of sAPPβ in medium was significantly reduced, no concomitant reduction in intracellular C99 was observed in cells expressing A673T APP. (2) Aβ level from cells expressing A2T C99 was roughly 50% of those expressing WT C99. Cell free assay using microsomal fraction indicated that Aβ produced from A2T C99 was significantly reduced, compared to that from WT C99. (3) Sucrose gradient centrifugation revealed that A2T C99 was less associated with lipid raft, compared to WT C99.

Conclusion: APP A673T mutation causes an improper subcellular localization of C99, which alters Aβ production in cells.

ADPD5-0567

CHARACTERIZATION OF AN ISOGENIC DISEASE MODEL OF ALZHEIMER'S DISEASE FROM HUMAN IPS CELL-DERIVED NEURONS

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Objectives: Mutations in the gene encoding beta amyloid precursor protein (APP) have been linked with the progression of Alzheimer's Disease (AD). Leveraging our ability to produce previously inaccessible human neurons from iPS cells, we made biologically relevant AD disease models incorporating two APP mutations of interest as part of our Disease and Diversity Products that represent an array of both healthy and disease-specific backgrounds.
Methods: Using a TALEN-mediated SNP alteration, we introduced APP A673V or APP A673T gene mutations into a 'control' iPS cell line (01279.107) from an apparently normal healthy Caucasian male donor (no family history of neurological disorders). Cortical neurons were differentiated from these three unique isogenic iPS cell lines. Gene expression was analyzed by target- and disease-focused PCR arrays. Levels of AD-related biomarkers (i.e., sAPPα, Aβ 1-40, and Aβ 1-42) were quantified using various HTS-compatible assays (i.e., HTRF and AlphaLISA). The network-level activity of neurons from each background was evaluated on multi-electrode array (MEA).
Results: Here, we present data characterizing the gene expression and functionality of these neurons. Results from the HTS-compatible assays and MEA showed differences in functionality between the allele variants. Additionally, we present their individual responses to pharmacological modulation.
Conclusions: These data illustrate how human neurons derived from isogenic iPS cell lines provide a biologically relevant disease model that can be used to study AD in a dish.

ADPD5-0633

DISTRIBUTION OF AMYLOID-BETA PROTEIN PRECURSOR AND SECRETASES IN CORTICAL SYNAPSES OF MICE: MODIFICATIONS IN EARLY ALZHEIMER'S DISEASE CONDITIONS.

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Background and Objectives: An unbalanced proteolytic cleavage of amyloid-β precursor protein (APP) can result in an increased amyloid β peptide (Aβ) production and, consequently, in AD onset. Although it is widely accepted that synaptic Aβ metabolism is crucial for AD initial damage, the distribution of APP and secretases in different nerve terminals remains to be clarified. This study aims to investigate if the cortical synaptic levels of APP and of the secretases involved in APP proteolysis, BACE1 and ADAM10, are affected under AD conditions, using a mice model of AD.
Methods and Results Western blot analysis revealed that APP was located mainly in the pre-synaptic active zone (53.1 ± 5.5%) and in post-synaptic regions (37.1 ± 2.6%), ADAM10 was enriched in post-synaptic regions (61.3 ± 4.3%,), whereas BACE1 was concentrated in extra-synaptic zone (72.5 ± 4.9%). Immunocytochemistry analysis revealed that APP and BACE1 were present in glutamatergic and GABAergic nerve terminals and these proteins were co-localized in about 30% of these nerve terminals. Moreover, in mice intracerebroventricularly injected with $A\beta_{1-42}$ that displayed hippocampal-dependent memory deficits and a decrease in long-term potentiation, the levels of APP and ADAM10 increased by 30%, whereas the levels of BACE1 were significantly reduced.
Conclusions: The results provide the first comparative analysis of the synaptic and sub-synaptic distribution of APP and secretases in cortical brain regions of mice and show that, in early AD, there are compensatory mechanisms to avoid Aβ overload.
Supported by 'FCT # PEst-C/SAU/LA0001/2013-2014# PTDC/SAU-NMC/114810/2009, FEDER (QREN), DARPA- W911NF-10-1-0059

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-0649

IDENTIFYING THE TOXIC ABETA OLIGOMERIC SPECIES BY CORRELATING NEUROTOXICITY AND ABETA BINDING TO NEURONS

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OBJECTIVES: Soluble Aβ oligomers (AβO) have been proposed as the key toxic Aβ species causing Alzheimer's disease (AD) brain pathology. The identity of the toxic oligomeric Aβ species remains unclear. We hypothesized that a specific AβO species binds to neurons inducing synaptic dysfunction and neuronal cell death. We identified the toxic AβO species that bound to neurons and correlated with neuronal death. The role of caspases in AβO-induced neurotoxicity was investigated as a possible mechanistic pathway of cell death. METHODS: Mouse (C57/BL6) primary cortical neurons (6 days in vitro) were treated with synthetic soluble Aβ40, Aβ42 and purified AβO (Aβ40; 1mer – 4mer prepared using PICUP technique) for up to 96 hours. Cell viability, Aβ binding to neurons and caspase (3, 6 and 8) levels were determined. RESULTS: Neurons treated with synthetic Aβ42 peptide caused neurotoxicity in a time dependent manner. This neurotoxicity significantly correlated with the presence of bound 3mer and 4mer AβO species. Treatment with purified AβO 4mer and 3mer oligomers were up to 50-fold more toxic than monomerised Aβ40 peptide. Soluble Aβ peptides induced cell death via caspase 3-dependent apoptotic pathway while the purified AβO induced cell death via non a caspase-dependent pathway. CONCLUSIONS: This study proposes that Aβ 4mer and 3mer are the most toxic of the lower molecular weight AβO species.

ADPD5-0656

BRILLIANT BLUE G DYE ATTENUATES ABETA OLIGOMER INDUCED NEUROTOXICITY BY DECREASING ABETA BINDING TO NEURONS

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OBJECTIVES: Brilliant blue G dye (BBG) is a primary component of the coomassie blue staining solution which is typically used for staining proteins on gels. But BBG has been shown to cross the brain blood barrier and act as a neuroprotective agent in various animal models including migraine, Huntington's disease, and Alzheimer's disease (AD). BBG has been shown to inhibit Beta Amyloid (Aβ) aggregation and its toxicity in cell culture models but the mechanism of how BBG in decreasing Aβ toxicity is unclear. Since Aβ oligomers (AβO) need to bind to neurons and cause significant neurotoxicity, we hypothesized that BBG attenuates AβO-induced neurotoxicity by decreasing Aβ binding to neurons. METHODS: Photo-induced cross-linking technique was used to generate and purify AβO (1mer-4mer). Mouse (C57/BL6) primary cortical neurons (6 days in vitro) were treated with purified AβO (with and without the presence of BBG) for up to 96 hours. Cell viability and Aβ binding to neurons were determined following treatments. RESULTS: AβO showed decreased neurotoxicity in the presence of BBG (at least 2 fold). This was accompanied with decreased AβO binding to neurons in the presence of BBG. CONCLUSIONS: This study showed that BBG attenuates AβO-induced neurotoxicity by decreasing AβO binding to neurons. Since BBG can enter the brain and inhibit Aβ binding and neurotoxicity, further studies are required to determine whether BBG has any therapeutic potential in treating AD.

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-0661

BEXAROTENE AND ASTAXANTHIN MODULATE CHOLESTEROL AND AMYLOID-BETA METABOLISM IN AN IN VITRO MODEL OF THE BLOOD-BRAIN BARRIER

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Using an *in vitro* model of the blood-brain barrier (BBB) we have shown that primary porcine brain capillary endothelial cells (pBCEC) express and release apoA-I that may assemble with cellular cholesterol to form HDL, a pathway that is enhanced by treatment with nuclear receptor agonists. These apo/lipoproteins may also interact with Aβ or its precursor protein (APP). Pharmacological modulation of cellular cholesterol metabolism could contribute to redirect APP synthesis and processing by cerebromicrovascular endothelial cells towards the beneficial, non-amyloidogenic pathway. This study aims to investigate the effects of a pharmacologic retinoid-X receptor (RXR) agonist, bexarotene, and PPAR-α agonist, astaxanthin, on pathways of APP processing, Aß production and transfer across the BBB, and on the (mechanisms of) formation and remodeling of HDL particles. Primary porcine (p)BCEC were incubated with bexarotene or astaxanthin. Protein and mRNA expression levels of apoA-I, apoJ, APP/Aβ, ABCA1, ABCG1 were measured. Furthermore, effects of bexarotene and astaxanthin on time-dependent [^{3}H]-cholesterol release from pBCEC were studied in the presence of apoA-I or HDL acceptor particles. Real-time PCR and immunoblotting analyses suggest a higher expression of both ABCA1, ABCG1 and APP and decreasing levels of Aβ oligomers. Cellular cholesterol efflux, the obligatory first step of reverse cholesterol transport, was enhanced after administration of [100 nM] bexarotene or [1 nM] and [10 nM] of astaxanthin with both acceptors. Our results, thus far, suggest that these two NR agonists exert beneficial effects on cholesterol and Aβ metabolism in cerebrovascular cells.

ADPD5-0691

INTRANEURONAL AMYLOID BETA OLIGOMERS CAUSE ABERRANT SPINE MORPHOLOGY AND DISRUPT AXONAL AND DENDRITIC TRAFFICKING INDEPENDENTLY OF TAU

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Objectives:
Mounting evidence indicates that extracellular amyloid β (Aβ) oligomers cause synaptic dysfunction and that this toxicity requires tau in the dendrites. Meanwhile, it has been suggested that intraneuronal accumulation of Aβ proceeds extracellular Aβ, and is an early event in Alzheimer's disease. It remains unclear whether intraneuronal Aβ also contributes to synaptic alteration, and if so, whether the toxicity requires tau.
Methods:
To address these questions, mouse/rat primary neurons were transfected with human APP with or without the Osaka (E693delta) mutation which induces intracellular accumulation of Aβ oligomers. The morphology of dendritic spines, and axonal or dendritic transport of BDNF, mitochondria, and transferrin receptor (a marker of dendritic recycling endosomes) were evaluated. For comparison, the effect of extracelluar Aβ on dendritic spines was examined by adding Aβ into untransfected neurons at concentrations comparable to those in culture media of wild-type APP-transfectants. To study the necessity of tau, primary neurons from tau-deficient mice were also analyzed following to APP transfection.
Results:
Neurons expressing APP Osaka, but not wild-type APP, accumulated Aβ oligomers within cells. APP Osaka-transfectants showed reduced numbers of total and mushroom-type spines, but wild-type APP-transfectants and Aβ-added untransfectants did not. The flux values of BDNF, mitochondria, and the transferrin receptor transport in axons and dendrites were reduced only in APP Osaka-transfectants. Intracellular Aβ-induced aberrant spine morphology was observed even in tau-deficient neurons.
Conclusions:
Intraneuronal Aβ oligomers disrupted synaptic integrity independently of tau, and this toxicity was accompanied by an impairment of axonal and dendritic trafficking.

ADPD5-0788

DIMERIZATION OF APP IS PRIMARILY MEDIATED BY ITS E1-DOMAIN AND REGULATED BY THE ACIDIC REGION

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Objectives

Alzheimer's disease (AD) is one of the most frequent dementias in the elderly population affecting more than 25 % of people in the age of 80 to 90 years. One key player in the generation of AD is the Amyloid Precursor Protein (APP). In addition to its role in Alzheimer's pathology many physiological functions, like stimulation of synaptogenesis and signal transduction in a receptor-like manner are discussed for APP. Dimerization of the protein has been described for APP and would influence most of its physiologic functions.

Methods

We studied the dimerization behaviour of the APP ectodomain by analysing the interaction of constructs comprising different parts of it employing a number of biochemical and biophysical methods like static light scattering, dynamic light scattering and analytical gelfiltration.

Results

We show that the dimerization of APP is primarily driven by the heparin induced dimerization of its E1 domain. Heparin induced dimerization has also been discussed for the E2 domain, but we see the concentration dependent self-dimerization of E2 and independent heparin binding, but no heparin induced dimerization here. Additionally, we show that the region in-between the two subdomains of APP influences the dimerization of E1 and with that of APP.

Conclusions

Our results led to a new model of APP dimerization that strongly depending on the cellular context features both, *cis*- and *trans*-dimers. This brings together a large number of previously mutually exclusive physiological functions and suggests that the trafficking of APP is essential for its dimerization and hence for its biology.

ADPD5-0793

IDENTIFYING THE MECHANISM OF DIFFERENTIAL PROCESSING OF AMYLOID PRECURSOR PROTEIN ISOFORMS

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Objectives
The expression profile of APP isoforms has been shown to change from the shorter APP695 isoform to the longer APP751/770 isoforms in the Alzheimer's disease (AD) brain. Amyloidogenic processing and Aβ production has been shown to vary between isoforms and we reported increased amyloidogenic processing and Aβ production from the APP695 isoform in neuronal cell lines (Belyaev et al, 2010). We sought to identify the molecular and cellular mechanisms underlying this differential processing of APP isoforms through studying their subcellular trafficking and interactome.
Methods
FLAG-tagged APP695 and APP751 isoform constructs were over-expressed in SH-SY5Y cells and their trafficking observed by fluorescence microscopy. Co-immunoprecipitation of APP isoforms was carried out and interacting proteins identified by mass spectrometry. Identified proteins were then targeted by siRNA knockdown and effects on APP processing observed by western blot and ELISA.
Results
A higher level of APP695 was observed in Rab11a positive endosomes, while a higher level of APP751 was observed in the trans-Golgi network and early endosomes. Several proteins appeared to interact differentially with the APP isoforms and affected APP processing to various extents including the APP interactor Fe65.
Conclusions
APP isoform interactomes show some significant variations and have identified several proteins potentially responsible for causing differences in isoform processing. Subtle differences were also observed in APP isoform trafficking, potentially affecting their processing through spatial separation from the proteases responsible for their cleavage.

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-0861

C-ABL PROMOTES APP/BACE1 INTERACTION FAVORING AMYLOIDOGENIC APP PROCESSING

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Objectives: Niemann-Pick type C (NPC) is a neurodegenerative disease that presents amyloid beta (Abeta) accumulation in the brain. Previously, we showed that c-Abl is activated in NPC models. Additionally, c-Abl interacts and phosphorylates the amyloid precursor protein (APP), however the relevance of this interaction has not been defined yet.
Methods: We evaluated i) APP processing following Abeta, betaCTF and sAPPalpha levels by *Western Blot* in: a) WT and NPC CHO cells expressing APP_{Swe} and treated with Imatinib (c-Abl inhibitor) and b) NPC mice injected with Imatinib for 4 weeks and ii) the interaction between APP, c-Abl and beta-secretase (BACE1) by co-immunoprecipitation and FLIM techniques in NPC CHO cells expressing c-Abl-DsRed and APP-GFP. A mutant APPY682A-GFP was used to investigate whether the binding of c-Abl to APP is dependent on the Y^{682}ENPT motif.
Results: We found that NPC models showed increased levels of Abeta. Imatinib inhibited the amyloidogenic processing of APP reducing Abeta and betaCTF and increasing sAPPalpha levels. NPC cells showed an increase of APP-c-Abl interaction compared with WT cells. Imatinib or the APPY682A mutation inhibited APP-c-Abl interaction, but more importantly, Imatinib significantly reduced the APP-BACE1 interaction.
Conclusions: Our results suggest that c-Abl interacts with APP, through the Y^{682}ENPT motif and promotes APP cleavage by BACE1 favoring Abeta accumulation and contributing to the pathogenesis of NPC disease.

ADPD5-0969

MITOCHONDRIAL BIOENERGETICS IS INVERSELY CORRELATED TO ER-MITOCHONDRIAL CONNECTIVITY IN ALZHEIMER DISEASE

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Perturbed mitochondrial function and altered mitodynamics have been described as possible triggers of neurodegeneration in Alzheimer disease (AD). However, while mitochondrial dysfunction is an undeniable early symptom of the disease, the pathogenesis of AD cannot be explained simply as a result of mitochondrial alterations; some other, currently-unknown, mechanism is likely at work.

We recently showed that presenilin 1 (PS1) and presenilin 2 (PS2), and γ–secretase activity itself, are present in mitochondrial-associated ER membranes (MAM), and that cells from AD patients with both the familial and sporadic forms of the disease have increased ER-mitochondrial connectivity and upregulated MAM function. For these reasons, we hypothesized that changes in MAM behavior might affect and perhaps regulate some mitochondrial activities. To explore this issue in the context of AD, we measured mitochondrial respiration in PS1/PS2 double knockout (DKO) mouse embryonic fibroblasts (MEFs), and found a profound decrease in OxPhos in these cells. In order to analyze the possibility that this phenotype was somehow related to the higher degree of ER-mitochondria apposition seen in AD-mutant cells, we measured mitochondrial respiration in mitofusin2-knockout MEFs (MFN2-KO) that have the opposite phenotype, namely, reduced ER-mitochondrial apposition and lower MAM activity. Remarkably, these cells showed a significant increase in OxPhos.

These data imply that there is a heretofore unappreciated inverse correlation between ER-mitochondrial apposition and mitochondrial bioenergetics, and provide new insight into the well-known defects in mitochondrial function in AD.

ADPD5-1056

SIMVASTATIN INFLUENCES APOJ AND AMYLOID-BETA METABOLISM AT THE BLOOD-BRAIN BARRIER

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Amyloid-β (Aβ) peptides accumulate in cerebral capillaries indicating a central role of the blood-brain barrier (BBB) in the pathogenesis of Alzheimer's disease (AD). Although a close connection between apolipoprotein-, cholesterol- and Aβ metabolism is evident, the interconnecting mechanisms operating in brain capillary endothelial cells (BCEC) are poorly understood. Apolipoprotein (apo)J, also known as clusterin, is present in lipoprotein particles and regulates cholesterol and lipid metabolism of brain which is disturbed in AD. ApoJ expression is increased in AD brains and ApoJ binds, prevents fibrillization, and enhances endocytosis of Aβ. Our central study aim is to define the involvement of apoJ and cellular cholesterol homeostasis in amyloid precursor protein (APP) processing/Aβ metabolism at the BBB.

Primary porcine (p)BCEC are incubated in the presence and absence of plasma-derived apoJ and modulators of cholesterol metabolism prior to analyses of APP/Aβ and apoJ mRNA and protein expression levels. Aβ transport studies are aiming to elucidate the role of apoJ and the HMGCoA reductase inhibitor simvastatin in Aβ clearance across the BBB.

Thus far, we found an increase of both apoJ and full-length APP mRNA and protein in response to treatment of pBCEC with 5 µM simvastatin. Further, simvastatin reduced cell-associated Aβ oligomer levels, whereas extracellular Aβ peptides were increased. Addition of purified apoJ [2µg/ml] increased APP, whereas apoJ silencing decreased APP and Aβ oligomer protein levels.

Our results, so far, suggest an important role of cellular cholesterol homeostasis and apoJ in modulating APP/Aβmetabolism at the posttranslational level in cerebrovascular endothelial cells.

ADPD5-1089

TRANSMEMBRANE INTERACTIONS IN APP STRUCTURE, FOLDING, AND PROCESSING BY GAMMA-SECRETASE

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Objective

Understanding the molecular mechanisms controlling APP processing by gamma-secretase represents a challenging task in AD research. Transmembrane (TM) interactions appear to have a central role here, both by driving the assembly and activation of the gamma-secretase, and the docking/fitting of the substrate prior to cleavage. We identified key structural determinants (GxxxG and GxxxG-like motifs) in APP/Presenilins TM domains (TMDs) and analyzed their role in APP amyloidogenic processing and γ-secretase activity.

Methods

APP/PS1/PS2 constructs (including FAD mutants) were generated by site-directed mutagenesis. Assembly and activation of gamma-secretase was studied by non-denaturating electrophoresis, co-immunoprecipitations, and combined to results of in vitro gamma-secretase assays. APP transmembrane/juxtamembrane regions were analyzed by structural approaches (FTIR/NMR spectroscopy). APP processing was monitored by electrochemiluminescence assays (ECLIA).

Results

GxxxG motifs in APP TMD were initially found to control the association and orientation of APP homodimers. We showed here they control the alpha-helix/beta-sheet structure of inhibitory and cholesterol-binding regions, impacting on gamma-cleavage and Abeta release. Mutation of PS1 and PS2 TMD8 motifs either abolishes PS endoproteolysis and gamma-secretase activity, or increase it in the case of FAD mutant. Non-denaturating conditions indicated that GxxxG-like motifs in PS TMD8 are key determinants for the geometry of the mature gamma-secretase controlling the switch between physiological and pathological conformations.

Conclusion

Our data suggest that GxxxG-like motifs in APP and PS are crucial structural determinants for the physiological and pathological processing of APP. They control both the fitness of APP for gamma-cleavage and the conformation of the active gamma-secretase complex.

ADPD5-1093

MECHANISMS OF BETA-AMYLOID IMPAIRMENT OF ERYTHROCYTE FUNCTION: A ROLE FOR PKC ALFA AND CASPASE 3

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Our attention is focused on the study of a new model based on the red blood cell (RBC) and on its interaction with Aß. RBCs are highly deformable to assist blood flow in the microcirculation, and in this context NO was proposed to be a regulatory factor of RBC mechanical properties since inhibitors of endogenous NO synthesis induces decreased erythrocyte deformability. For this reasons abnormalities in RBCs could contribute to AD by obstructing oxygen delivery to brain causing hypoxia. In our work, firstly we will focus on the morphology and nano-properties of RBC's membrane (i.e. roughness) by AFM (i.e. Atomic force microscopy), following to soluble Aß peptides exposure at different times, in order to characterize specific alterations induced by Aß. Secondly, considering that RBC membrane contains, among blood elements, higher acetylcholinesterase (AChE) levels, we can assume that there is a mechanism similar to the one which occurs at the neuronal level leading to an increase of Aß toxicity mediated by the binding with erythrocytic AChE. Since mechanical properties of RBC membrane are regulated by a number of molecular components of signalling and/or regulatory pathways, of these, particular interest has been addressed toward protein band 3, protein kinase C isoenzymes (PKC), endothelial nitric oxide synthase (eNOS) and caspase 3, due to their possible roles in the modulation of erythrocyte morphology, deformability and metabolic functions.

References:

Carelli-Alinovi C, et al. Clin Hemorheol Microcirc. 2014;
Misiti F et al.. Cell Biochem Funct. 2012

ADPD5-1130

SHEDDING OF APP LIMITS ITS SYNAPTOGENIC ACTIVITY AND CELL ADHESION PROPERTIES

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Objectives: The Amyloid precursor protein (APP) plays a central role in Alzheimer's disease and has essential synapse promoting functions. Synaptogenic activity as well as cell adhesion properties of APP presumably depend on trans-cellular dimerization via its extracellular domain. Since neuronal APP is extensively processed by secretases, it raises the question if APP shedding affects its cell adhesion and synaptogenic properties.

Methods: We performed cell clustering assays in Schneider (S2) cells with shedding deficient APP mutants and with a dominant-negative form of Kuzbanian (Kuz^{DN}). Processing of the mutants was analyzed in mammalian HEK293 cells as well as the amount of full length APP at the cell surface by cell-surface biotinylation. We used HEK293 cells expressing APP or APP mutants co-cultured with primary cortical neurons to assay their capacity to induce presynaptic differentiation.

Results: We generated APP mutants with a significant reduction in $sAPP_{total}$ secretion, which showed significantly increased clustering in Schneider cells, similar to APP wt co-expressed with Kuz^{DN}. The mutants also showed a highly significant reduction in $sAPP_{total}$ secretion in mammalian HEK293 cells and an increase in cell surface levels of full length APP. The shedding deficient APP forms promoted presynaptic differentiation of contacting axons to a much higher extent than APP wt and Neuroligin1.

Conclusions: Inhibition of APP shedding strongly enhances its cell adhesion properties and its synaptogenic activity suggesting that synapse promoting function of APP is tightly regulated by alpha-secretase mediated processing, similar to other synaptic cell adhesion molecules.

ADPD5-1200

AMYLOID BETA MONOMERS ARE REQUIRED TO SUSTAIN GLUCOSE UPTAKE DURING NEURONAL ACTIVATION

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Objectives: We have previously reported a neuroprotective activity of monomeric Aß$_{1-42}$, which was abrogated by inhibitors of insulin/IGF-1 receptor signaling (Giuffrida et al., J.Neurosci. 2009). Further investigation by our group has lead to the demonstration that Aß$_{1-42}$ monomers engage specifically type-I IGF receptors, thus promoting membrane translocation of Glut-3 and glucose uptake in primary neurons. We wanted to test the hypothesis that Aß$_{1-42}$ monomers could increase glucose uptake during neuronal activation.

Methods: Experiments were carried out in cultured cortical neurons stimulated KCl, and glucose consumption was assessed by either glucose meter or measuring the uptake of the fluorescent non-hydrolyzable glucose analogue NBDG. Causal relationship between Aß release during depolarization and glucose uptake was investigated by using a γ-secretase inhibitor or APP-null neurons. A set of experiments was carried out in CSF samples obtained from MCI and AD patients to obtain a source of native human Aß.

Results: Depolarization-induced glucose uptake was prevented by the addition of γ-secretase inhibitor IX, and it was re-established by exogenous Aß$_{1-42}$ monomers. In APP-null neuronal cultures, KCl-induced depolarization failed to enhance glucose uptake unless exogenous Aß$_{1-42}$ was added. Glucose uptake stimulated by high K^+ did not significantly differ between cultures incubated with MCI and AD CSF, although a trend to a reduction in AD-CSF treated cultures was seen. Addition of the anti-Aß 4G8 antibody (100 ng/50 µl CSF) significantly reduced glucose uptake at least in cultures treated with MCI CSF.

Conclusions: We conclude that Aß monomers are critical for neuronal glucose uptake under activation.

ADPD5-1217

UNEXPECTED ROLE OF VENULAR DEGENERATION IN THE PATHOGENESIS OF CEREBRAL AMYLOID ANGIOPATHY

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Most Alzheimer's disease (AD) patients exhibit accumulation of amyloid-beta peptide (abeta) on leptomeningeal and cortical arterioles, or cerebral amyloid angiopathy (CAA) that is associated with impaired vascular reactivity and accelerated cognitive decline. Despite widespread recognition of the significance of vascular dysfunction in AD etiology and progression, much uncertainty still surrounds the mechanism underlying AD vascular injury. Studies to date have focused on abeta-induced damage to capillaries and CAA-associated arterioles, without examining effects across the entire vascular bed. Here, we studied the differential regulation of vascular function in both the feeding (arteriolar) and draining (venular) vessels in a transgenic AD mouse model that develops progressive CAA. Unexpectedly, we found that CAA correlated with degeneration of mural cells on the penetrating venules but not on the penetrating arterioles. Further pharmacological depletion of venular mural cells using SU6668, a platelet-derived growth factor receptor-antagonist, resulted in increased tortuosity of the venules but not arterioles, exacerbation of CAA in the penetrating arterioles, and further alterations of the microvascular network cerebral blood flow response to hypercapnia. Together, this work shows hitherto unrecognized structural alterations in penetrating venules, demonstrates their functional significance and sheds light on the complexity of the relationship between vascular network structure and function in AD.

Funding was supported in part by CIHR MOP#102467 (JM, BS), Partnership for Stroke Recovery (JM, BS), CIHR Fellowship (AL), and Cryptic Rite Charitable Foundation (JM).

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-1220

EVIDENCE FOR A N-END RULE-MEDIATED PROTEASOMAL DEGRADATION OF SPECIFIC AICD SPECIES

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Objectives
Previous studies have shown a variable position of gamma-secretase cleavage of APP, providing an explanation for the generation of Abeta and AICD species of different length. While evidence for different toxic potentials of specific Abeta species exists, little is known about the different AICD species. Thus, the objective of the present work is to better understand the regulation and function of different AICD species.
Methods
Naturally-occurring AICD species were expressed by the use of the Ubiquitin-fusion technique in HEK cells. Epoxomicin was used to monitor proteasomal degradation and protein levels were accessed by Western blotting. Formation of AFT-complexes was observed by confocal microscopy of transfected cells.
Results
We show that exchange of the N-terminal residues of AICD (APP(VML646LLR)) abolishes AICD localization to nuclear AFT-complexes. Inhibition of the proteasome revealed that AICD derived from APP(VML646LLR) undergoes faster degradation than its wildtype equivalent. Together, these results suggest that AICD is subject to the N-end rule of proteasomal degradation, which relates the identity of a substrate's N-terminal residue to its degradation rate. Expression of specific AICD species was used to show significantly lower steady state levels, but significantly higher proteasomal degradation of AICD51 than AICD50.
Conclusions
Our data suggest, in line with the N-end rule of proteasomal degradation, that different AICD species have different proteasomal degradation rates. Our results also imply that different Abeta species are inherently correlated with different AICD levels. A possible contribution of different AICD species to the pathology of familial AD cases will be evaluated in future experiments.

ADPD5-1250

RHOMBOID-RELATED PROTEASE-4 PREVENTS THE AMYLOID PRECURSOR PROTEIN FROM AMYLOIDOGENIC PROCESSING

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Objectives: Alzheimer disease is a complex disorder and multiple cellular processes contribute to it. Cell stress, endoplasmic reticulum-associated degradation (ERAD), and the unfolded protein response (UPR) have previously been reported to be involved in Alzheimer disease. ERAD is required to eliminate misfolded proteins from the endoplasmic reticulum (ER) and includes many chaperones and processing enzymes. Recently, rhomboid-related protease 4 (RHBDL4) was shown to be a member of ERAD-mediating protein machinery and to degrade model substrates. Rhomboid proteases are a family of intramembrane proteases and were to our knowledge never before studied in the context of Alzheimer disease. We investigated if RHBDL4 alters processing of the amyloid precursor protein (APP).
Methods: Analysis of APP processing in cell culture models by western blot, MALDI-MS, and ELISA.
Results: We discovered that RHBDL4 efficiently cleaves APP and depletes full-length APP levels by about 90%. N- and C-terminal APP fragments were detected in cell lysates indicating that APP is cleaved while residing in the ER. Amyloid-beta levels were drastically reduced by about 50% in cell culture supernatants. Thus, RHBDL4 activity bypasses APP from amyloidogenic processing. RHBDL4 also cleaves APLP1 and APLP2, but not BACE1. Preliminary data from human Alzheimer disease brains indicate an upregulation of RHBDL4 mRNA levels, which might be a consequence of overall increased ERAD processing.
Conclusion: Further investigations of this novel APP-degrading pathway is necessary to reveal the pathophysiological impact of RHBDL4 on Alzheimer disease.

ADPD5-1254

OVEREXPRESSION OF MAOB IN PRIMARY NEURONS INCREASES ABETA PRODUCTION AND THE GAMMA SECRETASE/MAOB INTERACTION

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Objectives

The neurotoxic amyloid β peptide (Aβ) is formed by proteolytic processing of the amyloid precursor protein (APP) by BACE1 followed by γ-secretase. Although γ-secretase is an attractive drug target for AD, its regulation is poorly understood. Clinical trials with γ-secretase inhibitors have shown adverse side effects, since the enzyme can process many substrates in addition to APP, including Notch. We have identified several γ-secretase associated proteins (GSAPs) that regulate Aβ production but don´t affect Notch processing to any great extent and then characterized their interaction with γ-secretase using co-immunoprecipitation and proximity ligation assay (PLA). We have selected some of the GSAPs, including monoamine oxidase B (MAOB) for further studies.

Methods

We have in our previous studies shown that overexpression of MAOB resulted in increased Aβ production. Here we have studied whether overexpression of MAOB in primary neurons affects its interaction with γ-secretase. We expressed SNAP-tagged MAOB in primary neurons, followed by conjugation to the SNAP-tag specific stain SNAP-Cell Oregon Green. We then performed *in situ* proximity ligation assay (PLA) on the cells to study the interaction between MAOB and γ-secretase. The results were visualized by confocal microscopy.

Results

Transfected cells were selected by the incorporation of SNAP-Cell Oregon Green. The PLA signals were clearly increased in the transfected cells, verifying that overexpression of MAOB in primary neurons can increase the interaction with presenilin 1.

Conclusion

The results strengthen our hypothesis that Aβ production increases in MAOB overexpressing cells as a result from an increased association with γ-secretase.

ADPD5-1288

FURTHER CHARACTERISATION OF THE CELLULAR INTERACTION OF ABETA ASSEMBLIES AND PRION PROTEIN

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One of the hallmarks in Alzheimer's disease is the aggregation of amyloid-Beta peptide into plaques. Although plaques can cause neurodegeneration, mounting evidence suggests that ABeta oligomers are a major cause for toxic effects on neurons. A subset of ABeta oligomers interact with cellular prion protein (PrP) triggering a signalling cascade that impairs synaptic plasticity. Recently, our group showed that ABeta oligomers with a nanotube structure bind to PrP in the nanomolar range and inhibit long term potentiation in a PrP-dependent manner.
Objectives: To dissect the interaction between PrP- ABeta oligomers and find ligands that can block this interaction.
Methods: Using an array of biochemical tools and cell biology techniques we examined the interaction between PrP- ABeta oligomers.
Results: Given that PrP is not the only protein that binds ABeta oligomers on the cell membrane, we firstly set out to find experimental conditions that allowed us to measure ABeta binding in a PrP-dependent way. Subsequently, we assayed a collection of ligands that could potentially block the interaction.
Conclusions: Shedding light on mechanisms of interaction between ABeta and PrP could open the way to find the right target in order to inhibit the synaptotoxic effects triggered by ABeta and PrP interaction.
This work was funded by UK MRC and UCL grants.

ADPD5-1311

ACTIVITY-DEPENDENT ABETA OLIGOMERS PRODUCTION IMPAIRS SYNAPTIC FUNCTION AND DENDRITIC SPINE REARRANGEMENTS IN TG2576 MICE

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In Alzheimer Disease (AD), accumulation of almyloid-ß (Aß) contributes to synaptic dysfunction, dendritic spines loss and neural networks destabilization. These early events selectively occur in brain regions important for memory such as hippocampus and parahippocampal cortices and associate with initial AD symptoms, including impaired consolidation of new memories. Synaptic activity regulates Aß production and release and the activity-regulated cytoskeletal-associated (Arc) protein is an important player in this process. However, whether activity-regulated Aß contributes to AD progression is currently unknown.

In the present study, we collect evidences suggesting that activity regulated Aß might have a deleterious effect on synaptic function in Tg2576 mice, a well-recognized model in which the expression of a familiar-AD mutation favors Aß production. Neuronal activity was achieved by training early symptomatic Tg2576 mice in Contextual Fear Conditioning (CFC), a memory task commonly associated with structural and functional reorganizations of synapses in specific memory-related neuronal circuits.

We demonstrate that in Tg2576 mice, CFC results in increased levels of Aß oligomers as compared to mice under control conditions. Interestingly, CFC associates with increased number of dendritic spines and enhanced synaptic function in the hippocampal CA1 region and in the Anterior Cingulate Cortex (ACC) of wild type but not Tg2576 mice. Furthermore, we prove that Arc fluctuations participate in the regulation of Aß levels in Tg2576 mice.

Together, these data suggest that activity-related Aß might impact on synaptic rearrangements required for consolidation of long-term memories and encourage modulation of Arc expression in therapies aimed to slow down AD progression.

ADPD5-1337

AMYLOID PRECURSOR PROTEIN (APP) PROCESSING IS ALTERED BY GOLGI-LOCALIZED-GAMMA-EAR-CONTAINING ARF-BINDING (GGA) PROTEIN ISOFORMS 1, 2 AND 3 VIA THE GGA-GAE DOMAIN INTERACTION WITH BACE1

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Proteolytic processing of APP by BACE1 is the initial step in the production of amyloid beta (Abeta), which accumulates in senile plaques in Alzheimer's disease (AD). Essential for this cleavage is the transport and sorting of both proteins through endosomal/Golgi compartments. Golgi-localized gamma-ear-containing ARF-binding (GGA) proteins have striking cargo-sorting functions in these pathways. Recently, GGA1 and GGA3 were shown to interact with BACE1, to be expressed in neurons, and to be decreased in AD brain, whereas little is known about GGA2.

Since GGA1 impacts Abeta generation by confining APP to the Golgi and perinuclear compartments, we tested whether all three GGAs modulate BACE1 and APP transport and processing by overexpression and knock-down. As there are several potential binding sites, we addressed the question of which domain interacts directly with BACE1 by Co-Immunoprecipitation. To determine which GGA isoforms are most pathophysiologically relevant, we analyzed GGA levels in rat, and human postmortem brain tissue by in-situ hybridization, qPCR and Western blot.

We observed decreased levels of secreted APP alpha (sAPPalpha), sAPPbeta, and Abeta upon GGA overexpression, which could be reverted by knockdown. GGA-BACE1 co-immunoprecipitation was impaired upon GGA-GAE but not VHS domain deletion. Autoinhibition of the GGA1-VHS domain was irrelevant for BACE1 interaction. GGA1, 2, and 3 are differentially expressed and regulated in the brain.

Our data support a model in which all three GGAs contribute to endosomal/lysosomal transport of BACE1 and probably also APP via the GGA-GAE domain and might contribute to the development of AD pathology.

ADPD5-1356

HOMO- AND HETEROTYPIC DIMERIZATION OF APP, APLP1 AND APLP2 IS DIFFERENTLY REGULATED BY COPPER

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Objectives

APP and its mammalian homologs, APLP1 and APLP2, form homo- and heterotypic dimers, of which a minor fraction is stabilized by intermolecular disulfide bonds. Recently, we showed that copper promotes APP dimerization.

Methods

HEK293 cells were transiently transfected with APP/APLPs and incubated with increasing amounts of copper (0 – 250 µM), 4h prior analysis. APP/APLP interaction was analyzed by co-immunoprecipitation and in case of disulfide-bonded oligomers investigated under non-reducing conditions by gel mobility shift. To investigate the influence of copper treatment, cell viability and subcellular localization of APP/APLPs was analyzed by immunocytochemistry.

Results

Here we show that both, homotypic dimerization of APLP1 and APLP2, as well as heterodimerization of APP/APLP2 is promoted by increasing copper concentrations. In contrast, heterotypic interaction between APP and APLP1 is not influenced by copper, suggesting different interaction interfaces for homo- and heterotypic dimerization. Further, we show that APP family members form cysteine-bonded dimers that are strongly promoted by the addition af copper.

Conclusion

These data suggest that the influence of copper on the homotypic dimerization of APP/APLPs is mainly mediated by formation of stable intermolecular disulfide-bonded dimers. Differences in stability of covalently bond and non-covalently bond APP/APLP dimers suggest distinct pathophysiological functions.

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-1357

THE APP ADAPTOR PROTEINS FE65 AND FE65L1 ARE ESSENTIAL FOR LOCOMOTOR ACTIVITY, NMJ FORMATION AND LEARNING.

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Background
The Fe65-protein family, as the main intracellular interaction partner of the APP-family, plays a crucial role in proper positioning of neurons in the mouse brain. The Fe65/Fe65L1 dKO mice have phenotypes resembling those found in triple mutant mice lacking all APP family members. These data suggest a functional interplay between APP and Fe65 in brain development.
Methods
For analysis of Fe65/Fe65L1 KO mice we performed different immunohistochemical analyses and behavioral studies, such as grip strength measurements, balance beam, rota-rod, Morris Water Maze and fear conditioning followed by measurements of long term potentiation (LTP) and paired pulse facilitation.
Results
Here we report reduced grip strength and crucial impairments in locomotion for mice lacking the Fe65 and Fe65L1 proteins. Analysis of the neuromuscular junction (NMJ) formation in P5 pups and adult mice resulted in smaller pre- and post-synaptic areas and reduced apposition of the pre- and post-synapse, supporting deficits in the peripheral nervous system (PNS). Spatial learning analysis in the Morris Water Maze test and the fear conditioning test resulted in significant deficits in Fe65/Fe65L1 KO mice suggestive of hippocampal dysfunction. Further electrophysiological analyses revealed deficits in LTP in Fe65/Fe65L1 KO mice and impairments in paired pulse facilitation.
Conclusion
Fe65/Fe65L1 dKO mice resemble APP/APLP2 mutant mouse phenotypes with deficits in NMJ formation, locomotion and learning behavior suggesting that Fe65 protein family members are involved in APP/APLP synaptic signaling in the central nervous system (CNS) and in the PNS.

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-1370

A PAIRED RNAI AND RABGAP OVEREXPRESSION SCREEN IDENTIFIES RAB11 AS A REGULATOR OF BETA-AMYLOID PRODUCTION

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Alzheimer's disease (AD) is characterized by cerebral deposition of beta-amyloid peptides, which are generated from amyloid precursor protein (APP) by beta- and gamma-secretases. APP and the secretases are membrane associated, but whether membrane trafficking controls beta-amyloid levels is unclear. The objective of the study was to identify the specific trafficking mechanisms that regulate the production of beta-amyloid. Here, we performed an RNAi screen of all human Rab-GTPases,which regulate membrane trafficking, complemented with a Rab-GTPase-activating protein screen, and present a road map of the membrane-trafficking events regulating beta-amyloid production. We identify Rab11 and Rab3 as key players. We show that Rab11 controlled beta-secretase endosomal recycling to the plasma membrane and thus affected beta-amyloid production. Exome sequencing revealed a significant genetic association of Rab11A with late-onset AD, and network analysis identified Rab11A and Rab11B as components of the late-onset AD risk network, suggesting a causal link between Rab11 and AD. Our results reveal trafficking pathways that regulate beta-amyloid levels and show how systems biology approaches can unravel the molecular complexity underlying AD.

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-1396

DIFFERENT PROCESSING OF APP C-TERMINAL FRAGMENTS IN THE AMYLOIDOGENIC AND THE NON-AMYLOIDOGENIC PATHWAY

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The amyloid precursor protein (APP) is processed in two main ways: In the amyloidogenic pathway APP is cleaved by beta- and gamma-secretase to produce A-beta peptides. In the non-amyloidogenic pathway the consecutive actions of alpha- and gamma-secretase give rise to the shorter p3 peptides. The C-terminus of both types of peptides is produced by gamma-secretase in different lengths. The most prominent variants end on position 40 and 42 of A-beta. While gamma-secretase appears to cleave the APP C-terminal fragments produced by alpha- and beta-secretase with comparable overall efficiency, we do not know whether there are differences in the abundance with which gamma-secretase produces 40 and 42 C-terminal ends from the respective substrates.

We observed an overproportional decrease of total 42-ending APP-derived peptides over 40-ending peptides when beta-secretase activity was downregulated in wildtype mouse primary neurons. The downregulation of alpha-secretase on the other hand shifted the total 42/40 peptide ratio in the opposite direction. These effects were not present when we analyzed specifically A-beta produced in the amyloidogenic pathway. Our data point to a higher 42/40 ratio in the beta-secretase pathway and suggest different processing of the different APP C-terminal fragments by the gamma-secretase complex in neurons.

ADPD5-1400

REGULATION OF NEURONAL FUNCTION BY APP NUCLEAR SIGNALING

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Objectives: The central molecule in the pathologic cascade leading to Alzheimer's disease (AD) is the transmembrane amyloid precursor protein (APP). APP processing liberates the APP intracellular domain (AICD), which subsequently localizes with its binding protein Fe65 and the histone acetylase Tip60 in multiple nuclear complexes. These AFT spots are localized to transcription factories, loci of gene expression. APP regulates several processes such as dendrite growth and spine morphology that might be mediated by AICD nuclear signaling.

Methods: We use lentiviral vectors to express AICD in hippocampal neurons to analyze the effects on dendrite complexity. Via Sindbisvirus we are expressing AICD in hippocampal slice cultures and analyze spine density with confocal microscopy. Further, we perform stereotaxic injections of lentivirus into the hippocampus to analyze AICD-induced effects on CA1 pyramidal and dentate gyrus granule cell morphology. Virus-mediated expression of AICD is also used to analyze candidate target genes via qRT-PCR.

Results: We demonstrate that lentiviral-mediated AICD expression significantly increases dendritic branching in hippocampal neurons *in vitro* compared to control-treatment. To identify the regulated genes responsible for this effect we are analyzing candidate genes discovered in qRT-PCR screens of APP/APLP2 knock-out mice.

Conclusions: We provide experimental evidence for AICD-mediated effects on neuronal morphology with respect to dendritic branching and spine density.

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-1415

POST-TRANSCRIPTIONAL REGULATION OF APP EXPRESSION BY NEURONAL HU PROTEINS

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Amyloid-beta precursor protein (APP) is present in three main splice variants, the ubiquitous APP770 and APP751 and the neuronally enriched and less amyloidogenic APP695 (lacking exons 7 and 8). Hu (Human antigen) RNA-binding proteins - HUR and neuronal HU (nHU) HUB, HUC and HUD – are known to regulate alternative splicing events. Intriguingly, nHU are involved in cognitive processes and their levels, as well as those of APP695, are reduced in AD. We, thus, examined whether nHU are involved in splicing events generating APP695.

Methods: Protein and mRNA levels of Hu proteins and APP isoforms were determined by Western blot and RT-PCR, respectively, in lysates or RNA from various cell lines untreated, differentiated towards a neuronal phenotype or transfected with the appropriate plasmid constructs. Binding of nHU to *App* pre-mRNA was assessed by RNA-immunoprecipitation.

Results: We show that nHu expression correlates with APP695 expression in various cell lines and during neuronal differentiation. Moreover, over-expression of nHU results in a significant increase of APP695 mRNA and protein levels, whereas loss of endogenous HUC or HUD function leads to a significant decrease of APP695 levels. Consistently, we demonstrate that nHU interact with the endogenous *App* pre-mRNA. Finally, by co-transfecting artificial mini-gene constructs for APP exon 7 or exon 8 with plasmids expressing HUC or HUD, we identified the minimal flanking intronic sequences, necessary for APP exon 7 or 8 skipping by nHU.

Conclusion: Neuronal Hu proteins participate in the regulation of APP alternative splicing events leading to the generation of neuronal APP695.

ADPD5-1462

MODULATION OF BETA–AMYLOID/APP IN THE ENDOCYTIC PATHWAY

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Objectives
Alzheimer's-linked beta-amyloid (Aβ) is generated at synapses and accumulates in endosomal vesicles near synapses with the onset of synaptic dysfunction in AD. Previous evidence has suggested that sorting via the multivesicular body (MVB) pathway is impaired by endosomal Aβ accumulation in cultured AD-transgenic primary neurons. Our aim is to investigate the trafficking and processing of APP and its cleavage products in the endocytic pathway with a focus on the role of ESCRT (endosomal sorting complex required for transport) -mediated sorting in intracellular Aβ accumulation.
Methods
Different steps in the endocytic pathway, including exocytosis, MVB formation, lysosome acidification and autophagy are modulated in neuroblastoma cells and primary mouse neurons. Aβ and other AD-related proteins as well as synaptic proteins are analysed using WB and immunofluorescence. Differences in the endocytic pathway between wt and AD-transgenic primary neurons and neurons treated with synthetic Aβ are also investigated.
Results
Inhibition of the ESCRT component VPS4A or inhibition of the vacuolar H+ ATPase in lysosomes, leads to intracellular accumulation and decreased secretion of Aβ in N2a cells and primary neurons. However, stimulating autophagy partially rescues the dnVPS4A induced increase of intracellular Aβ back to control levels. In contrast, we confirm that inhibition of VPS4A increases α-synuclein secretion without altering the total pool of intracellular α-synuclein.
Conclusions
We previously reported progressive intraneuronal Aβ accumulation and impaired Aβ secretion in AD-neurons with time in culture. This is mimicked by inhibition of VPS4A and is consistent with indications of MVB/lysosomal dysfunction in AD.

ADPD5-1470

SYNAPTOTAGMINS REGULATE BACE1-MEDIATED PROCESSING OF AMYLOID PRECURSOR PROTEIN AND ABETA GENERATION

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Objective: Accumulation of β-amyloid peptide is the major pathological hallmark of Alzheimer's disease. However, proteins regulating Aβ generation at the synapse have not been characterized. Here, we sought to identify proteins that interact with the APP and regulate Aβ generation at the synapse.
Methods: We used affinity chromatography-coupled mass spectrometry to identify the APP ectodomain-interacting proteins in mouse brain. Co-immunoprecipitation and GST pull-down assays were performed to test for protein interaction. Co-localization with APP was verified by different image-based methodologies. Overexpression and knockdown studies were used to analyze changes in APP metabolism and Aβ generation.
Results: We identified the synaptotagmin (Syt) family of proteins as novel APP-ectodomain interacting proteins. Our results have shown that Syt-1, -2 and -9 interact with APP both *in vitro* and *in vivo*. We further demonstrated that Syt interaction site lies between E1 and KPI domains of APP. Stable overexpression of Syt-1/Syt-9 with APP elevated APP-CTFs and sAPPβ, with ~3 fold increase in secreted Aβ levels. Using Syt-1 knockout PC12 cells and Syt-1 specific siRNA, we have observed a ~50% reduction in secreted Aβ generation and also a significant decrease in APP processing. In addition, lack of Syt-1 expression in PC12 cells also led to a significant reduction in secreted sAPPβ levels as compared to the wild-type PC12, strongly suggesting that Syt-1 modulates BACE1-mediated APP cleavage.
Conclusion: Our data here clearly identify Synaptotagmins as novel APP-interacting proteins that regulate BACE1-mediated APP processing and Aβ generation and thus may play an important role in AD pathogenesis.

ADPD5-1630

GENOME-WIDE SIRNA SCREENING IDENTIFIES NEW MODULATORS OF THE APP METABOLISM

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Objectives:

Even if numerous players of the APP metabolism have been characterized over the last 20 years, a large part of the actors of this metabolism are still unknown. It is expected that these actors could interfere with secretase activity but also with APP trafficking and/or degradation. To identify those modulators in taking into account these different levels of interactions, we developed a Genome-Wide siRNA screening using a High Content Screening (HCS) approach.

Methods:

A HEK293 cell line stably over-expressing a Tagged-fluorescent APP was transitory transfected in 384 wells plates with the human siGENOME siRNA library to silence every human gene. Each plate contained controls for both intra-plate validation (siRNA-PS1 and siRNA-APP) and inter-plate normalization (non-targeting siRNAs). Plates were read 72 hours after transfection using an automated confocal microscopy system (In Cell 6000) and fluorescence intensities were quantified using the COLUMBUS software. Modulators of the APP metabolism were selected among those exhibiting the strongest variation in terms of fluroscence intensity (the 2.5 % highest and lowest variations). From the selected, enrichment pathway analysis were performed using the Ingenuity database.

Results:

Data for 18,049 different genes were generated after our different control processes and we finally selected 820 hits showing strong impact on the APP metabolism. Pathway enrichment analysis identified canonical-pathways such as IGF-1 signaling (P=1.1E-05), Cholesterol Biosynthesis (P=1.5E-05) and JAK/Stat Signaling (P=2.2E05).

Conclusions:

Characterization of these modulators could be useful for our in-depth understanding of the APP metabolism and could be potentially linked to Alzheimer’s disease following subsequent analyses.

ADPD5-1734

DEREGULATION OF PROTEIN SYNTHESIS IN ALZHEIMER DISEASE

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Alzheimer's disease (AD) is the most common senile dementia. Cognitive deficits are widely believed to result from progressive synaptic dysfunction and neurodegeneration, most likely caused by soluble oligomers of the amyloid peptide (AβO). Synaptic plasticity (for instance long term potentiation (LTP)) that underlies memory, involves changes of synapse efficacy associated with a variation in number, size and morphology of dendritic spines. AbO induce dendritic spine shrinkage and inhibit synaptic plasticity. These effects are likely to account for the memory defects associated with AD, but the mechanism remains obscure.

Using a non radioactive technique known as surface sensing of translation, (SUNSET) and based on the detection of the incorporation of puromycin into nascent peptide chains, we have observed that protein synthesis increases in dendrites of cultured hippocampal neurons upon chemical LTP induction (Forskolin, BDNF, NMDA and dopamine). This effect is severely blocked by AbO (500 nM, 3h). Interestingly, we observed at lower doses (100 nM, 3h) an increase of mRNA translation in nonstimulated neurons. Similar results have been obtained using cultures of neurons from Tg2576 mice expressing a pathogenic mutant of APP.

These effects of AbO on mRNA translation (inhibitory at 500 nM and excitatory at 100 nM) are correlated with changes in the activity of mTOR. By using a pharmacological approach to characterise the signaling pathways, we observed that AbO at 100 nM affect mTOR by activating the BDNF receptor TrKB and the PI3-kinase/AKT pathway. However, AbO used at higher doses seem to impair RNA translation by inactivating the ERK pathway.

ADPD5-1750

PROTON MYO-INOSITOL COTRANSPORTER IS A NOVEL GAMMA-SECRETASE ASSOCIATED PROTEIN THAT REGULATES ABETA PRODUCTION WITHOUT AFFECTING NOTCH CLEAVAGE

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Objectives

The neurotoxic amyloid β peptide (Aβ) is formed by proteolytic processing of the amyloid precursor protein (APP) by BACE1 followed by γ-secretase. Although γ-secretase is an attractive drug target for AD, its regulation is poorly understood. γ-Secretase associated proteins (GSAPs) could be of importance for substrate selection and regulation of amyloid β-peptide production.

Methods

We used affinity purification of γ-secretase in combination with mass spectrometry to identify novel GSAPs in microsomal preparations from human brain. We studied their effect on Aβ production and Notch cleavage using siRNA mediated gene silencing as well as an overexpression approach. Three of the proteins that reduced the Aβ levels; Probable phospholipid-transporting ATPase IIA (ATP9A), BDNF/NT-3 growth factor receptor precursor (NTRK2), and Proton myo-inositol cotransporter (SLC2A13), were selected for further studies, including co-immunoprecipitation and proximity ligation assay (PLA).

Results

Of the three proteins, SLC2A13 showed the most selective effect on APP vs Notch processing, the highest increase of Aβ production after overexpression and the greatest extent of association with γ-secretase based on co-immunoprecipitation as well as PLA.

Conclusion

SLC2A13 was identified as a novel GSAP that regulates Aβ production without affecting Notch cleavage. Further investigations will be performed to investigate whether the association between SLC2A13 and γ-secretase can be targeted for pharmaceutical intervention of AD.

ADPD5-1754

O-GLCNACYLATION SELECTIVELY AFFECTS APP PROCESSING IN NEURON-LIKE CELLS

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Objectives

Alpha-secretase processing of amyloid-beta precursor protein (APP) excludes the formation of the neurotoxic amyloid-beta (A-beta) peptide and generates a neuroprotective secreted fragment, sAPPalpha. We have previously shown that O-GlcNAcylation, a dynamic post-translational modification, stimulates alpha-secretase processing of APP, resulting in increased secretion of sAPPalpha concomitant with decreased A-beta formation. Further we investigated the mechanism behind the increased sAPPalpha secretion in response to PUGNAc, an inhibitor of the enzymatic removal of the GlcNAc moiety.

Methods

SH-SY5Y, SK-N-AS, HEK293, HeLa cells and rat primary neurons were cultured for 24 hours in the absence or presence of 50 µM PUGNAc. Cell lysates and conditioned medium were subject to western blot analysis with antibodies against APP. Co-immunoprecipitation assay was performed to analyze ADAM10-APP interactions. To analyze O-GlcNAcylation of proteins, O-GlcNAc immunoprecipitation or sWGA precipitation, followed by western blot using specific antibodies was performed as was click chemistry labeling of O-GlcNAcylated proteins.

Results

Our results show significant effects on sAPPalpha secretion in human neuroblastoma cell lines and rat primary neurons, but not in non-neuronal cells. In neuronal cells APP cell surface localization and its interaction with the alpha-secretase ADAM10 is enhanced.

Conclusions

O-GlcNAcylation-dependent APP alpha-secretase processing involves effects on APP trafficking, and ADAM10-APP interactions, and is most likely playing a specific role in neuronal cells.

ADPD5-1819

CELLULAR MECHANISMS OF LATE-ONSET ALZHEIMER'S DISEASE RISK GENES

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by senile plaques, neurofibrillary tau tangles and overt neuronal loss. According to the onset of disease one can distinguish two types of AD: early-onset and late-onset. However, while an increased production of Ab is seen in early-onset AD, an impaired clearance has been postulated to be the major cause for late-onset AD. In addition, while the aberrant phosphorylation and aggregation of tau protein appears to be solely downstream of Ab accumulation in early-onset AD, in late-onset AD pathological tau might also be formed independently and thereby contribute to disease onset and progression via non- Ab triggered pathways. In the recent few years, genome-wide association studies have identified a long list of SNPs associated with late-onset AD, revealing a large set of genes (Alzgenes) that could confer suceptibility to the disease. **H**ere we tested the role of the Alzgenes in a neuronal context, specifically investigating their effect on the Ab 42/40 ratio, on the total Tau levels and the Tau phosphorylation. In addition, we explored the possibility that the Alzgenes could specifically modulate the function of microglia. Not significant effects were detected in the neuronal system with respect to Ab x-40 and Ab x-42 neither to total tau expression nor tau-phosphorylation at Thr231. However, some Alzgenes appear to have a role in modulating microglial phagocytic activity. This supports the hypothesis that -at least some- risk gene variants can act in late-onset AD as a susceptibility factor for impaired Ab removal.

ADPD5-1967

SYNAPTIC AND NON-SYNAPTIC MITOCHONDRIAL PROTEOME AND FUNCTION REFLECTS THE PROGRESS OF BETA-AMYLOID EXPRESSION IN APP/PSI MODEL OF ALZHEIMER'S DISEASE

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The identification of early pathomechanism of Alzheimer's disease (AD) is in focus of interest for the discovery of potential markers for early diagnosis. Early synaptic dysfunction could be detected in AD transgenic mice models. The earliest detected clinical symptom of AD is the decrease in brain metabolism. It suggest early mitochondrial dysfunction as AD develops. In the present study we performed a brain synaptic and non-synaptic mitochondrial proteome analysis of the APP/PS1 double transgenic AD mouse model at 3, 6 and 9 month of age. Validation of correct separation of mitochondria was done by FACS, electron microscopy and Western Blot techniques. In parallel with proteomics studies we also recorded the functional changes in mitochondria via ROS metabolism and oxygen consumption. We investigated the mitochondrial protein changes with 2-D DIGE and mass spectrometry. We identified 45 mitochondrial protein changes, which reflects progressing effect of beta-amyloid on mitochondrial processes as glucose metabolism, oxidative stress and many others. Our data show, that beta-amyloid induced proteome changes in synaptic and non-synaptic mitochondria are different in all investigated ages. Proteome of synaptic mitochondria are more vulnerable than non-synaptic ones to Aβ insult particularly in early phase, when the mitochondrial functional parameters were still normal. Proteomics changes were in correspondence with ROS generation. We report here the first comprehensive, integrated mitochondrial protein-and-function data in correlation with Aβ progression in APP/PS1 mice. Our data are in good correspondence with *post mortem* human brain studies and also with mitochondrial protein data in AD obtained with biased strategy.

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-1968

THE BETA-AMYLOID EARLY EFFECT ON THE PROTEOME OF THE MITOCHONDRIA-ASSOCIATED ER MEMBRANE (MAM) IN APP/PS1 MICE BRAIN

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Development of Alzheimer's diseases (AD) is a long process and the key for better treatment is to identify its earliest molecular mechanisms for reversing the AD development in time. It is generally accepted that beta-amyloid intracellular accumulation is associated with AD progression. The earliest detectable changes in the brains of AD patients are the decreased metabolism. Recently it has been uncovered that Presenilin-1 and -2, processing Amyloid Precursor Protein, is located predominantly in a special sub-compartment of the endoplasmic reticulum (ER) functionally connected to mitochondria (Mit), called mitochondria-associated ER membrane (MAM). MAM has a lipid raft-like profile, involved in phospholipid and cholesterol biosynthesis, in Ca^{2+} signaling and homeostasis, and in mitochondrial function and dynamics. According to MAM hypothesis of AD early development, presenilins maintain MAM function and ER-Mit crosstalk. The increased presenilin level in AD induces metabolic dysfunction, which is an early detectable alteration in AD. Thus, MAM dysfunction is probably an early AD marker. In the present study we isolated MAM from the brains of APP/PS1 mouse model of AD and performed MAM proteome analysis of the 3 months old APP/PS1 mouse model. In this early phase there are no behavioral changes in the animals, furthermore there are no extracellular amyloid plaque. We validated the MAM preparations with EM and WB techniques. We investigated the protein changes with 2D-DIGE and mass spectrometry. We report here the first comprehensive, unbiased molecular study on early beta-amyloid effect on MAM proteome, which may explain some molecular features of early AD.

ADPD5-1982

DISSECTING THE ROLE OF MICRORNAS AND THEIR THERAPEUTIC POTENTIAL IN ALZHEIMER'S DISEASE

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A pivotal role for microRNAs (miRNAs) has been proposed in ageing and neurodegeneration, since studies demonstrate that several miRNAs significantly change their expression during senescense. The identification of "molecular signatures", such as miRNA profiles, may lead to the development of new therapies for Alzheimer's disease (AD). Hence, this study aims to modulate the levels of selected miRNAs predicted to target proteins involved in AD.

Through bioinformatic tools, we have identified miRNAs with high affinity to 3'UTR-APP and 3'UTR-BACE1 and performed a biochemical validation of these binding sites, through luciferase assay, upon co-transfection of HT-22 cells with miRNA mimics. The results demonstrate that the selected miRNAs target APP and BACE1, since there was a decrease in luciferase activity in the presence of these mimics. Additionally, a decrease in APP and BACE1 mRNA levels was observed upon transfection of HT-22 and HEK-293 cells with lentiviral constructions containing the selected miRNAs sequences.

Regarding the results and that miR-31-5p targets both APP and BACE1, we have selected this miRNA to evaluate its therapeutic potential. Therefore, we developed a lentiviral platform able to modulate the levels of the miRNA in the brain of a triple-transgenic animal model of AD, through stereotaxic injection. Our preliminary results show that miR-31-5p must be able to decrease the levels of APP and BACE1 *in vivo*. Given the high conservation of miRNAs across species, it is likely that new significant insights in ageing process may arise and support new diagnostic and therapeutic avenues to treat ageing-related diseases, such as AD.

ADPD5-2088

A NEW PUTATIVE RECEPTOR TRIGGERING SAPPALPHA NEUROTROPHIC AND NEUROPROTECTIVE PROPERTIES

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Objectives

The amyloid precursor protein (APP) can be cleaved by the alpha-secretase which releases the soluble fragment called sAPPalpha. This fragment has neurotrophic and neuroprotective properties. However, the mechanisms underlying such properties are not yet elucidated. To this end, we focused our interest in membrane proteins interacting with sAPPalpha and susceptible to act as a receptor or co-receptor.

Methods

We isolated mouse brain membranes. After lysis, membrane extracts were incubated with the recombinant protein sAPPalpha-Fc. The proteins attached to sAPPalpha-Fc were isolated on protein A-sepharose beads. After elution, proteins were identified by MS/MS. We identified a protein, called R-sAPPalpha (for patent reasons). The involvement of R-sAPPalpha in the properties of the sAPPalpha has been validated using a siRNA approach in primary neurons.

Results

We showed that siRNAs against the R-sAPPalpha abolished sAPPalpha-induced axonal growth. Moreover, our data strongly suggest that the activation of cellular signaling pathways triggered by R-sAPPalpha might participate in the neuroprotective effects of the sAPPalpha against beta-amyloid oligomers.

Conclusions

Taken together, our data suggest that R-sAPPalpha could be a new therapeutic target for Alzheimer’s disease.

02a. Cell, Molecular & Systems Biology.: APP, APLP, Abeta

ADPD5-2105

ALZHEIMER'S AMYLOID DEGRADATION BY SECRETED LYSOSOMAL ENZYMES

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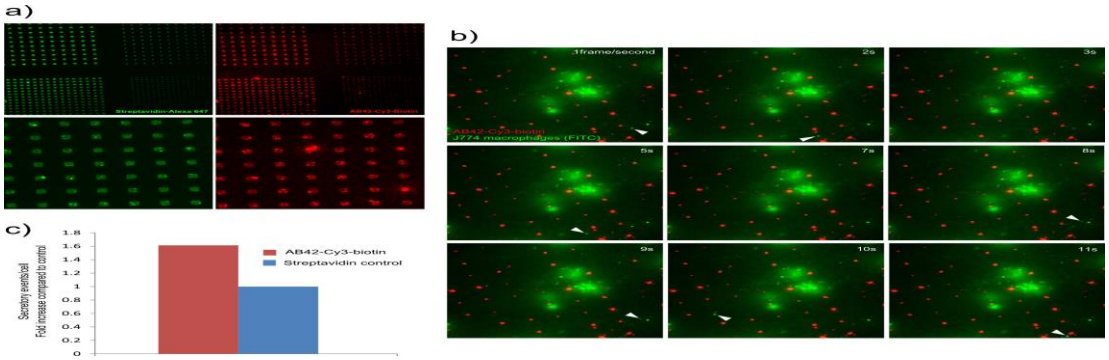
In Alzheimer's disease (AD), some brain regions associated with neurodegeneration present abundant amyloid-beta (Aβ) fibrillogenesis. It is unclear how microglia or macrophages in the brain can degrade Aβ fibrils (fAβ) and plaques that are significantly larger than these cells. A new mechanism is suggested by a study published by our lab in which it was found that macrophages could degrade large aggregates of LDL by creating an *extracellular,* acidic compartment that we called the 'lysosomal synapse" into which lysosomal contents are secreted (Haka et *al*. 2009).

To study the interaction of microglia and macrophages with fAβ, and in collaboration with the Cornell NanoScale Science & Technology Facility, we fabricate glass coverslips to present spatially defined, fluorescently labeled fAβ immobilized onto the surface (Fig.A). Cells, for which their lysosomes were labeled with FITC-dextran, are subsequently incubated on the fAβ-coated micro-pattern and imaged using TIRF microscopy, revealing rapid FITC flashes (white arrowheads in Fig.B) at the pattern surface which are indicative of lysosomal content release. As FITC exits the lysosomes, it undergoes fluorescence increase associated with the transition from the acidic lysosomal environment to the more alkaline pH of the extracellular environment. Measurements from a reduced number of samples indicate that macrophages incubated on fAβ-coated surfaces present higher secretory activity when compared to those exposed to streptavidin-coated surfaces (Fig.C).

These studies aim at exploring new ground for treatments based on the use of therapeutic agents to increase lysosomal activity, which could potentially lead to an enhancement in microglia fAβ lysosomal degradation.



ADPD5-2200

STUDY OF TRANSCRIPTIONNALLY ACTIVE/INACTIVE APP INTRACELLULAR DOMAIN (AICD) IN DIFFERENT CELL MODELS

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Objective

APP has been extensively studied for its pathological role in AD, but its physiological role remains poorly understood. Several studies indicated that APP regulates gene expression via APP/AICD signaling, but neither the exact signaling mechanism nor the downstream targets have been unambiguously established. We analyzed AICD production, accumulation and transcriptional activity in engineered cell models expressing different APP/CTFs (C-Terminal Fragments).

Methods

Transcriptionally active AICD was monitored by transactivation assays in mouse embryonic fibroblast (MEFs) knock out for Presenilins (PS1 and PS2) and MEFs rescued by wild-type PS or familial Alzheimer disease (FAD) mutants.
HEK293 and CHO cell model were transfected wit APP/CTFs constructs and treated with alkalizing drug to assess AICD detection. Fractionation protocol was used in a neuronal cell model to analyze AICD in nucleus and its phosphorylation status.

Results

Transcriptional activity of AICD is different depending on APP in cleavage by PS-1 or PS-2 γ-secretase. Mutations in PS affect differently AICD production and its transcriptional activity when compared to wt PS. Alkalizing treatment of cells increase, as previoulsy shown, AICD detection preferentially produced from full length APP instead of exogenous CTFs. Fractionation of neuronal cells showed that AICD in nucleus is mainly not phosphorylated at Thr668.

Conclusion

Our data suggest that AICD release from PS1 or PS2 γ-secretase complexes display different transcriptional properties. Alkilizing treatment suggest that an AICD pool is degraded via endosomal/lysosomal pathway, irrelevant to transcriptional properties in physiological condition.

ADPD5-2233

ANALYZING CLEARANCE IN APP TRANSGENIC MICE WITH MID-DOMAIN AMYLOID BETA-ANTIBODIES

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Objectives: The formation of amyloid plaques is a well-studied aspect of Alzheimer Disease (AD) pathogenesis, while mechanisms of amyloid-beta (Abeta) clearance are still fairly unknown. Although microglial/macrophage uptake and degradation of Abeta peptides has been demonstrated *in vitro*, its relevance *in vivo* remains controversial. New techniques and tools to examine Abeta clearance in brain is much needed.
Methods: A new mid-domain Abeta-antibody was characterized with dot-blot, epitope mapping and ELISA. Brain sections of tgArcSwe mice was used for immunofluorescent and immunogold staining at the light and ultrastructural microscopic level respectively. At the light microscopic level co-staining with microglial markers were done. ELISA was used for characterization of antibody binding epitope.
Results: We developed mid-domain antibodies as tools to better investigate degradation products of Abeta *in vitro* and transgenic models and AD. Antibodies were raised and purified against a 21-34 amino acid sequence in Abeta, but the binding domain was more limited. With the antibody we could detect Abeta-fragments and visualize amyloid beta-staining in brain microglia/macrophages at both the transmission EM data and at the light microscopic level.
Conclusions: Here we describe the *in vitro* characteristics of a new type of mid-domain Abeta-antibody that could become useful for further analysis of enzymatic Abeta-degradation and clearance *in vitro* and *in vivo*. Our data demonstrate uptake of Abeta occurring *in vivo* in transgenic mice, indicating a role of microglia/macrophages in Abeta-clearance.

ADPD5-2306

THERAPEUTIC EFFECT OF BERBERIS VULGARIS AND CALLUNA VULGARIS EXTRACTS ON LIPOPOLYSACCHARIDE (LPS)-INDUCED BRAIN INFLAMMATION

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Brain inflammation is important in aggravation of brain damage and causes neurodegenerative diseases that occur due to the formation reactive oxygen species and overexpress pro-inflammatory mediators of activated microglia. Lipopolysaccharide accounted as brain damage compound as it is induced injury in the rat brain. Both Berberis vulgaris L. and Calluna vulgaris L. have medicinal properties as they act as antioxidant and anti-inflammatory effects. Objective: The aim of the present study was to assess the mechanisms involved in the protective effect of B. vulgaris (Bv) and a C. vulgaris (Cv) extracts on LPS-induced brain inflammation and neurotoxicity. Methods: rats were orally administrated extracts (100 mg/kg/day) for three weeks and then intraperitoneally injected once with 4mg/kg LPS. Results: LPS administration significant increased brain lipid peroxidation (malondialdehyde level and xanthine oxidase activity) and decreased antioxidants status (superoxide-dismutase, reduced glutathione, glutathione-peroxidase, glutathione-S-transferase). Furthermore, oxidative stress stimulated the production of proinflammtory molecules. As a result, the brain acetylcholinestrase was activated that lead to brain glucose level diminished. Finally, β -amyloid plaques were formed as a result from low formation of (Aβ1-40) and insulin degrading enzyme activity and accumulation of (A β1-42) as a result of high expression levels of APP and β-secretase. we also show that production of β-amyloid plaques and neurodegenration are reduced by increasing the expressing of the NAD-dependent deacetylase SIRT1 in the brain. Conclusion: Our results suggest that the pre-treatment with extracts had the potential to suppress oxidative stress and pro-inflammatory cytokines and therefore prevent the neurotoxicity and amyloid plaques formation.

ADPD5-2327

MT5-MMP IS A NEW REGULATOR OF APP THAT PROMOTES AMYLOIDOSIS AND COGNITIVE DECLINE IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Objectives: Although membrane-type 5-matrix metalloproteinase (MT5-MMP) is a sheddase with emerging roles in brain pathophysiology, its implication in Alzheimer's disease (AD) remains elusive. Accordingly, we examined the impact of MT5-MMP invalidation in an experimental mouse model of AD.

Methods: We crossed the 5xFAD (Tg) mouse model of AD with MT5-MMP deficient mice (MT5-/-) to generate bigenic 5xFAD/MT5-MMP-/- mice (TgMT5-/-). We used molecular and cell biology techniques, as well as neuroanatomical assessment of amyloid lesions combined with LTP protocols and the 6-arm radial water maze.

Results: At early stages (4 months) of the pathology, the levels of amyloid beta peptide (Abeta) and its precursor the APP C-terminal fragment C99, where largely reduced in the brains of TgMT5-/-compared to Tg mice. Reduced amyloidosis in bigenic mice was concomitant with decreased glial reactivity and preservation of LTP and spatial learning, without changes in the activity of alpha-, beta-, and gamma-secretases. Beneficial effects of MT5-MMP invalidation were still present at 16 months of age in bigenic mice, which showed reduced amyloid burden and gliosis, and higher levels of synaptophysin. MT5-MMP expressed in HEKswe cells co-immunoprecipitated with APP, and significantly increased the levels of Abeta, C99 and a soluble APP fragment of 95 kDa (sAPP95). The latter was reduced in brain homogenates of TgMT5-/- mice, supporting the idea of in vivo APP processing by MT5-MMP.

Conclusions: MT5-MMP emerges as a new multimodal regulator of APP metabolism, whose invalidation limits the amyloidogenic processing of APP, and hence alleviates amyloid pathology and cognitive decline.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-0433
ENGULFMENT ADAPTER PTB DOMAIN CONTAINING 1 (GULP1) IS A NOVEL TAU INTERACTING PROTEIN

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Alzheimer's disease (AD) is the leading cause of dementia worldwide and characterized by senile amyloid β (Aβ) plaques and neurofibrillary tangles composed of hyperphosphorylated tau. Our group identified GULP1 as novel APP adaptor protein that shuttles between cytosol and nucleus modulating transactivation together with LRP1. Fe65, a well characterized APP adaptor protein, has recently been identified as a tau interacting protein. We here addressed the question whether GULP1 interacts with tau as well.
Co-localization of overexpressed and endogenous GULP1 and tau was analyzed in different cell lines showing co-localization in perinuclear region. In addition, we investigated the impact of GULP1 overexpression and siRNA mediated knockdown on tau expression and phosphorylation. We could demonstrate that GULP1 overexpression, especially in SH-SY5Y cells, changed the subcellular localization of endogenous tau and led to a clear reduction of endogenous total tau expression levels in an expression level dependent manner. To study the interaction between GULP1 and tau, co-immunoprecipitation experiments of overexpressed and endogenous proteins were performed demonstrating that tau was co-precipitated upon GULP1-precipitation.
Here, we provide first evidence that GULP1 is a novel tau interacting protein regulating tau expression in a dose dependent manner. The protein may also have an impact on tau phosphorylation and can thereby regulate cellular re-organization and cytoskeletal dynamics. As GULP1 has already been shown to interact with APP, it may function as linker between APP and tau.

02k. Cell, Molecular & Systems Biology: PrP

ADPD5-1945

ABETA INTERACTS WITH CELLULAR PRION PROTEIN INDUCING NEURONAL MEMBRANE DAMAGE AND SYNAPTOTOXICITY

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Objetives: A major feature of Alzheimer's disease (AD) is the accumulation of β-Amyloid peptide (Abeta) in the brain. Recent studies have shown that Abeta oligomers interact with the cellular Prion protein (PrPc). Therefore, this interaction might be driving some of Abeta toxic effects at the neuronal membrane level. The aim of this study was to evaluate the functional consequences of this interaction.
Methods: In the present study, using a combination of electrophysiological recording, immunofluorescence, calcium imaging and Western blots techniques, we report that Abeta binds to PrPc in the neuronal membrane and plays a role on the toxic effects induced by Abeta. Phospholipase C-enzymatic cleavage of PrPc from the plasma membrane attenuated the association of Abeta to the neurons. Furthermore, an anti-PrP antibody (6D11) decreased the association of Abeta to hippocampal neurons with a concomitant reduction in Abeta and PrPc co-localization. Interestingly, this antibody blocked the increase in membrane conductance, intracellular calcium and synaptic deficit induced by Abeta.
Conclusions: The data indicate that PrPc plays a role on the membrane perforations produced by Abeta, the increase in calcium ions and the release of synaptic vesicles that subsequently leads to synaptic failure. Future studies blocking Abeta interaction with PrPc could be important for the discovery of new therapeutic strategies for AD.
Supported by FONDECYT 1140473

02m. Cell, Molecular & Systems Biology: ApoE

ADPD5-0334

APOLIPOPROTEIN E4 AFFECTS TOPOGRAPHICAL CHANGES IN HIPPOCAMPAL AND CORTICAL ATROPHY IN ALZHEIMER'S DISEASE DEMENTIA: A FIVE-YEAR LONGITUDINAL STUDY

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Apolipoprotein E4 (*APOE4*) is a genetic risk factor for developing Alzheimer's disease (AD). Once AD manifests clinically, however, the effects of *APOE4* are less clear. Therefore, we investigated *APOE4* longitudinal effects on topographical changes in AD patient brain atrophy.

We prospectively recruited 35 patients with AD (19 *APOE4* carriers and 16 non-carriers), and 14 normal controls, then followed them for five years. We measured hippocampal deformities and cortical thickness. Hippocampal comparison between *APOE4* carriers and non-carriers with AD showed carriers had rapid changes in the head and body, while non-carriers had rapid changes in a small portion of the body. Cortical thickness comparison between *APOE4* carriers and non-carriers with AD dementia showed carriers had rapid thinning in the lateral frontal, temporal, and parietal regions, while no region showed more rapid cortical thinning in non-carriers than in carriers.

These findings underlined *APOE4* allele importance for designing and interpreting future treatment trials in patients with AD dementia.

ADPD5-0371

FUNCTIONAL AMYLOIDOGENESIS IS REGULATED BY APOLIPOPROTEIN E IN ENDOSOMES.

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Formation of inert mature amyloid fibrils constitutes a defense mechanism to clear the cellular toxicity of prefibrillar oligomers in pathological situations. Some genetic variants involved in this process predispose patients to heightened susceptibility to amyloid formation, such as of Apolipoprotein E (ApoE) in early onset Alzheimer's Disease. Amyloid structures can also serve physiological functions. Cells that produce so-called "functional amyloids" must therefore exploit specific mechanisms to avoid potential toxicity inherent in their formation.

To investigate the mechanisms of functional amyloidogenesis, we have combined cell biology methods, biochemistry and electron microscopy to analyze the amyloid formation by the premelanosome protein (PMEL) in mammalian pigment cells. Pigment cells have tuned their endosomes to assemble mature fibrils from the proteolytic fragments of PMEL using intraluminal vesicles (ILVs) as potential seeding platforms.

Using secreted ILVs, called exosomes, as reporters of endosomal processes, we show that exosomes and ILVs are associated with lipoparticles that are uniquely composed of Apolipoprotein E (ApoE). Within endosomes ApoE facilitates the loading of PMEL amyloidogenic fragments onto ILVs and regulates the formation of mature fibrils in melanocytic cell lines and pigment cells in vivo.

Our study establishes a paradigm for the mechanism by which ApoE regulates the assembly of mature amyloid fibrils under both benign and pathological conditions. The novel evidence that ApoE is a component of exosomes provides a breakthrough in the field of extracellular particles that might be exploited to reconsider the respective roles of each extracellular particle in amyloid associated pathologies.

ADPD5-1071

IMPAIRED AUTOPHAGY IN APOE4 ASTROCYTES

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Autophagy is the main cellular process involved in degradation pathways of organelles and damaged proteins. Recent studies suggest that Alzheimer disease (AD) is associated with activation of autophagy. Although it is not yet known whether autophagy plays a causative or protective role in AD, the prevailing view is that autophagy is first activated as a protective mechanism to remove cellular and protein debris but as the disease progresses, autophagy apparatus may become more clogged and dysfunctional. ApoE4 is the most prevalent genetic risk factor of AD, and affects more than half of the AD patient population. The mechanisms underlying the pathological affects of ApoE4 are not known.

Utilizing in-vitro and in-vivo models, the present study explores the possibility that autophagy plays a role in mediating the pathological effects of ApoE4. In vitro studies utilizing astrocytic cell lines prepared from ApoE3 and ApoE4 transgenic mice revealed that ApoE4 is associated with reduced levels of the microtubule-associated protein LC3-II and decreased levels of ubiquitin-binding scaffold protein p62. This suggest that ApoE4 can impairs autophagy and protein degredation. Additional studies revealed that the ApoE4 astrocytes are impaired in their ability to digest Amyloid-beta containing senile plaques in-situ suggesting that ApoE4 is associated with both biochemical and functional autophagy related impairments. Complementary studies utilizing ApoE3 and ApoE4 target replacement naïve mice revealed up regulation of p62 in hippocampal neurons of the ApoE4 mice.

Taken together these findings suggest that ApoE4 is associated with impaired autophagy, which may play role in exerting it's pathological affects.

ADPD5-1509

THE ROLE OF APOE IN REGULATING SYNAPSE FORMATION AND PLASTICITY

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Objective. In the human population, there exist three possible apoE alleles, e2, e3, and e4. A single copy of the APOE ε4 allele increases the risk of developing AD by about threefold, whereas homozygosity for ε4 increases AD risk ~12 fold. Here, we utilized patient iPS cell-derived astrocytes to investigate how the APOE4 genotype confers AD risk.
Methods. We utilized iPS cell-derived astrocytes from an apoE knockout line, and also patient-derived ApoE3/3 and ApoE4/4 lines to determine how apoE regulates synapse formation and whether the APOE4 polymorphism differentially affects synapse formation when compared to APOE3. Astrocytes were co-cultured with neurons and synapse formation/plasticity *in vitro* was quantified.
Results. We found that apoE plays an important role in synapse formation *in vitro*. Furthermore, synapse formation was dependent on APOE genotype.
Conclusions. Our results suggest that ApoE modulates synaptic formation and plasticity and that APOE4 confers AD risk by differentially affecting synapse formation and synaptic plasticity when compared to APOE3.

ADPD5-1649

RISK SCORING FOR ALZHEIMER'S DISEASE IN INDIVIDUALS WITH FAMILY HISTORY

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Introduction: The insight in the ethiopathogenesis of Alzheimer's disease(AD) suggests a slowly developing neurodegenerative process that starts 10 to 20 years before the clinical manifestation. Preventing or slowing the process of neuronal damage would be the state of the art in neuroscience, but the fist step is to identify individuals at risk, diagnose AD in the preclinical stage, develop preventive strategies and start treatment in the early disease course.

Objectives: Developing a model for estimating a predictive risk score for AD in individuals with family history for AD and .

Material and Methods: A battery of biochemical test for identifying LPL deficiency, serological biomarkers(alfa 2 macroglobulin, homocystein, complement factor H, interleukin-6) , APO E genotyping and neuropsychological tests (Mini Mental State Examination-MMSE, Clinical Dementia Rating, and the novel Syndrome Kurz test) are performed in 50 individuals with family history for AD.

Results: The early stage ongoing research that our team is performing, gives optimistic insight in the first results by combining the presence of the examined serological biomarkers, the neuropsychological tests results and ApoE genotyping, we estimate the risk score for developing AD.

Conclusion: Till now a list of potential blood biomarkers has been suggested but none of them has shown to be sensitive, nor specific in accurate preclinical AD diagnosing. We believe that by combing these tests, a risk score for individuals with family history of AD would be estimated, so that a prospective approach and preventive strategies could be developed.

ADPD5-0377

NEW TOOLS TO STUDY THE INTRACELLULAR TRAFFICKING OF GAMMA-SECRETASE COMPLEXES USING FLUORESCENCE MICROSCOPY

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Objectives: Gamma-secretase achieves the regulated intramembrane proteolysis of various substrates including APP, the cleavage of which leads to amyloid peptide production. The gamma-secretase protein complex is made of the four core-subunits presenilin (PS), nicastrin, APH1 and PEN2. With two PS variants (PS1 and PS2) and three APH1 variants (APH1aL, aS and b), the subunits combinations can potentially generate six different complexes. Our goal is to determine whether distinct gamma-secretase complexes traffic to similar or different cellular compartments and to identify the molecular motifs potentially involved in a specific targeting.
Methods: We generated different types of constructs to co-express specific combinations of gamma-secretase subunits and follow their intracellular trafficking using either bimolecular fluorescence complementation (BiFC) or more traditional fluorescent protein-tagging and fluorescence microscopy.
Results: We demonstrate that our constructs allow the formation of functional gamma-secretase complexes and their visualization. Using BiFC constructs, we detected PS1-containing complexes at the cell surface and in endosomes/lysosomes in addition to the endoplasmic reticulum (ER); however the majority of cells co-transfected with APH1b displayed only BiFC signal within the ER suggesting a higher retention of gamma-secretase complexes containing this variant.
Conclusions: Our new tools will be helpful to precisely characterize the successive steps of the subcellular trafficking of the different gamma-secretase complexes and to determine if distinct itineraries can be associated with a substrate specificity of particular gamma-secretase complexes.

ADPD5-0428

PROTEOGLYCAN NG2 IS SHED BY ALPHA SECRETASE; A PROCESS POTENTIALLY UNDERLYING THE ASSOCIATION BETWEEN LOWERED NG2 SHEDDING AND AMYLOID BETA 1-42 ACCUMULATION IN AD PATIENTS?

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Objectives: The NG2-proteoglycan is implicated in important processes including cell-proliferation and cell-survival of its host cells and the shed version (sNG2) is known to regulate angiogenesis. We have previously shown decreased level of sNG2 positively correlating with amyloid beta (Aß) 1-42 levels in cerebrospinal fluid (CSF) from patients with Alzheimer's disease (AD). We have also demonstrated a direct impact of Aß1-42 on NG2 shedding and a decreased NG2 immunoreactivity in hippocampal tissue from AD patients. Here we investigate whether NG2 and the amyloid precursor protein (APP) are sensitive to the same protease activity.
Methods: NG2 expressing fibroblasts isolated from (n=8) healthy donors and (n=3) AD patients were exposed to α-secretase inhibitor TAP1-0, γ-secretase inhibitor DAPT or respective vehicle. Levels of sNG2, APPalpha and APPbeta in cell culture medium were measured using electrochemiluminescence (ECL) technology (Mesoscale Discovery).
Results: Levels of sNG2 and APPalpha decreased ($p<0.001$), whereas APPbeta levels increased, after exposure to TAP1-0 compared to vehicle ($p<0.001$). No impact on sNG2, APPalpha or APPbeta levels was detected after DAPT exposure. The sNG2 levels were strongly and positively associated with APPalpha levels, but not with APPbeta, regardless of stimuli exposure ($r>0.800$, $p<0.01$).
Conclusion: Our results suggest that sNG2 and the non-amyloidgenic APPalpha are generated by the same proteolytic pathway, which may underlie the disturbed NG2 shedding in relation to Aß1-42 accumulation seen in AD patients.

ADPD5-0473

METALLOPROTEASE MEPRIN BETA IS A NEW LINK TO SPORADIC ALZHEIMER´S DISEASE

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Objective
N-terminally truncated amyloid β (Aβ) peptides have been described in brains of sporadic AD patients. These peptides are generated independently of BACE-1, but can be attributed to the β-secretase activity of the metalloprotease meprin β. Aim of this work was to characterize the role of meprin β in APP processing and, even more, to study its importance concerning sporadic versus familial Alzheimer´s Disease (AD).
Methods
Immunoprecipitated Aβ species were separated with 8 M urea SDS-PAGE. Aggregation propensity of Aβ species was analysed using ThT-binding assay. Analysis of protein interactions was performed by applying co-immunoprecipitation or a split-GFP based complementation assay. Sections of paraffin-embedded samples of frontal isocortex of AD patients and non-demented control patients were stained with hematoxilin/eosin (HE) and a Bielschowsky staining kit.
Results
We could show that APP mutations, associated with familial AD (FAD), affect meprin β cleavage and that meprin β is not able to generate N-terminally truncated Aβ from the Swedish mutant APP. Furthermore, we could show that N-terminally truncation promotes Aβ aggregation and, even more, enhances the aggregation of non-truncated species. Our *in vitro* data demonstrates that APP and meprin β interaction occurs prior to endocytosis. Moreover, we revealed increased levels of soluble APPα (sAPPα) in meprin β knock-out mice. Most interestingly, we were able to detect increased levels of meprin β in sporadic AD versus control brains.
Conclusion
We propose that, besides BACE-1, meprin β is a novel candidate that can be linked to the generation of N-terminally truncated Aβ in sporadic AD.

ADPD5-0560

SERUM STARVATION INDUCES BACE1 PROCESSING AND SECRETION

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Objectives

β-secretase (BACE1) is a type 1 transmembrane protease implicated in Alzheimer's Disease (AD) pathogenesis. Cleavage of Amyloid Precursor Protein (APP), initiated by BACE1 and followed by γ-secretase, leads to the formation of toxic Aβ peptides. Increased levels of BACE1 have been detected in the CSF of AD patients compared to age-matched healthy controls indicating that neurodegenerative conditions induce proteolysis-dependent secretion of BACE1. The aim of this study is to examine the mechanism by which serum deprivation stimulates proteolysis-dependent secretion of BACE1.

Methods and Results

HEK293 cells were incubated in the presence or the absence of serum. We observed that serum-starvation stimulated proteolysis-dependent secretion of BACE1 and that ADAM10 and ADAM17 may be involved in BACE1 processing. This is unexpected since BACE1 is localized mainly in lipid rafts while ADAM10 is localized mainly in non-lipid raft domains. We hypothesized that serum deprivation results in alterations in the lipid composition of the membrane or the activation of signal transduction pathways both of which can alter the localization of ADAM10 and BACE1. In support, we obtained results indicating that extraction of membrane cholesterol following incubation with methyl β cyclodextrin potentiated the effect of serum deprivation.

Conclusions

Serum starvation induces proteolysis-dependent secretion of BACE1.

02n. Cell, Molecular & Systems Biology: secretases

ADPD5-0651
THE RELATIONSHIP BETWEEN AGE-DEPENDENT ENDOCYTIC DISTURBANCE AND PRESENILIN-1

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Objectives
We previously showed that endocytic disturbance causes intraendosomal accumulation of β-amyloid precursor protein (APP) and Aβ. Therefore, endocytic disturbance may be involved in age-dependent AD pathology. Presenilin1 (PS1) is the catalytic core of gamma secretase complex that is required for Aβ cleavage. In this study, we investigated whether endocytic disturbance also affects PS1 level and localization.
Methods
Neuro2a cells were treated with siRNA against dynein heavy chain or lysosomal inhibitors to induce endocytic disturbance. We also examined various aged cynomolgus monkey brains for in vivo study.
Results
In contrast to APP, endocytic disturbance did not affect PS1 level or its subcellular distribution. Furthermore, co-immunoprecipitation analyses demonstrated that endocytic disturbance did not affect γ-secretase complex formation. In aged monkey brains, we confirmed that the level of PS1 unchanged in microsomal fraction, even though APP clearly accumulated as previously reported.
Conclusion
Age-dependent endocytic disturbance does not greatly affect PS1 level and its localization. These findings suggested that intracellular trafficking of PS1 would be different from APP.

ADPD5-0844

IDENTIFICATION AND VALIDATION OF NOVEL BACE1 SUBSTRATES IN VIVO

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The protease BACE1 cleaves the amyloid precursor protein (APP) and is a key drug target in Alzheimer's disease. Several pharmaceutical companies have recently entered clinical trials with BACE1 inhibitor drug candidates. However, studies showed that BACE1-deficient mice display several neurological phenotypes. This raises the concern that possible mechanism-based side effects of BACE1 inhibition in patients may result from reduced cleavage of other BACE1 substrates. To fully evaluate the therapeutic potential of BACE1 inhibition, it is essential to understand how the function of other substrates is altered in BACE1-deficient mice and whether their loss of cleavage contributes to the phenotypes observed in these mice.

Objectives: To identify and characterize novel BACE1 substrates under physiological conditions in vivo by comparing WT and BACE1 knock-out mice brains.

Methods: BACE1 substrates were identified using a mass spectrometry based proteomic workflow with spike-in SILAC mice and validated using western blotting and immunofluorescence.

Results: 17 novel and previously known BACE1 substrates such as; APP, APLP2, CHL1 and Contactin-2 were identified. A subset of substrates including the classical axon guidance molecule EPHA4 was validated in an independent set of brains. We show that EPHA4 is shed by BACE1 from embryogenesis to adulthood, although acting as a minor sheddase. Further validation of other substrates and functional aspects are currently in progress.

Conclusions: The identified BACE1 substrates points to a role for BACE1 in establishment and maintenance of neuronal connectivity, since a majority of identified proteins are involved in neural outgrowth axonal guidance and synapse formation.

02n. Cell, Molecular & Systems Biology: secretases

ADPD5-0868

UTILISING THE UNIQUE MULTIBAC BACULOVIRUS PROTEIN EXPRESSION SYSTEM TO EXPRESS AND GENERATE PURIFIED GAMMA-SECRETASE ENZYME COMPLEX

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Objective: γ-Secretase is a multi-subunit enzyme essential for processing a number of transmembrane proteins inclusing amyloid precursor protein (APP) and Notch. Although EM analysis of purified γ-secretase revealed a globular structure, higher resolution crystal structures for the complex or its components have not been elucidated. Such studies require large quantities of purified protein., Baculoviral expression is an approach to achieve such quantities... Previous studies have expressed multi-protein complexes by co-infecting cells with several viruses. With this approach, the logistical demands of maintaining known viral titres and establishing relative equal expression levels, renders large-scale complex production difficult. To circumvent these limitations, we used a baculoviral multi-gene vector (MulitBac) to generate γ-secretase.
Methods: Components of γ-secretase (PS1, APH1a, NCT and Pen-2) were cloned into pFBDM, MultiBac expression vector. Following integration into single baculovirus DNA, γ-secretase was expressed in insect cells. The complex was purified using affinity purification tags- octa-histidine and CBP on NCT and PEN-2 respectively.
Results: Expression of all components of γ-secretase was observed and the complex was active in processing APP and Notch (which was inhibited by γ-secretase inhibitors). Interactions between components were observed by co-immunoprecipitation studies. The complex was purified using His followed by CBP chromatography.
Conclusion: We have utilized the MultiBac expression system to successfully generate an active γ-secretase complex. This allows larger scale production and purification of the complex for further analysis. In conjunction with available automated multi-complex generation, this system allows the opportunity to generate γ-secretase variants with modified/mutated subunits in high throughput.

02n. Cell, Molecular & Systems Biology: secretases

ADPD5-0872

BACE1 REGULATES THE SURFACE LEVELS OF KV1.1/KV1.2 POTASSIUM CHANNELS

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Objectives: BACE1 regulates various neuronal functions by cleaving multiple substrates. Recently, we found that BACE1 cleaves contactin-2, a GPI-anchored protein in neurons. Since contactin-2 regulates K_v1.1/1.2 trafficking in axonal compartments, we asked whether BACE1-mediated cleavage of contactin-2 leads to altered trafficking of K_v1.1/1.2.

Methods: To analyze surface levels of contactin-2/K_v1.1/K_v1.2, we performed surface biotinylation on brain slices from BACE1 KO and WT mice. Given the selective expression of K_v1.1 in hippocampal neurons and K_v1.2 in cortical neurons, we also used mouse primary cortical/hippocampal cultures treated with a BACE1 inhibitor. To explore axonal colocalization of contactin-2/K_v1.1/K_v1.2 in myelinated CNS neurons, we performed immunostaining in paraffin sections of optic nerve and hippocampus from BACE1 KO and WT mice.

Results: We found that surface contactin-2/K_v1.2 levels are elevated in cortical slices of BACE1 KO mice as compared to WT mice and in cultured cortical neurons treated with a BACE1 inhibitor. We also found increased K_v1.1/contactin-2 surface levels in hippocampal neurons treated with a BACE1 inhibitor. Abnormal localization of K_v1.1/K_v1.2 was observed in optic nerve axon bundles, while K_v1.1/K_v1.2 levels were found increased in the CA3 region of BACE1 KO mice as compared to WT mice.

Conclusions: Our findings show that BACE1 regulates surface levels of K_v1.1/1.2 possibly by cleaving contactin-2 in neurons. Our data also suggest that axonal localization of contactin-2/K_v1.1/K_v1.2 is disrupted in BACE1 KO neurons. This study will help elucidate non-amyloidogenic functions of BACE1 in neurons, important for developing BACE1 inhibitors as a safe therapeutic strategy for Alzheimer's disease.

02n. Cell, Molecular & Systems Biology: secretases

ADPD5-0954

VALIDATION AND FUNCTIONAL ANALYSIS OF THE SEZ6 PROTEIN FAMILY - NOVEL SUBSTRATES OF THE ALZHEIMER PROTEASE BACE1.

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In Alzheimer's disease, the amyloid beta (Abeta) peptides are considered the main neurotoxic species. Abeta peptides are generated through proteolytic processing of the transmembrane amyloid precursor protein (APP) by the key rate-limiting enzyme BACE1. Thus, BACE1 is a main drug target for the treatment of AD. Although much effort has been made in order to inhibit BACE1 activity and therefore reduce Abeta production, possible side effects resulting from its inhibition have not yet been clarified. These side effects may occur from the cleavage inhibition of other BACE1 substrates. Importantly, besides APP, BACE1 cleaves other substrates whose loss of processing has been linked to phenotypes observed in BACE1-deficient mice. We have recently identified novel BACE1 substrates, including the Seizure protein 6 (Sez6) family. This family is particularly interesting given its role in brain development and the similarity between the phenotype of Sez6 and BACE1-deficient mice. Objectives: To evaluate how the expression and function of Sez6 and Sez6L is altered in BACE1-deficient mice and whether their loss of cleavage contributes to the phenotypes observed in BACE1-deficient mice. Methods: Biochemical analysis of primary neurons and BACE1-deficient tissue. Results: We have validated Sez6 and Sez6L as main BACE1 substrates both in vitro and in vivo. Moreover, we are investigating the function of the Sez6 protein family and alterations that their loss of BACE1-cleavage may induce in neurons. Conclusions: Our work allows a better understanding of BACE1 function and may allow to predict potential side effects resulting from therapeutic BACE1 inhibition.

02n. Cell, Molecular & Systems Biology: secretases

ADPD5-0971
UPREGULATED MAM FUNCTION IN ALZHEIMER DISEASE
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Alzheimer disease (AD) is characterized by neuronal loss, especially in the cortex and hippocampus, accompanied by accumulation in the brain of extracellular neuritic plaques containing b-amyloid (Ab) and of intracellular neurofibrillary tangles consisting of hyperphosphorylated tau protein. AD patients also present with other features that have received less attention, including aberrant cholesterol, phospholipid, and calcium homeostasis, and altered mitochondrial function and dynamics.
Presenilin-1 (PS1), presenilin-2 (PS2), and g-secretase activity, which processes the amyloid precursor protein (APP) to generate Ab, are all located predominantly in a specialized subcompartment of the endoplasmic reticulum (ER) that is physically and biochemically connected to mitochondria, called mitochondria-associated ER membranes (MAM). MAM is involved in the regulation of cholesterol and phospholipid metabolism, calcium homeostasis, and in mitochondrial function and dynamics. Our work shows that MAM is lipid raft-like domain where Abeta is produced and that cells from AD patients have massively upregulated MAM activity and increased ER-mitochondrial connectivity, resulting in altered cholesterol, phospholipid and calcium homeostasis, and aberrant mitochondrial dynamics, which may help explain many of the biochemical and morphological features of the disease.
Based on these findings, we believe that MAM dysfunction and altered ER-mitochondrial connectivity are early causative events in the pathogenesis of AD.

02n. Cell, Molecular & Systems Biology: secretases

ADPD5-1431

REGULATION OF BACE1 EXPRESSION BY TRANSLATION INITIATION FACTORS

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Objectives

BACE1 is the key enzyme in the production of the amyloid-beta (Abeta) peptides that are considered the cause of neuronal damage and death in Alzheimer's disease (AD). Changes in BACE1 expression/activity are a critical issue in disease progression and higher levels of BACE1 protein have been observed in the brain of AD patients. Previous work highlighted the complex regulation of BACE1 expression at the translational level and it has been proposed that translation initiation factors have a role in the modulation of BACE1 expression. We aim to investigate how translation initiation factors can affect the efficiency of BACE1 translation in neurons.

Methods

We employ primary neuronal cultures from the rat brain. We modulate the activity of translation factors by overexpression, by silencing and by modulating their phosphorylation. The expression of BACE1 is assessed by RT-PCR and Western blotting.

Results

We show that changes in the activity and expression of translation initiation factors can modulate the levels of BACE1 in neurons by acting at the level of the complexity of the BACE1 transcript leader. We also propose that neuronal-specific phosphorylation of translation factors could explain the difference in BACE1 translation efficiency between neurons and other cell types.

Conclusions

BACE1 translation in neurons can be under the control of translation initiation factors whose activity is regulated by the neuronal activity. A derangement of this control could underlie the pathological changes in BACE1 levels observed in AD.

02n. Cell, Molecular & Systems Biology: secretases

ADPD5-1668

IMPACT OF RTN3 DEFICIENCY IN MICE ON AMYLOID DEPOSITION AND TAU PHOSPHORYLATION

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Reticulon 3 (RTN3) is a richly neuronal expressing member of reticulon/Nogo protein family. Previous studies showed that RTN/Nogo interacts with BACE1 and negatively regulates BACE1 activity. To what extent and how critical RTN3 deficiency affects BACE1 activity is an intriguing question. In this study, we aimed to address this question by utilizing generated RTN3-null mice. Mice with complete deficiency of RTN3 grow normally and have no obviously discernible phenotypes. Morphological analyses of RTN3-null mice showed no significant alterations in cellular structure, although RTN3 is recognized as a protein contributing to the shaping of tubular endoplasmic reticulum. However, RTN3 deficiency in Alzheimer's mouse models facilitates amyloid deposition, further supporting an *in vivo* role of RTN3 in the regulation of BACE1 activity. Further biochemical analysis revealed that RTN3 deficiency increased protein levels of BACE1 via posttranslational event. This elevation of BACE1 levels correlated with enhanced processing of amyloid precursor protein at the β-secretase site. More intriguingly, RTN3 deficiency in mice induces hyperphosphorylation of tau proteins in different age groups. Since it has been shown that RTN3 monomer is reduced in brains of Alzheimer's patients, our results suggest that long-lasting reduction of RTN3 levels in elderly will contribute to Alzheimer's pathogenesis by having adverse effects on BACE1 activity and tau phosphorylation.

02n. Cell, Molecular & Systems Biology: secretases

ADPD5-2252
ACTIVITY-DEPENDENT AND GRADED BACE1 EXPRESSIN IN OLFACTORY EPITHELIUM IS MEDIATED BY RETINOIDS.
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Whether and how neuronal activity can modulate amyloid precursor protein (APP) metabolism and beta-amyloid peptide accumulation remains poorly understood but may be of potential medical relevance. It has been previously shown that in certain brain areas the beta-secretase (BACE1), the enzyme obligatory for amyloidogenic processing of APP, is elevated when neuronal activity decreases. However the mechanism of this process is not well examined. Here, using different transgenic mice and olfactory pathway as a model system, we show that BACE1 expression is controlled by activity-dependent expression of retinoic acid (RA) degrading enzyme Cyp26B1. Both genes form counter gradients along dorsomedial-ventrolateral axis and inversely depend on neuronal activity. Overexpression of Cyp26B1 or presence of dominant negative RA receptor selectively inhibit BACE1 expression in olfactory sensory neurons. We conclude that stimulus dependent neuronal activity by controlling RA catabolic enzyme which potentially leads to formation of RA gradient, can control expression of downstream genes such as BACE1. This results improves an understanding how topographic axonal connectivity is established during olfactory sensory map formation in healthy context. It also suggest potential mechanism how retinoids and neuronal activity can affect APP processing and beta-amyloid accumulation in pathogenic scenario.

02p. Cell, Molecular & Systems Biology: TTR

ADPD5-0840
ROLE OF TRANSTHYRETIN IN AMYLOID-BETA UPTAKE BY THE LIVER: IMPACT IN ALZHEIMERS DISEASE

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Objectives: Data from transgenic mice has shown Transthyretin (TTR) as a protective molecule in Alzheimer's disease (AD). The peripheral clearance of Amyloid-Beta (Abeta) is mediated mainly by the liver. In this study, we aimed at investigating the role of TTR in Abeta uptake by the liver.
Methods: We used SAhep cells, which does not express TTR, added fluorescent Abeta1-42 (FAM-Abeta1-42) in presence or absence of human recombinant TTR in the cell media, and measured FAM-Abeta1-42 internalization by Flow Cytometry. We also performed immunocytochemistry to observe the co-localization of TTR and FAM-Abeta1-42 in the cells using fluorescent microscopy. To confirm the results, we performed similar studies in primary hepatocytes derived from mice with different TTR genetic backgrounds (with 2 copies, 1 copy or without TTR gene).
Results: TTR promoted Abeta internalization in SAhep cells and the highest percentage of uptake was obtained in presence of 4-6 µg/ml of TTR. Fluorescent microscopy confirmed co-localization of Abeta and TTR in the cells. We were also able to optimize the isolation and establishment of primary cultures of hepatocytes derived from mice with different TTR genetic backgrounds. A preliminary experiment confirmed results obtained in the cell line. Further studies will be a key in confirming the importance of TTR in Abeta elimination by the liver.
Conclusions: TTR increases Abeta uptake by hepatocytes suggesting its participation in peripheral Abeta clearance at the liver. Given recent reports on decreased plasma TTR concentration in AD patients, restoring TTR levels might constitute a therapeutic approach for AD in future.

02q. Cell, Molecular & Systems Biology: protein degradation, proteasome & autophagy

ADPD5-1194

AUTOPHAGY ALTERATION UNDER ISCHEMIC CONDITION: A POSSIBLE LINK TO ALZHEIMER'S DISEASE

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Objectives. To understand brain ischemia injury contribution to Alzheimer's disease (AD) onset, looking for post-translational modifications, turnover alterations and AD hallmarks secretion such as tau and amyloid precursor protein (APP), associated to autophagy activation.
Methods. Rat cultured hippocampal neurons are subjected to oxygen glucose deprivation (OGD) followed by normal culture condition restoration (R), mimicking an ischemia/reperfusion (I/R) event. Afterwards AD hallmarks and autophagy markers expression and secretion are analyzed in the cells lysate and in microvescicles (MVs) isolated from culture medium. Confocal microscopy is employed to evaluate the potential re- and co-localization of tau and autophagic markers after treatment.
Results. In our cellular model subjected to OGD/R, we observed tau and APP post-translational and protein level modifications in parallel to the autophagic markers (LC3II and beclin-1) expression alterations. Moreover, OGD/R treatment elicits a time-dependent increase in tau and APP C-terminal fragments (CTF) secretion by means of a heterogeneous MV population, including a group of LC3II positive vescicles. Preliminary data obtained by confocal microscopy suggest that LC3 might undergo re-localization after OGD/R treatment.
Conclusions. The obtained results suggest that OGD treatment leads to multiple unconventional secretion mechanisms for tau and APP CTFs, in which autophagy activation seems to partially outcome in the exophagy secretory pathway. Although exophagy might be a defensive mechanism to prevent intracellular accumulation of misfolded proteins under conventional autophagy alteration, the secretion of misprocessed proteins has been recently associated to dendritic degeneration and neuronal death becoming a possible link to Alzheimer's disease.

02q. Cell, Molecular & Systems Biology: protein degradation, proteasome & autophagy

ADPD5-2035

ALLN INDUCES ACCUMULATION OF NOVEL AMYLOID PRECURSOR PROTEIN (APP) FRAGMENTS

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Objectives:
Alzheimer's disease (AD) is a progressive neurodegenerative disease, characterized by the deposition of amyloid β (Aβ) plaques. Aβ is a proteolytic product of its precursor, amyloid precursor protein (APP). Considerable evidence suggested a pivotal role for Aβ in AD pathogenesis, and APP proteolysis is therefore of extensive interest. Canonical APP proteolysis occurs via α-/ β- and γ-secretases. Here, we investigated the role of protein degradation system in APP proteolysis.
Methods:
HEK293 cells and other cell types underwent pharmacological treatments as indicated before collection and Western blot analysis. In vitro proteasome activity assay was performed using proteasome substrate Suc-LLVY-AMC. Transient transfection was performed using Turbofect transfection reagents.
Results:
By pharmacologically inhibiting protein degradation systems with ALLN, we observed an accumulation of heretofore undocumented APP fragments (CTFs). These changes are not due to cytotoxicity or novel transcription/ splicing events. Here, we name the novel CTF of 25 kD as η-CTF. Further examination reveals that η-CTF accumulation is mediated via cathepsin inhibition, and not proteasome or calpain inhibition.
Conclusion:
Collectively, our results suggest that a subpopulation of APP undergoes alternative processing by undetermined mechanism, and the resulting η-CTF is rapidly processed/ cleared by cathepsin. Given that impairment in protein degradation is a common feature in aging and neurodegeneration, we reason that failure of clearance of these η-CTFs could potentially be involved in the disease/ aging process.

ADPD5-1081

AMYLOID PRECURSOR PROTEIN REGULATION OF GDNF EXPRESSION CONTROLS NEUROMUSCULAR JUNCTIONS FORMATION

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Objectives: Beside its crucial role in Alzheimer's disease (AD) pathogenesis, different functions have been attributed to the amyloid precursor protein (APP) but its pivotal role is difficult to depict. We found that APP regulates GDNF (Glial cell-line Derived Neutrophic Factor) expression. Our work aims at investigating the role of APP-dependent GDNF expression in neuromuscular junctions.
Methods: Following transcriptome analysis, qRT-PCR, ELISA and reporter gene assays were performed on APPKO mouse embryonic fibroblasts (APP -/- MEFs) stably re-expressing APP695 and APP751. APP and GDNF expression have been monitored throughout skeletal muscle differentiation (C2C12 cells) upon APP silencing (small interfering RNA) and/or GDNF plasmid expression. We performed grip strength tests, mechanic measurements on isolated muscles and immunohistochemistry (IHC) of neuromuscular junctions (NMJs) on APP -/- mice. A co-culture model of C2C12 and cholinergic neurons (NG108-15) was set up to analyze the effects of APP silencing or GDNF expression on NMJs formation and neuronal maturation by immunocytochemistry (ICC).
Results: GDNF mRNA and protein levels together with GDNF transcriptional activity are down-regulated in APP -/- MEFs and restored specifically by APP751 isoform. GDNF and APP levels increase during muscular differentiation. Their overexpression or silencing favors or affects the process, respectively. APP-dependent GDNF expression controls the muscular phenotype and defective NMJs in APP -/- mice. In the co-culture model, GDNF restores the number of NMJs and neuronal maturation impaired by APP silencing.
Conclusions: APP controls GDNF expression with a critical involvement in neuronal and muscular differentiation underlying NMJs formation.

02r. Cell, Molecular & Systems Biology: growth factors

ADPD5-1984

PRONGF/P75NTR SIGNALLING IN HIPPOCAMPAL ADULT NEUROGENESIS: POTENTIAL TARGET FOR GENERATING NEW NEURONS IN ALZEIMER'S DISEASE

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Introduction

It has been established that a 10% of neurons generated in adult human brain, are able to establish synaptic connections that can be stabilized and incorporated into the neural network. In animal models of Alzheimer's disease, the induction of neurogenesis can reverse cognitive deficits of the disease. Previous studies demonstrate i) a significant increase stage - dependent of pro -NGF in human hippocampus and enthorinal cortex affected by AD ii) that a proNGF receptor, p75NTR is expressed not only in mature neurons but also in mitotic cells from mouse hippocampus, and that the injection of pro-NGF in adult mouse brain decreases hippocampal neurogenesis.

Methods

Immunoflurescence to detect proNGF, p75NTR, sortilin and markers of the different steps of maturation of new neurons in hippocampal preparations from C and AD affected human brains and in animal models.

Results

Adult new born cells in human Dentate Gyrus express p75NTR and are diminished in AD. Increased pro-NGF in adult hippocampal brain , may be responsible for the decrease of neurogenesis and the defective differentiation and synapse formation of new neurons blocking the effect of regeneration -inducing factors.

Conclusions

Disruption of proNGF/p75/sortilin signaling would be a relevant target in regenerative therapies for AD.

02s. Cell, Molecular & Systems Biology :GCPR, nicotinic, sigma-1 & other receptors

ADPD5-1171

GPR3-DEFICIENT MICE DISPLAY AN IMPAIRMENT IN COGNITIVE FUNCTION

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Objectives

G protein-coupled receptor 3 (GPR3) plays an important role in regulation of Aβ generation. In this study, we aim to determine the physiological function of GPR3 and understand the mechanism(s) regulating GPR3 function.

Methods

To establish the physiological function of GPR3, we determined the effect of the absence of GPR3 on cognitive and synaptic function, utilizing the Morris Water Maze (MWM) behavioral paradigm, immunohistochemical, and electrophysiological studies. In addition, we performed co-immunoprecipitation studies to identify a new binding partner for GPR3.

Results

The MWM paradigm showed that *Gpr3*$^{-/-}$ mice display reference memory and cognitive flexibility deficits. Furthermore, we observed a reduction in dendritic spine density in the hippocampi of *Gpr3*$^{-/-}$ mice and determined that GPR3 is localized to dendritic spines of hippocampal neurons, suggesting that GPR3 is involved in the regulation of synaptic function. We also identified a PDZ binding domain in the C-terminus of GPR3. Mutation of a serine residue in this domain significantly reduced β-arrestin recruitment to GPR3 and Aβ generation. Co-immunoprecipitation studies indicate that GPR3 interacts with PSD95 through the PDZ binding domain, suggesting that PSD-95 modulates the GPR3/β-arrestin-mediated Aβ generation through this PDZ binding domain.

Conclusions

These studies suggest that GPR3 is involved in the regulation of dendritic spine morphology and cognitive function. Furthermore, identification of a new binding partner for GPR3, the scaffolding protein PSD95, suggests that PSD95 may play a pivotal role in controlling the function of GPR3 at synapse.

02s. Cell, Molecular & Systems Biology :GCPR, nicotinic, sigma-1 & other receptors

ADPD5-2038

THE MEN1/MENIN TUMOR SUPPRESSOR COORDINATES CHOLINERGIC GENE TRANSCRIPTION, SYNAPSE FORMATION AND POSTSYNAPTIC CONSOLIDATION: A NOVEL PERSPECTIVE ON ALZHEIMER'S DISEASE

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Objectives: Loss of cholinergic synaptic connections and transmission in the hippocampus is a neuropathological hallmark of early Alzheimer's disease (AD), however, our current understanding of cholinergic synaptogenesis, maturation, plasticity, and overall function in the CNS is extremely limited. We have recently demonstrated that the MEN1 tumor suppressor gene, under the control of neurotrophic factor (NTF) signaling, regulates cholinergic synaptogenesis in the CNS. The objective of the present study was to define the underlying synaptogenic mechanisms, and whether MEN1 expression and function is also disrupted in AD.
Methods: Primary neuron culture with cholinergic invertebrate and murine hippocampal neurons was used in conjunction with electrophysiological and molecular analysis to determine the influence of MEN1/menin on nAChR expression and cholinergic synaptogenesis. Molecular analysis was performed using hippocampi from the 5xFAD mouse model of AD.
Results: Regulatory proteolysis produces menin fragments that perform unique albeit coordinated tasks during cholinergic synapse formation. The N-terminal fragment is targeted to the nucleus and upregulates expression of nAChR subunits. The C-terminal fragment is targeted to postsynaptic sites where it regulates postsynaptic consolidation. In mutant 5xFAD hippocampi the expression of NTF, MEN1 and nAChR subunits are down-regulated, and proteolytic cleavage of menin is reduced.
Conclusions: This study identifies menin as both a transcriptional regulator of nAChR in the CNS, and a candidate molecule for the postsynaptic clustering of nAChR at central cholinergic synapses. This NTF-MEN1-nAChR axis was found to be disrupted in a mouse model of AD, and suggests that this pathway is a putative target for therapeutic intervention.

02t. Cell, Molecular & Systems Biology: protein-protein interactions & connectome

ADPD5-1152

IP3R3-TOM70: A SCAFFOLD AT ER-MITOCHONDRIA CONTACT SITES WITH POTENTIAL IMPACT IN ALZHEIMER'S DISEASE PATHOGENESIS

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Objectives

Current Alzheimer's disease (AD) treatment is largely symptomatic. Hence, it is crucial to investigate pathomechanisms underlying progressive neurodegeneration in order to identify new targets for causal intervention strategies.

Recently, several lines of evidence point towards a role of ER-mitochondria contact sites between outer mitochondrial membrane (OMM) and ER membrane. A number of crucial metabolic processes that are significantly altered in AD are governed at this site.

New OMM and ER proteins composing the contact sites are constantly identified. Nevertheless, it's still unclear if such proteins are transiently or stably present at the contact sites and how their expression and/or distribution are/is regulated.

Methods

We performed subcellular fractionation of mouse brain and analysed the constituents of the fraction containing ER-mitochondria contacts by immunostaining. Newly identified components were verified by by immunocytochemistry, proximity ligation assay and co-immunoprecipitation.

Results

We have identified a so far unknown scaffold between ER and mitochondria by showing the interaction between IP3R3, on the ER-side and TOM70, a receptor component of the translocase of the OMM.

Conclusions

Here, we confirm for the first time the presence of a novel scaffold between ER and mitochondria in mouse brain tissue and primary hippocampal neurons. Considering that a considerable amount of the potentially neurotoxic amyloid beta peptide is produced at ER-mitochondria contact sites, we propose a scenario where changes in the contact sites cause an elevated production and mitochondrial import of amyloid beta peptide via the newly identified scaffold eventually leading to mitochondrial dysfunction-induced neurodegeneration.

ADPD5-1580

P75 MEDIATES THE CONVERGENCE OF AMYLOID PRECURSOR PROTEIN AND BETA SECRETASE 1

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Alzheimer's disease (AD) is the most common form of dementia. Amyloid beta (Abeta) accumulation in the brain is the hallmark of AD. Abeta is one of the cleavage products of amyloid precursor protein (APP) via sequential enzymatic processing of beta secretase 1 (BACE1) and gamma secretase. Thus Abeta production requires the interactions between APP and BACE1. After synthesis, APP and BACE1 are segregated and transported to different compartments. How APP converges with BACE1 is not known. We propose that neurons sense the neurodegenerative signals from environment which promote over production of Abeta and the p75 neurotrophin receptor (p75) on cell surface regulates the convergence of APP and BACE1. We tested the idea in neuronal cell lines and primary neurons of wild type and p75 knockout mice. We found that p75 interacts with both APP and BACE1 as determined by co-immunoprecipitation. p75 ligands Abeta and proNGF increase their interactions. This result is confirmed by FRET assays which display strong signals between FRET pairs of p75-CFP/APP-YFP and p75-CFP/BACE1-YFP. These FRET signals are significantly enhanced when cells are incubated with Abeta or proNGF. Abeta and proNGF also increase the FRET signals between BACE1-CFP and APP-YFP. Abeta also increases the colocalization of APP and BACE1 in primary neurons of wt mice but not in p75 knockout mice. Our data suggest that p75 is a key receptor that senses neurodegenerative signals which promote the convergence between APP and BACE1 and is a therapeutic target for drug development for AD.

ADPD5-1122

APPLICATION OF A GLOBAL (PHOSPHO)PROTEOMIC WORKFLOW TO ANALYSE FRESH/FROZEN BRAIN TISSUE IN TRANSGENIC MODELS OF NEURODEGENERATION

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Many neurodegenerative diseases are characterised by abnormal post-translational modification (PTM) of proteins leading to their aggregation and ultimately to neuronal cell death. One of the most common forms of pathological PTM is phosphorylation, with phosphorylated forms of tau, TDP-43 and alpha-synuclein all associated with disease. Additionally, the important role of cell signalling pathways in modulating key aspects of neurodegeneration such as re-activation of cell cycle in neurons and activation of immune cells, is increasingly recognised. Accordingly, there is a strong rationale to develop and apply global phosphorylation profiling to neuronal cell cultures and brain tissue in neurodegenerative disease research.

We have recently reported SysQuant®, a global phosphoproteomic workflow employing isobaric Tandem Mass Tags (TMT) and differential chromatography to measure fractions of enriched phosphopeptides and non-phosphorylated peptides derived from tryptic digestion of tumour cell lysates of up to 10 samples in a single run. SysQuant® can quantitate over 23,000 unique phosphorylation sites and more than 7,000 individual proteins.

When we applied SysQuant® to frozen brain tissue from a mouse model of human tauopathy (TMHT Mouse, QPS Austria) treated with vehicle or vehicle + kinase inhibitors, we were able to demonstrate significant, potentially protective effects on the phosphorylation of aggregating proteins and regulation of key pathways such as glycolysis/gluconeogenesis, calcium signalling, and oxidative phosphorylation along with all major neurodegenerative KEGG pathways.

Using SysQuant® we have generated a detailed, quantitative phosphoproteomic map for 3x3x3 animals treated with vehicle or one of two kinase inhibitors from different brain regions in approximately 8 weeks.

02u. Cell, Molecular & Systems Biology: network biology

ADPD5-1124

ALZHEIMER'S DISEASE AND TYPE 2 DIABTES: NETWORK ANALYSIS ACROSS CLINICAL BOUNDARIES

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Objectives: The concept that AD is fundamentally a metabolic disease that results in progressive impairment in the brain's capacity to utilize glucose and respond to insulin stimulation has recently gained increasing support. Based on this evidence and on the fact that Type 2 Diabetes Mellitus (T2DM) is a major risk factor for AD, we performed a cross-disease analysis to confirm the mechanistic links between these two multifactorial and chronic diseases of aging. Systems medicine approaches using network-based techniques in a context of protein-protein interactions were applied aiming at the thorough understanding of the mechanisms underlying these diseases and the potential comorbidities associated.
Methods: Disease signatures of AD and T2DM were obtained from transcriptomic data of post-mortem human brain and used in conjunction with a PPI network to construct two disease-specific networks. The overlapping AD/T2DM networks' proteins were then used to extract the most representative Gene Ontology biological process terms.
Results: This analysis revealed a major role of the autophagy process, a self-degradative process that is important for balancing sources of energy in response to nutrient stress, as the molecular basis of both diseases. In addition, AMP-activated kinase, a core signaling pathway in cellular homeostasis and crucial regulator of energy metabolism, was confirmed as a central dis-regulated process in AD and T2DM, in line with previous studies.
Conclusion: The present systems biology investigation identifies the autophagy pathway as a central dis-regulated process in neurodegenerative dementia and indicates the disease-specific molecules in the pathway that could be considered for therapeutic intervention.

02v. Cell, Molecular & Systems Biology: metabolomics

ADPD5-0533

METABOLIC PERTURBATIONS IN THE BRAINS OF PATIENTS WITH ALZHEIMER'S DISEASE DETERMINED BY GAS-CHROMATOGRAPHY MASS-SPECTROMETRY

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Objectives:

Defective energy metabolism is a fundamental component of Alzheimer's disease (AD). This study aimed to elucidate the molecular basis of the metabolic defects in different brain regions from AD patients compared with matched controls.

Methods:

Brain tissue was obtained from nine cases of confirmed clinical/neuropathological diagnosis of AD and eight controls, matched for age, sex and *post-mortem* delay. Metabolite levels were measured in *post-mortem* brain tissues from seven regions (hippocampus, entorhinal cortex, middle-temporal gyrus, sensory cortex, motor cortex, cingulate gyrus, and cerebellum) by gas-chromatography/time-of-flight mass-spectrometry.

Results:

Of 69 metabolites identified, 48 were significantly altered in AD in at least one brain region. The levels of free glucose, sorbitol, and fructose were dramatically elevated in all seven regions, as were glucose-6-phosphate, glycerol-2-phosphate, glycerol-3-phosphate, 1,2-butanediol, beta-hydroxybutyric acid, threitol, uric acid, margaric acid, and N-acetylglucosamine. Decreased levels of glycerol, methyl phosphoric acid, urea, and uracil were also a consistent feature across all brain regions. The amino acids leucine, aspartic acid, glycine, serine, and proline were decreased in the AD brain whereas phenylalanine, tryptophan, and tyrosine were elevated. Other metabolites, including those of Kreb's cycle, showed variable changes that were more brain region-specific.

Conclusion:

Multiple novel findings were uncovered by this study, including: 1) impaired carbohydrate metabolism via the main physiological pathways (glycolysis and glycogenesis), 2) shifts in the use of alternative, non-carbohydrate, fuel sources, and 3) dysregulation of amino acids critical for neurotransmitter homeostasis.

02v. Cell, Molecular & Systems Biology: metabolomics

ADPD5-0740

METABOLIC PROFILING OF CEREBROSPINAL FLUID IN DEMENTING DISORDERS

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Objectives
Our goal is to profile metabolic aspects of dementing disorders by quantifying metabolic changes in cerebrospinal fluid (CSF). We wi**ll als**o explore relations between multivariate metabolic profiles and data from clinical diagnostics. We believe that this large scale metabolomics study is likely to give insight regarding the metabolic attributes of dementing disorders and their molecular interrelationships.
Methods
Our sample set consists of over 750 CSF samples extracted from patients with a diagnosed dementing disorder. The sample cases in this study are thus divided into the following categories: Alzheimer's disease, Parkinson's disease, frontotemporal dementia, mild cognitive impairment, vascular dementia, amyotrophic lateral sclerosis, Lewy-body dementia and a cognitively normal control group. Conventional proton nuclear magnetic resonance (NMR) spectroscopy was utilized to measure all CSF samples.
Results
NMR-based metabolic profiling of the CSF samples yielded quantitative data on 30 molecular constituents, which belong in low-molecular-weight biomolecule groups such as amino acids, short chain fatty acids and energy metabolites.
Conclusions
We present a platform for metabolic profiling of CSF. The acquired metabolic profiles could set the foundation for understanding the metabolic features and pathophysiology of dementing disorders. Research into correlations of metabolic profiles with other biomarkers may open new views to understanding of pathomechanisms of neurodegenerative diseases.

02v. Cell, Molecular & Systems Biology: metabolomics

ADPD5-0767
PLATELET-DERIVED EXTRACELLULAR VESICLES - A TRIGGER FOR ALZHEIMER'S DISEASE?

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Platelets are the smallest of the three major types of blood cells. They are produced from very large bone marrow cells called megakaryocytes and contribute to hemostasis. During their lifetime they release small vesicular particles, so called extracellular vesicles (EV) which make up till 90 percent of the circulating EVs in the bloodstream. These platelets derived extracellular vesicles (PL-EVs) are involved in various cellular processes like autoimmunity, chronic inflammation and neurodegeneration. In this study we purified fractions from PL-EVs concentrates and analyzed there composition. By using LC-MS/MS and label-free quantification we have been able to detect the amyloid precursor APP, the hallmark protein of Alzheimer’s disease, enriched in distinct platelet fractions. This result indicates the importance of platelet function and regulation in Alzheimer's disease.

ADPD5-0760

TRANSCRIPTOMICS AND MECHANISTIC ELUCIDATION OF ALZHEIMER'S DISEASE RISK GENES IN THE BRAIN AND IN VITRO MODELS

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Objectives

Several risk genes for Alzheimer's disease (AD) have been identified, but their effects on disease pathogenesis remain elusive. Assessing the expression and splicing in the affected brain areas may help to identify possible functions of these genes. Protein-fragment complementation assay (PCA) can reveal their effects on disease-associated pathways.

Methods

The expression and splicing of genes involved in the pathogenesis or affecting the risk of AD was assessed in the post-mortem inferior temporal cortex samples from 60 subjects with varying degree of AD-related neurofibrillary pathology. PCA was performed in HEK293T cells.

Results

More advanced AD-related neurofibrillary pathology was associated with decreased expression of *FRMD4A* and increased expression of *MS4A6A*. Increased expression of two exons in *CLU* and *TREM2* was observed, with similar but non-significant trend in other exons, suggesting a global change in the expression rather than altered splicing. In expression quantitative trait loci analysis we did not detect significant effects of the risk alleles on gene expression or splicing. Using PCA, we found that down-regulation of *FRMD4A* associated with increased APP-beta-secretase interaction, increased amyloid-beta40 secretion and altered phosphorylation of tau.

Conclusions

Taken together, we show that the expression of *FRMD4A, MS4A6A, CLU,* and *TREM2* is altered in relation to increasing AD-related neurofibrillary pathology, and that *FRMD4A* may be associated with amyloidogenic and tau-related pathways in AD. We conclude that studying the gene expression in the brain and the effects of these genes on disease-associated pathways *in vitro* may provide mechanistic insights on how these genes contribute to AD pathogenesis.

ADPD5-1266

CURCUMIN ANALOGS IN ALZHEIMER'S DISEASE: BISDESMETHOXYCURCUMIN AS TRANSCRIPTOMIC REGULATOR

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Objectives: Several strategies have been studied in recent years to improve the activities related to the immune system in AD. The natural product mixture of curcuminoids, that improve certain defects in innate immune cells of AD patients, is of particular interest, as they may selectively enhance Abeta phagocytosis, attenuate APP maturation and alter gene transcription (Fiala et al.,2007; Gagliardi et al., 2012). Different compound have been tested and one of the most potent was bisdesmethoxycurcumin (BDC).
Methods: We analyzed 10 AD patients and 10 controls for phagocytic functions such as beta1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase (MGAT3) and vitamin D receptor (VDR) gene by Real Time PCR. PBMC from AD patients and controls have been treated with Abeta (5 ug/mL) + BDC (1nM). We also performed immunofluorescence experiments to morphologically evaluate the sub-cellular distribution and appearance of Abeta, in AD patients and controls untreated and treated with with Abeta and BDC.
Results: Our data showed that compared to controls, MGAT3 and VDR mRNA levels were up-regulated in lymphoblasts from AD patients treated with AB and BDC. Immunofluorescence of lymphoblasts showed the reduction of Abeta aggregates in AD patients treated with BDC compared to the samples not treated.
Conclusions: We demonstrated that BDC treatment may impact both gene expression and Abeta phagocytosis. MGAT3 is a gene essential for phagocytic functions and the overexpression of VDR suppressed amyloid precursor protein (APP) transcription in neuroblastoma cells (Wang et al., 2012). This preliminary data suggest a proof of concept for a future pharmacological intervention using curcumins.

02w. Cell, Molecular & Systems Biology: transcriptomics

ADPD5-1346

GENOME-WIDE TRANSCRIPTOME ANALYSIS IN OCTODON DEGUS: A NATURAL MODEL OF ALZHEIMER'S DISEASE

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Octodon degus, a small rodent endemic to Chile, has been found to naturally develop histopathological signs of Alzheimer's Disease: accumulation of beta-amyloid aggregates and hyper-phosphorylated tau protein, as well as cognitive decline in spatial memory and object recognition memory. The natural onset and development of neurodegeneration, without the need of genetic manipulation, validate O. degus as a suitable animal model for studying AD. The present work investigates the global gene expression profile of O. degus at the onset of the AD-like neuropathology. We developed a whole transcriptome analysis using RNA-sequencing on wild-type aged Octodon degus. To assess the quantity of soluble Aβ oligomers (Aβ42) we used MALDI-tof MS. For RNA-seq analysis, we used whole brain samples from both groups, according to their brain Aβ1-42 quantification. RNA-sequencing of paired-end libraries was done using ABI SOLID 5500xl system. We obtained an average throughput of 150 million reads per sample with 150X estimated depth coverage. Multi dimensional scaling plot were used to assess the biological coefficient of variation in the samples. Differential expressed genes according these criteria were analyzed using hierarchical clustering and Gene-Ontology (GO) enrichment analysis. Biological processes such us development and immune system response were enriched in GO-terms analysis. This work is the first genome-wide analysis in Octodon degus. We built a reference transcriptome for genetic O. degus research. The gene expression analysis reveals novel tentative biomarkers that could be useful for drug discovery in the fight to treat AD.

02w. Cell, Molecular & Systems Biology: transcriptomics

ADPD5-1728

GENETIC VARIANTS ASSOCIATED WITH ALZHEIMER'S DISEASE IN OCTODON DEGUS

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Octodon degus, a small south-american rodent endemic to Chile develops naturally aggregation of amyloid-β-peptides plaques and neuro fibrillary tangles containing hyperphosphorylated tau protein. These two AD features were also linked in O. degus with synaptic dysfunctions, spatial and object recognition memory loss and problems in activities of daily living. This natural AD animal model could represent one of the most promising tools for the study of AD at a pre-clinical level. While investigation with O. degus in the field of cognitive sciences is still in its early stages, we believe that O. degus provides an excellent opportunity for exploring biomarkers characterized in human patients. With this aim, we develop a whole-transcriptome analysis using RNA-sequencing in wild-type aged O. degus. Animals were analyzed at 3-years old by behavioural testing and for the presence of amyloid-β-peptides by MALDI-tof MS. RNA-seq data from AB SOLID 5500xl platform was processed for SNP discovery. For calling variants were used SAMtools and VCFtools. The criterion used was quality>30 (Phred scale) and read-coverage>10. We found 320109 SNPs that are only present in the aged O. degus AD onset. Focus in APP, PSEN1, PSEN2 and APOE genes, different SNPs in these genomic regions were identified only in the AD onset. This work provides a novel systematic characterization in Octodon degus of different SNP variants as a power tool for Alzheimer's research.

02w. Cell, Molecular & Systems Biology: transcriptomics

ADPD5-1735

DEREGULATION OF PURINE METABOLISM IN ALZHEIMER'S DISEASE

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Objectives

The neuroprotective role of adenosine and the deregulation of adenosine receptors in Alzheimer's disease (AD) have been extensively studied in recent years. However, little is known about the involvement of the purine metabolism in AD. The aim of our study is to identify possible alterations of the purine metabolism pathway in AD in human brain and to evaluate their functional effects by distinct "-omic" approaches.

Methods

First, gene expression was analysed in the entorhinal cortex of human controls and AD cases by whole-transcript expression arrays. Second, mRNA expression levels of twenty three purine metabolism genes was assessed by RT-qPCR in the entorhinal cortex, frontal cortex area 8 and precuneus of control samples and AD stages III-IV and V-VI of Braak and Braak, and controls. Finally, liquid-chromatography mass-spectrometry based metabolomics was performed in the entorhinal cortex.

Results

By whole-transcript expression arrays we identified differential levels of expression of a cluster of genes encoding enzymes involved in the purine metabolism pathway. RT-qPCR studies showed deregulation of *APRT, DGUOK, POLR3B, ENTPD3, AK5, NME1, NME3, NME5, NME7* and *ENTPD2* mRNAs, with regional and stage-dependent variations, in AD cases when compared to controls. Liquid-chromatography mass-spectrometry based metabolomics identified altered levels of dGMP, glycine, xanthosine, inosine diphosphate, guanine and deoxgyguanosine, all derived from this pathway.

Conclusions

Our results indicate stage- and region-dependent deregulation of purine metabolism in AD.

ADPD5-2242

IDENTIFYING THE EARLIEST MOLECULAR AND GENE EXPRESSION CHANGES ASSOCIATED WITH RISING AMYLOID-BETA IN TRANSGENIC MICE

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Objectives

By the time cognitive deficits are first detected in patients with Alzheimer's disease (AD) around 20% of the hippocampus has already been lost. Attempting to treat the disease at this late stage may be why clinical trials have not been successful. The objective of this study is to identify the earliest molecular changes that take place in mouse models of elevated Abeta, prior to deposition of plaques or neurodegeneration.

Methods

We use the Mouseac database (www.mouseac.org) that compares gene expression changes and histology, in the hippocampus, cortex and cerebellum at four ages, in four Amyloid mouse models, transgenic for familial mutations of *APP* and/or *PSEN1*. We use bioinformatics to identify the earliest genes/pathways altered during disease progression. In parallel, to detect the levels and species of soluble amyloid beta we use mass spectrometry, and to evaluate functional changes we use electrophysiology.

Results

Even before plaques develop in *APP/PSEN1* transgenic mice we detect differences in synaptic transmission in the hippocampus as early as 2 months of age compared to wild-type mice, concomitant with the rise of Abeta-42 levels. Our genome wide expression data and co-expression network analysis of the most varying genes reveals synaptic transmission also as the most significant gene module, and highlights several genes of interest that may provide novel targets, such as *Rims3* and *Atf5*.

Conclusions

Identifying the earliest changes that occur during the progression of Alzheimer's is essential for developing early interventions before neurodegeneration becomes irreversible.

02x. Cell, Molecular & Systems Biology: synaptic plastcity

ADPD5-1264

EFFECTS OF SAPP-ALPHA AND SAPP-BETA ON SYNAPTIC PLASTICITY IN PRIMARY HIPPOCAMPAL NEURONS AND HIPPOCAMPAL BRAIN SLICES

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Objectives:
In patients with Alzheimer's disease cerebral metabolism of amyloid precursor protein (APP) is altered affecting A-beta and potentially sAPP-alpha and/or sAPP-beta levels. So far studies have focused on A-beta or sAPP-alpha showing impairment or enhancement of synaptic plasticity, respectively. However, little is known about the function of sAPP-beta on synaptic plasticity. Here, we show a comparative study of sAPP-alpha and sAPP-beta regarding synaptic plasticity in hippocampal slices and primary hippocampal neurons on structural and physiological levels.
Methods:
In primary hippocampal neurons, LTP was induced chemically by repeated KCl treatment, intracellular Ca^{2+} levels were recorded simultaneously using a FLIPR system and the phosphorylation status of LTP dependent kinases was evaluated. Alterations of neuronal morphology after treatment with sAPP peptides were determined by Immunocytochemistry and fluorescence microscopy.
In parallel we evaluated the effects of the sAPP peptides on short-term and long-term plasticity in mouse hippocampal slices by paired-pulse facilitation and LTP.
Results:
Treatment of primary neurons with both sAPP peptides led to a significant increase in free intracellular Ca^{2+} concentrations after chemical LTP induction by repeated KCl treatment, indicating increased glutamate sensitivity. The morphometric analysis showed neurotrophic-like effects of sAPP-alpha and sAPP-beta treatment revealing a significant increase of dendrite numbers and branches of neurons.
Conclusion:
Results of these experiments reveal neurotrophic-like properties of both sAPP metabolites. This corroborates previous reports on sAPP-alpha. However, it also indicates a similar role of sAPP-beta on synaptic plasticity. Furthermore, results of sAPP peptides regarding functional plasticity by using hippocampal PPF and LTP will be discussed.

02x. Cell, Molecular & Systems Biology: synaptic plastcity

ADPD5-1868

CHOLINERGIC REGULATION OF HIPPOCAMPAL-DEPENDENT INFORMATION PROCESSING: IMPLICATIONS FOR ALZHEIMER'S DISEASE

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Pronounced degeneration in forebrain cholinergic neurons, which provide the major input to the cortex and hippocampus, is thought to underlie some of the cognitive and behavioural symptoms observed in Alzheimer's disease. Distinct hippocampal-dependent tasks recruit specific hippocampal circuitries, but whether cholinergic tone is involved in all modes of hippocampal information processing is unknown. Here we used mice with genetic and viral deletion of the vesicular acetylcholine transporter (VAChT), a critical protein required for acetylcholine (ACh) storage and release, to investigate the role of ACh in hippocampal-dependent information processing. Deletion of VAChT in forebrain neurons, or only on neurons projecting to the hippocampus, affected the performance of mice in several hippocampal-dependent tasks, including the spatial version of the Morris Water maze, tests of working memory and a touchscreen paired-associates learning task (currently utilized to identify individuals at high risk for developing AD). Mice with genetic elimination of VAChT were not able to learn the paired-associates task and the performance of mice with viral deletion of VAChT was correlated to hippocampal VAChT protein levels. These results suggest that cholinergic integrity is vital for acquisition in paired-associates learning. Spatial memory in the Morris water maze was only mildly affected in mice with reduced hippocampal levels of VAChT; in contrast, reversal learning was severely disturbed. Additionally, working memory was also compromised in mice with reduced levels of VAChT. These results provide a refined roadmap of how cholinergic signaling controls encoding and recall of information in tasks that recruit distinct hippocampal–dependent processes.

02y. Cell, Molecular & Systems Biology: modeling of disease progression

ADPD5-0391

ALZHEIMER'S DISEASE PROGRESSION PREDICTION ANALYSIS PLATFORM WITH EEG SIGNALS FROM NORMAL TO ALZHEIMER'S DISEASE PATIENTS

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Introduction

To investigate the possibility of progression from Mild cognitive impairment (MCI) to Alzheimer's disease (AD), we analyzed Electroencephalography (EEG) signal of patients diagnosed with AD and normal people. Previous studies have shown that multiple EEG biomarkers can predict conversion rate from MCI to AD with relatively low sensitivity (88%) and specificity (82%) (Poil, De Haan et al. 2013; Claudio, Francesco et al. 2014). By using real-time processing and multi-channel (32ch) EEG data, the progression rate from MCI to AD was predicted.

Methods

We developed time series-based machine learning algorithms to classify normal participants and AD. We applied support vector machine classifier with a non-linear chi-square kernel to make the final recognition. For evaluation, half/half random split validation setting was applied. Our dataset consists of brain EEG data of 140 subjects (AD and normal control). At each round, we randomly selected 70 samples (per class) as training data and 70 for testing. This random training/testing selection was repeated 100 times and their mean classification accuracy was computed.

Results

Our experiment confirmed that we are able to obtain reliable recognition performance: our approach showed 96.09% classification accuracy using 32-EEG-sensors attached subject's head. The performance was still reasonable (81.4586%) even with only 6 EEG sensors.

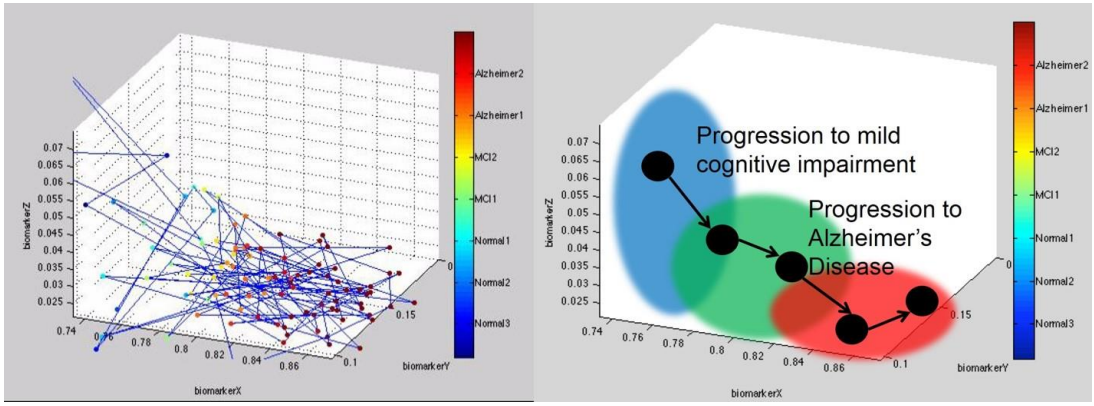

Figure 1. Predict progression result for normal, MCI and AD by EEG data

Conclusion

We performed the classification for diagnostics and prediction of AD progression with EEG data. These results imply that the prediction algorithm is sufficient for constructing big data platform to predict Alzheimer's disease progression.

02y. Cell, Molecular & Systems Biology: modeling of disease progression

ADPD5-0770

PROGRESSION OF ALZHEIMER'S DISEASE IN THE HUMAN HIPPOCAMPUS - NEW INSIGHTS AND PROSPECTS

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Alzheimer's Disease (AD) might be more complex than known so far. Regarding the last years of scientific research we conclude that the progression of AD in the human hippocampus is one of the key hallmarks of this neurodegenerative disease. Braak et al. (2000) observed that neuronal damage of the different regions of the human hippocampus (CA1, CA2, CA3, fascia dentata) occurs in a time dependent matter. Therefore we decided to analyse the content of the human hippocampal regions of interest by performing a differential proteomic study (label free LC-MS/MS approach) combined with a couple of functional analyses. Here we used the Laser-microdissection technique to separate the human hippocampal regions of interest by using 6 biological post mortem replicates. Afterwards we quantified the hippocampal proteoms and confirmed our findings with immunohistochemistry. Here we show new insights and prospects for the progression of AD in the human hippocampus by presenting novel candidate proteins.

02y. Cell, Molecular & Systems Biology: modeling of disease progression

ADPD5-1654

EFFECT OF BETA AMYLOID PEPTIDE ON CA1 PYRAMIDAL NEURON: IONIC CONDUCTANCE BASED MATHEMATICAL MODEL STUDY FOR POSSIBLE TREATMENT

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Evidence from a number of independent studies has demonstrated that generation of pathogenic β- amyloid (Aβ) peptides, one of the characteristic hallmarks of Alzheimer's disease (AD), can affect normal neuronal activity in various ways. In present scenario, the impact of Aβ amyloid peptide is not clearly understood. From ion channel hypothesis, we can assume that Aβ can affect the normal activity of a neuron; for example, making a neuron more excitable (by reducing the *A*-or *DR*- type K^+ currents) or less excitable (by reducing synaptic transmission and Na^+current). The aim is to establish a computational model with sufficient biophysical detail to quantitatively simulate CA1 cell electrical activities related to Aβ peptide and thereby inform future empirical investigations of physiological and pathophysiological mechanisms governing AD. In line with recent experimental evidence, we construct mathematical models for Na^+ current, voltage gated *DR*- type K^+ currents and synaptic current. The magnitudes and kinetics of each ionic current system in a borrowed CA1 cell with a specified surface area are described by differential equations, in terms of maximal conductances, electro chemical gradients and voltage-dependent activation/inactivation gating variables.

We have first modeled the different stages of AD by progressively modifying different active channels and synaptic properties of a realistic model neuron, where all parameters are adapted from experimental findings. We then tested sodium and potassium channel manipulations that could compensate for the effects of Aβ. The model points to possible pharmacological interventions of sodium and potassium ion channels in term of kinetic and activation properties.

02z. Cell, Molecular & Systems Biology: other

ADPD5-1222

IMPAIRED REELIN/APOER2 SIGNALING PATHWAY IN ALZHEIMER'S DISEASE

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Background: Reelin is a signaling protein that has a crucial role in synaptic function and plasticity, and also controls tau phosphorylation. Binding of homodimers of Reelin, the active form, to its receptor, ApoER2, relays the signal into the cell. We have previously demonstrated that β-amyloid peptide (Aβ) alters Reelin expression and its glycosylation pattern. An altered Reelin signaling might affect progression of Alzheimer's disease (AD) pathology. However, there is no consensus on whether Reelin levels are increased or decreased in brain regions affected by AD.
Methods: Reelin and Aβ interaction was assessed by co-immunoprecipitation. Reelin levels in AD and non-disease brain extracts were analyzed by western blotting and qRT-PCR. Presence of Reelin was also analyzed in pellets from cortex solubilized with guanidine. Reelin heteromers were characterized by sucrose density gradient. ApoER2 interaction with Reelin was measured by immunoprecipitation, and the presence of a soluble ApoER2 fragment in cerebrospinal fluid (CSF) by immunoblotting.
Results: We show that Reelin interacts with β-amyloid in brain extracts. Reelin increases at transcriptional level in AD brain extracts, together with accumulation of the Reelin protein associated to insoluble (guanidine-extractable) β-amyloid deposits. Reelin from AD cortex forms large complexes instead of homodimers and displays reduced affinity for ApoER2. Finally, the soluble ApoER2 fragment generated after Reelin binding, is found at lower levels in AD CSF respect to non-dementia samples.
Conclusions: Reelin levels trend to increase in brain from AD subjects, but Aβ compromises its binding to the receptor and its biological function resulting in impaired Reelin signaling.

02z. Cell, Molecular & Systems Biology: other

ADPD5-1272

DOWN SYNDROME BRAINS REVEAL CRITICAL CHANGES IN THE TEMPORAL AND SPATIAL EXPRESSION OF SINGLE GABAA RECEPTOR SUBUNITS DURING DEVELOPMENT

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Objectives: The Down syndrome (DS), the most common congenital cause of mental retardation, is characterized by a disturbed proliferation and migration of neurons and glia cells. Previous studies in animal models suggested that some $GABA_A$ receptor ($GABA_A$-R) subunits might play a pivotal role in the pathogenesis of DS. Thus, our aim was to investigate the most abundant $GABA_A$-R subunits (alpha1-3 and gamma2) in developing human DS brains, as they influence cell proliferation, migration, differentiation, and cell death during development.
Methods: The amount of immunoreactivity was quantified on digitalized coronal brain sections for each of four $GABA_A$-R subunits (alpha1-3 and gamma2) in the hippocampus (CA1 and subiculum) and in cortical structures (marginal zone and intermediate zone) of 30 foetal human DS cases and was compared to age-matched control brains (from 14 GW until 3 months of the early postnatal period).
Results: Here we provide the first detailed immunohistochemical investigation of $GABA_A$-R subunits in human DS brain tissue. Our data reveal spatial and temporal differences in the expression patterns of single subunits in DS cases: 1) alpha1, alpha2, alpha3 and gamma2 subunits showed selective depletion in hippocampal regions; 2) the pattern of changes of the alpha2 subunit in the hippocampus resembled strongly those of the gamma2 subunit; 3) in the DS cortex the change in temporal expression and/or amount was layer-specific.
Conclusions: Our data strongly imply complex involvement of the GABAergic system in the pathogenesis of DS in humans, extending the assumption from animal models and opening new conceptual possibilities.

02z. Cell, Molecular & Systems Biology: other

ADPD5-1463

BRI2 ECTODOMAIN INDUCES APOPTOSIS AND TAU TRUNCATION IN HUMAN NEUROBLASTOMA CELLS.

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Introduction

Alzheimer's disease (AD) is characterized by protein aggregates such as amyloid beta (Aβ) in plaques and hyperphosphorilated tau (p-tau) in neurofibrillary tangles (NFT). BRI2 regulates critical proteins involved in initial steps of the amyloid cascade hypothesis. We found aggregated BRI2 ectodomain associated with Aβ plaques in early stages of AD. Here we investigated the effects of aggregated recombinant BRI2 ectodomain ($rBRI2_{76-266}$) on important molecular pathways involved in early stages of AD, including apoptosis, the unfolded protein response (UPR), and the phosphorylation of glycogen synthase kinase 3 β (GSK3β) and tau.

Methods

Non-differentiated SH-SY5Y cells were exposed to $rBRI2_{76-266}$. Cell viability was assed by MTT assay. Apoptosis was examined by analysis of caspases 3 and 9 activities. Pro- and anti-apoptotic proteins Bcl-2 and Bax, truncated tau and the phosphorylation of GSK3β (P-GSK3β) were analysed by western blot. The mRNA of UPR related proteins (BiP, CHOP and Xbp-1) was analysed by qPCR and the levels of p-tau were determined using ELISA.

Results

$BRI2_{76-266}$ led to a 10% cell death, increased Bax/Bcl-2 ratio and increased activity of caspases 3 and 9, indicating an activation of the apoptosis pathway. UPR mRNA markers were not modified after incubation with $BRI2_{76-266}$. Incubation with BRI2 increased the levels of p-GSK3β and truncated tau.

Conclusions

Our results indicate that $rBRI2_{76-266,}$ which is prone to aggregate, can induce apoptosis and tau truncation. Since BRI2 is able to regulate APP processing and Aβ load, we propose that aggregated BRI2 ectodomain is a potential nexus between Aβ, tau pathology and neurodegeneration.

02z. Cell, Molecular & Systems Biology: other

ADPD5-1469

ALTERED GENE TRANSCRIPTION IN ALZHEIMER'S DISEASE TRHOUGH NFAT ACTIVATION

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by a progressive loss of synapses and neurons, especially in the hippocampus region. An important feature of AD is the presence of amyloid plaques, found in the extracellular region of patients' brains, composed mostly of the peptide beta-amyloid (Aβ). Exposed neurons to Aβ display higher levels of intracellular calcium, which could lead to the activation calcineurin A, a phosphatase that is able to regulate the transcription factor NFAT. Inhibition of NFAT leads to a prevention of both spine loss and dendritic simplification, even though it is not known which genes could have been regulated. The primary objective of the project is to define the molecular mechanisms of gene control by the transcription factor NFAT that may be involved in the AD. We already demonstrated that mice primary neuron culture treated with Aβ oligomers display NFAT translocation to the nucleus that can be completely reversed by ciclosporin A (CsA), an inhibitory drug of the NFAT activation pathway. We also have evidence that these primary cultures treated with Aβ peptides have a smaller amount of dendritic spines and pre-synaptic terminals (in relation to the post-synaptic terminals), suggesting a decrease of synapses, which is also reversed by CsA. Based on these results, we can assume that Aβ peptides are promoting a decrease of spines and synapses through activation of the calcineurin/NFAT pathway. We now seek to investigated genes that may be regulated by NFAT and are involved in the process of neurodegeneration in AD.

02z. Cell, Molecular & Systems Biology: other

ADPD5-1775

INHIBITION OF GSK3BETA ACTIVATION AND ABETA SECRETION BY PHARMACOLOGICAL MODULATION OF SPHINGOLIPID METABOLISM OCCUR INDEPENDENTLY OF GANGLIOSIDES DISTURBANCE IN A CELLULAR MODEL OF AD.

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Accumulating evidence implicate gangliosides and/or related-sphingolipids in the pathogenesis of Alzheimer's disease (AD). While gangliosides are thought to interfere with the proteolytic processing of the amyloid precursor protein (APP), it is not known whether a connection between these lipids and other important AD features such as deregulated insulin/Akt/GSK3 signaling occurs. Here, we analyzed the impact of aberrant gangliosides composition on GSK3 activation state and amyloid β peptide (Aβ) production in neuroglioma cells expressing the double Swedish mutation of human APP (H4APPsw) treated with several glycosphingolipid (GSL)-modulating agents. We found that both ceramide analogs D- and L-PDMP (1-phenyl 2-decanoylamino-3-morpholino-1-propanol), which have opposite effects on ganglioside synthesis, inhibited selectively GSK3β *via* Ser9 phosphorylation and reduced Aβ secretion, independently of the upstream insulin/Akt pathway. Concurrently, these two compounds strongly reduced the levels of long-chain ceramides. Moreover, the iminosugar N-butyldeoxynojirimycin (NB-DNJ) which also reduced cellular gangliosides levels but not that of ceramides, did not affect the phosphorylation state of GSK3β, but was able to reduce Aβ production in a different way from that of PDMP agents.
In sum, our data suggest that the regulation of both GSK3β activation state and APP processing might be independent of the altered cellular gangliosides composition, but rather implicated changes in ceramide levels. Nevertheless, this study provides novel information regarding the possibilities to target GSK3β and amyloidogenic processing of APP through modulation of sphingolipid metabolism. Furthermore, the anti-amyloidogenic effects of NB-DNJ reported here for the first time emphasizes a new potential therapeutic approach for AD.

02z. Cell, Molecular & Systems Biology: other

ADPD5-2299

PRIORITISING A SYSTEMS BIOLOGY APPROACH TO ALZHEIMER'S DISEASE

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To better understand the mechanisms of Alzheimer's disease (AD) conventional experimental methodologies have commonly targeted individual, or small sets of genes/proteins. However, the strong message from computational systems biology (CSB) approaches in other applications, such as cancer, indicate control is distributed across the cell network and rarely restricted to a single point. Successful drug interventions are therefore likely to be multi-targeted, meaning an unguided search for new treatments faces an almost infinite combination of possibilities. It is timely to consider exploiting CSB to decipher the true disease complexity.

Despite the earliest successes of CSB being in neuroscience, the AD field now lags significantly behind other disciplines, maybe partly due to the overwhelming challenge - clearly AD must be confronted from the outset as a multi-cellular system, which significantly adds to the complexity of analysis.

Identifying priority targets from an overwhelming choice is daunting, however computational approaches enhance intuition and empirical measurement, and can assist identification. We believe researchers are ready to embrace the opportunities of CSB with the abundance of data and a converging consensus of the role of amyloid-beta in the development of AD.

To create a roadmap for AD, we present key studies demonstrating the value of CSB. We establish a path to begin moving from overwhelming complexity to an iterative scheme of hypothesis generation, prediction and validation to identify the key elements of the interaction network implicated in AD. We finish by outlining how this knowledge could be used to help identify combination therapies to enable personalised treatment.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1215

RESVERATROL PROTECT RAT BRAIN FROM AGE-ASSOCIATED SEQUENTIAL PATHOLOGICAL CHANGES IN SPECIFIC HIPPOCAMPAL NEURONAL CIRCUITS.

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Background/Objective: Present study aims at investigating, age-specific degeneration of neuronal-circuits in hippocampal formation *(neural-layout of Subiculum-hippocampus proper-DG-EC)* and resultant cognitive impairment during aging that forms major risk factor for SAD like pathology. Further, neuroprotective effect of resveratrol on age-specific pathological changes in the hippocampal neuronal-circuits were evaluated. **Methods:**Radial-Arm-Maze (RAM) was performed to evaluate hippocampal-dependent spatial and learning memory. Nissl Staining of FC, subiculum, Hippocampal-proper (CA1→CA2→CA3→CA4), DG, amygdala, cerebellum, thalamus, hypothalamus, layers of temporal and parietal lobe of the Neocortex were examined for pathological changes in young and aged wistar rats, with and without resveratrol.**Results and Conclusion:** Extensive loss of glutamatergic and inhibitory GABAergic neurons were observed during aging. Moreover, Trisynaptic (EC layerII→DG→CA3→CA1) circuit forming new memory and monosynaptic circuit (EC→CA1) that strengthens old memories was disturbed. Loss of Granular neuron in DG and polymorphic cells of CA4 lead to decreased mossy fibers disturbing neural-transmission (CA4→CA3) in perforant pathway. Further, intensity of Nissl granules (SLM-SR-SO) of CA3 pyramidal neurons was decreased, disturbing the communication in Schaffer collaterals (CA3-CA1) during aging. We also noticed disarranged neuronal cell layer in Subiculum (PrS-PaS), interfering output from hippocampus to PFC, EC, Hypothalamus, and amygdala that interrupt thinking process. We conclude from our observations that neuronal-circuits of hippocampus (DG-CA4-CA1-Sub) were damaged leading to memory impairment that was supported by RAM analysis. Interestingly, resveratrol induced neurogenesis in DG and culminated the pathological events proving as good as therapeutic drugs against age associated neurodegeneration and memory loss.

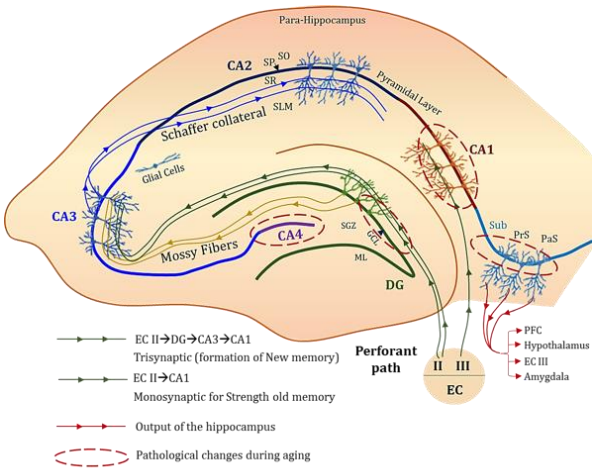

Figure:- A schematic representation of Age-associated sequential pathological changes in Hippocampal Circuits: CA, Cornu ammonis; DG, Dentate gyrus; SUB, subiculum; PrS, Presubiculum; PaS, Parasubiculum; GCL, granule cell layer; ML, molecular layer; SO, stratum oriens; SR, stratum radiatum, SP, Stratum pyramidal; SLM, Stratum lacunosum moleculare; EC, Entorhinal cortex; SGZ, Subgranular Zone

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1235

MODELING PRION-LIKE PROPAGATION OF NEURODEGENERATIVE DISEASE RELATED PROTEINS IN A IPSC DERIVED NEUROEPITHELIAL STEM CELL 3D CO-CULTURE SYSTEM

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Objectives: Establishment of a novel iPSC-based model for the investigation of neuron-to-neuron transmission of neurodegenerative disease related proteins.
Methods: Using a well characterized iPSC derived neuroepithelial stem cell (NES) line, neuronally differentiated cells were co-cultured in a 3D extracellular matrix (ECM) gel. Alexa-Fluor 700 conjugated amyloid-beta oligomers (oAb) was fed to neurons and subsequently taken up by these cells. Amyloid-beta loaded cells were then seeded onto a mixture of ECM gel with embedded GFP-labeled differentiated neuronal cells in standard lab plastics, or in microfluidically isolated (Xona Microfluidics) chamber slides.
Results: We have demonstrated the ability to track the spread of labeled oAb between mature iPSC derived neurons in vitro. This interneuronal transfer can be visualized with confocal microscopy in real time, as well as separated by FACS for downstream biochemical analysis. Additionally, the cells can be investigated with electrophysiological techniques. In this way, we have developed a powerful tool which can be used to investigate the immediate and long term effects of transfer of oAb from loaded neurons to healthy acceptor cells. This approach has also been applied to microfluidically isolated slides, allowing for the visualization of protein transfer between two distinctly isolated populations. Fluidic isolation allowed for investigation of different endocytic inhibitors (Latrunculin B, Dynasore) in their ability to diminish neuron-to-neuron propagation.
Conclusions: We have developed two new approaches to 3D co-culture modeling applicable to the investigation of neuron-to-neuron propagation of neurodegenerative related proteins using iPSCs. These models could be used to further understand potential mechanisms of protein transfer.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1300

AN INDUCED PLURIPOTENT STEM CELL MODEL FOR PHENOTYPIC SCREENING FOR INHIBITORS OF PRION-LIKE PROPAGATION OF BETA AMYLOID OLIGOMERS

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Objectives: The pathogenic progression of Alzheimer's disease spreads through interconnected brain areas causing neurodegeneration. Misfolded beta amyloid (Abeta) oligomer aggregates have been shown to propagate between neurons and act as seeds, recruiting new Abeta into the neurotoxic misfolded pool. One therapeutic strategy could be to decrease or stop this transfer of Abeta oligomers. We have previously shown that differentiated neuronal cells cultured in vitro are able to transfer Abeta between them. The aim of this study was to optimize a co-culture system with human induced pluripotent stem cells (iPSC) for high throughput screening of chemical libraries. This could potentially find compounds with an inhibitory effect on neuron-to-neuron Abeta transfer and the progression of Alzheimer's disease.
Methods: The acceptor subpopulation of neuronally differentiated iPS cells labeled with GFP is cultured on a layer of ECM gel in microtiter plates. Through endocytosis the donor subpopulation is loaded with Alexa-700-labelled Abeta oligomers, and subsequently seeded on top of the acceptor cells. The cells are co-cultured, and co-localization of GFP signal (acceptor cells) and Alexa700 (Abeta) is assessed. The possibility of automation of the different cell-culture steps was evaluated, as well as automated image acquisition and quantification.
Results: Neuron-to-neuron transfer of oligomeric Abeta was shown in the iPSC co-culture system. The co-culture system could be optimized for semi-automated handling. This system could thus be suitable for high throughput analysis.
Conclusions: We have developed a human iPSC based model of neuron-to-neuron Abeta oligomer transfer that can be used for phenotypic high throughput screening.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1375

AMYLOID-BETA ASSOCIATED BRAIN-DERIVED EXOSOMES PROMOTE AMYLOID SEEDING IN A MOUSE MODEL OF AMYLOIDOSIS

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Objective: Exosomes, bioactive secreted vesicles of endocytic origin, transport APP and APP metabolites into the extracellular space. The content of exosomes is protected from degradation due to the high stability of exosomal vesicles. We have previously shown that exosomes isolated from APP overexpressing Tg2576 mouse brains are enriched with the neurotoxic APP-C-terminal fragments (APP-CTFs), cleavage of which can result in the production of amyloid-beta (Abeta). Thus, exosomes are potentially capable of generating Abeta at sites distant from the cell that secreted the exosomes. Additionally, Abeta binds to the surface of the lipid-rich exosomal membrane, potentially forming a seeding site for amyloid. We investigated the ability of exosomes isolated from the brain of amyloid-depositing mice to induce Abeta accumulation in the brain of pre-depositing APP-overexpressing Tg2576 mice.
Methods: Brain exosomes from aged Tg2576 mice were isolated by a novel method developed in our laboratory, and injected into the hippocampus of pre-depositing Tg2576 mice. Injection of wild-type brain exosomes was used as control. Brains were analyzed three months post-administration for amyloid burden by thioflavin S staining and immunostaining with an anti-Abeta antibody.
Results: Greater amyloid pathology was observed in the hippocampus and adjacent cortex of Tg2576 mice administered with exogenous transgenic exosomes as compared to mice treated with wild-type exosomes.
Conclusions: This *in vivo* study demonstrates that brain-derived exosomes enriched with APP-CTFs and containing exosomal-surface Abeta can contribute to amyloid seeding in the brain.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1439

EXOSOSMES CAN TRANSFER OLIGOMERIC AMYLOID BETA FROM NEURON TO NEURON, CONTRIBUTING TO THE PROGRESS OF ALZHEIMER'S DISEASE

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Objectives:
Progressive accumulation of specific protein aggregates, is a defining feature of many major neurodegenerative diseases, including Alzheimer's disease (AD). Recent studies have suggested prion-like propagation of neurodegenerative proteins such as amyloid beta (Abeta) oligomers through the brain, thereby contributing to the development of the illness. The cellular mechanisms behind this transfer are not known. Recent reports suggest that secretory small vesicles called exosomes can contain normal Abeta. The objective is to investigate the involvement of exosomes, in the neuron-to-neuron transfer of the neurotoxic Abeta oligomers using a 3D co-culture system with neuronally differentiated human SH-SY5Y cells and induced pluripotent stem cells (iPSC).
Methods and Results:
Confocal microscopy of cells with fluorescent labeled oligomeric amyloid beta (oAbeta) and co-labeled with exosomal proteins (e.g. Flotillin-1, Alix) showed that Abeta aggregates were present in intracellular exosomes. Characterization and presence of Abeta in secretary exosomes isolated from conditioned media was confirmed by immunoblotting, EM, NTA and spectrofluorometer. In addition, these exosomes could later be fed to new cells, which internalized the oAbeta, showing that oAbeta can be transmitted from neuron-to-neuron via exosomes. Interestingly, the cellular uptake of oAbeta containing exosomes could be inhibited using dynasore, an inhibitor of dynamin dependent endocytosis.
Conclusion:
In conclusion, this study indicates that oAbeta transfers from neuron-to-neuron via exosomes and that the uptake is dynamin dependent. The results have important implications in understanding the disease propagation in AD pathogenesis.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1451

IMPACT OF EXCITATION ON NEURON-TO-NEURON TRANSFER OF BETA AMYLOID OLIGOMERS IN HUMAN NEURONAL PROGENITOR CELLS

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OBJECTIVE: The pathogenic progression of Alzheimer's disease spreads through interconnected brain areas causing neurodegeneration. Misfolded beta amyloid oligomer aggregates have been shown to propagate between neurons and act as seeds, recruiting new beta amyloid into the neurotoxic misfolded pool. The objective of this study is to investigate the effect of electrophysiologically and pharmacologically excitation on the neuron-to-neuron transfer of beta amyloid oligomer in differentiated human neuronal progenitor cells (HNPCs).
METHODS: HNPCs were differentiated with bFGF, EGF, LIF, and supplemented with N2. Differentiated cells and neuronal networks were characterized for neuronal markers (beta-III tubulin, Sv2) and assessed by two independent techniques, calcium imaging and basic electrophysiology tools such as voltage and current clamp in whole cell configuration.
RESULTS: Differentiated HNPCs express the neuronal marker beta-III tubulin and can trigger calcium responses spontaneously as well as in presence of acetylcholine. Spontaneous extracellular postsynaptic currents at -70 mV were detected in HNPC-derived neurons. The effect of electrophysiologically and pharmacologically excitation on the neuron-to-neuron transmission fluorophore labeled beta amyloid oligomers are currently investigated.
CONCLUSIONS: HNPCs can be differentiated into neurons that create neuronal network with intrinsic electrical activity. This activity could be mediated by acetylcholine and glutamate acting on nACh receptors and AMPA receptors, respectively. We propose that HNPC-derived neurons could help to understand the underlying mechanism of amyloid beta transference and the synaptic process that affects its propagation.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1751

CHOLINERGIC DYSFUNCTION OF BASAL FOREBRAIN AND MESOPONTINE TEGMENTUM IN ALZHEIMER'S DISEASE

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Cholinergic neurons of the basal forebrain are particularly vulnerable in Alzheimer's disease, and the consequent cholinergic neurotransmitter decline affects other neurotransmitter systems. Epidemiological studies have shown that sleep apnoea - stopping breathing during sleep is a risk factor for Alzheimer's disease. The neurons of another major cholinergic nucleus in the brain, the mesopontine tegmentum (MPT), project to upper motor neurons to control upper airway muscle tone during sleep, and are also implicated in initiating and maintaining rapid eye movement (REM) sleep, which is considered fundamental for learning consolidation and retention of memory. MPT neurons also project to the basal forebrain and produce nerve growth factor (NGF) and thus may support basal forebrain neuronal survival and function throughout life. We found that lesions of MPT cholinergic neurons by stereotaxic injection of saporin toxin conjugated to the specific urotensin II receptor peptide ligand produce a subsequent and selective degeneration of basal forebrain cholinergic neurons and a resultant decline in spatial memory. Our results indicate that loss of MPT-derived NGF due to MPT neuronal dysfunction may in turn cause dysfunction of basal forebrain neurons with negative flow-on effects on cognitive function and the development of Alzheimer's disease.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-2114

A MALFUNCTIONING Aß CLEARANCE SYSTEM AS A MAJOR FACTOR ASSOCIATED WITH ALZHEIMER'S DISEASE

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Objectives
Mechanisms underlying intra-brain misfolded proteins (MP) propagation/deposition remain essentially uncharacterized. Here, we aimed to a) create a biophysical model capable of describe and reproduce intra-brain spatiotemporal patterns of MP propagation/deposition, and b) clarify the effects of MP production, clearance and onset age on disease progression.
Methods
We proposed a stochastic epidemic spreading model (ESM) for spatiotemporal MP dynamics that considers propagation-like interactions between MP agents and the brain's clearance response, across the structural connectome. The validity/applicability of the proposed model was explored using 733 individual PET amyloid-ß (Aß) datasets from the Alzheimer´s Disease Neuroimaging Initiative (ADNI).
Results
The results suggest that it is not an increased Aß production but mainly a deficit in Aß clearance processes and an early Aß onset age that result in the formation of an excessive Aß deposition pattern. The malfunctioning Aß clearance system was also found significantly more related to the AD progression than Aß production. Additionally, our results highlight the strategic role of the MP outbreak regions and their anatomical connectional architecture on the disease's temporal progression, as well as the impact of individual genetic and demographic properties on intra-brain Aß propagation and deposition.
Conclusions
The proposed ESM allows for reconstructing individual lifetime histories of intra-brain MP propagation/deposition, and the analysis of factors that promote such propagation/deposition (e.g., MP production and clearance). The growing body of evidence supporting a reduced Aβ clearance in AD development, in line with our results, could imply a turning point for associated therapeutic mitigation strategies.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0464

MASS SPECTROMETRIC PROFILING OF ENDOGENOUS PEPTIDES DERIVED FROM THE POST-SYNAPTIC PROTEIN NEUROGRANIN IN PLASMA

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Objective: Neurogranin (Ng) is a 78aa 7618 Da soluble post-synaptic protein with high expression in particular brain regions, including cortex and hippocampus. We have previously shown that elevated cerebrospinal fluid levels of Ng clearly separates both Alzheimer's disease (AD) patients ($p<0.001$) and individuals that progress from mild cognitive impairment to AD ($p<0.001$) from controls. The specific peptide Ng48-76 was also found to be significantly increased in AD ($p=0.002$). In the present study, the objective was to characterize the Ng peptide profile in human plasma.

Methods: Plasma samples from healthy controls (n=37) and AD patients (n=38) were analysed by immunoprecipitation (IP) followed by MALDI-TOF/TOF mass spectrometry (MS). The IP was performed using in-house-generated monoclonal Ng antibodies targeting the C-terminal region of Ng. Endogenous plasma Ng levels were determined in parallel by MS by adding an isotopic labelled Ng peptide to the plasma prior any sample preparation and by an immunochemical method on Meso Scale Discovery platform.

Results: Mass spectrometric characterization of plasma Ng revealed that a variety of endogenous Ng peptides, such as Ng38-75 and Ng44-78, as well as full length protein are reproducibly detected in both controls and AD individuals. Plasma Ng concentrations were similar in AD patients and control individuals.

Conclusions: Plasma Ng is metabolized into several shorter endogenous peptides which can be repeatedly detected and quantified. Although total Ng concentration did not differ between AD patients and control individuals, some of the Ng fragments may have new biomarker potential, which will be examined in future studies on paired plasma and CSF samples.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0688

INVOLVEMENT OF NMDA RECEPTORS IN ABETA TOXICITY

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Objectives

Aβ oligomers correlate with the memory decline observed in Alzheimer's disease patients. N-methyl-D-aspartate receptors (NMDAR) are glutamatergic receptors that play an important role in learning and memory, but also in excitotoxic processes. Several studies have shown a link between NMDARs and Aβ toxicity. Most of these studies have been performed in neurons from young mice. However, NMDAR subunits show a change in their expression patterns during development. Hence, it is important to investigate the role of NMDAR in Aβ toxicity in adult mice.

Methods

To investigate the role of NMDARs in Aβ toxicity we overproduced Aβ by a virus-mediated approach in brains of mice with conditional knockout of NMDAR subunits. NMDARs were deleted by virus mediated Cre-recombinase expression in adult mice, in which NMDAR genes are flanked by *loxP*-sites (GluN1fl/fl and GluN2Bfl/fl). Aβ toxicity was assessed by electrophysiological and morphological analyses in virus infected dentate gyrus granule cells of adult mice.

Results

3 weeks after virus-mediated Aβ overproduction, we observed a reduction in miniature excitatory post-synaptic current (mEPSC) frequency without morphological abnormalities (e.g. no change in spine number). Deletion of GluN1 (i.e. absence of all NMDARs) or the GluN2B averted the Aβ-mediated decrease in mEPSCs.

Conclusions

Our work demonstrates the importance of NMDARs and more specifically of GluN2B-containing NMDARs in the context of Aβ toxicity in adult mice. The fact that Aβ overproduction reduces mEPSC frequency without affecting spine number indicates that an initial alteration in Aβ toxicity is a reduction in functional synapses.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0720

CALCIUM-FLUX-INDEPENDENT NMDA RECEPTOR ACTIVITY IS REQUIRED FOR ABETA OLIGOMER-INDUCED SYNAPTIC LOSS

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Synaptic loss is one of the major features of Alzheimer's disease (AD) and correlates well with the degree of dementia. NMDA receptors (NMDARs) have been shown to mediate downstream effects of beta-amyloid (Aβ). NMDARs can trigger intracellular cascades via Ca^{2+} entry, however also Ca^{2+}-independent (metabotropic) functions of NMDARs have been described. Here we show that transgenically produced Aβ as well as exogenously added synthetic Aβ oligomers induce dendritic spine loss and reductions in pre- and postsynaptic protein levels in slice cultures. Synaptic alterations were mitigated by blocking glutamate-binding to NMDARs using NMDAR-antagonist APV, but not by preventing ion-flux with Ca^{2+}-chelator BAPTA or open channel blocker MK-801 or memantine. Spine loss was mediated by active p38 MAPK. Aβ-induced p38 activation was reduced by APV but not by BAPTA, MK-801 or memantine treatment highlighting the role of glutamate binding to NMDAR but not Ca^{2+}-flux for synaptic degeneration. Our data suggest that Aβ-induced synaptic loss is signaled through metabotropic-like activation of NMDARs. This Ca^{2+}-independent NMDAR signaling may represent a therapeutic target for drugs designed to reduce synaptic deficits in AD.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0815

AFFINITY PROTEOMICS AS A TOOL TO CHARACTERIZE AND QUANTITATE SYNAPTIC PROTEINS IN BRAIN TISSUE AND CEREBROSPINAL FLUID

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Objectives
Our objective is to identify cerebrospinal fluid (CSF) biomarkers of synaptic damage in neurodegenerative diseases. Synaptic loss occurs early in e.g. Alzheimer's disease, and measurements of altered synaptic function could be important to monitor disease progression and drug effects. The SNARE complex regulates synaptic neurotransmitter release and changed expression of SNARE proteins (SNAP-25, syntaxin-1 and VAMP) has been found to alter synaptic function. In this study we developed an affinity proteomics approach to identify and characterize forms of SNAP-25 in post-mortem brain tissue and CSF.
Methods
SNAP-25 was immunopurified from homogenized and biochemically fractionated human brain tissue (15 AD and 15 controls) or CSF samples and trypsinized. Stable isotope labeled peptide standards were added and the mixtures were analyzed by liquid chromatography-mass spectrometry (LC-MS). A top-down MS approach was utilized to identify novel modified forms of soluble SNAP-25. Quantification was performed either by selected reaction monitoring (SRM) MS or by high resolution selected ion monitoring (HR-SIM) MS.
Results
We report a new strategy to study synaptic pathology by combining affinity purification and proteomics to characterize and quantitate SNAP-25 in brain tissue and CSF. Novel, soluble forms of SNAP-25 were identified in brain tissue and characterized by the same method. Soluble SNAP-25 were also detected and quantitated in CSF samples from individual patients.
Conclusions
The strategy we present make it possible to compare levels of SNAP-25 in individual patient CSF samples. This could be important for earlier diagnosis, assessment of disease progression, and to monitor drug effects in treatment trials.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0845

TARGETED APP EXPRESSION IN HIPPOCAMPUS DEMONSTRATES THAT SYNAPSES POSTSYNAPTIC TO NEURONS EXPRESSING APP ARE THE EARLIEST SITES OF INJURY

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Objectives: The mechanisms by which Abeta causes synaptic dysfunction in Alzheimer's disease (AD) are not well understood. It is difficult to determine from existing rodent models whether Abeta-induced synaptic injury is initiated by the pre- or post-synaptic neuron and how injury is propagated.
Methods: Targeting of APP to CA1 or CA3 neurons was achieved by combining Cre/loxP and Tet-Off systems, thus providing both temporal and spatial control of APP expression. Synapse loss was quantified by immunostaining of synaptic markers in dendritic layers of CA1 and CA3 neurons.
Results: Earlier studies demonstrated that LTP was impaired only in synapses where APP was expressed in pre- but not postsynaptic neurons. In one-year-old CA3-3xTg mice, where APP was expressed selectively in CA3, there was a significant 9% decrease in synaptophysin puncta only in axonal terminal field in stratum radiatum of CA1 neurons. In 18-month-old animals, significant reductions in synaptophysin (9-14%) were observed in dendrite fields of both CA1 and CA3 neurons indicating, a spread of injury to synapses that were both pre- or postsynaptic to neurons expressing APP. Consistent with these observations, preliminary studies in 10-month-old CA1-3xTg mice did not demonstrate any synapse loss CA1 dendritic fields.
Conclusions: These results showed that synapses most vulnerable to Abeta-induced injury are located postsynaptic to where APP was expressed. However, in older mice, even synapses presynaptic to dendrites of neurons expressing APP were susceptible to injury. Current efforts will determine the reversibility of synaptic loss by turning off APP expression.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0936

EXCITATORY SYNAPTIC FUNCTION IN MOUSE MODELS OF NEURODEGENERATIVE DISEASE AND IN ADULT HUMAN NEURONS

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Objectives: The aim of our research is to study how excitatory synapses are affected by amyloid beta (Aβ) and tau, how they affect NMDA receptors, and in particular the GluN2b subtype, which is implicated in synaptic plasticity.

Methods: Glutamate receptor composition at the synapse was determined using whole-cell voltage clamp and pharmacology (using the specific GluN2b blocker Ro-256981), in mice with a genetic deletion of murine tau ($Tau^{-/-}$) and transgenic expression of human tau protein in the $Tau^{-/-}$ background. GluN2b components were analyzed in $Tau^{-/-}$ mice, $Tau^{-/-}$ mice expressing wild type human tau ($Tau^{-/-+}$ H1 haplotype) or a disease-associated mutation ($Tau^{-/-+}$ N296H), prior to and following acute application of $A\beta_{1-42}$. Furthermore, to investigate the relevance of our findings to adult human neurons, glutamatergic synapse composition was studied using human cortical tissue resected during neurosurgery.

Results: A decrease in the NMDAR current was observed in $Tau^{-/-+}$ H1 mice under control conditions, which was not further reduced by Ro-256981. A GluN2b sensitive current was observed in $Tau^{-/-}$ and $Tau^{-/-+}$ N296H neurons, while $A\beta_{1-42}$ further reduced NMDAR current in $Tau^{-/-+}$ N296H neurons. Recordings from cortical layer II-III human neurons showed insensitivity to Ro-256981, suggesting an absence of GluN2b subtype at the human synapses analyzed.

Conclusions: Results suggest that $A\beta_{1-42}$ impairs NMDAR-GluN2b receptor function via a tau-dependent mechanism, and expression of human tau affects GluN2b expression at the synapse in transgenic mice. Further studies are required to understand the synaptic composition of cortical adult human neurons.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-1058

ASSESSMENT OF THE PRESYNAPTIC PROTEIN SNAP-25 AS A NOVEL CEREBROSPINAL FLUID MARKER FOR SYNAPTIC PATHOLOGY IN ALZHEIMER'S DISEASE

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Objectives
Synaptic pathology seems to be occurring early in Alzheimer's disease (AD) development. The cerebrospinal fluid (CSF) biomarkers reflecting synaptic pathology might therefore be valuable for earlier diagnosis, assessment of progression of disease and evaluation of treatment. We have developed an affinity mass spectrometry approach allowing reproducibly measurements of the low abundant proteolytic fragments of synaptosomal-associated protein 25 (SNAP-25) in CSF samples from individual subjects. Evaluation of SNAP-25 as a novel CSF biomarker for AD was assessed in three separate cohorts.

Methods
The CSF SNAP-25 proteoforms were immunoprecipitated, digested with trypsin and stable isotope labeled peptides were added. Quantification of SNAP-25 was performed by high resolution selected ion monitoring (HR-SIM-MS) on a Quadrupole-Orbitrap Mass Spectrometer (Q Exactive). SNAP-25 were measured in CSF samples from patients with prodromal AD (N=7), AD (N=9) and controls (N=9) (cohort I); non-demented controls (N=6) and AD (N=10) (cohort II) and finally in healthy controls (N=16) and AD (N=17).

Results
Levels of CSF SNAP-25 were found to be increased in early as well as later stages of AD in three independent case-control cohorts. Almost all of the measured tryptic peptides of SNAP-25 could differentiate AD from controls with an area under the curve of 0.800 to 1.000 depending on cohorts studied.

Conclusions
We have developed a sensitive method to analyze SNAP-25 levels in individual CSF samples. Levels of CSF SNAP-25 were found to be increased in early as well as later stages of AD in three independent case-control cohorts suggesting SNAP-25 to be a valuable marker.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-1108

EFFECT OF NEUROFILAMENT LIGHT GENE KNOCKOUT ON PATHOLOGICAL FEATURES IN THE APPSWE/PSEN1DE9 MOUSE MODEL OF ALZHEIMER'S DISEASE

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Objectives. Cortical neurons selectively containing neurofilament (NF) triplet proteins are vulnerable to degeneration in Alzheimer's disease (AD) and NFs accumulating in dystrophic neurites are an early feature of this condition. We examined the role of NFs by crossing the APP_{swe}/$PSEN1_{dE9}$ (APP/PS1) transgenic model onto a NF light (NFL) gene knockout background. APP/PS1 mice demonstrate pathological features that are characteristic of preclinical stages of AD.
Methods. F2 generation of mice from the APP/PS1 x NFL-/- cross were used for this study. APP/PS1/NFL-/- mice were compared to APP/PS1/NFL+/+ littermates, and as well as C57/BL/6 wildtype mice (n=5 animals per group). At 10 months of age, animals were terminally anaesthetised with sodium pentobarbitone (140mg/kg), brains fixed with 4% paraformaldehyde and processed for thioflavine-S staining and immunohistochemistry for phosphorylated NFs, synaptophysin, microglia (Iba1) and astrocytes (GFAP).Results. 10 month old APP/PS1/NFL-/- mice demonstrated a statistically significant ($p<0.05$) increase in thioflavine-S positive amyloid load compared to the APP/PS1/NFL+/+ mice (0.811±0.081% and 0.5673±0.026% respectively). There was reduced phosphorylated NF-labelled dystrophic neurites in APP/PS1/NFL-/- animals compared to APP/PS1/NFL+/+ mice. However, there was a statistically significant ($p<0.05$) increase in size, but not density, of synaptophysin labelled dystrophic neurites in APP/PS1/NFL-/- mice relative to APP/PS1/NFL+/+ mice. Microglia, but not astrocytes, were also increased in the APP/PS1/NFL-/- mice relative to APP/PS1/NFL+/+ animals.
Conclusions. NFL gene deficiency increases amyloid deposition, the size of a subtype of dystrophic neurite, as well as microglia, in the APP/PS1 model. This suggests an important role of this element of the cytoskeleton in pathological processes underlying AD.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-1210

SYNAPSE SPECIFIC LOSS OF ACTIVITY DEPENDENT TRANSLATION MEDIATED BY DECREASED AKT-MTOR SIGNALING PRECEDES ALZHEIMER'S DISEASE PATHOLOGY IN TRANSGENIC MOUSE MODEL

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Objectives

Synaptic dysfunction, seen structurally and functionally as loss of spines and LTP respectively, contributes to pathogenesis and progression of Alzheimer's disease (AD). The mechanisms underlying these dysfunctions, however, remain unclear. Activity-dependent protein translation at the synapse is essential for regulating long-lasting changes underlying synaptic remodeling and plasticity, such as those seen in LTP. We therefore examined activity-dependent protein translation and the underlying mechanisms at the synapse using a transgenic AD mouse model.

Methods

Synaptosomes and synaptoneurosomes were prepared from cortex of 3 and 9 months old wildtype and APPswe/PS1deltaE9 mice using differential gradient centrifugation and sequential filtration method, respectively. Activity-dependent translation was assayed following KCl stimulation in presence of S^{35}-methionine. Immunoblotting and immunoprecipitation was performed using standard protocols.

Results

Activity-dependent translation is severely diminished in synaptosomes from APPswe/PS1deltaE9 mice at 3 months of age prior to onset of behavioral dysfunction and pathology observed at 8-9 months. This is associated with synapse-specific loss of Akt-mTOR signaling as measured by loss of pAkt, pmTOR and downstream targets pS6K/p4EBP1 at 3 months of age. Further, Akt inhibition in wildtype synaptosomes is sufficient to abolish synaptic activity-dependent translation.

Conclusions

Synapse-specific loss of Akt-mTOR signaling and consequent disruption of activity-dependent protein translation seen in young adult mice, long before onset of symptoms indicate that the synapse is potentially an early target in pathogenesis of AD. Further, these deficits in young AD mice indicate that disease pathogenesis begins long before overt appearance of pathological symptoms, challenging the notion of AD being a disease of old-age.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-1239

EMERGENCE OF SYNAPTIC AND COGNITIVE IMPAIRMENT IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Objectives

Alzheimer's disease (AD) is the world's leading cause of dementia and yet we are still unclear as to the mechanisms behind memory impairment early in the disease. This study aims to uncover the relationship between cognitive dysfunction and early synaptic impairment to identify potential drug targets for this early phase of the disease.

Methods

We used a tet-off inducible AD mouse model expressing $APP_{Swe,Ind}$. Field recordings were made to measure synaptic transmission between hippocampal CA3-CA1 synapses and the T-maze was used to measure short-term memory. We used immunohistochemistry (Ab6E10) and Congo Red staining to assess the level of Abeta plaque load within these mice.

Results

$APP_{Swe,Ind}$ overexpression from birth leads to a significant impairment of basal synaptic transmission by 2 months of age. Surprisingly, levels of long-term potentiation (LTP) are similar between control and mutant mice.

To analyse how these synaptic changes emerge, we used this mouse model to allow short-term $APP_{Swe,Ind}$ overexpression in adulthood (6 weeks of age). Electrophysiological data show that three weeks of APP overexpression results in impaired LTP without affecting basal synaptic transmission. These mice are impaired in their short-term memory and we are establishing a test of long-term memory using a water maze.

Conclusions

We show that short-term inducible APP overexpression is sufficient to impair synaptic and cognitive function and that developmental expression can generate compensation mechanisms that require further study. Understanding the differences between the acute consequences of APP expression and compensatory mechanisms will guide us in the search for effective AD drugs.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-1671

SYNAPTIC DEPOSITION OF MISFOLDED TAU PROTEIN IN ALZHEIMER'S DISEASE EXAMINED BY FLUORESCENCE MICROSCOPY AND FLOW CYTOMETRY

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The accumulation of neurofibrillary tangles in Alzheimer's disease (AD) propagates with characteristic spatiotemporal patterns which follow brain network connections, implying trans-synaptic transmission of tauopathy. Since misfolded tau has been shown to transmit across synapses in AD animal models, we hypothesized that synapses in AD patients may contain misfolded tau. Generally, tau exists in axons; But tau also mislocalizes to dendrites in AD. We isolated synaptic terminals from the cortical tissue of AD subjects and immunostained for tau and synaptic markers, followed by examination with fluorescence microscopy and flow cytometry.

Immunofluorescence microscopy of bipartite synapses from AD subjects showed tau protein in 38.4% of presynaptic and 50.9% of postsynaptic terminals. The pre/post distribution for hyperphosphorylated tau was 26.9%/30.7%, and for misfolded tau 18.3%/19.3%. Within the temporal cortex, microscopic aggregates of tau, containing ultra-stable oligomers, were found to accumulate inside trillions of synapses, outnumbering macroscopic tau aggregates such as tangles by 10,000 fold. Non-demented elderly also showed considerable synaptic tau hyperphosphorylation and some misfolding, implicating the synapse as one of the first subcellular compartments affected by tauopathy. Misfolding of tau protein appeared to occur *in situ* inside synaptic terminals, without mislocalizing or mistrafficking. Misfolded tau at synapses may represent early signs of neuronal degeneration, mediators of synaptotoxicity, and anatomical substrates for transmitting tauopathy. We also developed four-channel flow cytometry to detect protein localization in isolated synaptosomes, which was applied to an amyloid mouse model of AD to examine the colocalization of Aβ and tau at synapses.

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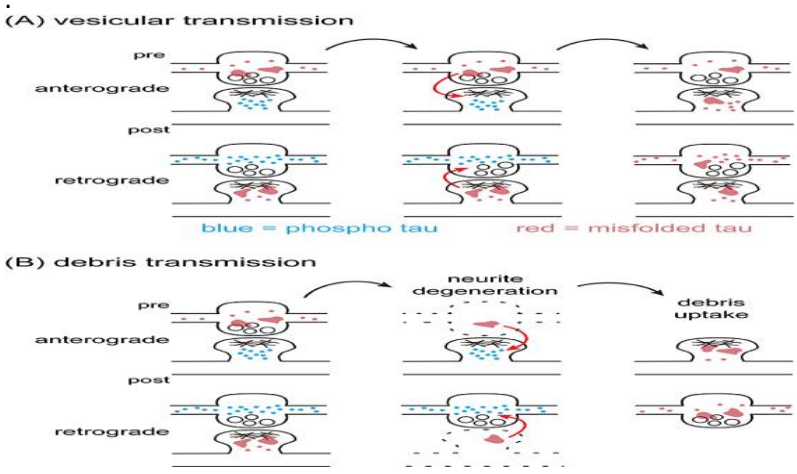

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-1736

ABETA FACILITATED MGLU5R-LTD IN HIPPOCAMPUS IN VIVO IS PROTEIN SYNTHESIS INDEPENDENT, BUT DEPENDENT ON LVA CALCIUM CHANNELS

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Acetylcholine muscarinic receptor-dependent long-term depression (LTD) can be induced by high intensity low frequency stimulation (LFS) in the hippocampus *in vivo*. In contrast, in the presence of amyloid ß –protein (Aß) a metabotropic glutamate-5 receptor–dependent LTD is facilitated, usurping the requirement for mAChRs. The mechanisms of these two forms of LTD remain to be elucidated.

Objectives:

Here we studied the roles of voltage-dependent calcium channels and protein synthesis in control, mAChR-LTD and Aß facilitated mGlu5R-LTD *in vivo*.

Methods:

EPSPs were recorded in the CA1 area of urethane-anaesthetized adult male Wistar rats. Control LTD was induced by 900 pulses at 1Hz (LFS-900) and Aß-facilitated LTD by 300 pulses (LFS-300). Agents were injected via a cannula implanted in the lateral ventricle.

Results:

We found that: (i) Injection of mibefradil (25 nmol), a blocker of low voltage activated calcium channels (LVA), prevented control LTD completely whereas i.c.v. injection of the L-type high voltage activated calcium channels blocker methoxyverapamil (100 nmol) had no effect. (ii) In animals co-injected with mibefradil and Aß, LFS-300 failed to induce LTD whereas LFS-300 induced robust LTD in animals co-injected with methoxyverapamil and Aß. (iii) The protein synthesis inhibitor emetine (434 nmol) blocked the late, but not the early, phase of control LTD. (iv) In contrast, LFS-300 induced robust and persistent LTD after co-injection of emetine and Aß.

Conclusions:

Our data indicate that LVA channels are required for the induction of both control, mAChR-LTD and Aß facilitated mGlu5R-LTD while protein synthesis is only required for mAChR-LTD.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-1740

AGE-RELATED PROTEOMIC ANALYSIS OF S-NITROSYLATION SITES OF POSTSYNAPTIC DENSITY PROTEINS IN MOUSE MODELS OF ALZHEIMER'S DISEASE.

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Alzheimer's disease (AD) is the most frequent neurodegenerative disorder, characterized by progressive loss of synapses and neurons, leading to deterioration of cognitive functions, learning and memory. The pathogenesis of AD is linked to oxidative/nitrosative stress caused by overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and an imbalance in the redox state of the cells. One of the consequences of the RNS interaction with proteins is their S-nitrosylation - a covalent incorporation of a nitric oxide moiety into cysteine thiol group. This modification is emerging as an important redox signaling mechanism which can regulate a broad range of physiological functions. Our research focused on systemic analysis of endogenous S-nitrosylation of proteins from postsynaptic densities (PSDs) - multiprotein structures responsible for the proper functioning of excitatory synapses. PSDs were isolated from brains of transgenic mice with neuronal expression of human mutant APP protein (which are an accepted model of Alzheimer's disease) and control FVB mice. To monitor the changes in protein S-nitrosylation during AD progression the study was carried out on 3-, 6- and 14-month-old mice. In the project we used methods enabling us to selectively enrich the PSD fraction in S-nitrosylated proteins and mass spectrometry analysis for their identification. As a result we obtained lists of peptides present in the samples and peptide sequences assigned to proteins that were S-nitrosylated in the brains of FVB control mice and APP transgenic mice modeling Alzheimer's disease of 3, 6 and 14 months of age.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-1766

GLOBAL ANALYSIS OF SYNAPTIC PROTEINS' S-SULFENYLATION IN MOUSE MODELS OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is characterized by progressive loss of memory and deterioration of cognitive functions. Although the hallmark lesions of the disease, amyloid plaques and intracellular neurofibrillary tangles were described —the molecular mechanisms underlying the disease are still unknown. Recent studies showed, synaptic dysfunction and the loss of synapses are early pathological features of Alzheimer's disease. The pathogenesis of this disorder is linked to the imbalance in production of reactive oxygen (ROS). One of the consequences of interaction ROS with proteins is S-sulfenylation. Cysteine S-sulfenylation is a reversible oxidation reaction that converts the thiol group of protein cysteine residues to a sulfenic acid group. S-sulfenylation, has long been regarded as a harmful cysteine modification, but is nowadays known as a messenger in signaling pathways. This modifications provides redox regulation of protein functions, but the global cellular impact of this transient post-translational modification remains unexplored.

The aim of our project is detection of changes in protein S-sulfenylation during progression of AD. In this work, we used selective cysteine sulfenic acid labeling technique combined with Western blot analysis, nano-LC MS/MS and bioinformatic approaches to globally map endogenously S-sulfenylated cysteines of brain synaptosomal proteins from wild type and transgenic mice overexpressing mutated human Amyloid Precursor Protein (hAPP).

This type of analysis help to defining the quantitative in S-sulfenylation of proteins among different biological states. The progress in the understanding of the molecular alterations underlying Alzheimer’s disease will be useful in developing successful preventive and therapeutic strategies able to block the neurodegenerative process.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-1791

PATHOPHYSIOLOGY OF HIPPOCAMPAL CA3 NEURONS IN THE APP/PS1 MOUSE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's Disease (AD) is characterized by progressive memory loss eventually resulting in dementia. Over the course of AD, insoluble amyloid plaques are formed but synapse loss is known be better correlated with the progression of the disease. The exact role of AB is not fully understood but recent evidence suggests that subtle alterations of synaptic transmission precede neuronal degeneration in the AD progression. Using APP/PS1 transgenic mice we evaluated synaptic function during the initial development of the disease. We characterized the structural and functional age-dependent deficits of glutamatergic synaptic transmission in hippocampal CA3 pyramidal area, a brain structure that plays a key role in memory formation, and that is poorly studied in AD mice models. At 6 months APP/PS1 animals display deficits in several hippocampal dependent behavioural tasks, but no histological features of AD. We looked for correlations of structural and functional impairments in this model of AD by combining patch-clamp electrophysiological recordings in acute hippocampal slices with STED microsccopy to study morphological changes. The originality of this approach lies in our ability to analyze in detail the deficits of individual types of synapses by examining parameters which have been little studied in adult/aged animals, such as synaptic imbalance in the expression of different glutamate receptors subunits and synaptic plasticity of NMDA responses. Our work provides insight and mechanistic understanding to the deregulation of synaptic function and alterations of dendritic spines of different types of synapses present in the same CA3 pyramidal cells and putative rescues strategies for rescue of synaptic dysfunction.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-2067

ABETA FROM YOUNG PS1/APP HIPPOCAMPUS INDUCED EARLY SYNAPTIC PATHOLOGY IN VIVO AND IN VITRO

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OBJECTIVES: We aim to investigate the effects of Abeta from young PS1/APP mouse model of Alzheimer`s disease (AD) on the synaptic integrity by different means. We analyzed the direct synaptotoxic effect of plaques in the hippocampus of this model and the repercussion of the soluble (S1) fraction in neuronal cultures.

METHODS: Hippocampal synapses were investigated by optic and electron microscopy. Primary neuronal cultures were incubated for 48 hours with 6 month-old PS1/APP and wild-type S1 fractions. Levels of several synaptic proteins were measured by Western-blots (WB).

RESULTS: Synapse number and synaptic-vesicles density were found to be significantly decreased in young PS1/APP mice, close to the Abeta deposits, in several hippocampal layers. Importantly, there was a correlation between these deficiencies and the distance to the plaques, which displayed oligomeric forms in their periphery. Some of the presynaptic elements were abnormally swollen and contained autophagic vesicles. In addition, we found by WB a decrease in several hippocampal synaptic markers as early as 4 months of age in this model and in neuronal cultures incubated with S1 fractions. These results correlated with early hippocampus-associated cognitive deficits.

CONCLUSIONS: Plaque-associated oligomeric Abeta induced an early deleterious effect on synapses along with memory deficits in young PS1/APP mice. Moreover, soluble Abeta derived from these transgenic mice reduced synaptic protein content in vitro. Therefore, this model produced synaptotoxic Abeta and may represent a valuable tool to test novel treatments to protect synapses as an early therapeutic approach for AD.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-0479

DISSECTING COMMON PATHO-MECHANISMS BEHIND AD AND PD BASED ON PATHWAYS USING COMPUTABLE MODELS

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Objective: One of the fundamental questions in NDD research is whether Alzheimer's disease (AD) and Parkinson's disease (PD) are different phenotypic variants of the same molecular aetiology or whether multiple aetiologies can converge into the same clinical phenotype. The concept of "shared pathways" between NDDs is attractive as it bears the hope that only a limited number of mechanisms could lead to major neurodegenerative diseases. Therefore identification of common patho-mechanistic pathways between AD and PD could raise the possibility of not only discovering common drug targets for therapeutics but also facilitates repurposing of existing drugs.
Methods: Preliminary cross-disease analysis of pathways was performed using the in-house pathway terminology system (PTS) in the SCAIView knowledge discovery search engine[1]. The top relevant pathways obtained from SCAIView were used to build cause-and-effect computable models for both diseases, based on published knowledge in literature applying BEL, the biological expression language.
Results: The autophagy pathway was found to be one of the shared pathways with maximum co-mentions in literature specific for Alzheimer's and Parkinson's diseases. We found Beclin 1 (BECN1) as one of the main co-players for assembling an interactome with stimulating and suppressive components, which regulates the initiation of the autophagosome formation in both diseases.
Conclusion: Cross-disease analysis using computable BEL models enables us to find novel drug targets based on shared pathways. We propose BECN1 as a novel therapeutic target common for both AD and PD by regulation of autophagy pathway.
[1] http://www.scaiview.com/scaiview-academia.html

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-0538

ASM REGULATES THE AUTOPHAGIC PROCESS BY CONTROLLING LYSOSOMAL BIOGENESIS IN ALZHEIMER'S DISEASE

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In Alzheimer's disease (AD) abnormal sphingolipid metabolism has been reported, although the pathogenic consequences of these changes have not been fully characterized. Here we show that acid sphingomyelinase (ASM) is increased in fibroblasts, brain and/or plasma from patients with AD and in AD mice, leading to defective autophagic degradation due to lysosomal depletion. Partial genetic inhibition of ASM (*ASM*$^{+/-}$) in a mouse model of familial AD (*APP/PS1*) ameliorated the autophagocytic defect by restoring lysosomal biogenesis, resulting in improved AD clinical and pathological findings, including reduction of amyloid-b deposition and improvement of memory impairment. Similar effects were noted after pharmacologic restoration of ASM to the normal range in APP/PS1 mice. Autophagic dysfunction in neurons derived from familial AD patient-induced pluripotent stem cells (iPSCs) was restored by partial ASM inhibition. Overall, these results reveal a novel mechanism of ASM pathogenesis in AD that leads to defective autophagy due to impaired lysosomal biogenesis, and suggests that partial ASM inhibition is a potential new therapeutic intervention for the disease. This work was supported by the Bio & Medical Technology Development Program (2012M3A9C6050107, 2012M3A9C6049913) of the National Research Foundation (NRF) of Korea funded by the Ministry of Science, ICT & Future Planning, Republic of Korea.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-1219

C99 ACCUMULATION, LYSOSOMAL DYSFUNCTION AND AUTOPHAGIC FAILURE IN ALZHEIMER DISEASE CELLULAR AND ANIMAL MODELS

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1-Objectives: Recently, we have shown, using the 3xTgAD mouse model (APPswe, PS1M146V, TauP301L), an early, age-dependent and hippocampus specific accumulation of the beta-secretase cleavage fragment C99 in Cathepsin B positive structures resembling autolysosomes. In the present work, we therefore investigated the relationship between C99 accumulation and autophagic function.
2-Methods: We used an *in vitro* model (SH5Y-APPswe cells) and the 3xTgAD mouse. Cells were treated with pharmacological agents affecting autophagy or with gamma-secretase inhibitors. Autophagy was evaluated by western blot analysis, Cathepsin dosage, electron microscopy and immunofluorescence. Animals were treated chronically with a gamma-secretase inhibitor.
3-Results: We found that autophagy plays a key role in the elimination of C99 and other APP C-terminal fragments (APP-CTFs). The pharmacological activation of autophagy or the overexpression of Cathepsin B both decreased APP-CTFs levels. In contrary, the pharmacological blockade of autophagy or the inhibition of lysosomal hydrolases (by Cathepsin inhibitors or lysosomal alkalization) lead to a large increase in APP-CTFs levels. Interestingly, we also found that APP-CTFs can themselves affect autophagic function, because the inhibition of gamma-secretase lead to the accumulation of APP-CTFs with associated loss of cathepsin activity, increase in autophagic markers and build-up of abnormal undigested autophagic vacuoles. Moreover, the chronical treatment of 3xTgAD mice with a gamma-secretase inhibitor lead to a massive increase in APP-CTFs in Cathepsin B positive structures and autophagic dysfunction.
4-Conclusion: Our data highlight a major role of autophagy in C99 clearance and also show a direct correlation between APP-CTF accumulation and autophagic dysfunction.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-1274

STUDYING THE BIOLOGY OF MISFOLDING PROTEINS IN CULTURE TO ELUCIDATE CSF BIOMARKER CHANGES IN ALZHEIMER'S DISEASE

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Objectives:
Alzheimer's disease is characterized by the abnormal aggregation of Beta-amyloid (Abeta) in plaques and tau in tangles. Plaques contain alpha-synuclein as their 2nd most abundant component. Our understanding of why these misfolding proteins show different changes in CSF in AD is incomplete. There is a complex dynamic equilibrium of extra- and intracellular pools of misfolding proteins involved in AD, which depends on production, secretion, uptake and degradation. Because the endosome-lysosome and autophagy systems regulate the biology of misfolding proteins linked with AD, these systems are modulated to elucidate biomarker changes in AD.
Methods: Endosomal, lysosomal and mTOR dependent autophagic pathways are modified in untransfected, human APP, Swedish mutant APP or alpha-synuclein transfected N2a neuroblastoma cells or primary neurons in culture and analyzed by Western blot and immunofluorescence microscopy.
Results: Reducing the extracellular levels of Abeta or alpha-synuclein decreases their respective intracellular levels supporting a dynamic equilibrium of extra- and intracellular levels of Abeta and alpha-synuclein. Modulating endosome-lysosome and autophagy pathways alters this equilibrium, although with these treatments Abeta and alpha-synuclein do not change in the same direction.
Conclusion: The extra- and intracellular pools of misfolding proteins affect each other in a manner consistent with a dynamic equilibrium between the respective pools. Modulation of endosome-lysosome and autophagy systems differentially changes the dynamics of extra- and intracellular pools of misfolding proteins/peptides. We hypothesize that these results in cell culture systems can provide insights into why Abeta is decreased while alpha-synuclein is increased in the CSF with AD.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-1477

IMPORTANCE OF SPHINGOSINE-1-PHOSPHATE-LYASE IN LYSOSOMAL METABOLISM OF THE AMYLOID PRECURSOR PROTEIN.

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Sphingolipids are components of cellular membranes and play important roles in cellular signaling. Lack of the sphingosine-1-phosphate (S1P) degrading enzyme S1P-lyase causes accumulation of S1P and its precursor sphingosine. We recently showed that S1P-lyase activity is involved in the lysosomal turnover of the amyloid precursor protein (APP) and its C-terminal fragments (APP-CTFs). Our data also indicated impaired lysosomal Ca^{2+} mobilization in S1P-lyase deficient cells. Interestingly, APP metabolism could be partially normalized by selective Ca^{2+} release (Karaca et al, 2014). We now analyzed a potential invovlement of PKC in APP processing when S1P-lyase was absent. Analysis of APP metabolism was carried out using biochemical and cell biological approaches in mouse embryonic fibroblasts from wild-type (WT) and S1P-lyase-knock-out (KO) mice. Lipid analysis was performed by mass spectrometry. The subcellular distribution of PKC and other proteins were analyzed by cell fractionation and fluorescence microscopy. S1P-lyase deficient cells show significant increase of intracellular S1P and sphingosine. Inhibition of sphingosine-kinases caused a selective increase in cytosolic levels of PKC. Furthermore, fluorescence microscopy revealed altered morphology of endo-lysosomal compartments in KO cells. Interestingly, this phenotype was partially mimicked by pharmacological inhibition of PKC. In particular, PKC inhibition causes a strong accumulation of APP-CTFs and altered maturation of the lysosomal protease cathepsin-D. These results point to an involvement of PKC in S1P or sphingosine mediated metabolism of APP. It will be interesting to further dissect the role of PKC in the regulation of APP metabolism by sphingolipids, and its functional role in the pathogenesis of Alzheimer's disease.

ADPD5-2223
IMPAIRMENT OF CHAPERONE MEDIATED AUTOPHAGY INDUCES DOPAMINERGIC NEUROGENERATION IN RATS

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Objective: To investigate the physiological role of Chaperone Mediated Autophagy (CMA) in the adult brain and to elucidate the consequences of CMA failure in the rat substantia nigra, the area mainly affected in Parkinson's disease.

Methods: We have generated adeno-associated viruses expressing shRNAs targeting Lamp2a, CMA's rate limiting step, as well as control scrambled shRNAs. These viruses were injected stereotaxically into the rat nigra and at 4 and 8 weeks post-injection we assessed the efficacy of Lamp2a down-regulation, endogenous alpha-synuclein levels, dopaminergic system integrity and relevant behavioural deficits.

Results: Using two different shRNAs against Lamp2a we detected a significant decrease in Lamp2a levels within the dopaminergic neurons, accompanied by intracellular alpha-synuclein-positive puncta, indicating that CMA, a major pathway for alpha-synuclein clearance, was compromised. These puncta were also positive for ubiquitin. Strikingly, down-regulation of Lamp2a led to a significant impairment of dopaminergic system integrity, characterized by a 60% loss of nigral dopaminergic neurons, and a similar reduction in striatal dopamine levels. Measurement of d-amphetamine-evoked rotations is currently underway.

Conclusions: Our study highlights for the first time an important physiological role of the CMA pathway in the brain. Furthermore, the model presented herein recapitulates main features of Parkinson's disease, including the severe loss of dopaminergic neurons and the accumulation of aggregated alpha-synuclein within surviving neurons. This fact, in conjunction with previous biochemical and neuropathological evidence linking impairment of the CMA pathway to the disease, suggests that this model may be useful in investigating relevant mechanisms and therapeutic interventions.

03e. Pathophysiology & Disease Mechanisms: proteasome and ubiquitin

ADPD5-1246

ABETA PLAQUE FORMATION AND IMPAIRED PROTEIN QUALITY CONTROL INTERACT VIA GAMMA-SECRETASE AND RESULT IN A BEHAVIOURAL PHENOTYPE CONSISTENT WITH AD

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Objectives: Immunization trials in AD patients have not been effective for curing or ameliorating dementia. Experimentally (line #85; APP_{Swe}-PSEN1Δexon 9), there is limited clearance of amyloid plaques. Therefore, more knowledge about the mechanism of Aβ plaque formation is a priority. We showed that misframed ubiquitin (UBB^{+1}) inhibits the ubiquitin proteasome system (UPS) resulting in ERAD dysfunction, mitochondrial clogging and impaired contextual behaviour (Dennissen, Prog. Neurobiol., 2012). Recently, GWAS studies, pathway analysis and proteomics identified protein ubiquitination as one of the key modulators of AD (Manavalan, Exp. Mol. Med., 2013). Crosstalk between a dysfunctional UPS and Aβ plaque formation has been surmised but never proven.

Methods: This issue was studied by crossbreeding 2 transgenic lines (lines #3413 with UBB^{+1} overexpression, line #85 with Aβ plaque formation at 4 months of age) and their crossbreed (#3413 x #85).

Results: In line 85, γ-secretase activity was decreased significantly at 3, 6 and 9 months of age, whereas α and β- secretases were unaffected. However, in the crossbreed (with a dysfunctional UPS) γ-secretase activity was enhanced at the age of 6 months; a critical period where Aβ plaque generation is attenuated. In the crossbreed, nest building and contextual memory are impaired. Our new data implicate a strong interaction between a dysfunctional UPS and Aβ plaque formation being mediated specifically via γ-secretase.

Conclusion: A striking inverse correlation is shown between γ-secretase activities and Aβ plaque load and will contribute to a better understanding of strategies to ameliorate or cure AD, via γ- secretase modulation.

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-0491

2-DEOXYRIBOSE-INDUCED APOPTOSIS REGULATION DISTINGUISHES LYMPHOBLASTS OF SPORADIC AND FAMILIAL ALZHEIMER'S DISEASE PATIENTS

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Objectives: We previously reported that control of cell cycle distinguishes lymphoblasts from sporadic and familial Alzheimer's disease patients (SAD and FAD). We found significantly increased basal p21 levels in SAD cells compared with FAD lymphoblasts. Since it is known that p21, besides controlling cell cycle, can regulate apoptosis, we checked whether p21 levels play a role in the cellular response of FAD and SAD cells to oxidative stress evoked by 2d-ribose (2dRib).

Methods: Cell viability after 2dRib and pifitrin (PFT–a) treatment was measured using MTT assay, mRNA levels were evaluated using real-time PCR and protein levels by immunoblotting. p21 levels in nuclear and cytosolic fractions were visualized using confocal laser scanning microscopy.

Results: FAD lymphocytes were more resistant to 2dRib-induced cell death than control or SAD cells. In response to 2dRib FAD cells showed significantly increased p21 mRNA and protein levels and preferentially cytoplasmic location of p21 as compared to SAD cells. Transcriptional activation of p21 was shown to be dependent on p53, as it can be blocked by PFT-a.

Conclusions: The increase in p21 transcription in FAD lymphoblasts and its cytoplasmic localization confer these cells a survival advantage, since PFT-a sensitized FAD cells to 2dRib-induced apoptosis. Thus, as some cellular mechanisms seem to be different in FAD and SAD cells, our data suggest a possibility for differential diagnosis of FAD and SAD based on p21 and p53, and individualized therapeutic approach for SAD and FAD.

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-0678

BACH1 OVEREXPRESSION IN DOWN SYNDROME CORRELATES WITH THE ALTERATION OF THE HO-1/BVR-A SYSTEM: INSIGHTS FOR TRANSITION TO ALZHEIMER DISEASE

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Objectives. Among the genes encoded on chromosome 21, Bach1 is a transcription repressor, which binds to antioxidant response elements (AREs) of DNA thus inhibiting the transcription of specific genes involved in the cell stress response including heme oxygenase-1 (HO-1). HO-1 and its partner, biliverdin reductase-A (BVR-A), are up-regulated in response to oxidative stress (OS) in order to protect cells against further damage. Since OS is an early event in Down Syndrome (DS) and might contribute to the development of multiple deleterious DS phenotypes, including AD pathology, we investigated the status of the Bach1/HO-1/BVR-A axis in DS and its possible implications for AD development.
Methods. Post mortem brain (frontal cortex) from DS patients with (mean age 59 ys) and without (mean age 25 ys) dementia, and from Ts65Dn mice, a Tg mouse model of DS, were analyzed. Western blot, immunoprecipitation and RT-PCR assays were performed to investigate Bach1/HO-1/BVR-A axis in the above samples.
Results. Our data show that the development of AD in DS subjects is characterized by (i) increased Bach1 total and poly-ubiquitination; (ii) increased HO-1 protein levels; and (iii) increased nitration of BVR-A followed by reduced activity. To corroborate our findings we analyzed Bach1, HO-1 and BVR-A status in Ts65Dn mouse model at 3 (young) and 15 (old) months of age.
Conclusions. The above data support that the dysregulation of HO-1/BVR-A system contributes to the early increase of OS in DS and provide potential mechanistic paths involved in the neurodegenerative process and AD development.

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-0707

NITRATED P53 AS AN EARLY BIOMARKER OF UNBALANCE REDOX STATUS IN ALZHEIMER'S DISEASE (AD)

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Objectives: An early Alzheimer's diagnosis is still missing because of the difficulty to establish a standardized method in periphery. On this basis the measurement of oxidative stress and p53 levels was made in immortalized B-lymphocytes AD patients to define a specific marker with prognostic value.

Methods: The levels of oxidative markers were measured (4-HNE, 3-NT and protein carbonyl) through western blot. The antioxidant activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GRD) were measured by enzymatic assays. To study p53 conformations, immunoprecipitation experiments with PAb1620 (for p53 wild-type) and PAb240 (for p53 unfolded) antibodies, were performed. The effect of peroxynitrite compound (SIN-1) on p53 conformation was evaluated with FACS analysis.

Results: We observed increased levels of HNE and 3-NT only in familiar AD, compared with controls. A reduced SOD and GRD activity was evident in familiar and sporadic AD. Furthermore a significant amount of p53 was found conformational altered in both pathological groups in comparison with controls, demonstrated by the high reactivity to PAb240 antibody. Immunoprecipitation experiments followed by the immunoblotting with anti 3-NT antibody, showed in both groups an increase of nitrated tyrosine residues in samples immunoprecipitated with PAb240. Interestingly, the nitration of tyrosine residues of p53 could be responsible of the p53 conformational change towards an unfolded phenotype, as demonstrated by the SIN-1 experiment. A correlation between unfolded p53 and SOD activity was also found.

Conclusions: The oxidative stress could alter p53 conformation. Nitrated-p53 might be considered as a potential early biomarker for Alzheimer's disease.

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-0733
HESPERIDIN: A NOVEL BIOFLAVONOID IN ATTENUATING LIPOPOLYSACCHARIDE-INDUCED COGNITIVE DEFICITS IN MICE.
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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by multiple cognitive deficits, behavioral abnormalities and mood changes. Neuroinflammation and oxidative stress have been established as fundamental components of AD pathogenesis. Microglia are activated in response to amyloid beta (Aβ) aggregates initiating a chronic inflammatory response in the brain. In addition, Aβ may result in mitochondrial dysfunction and augmented ROS levels. The present study was designed to evaluate the possible effect of different doses of the citrus flavonoid hesperidin (HDN) on male Swiss albino mice after receiving intraperitoneal (i.p) injections of lipopolysaccharide (LPS) at a dose of 0.8 mg/kg to induce AD. HDN was injected at three dose levels (2, 4, 8mg/kg, i.p.). Indomethacin (1 mg/kg, i.p., 5 days) as well as ascorbic acid (120 mg/kg, i.p., 7 days) were used as a positive control for their antinflammatory and antioxidant effects. Behavioral changes were evaluated using y-maze, 8-arm radial maze and novel object recognition tests, while molecular changes were assessed by measuring TNF-alpha (TNF-α), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione reductase (GSH) levels in the mouse brain. Moreover, immunohistochemistry was used to assess Aβ1-42 deposition in the mouse cortex and hippocampus. Results showed that HDN reversed LPS-induced cognitive impairment in mice and suppressed LPS-induced changes in molecular markers of inflammation and oxidative stress in a dose dependent manner. This was accompanied by a reduction in Aβ deposition in the brain. Thus, our study suggests that HDN is a promising memory enhancer with potential benefits in prevention of AD.

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-1186

CONFORMATIONALLY ALTERED P53 AFFECTS NEURON BIOLOGICAL FUNCTIONALITY

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Objective. In this study we investigated a possible contribution of p53 unfolded protein in neurodegeneration occurring in Alzheimer's Disease.
Methods. An in-vitro model of a stable transfected SH-SY5Y clone overexpressing APP751wt was used to study oxidative stress markers, such as HNE Michael-adducts and 3-Nitro-Tyrosine as well as unfolded p53. p53 conformation was evaluated using two specific monoclonal antibodies: PAb1620 (that recognizes p53wt) and PAb240 (direct towards unfolded p53). Furthermore, growth-associated protein 43 (GAP43) was examined at mRNA and protein levels.
Results. We found that SY5Y-APP clone expressed an increased amyloidogenic processing with enhanced expression of C-terminal fragments C99 and C83 and β-amyloid peptide. High oxidative markers and unfolded p53 conformation, due essentially to nitration of its tyrosine residues, were also observed in SY5Y-APP cells. In addition, this clone was less sensitive to acute oxidative insult because of p53 impairment. SY5Y-APP cells expressed reduced GAP-43 mRNA and protein levels in comparison with control, affecting cell differentation and morphology. Both H_2O_2-sensitivity and GAP-43 expression were restored by modulating p53 conformation towards a wild-type phenotype. In particular, Zinc-supplementation reverted p53 wild-type tertiary structure and increased cells sensitivity to acute cytotoxic injury and GAP-43 levels in SY5Y-APP clone.
Conclusion. We propose p53 oxidation/nitration as one of early molecular event in the estabilishment of neuronal dysfunction, leading to cognitive impairment and AD pathology. In particular, elevated oxidative environment may affect p53 conformation towards an unfolded structure. In this unfolded state, p53 is not able to exert its proapototic activity and physiological role in axonal outgrowth.

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-1207

EXTRACELLULAR AND INTRACELLULAR REDOX ALTERATIONS IN ALZHEIMER'S AND MILD COGNITIVE IMPAIRMENT PATIENTS

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Objectives This study investigated the alterations of extracellular and intracellular redox potential in peripheral blood cell (PBMC) and plasma of Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI) patients and cognitively healthy subjects (CHS).
Methods 120 individuals (42 AD, 36 MCI and 42 CHS) from Spain were enrolled. All subjects were examined by behavioural neurologists and classified according to recently reviewed criteria. Superoxide Dismutase (SOD), Catalases (CAT) and Glutathione Peroxidases (GPx), activities were measured by enzymatic assays. Unfolded p53 expression was analysed by ELISA using the specific antibody PAb240.
Results We found that among the three antioxidant enzymes, SOD activity was significant reduced both in plasma and PBMCs of MCI and AD patients compering them with CSH. On the other hand unexpected enhancement in GPx activity was observed especially in plasma of MCI group, suggesting a compensatory mechanisms in the tentative to take under control pro-oxidants. While an increased unfolded p53 was found in plasma in both AD and MCI, but only AD expressed high levels of it in intracellular compartment. Interestingly both PBMC-SOD activity and PBMC-unfolded p53 direct correlated with MMSE.
Conclusion. The concomitant measurement of extracellular and intracellular antioxidant enzymes activity pointed out the dynamical evolvement of unbalance redox potential in AD pathology. Based on our results, we suggest that the early diagnosis of AD may benefit from the combined determination of both the redox profile and unfolded p53 in both PBMC and the plasma.

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-1257

CELL PROLIFERATION RATE AND OXIDATIVE STRESS INVOLVED IN THE INVERSE ASSOCIATION BETWEEN ALZHEIMER DISEASE AND CANCER

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Introduction: We have proposed that deregulation of biological mechanisms involved in cell death/proliferation might explain the inverse association observed in epidemiological studies between Alzheimer's disease (AD) and a history of cancer. We reported that lymphocytes from AD patients have an increased susceptibility and those with a history of skin cancer a reduced vulnerability to oxidative death by H_2O_2 exposure (Behrens *et al.,* 2012).
Objective: To measure the proliferation rate cell and oxidative stress present in lymphocytes and fibroblasts from patients with AD, skin cancer history (CA) and healthy controls (HC).
Methods: Proliferation rate of cultured fibroblasts from patients older than 60 years with AD; CA; and HC, was measured by cell count. Reduced (GSH)/oxidized (GSSG) glutathione levels (fluorimetry) and lipid peroxidation (TBARs assay kit) were determined in lymphocytes.
Results: Cultured fibroblasts from AD patients had reduced proliferation rate while cancer fibroblasts had increased proliferation rate. Interestingly, the proliferation rate of cultured AD fibroblasts was increased by the addition of conditioned medium from cancer fibroblasts, and *vice versa*. Lipid peroxidation was increased and GSH/GSSG ratios reduced in lymphocytes from AD patients compared with CA and HC.
Conclusions: These results suggest that cell proliferation rate might be differently regulated in cancer and AD patients, in addition to the susceptibility to cell death due to higher basal oxidative stress levels in AD cells. In all, our results suggest that systemic circulating factors might be involved in the mechanism explaining the inverse association between cancer and AD. Fondecyt 1110189 (MIB), 3140273 (CSM).

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-1702

EFFECT OF EXERCISE ON REACTIVE OXYGEN SPECIES (ROS) PRODUCTION IN ALZHEIMER DISEASE BY ELECTRON PARAMAGNETIC RESONANCE (EPR)

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Objective: Oxidative Stress (OS) plays an important role in neurodegenerative diseases. OS may be evaluated measuring the Reactive Oxygen Species (ROS) production by Electron Paramagnetic Resonance (EPR), and/or the damage to biomolecules markers. Mini-invasive method detecting ROS concentration by EPR is now demonstrated suitable to monitor physiological and pathological conditions[1,2].
Methods: X-Band-EPR (~9GHz) ROS levels determination and enzymatic assays (Protein Carbonyls (PC), ThioBarbituric Acid Reactive Substances (TBARS) and Antioxidant Capacity (TAC) were utilized to evaluate OS in patients affected by Mild Cognitive Impairment (MCI), Alzheimer Disease (AD) and in healthy subjects (CTR). Incremental Exercise (IT) until voluntary exhaustion on cycle ergometer was performed.
Results: At rest, significantly (*; P<0.05) higher levels of ROS, TBARS and PC and lower TAC levels were calculated in AD respect to CTR (Table). At the end of exercise, an increase of ROS production was observed both in AD (p<0.0001) and CTR (p<0.001).
Conclusions: Our results confirm that neurodegenerations are related to an overproduction of OS. Moreover, strenuous aerobic exercise significant increased ROS production both in AD and CTR, while in AD the increase resulted more pronounced probably due to a loss in mitochondrial respiratory chain efficiency and/or antioxidant capacity.

References:

1. Mrakic-Sposta S. et al, Oxid Med Cell Longev. 2012;2012:973927;
2. Mrakic-Sposta S. et al, Oxid Med Cell Longev. 2014. In press.

	ROS (umol.min^{-1})	TBARS (uM)	PC (nmol.mg^{-1} protein)	TAC (mM)
MCI (n=19)	2.18±0.31	10.96±2.34	0.91±0.21	1.47±0.38
AD (n=18)	2.20±0.42*	11.78±3.4*	0.92±0.21	1.47±0.37
CTR (n=22)	2.12±0.29	9.97±2.65	0.78±0.22*	1.71±0.43*

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-1933

REGULATORY IMPACT OF LUTEOLINE ON MITOCHONDRIAL ENZYMATIC ELEMENTS AND POTASSIUM CHANNELS OF PC12 CELLS

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Objectives
Oxidative stress has been reported extensively in the involvement of mitochondrial impairment that contributes in pathological manifestations of Huntington and Alzheimer's diseases. Luteoline is a widely distributed flavonoid that encompasses the structures essential for flavonoid's antioxidant activity. In the present study we examined the dose-dependent protective effects of Luteoline against H_2O_2-induced inhibition of electron transport chain (ETC) complexes and tricarboxylic acid (TCA) cycle enzymes activities with the assessment of Ca2+-activated K+ (mitoBKca) channel protein level in rat pheochromocytoma (PC12) cells.

Methods
Undifferentiated PC12 cells were applied to undergo different treatments. Citrate synthase, aconitase, fumarase, malate dehydrogenase and alpha ketoglutarate dehydrogenase (alpha-KGDH) assays were performed to assess TCA enzymes activities. Measurement of ETC complexes activities were done by spectrophotometric evaluation. Western blotting were conducted to measure protein levels of different mitoBKca channels (alpha, beta-2 and beta-4).

Results
H_2O_2-exposed PC12 cells showed significant decrease in the activity of TCA and ETC enzymes. Pretreatment of PC12 cells with Luteoline (20 µM), followed by exposure to H_2O_2 (150 µM) caused about 53% increase in the mean activity of TCA and ETC enzymes, compared to H_2O_2-only treated cells. H_2O_2 pretreatment resulted in decrease of mitoBKca channels activity, however, Luteoline increased protein level of α, β2 and β4 subunits, compared to H_2O_2 group.

Conclusion
Overall, our data suggest that Luteoline can be a potential candidate in the treatment of oxidative stress-induced mitochondrial dysfunction, seemed to be due to restoration of TCA and ETC activity along with mitoBKca channels reactivation.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0312

EFFECTS OF THE NEUROSTEROID ALLOPREGNANOLONE ON MITOCHONDRIAL DYSFUNCTION IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) transgenic mice model (APPswe/PSEN1), present a decreased capacity to form allopregnanolone (APα), a natural neurosteroid, in the hippocampus, associated with deficits in hippocampal performance [1]. The APP-transfected SH-SY5Y cell line represents a commonly used model to study cellular and molecular mechanisms involved in AD. The effects of APα on mitochondrial activity will be compared to those of four chemically modified APα compounds (ANS).
We investigated the efficiency of APα and four ANS in restoring / ameliorating cellular bioenergetics. For that purpose, we determined ATP level, metabolic activity, mitochondrial respiration under physiological condition and reactive oxygen species (ROS) level under stress condition after a 24h treatment with APα or ANS at 500 nM in control and APP-transfected SH-SY5Y.
APα significantly improved ATP production, the capacity of respiration and cell proliferation in control as well as APP-transfected SH-SY5Y cells when compared to the respective untreated cells. Of note, among the four tested ANS, one of them was capable to ameliorate all this parameters. In addition, under stress condition, APα and this selected compound were able to reduce ROS level in both cell lines.
The screening of APα and ANS led to the identification of one promising molecule exerting beneficial effects on bioenergetics and mitochondrial homeostasis. These compounds will now be subjected to further in vivo investigations.
This work was supported by grant from Swiss National Foundation (SNF 31003A_149728), Interreg and Fonds Européen de Développement Régional.
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03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0329

THE ROLE OF GENETIC VARIATIONS IN THE MORTALIN GENE (HSPA9) IN ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE

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Objectives: To investigate whether the genetic variants in the mortalin gene (*HSPA9*), encoding a mitochondrial heat shock protein, are associated with the risk of AD and PD.
Methods: Direct sequencing using an automated DNA sequencer (ABI 3730, Applied Biosystems, CA, USA) for the entire coding region (17 exons) and exon-intron boundary of 50 bp was performed in 24 AD patients, 24 PD patients, and 24 controls. The genetic variants with minor allele frequency (MAF) > 1% from sequencing data were selected for the second-stage genotyping. Common genetic variants (MAF > 5%) were also selected using the HapMap and 1000 Genomes Project JPT and CHB samples. We genotyped eight *HSPA9* genetic variants in 400 AD cases and 500 controls, and 10 *HSPA9* genetic variants in 500 PD cases and 500 controls, using the Fluidigm high-throughput platform. Logistic regression analysis with additive coding schemes as a primary analysis was performed.
Results: The *HSPA9* genetic variant rs41295739 showed a significant association with AD (OR = 1.78, 95% CI = 1.18 – 2.68, p = 0.0062). This significant association was observed in additive, dominant, and allele genetic coding schemes. Other *HSPA9* genetic variants were not associated with AD. There was no significant association of each genetic variant in the *HSPA9* gene and PD.
Conclusions: Our results demonstrate that the *HSPA9* genetic variant rs41295739 is significantly associated with AD. However, other common and rare genetic variants in the *HSPA9* may not play a major role in the development of AD and PD.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0606
MITOCHONDRIAL FISSION ARREST IN ALZHEIMER'S DISEASE
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Mitochondrial dysfunction and altered cellular energetics have been implicated in the etiology of Alzheimer's Disease (AD). However, a mechanism linking mitochondrial behavior to the development of AD remains to be elucidated. Using 3D reconstruction electron microscopy (3D EM) of brain tissue from AD patients and animal models of familial AD (FAD), we demonstrate that AD neuronal mitochondria display a highly exaggerated fission arrest phenotype that resembles 'beads-on-a-string" (BOAS). The BOAS phenotype was mimicked in cultured neurons treated with cyclopentenone prostaglandin 2 (PGJ2) and in young mice under hypoxic conditions. Analysis of EM images, fission protein Drp1 activation, and kinetic modeling suggest that fission arrest occurs near the final stages of the process. Since fission is thought to provide a mechanism for quality control through the disposal of damaged mitochondria, we suggest that mitochondrial fission arrest may contribute residual mitochondrial functions extending a protective energetic margin that plays a role in neuronal cell survival.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0713

PERIPHERAL ALTERATIONS IN MITOCHONDRIAL BIOGENESIS: NEW POTENTIAL BIOMARKER FOR ALZHEIMER'S DISEASE

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Objective
For almost twenty years the amyloid cascade hypothesis has dominated Alzheimer's disease (AD). In Alzheimer's disease, mitochondria failure has been suggested by numerous studies.
The aim of this study was to investigate the mitochondrial biogenesis in its complexity identifying which factors of mitochondrial biogenesis can be altered/compromised in blood cells of patients affected by AD and Mild Cognitive Impairment (MCI).
Method
Lymphocytes from cognitive healthy subjects, MCI and AD patients, were isolated by Ficoll gradient density method. The expression of some markers of mitochondrial biogenesis were evaluated with quantitative rtPCR and immunoblotting. Mitochondrial DNA copy number was measured by means of qPCR. Cytochrome C oxidase and citrate synthase activity was also measured.
Results
All key factors re**gulatin**g mitochondrial biogenesis was found compromised in AD subjects, while MCI patients showed only specific alterations in the mitochondrial cascade. Copies number of mtDNA was significantly reduced in both AD and MCI. Interestingly, there are some correlations between these new findings and other major benchmarks of the disease
Conclusion
We demonstrated a defective program generating new mitochondria in PBMCs of AD patients, that resulted in turn in the reduction of mitochondrial control quality/content. These new findings point an intriguing relevance of mitochondrial biogenesis as an early peripheral marker for the detection of AD and MCI. In this contest, the selective and peculiar alterations in mitochondria cascade as well as in mitochondrial quality control might stand for risk factors, easy observable in blood cells, involved in AD progression.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0877

DISSECTING THE EFFECT OF NEUROSTEROIDS IN ALZHEIMER'S DISEASE: MODULATION OF A-BETA AND TAU-INDUCED MITOCHONDRIAL DYSFUNCTION

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We aimed to characterize the bioenergetic modulating profile of a panel of diverse neurosteroids of the sex hormone family (progesterone, estradiol, estrone, testosterone, 3α-androstanediol), under normal condition or under pathological Alzheimer's disease (AD) condition. More specifically, we assessed whether the selected neurosteroids could attenuate the toxic effects of amyloid-β (Aβ) and abnormal tau on mitochondrial function.
The effects of neurosteroids were investigated in native human neuroblastoma cells (SH-SY5Y cells) or in cells overexpressing either the human amyloid precursor protein (APP), wild-type tau protein (wtTau), or mutant tau (P301L). After 24 hrs of treatment, the effects of neurosteroids (concentration of 100 nM) were investigated on bioenergetic parameters, such as ATP production, mitochondrial membrane potential (MMP), mitochondrial respiration and glycolysis.
Under normal condition (in native SH-SY5Y), most of the steroids tested were able to improve bioenergetic activity by increasing ATP levels, MMP, glycolysis and basal mitochondrial respiration, at least in part through steroid nuclear receptor activation. Each neurosteroid appeared to have a specific bioenergetic profile. In addition, the majority of these steroids were effective in enhancing ATP levels, MMP and mitochondrial respiration in cells overexpressing APP and mutant tau, attenuating the mitochondrial dysfunction observed in these cell lines compared to the respective control cells.
Thus, our results provide new insights in re-defining the biological model of how neurosteroids control neuronal functions and lend further evidence to the neuroprotective effects of neurosteroids in AD pathology.
This work was supported by grant from the Swiss National foundation (SNF 31003A_149728) and Synapsis Foundation.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0918

EARLY ABNORMALITIES IN N-ACETYLASPARTATE METABOLISM IN THE 5XFAD MOUSE MODEL OF ALZHEIMER'S DISEASE

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N-acetylasparate (NAA) is a prominent amino acid derivative in the brain, linked to neuronal mitochondrial function and energetic integrity. NAA synthesis is energy intensive and requires TCA cycle-derived AcCoA and mitochondrial aspartate. Reductions in NAA constitute an index of neuronal metabolic integrity across the entire neurodegenerative spectrum, including AD.

Objectives: To analyze early reductions in NAA in the 5xFAD mouse model of AD from the perspective of transcriptional regulation of the gene encoding for the NAA synthetic enzyme Nat8L, with a view to defining pathological reductions in NAA as a coordinated neuronal response to energetic crisis.

Methods: High Performance Liquid Chromatography; Immunohistochemistry; *In-situ* hybrydization; RT-PCR.

Results: A significant reduction in neuronal *Nat8L* expression from 2-4 months of age in the 5xFAD brain is manifest in association with overall diminished energetic potential. Reduced neuronal *Nat8L* is most prominent in the hippocampus, and is preceded by an increase in the expression of the NAA-catabolizing enzyme aspartoacylase (ASPA) in oligodendrocytes. A parallel analysis of *Nat8L* during normal postnatal development identified a significant increase between 2 and 4 weeks of age, with the *aspa*-null *nur7* mouse presenting with the premature upregulation of *Nat8L* at 2 weeks of age.

Conclusions: This study suggests the active downregulation of *Nat8L* via the induction of oligodendrocytic *aspa* is a means by which energetic resources are conserved, and implicates the complete NAA metabolic cycle in the neuronal stress response.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0956

ALTERED MITOCHONDRIAL TRANSPORT IN ALZHEIMER'S DISEASE - HUMMR AS A TRAFFIC COP!

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Objectives: To unveil the role of the hypoxia up-regulated mitochondrial movement regulator (HUMMR - a protein that favors the anterograde movement of mitochondria in a hypoxia-inducible factor 1 (HIF-1alpha)-dependent process) on defective mitochondrial trafficking in Alzheimer's disease (AD).
Methods: Using human post-mortem brain cortex and hippocampus from AD subjects and differentiated SH-SY5Y cells (resemble mature neurons) exposed to amyloid-beta 1-42 ($Abeta_{1-42}$), HIF-1α and HUMMR protein levels and mRNA were evaluated by Western blotting and RT-PCR, respectively, and mitochondrial function and dynamics by fluorimetry and confocal microscopy.
Results: A progressive reduction in HIF-1alpha and HUMMR protein levels and mRNA was observed with increasing AD Braak stage. Furthermore, mature neurons treated with high levels of the amyloidogenic peptide $Abeta_{1-42}$ (10 µM; 24 hours) exhibited a marked reduction in mitochondrial membrane potential, loss of HUMMR co-localization with the mitochondrial marker MTCO1, reduced number of mitochondria presented in the axons and neuritic retraction.
Conclusions: These results suggest that during the initial phases of AD pathology, HUMMR promotes the anterograde movement of mitochondria in order to cope with the energetic demands within the synapses, acting as a cell quality control mechanism; however, with the progression of the disease this mechanism fails contributing to an energetic crisis and, consequently to neuronal loss.
Sónia C. Correia has a post-doctoral fellowship from the Fundação para a Ciência e a Tecnologia (SFRH/BPD/84163/2012). This work is supported by Alzheimer's Association (NIRG-13-282387).

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0964
MITOCHONDRIA-ER MEMBRANES AND NEURODEGENERATION
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Besides plaques and tangles, which typically accumulate late in disease progression, Alzheimer disease (AD) patients often present with other features that occur earlier in the course of the disease. One such feature is mitochondrial dysfunction (e.g. changes in bioenergetics and in organellar dynamics [e.g. shape, fusion/fission, and position]), but the reason for this is unclear.
Mitochondria communicate directly with the endoplasmic reticulum (ER), and this communication facilitates interorganellar signalling and mitochondrial behavior. We recently showed that presenilins and g-secretase activity regulate ER-mitochondrial connectivity, and that this connectivity is increased significantly in presenilin-mutant cells and in cells from both familial and sporadic AD patients. Importantly, mutations in mitofusin-2 (MFN2), a protein associated both with mitochondrial fusion and with ER-mitochondrial communication, also affect connectivity, but in a direction opposite to that of the presenilins.
We have found that changes apposition of mitochondria to ER in PS and MFN2-mutant cells is highly correlated with mitochondrial bioenergetics. Together with our other preliminary data, these results imply that there is an important role of ER-mitochondrial communication in the regulation of oxidative energy metabolism. We believe that this insight has direct relevance not only to the normal regulation of energy metabolism, but also to Alzheimer disease and related dementias (and to neurodegeneration in general), where such metabolism is deranged.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-1016

ALTERED PROTEOSTASIS ENVIRONMENT CAUSES MISMETABOLISM OF MITOCHONDRIAL PROTEINS IN A DROSOPHILA MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common form of senile dementia without effective therapy. Morphological alterations and functional declines in synaptic mitochondria are observed in the brains of AD patients. These changes are thought to cause synaptic failure and degeneration. Thus, protecting synaptic mitochondria may be a potential therapeutic strategy for AD. However, the mechanism that initiates a series of mitochondrial abnormalities in the synapses remains elusive.

Using a *Drosophila* model of Amyloid β42 (Aβ42) toxicity, we have previously reported that mitochondria-targeting GFP (mito-GFP) signals were reduced in the axons and dendrites of fly brain neurons before the onset of memory defects or neurodegeneration. In the present study, we investigated the mechanism underlying these mitochondrial changes. We found that the reductions in mito-GFP signals in the synapses were not due to either morphological alterations, damages or reductions in the number of mitochondria. Rather, our results suggest that reductions in the mito-GFP signals in the synapses are due to reductions in the stability of mitochondrial proteins and/or delivery of mitochondrial proteins to mitochondria in the cell body. Interestingly, we found that a specific, but not all, chaperone protein have negative impact on the stability of mitochondrial protein and causes age-dependent behavioral deficits.

This study shows a potential mechanism initiating mitochondrial abnormality in the synapses in the pathological environment with proteostasis imbalance such as AD. These results also suggest that a certain chaperon protein may be a potential therapeutic target to prevent mitochondrial dysfunctions.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-1070

AMYLOID-BETA-INDUCED IMBALANCE BETWEEN MITOCHONDRIAL NETWORK AND BIOENERGETICS.

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Objectives: Once perceived as solitary structures, mitochondria are now recognized as highly dynamic, interconnected organelles in order to maintain the bioenergetic homeostasis in response to metabolic, cellular and environmental changes. Both mitochondrial bioenergetics and dynamics are hallmarks of amyloid-beta (Aβ)-induced neuronal toxicity in Alzheimer's disease (AD). In our study, we addressed the questions of whether Aβ contributes to abnormal mitochondrial dynamics and how this impairment affects mitochondrial bioenergetic balance in AD.
Methods: For this purpose, we evaluated mitochondrial network morphology by confocal microscopy, expression of several genes involved in mitochondrial dynamics, as well as mitochondrial bioenergetic profile including ATP level and Oxygen Consumption Rate (OCR) by using a Seahorse Bioscience XF24 in cell cycle-controlled human primary skin fibroblasts under normal and Aβ conditions.
Results: We found that mitochondrial networks oscillated between 3 distinct states (fragmented, intermediate and tubular) under normal condition. Moreover, in between the switch of tubular to fragmented mitochondrial network, we observed a transient increase in ATP level which correlated with a higher OCR in the basal respiration as well as in ATP turnover and maximal respiration. In contrast, Aβ almost completely dampened the oscillations of mitochondrial dynamics which directly lead to a decline of mitochondrial metabolism including reduced ATP level and OCR.
Conclusion: We gained new insights into the deleterious cycle between abnormal mitochondrial dynamics which seems to contribute to the decay of mitochondrial energy metabolism in the pathogenesis of AD.
This work was supported by Swiss National Science Foundation (#31000_122572) and Synapsis Foundation.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-1259

METABOLISM AND PROTEIN INTERACTOME OF THE AMYLOID PRECURSOR PROTEIN IN THE MITOCHONDRIA ASSOCIATED MEMBRANES

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Objectives: Alteration of the physical association between the Endoplasmic Reticulum (ER) and the mitochondria, known as mitochondria-associated membranes (MAMs), has been proposed to contribute to Alzheimer disease (AD) pathogenesis. To further investigate this issue, we studied the subcellular distribution of amyloid precursor protein (APP) and its proteolytic products and secretases, and analyzed the protein "interactome" of the APP and its metabolites in the MAM.

Methods: Cellular distribution of APP and its catabolites were analyzed by subcellular fractionation. We studied APP catabolites and MAM co-localization by using live cell imaging and immunofluorescence. We tested enzymatic activities of beta- and gamma-secretases. Proteomic approaches and Nano-LC/MS/MS analysis were used to reveal the APP protein "interactome" in the MAM.

Results: We revealed that APP and its catabolites accumulate in the MAMs both in cellular models overexpressing mutated APP and in brains of AD transgenic mice. We evidenced also that while the gamma secretase complex is present and active in both the MAMs and the pure mitochondria, beta secretase enzyme is present and active only in the MAMs. We demonstrated an increased ER-mitochondria contact sites in neuroblastoma cells overexpressing mutated APP and revealed the APP protein "interactome" in the MAM.

Conclusions: We demonstrated that both APP and secretases are present in the MAM where APP catabolites are also detectable. Furthermore, we showed that APP interacts with key proteins of the MAM (i.e. enzymes of lipid metabolism). These data may highlight a hitherto unrecognized role of MAM dysregulation in the pathogenesis of AD.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-1701

EVALUATION OF SKELETAL MUSCLE OXIDATIVE METABOLISM IN ALZHEIMER'S DISEASE

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Objectives: The aim of this study was to evaluate whether in patients affected by Alzheimer's Disease (AD) βAP deposits in skeletal muscle impair oxidative metabolism.

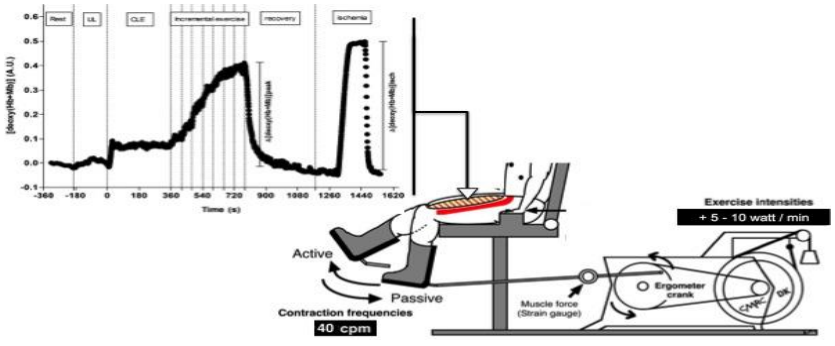
Methods: Eighteen AD (73±4.5 years, mean±SD) and twenty-six healthy control subjects (CTRL) (71.5±5.2 years) were investigated. Two incremental exercises were performed, in order to evaluate skeletal muscle oxidative metabolism: a cycloergometer (CE) and a one-leg knee extension (KE) exercise (see Figure). The following variables were determined: breath-by-breath pulmonary O_2-uptake (VO_2); cardiac output (CO); vastus lateralis muscle fractional O_2-extraction by near-infrared spectroscopy (Δ[deoxy(Hb±Mb)]); maximal voluntary contraction (MVC); total daily energy expenditure (TEE).

Results: Mean(±SE) values of the investigated variables obtained during CE and KE incremental exercise in the two groups of subjects (see Table). TEE was similar in AD and CTRL (2534±243.6 vs 2250±119.3 $kcal*day^{-1}$). As for MVC, no significant difference was found between CTRL and AD (440.5±39.5N vs 438.3±86.7N, respectively).

	CE		KE	
	AD	CTRL	AD	CTRL
Peak work-rate (W)	94.1±7.1*	128.3±8.5	50±5*	67.9±4.5
VO_2 peak ($mL*kg^{-1}*min^{-1}$)	22.0±0.8 *	26.4±1.1	10.7±0.7*	13.5±0.6
CO ($L*min^{-1}$)	14.4±0.5	15.3±0.8	10.93±0.49	11.62±0.91
Δ[deoxy(Hb±Mb)] (%)	51.05±5.8*	71.4±2.9	40.02±5.8*	61.01±4.7

*p<0.05

Conclusions: AD patients have a reduced exercise capacity compared to healthy control subjects, probably due to a reduced muscle fractional O_2-extraction capacity. The impairment of muscle oxidative function was evident also during KE, when cardiovascular constraints to oxidative metabolism were reduced.



03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-1710

INHIBITION OF AKT PHOSPHORYLATION DIMINISHES TRICARBOXYLIC ACID CYCLE ACTIVITY AND SUBSEQUENTLY RECOGNITION MEMORY DEFICIT IN RAT MODEL OF ALZHEIMER'S DISEASE

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Objectives: 3-methyladenine (3-MA), as a PI3K inhibitor, is widely used for inhibition of autophagy. Inhibition of PI3K class I leads to inhibition of Akt phosphorylation, a central molecule involved in diverse arrays of intracellular cascades in nervous system. Accordingly, in the present study, we aimed to determine the alterations of activity of specific mitochondrial enzymes of tricarboxylic acid cycle (TCA) in 3-MA-injected rats following amyloid beta (Aβ).
Methods: Activities of five critical enzymes Aconitase, α-ketoglutarate dehydrogenase (α-KGDH), malate dehydrogenase (MDH), citrate synthase (CS) and fumarase were determined using biochemical assays. Non-spatial memory was determined using novel object recognition test.
Results: Our data revealed that activities of three enzymes Aconitase, α-KGDH, and MDH reduced in the presence of 3-MA with or without Aβ insult. Although, activity of CS in 3-MA-administred rats decreased, this reduction was not significant relative to the control or Ab-injected rats and activity of fumarase did not show any changes in 3-MA-receiving rats with or without Aβ compared to the control rats. Decrease in enzyme activity in the rats receiving 3-MA and Aβ were more compared to the rats that received either alone; indicating the additive destructive effects of these two agents. In agreement with our molecular results, data obtained from behavioral test indicated that inhibition of Akt phosphorylation with or without Aβ injection impaired novel recognition memory.
Conclusions: Our results suggest that 3-MA amplified deleterious effects of Aβ by targeting central molecule Akt.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-1855

ABETA ATTACKS MITOCHONDRIAL-PROCESSING PEPTIDASE BETA SUBUNIT

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Reduction of glucose metabolism in certain regions of the brain is one of the earliest changes in Alzheimer's disease patients, which is partially attributed to the beta amyloid (Aβ)-induced dysfunction of mitochondria, the power house of the cell. How does Aβ affect the functions of mitochondria remains to be determined. Here, we present immunoprecipitation mass spectrum, co-immunoprecipitation and western blot results to show that Aβ attacks a mitochondrial enzyme, mitochondrial-processing peptidase beta subunit (PMPCP), which controls the entrance and maturation of majority of the mitochondrial proteins. Moreover, over expression of PMPCP unregulated intraneuronal Aβ accumulation, and enhanced the neural dysfunction in Aβ-expressing fruit flies. Currently, we are studying the effect of Aβ-PMPCP interaction on the processing of mitochondrial preproteins.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-2014

IMPAIRMENTS IN MITOCHONDRIAL RESPIRATORY COMPLEXES IN ANIMAL MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Objectives: Mitochondria play a fundamental role in Alzheimer's disease (AD) pathology. We have observed mitochondrial dysfunctions in AD brain neocortex, hippocampus, and have focused on characterization of mitochondrial electron transport chain complexes and oxidative stress.
Methods: Olfactory bulbectomy in mice was developed as an appropriate model of sporadic form of Alzheimer's disease. Mouse brain mitochondria were isolated from hippocampus and neocortex by differential centrifugation with Percoll gradient. Oxygen consumption was measured with the Clark-type electrode and activities of mitochondrial respiratory complexes by spectrophotometer. Lipid peroxidation level was estimated by the malondialdehyde-thiobarbituric acid, mitochondria-generated reactive oxygen species by Amplex Red. Level of soluble β-amyloid was detected by ELISA.
Results: Five weeks after bilateral olfactory bulbectomized mice displayed a significant deficiency in spatial memory, high level of soluble β-amyloid in extracts of neocortex and hippocampus as well as in mitochondria from those tissues. Impairments of the mitochondrial electron transport chain and energy metabolism has been found. We observed a significant decline in the respiratory rate, low value of respiration control ratio as well as reduced activities of cytochrome-*c*-oxidase (complex IV) and NADH:ubiquinone oxidoreductase (complex I). We have established the substantial lowering of CcOX complex content and increased ROS production. Complex I was a source of detrimental free radicals and a target for oxidative damage by them.
Conclusion: We have found mitochondrial impairments in sporadic type AD. There is link between inhibition of respiratory chain complexes, oxidative stress, and soluble β-amyloid accumulation in mitochondria.

03h. Pathophysiology & Disease Mechanisms: metabolism and insulin

ADPD5-0631

NEUROPROTECTIVE EFFECTS OF CHRONIC EXPOSURE OF SH-SY5Y TO LOW LITHIUM CONCENTRATION INVOLVE GLYCOLYSIS STIMULATION, EXTRACELLULAR PYRUVATE ACCUMULATION AND RESISTANCE TO OXIDATIVE STRESS

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Recent studies suggest that lithium protects neurons from death induced by a wide array of neurotoxic insults, stimulates neurogenesis and could be used to prevent age-related neurodegenerative diseases. In this study, SH-SY5Y human neuronal cells were cultured in the absence (C) or in presence (Li+) of a low lithium concentration (0.5 mM Li_2CO_3, i.e. 1mM lithium ion) for 25 to 50 weeks. In the course of treatment, growth rate of (C) and (Li+) cells was regularly analyzed using Alamar Blue dye. Resistance to oxidative stress was investigated by evaluating 1) the adverse effects of high concentrations of lithium (4-8 mM) or glutamate (20-90 mM) on cell growth rate, 2) the levels of lipid peroxidation (TBARS) and total glutathione, 3) the expression levels of the anti apoptotic Bcl-2 protein. In addition, glucose metabolism was investigated by analyzing selected metabolites in culture media and cell extracts by 1H NMR spectroscopy. As compared to (C), (Li+) cells multiplied faster and were more resistant to stress, as evidenced by lower dose-dependent decrease of Alamar Blue reduction and dose-dependent increase of TBARS levels induced by toxic doses of lithium and glutamate. Total glutathione content and Bcl-2 level were increased in (Li+) cells. Glucose consumption and glycolytic activity were enhanced in Li+ cells and an important release of pyruvate was observed. We conclude that chronic exposure to lithium induces adaptive changes in metabolism of SH-SY5Y cells involving a higher cell growth rate and a better resistance to oxidative stress.

03h. Pathophysiology & Disease Mechanisms: metabolism and insulin

ADPD5-0676

THE INTERPLAY BETWEEN OXIDATIVE STRESS AND INSULIN RESISTANCE DURING AGEING AND ALZHEIMER DISEASE

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Objectives: Insulin plays a key role in learning and memory. Given that, insulin resistance (i.r.) – defined as inadequate response to insulin by target cells due to a down-regulated expression of the insulin receptor (IR), and/or the insulin receptor substrate (IRS) proteins – has been proposed as pathogenic signaling pathway associated with Alzheimer disease (AD) onset/progression. As previously reported, increased oxidative/nitrosative stress levels (OS/NS) were associated with reduced insulin secretion and sensitivity, thus suggesting OS/NS may underlie i.r. in AD. Here, we aimed to understand the crosstalk between age- and AD-associated changes of (i) OS/NS and (ii) insulin signaling cascade.
Methods: We evaluated changes of (i) protein carbonyls, (ii) protein-bound 4-hydroxy-2-nonenal and (iii) 3-nitrotyrosine levels as well as (iv) IR and IRS1 protein levels and (v) IRS1 (Ser307) phosphorylation (pIRS1) in the hippocampus of 3-, 6-, 12- and 18-month old 3xTgAD mice and their WT littermate controls as effect of either age or AD pathology.
Results: An early elevation of OS/NS markers was found in 3xTgAD mice starting at 6 months of age, whereas WT mice showed increased OS/NS only at 18 months. Interestingly, age-associated changes of OS/NS parallel with the rise of pIRS1 both in 3xTgAD and WT mice. Further, a reduction of IR and IRS1 levels and increased pIRS1 with the progression of AD was observed.
Conclusions: Our findings suggest that the elevation of OS/NS and the impairment of insulin signaling cascade are early events in AD pathology and play a crucial role in disease progression.

03h. Pathophysiology & Disease Mechanisms: metabolism and insulin

ADPD5-0816
MARKERS OF INSULIN RESISTANCE IN RATS WITH MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is an untreatable neurodegenerative disease that deteriorates memory. In recent years, attention of scientists is focused on two key pathogenetic factors of AD. There are neuroinflammation and insulin resistance. However, the cellular and molecular mechanisms that connect insulin resistance, neuroinflammation and Alzheimer's pathogenesis remain largely unexplained.
The objective is to study the potential targets for the correction of insulin resistance in the brain tissue: IRAP, IL-18, and NLRP3.
Wistar rats (male; 200-250 g) were deeply anesthetized with chloral hydrate (0,35 mg/kg) and then fixed in the stereotaxic frame. Rats were randomly divided into two groups. Experimental group (administration of beta-amyloid 1-42 (5 µl) in Cornu Ammonis (CA1) of hippocampus bilaterally. A control group of sham-operated animals were injected with phosphate-buffered saline.
To investigate the expression of IRAP, NLRP3 and IL-18 in the hippocampus and olfactory bulb we performed immunohistochemical analysis of the brain samples taken from rats with Alzheimer's disease and sham- operated animals. In addition to this, we have carried out behavioral tests (Morris water maze, plus elevated maze).
We have measured expression of IRAP, NLRP3 and IL-18 in the neurons and astrocytes. We have shown disturbance of expression these markers after modeling AD. Since neuroinflammation and insulin resistance play an important role in the development of synaptic dysfunction and neurodegeneration, they can be regarded as potential targets for therapeutic intervention to prevent disease progression.

03h. Pathophysiology & Disease Mechanisms: metabolism and insulin

ADPD5-0874

HYPERGLYCEMIA MODULATES EXTRACELLULAR AMYLOID-BETA LEVELS AND NEURONAL ACTIVITY IN VIVO

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Objectives: Epidemiological studies suggest that type-2-diabetics, or those with elevated blood glucose levels independent of diabetes, have an increased risk for developing Alzheimer's disease (AD) and dementia. These observations suggest that abnormal glucose metabolism likely plays a role in the development of some aspects of AD. Thus, we investigated the effects of alterations in peripheral glucose homeostasis on neuronal activity and amyloid-β metabolism.

Methods: We teamed glucose clamps, a way to manipulate blood glucose levels, with in vivo microdialysis, a method to sample metabolites from the brain's interstitial fluid (ISF). This combination allows us to provide an acute hyperglycemic challenge to awake, freely moving mice while assessing real-time changes in Aß, glucose, and lactate within the brain's ISF.

Results: Acute hyperglycemia increases hippocampal ISF Aβ levels in young APP/PS1 mice, prior to the plaque deposition. In aged APP/PS1 mice with significant plaque pathology, a similar hyperglycemic challenge results in a further increase in ISF Aβ concentrations, suggesting that age- and pathology-dependent changes affect the brain's response to a metabolic insult. We identified inward rectifying, ATP-sensitive potassium (K_{ATP}) channels as a mechanistic link between hyperglycemia, neuronal excitability and Aß metabolism. Pharmacological manipulation of K_{ATP} channels results in altered neuronal activity and ISF Aβ levels. Furthermore, we found that hyperglycemia-dependent increases in ISF Aβ were blocked by K_{ATP} channel manipulation.

Conclusions: Taken together, this suggests K_{ATP} channel activation mediates the response of hippocampal neurons to elevated blood glucose levels by coupling metabolism with neuronal activity and ISF Aβ levels. Funding: NS080320(SLMR), NS080675(DH, MER)

03h. Pathophysiology & Disease Mechanisms: metabolism and insulin

ADPD5-1252

CHARACTERISING IMPAIRED INSULIN SIGNALLING IN HUMAN ASTROCYTES: A ROLE IN THE DEVELOPMENT OF ALZHEIMER'S DISEASE?

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Objectives: Our group has previously shown that astrocyte hyper-trophy and injury occur early in the progression of AD neuropathology and using microarray analysis, that the insulin/IGF-1 signalling pathway is downregulated as Alzheimer-type pathology develops. We have developed a model of impaired insulin signalling in human astrocytes to investigate how reduced signalling through this pathway affects astrocyte function and their ability to respond to stress.
Methods: Impaired signalling was induced in human primary astrocytes using a combined insulin+fructose treatment protocol and confirmed by immunoblotting for insulin receptor as well as the downstream targets Akt and ERK1/2. Cell growth/viability was determined using Cyquant and MTT assays. To assess the response of insulin-impaired astrocytes to cellular stress astrocytes were treated with sub-lethal concentrations of hydrogen peroxide.
Results: A combined insulin+fructose (4d) treatment protocol resulted in decreased expression of IR, but not IGF-1R, and decreased phosphorylation of the downstream target Akt but not ERK1/2. Decreased IR persisted after 7d but there is recovery in Akt activity suggesting other signalling pathways eventually compensate for the reduction in IR. Impaired insulin signalling had no effect on astrocyte growth/viability. The effect of oxidative stress on insulin impaired astrocytes is currently under investigation.
Conclusions: We have developed a model of impaired insulin signalling in human astrocytes using a combined insulin-fructose treatment protocol and are now investigating the impact of this on astrocyte function. This is important since astrocytes maintain brain homeostasis and deliver essential substances to neurons and impaired astrocyte function may play a role in AD progression.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0247

MINOCYCLINE MODULATION THE NEUROPROTECTIVE EFFECT OF COENZYME Q10 AGAINST AMYLOID BETA 1-42 INDUCED COGNITIVE DYSFUNCTION IN RATS: BEHAVIORAL AND BIOCHEMICAL EVIDENCE

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Objective- The aim of the study was to investigate the neuroprotective effect of CoQ10 and its modulation through minocycline against Aβ(1-42) induced cognitive dysfunction and biochemical alterations in hippocampus and cerebral cortex of rats.

Materials and Methods

Male SD rat were received bilateral, intrahippocampus Aβ(1-42) [1µg/µl] injection. Galantamine (2 mg//kg), CoQ10 (20, 40 mg/kg), Minocycline (50 mg/kg), were administered for period of 21 days. Various behavioral parameters (body weight, locomotor activity, elevated plus maze and Morris water maze) followed by biochemical estimations (LPO, GSH, Catalase, nitrite, SOD, AChE) were assessed in discrete areas of hippocampus and cortex.

Results

Bilateral intrahippocampus Aβ(1-42) administration significantly reduced body weight, impaired cognitive performance and oxidative damage as compared to sham treatment. CoQ10 (20, 40 mg/kg) treatment significantly attenuated body weight and improved cognitive performance in both tests (i.e. shortened transfer latency in elevated plus maze test, Morris water maze task as well as time spent in target quadrant), attenuated AChE level and oxidative damage (reduced lipid peroxidation, nitrite level and restoration of reduced GSH and Catalase activity) in both hippocampus and cortex as compared to control. Further, combination of CoQ10 (20, 40 mg/kg) with minocycline (50 mg/kg) significantly potentiated their protective effect as compared to their effect per se.

Conclusion

The present study highlight the involvement of possible microglia modulatory mechanism of CoQ10 against Aβ(1-42) induced cognitive dysfunction and oxidative damage.

Keywords

Alzheimer's disease, Ab(1-42), Coenzyme Q10, Minocycline, oxidative stress

Abbreviations

AD- Alzheimer's disease, CoQ10- coenzyme Q10, Ab- Amyloid beta

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0354
THE ROLE OF ASTROCYTES IN AIZHEIMER'S DISEASE
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Alzheimer's disease (AD) is a neurodegenerative disease characterized by the aggregation of amyloid β-peptide (Aβ) into β-sheet-rich fibrils. Although plaques containing Aβ fibrils have been viewed as the conventional hallmark of AD. The actual role of astrocytes in AD remains elusive, as they seem to adopt different functions dependent on disease progression and the extent of accompanying parenchymal inflammation. Increased production of amyloid β-peptide (Aβ) and altered processing of tau in Alzheimer's disease (AD) are associated with synaptic dysfunction, neuronal death and cognitive and behavioural deficits. Neuroinflammation is also a prominent feature of AD brain and considerable evidence indicates that inflammatory events play a significant role in modulating the progression of AD. The role of microglia in AD inflammation has long been acknowledged. Substantial evidence now demonstrates that astrocyte-mediated inflammatory responses also influence pathology development, synapse health and neurodegeneration in AD.Astrocytes may contribute to the clearance of amyloid β-peptide (Aβ) and restrict the spread of inflammation in the brain. Conversely, they may contribute to neurodegeneration in AD by releasing neurotoxins and neglecting crucial metabolic roles. Several anti-inflammatory therapies targeting astrocytes show significant benefit in models of disease, particularly with respect to tau-associated neurodegeneration. However, the effectiveness of these approaches is complex, since modulating inflammatory pathways often has opposing effects on the development of tau and amyloid pathology. An increased understanding of interactions between astrocytes and neurons under different conditions is required for the development of safe and effective astrocyte-based therapies for AD and related neurodegenerative diseases.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0454

MICROGLIAL RESPONSES TO ENVIRONMENTAL ENRICHMENT IN ALZHEIMER'S DISEASE MODELS

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Objectives

Emerging evidence suggests the central involvement of malfunctioning microglia and dysregulated neuro-inflammation in the progression of Alzheimer's disease, The interaction between soluble amyloid-beta oligomers (oAbeta)—the major cytotoxic species – and microglia is being studied intensively. Environmental enrichment (EE) has been examined in various mouse models as potentially beneficial against features of AD, and a few recent studies suggest possible cross-talk between EE and microglial biology. Our goal is to address whether and how the EE paradigm alters microglia and/or brain-entering monocytes and enhances resistance against oAbeta cytotoxicity in adult wild-type mice.

Methods

We perform stereotactic surgery to accurately microdeliver oAbeta species to hippocampus or lateral ventricle of WT mice housed under different conditions. To evaluate microglia, we perform a) immunohistochemistry on brain tissue using microglial-specific or functionally related protein markers followed by confocal microscopy and image analysis; b) microglial mRNA profiling, and c) *in vivo* microdialysis with a 1000 kDa MW probe.

Results

We observed a consistent ~20% increase in microglial density and associated morphological changes in the dentate gyrus of wt mice after 4+ weeks of EE. We also found decreased microglial responses of EE vs. control mice to oAbeta, shown by expression levels of a set of inflammatory genes measured by qPCR and Nanostring.

Conclusions

Prolonged exposure to a novel, enriched environment has an immunomodulating effect on microglia *in vivo* at the cellular and molecular levels, including changes in microglial density, morphology, and mRNA response profile to oAbeta, which may counteract Amyloid-beta toxicity in the brain.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0569

THE FUNCTIONAL ROLE OF FYNT TYROSINE KINASE IN REACTIVE ASTROCYTES: RELEVANCE TO ALZHEIMER'S DISEASE

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Introduction:
Fyn tyrosine kinase has been proposed to have a pathogenic role in Alzheimer's disease (AD). Fyn exists in two major alternative spliced isoform, FynB as the predominant isoform found in the brain while FynT in cells of hematopoietic origins. Our recent findings revealed that isoform FynT was selectively up-regulated in AD brain and associated with two neuropathological hallmarks: neurofibrillary tangles and reactive astrogliosis.

***Objective*:**
In this study, we would like to determine the functional role of FynT tyrosine kinase in reactive astrogliosis, particularly in modulating TNFα mediated NF-kB signaling pathway in association with neuroinflammation in AD.

Approaches:
(1) Primary rat astrocytes treated with β-amyloid were monitored for changes of FynT and FynB expression; (2) Immortalized normal human astrocytes (iNHA) stably expressing FynT constitutive kinase active (FynT-CA) or kinase dead (FynT-KD) were treated with or without TNFα and monitored up to 3 day for expression changes of astrocytic markers, proinflammatory cytokines and genes associated with NF-kB signaling pathway.

Results:
(1) β-amyloid induced selective up-regulation of FynT with no change of FynB expression in rat primary astrocytes; (2) The observation of TNFα-induced up-regulation of astrocytic marker and proinflammatory cytokines in FynT-CA clones was attenuated in FynT-KD clones; (3) FynT kinase activity modulate TNFα-induced biphasic expression of IL-1β and IL-6 at the late phase, which was likely associated with the auto-regulatory feedback loop of NF-kB signaling pathway.

Conclusion:
In summary, our findings suggested that FynT may be a potential neuroinflammatory mediator associated with astrocyte activation in the pathogenesis of AD.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0716

A SPATIAL ANALYSIS IN APP/PS1 MICE REVEALS THAT ASTROCYTES DO NOT MIGRATE TO AMYLOID-BETA PLAQUES

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Objectives. The clustering of GFAP immunopositive astrocytes around amyloid-beta plaques in Alzheimer's disease has led to the widespread assumption that plaques attract astrocytes. However, recent studies show that astrocytes stay put in injury. Here we re-examine whether astrocytes migrate to plaques by analyzing with mathematical functions and computer modeling the topology of astrocytes in 3D images obtained by 2-photon microscopy from living APP/PS1 mice and wild type littermates.
Methods. Astrocytes were identified with topically applied sulforhodamine 101, a fluorescent dye that, unlike GFAP, labels both reactive and non reactive astrocytes, whereas amyloid-beta plaques were labeled with methoxy-XO4. Changes in astrocyte position were examined by the *g(r)* function of astrocyte-to-astrocyte spacing, and by domain-size distributions modeled by Voronoi tessellation (L_c) in the astrocytes closest to the plaques.
Results. Cortical astrocyte topology fits a model akin to a liquid of hard spheres that exclude each other in a confined space. This supports the ordered spacing of astrocytes based upon domains. Plaques do not disturb this arrangement except at very large plaque loads but, locally, they cause a slight but statistically significant expansion of Voronoi domains up to three tiers of astrocytes around plaques. The expansions mean that plaques have pushed astrocytes away 1-4 microns according to simulations.
Conclusions. One, repulsive chemoactive agents may be essential to maintain astrocyte spacing. Two, astrocytes respond to plaque-induced neuropil injury by showing strained inter-astrocyte interactions and changing phenotype, including increased production of GFAP and development of a 'reactive' appearance, rather than proliferating or migrating towards plaques.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0757

NEUTROPHILS INDUCE ALZHEIMER'S DISEASE-LIKE PATHOLOGY AND COGNITIVE DECLINE VIA A MECHANISM DEPENDENT ON LFA-1 INTEGRIN

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Objectives: Inflammation is a pathological hallmark of Alzheimer's disease and understanding the underlying mechanisms may facilitate the development of new treatments. Our aim was to study the role of inflammation mechanisms in the pathogenesis of AD.

Methods: Confocal microscopy, intravital microscopy studies using two-photon microscopy (TPM), fear conditioning and Y maze tests were performed in animal models of AD. Adhesion assays were performed on integrin ligands and integrin affinity was measured using ImageStream technology. Neuropathological studies were performed on human samples and brains from AD mice.

Results: Using mice with five familial Alzheimer's disease (5xFAD) mutations presenting amyloid pathology, and 3xTg-AD mice with both amyloid and tau pathology, we found neutrophils extravasating in areas with amyloid beta (Abeta) deposits, releasing neutrophil extracellular traps (NETs) and producing IL-17. Abeta1-42 peptide triggered the LFA-1 integrin high-affinity state and rapid neutrophil adhesion. TPM experiments showed that LFA-1 integrin controls neutrophil extravasation and intraparenchymal motility. Neutrophil depletion or the inhibition of neutrophil trafficking using LFA-1 genetic ablation or an anti-LFA-1 antibody dramatically rescued memory loss in 5xFAD and 3xTg-AD mice. Interfering with neutrophil activity also reduced microglial density and activation, amyloid deposition, tau phosphorylation and restored synaptic protein loss. In Alzheimer's patients, neutrophils adhered and spread inside brain venules or migrated into the parenchyma and released NETs in larger numbers than in control subjects.

Conclusions: Our results demonstrate that neutrophils induce cognitive impairment and neuropathological changes suggesting that the inhibition of neutrophil trafficking may represent a new therapeutic strategy to address Alzheimer's disease.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0822
INFLAMMASOME FORMATION IN THE RAT BRAIN WITH MODEL OF ALZHEIMER'S DISEASE

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Neuroinflammation has a significant influence on the structural and functional plasticity of the brain (Dupret D., 2008). Alteration of neurogenesis is one of the key mechanisms of brain damage in Alzheimer's disease (AD) (Chuang T.T., 2010).
The objective is to study the molecular mechanisms of inflammasome formation in the neurodegeneration.
We used Wistar male rats aged 9 months. The animal model of AD was induced by injections of beta-amyloid into CA1 area. (Li X. et al., 2011). We tested memory consolidation and social recognition memory (five-trial social test). At two week postinjection brains were removed. Immunohistochemistry staining was used to determine NLRP1, NLRP3 expression in the neuronal and glial cells. We have evaluated expression of NLRP1 and NLRP3 in the cells expressing CD133 (stem cells), PNCAM (neuronal progenitor), NeuN (mature neurons), GFAP and S100beta positive cells (glial cells).
The changes in the functional activity of astrocytes in the in chronic neurodegeneration were associated with the production of inflammasomes and production of proinflammatory cytokines. It determines the nature of the participation of astroglial cells in the process of neurogenesis. Also we have investigated proliferation, migration activity of neuronal precursors and the efficiency of the integration of post-mitotic neurons in the synaptic chains depending on the nature of the expression different types of inflammasomes in experimental Alzheimer's disease.
The study of the molecular mechanisms of the effect of astrocytes and humoral inflammation mediators on neurogenesis may allow the identification of new target molecules for pharmacological correction of chronic neurodegeneration.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0944

MICROGLIA DO NOT MODULATE ABETA OR TAU PATHOLOGY IN THE ALZHEIMER DISEASE BRAIN, BUT CONTRIBUTE TO COGNITIVE IMPAIRMENTS

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Objectives: Chronic neuroinflammation is a key feature of the Alzheimer's disease (AD) brain. Much of this neuroinflammation is mediated by microglia that surround dense core plaques and react to their presence, but it is unknown if this response is protective (by limiting plaque growth), or harmful (by contributing to cognitive decline and/or tau hyperphosphorylation). We set out to directly determine the roles that microglia play in the disease process.
Methods: We have shown that microglia in the adult mouse brain are dependent upon colony-stimulating factor 1 receptor (CSF1R) signaling and that inhibitors rapidly eliminate >95% of all microglia CNS wide. 23-month-old 3xTg-AD mice were treated with a selective CSF1R antagonist for 3 months resulting in sustained elimination of ~90% of microglia for that duration. We then used behavioural testing followed by pathological analyses using confocal microscopy, ELISA and western blotting to measure microglia densities, plaque number and size, and levels of hyperphosphorylated tau species.
Results: Despite having no microglia for 3 months, Abeta/plaque levels were not altered in any form. Tau levels or hyperphosphorylation state were likewise unaltered by the absence of microglia. Cognitive testing revealed improvements in learning with chronic microglial-elimination, while levels of Il-1beta and TNFalpha were markedly reduced.
Conclusions: Together these results directly demonstrate that microglia in the aged AD brain do not contribute to the pathogenesis of Abeta or tau pathology but do contribute to the cognitive decline.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0951

AMYLOID-INDUCED PRO-INFLAMMATORY MECHANISMS AND ROS GENERATION IN ASTROCYTIC/GLIAL CELLS: RELEVANCE FOR THE INTEGRITY OF THE NEUROVASCULAR UNIT

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Objective: Vascular deposition of amyloid, a universal feature in Alzheimer's disease (AD), severely compromises the integrity of the neurovascular unit, a dynamic entity encompassing functional interactions among cells of the microvasculature, neurons, and astrocytic/glial populations. The complexity of the cellular mechanisms elicited by amyloid in astrocytic/glial cells and their relationship to the induction of pro-inflammatory conditions capable of affecting microvessel function/permeability remain to be fully elucidated. In this work we aimed to provide insight into the molecular pathways affected and identify potential new targets for drug discovery.
Methods: Astrocytic/glioma cells were challenged with wild-type Abeta and the E22Q vasculotropic variant associated with cerebrovascular deposition and hemorrhagic clinical phenotypes. A combination of FACs-analyzed bead arrays, ELISA, zymography, and confocal studies were employed to evaluate production of pro-inflammatory cytokines, activation of MMPs, and ROS generation whereas the vitamin-E analog Trolox was tested for prevention/amelioration of these detrimental cellular pathways.
Results: Oligomeric-Abeta triggered elevated production of the pro-inflammatory mediators IL-6, IL-8, and IFN-γ, enhanced activation of MMP-2, exacerbated ROS production, and cell death. In all cases, challenge with E22Q translated into a more pronounced response, in agreement with the high oligomerization tendency of the variant and the aggressiveness and early onset of the clinical phenotype. Trolox not only inhibited ROS production and MMP-2 activation, but also preserved cellular integrity and viability, highlighting the primary role of ROS in the initiation of amyloid-induced cell death pathways.
Conclusions: Our data emphasizes the detrimental role of astrocyte/glia-initiated Abeta-mediated pro-inflammatory pathways for the integrity of the neurovascular unit

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1085

THE EFFECT OF UBIQUILIN-1 ON ALZHEIMER'S DISEASE MOLECULAR MECHANISMS DURING NEUROINFLAMMATION

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Objectives: Ubiquilin-1 is a protein, which is involved in the regulation of levels, subcellular localization, aggregation and degradation of several proteins associated with the pathogenesis of Alzheimer's (AD) and other neurodegenerative diseases. Ubiquilin-1 also plays a role in unfolded protein response, endoplasmic reticulum and oxidative stress, and autophagy, mechanisms that are centrally involved in neurodegeneration.

Methods: The role of ubiquilin-1 during neuroinflammation was investigated in the co-cultures of E18 mouse primary cortical neurons and BV2 microglial cells. Neuroinflammation was induced by treating ubiquilin-1 overexpressing and control cells with lipopolysaccharide (LPS, 200ng/ml) and interferon-gamma (IFN-gamma, 5ng/ml) for 48 hours. As an outcome, neuronal viability, secretion of inflammatory cytokines, and levels of several AD-related proteins were analyzed.

Results: LPS and IFN-gamma treatment significantly induced the secretion of tumor necrosis factor alpha (TNFalpha) in neuron- BV2 cell co-cultures, when compared to untreated controls. Ubiquilin-1 overexpression further increased TNFalpha secretion and concomitantly significantly decreased the neuronal viability under neuroinflammation. Furthermore, ubiquilin-1 overexpression increased the levels of 3R-tau and BACE1 under neuroinflammation. LPS and IFN-gamma treatment also slightly, but not significantly, increased BACE1 levels in control cells without ubiquilin-1 overexpression.

Conclusions: These data indicate that ubiquilin-1 overexpression may aggravate the inflammatory response and neuronal cell death during acute neuroinflammation in co-cultures of primary cortical neurons and BV2-microglial cells. Together with previous reports, these results suggest that ubiquilin-1 may have diverse effects during different cellular stress conditions associated with neurodegeneration.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1111

TUMOR NECROSIS FACTOR-ALPHA AS A COMMON MEDIATOR IN ALZHEIMER'S DISEASE AND RHEUMATOID ARTHRITIS PATHOGENESIS IN TRANSGENIC MICE

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1. Objectives

TNF-α is a pro-inflammatory cytokine, involved in many autoimmune and neurodegenerative diseases, including rheumatoid arthritis (RA) and Alzheimer's disease (AD). In the brain TNF-α is synthesized by microglia and astrocytes. TNF-α is upregulated in cerebrospinal fluid and serum of AD patients, and colocalizes with amyloid plaques. TNF-α mediates the induction and maintenance of chronic inflammation in rheumatoid arthritis, and it has been reported that RA patients are protected from AD. To examine the role of TNF-α in AD and elucidate the molecular and cellular mechanisms involved we have used an established AD transgenic mouse model (5xFAD).

2. Methods

To assess the therapeutical potential of TNF-α inhibition in AD we have treated 5xFAD mice before the formation of amyloid plaques with infliximab, a TNF-α neutralizing antibody currently used in the treatment of RA patients. We have also genetically depleted TNF-α by mating 5xFAD with TNF-α knock-out mice.

3. Results

Our analysis has shown that inhibition of TNF-α can confer a protective effect in amyloid deposition. To examine the potential effect of TNF-α overexpression in AD we have employed a transgenic mouse that highly expresses human TNF-α (Tg197) and develops rheumatoid arthritis as well as neuroinflammation. Double transgenic mice (5xFAD/Tg197) are currently analyzed for amyloid deposition and brain inflammatory response, and treated with infliximab, which restores the arthritic phenotype, to evaluate the effect of anti-TNFα treatment in the AD-like pathology.

4. Conclusions

Our data provide evidence that modulating TNF-α expression can be a potent therapeutical approach in Alzheimer's disease treatment.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1127

LOSS OF REACTIVE ASTROCYTES IN APP23 MICE EXACERBATES DISEASE PROGRESSION AND ALTERS INFLAMMATORY PROFILE

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Objectives: Astrocytes actively contribute to neuronal functioning under normal and pathological conditions. In Alzheimer's disease (AD), they become reactive: clearing amyloid-beta and restricting the spread of neuroinflammation. However, they may also contribute to neurodegeneration by releasing neurotoxins and neglecting their crucial metabolic roles. We sought to establish the role of reactive astrocytes in AD by assessing the neuropathological consequences of ablating astrocytic proliferation in the APP23 mouse model.

Methods: APP23 mice were bred with GFAP-TK mice (APP/GFAP-TK), in which herpes simplex virus thymidine kinase (HSV-TK) is targeted to astrocytes via the GFAP promoter. At 9 months old, mice were infused into the right lateral ventricle with the antiviral drug, ganciclovir (GCV; $11\mu g \cdot \mu l^{-1} \cdot hr^{-1}$), or saline to ablate proliferating astrocytes. APP23 mice were also infused with GCV to control for the effect of drug infusion alone. Following this, changes in AD-like pathology were assessed.

Results: Ki67 staining confirmed that APP/GFAP-TK mice treated with GCV had a significant reduction in proliferating astrocytes in the cortex and hippocampus. The loss of proliferating astrocytes resulted in significantly increased levels of amyloid-beta 42, as measured by ELISA, concomitant with reductions in amyloid-beta staining with the pan-amyloid-beta antibody 6C3 in the cortex and hippocampus. In addition, APP/GFAP-TK mice treated with GCV showed significantly increased expression of IL1-beta in the cortex, suggesting that ablation of proliferating astrocytes leads to an enhanced pro-inflammatory response.

Conclusions: These results suggest that a loss of proliferating, reactive astrocytes in AD exacerbates disease progression, possibly via disruption of amyloid clearance and enhanced neuroinflammation.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1196

EFFECT OF AD-RISK VARIANTS ON THE PROCESSING AND BINDING PROPERTIES OF TREM2.

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Objective We recently showed that TREM2, a microglia receptor involved in the phagocytosis of apoptotic neurons, is implicated in LOAD. In our case control series, R47H TREM2 showed significant association with LOAD (OR: 4, $p = 6.6\times10^{-9}$). In the same series, we found that a second TREM2 missense variant, D87N, was also associated with LOAD (p=0.02). The goal of this study was to understand how the R47H and D87N variants affect the disease pathophysiology.
Methods BV2 and 293 cells expressing WT, R47 and D87N TREM2 were used to analyze TREM2 processing. This analysis included cell surface and total expression, shedding into the medium, and susceptibility to endoH and PNGaseF enzymes. For binding experiments 293 cells were transfected with constructs encoding WT, R47H, and D87N extracellular domains of TREM2. Medium supernatants from these transfectants were incubated with apoptotic N2a cells. Analysis of cells extracts and media was done by Western blot.
Results The Western blot profiles of the de- and fully glycosylated WT, R47H and D87N TREM2 were undistinguishably. Shedding of the TREM2 extracellular domain into the medium was reduced by the D87N but not the R47H mutation. On the other hand, when the binding of TREM2 extracellular domain to apoptotic neuronal cells was analyzed, it showed that only the R47H mutation significantly reduced the association of the TREM2 extracellular domain with apoptotic N2a cells as compared to WT.
Conclusion Our data suggest that AD-risk variants could affect TREM2 function either by impairing shedding or ligand binding in apoptotic neurons.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1227

CONTRIBUTION OF ASTROCYTIC INFLAMMASOME ACTIVITY IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

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Objectives: The proinflammatory cytokine IL1β is overexpressed in Alzheimer disease (AD) as a key regulator of neuroinflammation. Aβ peptide triggers activation of inflammasomes (protein complex responsible for IL1β maturation) in microglia, but nothing is known about activation of inflammasomes in astrocytes. We studied Aβ-mediated activation of astrocytic inflammasomes and its relevance in an AD mouse model.

Methods: ASC (a key protein in inflammasome complex) knockout mice were crossed with 5xFAD mice and tested for spatial reference memory using the Morris water maze, and amyloid load was quantified using thioflavine S staining. Astrocytes from ASC mice were cultured to measure IL1β production (ECLIA), to evaluate phagocytosis of labelled microbeads and to quantify inflammatory cytokines expression (qPCR).

Results: Cultured astrocytes primed with LPS and treated with fibrillar Aβ produced IL1β resulting from inflammasome activation mediated by Aβ phagocytosis and cathepsin B enzymatic activity. IL1β production was significantly decreased in ASC +/- astrocytes, in which a compensatory expression of TNFα and IL6 was measured. Moreover, ASC+/- astrocytes displayed a higher phagocytic activity as compared to ASC+/+ and ASC -/- cells. A significant decrease in amyloid load was measured in brain of 5xFAD mice carrying the ASC +/- genotype, most likely resulting from increased phagocytosis measured in ASC +/- astrocytes. In addition, rescue of spatial reference memory was observed in these mice as compared to 5xFAD mice.

Conclusion: All together, our results demonstrate that Aβ-mediated activation of astrocytic inflammasomes is involved in Aβ clearance and IL1β production in a well-established mouse model of AD.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1307

THE ROLE OF M1 AND M2 PHENOTYES ON THE UPTAKE AND NEURON-TO-NEURON TRANSMISSION OF AMYLOID-BETA OLIGOMERS

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Objectives: Neuroinflammation is characterized as playing an influential role in AD pathology; however the mechanisms surrounding this phenomenon remain poorly understood. Microglia are responsible for a majority of these effects, characterized by either pro- (M_1) or anti-inflammatory (M_2) phenotypes. In this study, we are investigating the effects of M_1 and M_2 conditioned macrophage media (as a peripheral analogue to microglia) on the uptake and transfer of oligomeric amyloid-beta (oAb).

Methods: Differentiated human SH-SY5Y cells were pre-treated with M_0, M_1, or M_2 conditioned media and the effect on oAb uptake into the cells was investigated. To study the effect on the neuron-to-neuron transfer of oAb we used a 3D gel co-culture system; acceptor cells were labeled with GFP, and donor cells were loaded with Alexa-700-labelled oAβ and seeded on top of the acceptor cells. oAb uptake and transfer was quantified by confocal microscopy and flow cytometry. The cytokine profile of the conditioned media will be analysed by MSD.

Results: Our preliminary data suggests that the uptake of oA-beta by neuron-like cells is inhibited by the pre-treatment of the M_2 medium. This system will be further employed to quantify neuron-to-neuron transfer of oA-beta.

Conclusions: Chronic activation of the M_1 phenotype is associated with neurotoxic activities and while M_2 plays a more protective role. Our results indicate that the M_2 phenotype could reduce the uptake of oAb in neuronally differentiated SH-SY5Y cells. Intervention in the microglial M_1/M_2 polarization pathway may be a suitable avenue for future treatments in AD.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1377

THE INFLUENCE OF ALZHEIMER'S DISEASE-LIKE AMYLOID PATHOLOGY ON THE IMMUNE SURVEILLANCE IN THE BRAIN

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Objectives. Chronic brain inflammation is a hallmark of Alzheimer's disease (AD); however, no protective autoimmunity against amyloid beta peptide (Abeta) is observed so far. Here, we tested the hypothesis that Abeta alters brain immune surveillance.
Methods. Antigen presenting cell (APC) and T cell numbers and phenotype were studied using e*x vivo* analysis via flow cytometry and confocal microscopy. Results from aged transgenic mouse models over-expressing amyloid precursor protein were compared with non-transgenic, age-matched littermates.
Results. In SweArc-transgenic mice we have observed reduced major histocompatibility class II (MHC-II) surface expression on APCs. Further analyses has revealed that most MHC-II accumulated intracellularly, suggesting an immature APC phenotype. Furthermore, we found a significant increase in brain $CD8^+$ T cell numbers with low expression of IFNgamma in parallel. We could confirm the altered MHC-II expression in brain APCs from a second model, the APP-PS1deltaE9. T cells here displayed a reduction of both IFNgamma and TNFalpha expression. No differences were observed neither in APC from cerebellum nor splenic T cells in transgenic and control animals, indicating a cerebrum and amyloid-specific effect.
Conclusions. Our preliminary results suggest that amyloid accumulation in the brain is accompanied by significant alterations in both MHC-II expression and T cell cytokine production. Further studies using both basic *in vitro* and *in vivo* approaches are underway to further explore the possibility that antigen presentation and immune surveillance are defective in the context of AD-like amyloid pathology. The results of these studies will help elucidating the role of the immune system in AD.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1450

ROLES OF PRO-RESOLVING LIPID MEDIATORS IN ALZHEIMER'S DISEASE, NEURONAL CELL SURVIVAL AND BETA-AMYLOID 42 PHAGOCYTOSIS BY MICROGLIA

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Objectives: Inflammation is a prominent feature in Alzheimer's disease (AD). Recent results suggest that chronic inflammation can be a consequence of a failure to resolve the inflammation. Resolution of inflammation is mediated by several families of lipid mediators, coined the specialized pro-resolving lipid mediators (SPMs) that include the resolvins, protectins, maresins and lipoxins. Reduced levels of SPMs have been shown in AD hippocampus, and this correlated with decreased cognitive state. The aim was to further characterize the resolution pathwayin AD, by analysing the entorhinal cortex (ENT), the area first affected in AD pathogenesis, and to investigate whether stimulating resolution of inflammation is beneficial in AD-related cellular models.
Methods: ENT tissue from postmortem AD brain was analyzed using liquid chromatography-tandem mass spectrometry (LC-MS-MS) approach. The effects of SPMs on neuronal cell death was analysed in a model of staurosporine (STS)-induced cell death in neuroblastoma SH-SY5Y cells. Effects of SPMs on microglial phenotype and phagocytosis of Ab were studied.
Results: The levels of docosahexaenoic acid (DHA) derived SPMs, including maresin 1 (MaR1), protectin D1 (PD1) and the D-series resolvins, were lower in ENT of AD-patients compared to age-matched controls. SPMs including lipoxin A_4 (LXA_4), MaR1, resolvin D1 (RvD1) and PD1 were found to be neuroprotective, while only MaR1 was effective in stimulating microglial phagocytosis of Ab_{42} and reducing inflammatory markers.
Conclusions: Our findings support a disturbance of the resolution pathways for local inflammation in the AD brain, and indicate that pro-resolving agents can be of potential therapeutic value for AD.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1545

THE ROLE OF GLIA IN THE PATHOLOGY OF THE NOVEL APP/ABETA K16N MUTATION

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We previously described a novel familial mutation affecting the alpha secretase cleavage site of APP (APP_{770} K687N/Abeta K16N) identified in a heterozygous early onset Alzheimer disease patient (Kaden et al, 2012). It has a unique phenotype, where the mutant Abeta42 K16N protein was toxic to neuroblastoma cells only in the presence of the Abeta42 wild-type peptide. It withstood neprilysin degradation, and protected Abeta42 wild-type protein from degradation. Collectively, we hypothesize that the Abeta K16N mutation likely increases the half-life of toxic Abeta species to perturb effective Abeta clearance, and to contribute to disease pathology. Microglia and astrocytes are the glial cells critical for both amyloid clearance in the CNS and neuroinflammation. Here, we studied the impact of the Abeta K16N mutation and its state of aggregation on glial cell function.

We assessed cell survival and glial activation in primary rat cultures by MTT reduction, immunocytochemical staining, flow cytometry, ELISA and Western blotting. Size exclusion chromatography and electron microscopy were used to examine the aggregation states of Abeta species.

Long-term treatment of primary glial cultures with Abeta42 K16N decreased viability only in the presence of the wild-type Abeta42. Also, glial cells were more activated by a mixture of wild-type and mutant Abeta, compared to treatment with Abeta42 K16N peptide alone. Interestingly, we found glia took up mutant Abeta42 peptide faster than wild-type Abeta42 alone or an equimolar-mixture of the two peptides. These results suggest this novel alpha-secretase mutation alters glial cell functions to potentially trigger a detrimental neuroinflammatory response.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1602

INCREASES BLOOD-BRAIN BARRIER VULNERABILITY TO SYSTEMIC INFLAMMATION AND REDUCED EXPRESSION OF IRON HOMEOSTASIS PROTEINS IN ALZHEIMER DISEASE

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There is an association between neurovascular dysfunction, inflammation and imbalance iron homeostasis in Alzheimer's disease (AD). It may occur as a consequence of a series of damage signals in the blood brain barrier (BBB), failure of immunomodulation, or be associated with excessive iron deposition in the brain.
Neuroimmunomodulation focuses on the link between neuronal damage and brain inflammatory process, mediated by failure of phagocytosis and the progressive activation of astrocytes and microglial cells. To investigate immunomodulatory processes in human AD brains, APP transgenic (APP-tg) mouse and controls brain samples were analysed by RT-PCR, Western blotting and immunohistochemistry (IHC). In APP-tg mice, Western blotting revealed an overproduction of proinflammatory cytokines (IL-6, IL-1b and TNF-a) and a reduction of both triggering receptor expressed on myeloid cells 2 (TREM2) and the iron exporter ferroportin. Both proteins were detected by IHC in microglia and macrophages in choroid plexus in normal brain but were reduced in AD model.
The iron regulatory protein hepcidin and its receptor ferroportin levels were decreased significantly in AD brains and in the later stages of the APP-tg mouse. In this model loss of pericytes and extensive endothelial disruption was seen confirming the presence of severe vascular pathology. Loss of vascular integrity is also associated with abnormal iron accumulation as ferritin, located in haempositive granules associated with blood vessels. The reduction in ferroportin and hepcidin in AD brain is likely to be associated with the pro-inflammatory state, which leads to impaired amyloid clearance process and enhanced senile plaque formation.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1639

ACCELERATION OF AMYLOIDOSIS BY INFLAMMATION IN THE AMYLOID-BETA MARMOSET MONKEY MODEL OF ALZHEIMER'S DISEASE

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Background: A pro-inflammatory state is associated and mentioned as potential target for treatment of amyloidopathy. The marmoset monkey has potential as an Alzheimer's disease (AD) model due to its natural amyloidosis. Moreover, the marmoset shows a human-like immune system and aging phenotype, which is partly due to exposure to environmental pathogens causing transient or chronic latent infections. Aim was to investigate the effect of inflammation on amyloidopathy in the marmoset AD model.
Methods: Four middle aged (5-8y) and two old (13-14y) common marmoset monkeys (*Callithrix jacchus*) of both sexes were intracranially injected with amyloid-beta (Ab) fibrils at three cortical locations in the right hemisphere (frontal, parietal, and sensorimotor cortices) and both hemispheres were injected with PBS (n=3) or LPS (n=3). The effect of inflammation on amyloidopathy was also investigated in an animal that died due to a systemic inflammatory condition, marmoset wasting syndrome (MWS), which is associated with chronic colitis. The pro-inflammatory effect of LPS and Ab was also tested in an *ex vitro* blood analysis (flow cytometry) for immune cell biomarkers (CD45RA and CD95). Campbell-Switzer silver staining and IHC analyses (Ab, Ab42, Ab43, Iba1, and GFAP antibodies) were used on mirror sections to assess amyloidopathy and immune reaction.
Results: The MWS and two LPS+Ab monkeys developed plaques and the LPS+Ab animals had an early-AD immune blood cell expression profile as seen in human AD patients.
Conclusion: A pro-inflammatory condition accelerates amyloidopathy in the marmoset, which indicates the possible importance of immune modulation to decrease the susceptibility for AD.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1700

NEUROINFLAMMATORY DEREGULATION IN AGING AND FIRST STAGES OF SPORADIC ALZHEIMER'S DISEASE ARE NOT LINKED TO BETA-AMYLOID PLAQUES AND NEUROFIBRILLARY TANGLES

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Objectives: Inflammation occurs with aging in several systems but practically nothing is known about inflammation in the aged brain. Although neuroinflammation is well known in advanced stages of sporadic Alzheimer disease (sAD) information about early stages and regional differences in sAD are lacking. AD-related transgenic mice models are used to test anti-inflammatory therapies, but there is no information regarding similarities and differences in neuroinflammation in mice and humans.

Methods: Functional genomics in the cerebral cortex of WT and APP/PS1 mice followed by validation analysis of mRNA expression by qRT-PCR analysis in mice and in entorhinal, orbitofrontal and frontal cortex in human control and sAD stages I-II/0(A), III-IV/A-B and V-VI/C. Studies of β-amyloid plaque burden, soluble Aβ40 and Aβ42, and membrane-associated β-amyloid by immunohistochemistry, ELISA and western blotting. Analyses of protein expression of specific cytokines were examined by western blot and immunohistochemistry.

Results: mRNA deregulation of several inflammatory mediators occurs in mice aged 12 to 20 months, and in humans between 50 and 65 years. Neuroinflammation extends to several cortical regions in sAD at stages I-II/0(A), III-IV/A-B, and V-VI/C. No relationship is found between regional neuroinflammation and plaque, soluble and membrane-associated β-amyloid in the frontal and entorhinal cortex at early stages of sAD.

Conclusions: Neuroimmunoaging is common in early old age in mice and humans. Neuroinflammation in AD and related models is not a mere acceleration of neuroimmunoaging. Characteristics of neuroinflammation differ in sAD and APP/PS1 thus alerting about possible different responses of anti-inflammatory therapies in APP/PS1 and humans with sAD.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1769

MODULATION OF MICROGLIAL RESPONSES BY A PUTATIVE TREM2 LIGAND

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Objectives

Microglial responses critically underpin pathological processes associated with progressive neurodegenerative diseases such as Alzheimer's disease and PD. Recent GWAS have identified mutations in the gene for triggering receptor expressed on myeloid cells (TREM2) as putative risk factors for late-onset AD (Guerreiro et al., 2013). TREM2 is a major microglia-specific gene, ranked #31 of the most highly expressed microglia receptors. TREM2 may act as a lock to repress microglial pro-inflammatory cytokine production, whilst promoting protective microglial responses such as chemotaxis and phagocytosis. However, a full appreciation of the function of TREM2 is needed to understand the role of TREM2 mutations implicated in neurodegenerative cascades. Because the endogenous ligand of this receptor is currently unknown, we took advantage of a putative TREM2 ligand to explore the signalling processes in microglia associated with this receptor (Daws et al., 2003).

Methods

All experiments were carried out in the BV2 microglial cell line or primary cultures of rat whole brain derived ex-vivo microglia. Either a TLR4 ligand, lipopolysaccharide, or a TLR2 ligand, lipoteichoic acid were used to activate microglia.

Results

The putative TREM2 ligand modulates iNOS and TNFα expression, phagocytosis and the profile of cytokine secretion. Intracellular superoxide generation was also regulated by the ligand.

Conclusions

Preliminary data suggest regulation of TREM2 in microglia can alter subsequent microglial signalling. Further experiments will explore the interplay of other receptors on TREM2 responses in microglia.

We acknowledge funding by Complement UK and Alexion.

R.Guerreiro et al., 2013. N Engl J Med
M.Daws et al., 2003. J Immunol

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1949

NEUROINFLAMMATION AND KININS: IN VITRO AND IN VIVO STUDIES

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Background: Neuroinflammation is expressed by activated microglial cells that release inflammatory markers, e.g. nitric oxide (NO) and tumor necrosis factor-alpha (TNF-alpha). Bradykinin (BK) and other kinins are known as potent peripheral mediators of inflammation and exert their effects via two kinin receptors: B_1 and B_2 receptors. In Alzheimer's disease (AD), where neurionflammation plays role, BK release in brain tissues is up regulated early during the course of the pathology. Hence, BK is believed to promote neuroinflammation. However, BK was recently also reported to exert anti-inflammatory roles.

Aims: The aims of this study were a. to examine the effect of BK on microglial production of NO and TNF-alpha under lipopolysaccharide-stimulated conditions. b. to check the involvement of BK in regulation of amyloid plaques burden and gliosis in AD transgenic model.

Methods: BV2 microglial cell lines were used. We also used 5 X familial AD (5XFAD) transgenic mice as an *in vivo* model.

Results: The results show that BK and selective BK agonists attenuated both microglial NO and TNF-alpha release that had been stimulated with LPS. R-715, a specific B_1R antagonist, but not HOE-140, a specific B_2R antagonist increased amyloid plaques levels in the cortex of 5XFAD mice. Moreover, B_1 receptor blockade resulted in increased gliosis in the cortex of these mice.

Conclusions: These results suggest that kinins protect the brain against neuroinflammatory processes such as the release of toxic factors from microglial cells. B_1 receptor is involved in the regulation of Aβ clearance and gliosis *in vivo*. (ISF grant No. 101/11-15).

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-2287

GLOBAL GENETIC REDUCTION OF THE PROSTAGLANDIN E2 RECEPTOR EP2 DOES NOT PREVENT NEURAL DYSFUNCTION IN HUMAN APP TRANSGENIC MICE

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Objectives: The prostaglandin E2 (PGE_2) receptor EP2 may be involved in Alzheimer's disease (AD). Conditional ablation of EP2 in microglia ameliorated memory deficits in hAPP/PS1 doubly transgenic mice (J. Clin. Invest. 125:350). To assess the therapeutic potential of global reduction in EP2 signaling, we crossed hAPP mice from line J20 onto a *Ptger2* (EP2)-deficient background and analyzed the six resulting genotypes: *Ptger2*$^{+/+}$, *Ptger2*$^{+/-}$, *Ptger2*$^{-/-}$, hAPP/*Ptger2*$^{+/+}$, hAPP/*Ptger2*$^{+/-}$ and hAPP/*Ptger2*$^{-/-}$.
Methods: Adult mice were tested in the elevated plus maze, Y-maze and Morris water maze. Aβ levels in brain tissues were determined by ELISA. Brain sections were stained with Thioflavin-S to detect amyloid plaques and with different antibodies to determine hippocampal levels of the neuronal activity-dependent proteins calbindin and cFos.
Results: Global reduction or ablation of EP2 did not ameliorate behavioral and cognitive abnormalities or neuronal reductions in calbindin and cFos in hAPP mice. It also did not alter Aβ levels in cortical/hippocampal homogenates, although ablation of EP2 reduced the burden of Thioflavin-S-positive amyloid plaques. In mice without hAPP, EP2 ablation caused deficits in the Y-maze, but not in the other behavioral tests.
Conclusions: We confirmed that EP2 deletion decreases plaque burden, indicating that PGE_2/EP2 signaling has a role in the deposition or removal of plaques. However, our findings suggest that EP2 receptors do not mediate hAPP/Aβ-dependent neuronal, behavioral and cognitive abnormalities in hAPPJ20 mice and, thus, provide no support for the hypothesis that global inhibition of PGE_2/EP2 signaling might be of benefit in the prevention or treatment of AD.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-0261

NOVEL FUNCTIONS FOR APOLIPOPROTEIN-D IN THE REGULATION OF BRAIN LIPID PEROXIDATION AND ALZHEIMER'S DISEASE PATHOLOGY

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Objectives: Apolipoprotein-D (apoD) levels are increased in affected brain regions in Alzheimer's disease (AD). ApoD has a lipid antioxidant function, however, the mechanisms involved and relationship to AD pathology are unclear. We addressed these questions using in vitro and in vivo approaches.

Methods: Recombinant apoD and Met to Ala mutants were assessed for antioxidant activity (conversion of radical-generating lipid hydroperoxides to inert lipid hydroxides) by HPLC. ApoD monomers and dimers were assessed by western blotting of postmortem control and AD hippocampus and cerebellum. Tissue lipid peroxidation markers (conjugated dienes and F2-isoprostanes) were assessed by HPLC and GC-MS methods. ApoD null mice and Thy-1 human apoD transgenic mice were crossed with APP/PS1 mice. Amyloid pathology was assessed at 11 months by immunostaining.

Results: We identified an antioxidant role for apoD Met-93 in the catalysis of lipid hydroperoxides to lipid hydroxides. The reaction generates Met sulfoxide that destabilizes apoD leading to dimer formation. In human AD hippocampus, apoD was increase by 32% ($p<0.01$) and apoD dimer was increased 2.1-fold ($p<0.01$) compared to controls. Similarly, a hippocampal-dependent increase in conjugated dienes and F2-isoprostanes was selectively detected in the AD hippocampus. In APP/PS1 mice, loss of apoD resulted in a 2.2-fold ($p<0.01$) increase in hippocampal plaque load. Conversely, transgenic expression of neuronal apoD reduced hippocampal plaque load by 33% ($p<0.01$).

Conclusions: ApoD lipid antioxidant activity involves Met-93. This antioxidant process may contribute to apoD dimer formation in the AD brain. Our studies also indicate apoD regulates amyloid pathology in a mouse model of AD.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-0399

INHIBITION OF GLUCOSYLCERAMIDE SYNTHASE (GCS) ENHANCES NEURONAL RESISTANCE TOWARDS AMYLOID-BETA-MEDIATED TOXICITY

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Objectives:
Gangliosides synthesized by GCS are essential components of cell membranes particularly enriched in neurons. During the recent years, they have emerged as molecules involved in the pathogenesis of Alzheimer´s disease (AD). In parallel decreased neuronal insulin receptor (IR) signaling was shown to be a hallmark of AD. We aim to study if altered IR signaling exerts neuroprotective effects on ganglioside-deficient neurons upon exposure to soluble amyloid-beta oligomers (ADDLs). Furthermore, we investigate if ADDL-binding to synaptic sites and subsequent cell viability depend on plasma membrane gangliosides.
Methods:
Neuronal hippocampal cells were treated with the ganglioside biosynthesis inhibitor Genz-123346, subsequent ganglioside depletion was determined by thin layer chromatography. The cells were incubated with ADDLs for 24h and cell viability was measured in an MTT assay. Intracellular IR signaling in the ADDL-treated ganglioside-depleted cells was analyzed by western blot. Co-localization and interaction of gangliosides with IR and ADDLs were investigated with immunofluorescence (IF) stainings and co-immunoprecipitation (co-IP).
Results:
We found that ganglioside-depleted neuronal cells displayed higher resistance towards ADDL stress. Furthermore, ADDL-exposure of control neurons lead to decreased IR levels and signaling, however, this effect was rescued on ADDL-treated ganglioside-depleted neurons. Ganglioside/ IR interactions could be confirmed by IF stainings and co-IPs. Moreover we showed that ganglioside/ IR interaction as well as ADDL binding localize to synaptic sites.
Conclusions:
IR levels and signaling can be modulated via ganglioside depletion resulting in less sensitivity to ADDL-induced neurotoxicity. These results open up the possibility of a potential novel therapeutic strategy for AD.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-0595

ALTERED LIPID COMPOSITION IN CORTICAL LIPID RAFTS AT EARLY STAGES OF ALZHEIMER'S DISEASE. RELEVANCE TO AMYLOID PROCESSING.

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Lipid rafts are membrane microdomains that provide a highly saturated and viscous physicochemical microenvironment facilitating protein-lipid and protein-protein interactions involved in cellular signal transduction. The presence of raft lipid alterations in the frontal cortex in early and late stages of Alzheimer's disease (AD) has been recently demonstrated.

The aim of this work was to investigate whether raft lipid matrix impairment may affect microstructure homeostasis and, consequently, modify the behaviour and dynamics of raft integrated proteins relevant to AD pathogenesis.

Our results show that changes in the raft lipid matrix in frontal cortex lipid rafts at the earliest stages of AD (AD I/II) affect, both, lipid classes and fatty acids, and were also detected in the entorhinal cortex, but not in the cerebellum. Paralleling these changes, lipid rafts from these brain areas displayed higher anisotropy for environment-sensitive probes, indicating an increased in membrane order and viscosity in these domains. Pathophysiological alterations were strengthened by redistribution of proteins related to amyloidogenic processing.

Overall, our results provide a mechanistic connection in these microdomains between lipid alterations, amyloidogenic processing of amyloid precursor protein and microstructural impairment initiated even at early stages of AD.

Supported by SAF2010-22114-C02-01/02, Seventh Framework Programme of the European Commission, grant agreement 278486: DEVELAGE. NF was hired from SEGAI.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-0738

LIVE-CELL MONITORING OF PROTEIN INTERACTIONS, PROTEOLYTIC PROCESSING AND LIPID RAFT LOCALIZATION OF PROTEINS INVOLVED IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

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Objectives: Amyloid-beta precursor protein (APP) and Tau play central roles in pathophysiology of Alzheimer's disease (AD). Coordinated regulation of proteolytic processing of APP and Tau phosphorylation/dephosphorylation is important for maintenance of neuronal health. Both processes are associated with lipid rafts (LR), nanoscale membrane microdomains. Our aim was to develop improved live-cell methods to provide new insights to these processes and to help in functional genomic and drug discovery efforts in AD.

Methods: *Gaussia princeps* luciferase-based protein-fragment complementation assay (PCA) was used for live-cell monitoring of protein-protein interactions. PCA-detection of APP-BACE1 interaction was combined with alkaline phosphatase-based detection of shed APP fragments from the media to form a multiple readout assay. For monitoring trafficking of proteins to LR, we developed a PCA reporter carrying one half of the luciferase protein fused to the LR localization signal from the Fyn kinase.

Results: The APP multiplex assay with four readouts is capable of delivering mechanistic details on trafficking and processing of APP in live cells. LR-localization assay showed dynamic localization of APP and Tau as well as several proteins regulating their function to lipid rafts. Changes in glucose and 1-carbon metabolism strongly influenced LR-localization of Tau. Of the tested late-onset AD risk genes, ABCA7 coexpression had the most pronounced effects on Tau localization to lipid rafts.

Conclusions: Monitoring protein-protein interactions and lipid raft localization of pathophysiologically central proteins with the new live-cell assay platform can provide new insights into disease mechanisms and functional genomics of AD.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-0787

INDUCTION OF HMGCR DEPENDENT CHOLESTEROL BIOSYNTHESIS IS REQUIRED TO IMPROVE SYNAPTIC PLASTICITY DRIVEN BY LXR/RXR ACTIVATION-MEDIATED CHOLESTEROL EFFLUX

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Objectives: Neuronal cholesterol homeostasis is essential for basic synaptic function, plasticity and behavior. The beneficial effects of Liver and Retinoic X Receptors agonists (LRAs) reported on synaptic and cognitive functions are based on their ability to increase cholesterol efflux. Here we report that neuronal activity and synaptic plasticity were improved by LRAs only when increased expression of cholesterol efflux regulatory protein ATP-binding cassette transporter ABCA1 was compensated by an increased expression of HMG-CoA reductase (HMGCR), the rate-limiting step enzyme of the cholesterol synthesis pathway.

Methods: Primary cultures of rat cortical neurons, mice deficient for *ApoE* (the main brain cholesterol transporter), and 5xFAD transgenic mice, a mouse model of Alzheimer disease with extensive memory impairments, were used to assess the importance of HMGCR expression in synaptic activity. Two LRAs, GW3965 and bexarotene, were utilized to induce cholesterol efflux and to promote compensatory HMGCR expression.

Results: In neuronal networks, induction of expression of ABCA1 by LRAs was concomitant with increased expression of HMGCR and the resulting neuronal activity improvement was not observed following HMGCR inhibition. We report that LRA-mediated cholesterol efflux did not increase HMGCR and synaptic plasticity in wild type mice and that impaired cholesterol supply in *ApoE* deficient mice was compensated by an increased HMGCR expression improving synaptic plasticity. Moreover, HMGCR needs to be enzymatically active since its inhibition inhibits bexarotene-mediated rescue of synaptic plasticity deficits in 5xFAD mice.

Conclusions: Our results suggest that expression of HMGCR induced by LRAs-mediated cholesterol efflux is needed to improve synaptic plasticity and cognition.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-0923

THE ROLE OF SCAVENGER RECEPTOR CLASS B TYPE I (SR-BI) IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

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Objectives
SR-BI is an HDL receptor, identified on astrocytes and vascular smooth muscle cells in Alzheimer's disease (AD) brain. We have previously shown that SR-BI reduction enhances amyloid plaque formation and exacerbates memory deficits in an AD mouse model (J20), suggesting that up-regulation of SR-BI could have a protective effect. To further elucidate the pathogenetic mechanism that involves SR-BI we will examine whether brain or peripheral expression of SR-BI is critical in AD pathogenesis.
Methods
We have generated a brain-specific and liver-specific AD transgenic mouse model to evaluate the effect of SR-BI up-regulation in the AD phenotype.
Results
We have expressed SR-BI in the J20 brain, using an astrocyte-specific promoter (GFAP) and bred the J20/GFAP-SRBI transgenic mice on the SR-BI knockout background. We have also generated J20/liver-specific SR-BI transgenic/ SR-BI knockout mice. We are currently analysing the amyloid phenotype of these mice, to evaluate the effect of SR-BI expression.
Conclusions
Our hypothesis is that SR-BI can exert an effect on the AD pathology either by regulating amyloid deposition in the brain, or by regulating cholesterol homeostasis, as SR-BI is involved in reverse cholesterol transport. To establish the protective role of SR-BI in AD and the mechanism involved we have generated transgenic mice that overexpress SR-BI in the brain and in the liver. Analysing these mice will help us establish a potential therapeutic role of SR-BI in AD.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1087

ARACHIDONIC ACID-SPECIFIC ACYL-COA SYNTHETASE 4 (ACSL4): A KEY ENZYME FOR NEURONAL DIFFERENTIATION AND DISTRIBUTION OF CRITICAL PHOSPHOLIPID SPECIES

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Background & Objectives
Western diets are characterized by excessive amounts of ω-6 fatty acid precursors and arachidonic acid (ARA). Competition between ARA and docosahexaenoic acid (DHA) for the incorporation into membrane phospholipids could contribute to lower DHA levels which are observed in Alzheimer's disease (AD) patients and could therefore favor the loss of synaptic plasticity and the neurotoxic effects of Aβ oligomers. Our objective was to test this hypothesis by analyzing the influence of the arachidonyl-specific acyl-CoA synthetase 4 expression (ACSL4) on neurite growth and ARA and DHA distribution into phospholipid species.
Methods
The murine hippocampal cell line HT22 was differentiated by phorbol ester, dibutyryl cAMP and NGF. The differentiation levels were quantified by morphological criteria and measurement of neuronal markers. ACSL4 expression levels were quantified in Western blot and quantitative RT-PCR experiments. MTT activities were measured to determine cell sensitivity to Aβ oligomers. Phospholipids species were analyzed by electrospray ionization/mass spectrometry.
Results
ACSL4 expression levels increased 3-4 fold in HT22 cells during the differentiation process and was further increased by 25% in presence of ARA. siRNA-mediated inhibition of ACSL4 expression did not modify HT22 cell neurite growth nor their sensitivity to Aβ oligomers. However, ARA uptake in HT22 cells reduced specific DHA-containing plasmalogen species and these modifications were abolished by reduction of ACSL4 expression.
Conclusions
ACSL4 expression is associated with neuronal differentiation/neurite growth and reduces DHA distribution into critical phospholipid species reported to be affected in AD.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1137

IMPACT OF HUMAN APP OVEREXPRESSION ON CEREBRAL CHOLESTEROL METABOLISM IN MICE

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Processing of APP and Abeta has been in the center of Alzheimer's disease (AD) research for decades. Beside many other variables, lipids, especially cholesterol and its derivatives, are discussed to contribute to AD pathogenesis. Several studies showed that cholesterol impacts on APP metabolism. But interestingly also the converse mechanism, the direct influence of Abeta on cholesterol metabolism was discovered. The presented study investigates whether human APP overexpression and changes in Abeta generation influence cholesterol metabolism in APP_{SL} mice, a well-established model of brain amyloidosis and AD?
Changes in cholesterol content and mRNA levels of cholesterol metabolism-associated genes were measured in brain tissue of APP_{SL} mice and non-transgenic littermates. To additionally investigate the impact of dietary cholesterol on these parameters, brain tissue of APP_{SL} and wild type (WT) mice that received a high-fat/high-cholesterol diet (HFD) for three months was examined as well.
Levels of free cholesterol were significantly reduced in cortices of APP_{SL} mice compared to WT animals. Therefore cholesteryl esters showed a tendency to be increased in APP_{SL} mice. Cortical mRNA levels of LDL-receptor, HMGCoA-reductase, ACAT 1 and 2 as well as ApoE were significantly altered in APP_{SL} mice compared to WT littermates both maintained on regular chow. The changes were more pronounced in mice on HFD. Changes of cortical cholesterol levels and mRNA expression patterns under normal diet and HFD conditions argue for an important role of APP in cerebral lipid metabolism, pointing towards a possible connection between APP overexpression and the amount and distribution of cerebral cholesterol.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1141

HIGH FAT DIET DECREASES PLASMA ABETA LEVELS IN MICE OVEREXPRESSING HUMAN APP

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Sporadic Alzheimer's disease (AD) is influenced by a set of non-genetic risk factors, whereas dyslipidemia seems to play a crucial role. Interestingly, it was shown in mice overexpressing human ApoB-100, that a shift in the plasma lipoprotein composition towards higher LDL-cholesterol levels, without dietary intervention, is able to induce cognitive decline. To see whether this phenotype could be more pronounced by additional cholesterol uptake, a high-fat feeding study was conducted with animals overexpressing human ApoB-100 and/or human APP_{SL}.

Animals of every genotype, ApoBxAPP, ApoB-100, APP_{SL} and wild type (WT), received either a standard chow or iso-caloric high-fat-diet (HFD) for 3 months. At an age of 6 months, all animals underwent several behavioral tests, including the Morris water maze (MWM) and contextual fear conditioning (CFC) task. Abeta levels were assessed in brain and plasma samples. Blood plasma was also analyzed for HDL-cholesterol, LDL-cholesterol and triglycerides.

HFD feeding leads to the expected changes of the plasma lipid profile resulting in more atherogenic conditions in all genotypes, with the strongest response in ApoB-100 mice. Behavioral data show, that HFD mainly affects emotional learning (CFC), but has no measureable impact on spatial learning (MWM). Interestingly, HFD leads to a significant decrease of Abeta1-40 levels in the plasma (48% in ApoBxAPP and APP_{SL} mice), while cerebral Abeta species increase in the brains of HFD-fed animals. Histological changes are subject of current investigations.

While HFD barely influenced behavioral aspects, it strongly affected cerebral Abeta clearance in both strains of human APP overexpressing mice, ApoBxAPP and APP_{SL}.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1323

MOLECULAR MECHANISMS OF BACE1 RECYCLING

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It is now over two decades that the amyloid cascade hypothesis was formulated and despite intensive research the molecular mechanisms that regulate intracellular transport and sorting of the amyloid precursor protein (APP) and the beta-site APP cleaving enzyme 1 (BACE1) remain to be perfectly understood. A comprehensive view of these events is yet essential as: (1) proteolytic cleavage of APP by BACE1 is the rate-limiting step in the production of the toxic amyloid beta-peptide (Abeta) that accumulates in the brain of patients suffering from Alzheimer's disease (AD), (2) Abeta production depends on the co-residence of APP and BACE1 in the same intracellular compartment. It is now clear that the overall endosomal machinery participates in regulating transport of APP and BACE1 and endosomes have been proposed as the main site of amyloidogenesis. Recent studies pinpointed the role of recycling endosomes in both intracellular trafficking of BACE1 and regulation of Abeta production by allowing a transient separation between APP and BACE1. Our work focuses at deciphering the molecular mechanisms that underlie BACE1 recycling pathway. Molecular and cell biology techniques such as mass spectrometry analysis and live-cell imaging as well as the use of in vivo AD models allowed us to discover new cellular factors involved in this process and to better understand how this transport route is a key step in the regulation of amyloidogenesis.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1676

DHA EFFECT ON NEURONAL SURVIVAL AND ZINC HOMEOSTASIS IN SY5Y AND NT2 CELLS.

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Docosahexaenoic acid deficiency (DHA) is reported to induce neuronal cell death in M17 cells through zinc-induced apoptosis. We hypothesize that the same phenomena is present in other cell types similar to M17. SY5Y and NT2 cells were used to test the hypothesis and following the exposure to DHA supplemented or deficient media, opposite effects were seen in those two cell lines. NT2 cells in response to DHA-deficient medium reacted similar to M17 cells, where the active caspase-3 levels were increased. DHA supplemented NT2 cells showed an increase in ZnT3 mRNA and protein levels similar to M17 cells. However, SY5Y cells did not show a consistency in both caspase-3 and ZnT3 mRNA level changes. Which was more consistent to human keratinocyte cell line HaCaT which was used as a control.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1824

ALZHEIMER'S DISEASE-CAUSING GENES LEAD TO THE REDUCTION IN CHOLESTEROL SYNTHESIS CAPACITY.

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Objectives
Many studies emphasize the importance of cholesterol in the build up of amyloid beta, regulating its production, aggregation and clearance. Despite cerebral cholesterol seems to be decreased in Alzheimer's disease, the reason behind this finding remains elusive. Here, we aim to elucidate how APP processing affects cholesterol metabolism in the brain.
Methods
Quantifying intermediates of cholesterol using isotope-dilution mass spectrometry. Elucidating the expression of key enzymes within the cholesterol synthesis pathway using RT-PCR and immunoblotting techniques. Utilizing dermal fibroblasts from AD patients and cell lines expressing the APP Swedish KM670/671NL double mutation (APP_{Swe}) and Presenilin 1 (PS-1) mutations.
Results
Decreased levels of SREBP2 in APP_{Swe}, was seen when the cells were challenged with cholesterol starvation, this however, was reversed in PS-1 expressing cells. Furthermore, HMG-CoA synthase and reductase along with LDL-receptor were observed to be reduced in PS-1 cells while showing no change or an increase in APP_{Swe}. Lower levels of cellular cholesterol in both APP_{Swe} and PS-1 were determined under lipoprotein starving conditions.
Conclusions
Aβ overproducing cells have compromised synthesis of cholesterol, affecting the function of brain processes such as synaptogenesis and neuroplasticity. In cholesterol starving conditions, cells expressing familial AD mutations such as APP_{Swe} have higher or standard expression of genes regulating key steps in cholesterol synthesis, which is the contrary with PS-1 mutations. Suggesting that even though both mutations lead to a decrease in cholesterol synthesis they achieve that through different mechanisms.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1963

ACTIVATION OF LXR/RXR RECEPTORS INCREASES CHOLESTEROL HOMEOSTASIS AND NEURONAL ACTIVITY IN PRIMARY CULTURES OF NEURONS EXPRESSING OR NOT HUMAN APP.

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Objectives: We have previously shown that there is a tight link between neuronal cholesterol homeostasis and neuronal activity. In primary cortical neurons expressing human APP (hAPP), cholesterol turnover was decreased and consequently neuronal activity was abolished. In this study, we have used Liver and Retinoic X Receptors agonists (LRAs) in order to restore cholesterol homeostasis and neuronal activity in primary cortical cultures of neurons expressing hAPP.

Methods: Primary cultures of rat cortical neurons with hAPP expression mediated by an adenoviral vector were used. Expression of genes and proteins involved in cholesterol homeostasis were assessed by quantitative RT-PCR and western blotting. Neuronal activity was analyzed by calcium imaging and patch clamp recording. Two LRAs, GW3965 (LXR agonist) and bexarotene (RXR agonist), were tested in their ability to improve neuronal cholesterol homeostasis.

Results: In primary cultures of neurons expressing or not hAPP, LRAs increase expression of ATP-binding cassette transporter *ABCA1,* HMG-CoA reductase (HMGCR), the rate-limiting step enzyme of the cholesterol synthesis pathway, and 24-cholesterol hydroxylase, involved in neuronal cholesterol hydroxylation. Consequently, neuronal activity was improved in control neuronal networks and restored in hAPP expressing networks. Furthermore, the LRA-dependent increase in neuronal activity was abolished by mevastatin, an HMGCR inhibitor, suggesting that HMGCR has to be enzymatically active to observe constitutive calcium oscillations.

Conclusions: Here, we show that LRAs-mediated increase in cholesterol turnover is able to rescue and improve neuronal activity in primary cortical cultures of neurons expressing of not hAPP only when HMGCR is enzymatically active.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-2058

INCREASING MEMBRANE CHOLESTEROL OF NEURONS IN CULTURE RECAPITULATES ALZHEIMER'S DISEASE EARLY PHENOTYPES

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Objective:

Cholesterol is linked to Alzheimer's disease (AD) pathology. We previously showed that cholesterol levels control the partition of APP and its secretases in lipid rafts, APP endocytosis and Aβ secretion. It still remained unclear whether sporadic AD could be initiated by a change in membrane cholesterol. To test this hypothesis, we triggered an acute increase of cholesterol at the membrane of cultured neurons and analysed cellular changes detected early in the development of the disease.

Methods:

Primary rat embryonic hippocampal and cortical neurons were treated with 1.4 mM MethybCycloDextrine (MbCD)-cholesterol for 30 min. Aβ38, Aβ40 and Aβ42 were measured using Meso Scale Discovery. Early endosomes were labelled using an anti-EEA1 antibody and observed by confocal and electron microscopies. APP vesicular transport was analysed followinf electroporation with APP_{751}mCherry plasmid. Gene expression was measured on total RNAs extracted from neurons.

Results:

By comparing the list of genes differentially expressed after cholesterol treatment with gene expression profiles of sporadic AD brain samples from different Braak stages we found that cholesterol induced changes that are reminiscent of early stages. After cholesterol treatment we found: i/ Enlarged and aggregated EEA1-positive early endosomes using confocal and electron microscopy; ii/ Reduced speed of vesicular anterograde transport of APP; iii/ Increased Aβ42 secretion.

Conclusions:

Increase of neuronal membrane cholesterol triggers APP processing, endosomal trafficking and axonal transport abnormalities. We propose that an increase in membrane cholesterol linked with age is one of the initial events that could trigger sporadic AD.

03l. Pathophysiology & Disease Mechanisms: translational regulation

ADPD5-0344

GENE EXPRESSION AND METHYLATION IN ALZHEIMER'S DISEASE

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Objective

To examine the correlation of methylation of 7 differentially expressed in Alzheimer's disease (AD) and the levell of gene expression to the risk of AD

Methods

80 AD patients and 180 age-matched control subjects were recruited for the study. Blood samples were collected from the subjects with consent and study was approved by institute ethic committee. Gene expression of CTSB, CTSD, DDT, TSC1, NRD1, UQCRC1 and NDUFA6 were quantitated by real-time RT-PCR. Methylation level was compared by Melting Curve Analysis-Methylation assay (MCA-Meth). Non-parametric Spearman's correlation was used to study the association between the methylation status and gene expression in the sample set. Independent t-test was performed to examine the association of methylation status and gene expression level in the group of AD patients and normal controls respectively. A nominal p-value of < 0.05 will be regarded as significant association.

Results

All 7 genes under investigation in this study showed differential gene expression levels among AD and normal controls. The gene expression of these 7 genes was significantly increased in AD patients and the increase ranged from 2.7-fold to 8.6-fold. UQCRC1 was highly methylated in AD patients but no significant difference in methylation status of CTSB, CTSD, DDT, TSC1, NRD1 and NDUFA6 between AD patients and normal controls.

Conclusions

UQCRC1 was highly methylated and expressed in AD and there might be methylation of this gene might serve as a regulatory mechanism for the expression of this gene and implicated in the role in AD pathogenesis.

03l. Pathophysiology & Disease Mechanisms: translational regulation

ADPD5-1720
TRANSLATIONAL REGULATION OF ADAM10 AND BACE1
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Objective: Anti-amyloidogenic processing of the amyloid precursor protein APP by α-secretase prevents formation of the amyloid-β-peptide (Aβ) which accumulates in senile plaques of Alzheimer disease patients. In contrast, cleavage of APP by β- and γ-secretase results in increased Aβ production. We recently demonstrated that the translation of the α-secretase ADAM10 as well as BACE1 is repressed by their 5'UTRs. We identified a translational inhibitory RNA-G-quadruplex secondary structure within the ADAM10 5'UTR, which is involved in translational repression of ADAM10. Furthermore we identified the cytoplasmic mRNA binding protein Unr as an ADAM10 5'UTR interactor, which is involved in translational regulation of ADAM10. We now extended our analysis and searched for BACE1 5'UTR binding proteins.
Methods: To identify BACE1 5'UTR binding proteins, we performed an affinity purification using internally biotinylated BACE1 5'UTR incubated with HEK293 cytosolic cell or mouse cytosolic brain extracts. Captured proteins were identified by LC-MS/MS. We analyzed the effect of the identified binding candidates on APP processing, by siRNA mediated knockdown or overexpression experiments in HEK293 cells.
Results: We identified HnRNP K and HnRNP E as the main BACE1 5'UTR binding proteins. Importantly, Unr does not bind to the 5'UTR of BACE1, however we observed significantly increased APPsβ and Aβ levels upon Unr overexpression, whereas BACE1 protein and mRNA level were not significantly altered.
Conclusion: In this study we demonstrate that the cytoplasmic protein Unr is able to modulate the expression and activity of ADAM10 and BACE1.

03l. Pathophysiology & Disease Mechanisms: translational regulation

ADPD5-1975

ESTABLISHING THE RELEVANCE OF EEF2K INHIBITION AS A NOVEL THERAPEUTIC STRATEGY IN AD

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Background: Burgeoning evidence implicates aberrant activity of AMP-responsive protein kinase (AMPK) and its downstream target the eukaryotic elongation factor-2 kinase (eEF2K) in Alzheimer's disease (AD) pathogenesis. Defects in neuronal energy metabolism and synaptic plasticity are thought to underlie cognitive dysfunction and neuronal loss in AD. AMPK is a metabolic sensor and is believed to couple neuronal energy metabolism to neural activity. Furthermore, eEF2K plays a pivotal role in the regulation of dendritic protein translation vital to synaptic strength and plasticity. Our previous data show that eEF2K confers resistance in tumor cells to nutrient deprivation and modulates apoptotic pathways (Leprivier, G. et al, Cell, 2013). These results point to eEF2K inhibition as a potential treatment strategy in cancers. Our current studies aim to investigate the role of AMPK-eEF2K axis in AD and establish its therapeutic relevance.
Objectives: To establish the relevance of eEF2K inhibition as a novel therapeutic strategy in AD.
Methods: APPPS1 mice, 3xTG-AD mice, AD patient brain samples, Primary neuronal cultures, Immunohistochemistry, Protein biochemistry, ELISA, Pharmacological agents, Gene silencing.
Results. eEF2K activity, measured by phospho-eEF2 (Thr56) immnuohistochemistry, is increased in AD patient brains compared with controls. Results from biochemical and immunohistochemical analysis in two AD transgenic mouse models (APPPS1 and 3x-Tg AD) will be presented. In addition, the effects of pharmacological agents and gene silencing approaches targeting AMPK-eEF2K axis on Aβ neurotoxicity in neuronal cultures will also be discussed.
Conclusions. Our data support that eEF2K inhibition represents a highly promising therapeutic target in AD.

03m. Pathophysiology & Disease Mechanisms: micro RNAs

ADPD5-1138

MICRO-RNA-29A AS A CANDIDATE BIOMARKER FOR ALZHEIMER'S DISEASE IN CELL-FREE CEREBROSPINAL FLUID

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Objective: The identification of reliable biomarkers for Alzheimer's disease (AD) remains a major challenge. In recent studies, microRNAs (miRNAs) miR-27a, miR-29a, miR-29b and miR-125b were described to be abnormally expressed in cerebrospinal fluid (CSF) of AD patients. We aimed to investigate if these miRNAs may indeed be confirmed as diagnostic markers. Additionally, we were interested in whether blood contamination, which can occur during lumbar puncture of CSF, affects the levels of these miRNAs in CSF, and if so, how fast this may occur.
Methods: We studied the expression levels of the four miRNAs by quantitative PCR in CSF samples of 18 AD patients, all characterized by NINCDS-ADRDA criteria, and 20 healthy controls. We further investigated the effect of blood contamination on miRNA levels in CSF.
Results: MiR-29a levels were increased in CSF of AD patients compared to controls (p=0.0001), and miR-125b levels were slightly increased in AD patients (p=0.025). Furthermore, miR-27a levels were similar in CSF of AD patients and controls, while miR-29b was not detectable in most samples. Analysis of CSF samples spiked with blood showed that miR-27a and miR-29a, but not miR-125b levels were strongly influenced by the number of blood cells in the sample. In addition, the presence of blood cells in CSF almost immediately affected CSF miRNA levels.
Conclusions: MiR-29a may be a candidate biomarker for AD, but only when used in cell-free CSF. Previous findings of altered CSF levels of miR-29b, miR-125b and miR-27a could not be confirmed.

03m. Pathophysiology & Disease Mechanisms: micro RNAs

ADPD5-1236
MICRORNA EXPRESSION IN ALZHEIMER'S DISEASE
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MicroRNA (miRNA) play an essential role in post-transcriptional gene regulation in the brain. Genetic variants located across the APOE locus are the strongest risk factors for late-onset AD described to date. A complex regulatory structure exists at the APOE locus that includes putative regulatory sites within the TOMM40 gene. Little is known about the influence of miRNA post-transcriptional regulation at this locus. The aim of this investigation is to demonstrate that miRNA that are predicted to target the APOE locus are expressed differently in brain of AD compared to controls and associated with APOE and TOMM40 brain expression. MiRNA array and miRNA qRT-PCR were used to measure miRNA expression in post-mortem brain from AD (n=21) or cognitively normal age-matched control (n=21) hippocampus (HP) and cerebellum (CB). Quantitative RT-PCR was used to measure APOE and TOMM40 mRNA. Western blots were used to measure brain APOE and TOMM40 protein. Linear regression was used to determine if APOE and TOMM40 mRNA expression correlate with protein or mRNA expression in AD (n=8) compared to cognitively normal age-matched controls (n=8) or in HP compared to CB. MiRNA were identified that were associated with AD or control brain APOE or TOMM40 expression levels. These results suggest that a complex regulatory structure at the APOE locus may be fine-tuned by miRNA post-transcriptional modulation according to brain region or disease status.

03m. Pathophysiology & Disease Mechanisms: micro RNAs

ADPD5-2003

MIRNAS AS THERAPEUTIC AGENTS FOR ALZHEIMER'S DISEASE: A PILOT STUDY

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Objectives: miRNAs are small non-protein-coding RNAs involved in the post-transcriptional regulation of gene expression. It is now well established that miRNAs can regulate several genes simultaneously, thus controlling biological or pathological "pathways". In this study, we aimed at identifying one or several miRNAs that could regulate simultaneously Aβ and Tau metabolism, providing a "silver bullet" for AD therapeutics. **Methods:** We used several bioinformatics programs to identify miRNAs that could potentially regulate APP and BACE1 expression as well as Tau phosphorylation. Transfection and luciferase assays were performed in different cell lines to validate these predictions. Candidate miRNA injection in the mouse brain was performed to validate our observations in vivo. APP, Aβ, BACE1, and Tau was analyzed by western blot analysis. The level of introduced miRNA was determined using qRT-PCR. Different brain tissues were used for iTRAQ proteomics analysis. **Results:** We identified various miR-15/107 family members (e.g., miR-16, miR-15a, miR-195) that can directly regulate the expression of APP and BACE1. Over-expression studies in HEK cell expressing APP Swedish mutation led to a strong decrease in Aβ levels. The same experiment in N2A and HT22 lines confirmed the effect of this family specifically on APP and BACE1. In vivo miRNA delivery results was also identified more potential targets of this family in vivo. **Conclusion:** Our data indicate that selected miR-16 family members can potentially function as combined endogenous regulators of Aβ and Tau in the mammalian brain opening a new vista to therapeutic strategies against AD.

03n. Pathophysiology & Disease Mechanisms: kinases and phosphatases

ADPD5-1000

HDAC2 TYROSINE PHOSPHORYLATION BY C-ABL INDUCES NEURONAL GENE REPRESSION IN ALZHEIMER DISEASES MODELS

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Objectives: HDAC2, is implicated in the epigenetic repression in Alzheimer's disease (AD), however the mechanisms involved in HDAC2 regulation are not fully elucidated. Here we demonstrated that c-Abl induces tyrosine phosphorylation of HDAC2 increasing its levels and inducing the repression of synaptic genes expression.
Methods: We used i) in vitro – neurons and HT22 cells exposed to AbetaO, and ii) in vivo- APPswe/PSEN1dE9 mice, AD models. We modulated c-Abl activity by using Imatinib, a c-Abl inhibitor, or by transfection with c-Abl plasmids. Then we measured the activity and protein levels of HDAC2, the recruitment of HDAC2 and the expression of target HDAC2 synaptic genes by ChIP and RT-PCR, respectively.
Results: Our data demonstrate that: i) in neurons, c-Abl inhibition with Imatinib prevents the AbetaO-induced increase in HDAC2 levels, ii) c-Abl knockdown decreases HDAC2 levels, while c-Abl overexpression increases it,, iii) c-Abl inhibition reduces HDAC2-dependent repression activity and HDAC2 recruitment to the promoter of several synaptic genes, increasing their expression, iv) c-Abl induces tyrosine phosphorylation of HDAC2, a novel previously unknown posttranslational modification, affecting both its stability and repression activity and v) treatment with Imatinib decreases HDAC2 levels in a transgenic mice model of AD.
Discussion: Our results support the participation of the c-Abl/HDAC2 signaling pathway in the epigenetic blockade of gene expression in AD pathology.
Support: Fondecyt 1120512, FONDEF D10I1077 and CARE-Chile-UC (PFB 12/2007). Gonzalez Z.M. acknowledges CONICYT

03n. Pathophysiology & Disease Mechanisms: kinases and phosphatases

ADPD5-1159

CDK5 INHIBITION AND ITS NOVEL FUNCTION IN NEURONAL DEVELOPMENT AND TREATMENT OF NEURODEGENERATION

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Objectives

Cyclin-dependent kinase 5 (Cdk5) is a multifaceted serine/threonine kinase protein playing an essential role in neuronal development. Cdk5 activity regulates such pivotal cellular processes as neuronal migration, neurogenesis, synaptic plasticity, behavior, cognition or dendritic outgrowth. The aberrant activation of Cdk5 results in hyperphosphorylation of its various substrates, like Amyloid Precursor Protein, Tau protein and neurofilaments and subsequently to neuronal death. Dysfunction of Cdk5 is associated with diverse neurological disorders including Alzheimer's disease or amyotrophic lateral sclerosis. Thus, we were interested if Dinaciclib, a novel Cdk5 inhibitor succesfully used as therapeutic agent in advanced malignancies, is able to promote neurite outgrowth and neurogenesis in primary rat hippocampal neurons.

Methods

For this purpose, primary embryonic rat hippocampal neurons were treated with Dinaciclib for 48 h on DIV1. Subsequently, number of neurites, total length of neurites and length of the longest neurite were determined. To address the effects of Dinaciclib on neurogenesis, we determined the percentage of BrdU-NeuN double-positive cells within the total number of primary rat hippocampal neurons. For these analyses, a software supported automatic quantification method was generated (Axio.Imager Z1 microscope, ImageProPlus).

Results

Dinaciclib did not affect neurogenesis. However, for the first time, beneficial effects of the novel Cdk5 inhibitor Dinaciclib on neurite outgrowth of primary rat hippocampal neurons were shown.

Conclusions

Our data indicate inhibition of Cdk5 activity to be a promising strategy to treat neurodegenerative diseases by promoting neurite outgrowth. Furthermore, these data identify Dinaciclib as a promising new drug candidate in neurodegenerative diseases.

03n. Pathophysiology & Disease Mechanisms: kinases and phosphatases

ADPD5-1368

TRUNCATION AND ACTIVATION OF DYRK1A BY CALPAIN I: A MOLECULAR MECHANISM LINKED TO TAU PATHOLOGY IN ALZHEIMER'S DISEASE

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Objectives: Abnormal hyperphosphorylation of tau and imbalanced expression of 3R-tau and 4R-tau are pivotally involved in the pathogenesis of Alzheimer's disease (AD) and other tauopathies. Dual-specificity tyrosine-phosphorylation-regulated kinase 1A (Dyrk1A) regulates alternative splicing of exon 10 and phosphorylation of tau. Overexpression of Dyrk1A contributes to tau pathology *via* suppression of exon 10 inclusion and hyperphosphorylation of tau in Down syndrome. However, the role of Dyrk1A in tau pathology in AD is not understood.
Methods. We employed *in vitro* proteolysis, enzyme kinetic analysis, *in vitro* kinase activity assay, and *in vivo* excitotoxicity mouse model to study the role Dyrk1A in tau pathology in AD.
Results. We found that truncation of Dyrk1A was increased and correlated with over-activation of calpain I as well as with an increase in the ratio of 3R-tau/4R-tau and hyperphosphorylation of tau in AD brain. Calpain I truncated Dyrk1A at its C-terminal *in vitro* and enhanced its kinase activity towards tau as seen by an increase in *Vmax*, but not *Km.* C-terminally truncated Dyrk1A was stronger than the full-length protein in leading to promotion of exon 10 exclusion and hyperphosphorylation of tau. Excitotoxicity induced by kainic acid (KA) caused Dyrk1A truncation, which coincided with an increase in 3R-tau expression and Thr212 phosphorylation of tau in mouse brains. Inhibition of calpain prevented these KA-induced changes.
Conclusions. Truncation and activation of Dyrk1A by Ca^{2+}/calpain I could be responsible for the increase in the ratio of 3R-tau/4R-tau through dysregulation of exon 10 splicing and the hyperphosphorylation of tau in AD brains.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-0292

FREE AND NANOENCAPSULATED CURCUMIN SUPRESS ABETA1-42-INDUCED SYNAPTIC TOXICITY AND COGNITIVE IMPAIRMENTS IN RATS: INVOLVEMENT OF CAMKII AND AKT/GSK-3 BETA SIGNALING PATHWAY.

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Increasing evidence demonstrates that β-amyloid (Aβ) is toxic to synapses, resulting in the progressive dismantling of neuronal circuits. Counteract the synaptotoxic effects of Aβ could be particularly relevant for providing effective treatments for Alzheimer's disease (AD). Several studies have been shown that curcumin is associated with anti-amyloidogenic properties. Here we developed curcumin-loaded lipid-core nanocapsules (Cur-LNC) in an attempt to improve the bioavailability and the neuroprotective effect of this polyphenol. Organotypic hippocampal slice cultures exposed to Aβ1-42 were used to study the neuroprotective effects of curcumin through a spectral analysis of multi-electrode array (MEA) recordings of spontaneous neuronal activity. Whereas, in the in vivo experiments the animals received a single intracerebroventricular injection of Aβ1-42 and they were administered either free curcumin or Cur-LNC intraperitoneally for 10 days. The analysis of MEA recordings of spontaneous neuronal activity *in vitro* showed an attenuation of signal propagation induced by Aβ before cell death and curcumin-induced alterations to local field potential (LFP) phase coherence. Curcumin-mediated attenuation of Aβ-induced synaptic dysfunction involved regulation of synaptic proteins, namely phospho-CaMKII. Our findings also demonstrated that administration of curcumin was effective in preventing behavioral impairments and Akt/GSK-3β signaling pathway disturbances triggered by Aβ *in vivo*, and Cur-LNC in a dose 20-fold lower presented similar neuroprotective results compared to the effective dose of free curcumin. Considered overall, our data suggest that curcumin is a potential therapeutic agent for neurocognition and its nanoencapsulation in LNC might constitute a promising therapeutic alternative in the treatment of neurodegenerative diseases such as AD.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-0457

INCREASED NEURONAL DNA DOUBLE-STRAND BREAKS AND DEFICITS IN DNA REPAIR MECHANISMS IN ALZHEIMER'S DISEASE AND A RELATED MOUSE MODEL.

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Objectives. We aim to determine the mechanisms that cause excessive neuronal DNA double strand breaks (DSBs) in Alzheimer's disease (AD) and the potential impact of these DSBs on cognitive functions. Physiological increases in neuronal activity increase neuronal DSBs in multiple brain regions, but these DSBs are rapidly repaired in wildtype mice [Nat. Neurosci. 16: 616–621 (2013)]. Human amyloid precursor protein (hAPP) transgenic mice, which simulate key aspects of AD have increased neuronal DSBs at baseline and delayed DNA repair.
Methods. We used immunohistochemical staining of brain sections from humans and mice to detect DSBs, comet assay to quantitate DNA fragmentation, western blot analysis to quantitate DNA repair factors, and behavioral tests to assess learning and memory. We used stereotactic injections of lentiviral vectors encoding shRNA to reduce neuronal expression of DNA repair factors in mouse brains. Scrambled shRNA served as a negative control.
Results. Humans with AD had a greater number of neurons with DSBs than non-demented controls. We are now comparing levels of neuronal DSBs across brain regions, at different stages of AD, and in other dementias. Compared with controls, AD patients and hAPP-J20 mice also had decreased hippocampal levels of the DNA repair factor BRCA1, but not of several other repair factors. Knocking down BRCA1 caused accumulation of neuronal DSBs in the dentate gyrus and learning and memory deficits in wildtype mice without causing neuronal apoptosis.
Conclusions. Impairments in DNA repair and abnormal persistence of DSBs may contribute to cognitive impairments in AD and related conditions.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-1076

BRAIN INTRACELLULAR SIGNALING, OXIDATIVE STRESS AND ALZHEIMER'S DISEASE-RELATED HALLMARKS UPON TYPE 2 DIABETES ARE DIFFERENTIALLY INFLUENCED BY GENDER

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Objectives: Aging, type 2 diabetes (T2D) and female gender are known risk factors for Alzheimer's disease (AD), but the underlying molecular mechanisms remain unclear. We hypothesized that gender affects insulin/IGF-1/estrogen-mediated signaling in T2D brain. We evaluated how gender may influence insulin/IGF-1/estrogen-related signaling and AD-like hallmarks in T2D rat brains.
Methods: We used brain cortical homogenates from middle-aged (8-month-old) male and female Wistar and T2D Goto-Kakizaki (GK) rats to evaluate insulin, IGF-1, cholesterol, sexual steroid hormones and amyloid-beta levels by ELISA, and density of signaling molecules by immunoblotting.
Results: Although male and female GK rats had higher glycemia than gender-matched controls, no significant changes occurred between genders. Despite the increased plasma estradiol levels in Wistar females than in males, its levels were similar between T2D females and males. T2D females had impaired brain steroid hormones' metabolism, lower IGF-1 levels and IGF-1 receptor density, whilst their blood and brain insulin levels and insulin receptor densities were increased. Therefore, a compensatory mechanism to maintain insulin receptor function and subsequently stimulating Akt activity may occur in GK females, thereby inhibiting BACE activity, and ultimately blunting brain amyloid-beta$_{1-42}$ accumulation and lipid and DNA oxidation.
Conclusions: Although brain steroid hormone cascade might be impaired in middle-aged T2D females, these appeared to be less vulnerable to oxidative stress and AD-like neuropathological markers associated to chronic T2D.
Supported by FEDER (Programa Operacional Factores de Competitividade – COMPETE) and Portuguese funds via FCT (projects: PTDC/SAU-TOX/117481/2010, Pest/SAU/LA0001/2011, PTDC/SAU-NMC/110990/2009; fellowships: SFRH/BPD/84473/2012, SFRH/BD/90036/2012, PTDC/SAU-NMC/110990/2009, QREN DoIT DIAMARKER PROJECT, *SFRH/BPD/84163/2012, SFRH/BD/73388/2010).*

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-1294
THE ROLE OF NMDA-DEPENDENT ERK SIGNALING IN AMYLOID-BETA REGULATION

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Objectives: The concentration-dependent accumulation of the peptide amyloid-beta (Abeta) as oligomers and plaques is thought to be a key event in Alzheimer's disease (AD) pathogenesis. Previously, our lab utilized *in vivo* microdialysis to find that high doses of *N*-methyl-D-aspartic acid (NMDA) result in reduced Abeta levels in the interstitial fluid (ISF) of wild-type mice and a mouse model of AD. We have shown that this NMDA-mediated decrease acts through the activation of extracellular-regulated kinase (ERK). Certain other receptors that activate ERK do not show this effect on Abeta, suggesting that NMDA-Rs act on ERK and Abeta through a selective pathway. The ERK subfamily includes two isoforms, ERK1 and ERK2, which are both ubiquitously expressed. Traditionally thought to have identical roles, recent findings have revealed distinct isoform functions. It is the objective of this study to determine if ERK isoforms have differential effects on Abeta production.
Methods: We will use shRNA against ERK1 and ERK2 packaged in AAV8 to knockdown expression in APP/PS1$^{+/-}$ mice. We will then use microdialysis to measure changes in ISF Abeta levels in the hippocampus in response to 40 microM NMDA treatment.
Results: We expect to find an ERK isoform-specific response to NMDA such that ERK1 but not ERK2 blocks the effect of NMDA treatment on Abeta production.
Conclusions: Given that ERK signaling is involved in widespread cellular processes, the ability to selectively target a single ERK isoform as a means to reduce Abeta generation could present a novel therapeutic option.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-1362

POSSIBLE ROLE OF STORE-OPERATED CALCIUM CHANNELS IN MEMORY LOSS CONNECTED WITH FAMILIAL ALZHEIMER'S DISEASE

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Familial Alzheimer's disease (FAD) which leads to memory impairments is caused by mutations in presenilin-1 (PS1) gene in approximately 40% of cases. PS1 is well known as a component of the gamma-secretase enzyme which cleaves APP to A-beta. To become a catalytic part of enzyme PS1 undergoes an endoproteolysis. It was shown that mutations in PS1 gene disrupt the endoproteolysis increasing uncleaved protein level in brain tissue of FAD patients. In our study we found effects of FAD PS1 mutants (PS1DE9, PS1 D247A) on activity of store-operated calcium (SOC) channels in mice hippocampal neurons and Neuro2a cell line. Increased uncleaved PS1 levels led to SOC channels hyperactivity detected with direct single-cell electrophysiological measurements and calcium imaging experiments with fura2-AM. The effects were caused by an impaired signal transduction from endoplasmic reticulum to SOC channels in plasmatic membrane. The impaired intracellular signal transduction by STIM1 sensor was revealed in live confocal imaging experiments and proved with STIM1 knock-down. Moreover, Drosophila melanogaster transgenes expressing human mutated PS1 in cholinergic nervous system feeding with pharmacological inhibitor of STIM1 sensor signal transduction 2APB led to rescue of the memory loss detected by courtship based assay with aged animals. Therefore hyperactive STIM1 signal transduction leads to increased SOC channels activity which could be the reason for memory loss in FAD.
This work was supported by the program of "Molecular and Cellular Biology" RAS, research grants from the Russian Basic Research Foundation, Russian Scientific Fund and the President of Russia Scholarship.

ADPD5-1983

P53 IN NEURODEGENERATION: IMPACT OF BETA-AMYLOID/P53 INTERFERENCE ON ALZHEIMER PATHOGENESIS

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Objectives: Zyxin and HIPK2 are proteins contributing to the activation of p53-regulated pathways. Zyxin is essential to maintain HIPK2 protein expression through interference with Siah-1 function. We described a link between zyxin-HIPK2-p53 signalling pathway and Alzheimer's disease (AD), finding that soluble beta-amyloid (Aβ) peptides modulate zyxin protein levels, fundamental in maintaining HIPK2 stability and in turn p53 wild-type conformation. The consequence is the loss of p53 transcriptional activity and failure to activate the proper apoptotic program when cells are exposed to *noxae*. Our aim is to describe the mechanisms of zyxin and HIPK2 regulation in presence of Aβ and its relationship to the correct control of cellular damage.
Methods: We examined the molecular mechanisms underlying zyxin and HIPK2 regulation in SH-SY5Y neuroblastoma cells and their counterpart stably transfected with wild-type APP751.
Results: We demonstrated that Aβ negatively modulates zyxin expression in protecting HIPK2 from Siah-1-mediated degradation, thus influencing the responsiveness to DNA-damaging agents. We further studied the alteration of cell death pathways induced by conformationally altered p53, mainly focusing on the regulation of autophagy. We investigated whether the loss of wild-type p53 conformation induced by Aβ could "free" mTOR, thus inducing abnormalities, which could contribute to system dysfunction.
Conclusions: These results may help to understand the pathogenesis of AD, through the dissection of events related to Aβ activities, shedding light on early events of AD pathogenesis related to an increase in the normal levels of Aβ and consequences related to disruption of the control of cell death via p53-dependent pathways.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-2146

MOLECULAR MECHANISM LINKING THE AMYLOID-BETA OLIGOMER AB*56 TO SPECIFIC TAU ALTERATIONS

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Objectives:

Although numerous neurodegenerative disorders including Alzheimer's disease (AD) and Parkinson's disease are characterized and neuropathologically defined by the presence of fibrillar protein aggregates, sustained evidence supports the concept that soluble, non-fibrillar forms of the amyloid proteins constituting these lesions might be the primary bioactive deleterious agents in these brain disorders. As such in AD, endogenous oligomeric forms of the amyloid-beta (oAβ) peptides appear to exist under various molecular arrangements, which include putative dimers, trimers and larger assemblies such as Aβ*56, and to differentially accumulate within the preclinical to clinical spectrum. Despite considerable efforts, little is known about the molecular signaling pathways induced by these soluble species thereby limiting our understanding of their role in the pathophysiology of AD.

Methods:

To address how Aβ*56 alters neuronal function, we combined biochemical, imaging and functional analyses of human tissue, transgenic mice modeling AD and mouse primary cultured neurons.

Results:

Coimmunoprecipitation approaches indicated that Aβ*56 forms a complex with NMDA receptors (NMDAR) in human and mouse tissue. Signaling pathway analyses revealed an aberrant activation of the Ca^{2+}-dependent calmodulin kinase CaMKIIα, triggered by a sublethal enhanced NMDAR-mediated Ca^{2+} influx. The observed CaMKIIα hyperactivity induced abnormal changes in tau *in vivo* and *in vitro*, consisting of selective hyperphosphorylation and missorting. Importantly, other forms of endogenous Aβ oligomers did not lead to similar changes.

Conclusions:

Our results demonstrate that distinct endogenous Aβ oligomers activate neuronal signaling pathways converging onto tau in a highly selective manner and support the notion of an abnormal excitatory neurotransmission observed in AD.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-0224

REGIONAL VARIATIONS IN THE EXPRESSION OF CAPILLARY LRP, RAGE AND P-GP IN NORMATIVE AND ALZHEIMER BRAINS.

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Senile plaques (SPs) accumulate pathogenic levels beta-amyloid (A-beta) in Alzheimer (AD) brains. This accumulation is facilitated by diminished efflux of A-beta by LRP, and / or enhanced influx of A-beta by RAGE, and / or diminished efflux of A-beta by P-gp. **Objectives**. In this study we investigated the expression of capillary LRP, RAGE and P-gp in brain samples of Alzheimer [AD] and normative brains [NM]. **Methods.** Superior temporal [ST] cortex, hippocampal [HC] and brain stem [BS] samples from 15 Alzheimer and 15 normative brains were selected from comparable sites. LRP, RAGE and P-gp positive capillaries and $Abeta_{42}$ plaques [SP] were quantified and statistical analysis of the non-parametric data was performed using the Mann-Whitney and Kruskal-Wallis tests. **Results.** In the ST, capillary expression of RAGE was greater in the AD condition ($p < .01$). For P-gp capillary expression there were no significant differences between the NM and AD conditions for any paired comparable sites. In the AD superior temporal cortex there was a positive correlation between the capillary expression of both LRP and RAGE with the presence of A-beta $_{42}$ senile plaques; but a negative correlation between P-gp expression and the presence of A-beta $_{42}$ senile plaques. **Conclusions.** These results indicate that regional variations in capillary LRP, RAGE and P-gp expression likely account for site-specific variations in SP lesion pathogenesis.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-0280
INTER-SITE VARIATIONS IN THE EXPRESSION OF CAPILLARY ENOS IN ALZHEIMERS BRAINS

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Fifteen Alzheimer [AD] and fifteen normative [NM] age-matched autopsy brains were examined within superior temporal cortex [ST] and brainstem [BS] samples. Endothelial nitric oxide synthase [eNOS] positive capillaries and β-amyloid $_{42}$ senile plaques were quantified in each site and in both conditions. The data was statistically analyzed using Mann-Whitney, Kruskal–Wallis and non-parametric Spearman's test.There was no significant difference in the expression of capillary eNOS between the AD and NM conditions in comparable sites. However, there was a significant difference in eNOS expression between superior temporal cortex and brainstem sites in both conditions [NM-p<.004; AD-p<.001].In both conditions the superior temporal cortex values were greater than those in the brainstem. In the NM superior temporal condition there was a positive correlation between eNOS expression and β_{42} senile plaque burden [<0.05]. In the AD superior temporal condition there was a negative correlation between eNOS expression and β_{42} senile plaque burden [<0.01].There were no significant correlations in either of the other two sites in either condition. The correlative results support the view that diminished capillary eNOS expression contributes to superior temporal cortex senile plaque pathogenesis. The inter-site comparative results demonstrate that capillary eNOS expression varies between sites in the brain, but not necessarily between NM and AD conditions. This inter-site variation in eNOS expression requires further study.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-0355

HYPERTHERMIA AND ENVIRONMENT POLLUTANT EXACERBATES AMYLOID-BETA PEPTIDE INFUSION INDUCED ALZHEIMER'S DISEASE PATHOLOGY. NEUROPROTECTIVE EFFECTS OF CEREBROLYSIN

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Military personals are often exposed to heat stress (HS) during combat operations in Middle East together with sand particles. HS induces breakdown of the blood-brain barrier (BBB) that is further aggravated by by SiO2 nanoparticles (SiO2 NPs) indicating more vulnerability of soldiers to development of Alzheimer's Disease (AD). This hypothesis was examined in a rat model of ameloid-beta peptide infusion (AbP 1-40, 250 ng/10 µl, i.c.v. daily) for 4 weeks.

Rats exposed to 1 h HS at 34°C in a biological oxygen demand (BOD) incubator for 30 days and also administered SiO2 NPs (50 mg/kg, i.p.) simultaneously. Control rats were kept at room temperature. After 30 days, AbP deposits in the brain and neuronal, glial or myelin pathology was examined.

AbP deposits within the cortex and hippocampus was 3- to 4-fold high in HS rats that further aggravated by 4 to 8 fold by SiO2 NPs treatment. Breakdown of the BBB to albumin, activation of astrocytes, myelin damage and neuronal injuries were also increased by 4 to 6 fold in HS and 6 to 10 fold in SiO2 NPs treated rats after AbP infusion. Cerebrolsyin (5 ml/kg, i.v. /day) treatment attenuated AD pathology in heat stressed rats intoxicated with SiO2 NPs, whereas only 2.5 ml/kg Cerebrolsyin is effective in control AD rats. This indicates for the first time that AD pathology depends on environmental factors and cerebrolysin is very effective in reducing AD pathology complicated with HS and SiO2 NPs.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-0470

BRAIN ENDOTHELIAL-SPECIFIC KNOCKOUT OF LRP1: A MODEL TO STUDY THE ROLE OF AMYLOID-BETA CLEARANCE ACROSS THE BLOOD-BRAIN BARRIER IN ALZHEIMER'S DISEASE

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The neurovascular hypothesis of Alzheimer's disease (AD) states that amyloid-beta accumulation is caused by impairment of low density lipoprotein receptor-related protein-1 (LRP1) at the blood-brain barrier (BBB). LRP1 is discussed to be the major brain-to-blood transporter of amyloid-beta. However, there are still controversies about the relevance of the BBB in AD pathology and LRP1's contribution in amyloid clearance due to embryonic lethality of global LRP1 knockout, and hence the lack of LRP1 knockout models.

Using a brain endothelial specific Cre-expressing mouse line, we generated a novel LRP1 knockout model to study LRP1-mediated amyloid-beta transport across the BBB. Here, we present a novel conditional brain endothelial-specific LRP1 knockout mouse to evaluate the neurovascular hypothesis. For the first time in an *in vivo* model, we can calculate the relevance of LRP1-mediated amyloid clearance across the BBB and distinguish from LRP1-mediated degradation within the brain. We show that deletion of LRP1 in murine brain capillaries strongly reduces amyloid-beta brain efflux. Furthermore, we demonstrate that major amounts of injected radiolabeled amyloid-beta are cleared via endothelial LRP1 across the BBB. In an AD mouse model, brain endothelial-specific knockout of LRP1 results in reduced plasma amyloid-beta, elevated brain amyloid-beta, vascular pathology, and memory deficits emphasizing the importance of systemic amyloid elimination via the BBB.

The collective data underline the important role of the BBB in AD pathology. The new knockout mouse provides a powerful instrument for investigating the functions of LRP1 at the BBB and will help to further clarify LRP1's relevance in health and disease

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-0739

IMPACT OF CHEMOTAXIS IN A MODEL OF BLOOD-BRAIN BARRIER IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

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Objectives

Alzheimer's disease (AD) is the most common form of dementia, posing a serious public health problem in worldwide. A central and peripheral inflammatory process is characterized in AD and is the subject of a debate on its beneficial or deleterious effects. Many data revealed the microglial senescence and suggested that a central distress could be support by peripheral blood mononuclear cells (PBMCs) going through the blood-brain barrier (BBB). At the molecular level, everything remains to be discovered. The objective of this study was to investigate the expression of some chemokines already known for their roles in the pathophysiology of AD in an *in vitro* human BBB model.

Methods

Two human cell lines constitute our BBB model: a human neuroglioma cell line **H4** from ATCC® and a human endothelial cell line **hCMEC/D3** from MTA with Inserm (PO Couraud, Institut Cochin, France). The study covers a total of 16 AD patients (MMSE between 10 to 25) by adding their PBMCs on BBB model. Chemokine levels were performed by using X MAP® Luminex assay after or not induction of amyloid stress with $Abeta_{1-42}$.

Results

We demonstrated that, regardless of amyloid stress, some chemokine expressions increased, certain strongly in endothelial cells. In contrast, others decreased in PBMCs, emphasizing negative environmental regulation of the BBB on PBMCs.

Conclusion

With this integrated human BBB model, we demonstrated the role of PBMCs from AD patients on the chemotactic environment at the BBB. The central origin of AD could be reviewed.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-0752

LONGITUDINAL CEREBROVASCULAR CHANGES IN A GENETIC MODEL OF ALZHEIMER'S DISEASE

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Clinical evidence suggests a role of cerebrovascular damage in the progression of Alzheimer's disease (AD). However, the causality between amyloid depositions and cerebrovascular dysfunction is uncertain. We have used a genetic mouse model of AD (5xFAD), previously demonstrated to reproduce hallmarks of the human pathology, to define: i) age-dependent cerebrovascular permeability and regional vessel distribution; ii) modification of blood-brain barrier cells, including pericytes; iii) topographical correlation between amyloid deposition and cerebrovascular dysfunction; iv) pro-inflammatory changes.

Fluorescent micro-angiography was performed on aging 5xFAD mice using FITC-Albumin. Confocal vessel reconstruction was performed with thioflavin-S or 6E10 staining to determine plaque-vessel topography. Pericytes were stained using PDGFRβ while GFAP and Iba-1 were used to assess inflammation. Distribution of vessel number, branching and length was performed using ImageJ. A parallel evaluation of human AD brains is being performed to validate clinical relevance of the experimental findings.

Blood-brain barrier dysfunction was observed in the hippocampus and cortices of 5xFAD mice. Changes included decreased microvessel number, shortening of vascular branches, irregular FITC-Albumin perfusion and albumin parenchymal leakages. PDGFRβ pericytes were redistributed towards the BBB interface, including a reactive increase of cell ramifications. Vascular and parenchymal amyloid depositions colocalized with BBB pathological changes and pericytes reactivity. Regional inflammatory GFAP/IBA1 activation was associated with vascular abnormalities.

Our results point to a multi-cellular contribution to AD. The pathological cerebrovascular changes observed reproduce clinical findings, supporting investigation at pre-AD stages. The latter is being performed to untangle the exact timing between cerebrovascular dysfunction and the progression of AD.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-0836

ROLE OF TRANSTHYRETIN IN A-BETA PEPTIDE BRAIN EFFLUX – INSIGHTS FROM IN VITRO AND IN VIVO STUDIES

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Objectives: Transthyretin (TTR) is the major A-beta (Ab) binding protein in the cerebrospinal fluid (CSF), rescuing the peptide, inhibiting its aggregation and toxicity. In vivo, TTR stabilization by iododiflunisal decreased brain Ab deposition and improved cognitive function. Ab levels were reduced in plasma and tending for reduction in the CSF, suggesting TTR promoted Ab clearance from the brain and periphery. This project aimed at investigating the influence of TTR in Ab transport across the blood- brain barrier (BBB), using a cellular model – human cerebral microvascular endothelial cells (hCMEC/D3).
Methods: We performed internalization and efflux assays of labelled (FAM-Ab-1-42) and non-labelled (Ab-1-42) Ab in the presence and absence of TTR. We also performed intracranial injections of FAM-Ab-1-42 in mice carrying one copy of TTR gene ($TTR^{+/-}$) and animals without TTR ($TTR^{-/-}$). Measurements of Ab concentrations were done by fluorometric analysis or by ELISA.
Results: Fluorometric measurements of FAM-Ab-1-42 in cell lysates indicated TTR increased FAM-Ab-1-42 internalization by hCMEC/D3 cells. In addition, permeability studies using hCMEC/D3 monolayers established in transwells filters, showed that only TTR added to the basolateral (brain) chamber was able to promote Ab efflux (added to the brain side), comparing to TTR added to the apical (blood) side. Our preliminary *in vivo* study demonstrated that, 30 minutes post injection, brains from $TTR^{+/-}$ mice retained less peptide than $TTR^{-/-}$ animals, indicating TTR favored Ab clearance from the brain.
Conclusions: TTR increases Ab internalization and transport across the BBB model, further supporting a role for TTR in Ab brain efflux.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-0997

THE RELATIVE CONTRIBUTIONS OF DEGRADATION AND BRAIN-TO-BLOOD ELIMINATION TO CEREBRAL CLEARANCE OF HUMAN AMYLOID-BETA PEPTIDE(1-40) IN MOUSE BRAIN

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Cerebral clearance of soluble amyloid-β peptide (Aβ) involves both degradation in the brain and elimination across the blood-brain barrier (BBB). The relative contributions of these processes are poorly understood. The purpose of the present study was to estimate the relative contributions of degradation and elimination processes to the clearance of microinjected human amyloid-b peptide (hAβ(1-40)) from mouse cerebral cortex using a newly developed UPLC/MS/MS quantitation method for intact hAβ(1-40) and a radiolabeled hAβ(1-40). The clearance rate constant of intact hAβ(1-40) in mouse cerebral cortex was determined to be 3.21×10^{-2} min^{-1} under conditions where saturable process in elimination across the BBB was expected to be saturated. Thus, this clearance rate constant should mainly reflect degradation. The [^{125}I]hAβ(1-40) elimination rate across the BBB under nonsaturating conditions was determined to be 1.48×10^{-2} min^{-1}. Inhibition studies suggested that processes sensitive to insulin and phosphoramidon, which inhibit known Aβ degrading enzymes such as neprilysin and insulin-degrading enzyme (IDE), are involved not only in degradation, but also in elimination of hAβ(1-40). Internalization of [^{125}I]hAβ(1-40) into cultured mouse brain capillary endothelial cells (TM-BBB4) was significantly inhibited by either insulin and IDE inhibitors, but was not inhibited by phosphoramidon. The internalization of [^{125}I]hAβ(1-40) by TM-BBB4 cells was reduced by IDE-targeted siRNAs. In conclusion, our results suggest dominant contribution of degradation to cerebral hAβ(1-40) clearance, and that elimination of hAβ(1-40) from mouse brain across the BBB involves an insulin-sensitive process, mediated by IDE expressed in brain capillary endothelial cells.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-1317

PLASMA LEVELS OF BETA-AMYLOID 1-42 ARE ASSOCIATED WITH EXTENT OF SLEEP FRAGMENTATION IN PSYCHIATRISTS ON-CALL

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Objectives: From mice there is evidence that the might be an association between sleep deprivation and plasma amyloid levels. In healthy subjects sleep fragmentation and low sleep efficiency are associated with impaired cognitive function, and impaired sleep quality predicts cognitive decline. Doctors with on-call duty represent shift working with utmost socioeconomic importance.
Hence, we examined whether plasma amyloid levels are associated with sleep duration or sleep fragmentation in Psychiatrists on-call.
Methods: Psychiatrists of Klinikum rechts der Isar, Technische Universität München, Munich, Gemany, underwent a venous blood draw at up to 8 consecutively nights with on-call duty at the beginning and at the end of their shift, respectively. Plasma beta amyloid 1-42 (Aβ42) concentrations were measured in quadruplicate with a commercially available ELISA.
Results: The generalized linear model solely including relative reduction of Aβ42 revealed a statistically significant reduction of Aβ42 over night (estimate 0.107, $p < 0.001$). The model with the outcome variable relative reduction of Aβ42 revealed for the variable number of sleep interruptions < 15 minutes a significant estimate of -0.037 ($p=0.001$), all other variables (inter alia sleep duration and total time of demand) were non-significant. This means that there is a significant decrease of the relative reduction of Aβ42 of 3.7% for every sleep interruption < 15 minutes.
Conclusions: The fact that reduction of plasma Aβ42 levels during night is decreased by the extent of sleep fragmentation raises the question whether on-call duty might be associated with an altered risk of developing Alzheimer's disease.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-1548

AMYLOID AND INSULIN TRAFFICKING AT THE BBB: QUID PRO QUO?

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Impaired brain clearance of amyloid beta (Abeta) peptides and perturbed brain insulin levels are closely associated with Alzheimer's disease (AD). Endowed with sophisticated cellular trafficking machinery and large interaction surface between blood and brain compartments, the blood brain barrier (BBB) effectively clears Abeta from the brain and serves as a major conduit for insulin delivery to the brain. Studies conducted in BBB cell culture models in vitro have shown that normal insulin signaling is necessary for receptor mediated insulin transport at the BBB. We observed that the insulin permeability at the BBB was significantly lower in AD transgenic mice (APP,PS1) compared to the age-matched wild type (WT) mice. Moreover, Abeta40 or Abeta42 exposure inhibited the uptake and permeability of insulin at the BBB to the levels observed in APP,PS1 mice . On the other hand, insulin altered the plasma membrane distribution of putative amyloid receptors such as LRP1 and RAGE; regulated vesicular trafficking machinery within the endothelial cell; and impacted BBB trafficking of Abeta40 and Abeta42. Following intravenous (IV) administration, insulin triggered differential trafficking of Abeta peptides at the BBB; the blood-to-brain permeability of Abeta40 was increased, whereas the Abeta42 permeability was decreased. Alternatively, the brain-to-blood efflux of both Abeta40 or Abeta42 was increased upon IV insulin administration. Hence, impaired insulin signaling, as observed in AD, could alter Abeta40:42 ratios and increase brain Abeta burden. The augmented Abeta levels could adversely affect brain energy metabolism, synaptic plasticity, memory and cognition.

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03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-1599

EXPRESSION OF REGULATORY PROTEINS IN CHOROID PLEXUS CHANGES IN EARLY STAGES OF ALZHEIMER´S DISEASE

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AIMS: The role of choroid plexus in Alzheimer´s disease (AD) is being increasingly recognized. Recent studies suggest that the choroid plexus has a more important role in physiological and pathological brain functions than previously appreciated.
METHODS: To obtain additional insight on choroid plexus function, we performed a proteomic analysis of choroid plexus samples from AD stages I-II (n = 16), III–IV (n = 16), and V–VI (n = 11), and 7 age-matched control subjects. We used differential 2D electrophoresis (2-D DIGE) coupled with mass spectrometry to generate a complete picture of changes in choroid plexus protein expression occurring in AD patients.
RESULTS: We identified 6 proteins which are significantly regulated in AD pathology, with central physiological functions including mitochondrial dysfunction and apoptosis regulation: 14-3-3 b/a, 14-3-3 e, moesin, proteasome activator complex subunit 1 (PSME1), annexin V, and aldehyde dehydrogenase (ALDH).
CONCLUSSION: The data presented here offer additional significance to the emerging importance of molecular and functional changes of choroid plexus function in the development of AD pathology.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-1838

HYPERTENSION INDUCED VASCULAR REMODELING AND INCREASED SUSCEPTIBILITY TO STROKE IN MOUSE MODEL OF CEREBRAL AMYLOIDOSIS

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Objectives: There is an increasing recognition that dysfunction in the cerebral vasculature can play a significant role in dementia, including AD where approximately 80-95% of the cases have cerebral vascular pathology The deposition of amyloid-beta peptides in the cerebral vasculature, known as cerebral amyloid angiopathy (CAA), is an important risk factor of intacerebral hemorrhage (ICH), and blood pressure controlling may significantly reduced the risk of CAA-related ICH. This study was designed to determine the effect of experimental hypertension on cerebral and cerebrovascular pathology in mouse model of amyloidosis.

Methods: This study aims to determine the effect of hypertension on Alzheimer's related pathology using an APP/Tg mouse model. Tg2576 mice and non-transgenic (nonTg) littermates were treated with an angiontensin II (AngII) infusion with ALZET Osmotic pumps (1000 ng/kg/min) and L-NAME (100 mg/kg/day) in drinking water to produce chronic hypertension. One week later, transient acute hypertension was induced by AngII injections (0.5 mg/g, twice daily). After appearance of stroke symptoms mice were analyzed for the AD related cerebral and vascular pathology.

Results: A similar increase in systolic blood pressure was observed in both Tg2576 and nonTg mice, however, compared with nonTg mice, Tg2576 mice developed signs of ICH with a markedly shorter latency. In addition, there was as an increase in inflammatory markers, CAA, and vascular remodeling factors pro-MMP-9 and p-selectin, which may explain increase in number and size of spontaneous microhemorrhages in hypertensive Tg2576 mice.

Conclusions: Hypertension, while been pharmacologically manageable, exacerbates Alzheimer's related pathology and vascular remodeling, leading to ICH.

03r. Pathophysiology & Disease Mechanisms: vasculature & neoangiogenesis

ADPD5-1275

PROTECTIVE MECHANISMS OF P66SHC DELETION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Objectives

According to the “vascular hypothesis”, age- and cerebrovascular-related risk factors play a critical role in AD pathogenesis, leading to early vascular dysregulation, which is considered to precede significant Abeta-plaque deposition, cognitive decline and onset of neurodegenerative changes. The mitochondrial adaptor protein $p66^{Shc}$ is involved in ageing/longevity and vascular disease. $p66^{Shc}$ knockout mice show a 30% prolonged life span and are protected against age-associated endothelial dysfunction. Moreover, $p66^{Shc}$ activation has been shown to be involved in Abeta-induced neurotoxicity *in vitro*.

Methods

To study the role of $p66^{Shc}$ deletion on AD-related pathology *in vivo*, we generated a novel APP transgenic mouse line lacking $p66^{Shc}$. The four genotypes (wildtype, PSAPP, p66 ko, p66 ko/PSAPP) of this novel p66 ko/PSAPP line were analyzed at three different ages.

Results

Behavioural testing revealed cognitive deficits in the PSAPP group at 15 months in the Y maze and Novel object recognition task (NORT) and already at 3 months in the Barnes maze. Strikingly, all observed impairments were rescued by $p66^{Shc}$ ablation. This rescue effect was independent of Abeta pathology since we observed only minor differences in Abeta-plaque burden. Interestingly, we found a “hypervascularity phenotype” in the PSAPP mice, which was reversed by $p66^{Shc}$ ablation. Furthermore, the PSAPP mice showed a trend towards increased blood brain barrier leakages.

Discussion

The results of this study suggest that protective effects of $p66^{Shc}$ ablation on Abeta-related cognitive dysfunction are most likely downstream of Abeta deposition and may be mediated by beneficial effects on vessel architecture and subsequent vascular dysfunction.

ADPD5-1296

THE EFFECT OF EXERCISE ON THE VASCULATURE OF THE SENSORIMOTOR CORTEX IN A MOUSE MODEL OF ALZHEIMER'S DISEASE.

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Objectives: There is growing interest in the role of brain vascular injury in the etiology and progression of AD. Further, exercise has been shown to elicit increased angiogenesis, reduction of amyloid-beta load and improved cognitive function in mouse models of AD. We thus set out to investigate the effect of exercise on the cortical microvessels in the TgCRND8 mouse model of AD.

Methods: Twenty nine 3-month old TgCRND8 and their non-transgenic littermates were given ad libitum access to a running wheel for 12 weeks until 6-months of age. They were then injected with Methoxy XO4 to label amyloid-beta and 24 **h**our**s thereafter** perfused with a mercox-BABB (Benzyl Alcohol-Benzyl Benzoate) solution containing Nile red. The brains were fixed in 4% PFA, progressively dehydrated, and cleared by BABB. Two photon fluorescence images were acquired over 4 partially overlapped regions in the motor and somatosensory cortex, at 0.5x0.5um in plane resolution. Following deconvolution and stitching of imaged volumes, the vasculature was tracked using a multi-scale automated segmentation algorithm.

Results: We evaluated vessel density, branch density, tortuosity and calculated a map of distances to nearest vessels for all of parenchyma in each animal. The preliminary data suggest that vessel density is decreased by ~20% in the motor cortex of the TgCRND8 mice compared to controls and that this effect is partially resolved with the 3-month exercise regimen.

Conclusions: This work provides further mechanistic insight into the effect of exercise on brain microvasculature and underscores the significance of vascular injury in AD progression.

ADPD5-1605

PRENATAL HIGH FAT DIET ALTERS THE NEUROVASCULAR UNIT AND CLEARANCE OF BETA-AMYLOID IN ADULT OFFSPRING

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Background: Cerebral amyloid angiopathy (CAA) is characterized by the accumulation of β-amyloid (Aβ) peptides in the walls of cerebral blood vessels. Failure of perivascular drainage of Aβ along cerebrovascular basement membranes contributes to the development of CAA. Mid-life hypercholesterolemia is a risk factor for the development of CAA. Maternal obesity is associated with the development of hypercholesterolemia in adulthood, suggesting that the risk for CAA may be influenced by early dietary environment. **Objectives**: We tested the hypothesis that early life exposure to a high fat diet results in changes to the cerebrovasculature and failure of Aβ clearance from the brain. **Results:** Using a mouse model of maternal obesity, we found that exposure to a high fat diet during gestation and lactation induced changes in multiple components of the neurovascular unit, including a downregulation in collagen IV, fibronectin and apolipoprotein E, an upregulation in markers of astrocytes and perivascular macrophages and altered blood vessel morphology in the brains of adult mice. Sustained high fat diet over the entire lifespan resulted in additional decreases in levels of pericytes and impaired perivascular clearance of Aβ from the brain. In humans, vascular Aβ load was significantly increased in the brains of aged individuals with a history of hypercholesterolemia. **Conclusions:** These results support a critical role for early dietary influence on the brain vasculature across the lifespan, with consequences for the development of age-related cerebrovascular and neurodegenerative diseases.

ADPD5-2293

OLIGOMERIC AMYLOID-BETA INDUCES CEREBROVASCULAR ABNORMALITIES IN THE APP E393Q TRANSGENIC MOUSE WITH ALZHEIMER'S DISEASE LIKE COGNITIVE DEFICITS

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Objectives: Cerebrovascular pathology is observed in patients with Alzheimer's disease (AD). However, it is unclear whether the soluble oligomeric or the fibrillar form of amyloid-beta, the AD neurotoxic peptide, is responsible for cerebrovascular pathology. Our study aims to provide evidence for the effect of soluble oligomeric amyloid-beta on cerebrovascular pathology, in the absence of amyloid plaques.
Methods: We used transgenic mice expressing the Dutch APP E693Q mutation (DU mice) that display oligomeric amyloid-beta but no amyloid plaques in the brain. We identified morphological changes in brain vasculature of DU mice as compared to wild-type (WT) controls, using collagen immunohistochemistry and quantitative stereology. We also examined the changes in expression of vascular endothelial cell tight junction proteins in DU mice, by Western blot.
Results: There was no change in the overall vascular length density between DU and WT mice. We found increased incidence of abnormal vessel morphology, such as twisted vessels and string vessels, in the hippocampal formation of the APP E693Q mice as compared to WT controls. The expression of the tight junction protein zona occludens-1 [MV1] was altered in the hippocampus, but not the cortex, of DU mice as compared to WT. Future studies include characterization of other tight junction proteins, including claudin-5, occludin and VE-cadherin.
Conclusions: Our results indicate that the presence of soluble oligomeric amyloid-beta is sufficient to induce vascular abnormalities in the brain and may represent early changes occurring in AD prior to amyloid deposition.

[MV1]We only have n=2 for the Western data of ZO-1.

03s. Pathophysiology & Disease Mechanisms: neurogenesis and stem cells

ADPD5-0468
INITIATION OF ABETA DEPOSITION IMPAIRS ADULT HIPPOCAMPAL NEUROGENESIS

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Objectives
Alzheimer´s disease (AD) is characterized by the accumulation of Abeta which ultimately forms plaques. These Abeta deposits can be induced in young pre-depositing APP transgenic mice by intracerebral injection of Abeta-containing brain homogenate. Although previous studies reported a decline of neurogenic capacity in the brain of AD patients and AD mouse models it still remains unknown whether this impairment is specifically altered by Abeta plaques.
Methods
In order to determine the impact of amyloid-beta pathology on neural progenitor cells in the hippocampus we performed intracerebral injections of brain homogenate from aged transgenic mice into young pre-depositing APP transgenic mice. By using immunohistochemical stainings and western blot analysis we quantified Ki67 positive cells as a marker for proliferation, counted the number of doublecortin positive cells as a marker for neurogenesis and measured the Abeta content with an antibody against Abeta.
Results
We observed a dramatic decrease in proliferation and neurogenesis in mice that had been injected with Abeta-containing brain homogenate when compared to controls. Furthermore, the number of granular cells was significantly decreased, and signs of neuronal cell death occurred primarily in the vicinity of induced Abeta deposits. Additionally doublecortin positive cells differed in their morphology displaying less elaborated dendrites and developing shorter dendrite length indicating that the generation and maturation of newborn neurons is affected.
Conclusions
In summary our data suggest that induced Abeta deposition can lead to impaired adult hippocampal neurogenesis and contributes to neuronal vulnerability. We hypothesize that this might result in learning and memory deficits.

03s. Pathophysiology & Disease Mechanisms: neurogenesis and stem cells

ADPD5-1515

THE UCI ADRC IPS CELL CORE: A NOVEL RESOURCE TO EXAMINE RELATIONSHIPS BETWEEN GENOTYPE AND PHENOTYPE IN LATE-ONSET ALZHEIMER'S DISEASE

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Objectives:

Twin studies have shown that late-onset Alzheimer's disease (AD) is highly heritable and GWAS studies have begun to uncover both common and uncommon genetic variants that influence disease risk. However, the precise relationship between these genetic markers and disease-associated phenotypes will likely be challenging to unravel. Recent advances in cellular reprogramming provide an innovative new way to examine these relationships by studying patient-derived induced pluripotent stem (iPS) cells. Yet given the variable penetrance and effect size of many of these genetic variants, it will be important to examine multiple well-characterized iPS cell lines.

Methods:

Here, we describe the creation of a federally-funded iPS Cell Core tasked with generating and distributing iPS cells from a well-characterized cohort of AD, MCI, and control patients. Skin biopsies are routinely collected from the UCI Alzheimer Disease Research Center patient cohort and iPS cells are generated via non-integrating Sendai viral reprogramming.

Results:

Stably reprogrammed iPS cells are expanded, tested for pluripotency and sterility, and karyotyped before banking. For each line, corresponding de-identified clinical and biomarker datasets and SNP analysis of 31 GWAS-associated genes are also available. For several of these lines neural stem cells have also been generated and banked. Using these iPSC-derived cells the iPS cell core has also now established protocols to generate neurons, astrocytes, endothelial cells, and microglia.

Conclusion:

The UCI ADRC iPS cell bank aims to provide an invaluable new resource to help researchers around the world to study the relationships between AD-associated genetic polymorphisms and disease-associated phenotypes.

03s. Pathophysiology & Disease Mechanisms: neurogenesis and stem cells

ADPD5-1942

THE AMYLOID PRECURSOR PROTEIN EXPRESSED IN THE CHOROID PLEXUS IS A KEY REGULATOR OF ADULT NEUROGENESIS

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Physiologically, the Amyloid Precursor Protein (APP) is cleaved by an alpha-secretase, releasing soluble APP (sAPP) an important regulator of adult neurogenesis. This cleavage prevents two others in positions beta and gamma that generate the ßA4 toxic peptide, a hallmark of Alzheimer Disease.

Next generation RNA-sequencing has revealed that *APP* is the 16th most expressed genes in the choroid plexus, more than in the hippocampus and cortex, suggesting that it may be a major source of sAPP and ßA4 in the cerebrospinal fluid (CSF). If so, adult neurogenesis in the SVZ and hippocampus may be regulated by the choroid plexus and impeded in mutations favoring ßA4 production.

The choroid plexus is easily accessible and can be target by viral vectors. This property was exploited to develop APP loss and gain of functions specifically in this structure and in the CSF. This has allowed us to confirm that modifying *APP* expression specifically in the choroid plexus is sufficient to modify adult neurogenesis.

In the course of this presentation we shall describe the adult neurogenesis model summarized above and discuss the preliminary experiments aimed at down-regulating mutated *APP* in the choroid plexus to replace it by the wild type gene.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-0279
IRON INDUCED CHANGES IN MICROGLIA INCREASE APP PROCESSING IN CO-CULTURE.

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As the brain ages, both glia and neurons undergo changes in numerous processes especially those associated with maintaining the redox balance. Microglia have also been shown to increase storage of iron. Microglia with high iron content are often dystrophic and such microglia may play a significant role in enhancing or exacerbating pathological conditions in the brain. We created a model of iron induced dystrophic microglia by growing microglial cell lines in high iron concentrations. Iron –fed microglia showed increased iron content and ferritin levels. Co-culture with such microglia induced neuronal loss in both primary neuronal cells and cell lines in culture. We used an assay for beta-amyloid formation that demonstrated that co-culture with microglia in general decreased APP processing, but if the microglia were activated with LPS or grown previously with high iron, there was a significant increase in APP processing. Increased exposure of cells to iron-fed microglia resulted in loss of cells. The cells killed by the microglia were predominantly those cells that showed higher APP processing. We are currently in the process of identifying the cytokines generated by the microglia that mediate these changes.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-0331

ASTROCYTIC ADENOSINE RECEPTOR A2A REGULATES MEMORY

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Objectives: To test the hypothesis that the G_s-coupled adenosine receptor A2A expressed by glial cells contributes to Alzheimer's disease (AD) pathogenesis. In particular, we determined if 1) A2A receptors are expressed by glial cells in humans with AD and in mouse models relevant to this condition, and 2) glial A2A receptors and G_s-coupled signaling affect learning and memory, and contribute to AD-related cognitive deficits.
Methods: To examine glial A2A receptor expression, we performed immunolabeling, western blotting, and qPCR in postmortem brain tissue from human cases with AD and transgenic mouse models of AD. To examine the roles of glial A2A receptors and G_s-coupled signaling in cognitive function, we conducted behavioral testing in transgenic mice with conditional genetic ablation of A2A receptors or inducible expression of a modified G_s-coupled receptor amenable to chemogenetic manipulation.
Results: A2A receptor levels were increased in hippocampal astrocytes of humans with sporadic AD and these increases correlated with disease progression. Astrocytic A2A receptor levels were also increased in the hippocampus of aging transgenic mice expressing human amyloid precursor protein (hAPP). Conditional genetic ablation of astrocytic A2A receptors enhanced long-term memory, but not learning, in young and aging mice without hAPP expression. Ablation of astrocytic A2A receptors enhanced memory also in aging hAPP mice without affecting their learning deficits. Chemogenetic stimulation of astrocytic G_s-coupled receptor signaling reduced long-term memory without affecting learning or short-term memory.
Conclusions: Astrocytic G_s-coupled A2A receptors negatively regulate memory and AD-linked increases in astrocytic A2A receptor levels may contribute to memory loss.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-0643

THE RESPONSE OF OLIGODENDOCYTES AND THEIR PROGENITORS TO THE ALTERED CHEMISTRY OF THE ALZHEIMER'S DISEASE BRAIN

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Objectives
White matter pathology occurs in Alzheimer's disease (AD), but its cellular basis remains unknown. We report the use of immunocytochemical methods to test the hypothesis that oligodendrocyte progenitor cells (OPC) and their descendents are directly suceptible to Aβ toxicity and inflammation in AD.
Methods
R1.40 transgenic mice carrying the human APP gene with the Swedish mutation were used as an AD model. The number of OPC (NG2-positive) and mature oligodendrocytes (myelin basic protein [MBP]-positive) cells were quanitified in 18-20 month old R1.40 mice and their C57BL/6-wildtype littermates. The effects of fibrillar Aβ peptide and lipopolysaccharide (LPS)-induced inflammation on the numbers and morphology of OPC and mature oligodendrocytes (mOL) were quantified in mixed cultures of embyronic neocortical neurons (wildtype-DIV14).
Results
In immunostained sections of R1.40 hippocampus, the densities of OPC and mOL were 35% and 19% lower respesctively than in wildtype ($p < 0.05$). *In vitro*, both Aβ peptide and LPS reduced the number of OPC and mOL in a concentration-dependent fashion. At 10 μM, Aβ reduced OPC and mOL by 82.2% and 60.3% respectively after 24 h of treatment ($p < 0.001$). Both treatments also induced a substantial change in the normal morphology of cultured OPC and mOL.
Conclusions
OPC and mOL are both vulnerable to Aβ-toxicity *in vivo* and *in vitro*. The effects are especially dramatic *in vitro* and warrant further investigation into the contribution of amyloid and inflammation to the observed early white matter pathology in AD.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-0754

ACCUMULATION OF AMYLOID-BETA RESULTS IN GIANT ASTROCYTIC VACUOLES AND SECONDARY NEURONAL CELL DEATH

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Objectives
A growing body of research suggests that astrocytes play an important role in the pathophysiology of Alzheimer's disease (AD). Reactive astrocytes, surrounding amyloid plaques have been demonstrated to engulf amyloid-beta (Aβ), but their effectiveness in Aβ degradation remains elusive. The aim with this study was to clarify the role of astrocytes in Aβ clearance by studying Aβ uptake, degradation and toxicity in a co-culture system of neurons, astrocytes and oligodendrocytes.

Methods
Neural stem cells from embryonic mouse cortex were differentiated to neurons, astrocytes and oligodendrocytes and exposed to Cy3-labeled Aβ1-40 monomers or Aβ1-42 protofibrils for 24h. The cultures were then thoroughly washed and followed for a period of 12 days. Uptake, degradation and toxic effects of Aβ were studied by time-lapse microscopy, immunocytochemistry and ELISA.

Results
Our data show that monomeric Aβ1-40 is effectively degraded by the cells, but Aβ1-42 protofibrils are accumulated for long times. Immunostainings with specific cell markers, demonstrated that astrocytes and oligodendrocytes engulf large amounts of Aβ, while neurons ingest very little. Interestingly, Aβ1-42 protofibril exposure results in extremely large astrocytic vacuoles. These vacuoles never appear in control cultures, indicating that they may be a way for the astrocytes to cope with the high Aβ load. Aβ has no direct toxic effect on neurons and glia, but causes secondary neuronal cell death several days after the Aβ removal.

Conclusions
The engulfment of Aβ1-42 protofibrils by glial cells results in Aβ accumulation, formation of pathological vacuoles in the astrocytes and secondary neuronal cell death.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-0758

MILD TRAUMATIC BRAIN INJURY IN APP/PS1 KNOCK-IN MICE INDUCES ALTERED GLIAL RESPONSES AND ACCELERATED COGNITIVE DEFICITS

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Objectives: Epidemiological studies show a history of head injury is associated with earlier onset, and increased Alzheimer's disease (AD) pathological and cognitive changes. Currently, little is known about how a single head injury accelerates onset of AD; yet, clinically, neuroinflammation has been found to be chronically elevated after a single head injury. As neuroinflammation can affect AD neuropathology and cognitive impairment, we tested whether an altered inflammatory response following a traumatic brain injury might be a contributing factor.
Methods: APP/PS1 knock-in (KI) mice and wild-type (WT) controls received a closed head injury (CHI) at 8 months of age, prior to cognitive deficits in the KI mice, and then various endpoints were measured at 9h, 1d, 7d, 1m, and 2m post-injury.
Results: At 1m post-injury, injured KI mice exhibited a significant impairment in radial arm water maze compared to sham KI mice or injured WT mice. Unexpectedly, we found that the temporal astrocyte and cytokine response in the injured KI mice was delayed compared to the injured WT mice. However, once activated, the glial injury response in the KI mice failed to resolve compared to the injured WT mice, resulting in chronic glial activation.
Conclusions: In agreement with clinical findings, our experimental model suggests that a single head injury can accelerate cognitive impairment, and that the mechanism may involve a dysregulated neuroinflammatory response.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-0825

HIPPOCAMPAL INCREASE IN TNF-ALPHA CORRELATES WITH TRANSLOCATOR PROTEIN AND MICROGLIA MARKER IBA1 UP-REGULATION DURING THE ASYMPTOMATIC STAGE OF ALZHEIMER'S DISEASE

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OBJECTIVES: Our main goal was to assess glia activation during the asymptomatic stage of Alzheimer's disease. The hippocampal expression of TNFalpha and mitochondrial translocator (TSPO) protein were used as out-read of glia activation. To determine whether both microglia and astrocytes contribute to putative glia activation, the expression of their respective (Iba1 and GFAP) phenotypic markers was correlated with the level of TSPO and TNFalpha.
METHODS: Transgenic APPswePS1dE9 and the littermate non-transgenic female mice aged 3 months (n=6-11 per genotype) were used. Amyloid precursor protein (APP), TNFalpha, TSPO, GFAP and Iba1 expressions were quantified in the hippocampus by western blot. TNFalpha and TSPO were additionally assessed by ELISA and [3H]-PK11195 autoradiography, respectively.
RESULTS: Considered globally, the inter-individual variability was important independently of the genotype and no statistically significant difference was found between mean values for any of the studied parameters. However, linear regression analysis indicated that the increase in APP correlates positively with both TNFalpha ($r=0.878$, $p=0.0002$) and GFAP ($r=0.913$, $p=0.0001$) which are also mutually correlated ($r=0.907$, $p=0.0001$ for GFAP *versus* TNFalpha). By contrast, APP is not correlated with Iba1 ($r=0.372$, $p=0.2338$). Moreover, TSPO induction is not correlated with APP ($r=0.362$, $p=0.2469$), but is correlated positively with Iba1 ($r=0.768$, $p=0.0035$) which is in turn correlated with TNFalpha ($r=0.586$, $p=0.0452$).
CONCLUSIONS: Both astrocytes and microglia contribute to the increase in TNFalpha during the early stages of Alzheimer's-like pathogenesis though TSPO appears selectively up-regulated only in microglia.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-0846

IMPACT OF PERIPHERAL MYELOID CELLS ON ABETA PLAQUE BURDEN

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Alzheimer's disease (AD) is a primary neurodegenerative disorder characterized by neuronal loss, neurofibrillary tangles and accumulation of β-amyloid (Aβ) plaques. While central nervous system (CNS) resident microglia are believed to be ineffective at phagocytosing and clearing Aβ, evidence from transgenic AD mouse models suggests that peripheral myeloid cells constitute a heterogenous cell population with greater Aβ clearing capabilities. To assess the ability of peripherally-derived macrophages to reduce Aβ plaque pathology, we exchanged the pool of resident microglia with peripherally-derived myeloid cells using APPPS1 mice crossed to CD11b-HSVTK mice (APPPS1-TK), which allow for selective ablation of CD11b+ cells. We found that under certain experimental conditions, depletion of microglia is invariably followed by rapid and large-scale infiltration of peripheral macrophages. Using this approach to exchange the CNS myeloid cell pool, we evaluated the effects of microglial depletion and repopulation by peripherally-derived myeloid cells on Aβ plaque load in 150 day-old APPPS1-$TK^{+/-}$ mice. While there was no change in plaque burden *per se* in these animals compared to APPPS1-$TK^{-/-}$ animals that were not depleted of resident microglia and had little or no invasion by peripheral myeloid cells, additional manipulation such as non-specific or Aβ-targeted stimulation revealed that peripherally recruited myeloid cells have greater amyloid clearing capacity than resident microglia in response to activating signals, highlighting the therapeutic potential of cell-based therapy for Alzheimer's disease.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-0911
IRAK-M REMOVAL PROMOTES MICROGLIA-MEDIATED AMYLOID PHAGOCYTOSIS AND IMPROVES COGNITIVE IMPAIRMENT IN PSAPP MICE

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Objectives: Amyloid-β (Aβ) engages toll-like receptors (TLRs) to stimulate 'host defense' mechanisms, resulting in pro-inflammatory activation of microglia, the brain's innate immune cells. TLRs transduce their signals through MyD88 and the serine/threonine IL-1 receptor-associated kinase (IRAK). The IRAK family of kinases generally upregulate TLR signaling, with the notable exception of the inhibitory kinase IRAK-M. IRAK-M expression is specific to cells of monocytic lineage, including microglia, and plays a critical role in the maintenance of innate immune homeostasis by dampening inflammation.
Methods: We crossed mice deficient in IRAK-M (IRAK-M$^{-/-}$) with mice over expressing mutant human amyloid precursor protein, the PSAPP mouse model of cerebral amyloidosis. We then assayed behavioral impairment and evaluated the state of immune cell activation and cerebral Aβ burden and in age and sex-matched littermates from PSAPP-IRAK-M^{+} and PSAPP-IRAK-M$^{-/-}$ mice at 15 months of age.
Results: Transgene-associated behavioral deficits were reversed in PSAPP-IRAK-M$^{-/-}$ mice including hyperactivity and spatial working and reference memory. Immunohistochemistry for the macrophage/microglia-specific protein Iba1 disclosed statistically significant increased expression in PSAPP-IRAK-M$^{-/-}$ *vs.* PSAPP-IRAK-M^{+} mice in the hippocampus, cingulate cortex and entorhinal cortex (up 38-42%), brain regions associated with AD-type pathology in humans. Additionally, *in vivo* quantification of Aβ encapsulation within Lamp1^{+} lysosomes revealed a phagocytic mechanism of IRAK-M$^{-/-}$ microglia-mediated Aβ clearance.
Conclusions: Collectively, these data suggest that IRAK-M negatively regulates brain innate immunity in a mouse model of cerebral amyloidosis. Disinhibition of IRAK-M may represent a means to target microglial remodeling of amyloid pathology.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-1084

SILENCING OF UPREGULATED KV3.4 POTASSIUM CHANNEL SUBUNIT REDUCES BETA-AMYLOID LEVELS IN TG2576 AD MICE

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Astrocyte dysfunction emerges early in Alzheimer's disease (AD) and may contribute to its pathology and progression. In the present study we investigated the expression and functional activity of the voltage-gated potassium channel subunit $K_V3.4$, that has been recognized to be relevant for AD pathogenesis, in astrocytes.

To this aim we evaluated: 1) the $K_V3.4$ expression by means immunofluorescence and western blot analysis and 2) the functional activity of the $K_V3.4$ by means patch-clamp. The protein expression and the activity of $K_V3.4$ were significantly upregulated in rat primary astrocytes exposed to β-amyloid peptide 1-42 (Ab_{1-42}). Furthermore, $K_V3.4$ expression was intensely upregulated in the astrocytes of the hippocampus, corpus callosum, and cerebellum of six-month-old Tg2576 AD mice at the early stages of AD. Coexpression and co-immunoprecipitation studies revealed a large overlap of $K_V3.4$ with, and direct binding to, the astrocyte-specific intermediate filament GFAP. Conversely, the selective knockdown of $K_V3.4$ expression significantly downregulated both GFAP and Ab protein levels in the brain of Tg2576 AD mice.

In conclusion, our results demonstrate that $K_V3.4$ have a critical role in astrocyte activation during the early stages of Tg2576 AD mice. Therefore, modulating astrocyte activity through a regulation of $K_V3.4$ channel functioning might open new avenues for developing innovative therapies capable of actually slowing down the progression of AD at its earliest stages.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-1120

TMT CALIBRATOR PLUS REVEALS MARKERS OF MICROGLIA ACTIVATION IN AD CSF

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An early event in neurodegenerative pathology involves activation of microglial cells that will secrete distinct proteins into the brain and possibly then into CSF. The challenge is how to find these molecules. Traditional unbiased proteomic approaches have struggled to detect low abundance markers due to the high dynamic range of CSF proteins. To improve biomarker discovery in peripheral fluids, we developed TMTcalibrator+; a novel MS workflow.

Using isobaric Tandem Mass Tags (TMT) allows tissue/cell lysate samples to be labelled and mixed into labelled samples of peripheral biofluids at a concentration sufficient to ensure the majority of MS/MS acquisitions are made on tissue/cell-derived peptides. We used a cultured microglial cell-line to represent activated microglia in Alzheimer's disease (AD) brain. Peptide preparations from microglial cells were TMT labelled to form a reference peptide mix. CSF samples from three patients with AD, and three non-AD controls were digested and labelled with the remaining TMT tags. All labelled digests were pooled together to form the TMTcalibrator+ analytical sample for analysis by LC-MS/MS.

By comparing the levels of each TMT reporter ion generated upon fragmentation, we can determine which microglial proteins are found in CSF and which are differentially expressed in AD. We have identified multiple up-regulated peptides in the CSF of AD patients belonging to proteins originating from microglia.

Application of our pioneering TMTcalibrator+ MS approach has allowed the identification of low abundant proteins in the CSF of AD patients which warrant further investigation as they may represent early events in the disease process.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-1625

IMMUNOHISTOCHEMICAL ANALYSIS OF WATER CHANNEL PROTEIN AQUAPORIN AND GLUTAMATE TRANSPORTER GLT-1 EXPRESSION IN ALZHEIMER'S DISEASE

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Objectives: We have previously reported marked changes in the expression of astrocytic aquaporin 4 (AQP4) and AQP1 in relationship to amyloid β peptide (Aβ) deposition in human Alzheimer's disease (AD) brains (Hoshi A et al, JNEN, 2012). GLT-1, the dominant astrocytic glutamate transporter in the cerebral cortex seems to be significantly reduced in AD. No studies have demonstrated any correlation between the expression of AQP and that of GLT-1 in AD. We, therefore, investigated the expression of AQP in association with GLT-1 in autopsied brains affected by AD.
Methods: Expression of AQP4, AQP1, and GLT-1 in the temporal lobes of 8 patients with AD and 5 age-matched controls were examined immunohistochemically.
Results: In the AD group, AQP4 immunoreactivity appeared more intense and number of AQP1-positive astrocytes was larger with decreased GLT-1 expression than those observed in the control group. Intriguingly, double immunofluorescence of AQP4 and GLT-1 showed numerous Aβ plaque-like AQP4/GLT-1 expressions. On the other hand, AQP1/GLT-1 double positive structures were rarely observed in the AD group.
Conclusions: There are no reports regarding to Aβ plaque-like AQP4/GLT-1 expressions in AD. These findings suggest a pathomechanism of senile plaque formation in association with reactive astrocytes expressing AQP4/GLT-1. Additionally, in AD brains, AQP1 positive astrocytes seem to appear in some population of astrocytes with reduced GLT-1 expression. In conclusion, AD neurodegenerative processes may progress mediated with disruption of water and glutamate homeostasis.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-1704
P2Y12 MEDIATES AMYLOID-BETA CLEARANCE BY MICROGLIA
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Alzheimer's disease (AD) is a degenerative brain disorder affecting around 10% of people older than 65 years. It is generally thought to be caused by the extracellular accumulation of amyloid-beta, Aβ, in the brain, causing neurodegeneration and memory loss at its later stages. It has previously been shown that microglial cells are involved in the pathogenesis of AD and that microglia are part of clearing and degrading Aβ from the brain. However, competing theories claim that microglia might actually contribute to the neurodegeneration in AD. In this study, we investigate how injected Aβ is cleared from the brain by microglia in zebrafish larvae using *in vivo* imaging. Our experiments demonstrate that microglia has neuroprotective features against Aβ toxicity. Our results also show that the p2y12 receptor plays an important role in the clearance of Aβ. In conclusion, our study indicates that microglia could serve as a drug target for future AD treatments.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-1947

NLRP3 INFLAMMASOME IS EXPRESSED AND FUNCTIONAL IN BRAIN MICROGLIA BUT NOT IN ASTROCYTES

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Neuroinflammation is the local reaction of the brain to infection, trauma, toxic molecules or protein aggregates. The brain resident macrophages, microglia, are able to trigger an appropriate response involving secretion of cytokines and chemokines, resulting in the activation of astrocytes and recruitment of peripheral immune cells. IL-1 beta plays an important role in this response; yet its production and mode of action in the brain are not fully understood and its precise implication in neurodegenerative diseases needs further characterization. Our results indicate that the capacity to form a functional NLRP3 inflammasome and secretion of IL-1 beta is limited to the microglial compartment in the brain. We were not able to observe IL-1 beta secretion from astrocytes, nor do they express all NLRP3 inflammasome components. Microglia are able to produce IL-1 beta in response to different classical inflammasome activators, such as ATP, Nigericin or Alum. Similarly, microglia are able to secrete IL-18, IL-1 alpha and HMGB1, three other inflammasome-linked pro-inflammatory factors. Cell stimulation with neurodegenerative disease-related peptides, such as alpha-synuclein, did not result in the release of active IL-1 beta by microglia despite their pro-inflammatory effect. Amyloid-beta peptides are able to activate the NLRP3 inflammasome and IL-1 beta secretion occurs in a P2X7 receptor-independent manner. Thus microglia-dependent inflammasome activation can play an important role in the brain and especially in neuroinflammatory conditions.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-0253

STUDY ON NEUROPROTECTIVE POTENTIAL OF MALAYSIAN TUALANG HONEY IN ALZHEIMER'S MODEL OF RATS.

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Objectives: One of the mechanisms which is thought to play a significant role in neurodegenerative disorders including Alzheimer's disease (AD) and dementia is oxidative stress and neuroinflammation leading to neuronal cell death. Since, honey has an antioxidant and anti-inflammatory activity, the present study was planned to study the neuroprotective capacity of Tualang honey in Alzheimer's model of rats.
Methods: Reduction in cerebral blood flow (CBF) due to aging has been linked to AD. Experimentally, chronic cerebral hypoperfusion due to reduced CBF can be created by permanent bilateral occlusion of common carotid arteries (2VO) in rats. Thirty Sprague Dawley rats weighing 200-250 g were divided into three groups (n=10). Group A – Sham control; Group B – 2VO; and Group C – 2VO+H (treated daily with honey, 1.2 g/kg freshly diluted with distilled water, orally by gastric gavage every morning following 2VO). On 10^{th} week, all the rats were euthanized and hippocampi were dissected out. Viable neuronal cells were counted in the hippocampal CA-1 region.
Results: In Group – B (2VO), damaged, distorted, irregular cells with shrunken cytoplasm and dark pyknotic nuclei were seen as compared to Sham control Group – A ($p<0.001$). Treatment of rats with honey restored the hippocampal CA-1 cells to their normal structure and revealed the reduced loss of neuronal cells in 2VO+H rats as compared to untreated 2VO rats ($p<0.001$).
Conclusions: This study shows that Malysian Tualang honey might have a therapeutic benefit in the management of chronic cerebral hypoperfusion induced neurodegenerative disorders like Alzheimer's disease.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-0294

AN IN VITRO MODEL OF ALZHEIMER'S DISEASE: AN ATTEMPT TO EXPEDITE THE SEARCH FOR NEW DRUG ENTITIES.

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Objective: The available medications for Alzheimer's disease (AD) are not able to prevent the disease progression. Therefore, a rapid screening test model is required to mitigate the cost and time required for screening new drugs. Streptozotocin (STZ, intracerebroventricularly) induces AD like pathology in rodent brain. The present study was performed to assess the potential of STZ treated neuronal cell line N2A as the experimental test model for the screening of drugs for AD.
Methods: AD related pathological markers like tau phosphorylation, amyloid aggregation, choline levels were estimated in STZ (10, 50, 100 and 1000 microM) treated N2A cells. Also, cell viability, cytotoxicity, glucose uptake, mitochondrial membrane potential, caspase-3 expression and DNA fragmentation were assessed in STZ treated N2A cells. STZ treated cells were also co-treated with clinically used anti AD drug donepezil (1microM) to validate the test model.
Results: The STZ treated neuronal cells exhibited the increased phosphorylation of tau protein, amyloid aggregation and decreased choline level which are the widely accepted pathological markers of AD. The STZ treatment also caused significant decrease in cell viability, inhibited glucose uptake, elevated mitochondrial stress and increased expression of caspase -3 and DNA damage. STZ induced AD related pathological markers were inhibited with donepezil treatment, but did not attenuate the STZ induced apoptosis.
Conclusion: Finding suggests that STZ treated N2A cells express the pathological markers hence could be used as *in vitro* rapid screening test model to screen new anti-AD drug entities.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-0309

A DEFENSIVE WARBUR-LIKE EFFECT PROTECTS NEURONS IN THE EARLY PHASE OF APOPTOSIS

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OBJECTIVES: Cerebellar granule neurons, undergoing low potassium induced apoptosis, were used as a cell model system for Alzheimer Disease (AD) to investigate the metabolic changes that occur during the entire time frame of cell death.
METHODS: Enzymatic assays were performed in the presence or absence of specific inhibitors of glucose metabolism. The expression levels of some glycolytic enzymes were detected by immunoblotting.
RESULTS: Cerebellar granule cells apoptosis can be divided into an early (reversible) and a late (irreversible) phase. In the early phase, several events, such as antioxidant system upregulation, proteasome activation and cytochrome *c* release, occur in an attempt to counter the death program. At this stage, aerobic glucose metabolism is enhanced with upregulation of the key proteins internalizing and metabolizing glucose, *i.e.* glucose transporter, hexokinase and phosphofructokinase. Concomitantly, mitochondria activity partially decreases with reduction in oxygen consumption and L-lactate accumulation. In the late phase, caspases become active, mitochondrial impairment goes further with alteration of the adenine nucleotide translocator and aerobic glycolysis is forced through the anaerobic pathway.
CONCLUSIONS: Results so far obtained point to a scenario in which a Warburg-like effect, with enhanced glycolysis and low-active mitochondria, takes place in the early phase when cells actively counter the death process in a manner resembling the apoptotic resistance mechanism of surviving neurons in the AD brain. However, in the late phase, a metabolic shift towards anaerobic glycolysis, mainly due to mitochondrial impairment, occurs and can exacerbate the pathophysiological processes associated with AD thus leading inevitably neurons to death.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-0487

CYTOSOLIC RELEASE OF LYSOSOMAL ENZYMES TRIGGERS NEURONAL DEATH IN ALZHEIMER'S DISEASE PATHOLOGY

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Objectives
Up-regulation of certain lysosomal enzymes within lysosomes can prevent sub-lethal damage, whereas sustained release of the enzymes into cytosol can induce cell death via cytochrome c release from mitochondria. However, very little is known about the subcellular distribution of lysosomal enzymes and their significance in Alzheimer's disease (AD). Since endosomal-lysosomal system is critical in the generation of β-amyloid (Aβ) peptides, which play important roles in the degeneration of neurons and development of AD pathology, we hypothesize that release/activation of lysosomal enzymes may participate in Aβ-mediated toxicity and development of AD pathology.
Methods
We used oligomeric human $A\beta_{1-42}$-induced primary mouse cortical neuronal death model to evaluate the levels/activities and subcellular distributions of lysosomal enzymes i.e. cathepsins B and D and related neuronal death mechanisms during neurodegeneration. Experiments are also being carried out in the cortex of TgCRND8 mouse model of AD.
Results
We found levels of cathepsins B and D were increased with time during the oligomeric $A\beta_{1-42}$-induced neuronal death. The increased cytosolic release of cathepsins B and D was associated with increased expression of pro-apoptotic molecular markers. In parallel, levels of cathepsins B/D and their cytosolic release were found increased in the cortex of TgCRND8 mice compared to age-matched control mice, and these changes were associated with increased neuronal loss.
Conclusions
Cathepsins B/D were increased in $A\beta_{1-42}$-mediated neuronal death and the cortex of mice exhibiting AD-related pathology. Increased cytosolic release of cathepsin B/D may be associated with $A\beta_{1-42}$-mediated neuronal death.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-0692

LIPOPROTEIN RECEPTORS IN REGULATION OF NEURONAL CELL DEATH

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Objectives: Lipoprotein receptors are multifunctional receptors that hold key functions in brain development, plasticity, cholesterol homeostasis and neuronal viability. Early Alzheimer's disease pathology is characterized by Reelin depletion, altered brain lipoprotein receptor expression and neuronal hypometabolism. Apolipoprotein receptor 2 (ApoER2) and very-low density lipoprotein receptor (VLDLR) are the primary Reelin and ApoE receptors in the brain, but they also interact with clusterin/ApoJ, APP and Tau. However, the role of ApoER2 and VLDLR in AD development and neurodegeneration remain poorly characterized. The objective of this study was to elucidate the role of lipoprotein receptors in the regulation of neuronal survival.
Methods: Primary neuron cultures were used in combination with lentiviral RNAi and various inducers of cell death to study the role of lipoprotein receptors in neuronal degeneration. Cell viability was assessed by immunofluorescence, Western blotting, and cell toxicity and mitochondrial assays.
Results: (1) Induction of the endogenous lipoprotein receptor inhibitor PCSK9 and concomitant downregulation of ApoER2 is used as a developmental switch to trigger neuronal apoptosis in cerebellar granule neurons. (2) A potential PCSK9 inhibitor and a widely used nutraceutical berberine caused robust mitochondria- and NMDA receptor-dependent neuron death. (3) At subtoxic nanomolar concentrations, berberine sensitized neurons to rotenone and glutamate toxicity. (4) VLDLR expression was specifically increased in hypoxic conditions through HIF-1alpha and Nrf2-pathways. Although berberine neurotoxicity does not directly involve lipoprotein receptors, mitochondrial hypofunction is linked to transcriptional regulation of VLDLR.
Conclusions: ApoER2, VLDLR and their inhibitor PCSK9 contribute to the regulation of neuronal cell death via multiple mechanisms.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-0794

ROLE OF NMDA RECEPTOR SUBUNITS IN NEURONAL TOXICITY OF AMYLOID BETA OLIGOMERIC FRACTIONS

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Number of studies have clearly indicated that amyloid β oligomers (Aβo) toxicity is mediated, at least in part, by glutamate-mediated excitotoxicity, which could involve activation of the NMDA receptors (NMDAR), leading to elevated intracellular Ca^{2+} and consequent stimulation of a cascade of enzymes resulting in cell death. This is supported by recent data showing that inactivation of the NMDA receptor by antagonists, could protect neurons from amyloid toxicity. Taken together, these studies suggest that glutamate receptor activation may be involved in part for amyloid β–induced cell death. In this study, we investigate the sequence of acute toxicity linked to Aβo (1-42) in primary cortical neurons. In presence of NMDAR antagonists (memantine, ifenprodil, MK801), Ca^{2+} influx was recorded immediately after Aβo administration and hyper-phosphorylation of Tau protein was assessed by western blot. The role of 2A/2B subunits was deeply investigated. For this purpose we used siRNA of NMDAR2A and 2B. We showed that MK801 and the specific inhibitor of NMDAR2B (ifenprodil) could fully protect neurons from Aβo injuries. Memantine (NMDAR2A) showed moderate inhibition proving that the 2A subunit was partially involved in the neurotoxic process. Moreover, a clear Ca^{2+} influx was observed in the few minutes after Aβo application, interestingly in Ca^{2+} free medium, no toxic damage was observed proving indirectly the role of the Ca^{2+} influx in the Aβo toxicity. In conclusion, we showed that the Aβo acute toxicity mainly involved the NMDAR2B activation inducing a massive entry of Ca^{2+} driving the apoptotic pathway and tau hyperphosphorilation.

03v. Pathophysiology & Disease Mechanisms: metal ions

ADPD5-2097

DETAILED QUANTITATIVE STUDIES OF CU(II) BINDING TO ABETA1-16: BEYOND THE COMPONENTS MODEL.

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Objectives

Complexation of Cu(II) by Abeta(1-40/42) peptides accelerates their aggregation and is considered to contribute significantly to neuronal damage in AD brains. Detailed knowledge of Cu(II)/Abeta interactions is important for designing AD therapeutic strategies. Abeta(1-40/42) bind one Cu(II) ion specifically, in a pH-dependent fashion. Previous spectroscopic studies on the Abeta(1-16) model peptide led to a proposal of four Cu(II) binding modes (so-called Components I-IV) in the pH range 6-11. A rigorous study performed at pH 7.4 (containing Components I and II) yielded conditional log K values of 10.4 for Abeta(1-40) and 10.1 for Abeta(1-16). Such data were not available for other pH values, because previous attempts to determine them did not agree well with spectroscopic results. We aimed to obtain a consistent binding model, ready for predictions of Abeta peptide interactions with Cu(II) ions at various pH that can be encountered in the brain.

Methods

The model Abeta(1-16) was synthesized using standard Fmoc methodology. Its protonation and Cu(II) affinity constants were determined by potentiometry in a broad pH range and verified quantitatively by electronic absorption spectroscopy and EPR.

Results

The combination of potentiometry and spectroscopic methods revealed the presence of eleven peptide and complex species over the studied pH range (3-11). Absolute protonation and affinity constants were obtained for each of them. The value at pH 7.4 was consistent with previous spectroscopic studies.

Conclusions

We obtained a correct set of data, which expands the understanding of Abeta/Cu(II) interactions beyond a four component model and a single affinity constant.

03v. Pathophysiology & Disease Mechanisms: metal ions

ADPD5-2098

EXTREMELY HIGH CU(II) AFFINITY AND REDOX PROPERTIES MAKE ABETA 4-X KEY FACTORS IN COPPER-RELATED NEUROTOXICITY IN AD

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Objectives
The N-terminally truncated Abeta(4-40/42) peptides are abundant in healthy and AD brains. The Abeta(4-42) peptide predominates in plaques present in human AD brains, but not in most transgenic mouse models, which until very recently have expressed only human Abeta(1-40/42). The deletion of the DAE tripeptide creates a novel N-terminal FRH sequence in Abeta(4-40/42) peptides. Peptides containing such sequences are known to bind a Cu(II) ion avidly, with conditional log K values at pH 7.4 in the range of 12.0-14.6, much higher than that determined for the Abeta(1-40) peptide (log K = 10.4 at pH 7.4). Our aim was to verify this concept experimentally.
Methods
The model Abeta(4-16) was synthesized using standard Fmoc methodology. Its protonation and Cu(II) affinity constants were determined by potentiometry in a broad pH range and verified by spectroscopic techniques: electronic absorption, CD and fluorescence.
Results
The combination of potentiometry and spectroscopic methods revealed the presence of several complex species over the studied pH range (3-11). For the 1:1 Cu(II):peptide stoichiometry the sole binding site is provided by the FRH sequence. The conditional log K value at pH 7.4 under these conditions was 14.9. At a 2:1 Cu(II) excess over the peptide, another Cu(II) complex could be detected with a conditional log K value at pH 7.4 of ca. 8.0.
Conclusions
Extrapolating from the model peptide, our results indicate that Abeta(4-40/42) peptides can bind one Cu(II) ion with an extreme, femtomolar affinity. Such complexes will resist all chelating drugs currently considered for therapeutic intervention in AD.

03w. Pathophysiology & Disease Mechanisms: calcium homeostasis

ADPD5-0264
STIM2 PROTEIN IS DOWNREGULATED IN CONDITIONS OF AMYLOID SYNAPTOTOXICITY

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In our recent breakthrough study we observed that reduced postsynaptic neuronal store-operated calcium entry (nSOC) is responsible for mushroom spine loss in knock-in mice expressing mutant human presenilin 1 (PS1-M146V-KI)- mouse model of Alzheimer's disease (AD). We have shown that key player of SOC STIM2 but not STIM1 is downregulated in the hippocampus of PS1-KI mice and that hyperexpression of STIM2 protein rescues synaptic nSOC and mushroom spine loss in PS1-KI hippocampal neurons (Sun at al (2014) **Neuron**. 82:79-93). However, PS1-M146V-KI mice do not express human amyloid precursor protein and do not form Ab40 and Ab42. Since amyloid hypothesis is still considered to be dominant the next question arises: does similar pathological STIM2-nSOC process take place in conditions of amyloid toxicity? At the current study we have developed in vitro and in vivo models of low amyloid synaptotoxicity. In our studies we observed decrease in STIM2 protein levels in vitro and in vivo models of amyloid synaptotoxicity. Moreover, in in vitro and in vivo conditions we detected reduction of mushroom spines fraction. We observed that overexpression of mSTIM2 significantly increase percentage of mushroom spines in neurons treated with Ab42 in in vivo conditions. This indicates that STIM2 overexpression is able to protect mushroom spines from amyloid toxicity.

Our results suggest that downregulation of STIM2-nSOC pathway may play a general role in AD-related synaptic pathology and that modulators/activators of nSOC may be considered as potential therapeutic agents for treatment of AD patients.

03w. Pathophysiology & Disease Mechanisms: calcium homeostasis

ADPD5-0443

INTRACELLULAR CA2+ STORES CONTRIBUTE TO IN VIVO NEURAL NETWORK DYSFUNCTION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Objectives

In vitro studies suggest that Alzheimer's disease-related mutations in presenilin genes cause a dysfunction of intracellular Ca^{2+} stores in neurons. However, little is known about the role of intracellular Ca^{2+} stores *in vivo* as well as their dysfunction in the amyloid-depositing brain. Here we analyzed the *in vivo* properties of intracellular Ca^{2+} stores in amyloid-depositing mice and their contribution to network-driven neuronal activity.

Methods

Functional properties of intracellular Ca^{2+} stores were analyzed in cortical neurons of 10-14 months old $APP_{Swe}PS_{G384A}$ mice and age-matched wild type (WT) littermates using *in vivo* two-photon Ca^{2+} imaging. Ryanodine receptor (RyR)-mediated Ca^{2+} release from stores was evoked by brief pressure application of RyR agonist caffeine. Network-driven spontaneous Ca^{2+} transients were measured under control conditions and in the presence of Cyclopiazonic acid (CPA), which reversibly empties intracellular Ca^{2+} stores.

Results

Amplitudes of caffeine-induced Ca^{2+} transients were similar in both mouse strains but the duration of the transients at a half amplitude (T/2), decay time constant tau, and normalized area under the transient were significantly higher in amyloid-depositing mice compared to WT littermates ($p<0.05$; Mann-Whitney test). CPA significantly reduced the frequency of spontaneous Ca^{2+} transients in mutant mice ($p<0.01$). In addition, hyperactivity of neurons in the vicinity of amyloid plaques (see Busche et al., 2008) decreased dramatically upon store depletion.

Conclusions

Our *in vivo* data reveal a dysfunction of intracellular Ca^{2+} stores in amyloid-depositing mice and show that they are critically involved in controlling the network-driven neuronal activity.

03w. Pathophysiology & Disease Mechanisms: calcium homeostasis

ADPD5-0446

CHANGES IN CALCIUM-ASSOCIATED PROTEINS DURING THE PROGRESSION OF ALZHEIMER'S DISEASE

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Objectives: Disrupted neuronal calcium homeostasis is implicated in the development of Alzheimer's disease (AD). In late-stage AD brain, we have previously shown that elevated beta-amyloid burden was associated with increased calpain-1 activation and calpain-mediated cleavage and inactivation of the sodium calcium exchanger (NCX)3. The purpose of the present study was to investigate temporal changes in markers of calcium dysregulation during the progression of AD.
Methods: Frozen post mortem human temporal cortex from control and Braak Stage II-VI AD brain was obtained from the MRC London Brain Bank for Neurodegenerative diseases. Hallmark AD pathologies and calcium-associated protein amounts in each tissue were determined by western blotting and ELISA. Correlative relationships between specific protein changes were determined following regression analysis.
Results: Increased amounts of active calpain-1 were detected in early stage AD (Braak II) brain, and this change was sustained throughout disease progression. The increase in calpain activity was associated with elevated cleavage of its substrates, including NCX3. Moreover, calpain-1 activity correlated with total tau amounts and also with activity of the key tau kinase, glycogen synthase kinase (GSK) 3, which can be activated upon N-terminal calpain cleavage. Calpain-1 alterations were also associated with changes in the amounts of pre- and post-synaptic proteins, suggesting a relationship with synapse health in AD.
Conclusions: This study identified a close association between calpain, tau kinases activities and tau amounts, and supports the hypothesis that calcium dyshomeostasis occurs in early, pre-symptomatic stages of AD and is sustained throughout disease progression.

ADPD5-0241

STRUCTURAL DEGENERATION OF NEURONAL DENDRITES IS FUNCTIONALLY LINKED TO CELLULAR EXCITABILITY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Dendritic architecture and neuronal electrical properties are inseparably linked, defining the dendritic integration of synaptic signals, their propagation and their capability to evoke action potential output. The diversity of dendritic architectures enables neurons to fulfill their specialized circuit functions during cognitive processes. It is well known that dendritic integrity is impaired in patients with Alzheimer's disease and in animal models. It is unknown, however, whether such structural degeneration translates into neuronal dysfunction. We used *in vivo* whole-cell patch-clamp recordings, high-resolution STED imaging and computational modeling of CA1 pyramidal neurons in a mouse model of Alzheimer's disease to reveal that dendritic structural degeneration and neuronal hyperexcitability are critically linked. We demonstrate that a structure-determined amplification of synaptic input to action potential output conversion may constitute a novel mechanism underlying network dysfunction with a potential relevance for other neurodegenerative diseases with dendritic pathology.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-0634

SIMPLE REACTION TIME IS MODIFIED IN ALZHEIMER'S DISEASE: AN INDIRECT MEASURE OF THE CHOLINERGIC LOSS?

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Objectives: We studied simple reaction time (sRT) in Alzheimer's (AD) patients before and after 2 months of treatment with 10 mg of donepezil in order to assess the alertness of their motor system.
Methods: 8 patients in early AD (70 +/- 6,3 years old) were compared to 15 normal subjects (71,1 +/- 4,2 years old)
Patients and normal subjects pushed their index on a button as quickly as possible after a randomly computer-generated visual red flash, determining the sRT. A surface electrode placed on the extensor indicis proprius recorded the EMG activity determining the onset of muscular movement time (sMT). The latency of sRT and of sMT were recorded by a CED1401 system.
Results:
Mean sRT was 207,3 +/- 25,5 ms in normals and 253,0 +/- 32,5 ms in AD patients before donepezil ($p < 0,001$)
Mean sRT was 229,9 +/- 30,6 ms in AD after donepezil ($p < 0,01$ compared to normals ; $p < 0,02$ compared to AD before donepezil)
Mean sMT was 159,3 +/- 29,3 ms in normals and 203,8 +/- 29,0 ms in AD patients before donepezil ($p < 0,001$)
Mean sMT was 180,8 +/- 32,2 ms in AD patients after donepezil ($p< 0,01$ compared to normals ; $p < 0,01$ compared to AD before donepezil)
Conclusions: sRT and sMT are significantly increased in AD patients compared to normal subjects of the same age. Donepezil improves the simple reaction time and the simple movement time in Alzheimer's disease patients supporting a cholinergic modulation of motor alertness.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-0648

BRIVARACETAM, BUT NOT ETHOSUXIMIDE, REVERSES MEMORY IMPAIRMENTS IN ALZHEIMER MOUSE MODEL

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Introduction:

Recent studies have shown that several strains of transgenic Alzheimer's disease (AD) mice overexpressing the amyloid precursor protein (APP) have cortical hyperexcitabilty, and suggested that this aberrant network activity may be a mechanism by which amyloid-b causes more widespread neuronal dysfunction. Specific anticonvulsant therapy reverses memory impairments in various transgenic mouse strains, but it is not known whether reduction of epileptiform activity might serve as a surrogate marker of drug efficacy in an AD mouse model.

Methods:

Transgenic AD mice (APP/PS1 and 3XTg-AD) were chronically implanted with dural EEG electrodes, and epileptiform activity was correlated with spatial memory function and transgene-specific pathology. The anticonvulsant drugs (AEDs) ethosuximide and brivaracetam were tested for their ability to suppress epileptiform activity, and to reverse memory impairments and synapse loss in AD mice.

Results:

We report that in two transgenic mouse models of AD (APP/PS1 and 3xTg-AD), the presence and severity of spike-wave discharges (SWDs) correlate with impairments in spatial memory. While both AEDs reduce mouse SWDs, only brivaracetam reverses memory impairments in APP/PS1 mice, without altering Ab metabolism or synaptic density.

Conclusions:

Our data confirm an intriguing role of anticonvulsant drugs targeting Synaptic Vesicle Protein 2A (SV2A) across AD mouse models. Chronic ethosuximide dosing did not reverse spatial memory impairments in APP/PS1 mice, despite reduction of SWD. Our data also indicate that SWDs are not a reliable surrogate marker of target appropriate target engagement in APP/PS1 mice.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-1178

BRAIN STRUCTURAL, FUNCTIONAL, AND COGNITIVE CORRELATES OF RECENT VERSUS REMOTE AUTOBIOGRAPHICAL MEMORIES IN MILD COGNITIVE IMPAIRMENT

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Deficits in autobiographical memory appear earlier for recent than for remote life periods over the course of Alzheimer's disease (AD). The present study aims at further our understanding of this graded effect by investigating the cognitive and neural substrates of recent versus remote autobiographical memories in patients with amnestic Mild Cognitive Impairment (aMCI).

20 aMCI patients and 25 Healthy controls (HC) underwent neuropsychological tests assessing remote (20-to-30 years old) and recent (the ten last years) autobiographical memory as well as episodic and semantic memory, executive function and global cognition. All patients also had a structural MRI and an FDG-PET scan.

Correlations were assessed between each autobiographical memory score and the other tests as well as grey matter volume and metabolism. Within the aMCI, the remote period correlated with semantic autobiographical memory, episodic memory retrieval whereas the recent period only correlated with episodic memory retrieval. Neuroimaging analyses revealed significant correlations between the remote period and temporal pole and temporo-parietal cortex volumes and anterior cingulate gyrus metabolism, while the recent period correlated with hippocampus volume and posterior cingulate, medial prefrontal and hippocampus metabolism. The brain regions related with the recent period showed greater atrophy/hypometabolism in MCI patients compared to HC than those involved in remote memories.

Recall of recent memories essentially rely on episodic memory processes and brain network while remote memories also involve other processes such as semantic memory. This is consistent with the semantization of memories with time and may explain the better resistance of remote memory in early AD.

ADPD5-1845

CELLULAR AND SYNAPTIC CORRELATES OF LEARNING AND MEMORY

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Hippocampus dependent learning and memory requires coordinated firing of neurons that form a memory trace during encoding. The reactivation of neurons belonging to a memory trace is thought to take place during memory retrieval. Moreover, changes on the synaptic level maybe required to strengthen or weaken connections between neurons that form a memory trace. We wanted to find out if memory traces do exist in the hippocampus of living mice and how they are represented on the synaptic level. Therefore, we combined a hippocampus dependent learning and memory test with longitudinal two-photon in vivo imaging of CA1 neurons in the hippocampus. To monitor neuronal activity we used the immediate early gene c-fos in a mouse model that expresses GFP under the c-fos promotor. Fear conditioning induced structural changes on the synaptic level were analyzed in excitatory and inhibitory neurons. To identify mechanisms of learning and memory impairment, we analyzed a mouse model of amyloidosis. By monitoring neuronal activity of CA1 neurons in fosGFP mice throughout fear conditioning, we found a large fraction of neurons that are constantly active plus a smaller fraction that is specifically reactivated and stabilized. In APPPS1 transgenic mice already the memory formation was found to be impaired. On the synaptic level, we found an increased density of lost spines on excitatory radial oblique dendrites that may reflect altered network connectivity due to learning and memory. Summarizing, we identify cellular and synaptic correlates of learning and memory that are impaired in mouse model of Alzheimer's disease.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-2227

EXCITABILITY OF HIPPOCAMPAL CELLS AND THE ROLE OF SEIZURES IN EARLY STAGES OF ALZHEIMER'S DISEASE

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A complication of Alzheimer's disease (AD) is the occurrence of epileptic seizures, but whether this hyper-activity contributes to cognitive impairment in AD has not yet been clarified. To answer this question, we performed experiments on the Tg2576 mouse model, bearing the human APP Swedish mutation, at both a pre-symptomatic and a symptomatic age. We characterized, by *in vitro* slice electrophysiology, the excitability properties of CA1 pyramidal cells to pinpoint neuronal signaling alterations underlying both epileptiform activity and hippocampal dependent memory deficits at their onset. We found significant differences in excitability properties of cells from Tg2576 vs wild-type mice at both stages, and indications that some of the electrophysiological alterations at the *onset* of cognitive deficits could actually be homeostatic responses to previous changes. Furthermore, experiments using anti-epileptic drugs in slice suggest that these could actually exacerbate some of these homeostatic responses.
In a different set of experiments, we induced seizures *in vivo* at the pre-symptomatic stage to investigate whether these play a role in inducing cognitive impairment specifically in the AD phenotype. Our data suggest that this is indeed the case.
Overall our *in vitro* and *in vivo* data provide a framework to elucidate the contribution of seizures in determining cognitive deficits in AD and for a use of anti-epileptic drugs based on the knowledge of the underlying neuronal signaling alterations at the different stages of the disease.

03y. Pathophysiology & Disease Mechanisms: aging

ADPD5-0288

THE ROLE OF AGEING IN NEURONAL VULNERABILITY IN DROSOPHILA.

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Objectives: The incidence of neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD) increases markedly with advancing age. Recent research using model organisms, such as worms, flies and mice, has revealed that the aging process itself can be ameliorated by the manipulation of single genes. This provides an exciting new avenue for discovering much-needed treatments that can prevent or delay the progression of several age-related neurodegenerative diseases simultaneously, because aging is the major risk factor for all of them.
Methods: Models of neurodegenerative diseases in the fruit fly *Drosophila* are well established. Flies, due to their short lifespan and ease of genetic manipulation, provide a useful tool for studying the interaction between aging and disease. Our previous work has shown experimentally that older flies are intrinsically more susceptible to Aβ42 toxicity in neurons, suggesting that the aging process itself does indeed increase vulnerability to neurodegeneration. We have now examined the role of several candidate longevity-assurance processes, including cellular detoxification, defence against oxidative stress and proteasomal degradation, on Aβ42 toxicity in our fly model.
Results: We found that Aβ42 expression reduced activity of cncC, a *Drosophila* homologue of Nrf-2, which activates transcription of several antioxidant and cellular detoxification genes. Moreover, genetic enhancement of cncC protects against toxicity in Aβ42-expressing flies.
Conclusions: Our future studies will examine the mechanisms by which these pathways mediate both their lifespan-extending effects and protection against amyloid toxicity, with the aim of discovering new targets for disease-modifying therapies for neurodegenerative conditions.

03y. Pathophysiology & Disease Mechanisms: aging

ADPD5-0517

RESVERATROL PROTECTS THE BRAIN FROM SPORADIC ALZHEIMER'S DISEASE (SAD) LIKE PATHOLOGY BY REDUCING EXTRACELLULAR ACCUMULATTION OF AMYLOID-BETA VIA ALPHA-SECRETASE ACTIVATION

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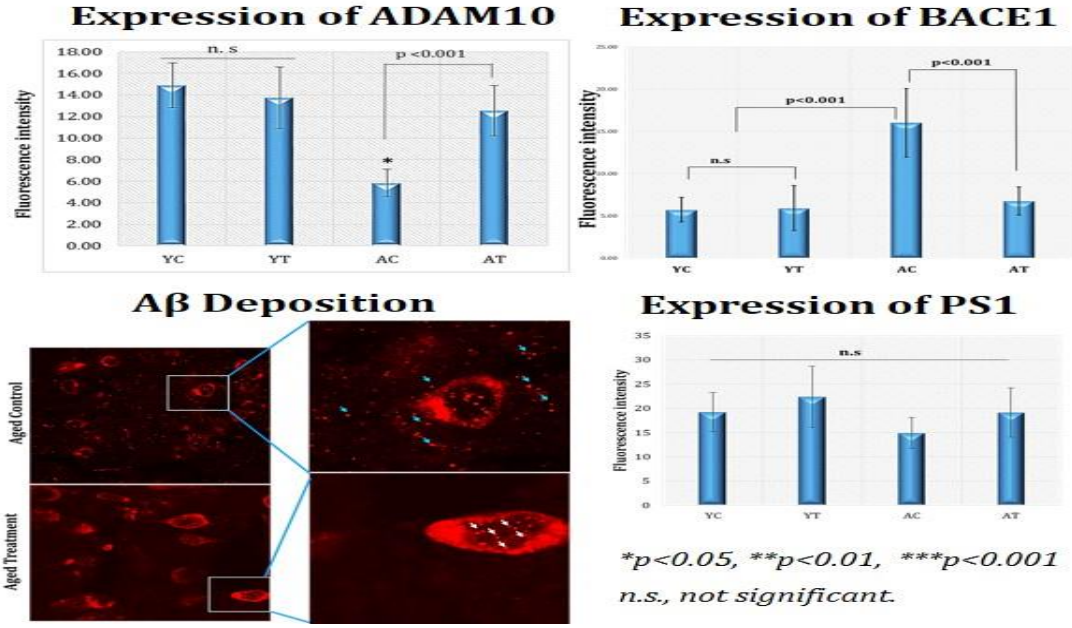
Background: Cerebral deposition of amyloid-β (Aβ) is the beginning of amyloid cascade leading to Alzheimer's disease (AD). Approximately 95% of AD accounts from SAD during aging. $A\beta_{42}$-peptides and plaques, generate from APP through sequential actions of β- and γ-secretases, whereas cleavage by α-and γ- secretases prevent toxic Aβ formation.
Objectives: Although localization of APP and secretases (ADAM10, BACE1 and PS1) are key proteins for regulating amyloidogenic pathway, it is unclear, whether aging promotes altered levels of expression and its subcellular distribution. Present study thus aimed to elucidate the cellular effect of resveratrol, on localization of AD related proteins during aging.
Methods: Using double immunofluorescence technique, the expression and subcellular localization (ER, Golgi and Mitochondria) of ADAM10, BACE1, PS1, APP and Aβ were examined in young and aged wistar rat brain regions with and without resveratrol.
Results: Absolute weight of the aged brain was increased (~26.5%), whereas the relative weight was decreased (~52%). We further identified decreased levels of ADAM10 (~50%) and elevated levels of BACE1 in aged rats. Although cell surface PS1 level was reduced, total expression remains unchanged, providing clue for enhanced Amyloidogenic pathway facilitating extracellular Aβ accumulation during aging. Resveratrol treatment significantly reduced BACE1 elevation and increased the expression of ADAM10 levels, further normalized cell surface PS1 expression.
Conclusion: From our results we conclude that aging promotes altered expression and subcellular distribution of secretases that may favor SAD, while resveratrol treatment exhibited positive influence on non-amyloidogenic pathway.



03y. Pathophysiology & Disease Mechanisms: aging

ADPD5-0870

PROTECTIVE ROLE OF RESVERATROL ON SMS1 ALTERATION LINKED MITOCHONDRIAL PRESENILIN-1 EXPRESSION IN RAT BRAIN REGIONS DURING AGING

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***Background*:** APP processing by γ-secretase strongly depends on cellular membranes, which are highly sensitive to even slight alterations in membrane environment. Sphingomyelin synthase (SMS1) is one such determinant of mitochondrial membrane composition. Mitochondrial dysfunction has long been associated to SAD, while the mechanism remains unclear. Decreased SMS1 expression has also been reported during aging facilitating ceramide mediated mitochondrial apoptosis.
Objective: SMS1 mediated alteration in sphingomyelin levels may influence altered function of membrane bound enzymes in particular PS1 in case of SAD, promoting Aβ formation during aging. Thus we intend to study the influence of SMS1 over mitochondrial PS1 activity during aging and to exhibit the role of sirtuin in regulating these altered metabolic events.
***Methodology*:** 20 mg/kg body weight of resveratrol as sirtuin enhancer was treated for young and aged male wistar rats for 15 days. Rat brain regions were assessed for mitochondrial abnormalities. SMS1, SMS2 levels were analyzed to determine sphingolipid alterations. Mitochondrial (APP, PS1, CTFs) and SIRT1 levels were analyzed using western blotting.
***Results*:** SMS2 expression was increased and in contrast SMS1 and SIRT1 levels were decreased significantly in aged rats. Mitochondrial APP was found to decrease with a significant increase in CTFs. Surprisingly, there wasn't any significant increase in Aβ formation. Mito-PS1 activity displayed a significant increase of $p<0.001$ compared to young rat brain regions.
***Conclusion*:** The current study thus provided a rationale relationship between SMS1 and altered mitochondrial PS1 activity during aging. Resveratrol found to exhibit a homeostatic effect in regulating age associated key metabolic events.

03y. Pathophysiology & Disease Mechanisms: aging

ADPD5-1458

THE TYROSINE PHOSPHATASE STEP REGULATES COGNITIVE FUNCTION DURING AGING

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1. Objectives

There is no effective treatment to prevent memory decline in the elderly or patients diagnosed with mild cognitive impairment (MCI). The striatal-enriched protein tyrosine phosphatase (STEP) occupies a key position in the cascade of events involved in memory by controlling the activity of proteins implicated in memory, such as ERK and the GluN2B subunit of the NMDAR. The objective of this study is to uncover the role of STEP in age-related memory deficits across different species.

2. Methods

Memory performances of aged rats and mice (STEP KO, Het, and WT) were tested in the Morris water maze and Y maze tasks. Cognitive deficits of rhesus monkeys were evaluated in the paired associate learning test and delayed matching to sample task. Protein Levels of STEP and its substrates were quantified by western blots, and STEP activity was analyzed by immunoassay.

3. Results

We found an elevation in the levels and activity of STEP in aged memory-impaired mice and rats. We also observed increased STEP expression in aged rhesus monkeys and humans with MCI. These increases are linked to enhanced dephosphorylation of the STEP substrates GluN2B and ERK. STEP accumulation with aging appears to involve dysfunction of the ubiquitin-proteasome system. Genetic reduction of STEP levels alleviates age-related memory decline.

4. Conclusions

Elevated STEP levels that occur with advancing age constitute a molecular mechanism common to several species that contributes to age-related cognitive deficits, and may represent a target for pharmacological therapy to enhance cognition in the elderly and MCI patients.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-0549
REVERSE SIZE EFFECTS IN A PATIENT WITH POSTERIOR CORTICAL ATROPHY; A CASE REPORT

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Background: We herein report the case of a PCA patient who showed a greater difficulty perceiving the large letters than the small letters.
Case report: A 53-year-old, right-handed female housewife was admitted to the hospital because of four-years visual symptoms, including difficulties in reading a text and writing letters, problems with driving or parking a car, color recognition impairment and difficulties performing dual tasks at the same time. The findings of neuropsychological examinations, such as the MMSE24/30, Digit Span(forwards)9, (backforwards)4, Tapping Span (forwards)1, (backforwards)0, WAIS-III, VIQ91, PIQ47, FIQ66, VC95, PO50, WM76, PS50, showed the greater visual problems than the verbal problems. When she was asked to read a Japanese Kanji, she could read it if it was shown in a small size, but, she could read only part of the Kanji shown in a large size. She read large “H” as “I”, but could read “H” shown in a small size. Occipitoparietal atrophy, particularly in the right side, was observed by MRI, and marked hypoperfusion was noted in the same regions by SPECT.
Discussion and Conclusions: The reverse size effect (the ability to better read small than large prints) is a rare phenomenon in PCA(Crutch et al. 2011). The difficulty in reading large written Kanji observed in this patient is consistent with the reverse size effect described by Crutch et al. The responsible region is suspected to have been in the right occipitoparietal region.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1123

SEVERE DEPRESSION EFFECTS ON CLINICAL DEMENTIA STAGING

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Objectives
Neuropsychological test has a distinct role in the detection and monitoring of cognitive and functional changes, so it provide unique value as biomarkers of dementia. Clinical dementia rating (CDR) is another tool to evaluate the severity of symptoms of cognitive decline. And the two scale is known to have relevance each other. We aimed to assess the dissociation between neuropsychological test and CDR-SOB according to severe depression in MCI to early stage of dementia.
Methods
From 3873 patients who were assessed at the Seoul National University Bundang hospital, 364 patients with clinically questionable impairment (CDR-SOB 0.5-2.0) and 54 patients with very mild dementia (CDR-SOB 2.5-4.0) were identified as severe depressed status. Detailed neuropsychological test scores were compared with those of patients whose geriatric depression score were normal range.
Results
In clinically questionable impairment group, severe depressed patients showed significantly lower scores in immediate recall, word fluency and stroop test ($p<0.01$). On the other hands, in clinically very mild dementia group, severe depressed patients showed significantly higher scores in memory discrimination test($p=0.027$) and had tendency to higher level in delayed recall test ($p=0.07$).
Conclusion
Whether severe depression is present or not may affect the clinical assessment of cognitive function showing a gap between neuropsychological value and clinical dementia scale.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1136

THE DEVELOPMENT OF ALZHEIMER DISEASE IN THE CONTEXT DOWN SYNDROME: MODELING THE MECHANISM

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People who have Down syndrome, which is caused by trisomy of chromosome 21, are at significantly increased risk of developing Alzheimer's disease (AD). An additional copy of the chromosome 21 encoded gene, APP, is known to promote the development of AD. However, the effect of trisomy of the other ~300 chromosome 21 genes on disease is unclear. Here we show, using mouse models, that trisomy of chromosome 21 (in the absence of APP duplication) makes a significant contribution to the development of AD. Trisomy of chromosome 21 significantly enhances ABeta deposition and associated synaptic transmission and cognitive deficits, and reduces survival. We show that trisomy of chromosome 21 alters the development of ABeta pathology by a novel mechanism that modulates the production and resultant aggregation of ABeta. These data suggest that people who have Down syndrome have an increased risk of developing Alzheimer's disease not only because they have an additional copy of APP but because trisomy of a further chromosome 21 encoded gene, or genes, modulates disease. We now aim to identify this gene or genes, to provide further novel mechanistic insight into the development of Alzheimer's disease.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1181

HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS DYSREGULATION IN ALZHEIMER'S DISEASE USING IN-VIVO AND IN-VITRO MODELS.

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The function of the hippocampus, a key structure responsible for memory encoding, is affected early in Alzheimer's disease (AD) leading to progressive and irreversible memory loss. The molecular mechanisms underpinning this memory loss remain elusive but are crucial to identify for the development of effective therapies. There is strong evidence that both amyloid-beta (Ab) and the main stress hormone, cortisol (corticosterone (CORT) in mice), are abnormally elevated during the early phase of AD. CORT signaling is regulated by the Hypothalamic–pituitary–adrenal (HPA) axis. Using a hAPPswee-overexpression mouse model of AD, which accumulates Ab in the hippocampus, we characterized the onset and extent of HPA dysfunction during the early phase of hippocampus-dependent memory impairments. We show that the first alterations are represented by CORT increase in the plasma and loss of the feedback mechanism induced by dexamethasone treatment, while ACTH levels and the weight of HPA-axis related organs remain normal. We are also investigating the relationship between Ab, glucocorticoid receptors (GRs) and synaptic function in cultured hippocampal neurons. Our data suggest a complex relationship between synaptic GRs, synaptic AMPA receptors and presence of Ab. Together, these data should help clarify the mechanistic relationship between APP misprocessing and HPA axis dysfunction in AD.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1554

CEREBROSPINAL FLUID THYROID HORMONES IN AD PATIENTS: A MASS-SPECTROMETRY STUDY.

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Background: Thyroid hormones might play a role in AD pathogenesis. In the last decades several studies have assessed thyroid hormone (TH) levels in serum and CSF in AD patients, with conflicting results. Mass spectrometry allows quantifying precisely their levels, and better discriminating triiodothyronine (T3) from reverse T3 (rT3) [originating from the conversion of thyroxin (T4) by Type II and Type III deiodinase, respectively], differently from the immunoenzymatic methods used so far.

Methods: We assessed nine consecutive patients with a diagnosis of probable AD (according to NINCDS-ADRDA revised criteria) submitted to cerebrospinal fluid (CSF) analysis (for Ab1-42, tau and phospho-tau analysis), full neuropsychometric evaluation, neuroimaging investigations with fluoro-deoxy-glucose positron emission tomography and brain magnetic resonance imaging. T3, rT3, T4 levels were assessed by mass spectrometry in CSF and serum (in this case, discriminating between protein-bound and unbound forms, differently from other techniques). The same hormones were assayed in CSF of non-demented patients.

Results: Serum and CSF levels of free and total TH were similar in AD patients and controls. CSF rT3/T3 ratio was lower in AD than in controls. We also performed a correlation analysis between CSF TH levels, CSF biomarkers and neuropsychometric/ neuroimaging data.

Conclusion: Our data show a reduced ratio between rT3/T3 in AD CSF, with mass spectrometry, a technique giving reliable values as compared with current routine lab techniques. Speculative hypothesis on their role, based on correlative data are provided.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-2218

POSSIBLE LOWER PLASMA AND BRAIN LEVELS OF NUTRIENTS THAT RATE-LIMIT NEURONAL PHOSPHOLIPID SYNTHESIS IN ALZHEIMER'S DISEASE: A RETROSPECTIVE COHORT STUDY

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Objectives

Synapse loss and synaptic dysfunction in Alzheimer's disease (AD) are linked to membrane loss and altered membrane composition. Neurons require specific nutrients for the formation and maintenance of synaptic membranes. These nutrients fuel the metabolic pathways that synthesize new phospholipids, the main constituents of membranes. While AD patients may benefit from higher availability of the phospholipid-synthesizing nutrients that can support synapse formation and function, many patients have lower plasma levels of these nutrients. Such compromised nutritional status may progress during the course of the disease and may already be evident in earlier stages of AD. We aimed to investigate the role of these potential biomarkers in the pre-dementia stages of AD as well as in AD.

Methods

We currently investigate whether blood and cerebrospinal fluid (CSF) levels of phospholipid-synthesizing nutrients differentiate between subjects with subjective memory complaints (n=150), mild cognitive impairment (n=150), or AD (n=150) in a retrospective cohort study. Subjects were selected from the memory clinic based Amsterdam Dementia Cohort of the VUmc Alzheimer Center.

Results

Analyses of paired CSF, serum, and plasma samples of the Amsterdam Dementia Cohort are ongoing. Among others, levels of the following nutrients are being measured: plasma and CSF choline, folic acid, uridine, and homocysteine and plasma polar lipid profile.

Conclusions

The current study may further elucidate the role of these nutrients in AD pathology by identifying their levels in plasma and CSF at different stages of the disease. In addition, these nutrients may have potential to serve as (modifiable) biomarkers for AD.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-0326

DETECTION OF ANTI ABETA40-ANTIBODIES IN THE BRAIN OF TWO TRANSGENIC MICE MODELS OF AD AFTER ACTIVE IMMUNOTHERAPY

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Active immunization against Aβ40 of two transgenic mice models of AD (tg2576 and APPswe/PS1dE9) produces a marked Aβ40 level increase and anti-Aβ40 antibody titers in plasma.

We explored here this increase in Aβ40 levels and ascertain whether self-generated anti-Aβ40-antibodies reach brain tissue.

ABtest40 cannot detect Aβ40 bound to self-antibodies generated by immunization; unless they are displaced by competing xeno-antibodies. To determine whether plasma Aβ40 levels increase was caused by free Aβ40 or peptide bound to self-anti-Aβ40-antibodies we devise an ELISA for the detection of Aβ40-antibody complex. This ELISA was run either with or without the addition of label-free competing anti-Aβ40-xeno-antibodies. Lack of signal differences between the two runs of each sample would denote lack of displacement meaning that the signal obtained in ABtest was due to free Aβ40.

Antibody titers in plasma and the concentration of anti-Aβ40 IgG in brain extract from twelve vaccinated mice were assessed by ELISA in plates coated with Aβ40.

On average, the plasmatic signal for Aβ40-antibody complex dropped just 11 % when the samples were run in the Aβ40-antibody complex assay with competing antibodies. This drop did not reach statistical significance ($p = 0.120$).

Relevant concentrations of anti-Aβ40-antibodies were found in brain extracts correlating with plasma antibody titers (tg2576 $R^2 = 0.654$, $p < 0.001$; APP/PS1 $R^2 = 0.723$, $p < 0.001$)

The increment in plasma Aβ40 concentration after vaccination is mainly due to accrued free peptide. These results indirectly favor the sink hypothesis together with a central action of specific antibodies in the brain.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-0404

A NOVEL ANTIBODY EFFICIENTLY NEUTRALIZES ALZHEIMER'S DISEASE-RELATED IMPAIRMENTS IN SPATIAL MEMORY AND SYNAPTIC PLASTICITY IN RATS

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Objectives: Although the immunotherapy for Alzheimer's disease (AD) showed exciting results, both active and passive immunization against amyloid β protein (Aβ) in human AD have been suspended because of serious side effects which partly arise from the damage of physiological amyloid precursor protein (APP). Our previous studies indicated that the sequence 31-35 in Aβ is a shorter active center responsible for the Aβ neurotoxicity. In the present study, we prepared a novel antibody specifically targeting the sequence 31-35 of Aβ, observed the effects of the antibody on $Aβ_{1-42}$-induced impairments in spatial cognitive behavior of rats, and investigated its possible electrophysiological and cellular mechanisms. **Methods:** Morris water maze, neuronal culture and *in vivo* hippocampal field potential recording techniques were used in the study. Anti-Aβ31-35 antibody and Aβ1-42 were injected into the lateral ventricle of SD rats two weeks before performing spatial cognitive test. Anti-Aβ31-35 antibody in co-application group was delivered 15 min before Aβ1-42 injection. **Results:** Anti-Aβ31-35 antibody efficiently protected against the Aβ1-42-induced impairments in spatial memory and hippocampal long term potentiation (LTP) of rats. The cytotoxicity induced by Aβ1-42 in cultured cortical neurons was also potently inhibited by the anti-Aβ31-35 antibody in a dose-dependent manner. **Conclusions:** These results suggest that the anti-Aβ31-35 antibody is probably an effective immunotherapy approach for AD, and the sequence 31-35 of Aβ may be a new therapeutic target.

Keywords: Anti-Aβ31-35 antibody; Amyloid-β protein; Spatial learning and memory; Long-term potentiation; Neuronal cytotoxicity

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-0564

NT4X-167; NOVEL N-TERMINAL ABETA SPECIFIC ANTIBODY, PREVENTS IN VITRO AND IN VIVO TOXICITY OF HIGHLY TOXIC ABETA4-42 SPECIES

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Objectives: The novel monoclonal antibody (NT4X-167) specifically reacts with N-truncated ABeta at position 4 of ABeta. It binds N-truncated ABeta under native and denaturing conditions but only rescues *in vitro* toxicity of $ABeta_{4-42}$ and not that of pyroglutamate ABetapE3-42. One objective was to determine whether the NT4X-167 antibody and its respective Fab fragment could prevent working memory deficits caused by $ABeta_{4-42}$ injection in wildtype mice.
Methods: Cell toxicity assay, *in vivo* toxicity, Y-Maze, mAB antibody production, immunostaining
Results: The novel $ABeta_{4-x}$ immunoreactive antibody NT4X-167 detected high molecular weight aggregates derived from N-truncated ABeta species. Phenylalanine at position four of ABeta was imperative for antibody binding. Both full-length and the Fab fragment of the antibody were able to prevent the *in vitro* toxicity caused by ABeta4-42 in rat primary cortical neuron cultures. Intracerebroventricular ABeta4-42 injection into wildtype mice induced a behavioral deficit, shown as a reduction in alteration rate in a Y-Maze, which was prevented using the NT4X-167. The Fab fragment of the antibody, at a higher dosage, was also able to prevent the behavioral deficit in a replicate experiment.
Conclusions: NT4X-167 and Fab demonstrate binding and prevention of ABeta4-42 toxicity *in vitro*. NT4X full length antibody and Fab prevent $ABeta_{4-42}$ induced behavioral deficits in wildtype mice. Functionally, the full length antibody and its respective Fab fragment exhibit the same profile. It would thus be of great interest to study the structural binding characteristics of the NT4X-167, represented by its Fab fragment, to the highly toxic N-truncated ABeta4-x species.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-0591

ANTIBODIES DRAMATICALLY INCREASE THE NEUROTOXICITY OF AMYLOID BETA OLIGOMERS IN PRIMARY NEURONAL-GLIAL CULTURES BY ACTIVATING MICROGLIA

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Objectives. Beta amyloid (Aβ) oligomers are thought to contribute to the pathogenesis of Alzheimer's disease (AD). The success of anti-Aβ immunotherapies in preclinical studies led to the initiation of anti-Aβ in clinical trials, but halted due to brain inflammation, the mechanisms of which are poorly understood. In the present study we aimed to investigate the effects of antigen-antibody complexes formed by oligomeric Aβ and specific monoclonal antibodies in primary neuronal-glial cultures. Methods. Monoclonal antibodies generated against synthetic Aβ1-42 were preincubated with Aβ1-42 then added to cultures. The viability of neurons was examined by fluorescence microscopy. Results. We found that antibodies dramatically increased the neurotoxicity of Aβ oligomers. Complexes of antibodies plus monomeric Aβ had no effect on neuronal viability. The neurotoxicity of antibody-oligomeric antigen complexes was abolished by removal of the Fc region from the antibodies or by removal of microglia from cultures, and was accompanied by inflammatory activation and proliferation of microglia. However, the preincubation of cultures with inhibitors of NADPH oxidase and NO synthase did not prevent neuronal death caused by antibody-oligomeric Aβ. Conclusions. Antibody-antigen complexes formed by Aβ oligomers or other oligomeric antigens and their specific antibodies exert strong toxic effects on neuronal cells via Fc-dependent microglial activation. Our study provides new insight into the mechanism of possible Aβ neurotoxicity in the presence of Aβ-specific antibodies in AD brain and also may be important clinically in the development of safer vaccines for AD treatment.
This work was supported by the Research Council of Lithuania (grant LIG-04/2012).

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-0674
CONFORMATIONAL SPECIFICITY AND DIVERSITY OF THE IMMUNE RESPONSE TO ABETA42 AMYLOID FIBRILS

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Objectives: Abeta forms multiple conformationally distinct types of aggregates, so the objective was to isolate as many different monoclonal antibodies against distinct Abeta epitopes as possible.
Methods: Monoclonal antibodies were isolated from rabbits vaccinated with Abeta42 fibrils and 23 unique antibodies were isolated.
Results: These antibodies define 18 distinct immunological profiles of Abeta and. display a strong preference for aggregates over monomer, indicating that they recognize conformational epitopes. Most antibodies recognize N-terminal linear segments of Abeta, although many recognize a discontinuous epitopes. Many antibodies that recognize linear Abeta segments also recognize generic fibril epitopes contained in fibrils from unrelated amyloid sequences, indicating that linear epitope mapping is not a reliable indicator of sequence specificity. The antibodies display strikingly different patterns of immunoreactivity in Alzheimer disease and transgenic mouse brain. One antibody stains unique nuclear deposits in early AD and another is specific for a subset of vascular amyloid, indicating that the conformational differences are observed in human and Tg mouse brains.
Conclusions: The humoral immune response to Abeta42 fibrils is diverse and reflects the structural polymorphisms in fibrillar amyloid structures. No single antibody recognizes all of the different aggregation states under all conditions. Reliance on only a few monoclonal antibodies may restrict scientists to the observation of only a subset of the amyloid involved in pathology and also may not target all of the pathological aggregates necessary to be therapeutically effective.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-1153

LONGITUDINAL MRI STUDY TO MONITOR BLOOD BRAIN BARRIER DISRUPTION FOLLOWING ABETA IMMUNOTHERAPY IN A PDAPP MOUSE MODEL

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Amyloid-related imaging abnormalities (ARIA) have been reported in Alzheimer disease (AD) patients. Moreover, an increased risk of ARIA upon β-amyloid (Aβ) immunotherapy has been reported. It has been proposed that reduced vascular integrity caused by aggressive lowering of central and vascular Aβ might be involved. In the current study we applied T_1 mapping MRI, in combination with Gadolinium (Gd, Dotarem®) injection, to probe for blood-brain-barrier (BBB) integrity applying 3D6 (the murine equivalent of bapineuzumab) immunotherapy in PDAPP mice.

PDAPP mice (12 months) were treated weekly with either 3D6 or saline. Wild type animals served as controls and were treated with saline for a period of 5 weeks. T_1 weighted images were acquired weekly on a 7T small animal MRI system, before (baseline) and after intravenous administration of Gd (0.2 mmol kg^{-1}). T_1 values were quantified.

No differences in cortical T_1 values were observed at baseline. Interestingly, five mice (out of 12) showed a considerable larger drop in T_1 values (~300ms) after 2-5 weeks of treatment with 3D6, which was not observed in the saline-treated PDAPP group. This suggests increased vascular permeability, probably induced by the clearance of vascular Aβ.

This study shows for the first time *in vivo* increased cerebral vascular permeability upon Aβ immunotherapy. The time course of these events was documented and the methodology may be useful to assess ARIA risk in the development of other Aβ lowering compounds. Hence, our findings are important to increase our understanding of ARIA observed in AD patients upon Aβ lowering therapies.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-1382

AN IMMUNOLOGICAL EPITOPE SPECIFIC FOR TOXIC OLIGOMERIC AMYLOID-BETA IN ALZHEIMER'S DISEASE

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Oligomers of the amyloid-beta (Abeta) peptide play key roles in neurotoxicity and region-to-region spreading of Alzheimer's disease (AD) neuropathology. Here we describe a novel immunological epitope that is accessible to antibody binding only when Abeta is in an oligomeric form, termed cSNK.

Specificity of a monoclonal antibody raised against the cSNK epitope, 5E3, was evaluated by surface plasmon resonance, dot blotting/immunoblotting, immunohistochemistry, and atomic force microscopy. Therapeutic potential of the 5E3 antibody was assessed in *in vivo* assays of oligomer behavioral toxicity and acute treatment of aged AD mouse models. Immune responses elicited by cSNK vaccine formulations were evaluated in young mice.

The oligomer-specific epitope consists of a constrained turn at residues 26-28: cyclized serine-asparagine-lysine (cSNK). A monoclonal antibody raised against this novel epitope (5E3) binds to synthetic Abeta oligomers (ABO) with high affinity (K_D = 2.02 nM), but is unreactive to monomeric and fibrillar Abeta, as well as to amyloid plaques. 5E3 specifically recognizes soluble analyte in high-speed clarified saline brain extracts and cerebrospinal fluid from AD patients, while displaying negligible reactivity to healthy control samples. Pre-clinical evaluation indicated that the antibody is effective at blocking ABO toxicity and reducing ABO levels in CSF and brains of treated APP/PS1 and Tg2576 mice, with no apparent effect on pre-existing plaques. Moreover cSNK vaccinated mice produced an ABO specific immune response, without producing a response targeting Abeta monomers.

Together, the data demonstrates that cSNK is a unique ABO specific epitope with clear therapeutic and diagnostic potential.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-1473

THERAPEUTIC POTENTIAL OF REGULATORY T CELLS IN ALZHEIMER'S DISEASE

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OBJECTIVES

Besides innate immune responses mediated by microglia and monocyte-derived macrophages, recent reports suggest that adaptive immunity, and particularly T cell responses, may be implicated in the pathophysiology of Alzheimer's disease (AD). However, the role of different T cell populations in disease progression remains poorly defined. We previously showed that regulatory T cells (Tregs) critically control the magnitude of Abeta-specific CD4+ T cell responses in both physiological and pathological settings. Here we analyzed the impact of Tregs on disease progression in a murine model of AD.

METHODS

Preclinical studies were carried in the APPPS1 mouse model. Treg cells were either depleted using anti-CD25 antibodies, or selectively amplified through low-dose IL-2 treatment. Impact of Treg modulation on neuropathology, disease-related gene expression, and cognitive functions was assessed.

RESULTS

Depletion of Tregs accelerated the onset of cognitive deficits in APPPS1 mice, without altering Abeta deposition. Early cognitive impairment was correlated with reduced recruitment of microglia towards amyloid plaques, and altered disease-related gene expression profile in the brain. Conversely, targeted amplification of Tregs enhances microgliosis and the recruitment of microglia around amyloid deposits, and delays the onset of cognitive deficits in APPPS1 mice.

CONCLUSION

Treg cells play a beneficial role in the pathophysiology of AD, and new immunotherapy strategy targeting Tregs proves efficient at delaying disease progression in preclinical studies. Altogether, these data suggest the therapeutic potential of such Treg-based innovative immunotherapy approaches for the treatment of AD.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-1500

ALZHEIMERS' DRUG DELIVERY ACROSS BLOOD-BRAIN BARRIER BY PLGA NANOPARTICLES

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Over the last few decades, attempts were made in order to prevent the beta amyloid peptide (Aβ) aggregation. One approach achieved was the development of a peptide $iAβ_5$ capable of inhibit these aggregation. Though, due to the blood brain barrier (BBB) this potential drug for the treatment of Alzheimer's disease (AD) can not reach the brain in sufficient concentrations.

To circumvent this problem a novel carrier-based brain drug delivery system made of PLGA polymer with surface functionalized with transferrin receptor monoclonal antibody (OX26) and anti-Aβ (DE2B4) was prepared to delivery encapsulated $iAβ_5$ to the brain. The immuno nanoparticles (NPs) were synthetized by the nanoprecipitation method, resulting in a particle size with around 160nm, making them a suitable vehicle for intravenous administration.

The porcine brain capillary endothelial cells were used as a model of the BBB. Uptake of immune NPs was substantially higher compared to the non functionalized nanoparticles. Furthermore, the peptide release behavior indicates a controlled delivery of the peptide at 37ºC.

In conclusion, these Trojan horses have a higher probability to cross BBB, bind to Aβ peptide and deliver the drug tested in the local of action. By this way, they represent a novel platform technology for treatment of central nervous system (CNS) disease.

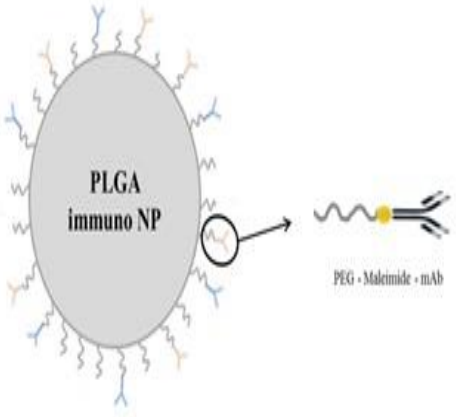

Figure 1. Schematic representation of developed PLGA NPs conjugated with two different types of antibodies.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-2291

FUNCTIONAL EFFICACY OF PASSIVE IMMUNIZATION AT TREATMENT-SENSITIVE STAGES IS NOT INCREASED BY EARLIER START OF THE TREATMENT IN AD MODEL

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As a number of clinical trials with passive anti-Abeta immunization are under way, the critical mechanisms required for the efficacy of this approach are still poorly understood. In this study, we compared functional efficacy of three similar monoclonal anti-Abeta antibodies (AB) raised against an internal domain of Abeta (Abeta13-28). As detected by a surface plasmon resonance analysis the ABs had no significant differences in affinities to monomeric or oligomeric Abeta. Comparative efficacies of the ABs in amelioration of Abeta accumulation and cognitive deficits were tested after one month of i.p. treatments in 12-mo-old APPswe/PS1dE9 mice that had already developed plaques and cognitive deficits in episodic-like memory. All three ABs increased plasma Abeta concentrations, two ABs decreased levels of Abeta accumulated in brain, and only one of the latter ABs was also effective significantly ameliorating cognitive deficits. To test whether earlier start of the treatment increases efficacy in restoring cognition, 12-mo-old APPswe/PS1dE9 mice were tested after 6 month-long therapy with the cognitively-effective AB. This longer treatment initiated at the onset of plaque deposition showed efficacy equal to that after just one-month-long treatment. We proceeded to test whether longer duration of the treatment has any benefits in older, 18-mo-old, APPswe/PS1dE9 mice. In the older mice, despite of the presence of duration-dependent amelioration in Ab load, no cognitive benefits were detected after one- or six-month-long treatments. Taken together, these findings suggest that efficacy of passive immunization at treatment-sensitive stages might not be increased by earlier start / longer duration of the treatment.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-0256

AS A MODEL OF EXTRACORPOREAL BLOOD A-BETA REMOVAL SYSTEM FOR ALZHEIMER'S DISEASE THERAPY: PROSPECTIVE STUDY OF HEMODIALYSIS PATIENTS

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Objectives;
We have been developing Extra-corporeal Abeta Removal System (EARS) as a novel therapeutic method of Alzheimer's Disease. We followed hemodialysis patients prospectively to investigate whether the repeated removal of blood Abeta affect plasma Abeta concentrations, cognitive functions and Abeta burden in the brain.
Methods;
This study was approved by the institutional review boards of Fujita Health University and the hospitals involved. The plasma Abeta concentrations by ELISAs (WAKO) and the cognitive functions by MMSE were investigated in 30 patients on hemodailysis (age,71.8 ± 3.3 yo; duration of dialysis, 12.9 ± 8.8 years) at Baseline and 18 or 36 months later (2ndline). Postmortem brain cortices of 16 hemodailysis patients (75.8 ± 9.8 yo) and 16 non-hemodialysis controls (79.0 ± 12.5 yo) were retrospectively analyzed with anti Abeta antibodies.
Results;
Hemodialyzers removed blood Abeta effectively and triggered the influx of Abeta from outside of the blood. This Abeta-influx ceased in association with the end of hemodialysis. From Baseline to 2ndline, plasma $Abeta_{1-40}$ decreased (750.3pg/ml to 689.4 pg/ml, p=0.031), but plasma $Abeta_{1-42}$ increased (41.5 to 69.6pg/ml, p<0.001). Individual MMSE scores were slightly improved (p=0.057, Wilcoxon signed-rank-test). The brains of hemodialysis patients contained significantly fewer plaques than controls. Brain Abeta imaging of hemodialysis patients will also be discussed.
Conclusions;
Heamodialysis removed blood A-beta effectively and improved or maintained cognitive functions. It might trigger the Abeta-influx from the brain into the blood.
This work was partly supported by KAKENHI (23500531, 26282126), Smoking Research Foundation, and Suzuki Memorial Foundation.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-0313

RETINOIC ACID SKEWS THE T HELPER CELL RESPONSE TOWARDS AN ANTI-INFLAMMATORY TH2 PHENOTYPE IN AMYLOID-BETA PEPTIDE IMMUNIZED MICE

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Objectives: The beneficial effects of Abeta immunotherapy may be counteracted by inflammatory responses. Taking into account a chronic inflammatory environment in the AD brain, therapeutic strategies that would not result in a worsening of this phenomenon should be designed, and the induction of the Th2 response by immunotherapy should be more beneficial and safer than a Th1 response. In this study we evaluated antibody production and T cell response in mice after immunization with Abeta in the presence of all-trans retinoic acid (ATRA) as an immunomodulator.
Methods: C57BL/6J wild type and 3XTg-AD mice were immunized with four major Abeta pathological species present in human AD brain (Abeta 1-42, AbetaN3(pE), Abeta 11-42 and AbetaN11(pE)) using saponin as an adjuvant and ATRA. ELISA and cell proliferation assay followed by flow cytometric analysis and supernatant cytokine measurement were performed using standard protocols.
Results: We have demonstrated that ATRA skews anti-Abeta antibody and cytokine production to the potentially safer anti-inflammatory Th2 phenotype in mice immunized with full length Abeta 1-42, but not with N-truncated/pyroglutamate modified peptides in the presence of saponin adjuvant. Interestingly, we didn´t observe proliferation of Abeta 11-42 or AbetaN11(pE) immunized mouse T cells in vitro in the presence of any Abeta peptide/epitope probably because of the loss of the known T cell epitope mapped to the central region of Abeta 1-42 in previous studies.
Conclusions: Our results may have implication in future design of new immunotherapeutic protocols for AD minimizing the risk of neuroinflammation-related toxicity.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-0321

SYNTHETIC FRAGMENT OF RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS PREVENTS MEMORY LOSS IN MICE WITH EXPERIMENTALLY INDUCED ALZHEIMER'S DISEASE

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It is known that oligomeric Abeta binds receptors on neuronal cell surface and this interaction can mediate cell death and amyloid plaque formation during AD. We proposed that short receptor fragments representing the potential binding sites of Abeta are able to bind Abeta and to prevent its interaction with the receptors.Thus administration of these receptor fragments will decrease brain level of Abeta and improve the memory state.

We have selected and synthesized the 14 peptide fragments from four potential receptors of Abeta:alpha-7 acetylcholine receptor, prion protein, p75 neurotrophin receptor and receptor for advanced glycation end products. Synthetic peptides were intranasally administrated into animals with experimentally induced form of AD - bulbectomized mice. Then memory of mice was examined in the water Morris test. We have found that administration of only one fragment of receptor for advanced glycation end products effectively prevented the murine memory from impairment and decreased Abeta level in the brain of experimental mice. The revealed synthetic fragment seams perspective for development of new medicine for AD therapy. Supported by RFBR Grants No. 13-04-40106-H, 13-04-00633,15-04-01360

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-0324

ABETA-INDUCED ALTERATIONS IN SYNAPTIC EXPRESSION OF PHOSPHORYLATED NMDA RECEPTOR SUBUNITS IN RAT HIPPOCAMPUS

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Aβ synaptotoxicity is NMDA receptor-dependent, and post-translation phosphorylation of NMDA receptor is a reversible modification regulating protein activity, location, mobility and protein-protein interaction. Based on our previous results, we tried to further explore Aβ-induced alterations in synaptic expression of phosphorylated NMDA receptor subunits in rat hippocampus, and found that Aβ-induced decreases in protein levels of pTyr1472-NR2B and Fyn, but no changes in protein levels of pTyr1325-NR2A and Src during the early stage of Aβ treatment. The results suggest changes of pTyr1472-NR2B are more significant in Aβ synaptotoxicity. Furthermore, we surprisedly found Aβ induced decreases in puncta densities of synaptic pTyr1472-2B and extrasynaptic pTyr1325-NR2A, which imply complex alterations happened in synaptic and extrasynaptic locations during the early stage by Aβ treatment. This study is to investigate the cellular and molecular mechanisms of synaptic alterations during the development of Alzheimer's disease, and will contribute to prevention and early therapy of Alzheimer's disease.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-0325

ACUTE AMYLOID-BETA TREATMENT INDUCES EARLY SELECTIVE ALTERATIONS OF NMDA RECEPTOR SUBUNITS IN PRIMARY HIPPOCAMPAL GRANULE NEURONS

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Soluble amyloid-β oligomers are thought to induce synaptic dysfunction during the early stage of Alzheimer's disease (AD). Different NMDA receptor (NMDAR) subunits have been linked with Aβ-induced synaptotoxicity. In this report, we revealed Aβ-induced selective reduction in protein levels of NR2B, but no changes in protein levels of NR1, NR2A and SAP102 during the early stage of Aβ treatment. The results suggest the change of NR2B is more striking in Aβ synaptotoxicity. Furthermore, we surprisedly found Aβ could induce significantly reduced puncta densities of synaptic NR2A and SAP102, which imply complex alterations happened in synaptic and extrasynaptic locations by 1h Aβ treatment. Our study indicates the complex effects of Aβ on different NMDAR subunits and synaptic associated proteins during the early stage of Alzheimer's disease. This may explain the difficulty and the great challenge to seek appropriate drug targets for preventing synaptic dysfunction and loss related to abnormal NMDARs in the early stage of AD.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-0499

COLIVELIN AMELIORATES AMYLOID BETA PEPTIDE-INDUCED IMPAIRMENTS IN SPATIAL MEMORY, SYNAPTIC PLASTICITY AND CALCIUM HOMEOSTASIS IN RATS

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Objectives: Amyloid β peptide (Aβ) has been thought to be neurotoxic and responsible for the impairment of learning and memory in Alzheimer's disease (AD). It is reported that the Aβ induced cell death can be effectively prevented by Colivelin (CLN), a most potent Humanin derivative. In this study, we further investigated the neuroprotective effects of CLN against Aβ25-35-induced impairments in spatial learning and memory, synaptic plasticity, and intracellular calcium levels. **Methods:** SD rats pretreated with Aβ25-35 and CLN via intrahippocampal injection were first subjected to Morris water maze test to examine the spatial learning and memory. After behavioral testing, *in vivo* hippocampal field excitatory postsynaptic potential (fEPSP) was evoked to record the long term potentiation (LTP) induced by high frequency stimulation. At last, intracellular calcium level in primary cultured hippocampal neurons was examined by using confocal calcium imaging technique. **Results:** CLN alone, even at a high concentration, had no significant effect on the escape latency and the time in the targeted quadrant compared to vehicle-injected animals, but CLN (0.2 nmol) pretreatment effectively prevented Aβ25-35 (4nmol)-induced deficits in spatial learning and memory of rats. The suppression of *in vivo* hippocampal LTP by Aβ25-35 was nearly completely prevented by CLN, while paired-pulse facilitation (PPF) was not affected. In addition, CLN pretreatment also effectively inhibited Aβ25-35-induced calcium overload. **Conclusions:** CLN has significant *in vivo* neuroprotective properties against Aβ, and CLN might holds great promise for the treatment and prevention of AD.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-0732

RECEPTOR FOR ADVANCED GLYCATION ENDPRODUCTS AS A PROMISING TARGET FOR AMYLOID BETA PEPTIDE INDUCED BLOOD BRAIN BARRIER DYSFUNCTION

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Objectives

Recent studies have demonstrated that receptor for advanced glycation endproducts (RAGE) is promising therapeutic target in β-amyloid diseases. In this study, we examined the effects of antiRAGE primary antibody (Ab_{RAGE}) and azelnidipine (a dihydropyridine-based calcium channel blocker) on $A\beta_{42}$-induced reactive oxygen species (ROS) generation, activation of NADPH oxidase, phosphorylation of ERK1/2 and cPLA2α, and expression of P-selectin on the surface of the cerebral endothelial cells (CECs).

Methods

In this study, immortalized CECs (bEnd3) were applied. To confirm Aβ-RAGE and azelnidipine -RAGE binding, we examined the quantitative immunofluorescence microscopy (QIM) of cellular surface RAGE for bEnd3 cells pretreated with azelnidipine or $A\beta_{42}$and stained with Ab_{RAGE}. To quantify the ROS production and surface P-selectin expression, we applied fluorescence microscopy method. Confocal immunofluorescence microscopy of double immunofluorescent labeled gp91-phox and p47-phox subunits was performed to characterize NADPH oxidase complex assembly. Western blot analysis was applied to characterize phosphorylation of ERK1/2 and cPLA2α.

Results

We report that both $A\beta_{42}$and azelnidipine competed with Ab_{RAGE} to bind to RAGE on the surfaces of CECs. In addition, Ab_{RAGE} and azelnidipine abrogates $A\beta_{42}$-induced ROS production and co-localization between the cytosolic (p47-phox) and membrane (gp91-phox) subunits of NADPH oxidase. Moreover, Ab_{RAGE} and azelnidipine suppressed $A\beta_{42}$ induced phosphorylation of ERK1/2 and cPLA2α in CECs, and accumulation of P-selectin on the surface of the cells.

Conclusions

This study demonstrated that blocking of RAGE can, at least partially, neutralize toxic effect of Aβ on the CECs *in vitro*. Therefore, there is a need to investigate new promising Aβ/RAGE axis blockers.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-0765

AZD3293 A NOVEL BACE1 INHIBITOR: PHARMACOKINETICS AND EFFECTS ON PLASMA AND CSF A-BETA PEPTIDES FOLLOWING MULTIPLE-DOSE ADMINISTRATION IN ALZHEIMER'S DISEASE PATIENTS

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Objective: To evaluate the effects of AZD3293 (a novel BACE inhibitor) on plasma and CSF Aβ biomarkers in AD patients.

Methods: A Phase 1, randomized, double-blind, placebo-controlled, 2-part multiple ascending dose study (NCT01795339) was conducted in healthy young and elderly adults (Part 1) and AD patients (Part 2). Results of Part 1 were previously reported. In Part 2, mild to moderate AD patients received 15 mg, 50 mg, or 150 mg AZD3293 or placebo (total N=16) as a single dose on Day 1 followed by multiple daily doses on Days 3 through 14. Plasma was collected for pharmacokinetic and biomarker analysis. CSF was collected on Day -1 (baseline) and at Day 14 (steady state) for biomarker analysis.
Results: AZD3293 was safe and well-tolerated in this small cohort of AD patients. At steady-state, maximal plasma concentrations of AZD3293 were achieved at 1-3 h and mean effective half-life was 16-21 h. Significant plasma and CSF Aβ1-40 and Aβ1-42 reduction was seen at all doses tested compared to placebo. At Day 14, levels of plasma Aβ1-42 were reduced to the maximal extent measureable. Levels of CSF Aβ1-42 were reduced by 52%, 76%, and 90% for the 15 mg, 50 mg, and 150 mg AZD3293 dose groups, respectively. Results for Aβ1-40 levels were comparable to the Aβ1-42 results. PK and biomarker results in AD patients were comparable to those observed in Part 1.
Conclusions: AZD3293 is safe and well-tolerated, and potently inhibits Aβ in the periphery and CSF in AD patients.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-0892

ANTI-PGLU-3 ABETA MAB IG ISOTYPE AFFECTS PLAQUE CLEARANCE

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Objectives:

Pyroglutamate-3 Aβ (pGlu-3 Aβ) is an N-terminally truncated and modified Aβ species found in Alzheimer's disease (AD) brain (Saido et al., 1995; Lemere et al., 1996). Following truncation of the first 2 Aβ residues, pGlu-3 Aβ is formed by the cyclization of glutamate at residue 3 by glutaminyl cyclase and is associated with increased aggregation and neurotoxicity (Russo et al., 2002; Schilling et al., 2006; Nussbaum et al., 2012). Antibody Ig isotype and specificity for deposited Aβ may influence plaque removal and initiate clearance through Fc receptor-mediated microglial phagocytosis (Bard et al., 2000; DeMattos et al., 2012). Here, we compared the effector functions of different Ig isotypes of the same anti-pGlu3 Ab mAb.

Methods:

An *ex vivo* phagocytosis assay was used to compare different Ig isotypes (IgG1, IgG2a, and IgG2a variant) of the same pGlu-3 Aβ mAb (07/1): frozen brain sections from plaque-rich aged APP/PS1ΔE9 mice were pre-incubated with an Aβ mAb for 1h and then covered with primary microglia or N9 cells for 24h. Plaque clearance was measured by immunofluorescent staining and Aβ ELISA.

Results:

In general, the 07/1 IgG2a anti-pGlu3 Aβ mAb provided the most Aβ clearance with the greatest effect on reducing Aβx-40 and pGlu-3 Aβ levels; intermediate plaque clearance was observed by the 07/1 IgG2a variant mAb. Further analyses will determine cytokine levels and inflammatory changes that may be associated with effector function.

Conclusion:

We conclude that pGlu-3 Aβ mAb Ig isotype strongly affects microglial-mediated clearance of Aβ.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1028

SPINOGENIC AMYLOID-TARGETING BENZOTHIAZOLES (ATBAS) AS POTENTIAL THERAPEUTICS FOR AMYLOID-ASSOCIATED NEURODEGENERATIVE DISEASES

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Objectives: We present the rational design and evaluation of amyloid-binding benzothiazoles that exhibit advantageous properties for potential treatment of neurodegenerative diseases including Alzheimer's Disease (AD). In particular, the therapeutic potential of this class of compounds towards neuroprotection and improving cognitive function is discussed.
Methods: Design of novel ATBAs included consideration of a balance between hydrophobic/hydrophilic characteristics in order to improve the therapeutic window for this class of compounds. Compounds were evaluated for their potential to protect against amyloid-beta induced cellular toxicity as well as for their effect on dendritic morphology in primary neurons.
Results: All new ATBAs were found to be less toxic to SH-SY5Y neuroblastoma cells and had lower propensity to lyse membranes than their parent compound, thereby increasing biocompatibility. In addition, the compounds retained their amyloid-binding capability, were found to have cytoprotective properties, and increased dendritic spine density in primary hippocampal neurons.
Conclusions: This class of ATBAs has shown promise for their ability to be both neuroprotective and spinogenic, while also exhibiting low toxicity. These characteristics allow for ATBAs to potentially serve as therapeutics for not only amyloid-associated neurodegenerative disease like AD but for protection against general cognitive decline as well.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1173

ABETA PROTOFIBRIL SELECTIVE ANTIBODY MAB158 DISPLAY ACUTE EFFICACY AND REDUCE BRAIN ABETA PROTOFIBRILS IN AGED APPARCSWE TRANSGENIC MICE

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Objectives

The objective of the present study was to investigate the efficacy of an Abeta protofibril selective murine monoclonal antibody, mAb158, on reducing soluble toxic species of Abeta in aged APPArcSwe transgenic mice using an acute dose setting.

Methods

A transgenic mouse model, APPArcSwe, that overexpresses human APP with the Swedish (K670N/M671L) and Arctic (E693G) mutations, was used to study acute efficacy with mAb158 on brain and CSF large oligomer/protofibril Abeta lowering. Fourteen to sixteen months old APPArcSwe transgenic mice received a single intraperitoneal injection of mAb158 at 50 mg/kg body weight, or sterile PBS as negative control. Animals were sacrificed two or three weeks after injection, and analyzed for brain and CSF exposure and Abeta protofibril levels.

Results

As expected, the exposure was lower in plasma, CSF and brain in the group that was sacrificed three weeks after injection compared to the group that was sacrificed two weeks after injection. The Abeta protofibril levels in brain were reduced by 38-51% in animals sacrificed after two weeks and by 27-50% in animals sacrificed after three weeks, depending on the extraction method and assay used.

Conclusions

In this study, brain Abeta protofibril lowering was confirmed already after a single dose of mAb158 in APPArcSwe transgenic mice. These results support Abeta protofibril immunotherapy as effective in reducing soluble toxic species of Abeta in brain and hence potentially as a useful therapeutic intervention in Alzheimer's disease.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1221

STABLE AMYLOID OLIGOMERS AS TRACTABLE DRUG TARGETS AND VERSATILE RESEARCH TOOLS IN AD AND PD.

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Accumulating evidence supports a major role for soluble oligomers as toxic entities in Alzheimer's, Parkinson's and other diseases. Unfortunately the instability and limited characterization of oligomer preparations have seriously impeded research and development in this field.

Here we showcase the utility of reproducibly generated stable oligomers of several peptides including amyloid beta (1-42 and pGlu3-42) and alpha-synuclein. Crossbeta stable oligomers are free from monomers and fibrils and demonstrate representative properties and functionality for the non-stable oligomeric analogues. For example, the stable oligomers induce depression of Long Term Potentiation (LTP) after administration *in vivo* in the rat brain. When stored frozen the oligomers have a shelf life of more than 6 months.

The availability of the stable oligomers opens up previously inaccessible avenues for R&D. For example, it enabled high throughput screening of drug-like compound libraries (100k+300k compounds). This resulted in the identification of multiple oligomer binding hits that inhibit oligomer binding to a receptor implicated in AD. We present *in vivo* proof of concept by showing that our front-runner compound when given systemically effectively protects against the oligomer induced depression of LTP in rat.

In another application we show that Crossbeta oligomers are excellent reference standards in biomarker assays. Several groups have demonstrated that Abeta oligomers can be detected in CSF of AD patients, thus potentially providing a much needed early AD biomarker for clinical studies and/or diagnosis. Yet, the variation and sensitivity of these assays has been notoriously difficult. Here we show that Crossbeta oligomers abrogate these problems.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1279

A NANOMEDICINE-BASED THERAPEUTIC APPROACH RESTORES MEMORY AND AMELIORATES AMYLOID PATHOLOGY IN ALZHEIMER'S MOUSE MODELS

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Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by deposition of the β-amyloid (Ab) peptide in the brain. Although many Ab-centric therapies have been attempted, they all failed and no efficacious therapy is available yet.
Objectives. Main objectives of this study were to investigate the efficacy of multi-functional liposomes (Lip), designed to target the brain, to 1. promote disaggregation of brain Ab assemblies in AD mouse brain, 2. reduce brain Ab burden and 3. restore mouse memory, at a post-symptomatic stage. 4. Arrest pathology progression at a pre-symptomatic stage.
***Methods*.** Functionalized Lip with a peptide derived from apolipoprotein-E receptor-binding domain (mApoE) for blood-brain barrier targeting, and with phosphatidic acid (PA) for Ab binding (mApoE-PA-Lip) were administered (I.P.) for 3 weeks in post- and 4 months in pre-symptomatic APP/PS1 mice subsequently tested in the novel object recognition memory test (NORT). At the end mouse brains were collected and analyzed through histology and biochemistry for Ab deposition.
Results. Administration of mApoE-PA-Lip decreased total insoluble brain Ab_{1-42}, total plaque area and Ab oligomers. Plaque reduction was confirmed by PET imaging with [^{11}C]-PIB. The reduction of brain Ab was associated with its increase in liver and spleen. Treatment also restored mouse impaired memory to normal at post-symptomatic ages and prevented its loss when administered at pre-symptomatic ages.
Conclusions. These data suggest that bi-functionalized Lip destabilize brain Ab aggregates and promote peptide removal from the brain and its peripheral clearance. This innovative therapeutic approach can be considered as a new candidate for AD treatment.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1325

YOUTHFUL SYSTEMIC MILIEU AMELIORATES ALZHEIMER'S DISEASE PATHOLOGY

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Objectives: Alzheimer's disease (AD) is characterized by memory loss, neuronal death, synaptic alterations, brain inflammation and the presence of amyloid plaques. Compelling evidence suggest that young blood conveys potent factors capable to ameliorate aging-related cognitive decline. To study if young systemic milieu reverses AD pathology, we developed a technique of whole blood exchange (BE) which replaces >50% of the original blood volume by normal blood from mice having same genetic background.

Methods: TG2576 (TG) mice received BE from 3 to 17 months of age (Group I) to test its preventive effect, and from 13 to 17 months of age (Group II) to test the effect of BE on pre-existing brain plaques. Spatial memory was tested by Barnes maze at 13 or 17 months of age in groups I and II, respectively.

Results: Histological analysis found age-matched Sham TG in group I showed intense amyloid plaques predominantly in the cerebral cortex and hippocampus. In contrast, BE mice showed significant reductions in these regions. Group II confirmed that BE significantly reduced brain amyloid deposition, even when the treatment was initiated after the development of cerebral plaques. Such decreases in brain plaques reflected in improved performance in spatial memory test in both groups. Brain clearance of amyloid-beta across the blood-brain barrier (BBB) remained unchanged in BE mice compared to age-matched Sham mice.

Conclusions: Young blood clearly affects brain environment and ameliorates brain pathology in AD model mice. This study draws topical interests in seeking disease-modifying treatment for neurodegenerative diseases.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1358

NEUROPROTECTIVE EFFECTS OF SCYLLO-INOSITOL TREATMENT ON DISEASE-BEARING MOUSE MODEL OF AD

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Objective: To determine the neuroprotective role of *scyllo*-inositol treatment in an Alzheimer's disease model after the onset of cognitive and pathological deficits.
Methods: We undertook a microarray study on the hippocampi of TgCRND8 mice treated with *scyllo*-inositol for 30 days after the onset of spatial cognitive dysfunction and significant amyloid load. These data identified two different neuronal pathways that contribute to neuronal function, neurogenesis and GABAergic neuronal signalling as well as upregulation of the hormone, Proopiomelanocortin (POMC) . We subsequently undertook pathological studies to characterize these two neuronal processes in the presence and absence of scyllo-inositol and a function of POMC expression.
Results: We found a profound deficit in neurogenesis and in GABAergic interneurons with increasing amyloid load. The deficits in neurogenesis early in pathology correlate directly with Amyloid-beta peptide load, whereas later in pathology are more directly related to the loss of neurotrophic support. We also report that GABAergic interneuron loss is of the somatostatin expressing cells and protection from loss is correlated with POMC expression. We did not find deficits in the cholinergic neuronal population at the ages that we examined.
Conclusions: *scyllo*-inositol treatment of disease-bearing mouse model of AD protects adult neurogenesis and GABAergic interneurons within the hippocampus

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1393

TRANSTHYRETIN-DERIVED PEPTIDES AS AMYLOID-BETA AGGREGATION INHIBITORS: POTENTIAL USE AS THERAPEUTICS FOR ALZHEIMER'S DISEASE

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Objectives: The beneficial effect of the human tetrameric protein Transthyretin (TTR) over Amyloid-beta (Ab) totoxicity has been reported both *in vitro* and *in vivo.* However, the mechanism of protection remains unclear. We pursued to characterize such mechanism, and identify the minimum peptide sequence of TTR capable of preventing Ab from aggregation.
Methods: Mutational analysis of TTR was performed to elucidate the mechanism of protection. Based on the resulting model, peptide inhibitors were designed using TTR sequence as a reference. The toxicity of Ab was then measured to assess effectiveness of the peptide inhibitors.
Results: Mutational analysis of TTR revealed that although TTR fibrils are not capable of preventing Ab from being toxic, the ability to aggregate is required for TTR in order to be protective. An amyloidogenic 13-residue segment of TTR prevented Ab aggregation and toxicity only when was unable to self-associate by addition of a charged tag.
Conclusions: We propose a protection model where a small hydrophobic segment of TTR is exposed only when the tetramer dissociates into monomers, and binds to Ab preventing its aggregation. In order to use the segment as inhibitor, the addition of a charged tag was needed, to hinder self-association and allow Ab binding. Comprehensive analysis revealed that the peptide inhibitors were efficient at hindering Ab aggregation and toxicity. This work provides a novel methodology for Ab aggregation inhibition that is likely to initiate an innovative strategy for the development of a therapy for Alzheimer's Disease.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1496

REGION AND CELL SPECIFIC EXPRESSION OF ISOGLUTAMINYL CYCLASE AND ITS SUBSTRATE CCL2 IN MOUSE AND HUMAN BRAIN

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Objectives: Main hallmarks of brains of patients affected by Alzheimer's disease (AD) are pathogenic deposition of Abeta peptides and chronic inflammation. Both features may be fueled by the action of glutaminyl cyclases, QC and isoQC, catalyzing the conversion of N-terminal Gln/Glu-residues of Abeta peptides and chemokine (C-C motif) ligand 2 (CCL2) into pyroglutaminated (pE) forms. We here focused on the regional and cellular expression of isoQC and its substrate CCL2 in brain tissue of wild type mice and humans compared to inflammatory conditions seen in aged APP transgenic mouse brain and *post mortem* brain tissue from AD patients.
Methods: We used enzymatic activity assays and immunohistochemistry of mouse and human brain tissue followed by confocal laser scanning analyses to reveal brain region and cell type-specific expression of isoQC and its putative pro-inflammatory substrate CCL2.
Results: In mouse brain, isoQC was ubiquitously expressed in neurons of neocortical, hippocampal and subcortical structures, localized to the endoplasmic reticulum and Golgi apparatus and co-expressed with its substrate CCL2. In APP-transgenic Tg2676 mice and in AD, isoQC and CCL2 proteins were found to be co-induced in Abeta plaque-associated reactive astrocytes. In brains of AD patients, correlations of isoQC and CCL2 with pGlu-Abeta load and with the decline in mini mental state examination were established.
Conclusions:The wide-spread isoQC expression in brain suggests a number of yet unidentified physiological functions. Additionally, the neuronal co-expression with the pro-inflammatory chemokine CCL2 and the co-induction of both proteins in reactive astrocytes indicates an involvement in pathogenic conditions in AD.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1511

PYROGLUTAMATE ABETA42 CORRELATES WITH INCREASED PIB BINDING IN POSTERIOR CINGULATE CORTEX AND PRECUNEUS OF SUBJECTS WITH MCI AND ALZHEIMER'S DISEASE

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Objectives: Positron emission tomography imaging demonstrates increased retention of [C-11] Pittsburgh compound B (PiB) in the default mode network (DMN) in Alzheimer's disease (AD). This likely reflects accumulation of fibrillar amyloid-beta (Abeta). N-truncated and pyroglutamate-modified Abeta forms, e.g., Abeta(pE3-42), are particularly fibrillogenic. We hypothesized that Abeta(pE3-42) concentration corresponds to regional PiB retention in the DMN.
Methods: We quantified [H-3]PiB binding and formic acid-extracted Abeta(pE3-42) in the precuneus (PreC, BA7) and posterior cingulate cortex (PC, BA23), regions in the DMN, from cases with a premortem clinical diagnosis of no cognitive impairment (NCI, n=19), mild cognitive impairment (MCI, n=20), or AD (n=24).
Results: Compared to NCI and MCI, AD cases had higher Abeta(pE3-42) concentration (PC, p2= 0.0039; PreC, p2<0.0001) and higher [H-3]PiB binding (PC, p2=0.001; PreC, p2=0.0001). Overall, higher PreC and PC Abeta(pE3-42) levels correlated with greater [H-3]PiB binding (PC, R=0.7938, p2<0.0001; PreC, R=0.7455, p2<0.0001). When AD cases were divided into mild-moderate (N=18) and severe (N=6) AD, the two groups were comparable by Abeta(pE3-42) levels in both regions, while [H-3]PiB binding was higher in the PreC, but not the PC, of the severe AD compared to the mild-moderate AD group.
Conclusions: Although Abeta(pE3-42) and PiB binding correlated closely in PreC and PC, these data suggest regional differences in the progression of amyloid pathology in the DMN of AD dementia cases. Abeta(pE3-42) may be a particularly good substrate for PiB, and a clinically relevant biomarker.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1660

POTENTIAL NANOTHERAPEUTIC APPROACH FOR ALZHEIMER'S DISEASE: IN VITRO CLEARANCE OF BETA-AMYLOID ACROSS THE BLOOD-BRAIN BARRIER BY MULTI-FUNCTIONAL LIPOSOMES.

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The reduction of amyloid-beta peptides (Ab) is considered a primary therapeutic target to minimize the neurodegeneration in Alzheimer's disease (AD), a pathology characterized by accumulation of Ab in the brain. A possible strategy to reduce Ab levels is to exploit the peripheral-sink effect that is based on the removal of plasma Ab, drawing out soluble Ab from the brain. *Objectives.* The aim of the present study was to investigate the potential effect of multi-functional liposomes binding Ab on the peptide exchange across an *in vitro* model of the blood-brain barrier (BBB). *Methods.* Liposomes were prepared by extrusion procedure and bi-functionalized with phosphatidic acid and with a modified ApoE-derived peptide (mApoE-PA-LIP) for Ab binding. An *in vitro* BBB model made of human brain endothelial cells (hCMEC/D3) was set up on a transwell system and soluble small Ab42 assemblies were added to the basolateral compartment (brain side). The Ab translocation across the BBB was evaluated both alone or with mApoE-PA-LIP present in the apical compartment (blood side). Also the permeability of liposomes from basolateral-to-apical compartment in the presence of Ab was analyzed. *Results.* The presence of mApoE-PA-LIP strongly enhanced (5-fold) the basolateral-to-apical transcytosis of Ab across the BBB model, compared to the peptide alone, in a lipid dose-dependent manner. Moreover, the transcytosis of Ab was due to a peripheral-sink effect since mApoE-PA-LIP were not able to transport the peptide across the cellular monolayer. *Conclusions.* This study provide a rationale for the use of Ab-binding particles as a potential therapeutic strategy for AD.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1678

A NOVEL ALZHEIMER'S DISEASE VACCINE CLEARS ABETA PLUGS, SUPPRESSES T CELL INFLAMMATIONS IN BRAINS AND IMPROVES COGNITIONS IN ANIMAL MODEL

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Background

Alzheimer's Disease (AD) is an age-dependent neurodegenerative disease that commonly results in brain degeneration associated with severe impairment of cognitive function. Amyloid β-peptide (Aβ), particular the Ab42, plays a key role in the pathology of Alzheimer's disease and becomes a potential target for vaccines. Aβ42 (AN-1792) vaccine phase II trials were halted when 18 of 298 patients immunized with AN-1792 presented with brain inflammations likely due to T cells-mediated infiltrations and inflammations in brains. We developed a co-immunization protocol to induce high levels of antibodies and iTreg cells previously. In this study, we aimed to demonstrate that a co-immunization protocol with Aβ42 protein and its DNA vaccine could clear the Ab42 plaques and prevent inflammations in brain in a murine AD model.

Methods

After we co-immunized the Abeta transgenic mice, behavior improvements, Aβ42 plaques clearances and T cell infiltrations and inflammations in brain were determined.

Results

The co-immunization could induce high levels of anti-Ab42 antibodies and iTreg cells, and reduce Aβ plaques and significantly ameliorate cognitive deficits. Importantly, induced iTreg suppresses T cell-mediated brain inflammations and infiltrations. This study demonstrates that the co-immunization protocol can improve animals' cognitions and behaviors. More importantly, such protocol could eliminate T cell infiltrations and inflammations in the brain of these tested animals.

Conclusions

The co-immunization protocol could clear Abeta plaques and suppress T cell inflammations in brains in animal model. Such protocol could leads to the development of a safer immunotherapeutic/preventive strategy against AD in humans.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1714

FDA APPROVED ASTHMA THERAPEUTIC AGENT IMPACTS AMYLOID BETA IN THE BRAIN IN A TRANSGENIC MODEL OF AD

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Objectives:
Cromolyn Sodium is an FDA-approved drug used in the treatment of asthma. Because of its structural similarity with fisetin, which inhibits amyloid aggregation, we hypothesized that Cromolyn Sodium prevents Aβ fibrillization and Aβ metabolism. In this study, we evaluated the efficacy of Cromolyn Sodium on amyloid pathology in a mouse model of Alzheimer's disease.
Methods:
We first performed Aβ fibrillization and oligomerization assays *in vitro*, using Thioflavin T fluorescence and a split-luciferase complementation, respectively. We also intraperitoneally administered Cromolyn Sodium to APP/PS1 mice daily for one week, and then measured the levels of soluble and insoluble Aβ within the cerebral parenchyma and the interstitial fluid (ISF) using *in vivo* microdialysis technique. Finally, to elucidate the mechanisms, we examined the efficiency of Aβ uptake by microglial cells using double immunostaining between Iba1 and Aβ in brain and the *in vitro* microglial Aβ uptake assay.
Results:
In vitro, Cromolyn Sodium inhibits the aggregation of monomeric Aβ into oligomers and fibrils. *In vivo*, the drug rapidly decreases the amount of soluble monomeric Aβ in the brain by ~50% and within the ISF by ~30%. The half-life of ISF Aβ is significantly reduced in Cromolyn Sodium treated mice. Finally, we revealed that Cromolyn sodium increases the percentage of Iba1 immunoreactivity co-localizing with amyloid in mice and microglial Aβ uptake *in vitro*.
Conclusions:
Cromolyn Sodium prevents Aβ oligomerization. This effect favors Aβ clearance from CNS, which are in part attributed to an increased efficiency for uptake and degradation of Aβ by microglial cells.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1727

THE GLUTAMINYL CYCLASES QC AND ISOQC ARE DIFFERENTIALLY EXPRESSED IN BRAIN REGIONS AFFECTED IN ALZHEIMER'S DISEASE

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Objectives: Glutaminyl cyclases (QCs) catalyze the physiological formation of pyroglutamate (pGlu) from glutamine precursors at the N-terminus of a number of peptide hormones, neuropeptides and chemokines. However, QCs are also implicated in the pGlu modification and stabilization of pathogenic Abeta variants and of pro-inflammatory CCL2. These pGlu-modified peptides are resistant to proteolytical degradation and accumulate in brains of Alzheimer's disease (AD) patients. For the generation of respective animal models and for pharmacological treatment studies the characterization of the mouse strain and brain region-specific expression of QC and isoQC is indispensible.
Methods: We used enzymatic activity assays and specific antibodies to detect both QC and isoQC variants by immunohistochemistry in nine different mouse strains.
Results: The highest enzymatic QC/isoQC activity was detected in ventral brain, followed by cortex and hippocampus. Immunohistochemical stainings revealed that QC/isoQC activity in cortex mostly arises from isoQC expression. For most brain regions, the highest QC/isoQC activity was detected in C3H and FVB mice, whereas low QC/isoQC activity was present in CD1, SJL and C57 mice. Quantification of QC- and isoQC-immunoreactive cells by unbiased stereology revealed a higher abundance of isoQC- than of QC-immunoreactive neurons in Edinger-Westphal nucleus and in substantia nigra. In the locus coeruleus, however, there were comparable densities of QC- and of isoQC-immunoreactive neurons.
Conclusions: These observations are of considerable importance with regard to the selection of appropriate mouse strains for the study of QC/isoQC relevance in mouse models of neurodegeneration and neuroinflammation and for the testing of therapeutical interventions in these models.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1774

HIGH-THROUGHTPUT PHENOTYPING AND PATHOLOGY OF THE APP/PS1 DOUBLE TRANSGENIC MODEL

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Objective: Double transgenic mice from the cross between Tg 2576 (mutant APP) and a mutant presenilin (PS1) line develop increased amyloid- beta 40 and 42 levels and early amyloid pathology in the cerebral cortex and in the hippocampus. The usefulness of new behavioral technologies for detection of early symptoms in relation to biochemical changes is presented and discussed.
Methods: PhenoCube® NeuroCube® and SmartCube® are high-throughput platforms that assess circadian, cognitive, motor behavior, anxiety, gait, and other domains using PGI's proprietary Computer Vision automated scoring system and machine learning algorithms to define phenotypic signatures. Other standard test follow published protocols. Immunohistochemistry with fluorescence or with DAB labeling was used to detect epitopes of interest in hippocampus and cortex. Levels of amyloid- beta 40 and 42 were measured by ELISA (EMD Millipore, Billerica, MA); data was normalized to the total protein content.
Results: Transgenic mice are hyperactive and show memory deficits as early as 12 weeks of age as assessed in our high-throughput phenotypic platforms and in standard tests assessing spontaneous alternation, fear conditioning and spatial learning. We also present an assessment of inflammatory markers (Iba1, FAP), and accumulation of phospho-tau, amyloid-beta oligomers and plaques in these transgenic mice.
Conclusion: The possibility to evaluate behavioral deficits with the novel high-throughput technology early in life forms a basis to test therapeutic interventions at early stages of developing brain pathology. This may allow collecting important data for human trials in prodromal AD.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-1795

IDENTIFICATION OF THYROTROPIN-RELEASING HORMONE AS HIPPOCAMPAL GLUTAMINYL CYCLASE SUBSTRATE IN NEURONS AND REACTIVE ASTROCYTES

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Objectives: Abeta peptide variants with an N-terminal truncation and pyroglutamate modification were shown to be highly neurotoxic and prone to aggregation. This modification of Abeta is catalyzed by glutaminyl cyclase (QC) which was initially described in hypothalamus, where thyrotropin-releasing hormone (TRH) is a physiological substrate. Recently, in the hippocampus a novel neuroprotective function of TRH has been reported following excitotoxicity and Abeta-mediated neurotoxicity. Functionally matching this finding, we recently demonstrated QC expression by interneurons of mouse hippocampus.
Methods: We used brain tissue of Tg2576 mice and primary astrocytes for analysis by qPCR and immunohistochemistry to reveal brain region and cell type-specific expression of QC and TRH.
Results: We detected neuronal co-expression of QC and TRH in the hippocampus of adult wild type mice. In neocortex of aged but not of young mice transgenic for amyloid precursor protein an increase of QC mRNA levels was found compared to wild type littermates. This was not observed in hippocampus, which is later affected by Abeta pathology. However, in hippocampus of transgenic mice a correlation between QC and TRH mRNA levels was revealed. Interestingly, the enzyme QC and its substrate TRH were detected in reactive astrocytes in proximity of Abeta deposits and co-regulated in primary astrocytes challenged by pro-inflammatory stimulation.
Conclusions: Functionally, the expression of QC in astrocytes could play a role in neuroprotection by activation and release of TRH due to diminished excitotoxicity and in neurodegeneration due to the formation of pGlu-Abeta.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-2179

TREATMENT WITH D3-DERIVED D-ENANTIOMERIC PEPTIDES PREVENTS DETERIORATION OF MOTOR PHENOTYPE INDUCED BY PGLU-ABETA

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1. Objectives

Until today, treatment of Alzheimer's disease (AD) can only be provided palliatively. Our group has developed the D-enantiomeric peptide D3, which has been shown to ameliorate cognition and to reduce beta-Amyloid (Abeta) plaque load in transgenic AD mouse models. Here we tested D3 and a very potent derivative in the mouse model TBA2.1 that produces N-terminally truncated and pyroglutamated Abeta (pGlu-Abeta). In contrast to other AD mouse models these mice suffer from severe neurodegeneration in the hippocampus and cerebellum. After initial characterization of the motor phenotype we treated the mice with D3 and the derivative to assay their therapeutic potentials in the TBA2.1 model.

2. Methods

TBA2.1 mice were characterized regarding motor balance, motor phenotype and Abeta deposits in the brain. For treatment studies we administered D3, its derivative or placebo intraperitoneally over 4 weeks. Motor balance, motor phenotype and Abeta deposition were analyzed before and after treatment.

3. Results

Cross-sectional characterization of homozygous TBA2.1 mice revealed a progressive phenotype and reduced motor balance compared to wildtype performance starting from 3 months of age. Treatment with both, D3 and its derivative, stabilized motor balance and the D3 derivative was also able to inhibit deterioration of the motor phenotype. Abeta deposition, however, seemed to be unchanged compared to placebo group.

4. Conclusions

Although Abeta deposition does not seem to correlate with the neurological and motor phenotype in TBA2.1 mice these treatment studies indicate that the D3 derivative is an even more effective agent than D3.

04b. Therapeutic Targets & Mechanisms for Treatment: Abeta, truncated & pGlu-Abeta

ADPD5-2308

IDENTIFICATION OF A COMPOUND WHICH DISRUPTS BINDING OF AMYLOID-BETA TO THE PRION PROTEIN USING A NOVEL FLUORESCENCE-BASED ASSAY

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Objectives

The prion protein (PrP) has been implicated both in prion diseases such as Creutzfeldt-Jakob disease (CJD), where its monomeric cellular isoform (PrP^C) is recruited into pathogenic self-propagating polymers of misfolded protein, and in Alzheimer's disease (AD) where PrP^C may act as a receptor for synaptotoxic oligomeric forms of amyloid beta. There has been considerable interest in identification of compounds which bind to PrP^C, stabilising its native fold and thereby acting as a pharmacological chaperone to block prion propagation and pathogenesis. However, compounds binding PrP^C could also inhibit the binding of toxic amyloid beta species and may have a role in treating AD, a highly prevalent dementia for which there are currently no disease-modifying treatments. However, the absence of a unitary, readily measurable, physiological function of PrP makes screening for ligands challenging, and the highly heterogeneous nature of amyloid beta oligomer preparations makes conventional competition binding assays difficult to interpret.

Methods

We used site-specific fluorescent labelling recombinant prion protein (PrP) to identify compounds which bind to PrP and inhibit both amyloid beta binding and prion propagation.

Results

Here we present results from the development of a high throughput screen capable of identifying small molecule inhibitors of amyloid beta binding to the prion protein.

Conclusions

The fluorescence-based assay allows high throughput screening and identification of compounds capable of disrupting the binding of amyloid-beta to the prion protein.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1917

NEUROCOGNITIVE PROTECTIVE EFFECT OF S-NITROSOGLUTATHIONE (GSNO) IN ALZHEIMER'S DISEASE

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Objective: S-nitrosoglutathione (GSNO) is an endogenous nitric oxide carrier molecule involved in physiological regulation of vascular function. We have previously reported that treatment of rats subjected to permanent bilateral common carotid artery occlusion (pBCCAO) producing a state of chronic cerebral hypoperfusion (CCH) with GSNO improved spatial learning and memory functions and decreased amyloid-β (Aβ) accumulation and decreased induction of vascular inflammation in the brains. To further investigate the effect of GSNO on neuroprotection and underlying mechanisms under CCH and AD conditions, we designed the experiments as followings:
Methods: The effect of GSNO treatment on neuronal survival, tau-hyperphosphorylation, and activities of tau-kinase and its regulators were analyzed in *in vitro* (primary rat cortical neuron cultures), *ex vivo* (purified synaptosomes), and in vivo (rats subjected to pBCCAO and APPSw/PS1dE9 mice).
Results: GSNO administration significantly attenuated the impaired spatial learning process and neurodegeneration in rats with pBCCAO and APPSw/PS1dE9 mice. GSNO treatment decreased pathological tau hyperphosphorylation (Ser202/396/404 and Thr205) in the brains of tested animals, and primary cultured neurons and purified synaptosomes treated with Aβ peptide. In these experimental models, GSNO inhibited GSK-3β activities and Cdk5 activities which play critical role in tau hyperphosphorylation. Mechanistically, GSNO inhibited GSK-3β activity via inhibiting phosphorylation of GSK-3β (Tyr279) and Cdk5 activity via inhibiting calpain-mediated p35 proteolysis leading to generation of p25.
Conclusions: Taken together with our previous report, these data suggest the therapeutic potential of GSNO as neuro- and cognitive-protective agent for CCH/AD.

04g. Therapeutic Targets & Mechanisms for Treatment: kinases

ADPD5-0394

LEUCETTINES, A CLASS OF DYRKS/CLKS DUAL INHIBITORS: IMPLICATIONS FOR TREATMENT OF ALZHEIMER'S DISEASE AND DOWN SYNDROME

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Objective

The protein kinases DYRKs (dual specificity tyrosine-phosphorylation-regulated kinases) and CLKs (cdc2-like kinases) are involved in Alzheimer's disease (AD) and Down syndrome (DS). We wanted to investigate the structure-activity relationship (SAR) in Leucettines, a class of pharmacological inhibitors of DYRKs/CLKs, derived from the marine sponge natural product Leucettamine B (1, 2, 3).

Methods

78 Leucettines were synthesized and tested for their inhibitory action on 11 purified recombinant kinases (CLK 1-4, DYRK1A, 1B, 2, 3, GSK-3, CK2, Pim1). Leucettines were also tested in four cellular assays: (i) modulation of CLK1 pre-mRNA splicing, (ii) protection towards glutamate -induced cell death in HT22 hippocampal cells, (iii) induction of autophagy and (iv) phosphorylation of Tau on Thr212.

Results

The enzymatic SAR highlights an excellent fit with previously determined Leucettine/DYRK co-crystal structures and suggests directions for improvements in potency and selectivity.

The cellular SAR highlights correlations between inhibition of specific targets and some but not all cellular effects.

Conclusion

Leucettines warrant further development as potential therapeutics against neurodegenerative diseases on the basis of their multiple molecular targets and cellular effects.

(1) *J. Med. Chem. 54* (2011), 4172.
(2) *J. Med. Chem. 55* (2012), 9312.
(3) *Mol. Pharmacol. 85* (2014), 441.

04g. Therapeutic Targets & Mechanisms for Treatment: kinases

ADPD5-0396

DYRK1A INHIBITOR PREVENTS COGNITIVE IMPAIRMENT IN TWO MICE MODELS OF DOWN SYNDROME: IMPLICATIONS FOR TREATMENT OF ALZHEIMER'S DISEASE

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Objective

Analyzing the potential of pharmacological inhibitors of DYRK1A (dual specificity tyrosine-phosphorylation-regulated kinase 1A) in Alzheimer's disease (AD) and Down syndrome (DS).

Methods

After the identification of the marine sponge Leucettamine B as a DYRKs inhibitor (1), a structure-activity relationship was established based on 500+ analogues (Leucettines), leading to an optimized product, Leucettine L41, which was tested in cellular and animal models.

Results

Leucettines were co-crystallized with DYRK1A, and 4 other kinases, and their selectivity was extensively studied (2). L41 displayed neuroprotective effects on glutamate-induced cell death in immortalized mouse hippocampal cells and enhanced autophagy without inducing cell death (3). L41 inhibited phosphorylation of Tau Thr212. L41 was tested in tgBACDyrk1a mice (a model expressing three DYRK1A gene copies) and in Ts65Dn mice (a partial trisomy model). Both models overexpressed DYRK1A (about 1.5-fold increase in mRNA and protein levels and in catalytic activity) and displayed cognitive impairment related to DS and AD. L41 treatment of mice led to normalization of the DYRK1A activity and fully corrected the novel object recognition deficit. No effect was observed with kinase-inactive, control Leucettines or on wild-type animals. Extensive proteomic and phosphoproteomic studies elucidate on the molecular actions of L41 in the brains of these mouse models.

Conclusion

The multi-target selectivity of Leucettines may account for their neuroprotective and positive behavioral effects. This family of kinase inhibitors warrants further optimization as potential therapeutics against AD and DS.

(1) *J. Med. Chem. 54*, 4172.
(2) *J. Med. Chem. 55*, 9312.
(3) *Mol. Pharmacol. 85*, 441.

04g. Therapeutic Targets & Mechanisms for Treatment: kinases

ADPD5-1027

A PHASE IB MULTIPLE ASCENDING DOSE STUDY OF THE SAFETY, TOLERABILITY, AND CNS AVAILABILITY OF AZD0530 (SARACATINIB) IN ALZHEIMER'S DISEASE

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Objectives
Recent findings strongly implicate oligomeric Amyloid–beta in the pathogenesis of Alzheimer's disease. We recently described a putative signaling cascade whereby oligomeric Amyloid-beta binds to neuronal cellular prion protein (PrPC), activating the intracellular kinase Fyn leading to synaptotoxicity. Fyn has long been implicated in AD pathophysiology, but has not been previously tested as a therapeutic target in this disease. Herein, we present a Phase Ib trial of AZD0530, a Src family kinase inhibitor specific for Fyn and Src kinase, for the treatment of patients with mild-moderate AD.
Methods
The study was a single site, multiple ascending dose, randomized, placebo-controlled, double-blind Phase Ib trial of AZD0530 in patients with AD and MMSE ranging from 16-26. A total of 24 patients were enrolled in 3 sequential groups, and treated with AZD0530 or placebo for one month, at doses of 50, 100, or 125mg. Primary endpoints were safety, tolerability, and CSF penetration of AZD0530. Secondary endpoints included changes in neuropsychological performance (ADAS-Cog, MMSE, ADCS-ADL, NPI, and CDR-SOB), and changes in brain glucose metabolism measured by 18FDG-PET.
Results
AZD0530 was generally safe and well tolerated across doses. Plasma:CSF ratio of AZD0530 was 0.4. One-month treatment with AZD0530 did not affect cognitive or behavioral function, activities of daily living, or 18FDG-PET measures.
Conclusions
AZD0530 is safe and well tolerated in patients with mild-moderate AD, achieving good CNS penetration with oral dosing. Targeting Fyn kinase is a promising therapeutic approach in AD, and our current data support a larger Phase IIa clinical trial.

04g. Therapeutic Targets & Mechanisms for Treatment: kinases

ADPD5-2300

PROTECTIVE EFFECTS OF SALVIA ROSIFOLIA AND S. HUBERI ON BETA AMYLOID PEPTIDE INDUCED NEUROTOXICITY THROUGH GLYCOGEN SYNTHASE KINASE-3 BETA INHIBITION

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Objectives

S. officinalis and salvianolic acid has been shown to protect from neurotoxicity in several studies. The aim of this study is to investigate the protective effects of endemic *Salvia* species*; S. rosifolia* and *S. huberi* against amyloid beta (A*β*1-42) induced toxicity on SHSY5Y neuroblastoma cell lines and to clarify its protective effect is due to its GSK-3*β* inhibitory activity or not.

Methods

The methanol extracts of both plants were fractioned between water, petroleum ether, n-butanol. The sub-extracts were examined for *in vitro* GSK-3*β* inhibitory activity due to manufacturer's procedures. The n-butanol extracts' GSK-3*β* inhibitory activity were further high compared to other extracts so it was fractioned via Polyamide CC with various polarities of H_2O:MeOH. Five fractions were obtained (FR1-5).
SHSY5Y cells were incubated at 37 °C and %5 CO_2 and %88 humidity and real time cell profiles obtained by X-celligence system. Cell indexes have been recorded and the cell response profiles obtained every 15 minutes during 60 hours.

Results

Enzyme activity studies showed that both n-butanol extracts, FR5 for *S. rosifolia* and FR4 for *S. huberi* have higher inhibitory activity compared to others.
FR5 and FR4has protective effect on amyloid beta induced toxicity on SHSY5Y cells due to X-celligence profiles.

Conclusions

Fractions among the ones tested for both amyloid beta toxicity and GSK-3*β* inhibitory reflecting that the *S. rosifolia* and *S. huberi* might show its neuroprotective effect over their GSK-3*β* inhibitory activities. **Acknowledgement**
This study was supported by the scientific and technological research council of Turkey (TÜBİTAK 112S553).

04h. Therapeutic Targets & Mechanisms for Treatment: secretases & other proteases

ADPD5-0653

NEURONAL ILEI REDUCES AMYLOID-BETA PRODUCTION BY DESTABILIZING AMYLOID PRECURSOR PROTEIN

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Objectives: Amyloid-beta (Abeta) peptide is produced through beta- and gamma-secretase-mediated sequential proteolysis of amyloid precursor protein (APP). Although gamma-secretase is a major target for therapeutic intervention for Alzheimer's disease (AD), non-selective inhibition of its activity caused serious adverse effects due to blockade of Notch signaling and accumulation of neurotoxic APP-C-terminal fragments (CTFs). We searched for additional proteins that affect Abeta production by interacting with the gamma-secretase complex, and identified a secretory protein named ILEI (also known as FAM3C). In this study, we examined the expression and function of ILEI.
Methods: We examined the effect of ILEI expression on Abeta production using cultured cells and transgenic mice. ILEI expression in the brain was studied with western blotting and immunohistochemistry.
Results: ILEI destabilizes APP-CTFs by binding to the gamma-secretase complex and interfering with its chaperone properties. Notch processing and gamma-secretase activity are not affected by ILEI. ILEI is predominantly expressed in neuronal cells, and transforming growth factor-beta induces ILEI in the brain. The level of secreted ILEI is declined with age in the monkey brain, and the protein and mRNA levels of ILEI are decreased in autopsy brains of AD patients. Transgenic overexpression of ILEI significantly reduces the brain Abeta burden and ameliorates the memory deficit in AD model mice.
Conclusions: ILEI destabilizes APP-CTFs and reduces Abeta production. Decrease in ILEI expression may be a risk factor for Abeta accumulation in the brain, and ILEI may be a plausible target for the development of disease-modifying therapies for AD.

04h. Therapeutic Targets & Mechanisms for Treatment: secretases & other proteases

ADPD5-0976

ACTIVATION OF ADAM10 AS A THERAPEUTIC STRATEGY FOR ALZHEIMER'S DISEASE: SHEDDING OF PRION PROTEIN REDUCES ABETA OLIGOMER BINDING AND TOXICITY

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Objectives

The cellular prion protein (PrP^C) is a neuronal receptor for amyloid-beta (Abeta) oligomers, mediating neuronal impairment via the activation of Fyn kinase and phosphorylation of tau. The alpha-secretase, ADAM10 cleaves the amyloid precursor protein (APP), releasing sAPPalpha thereby precluding the formation of Abeta, also sheds PrP^C from the cell surface. We hypothesised that modulation of the shedding of PrP^C by ADAM10 would alter the binding and neurotoxicity of Abeta oligomers.

Methods

SH-SY5Y cells were treated with ADAM10 targeted siRNA, ADAM10 inhibitor or pharmacological agents (e.g. carbachol). ADAM10 activity was monitored by detection of sAPPalpha/ beta by immunoblotting or by using the MESO scale multiplex immunoassay. Immunofluorescence microscopy was used to monitor cell surface PrP^C and Abeta oligomer binding following treatments. Phosphorylation of Fyn kinase was monitored by immunoblotting,

Results

Inhibition of ADAM10 resulted in increased Abeta oligomer binding to cells as a direct result of increased cell surface PrP^C. Conversely, up-regulation of ADAM10 activity promoted shedding of PrP^C, and reduced binding of Abeta oligomers. The specificity of each activator for ADAM10 was tested by monitoring sAPPalpha production following inhibition of ADAM10. Carbachol and resveratrol differentially modulated ADAM10 activity, with resveratrol showing a greater specificity. Modulation of ADAM10 activity also altered the ability of the Abeta oligomers to activate Fyn kinase.

Conclusions

Developing specific agonists of ADAM10 will not only preclude Abeta formation and up-regulate neuroprotective sAPPalpha, it will also prevent the binding of Abeta oligomers to PrP^C and prevent the aberrant activation of signalling pathways which lead to neurotoxicity.

04h. Therapeutic Targets & Mechanisms for Treatment: secretases & other proteases

ADPD5-1082

MTDL BASED DERIVED COMPOUNDS AS NOVEL THERAPEUTIC AGENT, EFFECTIVE AGAINST AD PROMOTING ENZYMES IN APP OVEREXPRESSING CHO CELL LINES

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Background: AD is the resultant of multifactorial process that makes single-target strategy difficult to shed desirable therapeutic effect. The current study utilized Multi-target-directed ligands (MTDL) to generate novel therapeutic compound that targets multiple proteins involved in AD.

Objective: Until now, there is no effective therapeutic compounds, that target multiple factors, viz., Apo-E4, ACAT, BACE-1 and Acetylcholinesterase, that are possible promoters of AD pathophysiology and memory loss. The present study thus aim at screening novel Multi-target-directed compounds from medicinal plants that thought to inhibit AD promoting enzymes.

***Methodology*:** We applied computer assisted methodology unifying molecular docking studies to identify potent inhibitors against AD promoting enzymes from medicinal plants clinically proved to have memory enhancing properties. 616 compounds were retrieved from TCM database and docked into active site of AD promoting enzymes using Glide. The bio-potentiality of these best hit compounds were assessed in CHO-APP cell lines.

Results: Two best hits were arrived from Insilco experiments that had a great glide score and binding energy with more number of hydrogen bonding with active site amino acids of AD promoting enzymes. Interestingly, these best hit compounds could decrease the pathological expression of extracellular Aβ peptide, inhibiting BACE1 and simultaneous repression of AChE activity.

***Conclusion*:** Our findings strongly suggest that these two hit compounds had best inhibiting activity against BACE1 and Aβ generation. Further studies with other enzymes might prove the efficacy of these hit compounds as novel multiple target compound.

04h. Therapeutic Targets & Mechanisms for Treatment: secretases & other proteases

ADPD5-1179

SCREENING FOR COMPOUNDS THAT CAN DECREASE THE ACTIVITY OF BACE BY USING A NOVEL APP/BACE DROSOPHILA MODEL OF ALZHEIMER'S DISEASE

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Objectives: Production of Amyloid-β (Aβ) peptides caused by the proteolytic cleavage of the transmembrane amyloid β precursor protein (AβPP) through proteolysis by the β-secretase activity of the β-site AβPP-cleaving enzyme (BACE) and by the intramembraneous enzyme γ-secretase is a key molecular event in the pathogenesis of Alzheimer's disease (AD). Therefore, screening for compounds that can reduce the activity of these enzymes is an important approach to find possible treatments for AD. We are aiming to find molecules that can decrease/inhibit BACE activity.
Methods: To achieve this, we use *Drosophila melanogaster* (fruit flies) co-expressing human AβPP (hAβPP) and human BACE (hBACE). *Drosophila* has endogenous orthologs of both hBACE and human γ-secretase but only the ortholog of γ-secreatse is able to process hAβPP normally. Therefore, hAβPP needs to be co-expressed with hBACE in the flies to achieve a complete cleavage of hAβPP to generate Aβ peptides. The processing of hAβPP in this fly model have been characterized using biochemical and histochemical assays and the pathological effects have been examined by analysing the disruption of eye morphology and the longevity and locomotor behavior of the flies.
Results: Early evaluation of our AD fly model shows a strong toxic effect when co-expressing hAβPP and hBACE in the fly that can be used as a phenotypic marker for BACE activity.
Conclusion: We have succeeded to establish a novel fly model of AD that can be used to screen for BACE inhibitors in a search for lead compounds for drug development.

04h. Therapeutic Targets & Mechanisms for Treatment: secretases & other proteases

ADPD5-1456

LYSOSOMOTROPISM OF BASIC BACE1 INHIBITORS CONTRIBUTES TO INCREASED CELLULAR POTENCY AGAINST CATHEPSIN D AND OCULAR TOXICITY

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Objectives: The development of BACE1 inhibitors (BSIs) for the treatment of AD has been plagued by ocular toxicity in preclinical animal models. We hypothesized that ocular toxicity was caused by off-target inhibition of Cathepsin D (CatD), a closely related homolog of BACE1, and an enzyme highly enriched in lysosomes of the retinal pigment epithelium (RPE). However, many of our toxic compounds showed CatD selectivity in purified enzyme assays. Many drug candidates contain a basic functional group that results in lysosomotropism--the accumulation of drug in the acidic lysosomes. We thus hypothesized that our basic BSIs were lysosomotropic.
Methods: We generated a clickable photoprobe analog of a lead Pfizer BSI that exhibited ocular toxicity in rats and performed photoaffinity labeling studies in live cells.
Results: Firstly, radiolabeled BSIs were shown to accumulate in acidic compartments in RPE cells. Secondary, long-term administration of the basic BSIs potently increased cellular and tissue protein levels of CatD. This stabilization to proteolytic degradation has been shown for many Cathepsins following enzyme inhibition. Thirdly, our BACE1 photoprobe potently bound to CatD in RPE cells. Finally, we created the first cellular assay for CatD in live RPE cells using the photoprobe. Many BSIs showed >100-fold potency increases for CatD in live cells compared to purified enzyme – resulting in a significant lowering of the BACE1/CatD selectivity window.
Conclusions: Basic BSIs have reduced selectivity against cellular CatD compared to those predicted using purified enzyme assays and cellular CatD potency must be used to predict off target engagement *in vivo.*

04h. Therapeutic Targets & Mechanisms for Treatment: secretases & other proteases

ADPD5-1459

CHRONIC ADMINISTRATION OF A GAMMA SECRETASE MODULATOR HALTS AMYLOID PLAQUE ACCUMULATION IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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Objectives: Amyloid-β (Aβ) plaque deposition plays a central role in the pathogenesis of Alzheimer's disease (AD), therefore inhibiting the production of amyloidogenic Aβ peptides represents a potential therapeutic strategy. Unlike inhibitors, γ-secretase modulators (GSMs) shift the generation of longer, amyloidogenic Aβ42 and Aβ40 towards shorter, non-amyloidogenic forms. Present study evaluated plaque-lowering efficacy of a novel GSM compound in transgenic mice expressing human mutant amyloid precursor protein and presenilin-1.
Methods: In vivo 2-photon fluorescence microscopy was used to serially image amyloid plaques through a thinned-skull cranial window in the somatosensory cortex of PS1(G384A)/APP(K670N/M671L) transgenic mice during GSM compound (50 and 100 mg/kg) or vehicle treatment for 2 months starting at age 6-months. Individual plaque volume was quantified by a custom image analysis package. At the conclusion of the study, brain Aβ levels were determined by DELFIA.
Results: Parenchymal and vascular amyloid plaque load increased over 2 months in vehicle-treated PS1/APP mice by 1.8-fold and 3.4-fold, respectively, compared to baseline at age 6-months. Chronic treatment with the GSM compound resulted in near complete inhibition of both parenchymal and vascular plaque growths. Biochemical analysis of brain homogenates showed that GSM treatment resulted in significant reductions of SDS soluble Aβ42 and Aβ40 (58% and 47% of vehicle, respectively), and GuHCl solubilized Aβ42 and Aβ40 (71% and 58% of vehicle, respectively).
Conclusions: Present studies demonstrate that chronic administration of a GSM compound effectively halts parenchymal and vascular amyloid plaque accumulation. These results provide robust support for the utility of GSMs for disease-modifying treatment of AD.

04h. Therapeutic Targets & Mechanisms for Treatment: secretases & other proteases

ADPD5-1973

OVEREXPRESSION OF TRPC6 IMPROVED LEARNING AND MEMORY IN AD MOUSE MODEL

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Alzheimer's disease is characterized with progressive synaptic dysfunction and memory impairment. How to prevent synapse loss and memory decline remains challenge for the disease. Transient receptor potential (Trp) channels are a large family of nonselective cation channels, which have been reported to mediate extracellular calcium influx to induce cellular responses in a number of biological processes. Previous work in our lab found that TRPC6 promoted dendritic spine formation and enhanced spatial learning and memory of mice. In this study, we explored the possible roles of TRPC6 in AD. We crossed TRPC6 transgenic mice with APP/PS1 mice and found that overexpression of TRPC6 in neurons reduced spine loss and alleviated impairment of spatial working memory in the AD mouse model. Additionally, we found that the amyloid plaque load in APP/PS1/TRPC6 mouse brain was greatly reduced. Thus, overexpression of TRPC6 in AD mouse model could diminish the pathological deterioration and prevent the memory decline.

04h. Therapeutic Targets & Mechanisms for Treatment: secretases & other proteases

ADPD5-2084

KOREAN MEDICINAL PLANT EXTRACTS EXHIBIT POTENTIAL THERAPEUTIC VALUE AS ALPHA-SECRETASE ENHANCERS

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One crucial element in the pathogenesis of Alzheimer's disease is the proteolytic cleavage of the amyloid precursor protein (APP). While cleavage by the beta-secretase BACE-1 leads to generation of neurotoxic Abeta peptides and finally to disease-characteristic plaque formation (amyloidogenic processing), cleavage by the alpha-secretase ADAM10 in turn leads to generation of neurotrophic and neuroprotective sAPPalpha fragments.

Since current treatment options are only of symptomatic nature, efforts in developing novel drugs targeting disease mechanisms are urgently needed. Instead of targeting the amyloidogenic pathway which remains fruitless until today in human application, an approach focusing on potential alpha-secretase enhancers has emerged during the last years. Also, medicinal plant extracts used by indigene people have come into focus of current drug research for their abundance of potential novel lead substances.

Here we employed a promoter-based screening approach using 313 Korean medicinal plant extracts that yielded four potential candidates selectively enhancing ADAM10 promoter activity in a neuroblastoma cell line without affecting APP. *In vivo* studies with wild type mice proved the extracts were harmless and one of the four candidates was able to induce ADAM10 gene expression in peripheral tissue but not in the brain. *In vitro* blood-brain-barrier (BBB) studies delineated a potential hindrance of transport into the brain explaining lack of effect in the respective tissue. We were able to fractionate the most promising candidate extract to further decipher potential active compounds responsible for ADAM10 enhancement, which can be a future basis for modulating them and achieving penetrance into the brain.

04i. Therapeutic Targets & Mechanisms for Treatment: other enzymes

ADPD5-0427

DESIGN, SYNTHESIS AND EVALUATION OF SOME NEW SEMICARBAZONES OF P-AMINOBENZOIC ACID AS AN ANTIAMNESIC AND COGNITION ENHANCING AGENTS

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Objective: Discovery of new drug to treat Alzheimer's disease is challenging as characterised by selective loss of cholinergic neurons and accumulation of β-amyloid protein in selective brain areas.
Methods: Some new semicarbazones of p-aminobenzoic acid was synthesiezed, characterised and evaluated for antiamnesic, cognition enhancing and anticholinesterase activity through their respective in-vitro, in-vivo and computational models.
Results: The IC_{50} value and selectivity of synthesized compounds showed a comparable activity of **19** (0.046±0.016 μM and 11.96±0.64), (260) with reference standard donepezil (0.04±0.012 μM and 15.24 ± 0.86 μM), (381) for AChE and BChE inhibition respectively, whereas its enzyme kinetic study revealed a non-competitive inhibition for both AChE and butyrylcholinesterase (BChE) enzymes (Ki = 0.041± 0.60 and 8.46± 0.66 μM). Significant inhibitions in AChE activity by all the synthesized compounds were found in specific brain regions i.e. prefrontal cortex, hippocampus and hypothalamus. Docking studies predicted the binding modes of these compounds in AChE and BChE active sites and showed an affinity with the key peripheral anionic site residues Trp-286, Tyr-124 and Tyr-341 of AChE and Leu-286 and Val-288 of BChE, which were further processed for molecular dynamics simulation for calculating binding free energies using Molecular Mechanics-Generalized Born Surface Area (MM/GBSA).
Conclusion: The newly synthesized derivatives have illustrated an enhanced cognition effect

04i. Therapeutic Targets & Mechanisms for Treatment: other enzymes

ADPD5-1318

NOVEL STRATEGY FOR SYMPTOMATIC AND DISEASE-MODIFIYING TREATMENT OF ALZHEIMER´S DISEASE

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Objective: The goal of this study was the identificationof a novel mode of action to treat Alzheimer´s disease. We postulated that the simultaneous inhibition of specifc histone deacetylase (HDAC2 and/or HDAC6) and a non-epigenetic target, both involved in independent pathways related to AD pathology, could restore memory deficits in AD mouse models.
Methods: We use in vitro (neuronal cultures) and in vivo AD models (Tg2576 mice). The effects of treatments on AD-related cognitive decline are evaluated using the fear conditioning and Morris water maze tests. After behavioural testing, animal brains are used to analyze amyloid and tau pathology. Spine density in apical dendrites inpyramidal neurons is determined using Golgi-Cox method. Affymetrix microarrays technology is used to analyze gene expression analysis.
Results: We demonstrated that beneficial effects on reversing AD-phenotype, including memory deficits, synaptic loss and amyloid and tau pathology, are only achieved when both pathways are target simultaneously. A lead compound (CM-414) hitting both targets simultaneously was synthesized and tested in vivo, confirming the beneficial effects observedby using reference compounds. Microarray expression analysis identified differential genes involved in the beneficial effect observed in treated animals.
Conclusions: A novel dual approach, hitting two different pathways, involved in memory formation and other AD-related features, leads to a synergistic therapeutic effect in the Tg2576 mouse model.

04i. Therapeutic Targets & Mechanisms for Treatment: other enzymes

ADPD5-1334

WHOLE BRAIN CHOLINESTERASE INHIBITION - INVITRO AND EXVIVO ESTIMATION USING DESMODIUM GANGETICUM AND ITS CONSTITUENTS

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Objectives: Present study was conducted to explore the phytopharmaceuticals from *Desmodium gangeticum* as possible leads for Alzheimer's disease therapy.
Methods: Major phytoconstituents in *D.gangeticum* Petroleum ether extract (DG), Quercetin, Rutin, Gallicacid, Kaempferol and Stigmasterol were identified using LC-MS/MS. They were screened for *invitro* Acetylcholinesterase inhibition (AChEI) and Butyrylcholinesterase inhibition (BChEI) along with DG in male Sprague Dawley (SD) rat brain homogenate, IC_{50} values were calculated. Also *exvivo* enzyme inhibitory assays were performed in SD rats with 6 per group, received 10 mg/kg dose PO, after 1 h animals were sacrificed and brain homogenates were used for assays.
Results: Quercetin exhibited greater *invitro* AChEI and BChEI with IC_{50} 18.31±0.34 and 62.80±2.76 µM respectively. Rutin, Gallic acid, Kaempferol, Stigmasterol and DG showed AChEI and BChEI IC_{50} of 86.14±0.92 & 300.11±7.98 µM, 41.20±1.12 & 78.36±1.92 µM, 128.19±1.94 & 142.01±1.87 µM, 302.09±8.34 & 384.12±6.34 µM, 20.30±0.79 & 51.04±1.02 mg respectively. The percentage inhibition for above mentioned treatments in *exvivo* AChEI and BChEI was found to be respectively 28±0.34 & 11±0.12, 12±0.71 & 08±0.32, 06±0.57 & 01±0.39, 10±0.61 & 02±0.98, 18±0.76 & 16±0.98, 06±0.94 & 02±0.56. Quercetin exhibited significant *exvivo* inhibitory activity indicating higher affinity for enzyme and permeability into brain.
Conclusion: Phytoconstituents of *D.gangeticum* showed significant AChEI and BChEI in both assays, also have proven antioxidant and anti inflammatory activity which is vital in prevention of Alzheimer's disease. These can be forwarded as leads for screening in *invivo* Alzheimer's models and possible mechanism to be studied further

04i. Therapeutic Targets & Mechanisms for Treatment: other enzymes

ADPD5-1691
FUNCTIONAL INTERACTOMICS IN ALZHEIMER'S DISEASE
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Objectives: Proteins often use complex networks of interactions to produce a sophisticated signaling network. Deciphering the structure and dynamics of complex network of protein-protein interactions in AD is important for understanding many aspects of disease in depth. Our quest is for defining the functional interaction of AD brain proteins and the altered expression of interacting components in a complex using an ESI Q-TOF–MS/MS and Orbitrap approach.
Methods: The protein complexes were isolated from the autopsied human brain tissue cortical region (n=8 each) of Alzheimer's patients and Control subjects. Expression profiling was carried out on Blue Native (BN) PAGE in the first dimension followed by two-dimensional SDS-PAGE. Software analysis of differentially expressed proteins, Mass Spectrometric analysis and validation by western blotting were performed. The protein-protein interactions in a single complex were further confirmed by STRING and MINT databases.
Results: Thirteen protein complexes were isolated and separated on BN-PAGE gel. Each complex was further resolved into five to eight components on SDS-PAGE. A number of protein components in the second dimension were found to be differentially expressed between the AD and control brain. Mass spectrometric analysis in conjunction with protein-protein interaction database reveals significant interaction of proteins in a single complex that might be involved in the pathophysiology of the disease.
Conclusion: Current study expressed remarkable results that propose unique possibilities to investigate the interaction between protein in complexes in more detail with their modifications in AD for therapeutics and biomarker discovery.

04i. Therapeutic Targets & Mechanisms for Treatment: other enzymes

ADPD5-1759

[60]FULLERENE DERIVATIVE IS NEUROPROTECTIVE AGAINST AMYLOID-BETA PEPTIDE THROUGH MODULATION OF ADENOSINE AND METABOTROPIC GLUTAMATE RECEPTORS

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Amyloid-beta peptide (Aβ) is widely known as an important hallmark of Alzheimer's Disease (AD). It is aggregated into senile plaques which are responsible for neurodegeneration and cell death characteristic of AD. Although the mechanisms for its formation and toxicity are still unclear, Aβ is considered a potential target for therapeutic agents. [60]Fullerenes have been observed to be neuroprotective due to its antioxidant and radical scavenger properties. These effects have been associated to neurotransmitter receptor modulations. Some of these receptors, as adenosine and metabotropic glutamate receptors, have been shown to be affected in AD. mGlu receptors are significantly decreased in frontal cortex from AD brain in parallel to progression of disease. On the contrary, adenosine receptors are significantly increased in the same area even since early stages. The aim of the present work was to determine the possible neuroprotective effect of t3ss, a fullerene hydrosoluble derivative, and the role of adenosine and mGlu receptors in this neuroprotection. To this end, SH-SY5Y and SK-N-MC cells were treated with Aβ at different concentration and time exposure. Cell viability, radioligand and quantitative real time PCR assays were performed with treated and control cells. Results showed that Aβ caused a significant cell death affecting also adenosine and mGlu receptors expression. In addition, t3ss prevented the cell death elicited by Aβ and caused modulation of these receptors. These results suggest [60]fullerenes as neuroprotective nanoparticles against Aβ toxicity involving modulation of GPCRs and open new future perspectives to be considered in the treatment of AD.

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-0647

THE COMPARATIVE EFFICACY OF CHOLINESTERASE INHIBITORS IN PATIENTS WITH MILD TO MODERATE ALZHEIMER'S DISEASE: A BAYESIAN NETWORK META-ANALYSIS

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Objectives
To provide comparative evidences and hierarchies for efficacy between three cholinesterase inhibitors (ChEIs); donepezil, galantamine, and rivastigmine in patients with mild to moderate Alzheimer's disease (AD)

Methods
Article search was done using PubMed and The Specialized Register of the Cochrane Dementia and Cognitive Improvement Group. Previously published systematic reviews and meta-analyses were also manually reviewed. We included randomized, double-blind, placebo-controlled trials and head-to-head randomized trials evaluating the efficacy of ChEIs in AD patients. Comparisons evaluating high dose or low dose treatment were excluded. We examined Alzheimer's Disease Assessment Scale (ADAS-Cog), Neuropsychiatric Inventory (NPI), and Clinician's Interview-Based Impression of Change scale (CBIC+) as efficacy of treatment.

Results
We extracted data from 18 studies that provided sufficient data and met our inclusion criteria. Network meta-analysis was performed based on 7 arms of drug/dose treatment conditions, including donepezil 5mg & 10mg, galantamine 16mg & 24mg, rivastigmine 6-12mg, rivastigmine-patch 18mg, and placebo control. The analysis showed that all treatments were significantly more efficacious than placebo in cognitive improvement measured by ADAS-Cog. All treatments except rivastigmine-patch were significantly more efficacious than placebo in global assessment measured in CBIC+. Across all conditions, no significant efficacy was observed in behavioral improvement measured by NPI. Derived hierarchies in the efficacy of treatment conditions showed no consistency or clear-cut superiority in all 3 efficacy measures.

Conclusions
The Bayesian network meta-analysis incorporated direct and indirect comparisons. Our results suggested that the three drugs should have significant efficacies on cognition and global assessment, and these efficacies should be relatively similar.

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-1100

TRANSCRIPT PROFILING OF NEUROPEPTIDES IN ALZHEIMER'S DISEASE: FOCUS ON THE GALANIN SYSTEM

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Objective. *The neuropeptide galanin (GAL) and its G-protein-coupled receptors (GALR1-3) are widely distributed in the mammalian brain and modulate several neurotransmitter systems. Less is known about AD and galanin but analysis of postmortem AD brains shows a marked galaninergic hyperinnervation of the cholinergic forebrain neurons. Several studies show that GAL levels increase throughout the cortex in AD and that GALR binding sites are amplified in various brain regions during the course of the disease.* ***Method****. In this study we aim to quantitate the transcript levels of galanin and its receptors in brain regions showing AD pathology using qRT-PCR. Brain regions including hippocampal formation (HiFo) and entorhinal cortex (EC) were analyzed to elucidate differential expression patterns related to region and degree of degeneration. In a preliminary study post mortem brain tissue of 10 cases each with Braak 0-II, Braak III/IV and Braak V/VI have been analyzed.* ***Results.*** *We found that galanin and GalR1/2 mRNA are expressed in both the HiFo and adjacent EC of controls and a significant but transient increase was observed in the EC for GalR1 (Braak III/IV), perhaps suggesting an attempt to counteract degeneration. Transcript levels for galanin, GalR2 and GalR3 were significantly elevated in Braak V/VI samples of the HiFo, and GalR2 from the EC, suggesting involvement of galanin system in severe AD in this region.* ***Conclusion.*** *We hypothesize that there are significant changes in the galanin system in AD and that further exploration in selectively vulnerable brain regions may help explore therapeutic possibilities.*

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-1371

THE ROLE OF CHOLINESTERASES IN ALZHEIMER'S DISEASE: SCREENING OF TARGET COMPOUNDS

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Background: Alzheimer's disease (AD) is the most common form of dementia and causes a progressive and irreversible neurodegeneration. The loss of cholinergic neurons leads to the progressive reduction of acetylcholine (ACh) in the brain and resulting cognitive impairment in AD. ACh is hydrolyzed by both acetylcholinesterase (AChE) and butirylcholinesterase (BuChE). It was found that in the course of the disease, levels of AChE in the central nervous system (CNS) decrease, inversely to BuChE levels, so both enzymes represent legitimate therapeutic targets for ameliorating the cholinergic deficit characteristic of AD.

Objective: Screen a library of new isoquinoline, indolinone and benzoazepinone derivatives for their ability to inhibit AChE and BuChE activities, using galantamine and rivastigmine as standards.

Methods: The enzyme activities and inhibition studies were carried out using spectrophotometric techniques, based on the Ellman's method, with acetylthiocholine (ATCI) and butirylthiocholine (BTCI) as substrates, for AChE and BuChE, respectively. The data were complemented with modeling to analyze the structure-activity relationship.

Results: Our results show that the tested compounds are competitive inhibitors for AChEs and BuChEs, as the benchmarks galantamine and rivastigmine. The isoquinoline and indolinone derivative compounds showed strong anti-cholinesterases activities, with IC50 values ranging from 0.4 to 400 micromolar.

Conclusions: The results presented are promising and provide a pathway for the design of new AChE and BuChE inhibitors.

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-1931

NEUROPROTECTIVE EFFECT OF THYMOQUINONE AGAINST LIPOPOLYSACCHARIDE- INDUCED BEHAVIORAL AND NEUROTRANSMITTER ALTERATION IN AN ANIMAL MODEL

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Objectives: The aim of this study was to investigate the neuroprotective effect of Thymoquinone (TQ) against Lipopolysaccharide (LPS) induced cognitive impairment. Two positive controls were used Zileuton (Zil) and Deprenyl (Dep) as being anti-inflammatory and antioxidant compounds respectively.
Materials and Methods: 96 Sprague Dawley male albino mice (25-30 gm) were used. All the drugs were injected intraperitoneally once daily for 7 successive days after a single injection of 0.8mg/kg LPS. Animals were divided randomly into 8 groups: Negative controls (1%Saline, 1%DMSO and 1%Tween/ body weight), LPS (0.8mg/kg), Zil, Dep (10mg/kg), and TQ (2.5 and 5mg/kg). Animals were subjected to neurobehavioral tests including Y-maze test, Novel object recognition test and Open field test which assess spatial and recognition memories and locomotor activity respectively. Animals were sacrificed by cervical dislocation and the whole brains were isolated for the determination of Monoaminergic neurotransmitters Dopamine, Norepinephrine and Serotonin using High performance liquid chromatography with fluorescent detection.
Results: LPS altered animals' behavioral activity; it significantly reduced spatial and recognition memories and motor activity. Also, it significantly suppressed Dopamine, Norepinephrine and Serotonin levels. Both doses of TQ significantly improved spatial and recognition memories and motor activity as well as Dopamine, Norepinephrine and Serotonin levels. Moreover, TQ 5 mg/kg significantly improved animals' behavior and neurotransmitter levels not only when being compared to TQ 2.5 mg/kg, but also when being compared to the two positive controls.
Conclusion: TQ proved to be a promising neuroprotective agent in restoring memory and neurotransmitter levels owing to its antioxidant and anti-inflammatory properties.

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-2004

DETECTION, EARLY DIAGNOSIS AND TREATMENT OF ALZHEIMER'S DISEASE

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We have been observing 20 patients with Alzheimer's disease and 48 patients with vascular dementia of Alzheimer's type for 8 years. Setting the diagnosis of Alzheimer's disease, great attention was paid to the family anamnesis on the given disease, as well as to the arterial pressure, (hyper)lipemia, diabetes, overweight and hypodynamia

The main methods of the research were the tests, questionnaires, where the level of dementia has been revealed by the scale of Gamilton for revealing emotional and behavioral disorder, as well as objective research of neurological status, MRI of the brain were conducted.

In the process of the research it was revealed that 16 patients with Alzheimer's disease and 14 patients with vascular dementia were at the moderately severe stage and 4 patients had severe dementia.

With this aim the observation of the Alzheimer's disease has been conducted. For this purpose we prescribed the preparation Nitrendipine (Nitresan) in a dose of 10mg a day. For reducing the evidence of the main symptoms we have successfully used the preparations, which optimize synaptic transmission: inhibitor Donepezil (Alzepil) in a dose of 5- 10 mg. a day, as well as noncompetitive reversible antagonist – NCRA - glutamate receptor – memantin (carrier) 10-20 mg. a day.

As a result of detection, patients' diagnostics and treatment we were able to increase the quality of patients' life and prolong it; to reduce the cognitive deficiency and to reintegrate the patient to the family and society life in early stages.

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-2145

IMPACT OF DONEPEZIL AND MEMANTINE ON BEHAVIORAL AND PSYCHOLOGICAL SYMPTOMS IN PATIENTS WITH ALZHEIMER'S DISEASE

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Introduction: Behavioral and psychological symptoms of dementia (BPSD) are common in Alzheimer disease (AD). Donepezil and memantine are both used for the treatment of moderate AD. This study evaluated the efficacy of donepezil and memantine on BPSD in patients with moderate AD.
Methods: A prospective, longitudinal, randomized, 6-month clinical trial included 57 patients with moderate AD. The Neuropsychiatric Inventory (NPI) was performed to assess the prevalence and severity of BPSD at baseline and after 6-month treatment with memantine (n=29) and donepezil (n=28).
Results: .Baseline characteristics of participants including age, sex, mean length of education and disease duration were comparable. There was also no difference considering baseline Mini-Mental State Examination (MMSE) and Hachinski Ischemic Score (HIS) scores The NPI total scores improved from baseline to month 6 in both groups, with greater change in memantine group ($p<0.001$) comparing to donepezil group ($p=0.008$). Patients treated with memantine had significantly lower NPI final scores than patients treated with donepezil ($p=0.029$). Analyses of the NPI domains revealed that memantine treatment produced statistically significant improvement in agitation/aggression and anxiety NPI items ($p=0.01$; $p=0.03$ respectively).
Conclusion: The data suggest that specific drugs for AD, especially memantine, may be effective in treating BPSD in patients with moderate AD. The major benefits were observed on the symptoms of agitation/aggression and anxiety in memantine group

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-2199

SYNTHESIS OF A KEY INTERMEDIATE TACRINE-DONEPEZIL HYBRID AS A DUAL BINDING SITE ACHE INHIBITOR

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1. Objectives: Nowadays, novel AChE inhibitors are being designed to interact with both active and peripheral binding sites of AChE. It is known that the active site is responsible by acetylcholine hydrolysis and peripheral site is being related with the aggregation and deposition of the neurotoxic beta-amyloid peptide (Abeta), main cause of AD[1]. In this work, we reported the synthesis and biological evaluation of a key intermediate in the total synthesis of a new tacrine-donepezil hybrid, which was shown by preliminary studies of docking to be a potential AChE dual binding site inhibitor.

2. Methods: Synthesis of the key intermediate of tacrine-donepezil hybrid. Biological human AChE in modified Ellman's assay[2] and also Blood-brain barrier penetration using parallel artificial membrane permeability assay (PAMPA-BBB) [3].

3. Results: The key intermediate presented an IC_{50} 4.88±0.45 μM in Ellman's assay and also was able to cross the blood brain barrier.

4. Conclusions: The key intermediate of the total synthesis of tacrine-donepezil was performed and will be transformed into the designed hybrid in two steps. In addition, the biological assays will be carried out, including the Tioflavine T test to evaluate the capacity of the hybrid to avoid the beta amyloid aggregation induced by AChE peripheral site.

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04m. Therapeutic Targets & Mechanisms for Treatment: wnt signalling

ADPD5-0444

WNT SIGNALING PREVENTS MITOCHONDRIAL MEMBRANE PERMEABILITY INDUCED BY AMYLOID-BETA OLIGOMERS, THROUGH THE PERMEABILITY TRANSTION PORE INHIBITION IN HIPPOCAMPAL NEURONS.

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Objectives: The canonical *Wnt* signaling, activated through its Wnt3a ligand, has been described as a neuroprotective pathway against A-beta toxicity, however the mechanism has not been explored. Mitochondrial permeability transition pore opening (mPTP) occurs under apoptotic agent exposure, such as A-beta. The aim of this work is to determine whether the protective effect of Wnt signaling pathway is mediated by the inhibition of mPTP opening in hippocampal neurons exposed to A-beta oligomers (ABo).

Methods: Live cell imaging was performed in hippocampal neurons treated with *Wnt3a*. ABo toxicity was evaluated measuring mitochondrial calcium levels (Rhod2-AM), mitochondrial membrane potential with Mitotracker Orange and mPTP opening with calcein-Co^{2+} labeling. Also, we carried out electron microscopy to observe structural changes and integrity of the mitochondrial membranes.

Results: *Wnt3a* prevents a cascade of mitochondrial events that leads to neuronal death induce by Abo, such as: (a) mitochondrial calcium increase, (b) mitochondrial membrane potetial loss, (c) cytochrome-c release, (d) the mPTP opening and (e) mitochondrial swelling, to finally prevents the cell death. We propose that this mPTP inhibition could be mediated by interaction of GSK3-beta with some of the mPTP protein components.

Conclusions: The activation of the canonical *Wnt* signaling protects neurons against ABo damage. Under ABo exposure mitochondria undergoes several changes, such as, dissipation of the membrane potential, release of apoptotic factors and membranes permeabilization through mPTP opening. We observed that these events were prevented by Wnt3a ligand, protecting mitochondria against ABo and therefore preventing cell death.

Supported by Conicyt-CARE (PFB 12/2007) to NCI

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-0378

LONG-TERM TREATMENT WITH GINKGO BILOBA EXTRACT EGB 761 IMPROVES ALZHEIMER'S DISEASE-RELATED SYMPTOMS AND PATHOLOGY

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OBJECTIVES: Alzheimer's disease (AD) is a neurodegenerative disease characterized by extracellular deposits of amyloid beta peptide (Abeta) and microglia-dominated neuroinflammation. The therapeutic options for AD are still very few up to now. In this study, we aimed to investigate preventive or therapeutic effects and underlying molecular mechanisms of *Ginkgo biloba* extract EGb 761 in TgCRND8 AD mice. METHODS: We fed APP-transgenic mice with EGb 761 as a supplement in the diet for 2 or 5 months. Neuronal function, neuroinflammatory activation and Abeta pathology are investigated in this AD mouse model. RESULTS: We observed that the treatment of EGb 761 for 5 months but not for 2 months significantly improved cognitive function as demonstrated by the Barnes maze and attenuated the loss of synaptic structure proteins like PSD-95, Munc18-1 and SNAP25. Five- but not two-month EGb 761 treatment also inhibited microglial inflammatory activation in the brain. As a potential anti-inflammatory mechanism, EGb 761 could activate autophagy and thereby degrade NLRP3 inflammasome in microglia. Moreover, long-term EGb 761 treatments potentially reduced cerebral Abeta pathology through inhibiting BACE1-secretase activity and Abeta aggregation and even protect neurons via activating ERK1/2 and NF-kappaB signaling. CONCLUSIONS: Ginkgo extract EGb 761 as a nature-produced compound might efficiently prevent AD pathogenesis.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-0805

DASATINIB MODULATES M1/M2 POLARIZATION AND IMPROVES COGNITIVE DEFICITS IN A MOUSE MODEL OF NEUROINFLAMMATION

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Objectives: Alzheimer's disease (AD) is the most common age-related disorder which is characterized by cognitive deficit and neuroinflammation. Activated microglia produce a robust inflammatory response causing secondary neurodegeneration. Microglial activation has been classified into a classical proinflammatory M1 and an alternative neuroprotective M2 phenotype. LPS, a known proinflammatory stimulus, was shown to cause AD-like pathology. An *in vivo* LPS model was used in this study to investigate the effects of dasatinib, a tyrosine kinase inhibitor, on the cognitive functions and the microglial activation phenotypes.

Methods: Adult male Swiss albino mice were treated with intraperitoneal injection (i.p) of dasatinib (5 mg/kg) 1 hour prior to i.p administration of LPS (250 µg/kg). Four hours following LPS, the effects of dasatinib on spatial working and recognition memory were determined using Y-maze and novel object recognition tests respectively. The effect of dasatinib on mRNA levels of the M1 marker CD86 and the M2 marker arginase-1 was assessed in brain tissue using RT-qPCR.

Results: Behavioral tests showed that peripheral LPS challenge significantly impaired the cognitive functions, which was reversed by dasatinib pre-treatment. Moreover, LPS strongly upregulated both CD86 and arginase-1 gene expression with a higher induction of CD86. Dasatinib could significantly attenuate the LPS-mediated effect.

Conclusions: The present study shows for the first time that dasatinib modulates M1 and M2 microglial activation phenotypes in a mouse model of neuroinflammation. This correlates with a remarkable enhancement of the cognitive functions, suggesting a possible therapeutic role of dasatinib in controlling AD progression.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-1497

TUDCA TREATMENT AMELIORATES INFLAMMATION IN ALZHEIMER'S DISEASE (AD) MODELS

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Objectives:
Our aim was to evaluate the therapeutic effects of tauroursodeoxycholic acid (TUDCA) when administrated after the onset of amyloid pathology in APP/PS1 mice, focusing on neuroinflammation.

Methods:
We injected APP/PS1 mice and wild-type littermates 7 months old with 500 mg/kg body weight TUDCA intraperitoneally every 3 days for 3 months. One hemisphere of the brain was processed for immunohistochemistry (IHC) and immunostained for beta-amyloid (Abeta) and astrocytes or microglia markers. The other hemisphere was processed for RNA and protein extraction, and analyzed by qRT-PCR and Western blot (WB). To evaluate whether TUDCA specifically inhibits microglial pro-inflammatory response, we used microglia cell lines exposed to lipopolysaccharide (LPS) and Abeta species, followed by qRT-PCR and WB analysis.

Results:
TUDCA-treated transgenic mice brains presented reduced amyloidogenic proteolytic fragments of amyloid precursor protein (APP), along with decreased amyloid plaque burden and Abeta levels. Notably, gliosis was significantly decreased by TUDCA, as evaluated by IHC and WB, with a concomitant decrease in the expression of TNFalfa. Importantly, increased GSK3beta activity, an important event in glial activation, was strongly associated with Abeta accumulation and markedly abrogated by TUDCA. Finally, TUDCA-treated microglial lines exposed to LPS or Abeta presented reduced NFkappaB and GSK3beta activation.

Conclusion:
Overall our results suggest that TUDCA modulates microglial anti-inflammatory mechanisms in a pleiotropic manner, highlighting TUDCA as a potential therapeutic option for the treatment of neuroinflammation-related pathologies, including AD.
(Supported by PTDC/SAU-NMC/117877/2010 and PTDC/BIM-MED/0251/2012, and fellowship SFRH/BPD/47376/2008, from FCT, Portugal)

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-1932

GLYCYRRHIZIN ATTENUATES OXIDATIVE STRESS AND NEUROINFLAMMATION: NOVEL APPROACH TARGETING ALZHEIMER'S DISEASE

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Objectives: Alzheimer's disease (AD) pathogenesis is still intangible but substantial facts demonstrate that oxidative stress and neuro-inflammation are the most prominent features of AD. The aim of this study was to explore the effect of Glycyrrhizin (Gly) on oxidative damage and neuro-inflammation induced by Lipopolysaccharide (LPS). Zileuton (Zil) a 5-lipoxygenase (5-LOX) enzyme inhibitor and Deprenyl (Dep) a potent antioxidant drug were used as positive controls.
Methods: 96 Sprague Dawely male albino mice (25-30 gm.) were randomly divided into 8 groups: Negative controls: Saline and DMSO (1%/ body weight), LPS (0.8 mg/kg), Zil and Dep (10mg/kg), and Gly (5, 10 and 20 mg/kg). Drugs were administered once per day via intraperitoneal route for 7 successive days after single injection of LPS. Lipid peroxidation end product thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) levels and superoxide dismutase (SOD) activity were determined biochemically. 5-LOX level was determined by ELISA technique. Amyloid beta (Aβ) deposition in the dentate gyrus of the hippocampus and the cerebral cortex were determined by immunohistochemistry.
Results: LPS significantly increased TBARS & 5-LOX levels, and suppressed GSH levels and SOD activity. Also it stimulated the accumulation of Aβ in the two different brain areas. Gly elevated significantly the levels of TBARS & 5-LOX and increased GSH levels and SOD activity. Moreover, Gly prevented the accumulation of Aβ.
Conclusion: Gly might be an auspicious drug for the control of memory insult and neuronal aging due to its anti-inflammatory & antioxidant properties.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-1979

NEUROPROTECTIVE EFFECTS OF AMINOPROCALCITONIN INHIBITION IN EXPERIMENTAL MODELS OF ALZHEIMER'S DISEASE

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Background: Neuroinflammation, a prominent feature in neurodegenerative disorders, has been associated with Alzheimer's disease (AD). Growing evidence suggests that inflammatory responses cause brain atrophy and play a prominent and early role in the progression of AD. Recent findings show that aminoprocalcitonin (NPCT), a neuroendocrine peptide produced in the periphery and in the brain, plays a critical role in the development of systemic inflammatory response, sepsis and multiple organ dysfunction syndrome. However, the presence, possible function, regulation and mechanisms by which NPCT could be involved in AD neuropathology remain unknown.
Objective: The aim of this study was to explore the expression of NPCT and its interaction with amyloid-b (Aβ), pro-inflammatory, and neurogenic effects.
Method: We used *in vitro* (neuronal cultures) and *in vivo* (brain autopsy samples from AD patients, and APP/PS1 transgenic mice) approaches to explore our objectives.
Results: We found Ab-induced NPCT up-regulation in cortical and hippocampal brain areas of APP/PS1 mice and AD patients. Additionally, Ab-induced cytotoxicity was blocked by anti-NPCT treatment, accompanied by reduced levels of pro-inflammatory cytokines. Remarkably, anti-NPTC therapy resulted in a significant improvement in behavioral status of APP/PS1 mice.
Conclusions: Our results indicate a central role of NPCT in the pathogenesis of AD and suggest this protein as a potential diagnostic and therapeutic target for this disease.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-2169

GAMMA-LINOLENIC ACID ACTS ON AB25-35-INDUCED INFLAMMATORY RESPONSES MODULATING NF-KAPPA B SIGNALING PATHWAY IN PC12 CELLS

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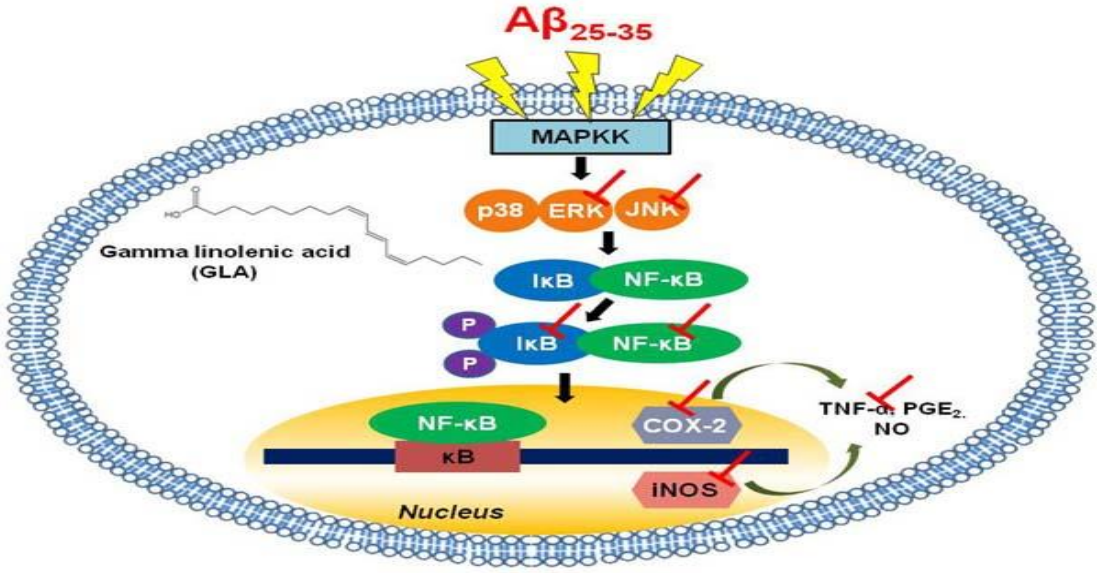
Objectives: Alzheimer's disease (AD) is a neurodegenerative disorder initiated by the aggregation of amyloid beta (Aβ) peptide. The activation of the nuclear factor kappaB (NF-κB) is one of the signaling pathways through which Aβ exerts its neurotoxicity. The present study investigated the effect of gamma-linolenic acid (GLA) on $A\beta_{25-35}$-induced inflammatory damage and their underlying molecular mechanism of neuroprotective action

Methods: The cells were treated with GLA at different doses for 1 h and then added by $A\beta_{25-35}$ for another 24 h. Subsequently, the expressions of the TNF-α, iNOS, COX-2, NF-κB, IκB, p38, ERK and JNK were determined by Western blotting and the NO and PGE_2 were examined by ELISA.

Results: GLA attenuated levels of NO, PGE_2 and TNF-α, and induced down regulation of COX-2 and iNOS protein expression. GLA suppressed NF-κB p65 and IκB, and reduced the activation of JNK and ERK MAPK, whereas no changes in the activation of p38 could be observed.

Conclusion: This is the first report proving that GLA attenuates $A\beta_{25-35}$-stimulated neuroinflammation *via* inhibition of the NF-κB signaling pathway.



04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0228

EDARAVONE AMELIORATES STREPTOZOTOCIN (STZ) - INDUCED COGNITIVE IMPAIRMENT IN SPORADIC MODEL OF ALZHEIMER'S DISEASE IN RATS

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Objectives:

This study evaluates the effect of edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a neuroprotective, in a sporadic model of Alzheimer's disease in rats.

Methods:

Male Wistar rats were divided into four groups: (i) Control wherein no treatment was given nor any procedure was done; (ii) Sham animals were injected artificial CSF intracerebroventricularly (i.c.v.); (iii) Streptozotocin (STZ) group wherein STZ (3 mg/kg, dissolved in artificial CSF) was injected i.c.v. (0.8 posterior, 1.5 mm lateral from bregma, and 3.6 mm ventral to dura) and (iv) STZ plus edaravone wherein edaravone (3 mg/kg, oral) was administered for 28 days after STZ. Cognitive impairment was evaluated using Morris water maze (MWM), elevated plus maze (EPM) and passive avoidance tests (PAT) on days 0, 14 and 28 after STZ injection. Rat brain malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), superoxide dismutase (SOD), acetylcholinesterase (AChE) and butylcholinesterase (BuChE) were estimated at the end of 28 days.

Results:

STZ caused significant impairment of learning and memory as assessed by MWM, EPM, and PAT compared to control. Edaravone ameliorated the cognitive deficits induced by STZ. GSH level and SOD activity decreased while levels of MDA and NO increased significantly in STZ rats. Edaravone restored their levels towards normal. Edaravone attenuated the significantly higher AChE and BuChE levels in STZ group. There were no differences between control and sham groups in any of the studied parameters.

Conclusion:

Edaravone protects against STZ-induced cognitive impairment in sporadic model of Alzheimer's disease in rats probably by modulating cholinergic status and reducing the oxidative stress.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0679

THYMOL FROM ZATARIA MULTIFLORA BOISS. PREVENTS NEUROTOXICITY INDUCED BY AMYLOID BETA IN PC12 CELLS

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Objective: Essential oil of Zataria multiflora Boiss. (ZM), a member of Lamiaceae family, has been shown ability to reduce symptoms of Alzheimer's disease (AD) and affect the disease mechanism in animal and cellular models of AD. In this study, the effect of thymol as a major constituent of ZM essential oil on amyloid β (Aβ)-induced toxicity in cultured rat pheochromocytoma (PC12) cells was investigated.
Methods: PC12 cells were incubated with $A\beta_{25-35}$ (50 µM) in the presence or absence of thymol (10, 20, 50 µM). The activity of cells was measured by methyl thiazolyl tetrazolium (MTT) method. Flurospectrophotometer was employed to observe intracellular reactive oxygen species (ROS) production.
Results: The results showed that after incubation with $A\beta_{25-35}$, the cell viability was decreased; in contrast, the level of ROS was increased in PC12 cells. Co-incubation of thymol prevented the cytotoxicity induced by $A\beta_{25-35}$ in cells and attenuated intracellular ROS.
Conclusions: In conclusion, thymol significantly protects the PC12 cell from Aβ-induced injury through the mitochondria dysfunction and oxidative damage. It seems that antioxidant activity of thymol might contribute to its beneficial effects in this model. Our findings suggest that thymol may be a potentially valuable source of natural therapeutic agents for the treatment of AD. However, further investigations are necessary to establish its efficacy and potential toxicity in clinical trials.
Key Words: Alzheimer's Disease; Amyloid β; Thymol; Antioxidant Activity.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0680

CARVACROL FROM ZATARIA MULTIFLORA BOISS. PREVENTS NEUROTOXICITY INDUCED BY AMYLOID BETA IN PC12 CELLS

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Objective: Essential oil of Zataria multiflora Boiss. (ZM), a member of Lamiaceae family, has been shown ability to reduce symptoms of Alzheimer's disease (AD) and affect the disease mechanism in animal and cellular models of AD. In this study, the effect of carvacrol as a constituent of ZM essential oil on amyloid β (Aβ)-induced toxicity in cultured rat pheochromocytoma (PC12) cells was investigated.
Methods: PC12 cells were incubated with $A\beta_{25-35}$ (50 μM) in the presence or absence of carvacrol (10, 20, 50 μM). The activity of cells was measured by methyl thiazolyl tetrazolium (MTT) method. Flurospectrophotometer was employed to observe intracellular reactive oxygen species (ROS) production.
Results: The results showed that after incubation with $A\beta_{25-35}$, the cell viability was decreased; in contrast, the level of ROS was increased in PC12 cells. Co-incubation of carvacrol prevented the cytotoxicity induced by $A\beta_{25-35}$ in cells and attenuated intracellular ROS.
Conclusions: In conclusion, carvacrol significantly protects the PC12 cell from Aβ-induced injury through the mitochondria dysfunction and oxidative damage. It seems that antioxidant activity of carvacrol might contribute to its beneficial effects in this model. Our findings suggest that carvacrol may be a potentially valuable source of natural therapeutic agents for the treatment of AD. However, further investigations are necessary to establish its efficacy and potential toxicity in clinical trials.
Key Words: Alzheimer's Disease; Amyloid β; Carvacrol; Antioxidant Activity.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0773

TOTAL OLIGOMERIC FLAVONOID FRACTION OF CYPERUS ROTUNDUS AMELIORATES AMYLOID BETA 25-35 INDUCED ALZHEIMER'S DISEASE PATHOGENESIS IN SH-SY5Y HUMAN NEURONAL CELLS

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Abstract

Objectives

The objective of the study is to determine the protective effect of TOF fraction of *Cyperus rotundus* against amyloid peptide induced neurotoxicity in SH-SY5Y human neurons.

Methods

The amyloid beta 25-35 induced toxicity in SH-SY5Y cells and the protective activity of TOF was evaluated by MTT assay and LDH assays. The ROS and MMP were evaluated by spectrofluorometer assays. The antioxidant enzymes levels and lipid peroxidation was evaluated by spectrophotometer method. Further, the gene expression of apoptotic marker proteins such as Bcl-2 and Bax and neuronal markers such as BDNF and TH were evaluated by qRT-PCR analysis.

Results

The pretreatment of SH-SY5Y cells with total oligomeric flavonoid fraction (TOF) of CR effectively inhibited cell damage. TOF effectively inhibited amyloid beta 1-35 induced LDH leakage and neuronal DNA damage. TOF also regulated antioxidant activity and restored the antioxidant enzymes levels such as such as SOD, CAT, GPx, GR and glutathione and inhibited ROS generation. The TOF pretreatment also restored amyloid beta 1-35, induced cellular and mitochondrial damage and also restored the expression of BDNF.

Conclusion

TOF pre-treatment effectively inhibited amyloid beta 25-35 induced neurotoxicity in SH-SY5Y cells. Taken together, our findings suggest that TOF might be developed as an agent for neurodegeneration prevention or therapy.

Keywords: Alzheimer's disease, Amyloid beta peptide, *Cyperus rotundus*, neuroprotection, SH-SY5Y neurons, total oligomeric flavonoid fraction.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-1088

PLASMA VITAMIN C LEVELS ARE ASSOCIATED WITH INCREASED RISK OF GROWING INTIMA-MEDIA THICKNESS IN AGED SUBJECTS

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Objective: To explore the relationship between plasma levels and activities of the major antioxidant molecules and the carotid intima-media thickness (IMT) in a consecutive sample of healthy older subjects.
Methods: 192 subjects aged 65 years and older consecutively presenting to a Geriatrics Department for routinary check-up were admitted in this study. All patients underwent high-resolution B-mode ultrasonography for IMT measurement of the carotid artery. Plasma levels of vitamins A, C, E, of uric acid as well as activities of the plasma antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured in all patients by HPLC with electrochemical detection (vitamin C and uric acid), HPLC with UV detection (vitamins A and E) and by spectrophotometry (SOD and GPx activity).
Results: Plasma levels of only vitamins C and E significantly decreased among participants from the first to the fourth IMT quartile, with a linear slope only for vitamin C. Results were independent of age, gender, smoking habit, hypertension, CVD, stroke, diabetes, antiplatelet treatment and uric acid levels. Compared to participants in the lowest vitamin C quartile, the probability to have IMT>1.2 mm significantly decreased among persons from the second to the fourth quartile independent of confounders. This could not be shown for the other compounds measured.
Conclusions: Vitamin C plasma levels but not of other antioxidant micronutrients and enzymes appear to be selectively associated with the risk of growing IMT. An adequate vitamin C status might be particulalrly important for protection against the clinical manifestations of vascular and cognitive ageing.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-1635

NEUROPROTECTIVE EFFECTS OF DIARYLPROPIONITRILE AGAINST BETA-AMYLOID PEPTIDE-INDUCED NEUROTOXICITY IN RAT CULTURED CORTICAL NEURONS

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Alzheimer's disease is a major cause of dementia in the elderly that involves a β-amyloid peptide (Aβ)-induced cascade of an increase in oxidative damage and inflammation. The present study demonstrated the neuroprotective effects of diarylpropionitrile (DPN), a non-steroidal estrogen receptor β selective ligand, against 10 μM $A\beta_{1-42}$-induced oxidative stress and inflammation in primary rat cortical cell culture. Pre-treatment with 1-100 nM DPN significantly decreased neuronal cell death by increasing cell viability through a significant attenuation in the reactive oxygen species level, downregulation of pro-apoptotic activated caspase-3 and Bax, and upregulation of anti-apoptotic Bcl-2, thereby mitigating apoptotic morphological alterations. DPN pre-treatment decreased the expression levels of pro-inflammatory cytokines IL-1β and IL-6 through attenuation of $A\beta_{1-42}$-induced phosphorylation of mitogen-activated protein kinases JNK and p38. In addition, DPN enhanced ERK1/2 and Akt phosphorylation depressed by $A\beta_{1-42}$. These findings suggest that DPN protects neurons from $A\beta_{1-42}$-induced neurotoxicity through a variety of mechanisms, ranging from anti-oxidation, anti-apoptosis, through to anti-inflammation.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-1661

CURCUMIN/MELATONIN HYBRID AS NEUROPROTECTANT FOR AD

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In our effort to develop effective neuroprotectants as potential treatments for Alzheimer's disease (AD), hybrid compounds of curcumin and melatonin, two natural products that have been extensively studied in various AD models, were developed. A lead hybrid compound was discovered to show significant neuroprotection with nM potency (EC_{50} = 27.60 ± 9.4 nM) in MC65 cells, a cellular AD model. Mechanistic studies demonstrated that this lead compound might interfere the interactions of AβOs within the mitochondria. Furthermore, this lead compound was confirmed to cross the blood-brain barrier (BBB) and deliver a sufficient amount to brain tissue after oral administration. In vivo studies in APP/PS1 mice after three months treatment demonstrated that this lead compound reduced Aβ plaques by 20%. it also reduced the oxidative stress as demonstrated by the reduction of 8OHG and HNE staining. More importantly this lead compound enhanced the mitochondria complex I activities, which is consistent with our in vitro mechanistic results. Collectively, these results strongly support the hybridization approach as an efficient strategy to help identify novel scaffolds with a desired pharmacology, and strongly encourage further optimization of this lead compound to develop more potent neuroprotectants for AD.

04p. Therapeutic Targets & Mechanisms for Treatment: neurotrophic factors

ADPD5-0359

NANOWIRED DELIVERY OF CEREBROLYSIN REDUCES ALZHEIMER'S DISEASE PATHOLOGY FOLLOWING AMYLOID BETA INFUSION AGGRAVATED BY CONCUSSIVE HEAD INJURY

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Concussive head injury (CHI) is quite frequent in Military personnel during combat operation. CHI induced breakdown of the blood-brain barrier (BBB) causing brains to be more vulnerable to peripheral toxins and other adverse reactions. Thus, this is likely that CHI may induce brain damage leading to enhanced reaction with additional amyloid beta peptide (AbP) exposure causing adverse Alzheimer's Disease (AD) pathology. This hypothesis was tested in this investigation.

AD like pathology was induced by AbP infusion (50 ng/10 µl, i.c.v. daily for 4 weeks) in control and CHI rats. CHI was inflicted by an impact injury (0.224 N) on the right skull without fracture to simulate clinical conditions.

We found that ABP infusion in CHI cases results in 8 to 12 fold higher deposition of ABP in various brain areas and accumulation of tau proteins. A significantly higher increase in CSF tau proteins was also seen in CHI group than normal rats induced with identical AbP. In these groups of animals BBB and blood-CSF barrier (BCSFB) was also broken down for Evans Blue albumin. Brain edema, neuronal injuries, activation of astrocytes closely corresponds to the tau protein concentrations in the brain. Interestingly TiO2 nanowired delivery of cerebrolysin, a balanced composition of neurotrophic factors and peptide fragment (2.5 ml/kg, i.v. daily for 3 weeks after 1 week of AbP infusion), resulted in marked neuroprotection in CHI induced exacerbation of AD pathology. This indicates that CHI aggravates AD pathology and nanowired cerebrolysin has a therapeutic value, not reported earlier.

04p. Therapeutic Targets & Mechanisms for Treatment: neurotrophic factors

ADPD5-0442

FUNCTIONAL MAPPING OF THE P75 NEUROTROPHIN RECEPTOR IN A MOUSE MODEL OF AD

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P75 neurotrophin receptor (p75) may induce opposing effects depending on co-receptor partners and associated ligands. The p75 is a well known mediator of β-amyloid (Aβ) neurotoxicity and neuronal apoptosis induced proNGF, i.e. two agents implicated in Alzheimer's disease (AD). Elucidation of the mechanisms that modulate p75-mediated signaling in AD can be useful tool for target therapy.
To create a functional map p75 extracellular domain to normal state and AD, we blockaded the different parts of p75 by binding with antibodies, and investigated the effects of immunization on spatial memory and morphology of neurons in a chronic AD mouse model – bulbectomized (OBE) mice and sham-operated (SO) animals. The mice were immunized with synthesized eight potentially immunogenic fragments of the extracellular domain of p75.
We showed that immunization with two fragments (III and IV) was effective in preventing impairment of spatial memory in OBE mice. Aβ reduction in the brains of these animals until to a level in SO mice was found after immunization by peptide III. . Peptide IV was effective protector against destruction of neurons in the cortex and hippocampus , but was not much visible impact on level of Aβ. Other fragments did not restore the spatial memory in OBE mice. In contrast to OBE mice the immunization with fragments I- IV reduced the neuronal density and impaired spatial memory in SO mice.
Our results provide new insights into the functions of different parts of this enigmatic receptor in normal and pathological state.
Supported by RFBR Grant No. 13-04-40106-H.

04r. Therapeutic Targets & Mechanisms for Treatment: protein aggregation

ADPD5-0839

APOJ/CLUSTERIN PEPTIDE AS A NOVEL THERAPEUTIC AGENT FOR ALZHEIMER'S DISEASE

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Objectives: Apolipoproteins are essential structural components of lipoproteins and mediate a variety of biological functions. ApoJ (a.k.a. clusterin) is an apolipoprotein associated with high density lipoproteins (HDL) in the plasma and brain. Recent genetic studies identified the gene of apoJ (CLU) as one of the top-ranking loci associated with late-onset Alzheimer's disease (AD) after apoE4, a primary genetic risk factor for AD. ApoJ is a multifunctional protein; it binds amyloid-beta protein (abeta), inhibits abeta aggregation, promotes abeta clearance across the blood-brain barrier (BBB), and modulates inflammatory and immune functions in the brain. A 10-amino acid peptide derived from an integral sequence of apoJ, apoJ[113-122], mimics the properties of apoJ. It reduces atherosclerosis in apoE-null mice and improves anti-inflammatory properties of plasma HDL in monkeys. The present study was designed to investigate the therapeutic potential of apoJ[113-122] for AD.
Methods and Results: ApoJ[113-122] was used for several studies in vitro. Our results show that apoJ[113-122] inhibits the aggregation of abeta, protects neuronal cells from abeta-induced toxicity, and reverses abeta-induced disruption of microtubule dynamics in neuronal cells. In addition, apoJ[113-122] treatment enhances synaptic plasticity in hippocampal slices from wild-type mice and promotes anti-inflammatory activity of HDL in the plasma of APP/PS1 mice, a model of AD. Moreover, apoJ[113-122] effectively crosses the BBB in an in vitro model.
Conclusions: Our findings render apoJ[113-122] a promising therapeutic agent for AD. Animal experiments are underway to test its ability to mitigate cognitive impairment and neuropathology in the APP/PS1 mouse model of AD.

04r. Therapeutic Targets & Mechanisms for Treatment: protein aggregation

ADPD5-2247

DESIGN AND OPTIMIZATION OF SMALL MOLECULE MISFOLDING INHIBITORS TARGETING BOTH BETA-AMYLOID AND TAU.

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Objectives: Aberrant protein aggregation of both beta-amyloid *and* tau is often implicated in the development of AD. The clinical presentation of AD is heterogeneous (e.g. extrapyramidal symptoms in some people with AD and dementia symptoms in some people with PD). The clinical overlap amongst neurodegenerative disorders implies that AD is more of a syndrome than a disease and that the suppression of other aberrantly misfolded protein may afford additional therapeutic benefits. Accordingly, we are seeking to design and develop brain-penetrable small molecule new chemical entities as anti-protein misfolding agents targeting both beta-amyloid and tau. Based upon extensive in silico modelling a family of novel compounds has been identified. A representative compound is TRV 101.
Methods: Efficacy of TRV 101 was measured in a variety of *in vitro* assays including biotin- beta-amyloid and biotin- tau oligomerization assays. TRV 101 binding to beta-amyloid and tau 4NR2 was measured by Surface Plasmon Resonance (SPR). Additionally, in vitro ADMET data along with mouse pharmacokinetics/ bioavailability (plasma and brain) was collected.
Results: TRV 101 showed anti-oligomerization activity against both beta-amyloid and tau. TRV has optimum drug like properties, demonstrating favourable in vitro ADMET, high brain penetrance and oral bioavailability, and benign in a 44-receptor panel test.
Conclusions: We have developed a new class of compounds capable of inhibiting oligomerization of both beta-amyloid and tau proteins. Our small molecules have appropriate pharmacokinetic profiles and able to reach the target tissue. Our compounds are currently being tested in animal models of both beta-amyloid and tau pathologies.

04s. Therapeutic Targets & Mechanisms for Treatment: misfolding and chaperones

ADPD5-1224

INHIBITION OF ABETA AGGREGATION AND TOXICITY BY BRICHOS IN A DROSOPHILA MODEL

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Objective

The BRICHOS domain is present in several protein families associated with respiratory distress, amyloid disease, dementia and cancer. It has been suggested that BRICHOS domains bind to aggregation-prone regions in their respective precursor protein, and thereby protect them from misfolding. The BRICHOS domains of Bri2 (associated with dementia) and proSP-C interact with other amyloid forming peptides like Aβ (associated with AD). Both proSP-C and Bri2 BRICHOS delay the amyloid fibril formation of Aβ40 and Aβ42 *in vitro*, even at sub-stoichiometrical amounts of BRICHOS.

Methods

To further examine the effect of BRICHOS on Aβ *in vivo*, we have developed two *Drosophila* models, one expressing proSP-C BRICHOS and the other expressing Bri2 BRICHOS. By crossing the BRICHOS flies with Aβ42 transgenic flies, and driver flies for expression in the neurons or in the fly eye, we can analyze the toxic effect of Aβ42 with or without BRICHOS co-expression.

Results

Neuronal expression of Aβ42 alone in flies results in peptide aggregates detectable with confocal microscopy, reduced life-span and decreased locomotor activity, while expression of BRICHOS alone causes no detectable effects. Co-expression of both proSP-C and Bri2 BRICHOS with Aβ42 increases life-span and improves locomotor activity. Expression of Aβ42 in the eyes leads to degeneration of the ommatidia, and co-expression of BRICHOS efficiently decreases the toxicity seen in the eyes by Aβ42.

Conclusions

This strongly suggests that the proSP-C and Bri2 BRICHOS domains, decrease Aβ42 induced toxicity *in vivo,* which holds promises for targeting this chaperone as a treatment for AD.

04t. Therapeutic Targets & Mechanisms for Treatment: gene therapy

ADPD5-0884

CRTC1 OVEREXPRESSION RESCUES BETA-AMYLOID-INDUCED HIPPOCAMPAL-DEPENDENT MEMORY DEFICITS IN MICE

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Objectives: Cognitive decline is associated with gene expression changes in the brain, but the specific gene programs and transcriptional mechanisms underlying memory impairments in cognitive disorders are largely unknown. We aim to elucidate relevant transcriptional mechanisms responsible for early synaptic dysfunction and memory loss in Alzheimer's disease (AD).

Methods: To elucidate transcriptional mechanisms relevant for memory loss in AD we performed extensive pathological, behavioral, transcriptome and biochemical analyses in control and mutant β-amyloid precursor protein ($APP_{Sw,Ind}$) transgenic mice during aging.

Results: Genome-wide transcriptome analysis revealed deregulation of a gene network related with neurotransmission, synaptic plasticity, and learning/memory in the hippocampus of $APP_{Sw,Ind}$ mice after spatial memory. $APP_{Sw,Ind}$ mice show changes on a transcriptional program dependent on the CREB-regulated transcription coactivator-1 (CRTC1). Interestingly, synaptic activity and spatial memory induces CRTC1 dephosphorylation and nuclear translocation leading to CRTC1-dependent transcription in the hippocampus, and these events are impaired in $APP_{Sw,Ind}$ mice at early pathological and cognitive decline stages. Adeno-associated viral-mediated CRTC1 expression in the hippocampus of $APP_{Sw,Ind}$ mice efficiently reversed Aβ-induced spatial learning and memory deficits by restoring a specific subset of CREB/CRTC1 target genes. We are currently applying similar gene therapy approaches targeting CRTC1 in other faithful mouse models of brain diseases.

Conclusions: Our results reveal a critical role for CRTC1-dependent transcription on memory processing in normal and pathological conditions and provide evidence that targeting CRTC1 can reverse memory loss in AD.

This study was supported by SAF2013-43900, CIBERNED CB06/05/0042, BrightFocus Foundation (A2014417S) and Generalitat de Catalunya SGR0984 grants.

04t. Therapeutic Targets & Mechanisms for Treatment: gene therapy

ADPD5-1839
TARGETING GANGLIOSIDES FOR NEUROPROTECTION, COGNITIVE ENHANCMENT, AND REDUCTION OF AMYLOID AND ASSOCIATED NEUROPATHOLOGY

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1. Objectives. Given evidence showing a critical role for gangliosides in Abeta aggregation and apoptosis, we sought to understand their role *in vivo*. We summarize here five translational studies and present new data using treatments chosen to decrease levels of the neurotoxic GD3 ganglioside, increase the neuroprotective GM1 ganglioside, and reduce gangliosides that serve as high-affinity substrates for Aβ aggregation (GD1b & GT1b).
2. Methods. We first cross-bred GD3 synthase (GD3S) knockouts with APP/PSEN1 transgenics and assessed cognition and neuropathology. We then examined shRNA-mediated knock-down of GD3S in 5xFAD transgenics. We then infused sialidase (VCS) from *v. cholerae* intraventricularly in aged 5xFAD transgenics over an 8-week period. We followed this by creating a recombinant adeno-associated viral (rAAV) vector encoding the ganglioside-specific Neu3 sialidase, injected intrahippocampally in 4-month-old 5xFAD transgenics. Finally, we administered the milk protein glycomacropeptide (GMP) through the diet for 7 months to 5xFAD transgenics.
3. Results. Knockout and knock-down of GD3S both improved memory and reduced Aβ aggregation, and rAAV.GD3S.shRNA prevented neuronal loss in the 5xFAD transgenics. VCS did not significantly improve memory in aged transgenics, but reduced neuronal loss and Aβ on the ipsilateral (infused) side. VCS also significantly reduced Aβ on the contralateral side, but to a lesser extent. Assessment of the effects of rAAV.Neu3 are ongoing. Dietary GMP improved memory and dramatically reduced soluble and insoluble Aβ. Assessment of neuronal loss is ongoing.
4. Conclusions. Altering brain ganglioside profiles can improve memory and reduce Alzheimer-related neuropathology, and warrants further investigation as a novel therapeutic strategy.

04v. Therapeutic Targets & Mechanisms for Treatment: synaptic plasticity and repair

ADPD5-0609

LITHIUM MICROEMULSION OF LOW DOSE RESTORES NEUROPLASTICITY, NEUROGENESIS, AND RESCUES COGNITIVE DEFICITS IN A TRANSGENIC RAT MODEL OF ALZHEIMER'S DISEASE

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Objectives

Lithium, a drug often prescribed for bipolar disorder, has disease-modifying properties in patients at risk for developing Alzheimer's disease. However, the exact mechanisms underlying these effects are poorly understood. In addition, conventional lithium therapy has many serious side effects that make it inappropriate for long-term treatment.

Methods

We evaluated a new lithium water-in-oil microemulsion of low dose on transgenic rats with AD-like amyloid beta pathology. Separate cohorts of McGill-R-Thy1-APP transgenic rats, representing pre and post-plaque stages of the amyloid beta pathology, received either vehicle or low-dose lithium for 2 and 4 months, respectively. After treatment, animals were assessed on learning and memory tasks and for markers of synaptic plasticity and inflammatory processes.

Results

We found that lithium-treated transgenic rats showed significant recovery in cognitive performance, including on the Morris water maze, novel object recognition, and fear conditioning. The new lithium microemulsion reduced soluble levels of human Abeta42 peptides, but did not affect soluble human Abeta40. Insoluble human Abeta42 was undetectable at this age. Neurogenesis, which was significantly reduced in transgenic compared to wild type rats, was restored to wild type levels after treatment. These changes occurred in parallel with a modulation of inflammatory processes. Most importantly, the present preparation, dosage and administration route did not provoke any adverse effect.

Conclusion

The new lithium microemulsion of low-dose allows delivery of significantly lower, yet pharmacologically active, amounts of lithium. These results show that this new formulation of lithium has a therapeutic effect in transgenic rats with AD-like amyloid beta pathology.

04v. Therapeutic Targets & Mechanisms for Treatment: synaptic plasticity and repair

ADPD5-0876

A BLOOD-BASED GLUTAMATE SCAVENGER, OXALOACETATE, ABROGATES ABETA-MEDIATED HIPPOCAMPAL SYNAPTIC PLASTICITY DISRUPTION IN VIVO

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Abeta-mediated inhibition of long-term potentiation may be caused by triggering an increase in brain extracellular glutamate concentration, possibly by inhibiting glutamate uptake or promoting its release.

Objectives:

Determine the ability of intravenous administration of a blood-based glutamate scavenger to abrogate LTP disruption caused by Abeta or by an excitatory amino acid transporter inhibitor, DL-*threo*-beta-benzyloxyaspartic acid.

Methods:

Electrically evoked field EPSPs were recorded in the CA1 area of the dorsal hippocampus of urethane (1.5 g/kg, i.p.) anaesthetized adult male Wistar rats. A cannula was inserted in the lateral ventricle. LTP was induced by repeated-200 Hz high frequency stimulation.

Results:

Intracerebroventricular injection of either protofibril-enriched Abeta1-42 (350 pmol) or TBOA (6 nmol) 1 h prior to the conditioning stimulation inhibited LTP (117.3±4.2% n=5; 111.5±3.7% n=5, $P<0.05$ compared with vehicle 133.1±1.3% n=5). In contrast, Abeta no longer inhibited LTP in rats pretreated 1 h (132.8±2.8% n=5), but not 2 h (114.7±2.1% n=5), with oxaloacetate (35 mg/kg, i.v.), a dose that on its own did not affect control LTP. Indeed i.v. administration of oxaloacetate 45 min after Abeta also abrogated the inhibition of LTP by Abeta (131.8±6.9% n=5, $P>0.05$ compared with vehicle). Furthermore, post-injection of oxaloacetate i.v. similarly prevented the synaptic plasticity disruptive effect of TBOA (136.5±3.2% n=5, $P>0.05$ compared with vehicle).

Conclusion:

These findings indicate that reducing blood glutamate concentration can abrogate the synaptic plasticity disruptive effects of Abeta or TBOA in the brain within a defined time window. Oxaloacetate appears to promote rapid glutamate homeostasis across the blood-brain barrier.

04v. Therapeutic Targets & Mechanisms for Treatment: synaptic plasticity and repair

ADPD5-1329

THE RATIONALE TO TEST A SPECIFIC NUTRITION COMBINATION IN THE LIPIDIDIET CLINICAL STUDY1 IN PRODROMAL ALZHEIMER'S DISEASE

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Objectives:
The LipiDiDiet study in prodromal AD (Dubois 2007) investigates the long-term effects of a specific nutritional intervention (Souvenaid) targeting the formation and function of neuronal membranes and synapses. The rationale for this study is based on effects of specific nutrient enrichment in basic science models and clinical trials in AD.
Methods:
Synaptic loss has been recognized as strongest structural correlate with memory impairment in AD and is apparent already early in the disease, including MCI. Synapses largely consist of neuronal membranes which are mainly composed of phospholipids. Phospholipid synthesis depends on the availability of rate limiting nutritional precursors and cofactors. Basic science studies indicate that their increased intake enhances synaptogenesis. However, lower plasma levels of these nutrients are widely observed in AD, eg lower levels of uridine and docosahexaenoic acid are found in early AD patients compared with controls.
Results:
Recent preclinical studies in the LipiDiDiet project confirmed beneficial effects of the intervention on cognitive performance and neuroimaging markers in a mouse model (APP/PS1) of AD. The clinical studies so far in AD demonstrated that the nutritional intervention is safe and improved memory performance in mild, but not moderate, AD and improved EEG measures of functional connectivity.
Conclusions:
Mechanistic and clinical studies suggest potential utility of increasing intake of specific nutrients in the earliest stages of AD. The LipiDiDiet study is designed to test the hypothesis that the investigational intervention Souvenaid improves cognitive outcome and disease markers in prodromal AD.
[1]Funded by EU FP7 project LipiDiDiet, Grant Agreement N°211696

04v. Therapeutic Targets & Mechanisms for Treatment: synaptic plasticity and repair

ADPD5-2075

A CNS-PERMEABLE HSP90 INHIBITOR RESCUES SYNAPTIC DYSFUNCTION AND MEMORY LOSS IN ALZHEIMER'S MICE VIA HSF1-MEDIATED SRTESS RESPONSE

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Objectives. Inhibition of heat-shock protein Hsp90, such as via pharmacological inhibitors, is being investigated as a treatment option for neurodegenerative diseases such as Alzheimer's disease (AD) and tauopathies**.** However**,** a pharmacologically feasible Hsp90 inhibitor with CNS permeability and safety is lacking.
Methods. Using a combination of experimental approaches of biochemical, cell biology, electrophysiology and animal models, we tested a CNS-permeable compound in AD-related functions.
Results. We recently discovered that a commercial Hsp90 inhibitor 17-AAG not only elicits a heat shock-like response but also upregulates several presynaptic and postsynaptic proteins, such as synapsin I, synaptophysin, and PSD95 via transcriptional activation in neurons. We now demonstrate that a proprietary Hsp90 inhibitor compound (NXD30020, NXD) displays similar actions on HSPs and on synaptic protection as 17-AAG. Chronic treatment with NXD completely rescues memory deficits induced by soluble amyloid β-peptides Abeta as well as in symptomatic Tg2576 AD mice without causing systemic toxicity. Despite the short shelf-life of NXD in mouse brain, a single intraperitoneal injection of NXD induces rapid and long-lasting (2-3 days) nuclear activation of the heat shock transcription factor HSF1, and increases expression of synaptic proteins including BDNF, most prominently in the CA1 of hippocampus. Furthermore, we found that this master stress response regulator HSF1 is crucial for the initial memory consolidation in contextual fear test.
Conclusions. The activated HSF1 may largely mediate the beneficial effects of Hsp90 inhibitors on synapses and cognition. Taken together, this work implicates a previously unappreciated role of HSF1 in synaptic functions and memory.

04w. Therapeutic Targets & Mechanisms for Treatment: adult neurogenesis

ADPD5-0984

PRESENILIN-1 FAMILIAL ALZHEIMER'S DISEASE MUTATION ALTERS HIPPOCAMPAL NEUROGENESIS AND MEMORY FUNCTION IN CCL2 NULL MICE

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1. Objectives

Familial Alzheimer's disease (FAD)-linked presenilin 1 (PS1) mutations affect adult neurogenesis. However, how neurogenesis is affected is a point of conflict. Some studies show impaired cell proliferation and survival while others demonstrate increased cell proliferation. We sought to define the mechanisms underlying FAD PS1 mutations in hippocampal neurogenesis by engaging multidisciplinary behavioral, molecular and biochemical approaches.

2. Methods

PS1 FAD mutant (M146L)-overexpressing mice were crossed with a CC-chemokine ligand 2 knockout (CCL2KO) to generate double transgenic (Tg) PS1/CCL2KO mice. Non-Tg, single Tg mice (PS1 and CCL2KO) were used as controls. Morris water maze tasks were utilized to assess learning and memory function. Immunohistological and biochemical assays were employed on brain tissues and neural progenitor cells, respectively to uncover pathways of neural dysfunction.

3. Results

PS1/CCL2KO mice developed age-dependent deficits in learning and memory, impaired hippocampal neurogenesis, altered expression of synaptic plasticity-related molecules and reduced non-canonical ErbB4 processing. Associated increases in hippocampal N-acetyl-glutamic acid and ornithine were observed in the PS1/CCLKO mice implicating a role for altered ammonia metabolism through urea cycle activation.

4. Conclusions

The findings show that CCL2 affects both chemotaxis and cell fate for hippocampal neurogenesis. These act in concert with PS1 FAD mutations. In parallel, the PS1/CCL2KO mice serve as a model of hippocampal degeneration in AD and for other neurodegenerative disorders.

04w. Therapeutic Targets & Mechanisms for Treatment: adult neurogenesis

ADPD5-1425

BEYOND DRUG DELIVERY TO THE BRAIN: THE EFFECTS OF MRI-GUIDED FOCUSED ULTRASOUND ON BRAIN PLASTICITY

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Objective: The use of transcranial MRI-guided focused ultrasound (MRIgFUS) is known for the delivery of therapeutics to targeted areas of the brain. Here, we evaluated the effects of FUS on brain plasticity, particularly with regards to the stimulation of adult neurogenesis and dendritic complexity.

Methods: We used non-transgenic rodents, and a transgenic mouse model of amyloidosis. Animals were anesthetized and secured in the FUS system. The hippocampus was located by MRI and targeted with the ultrasound. MRI images were capture to detect the increase in permeability of the blood-brain barrier due to FUS in presence of microspheres. One of the best known modulator of adult neurogenesis is exercise. As such, we compared the impact of FUS with running on adult neurogenesis. The impact of these treatments was evaluated on cognitive functions, neural and vascular plasticity.

Results: Transcranial MRIgFUS efficiently reduced amyloid load in transgenic animals, even at advance stages of the pathology. In both non-transgenic and transgenic animals, MRIgFUS significantly increases adult neurogenesis and dendritic complexity, similarly to what was observed with exercise. Furthermore, MRIgFUS was found to improve cognitive functions.

Conclusions: FUS applications can reduce amyloid pathology, increase neural plasticity and improve cognition. MRIgFUS has the potential to fulfill the long sought-after goal of non-invasive drug delivery to the brain and, in addition, inducing brain plasticity.

04w. Therapeutic Targets & Mechanisms for Treatment: adult neurogenesis

ADPD5-1762

BEXAROTENE INCREASES NEURONAL DIFFERENTIATION IN VITRO AND IN VIVO

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Retinoid X receptors (RXRs) are ligand-activated transcription factors that bind to DNA as homodimers, or heterodimers with other nuclear receptors. We and others have shown that RXR ligand Bexarotene improves memory in mice expressing human apolipoprotein E (APOE).

To identify direct RXR targets genome-wide we applied chromatin immunoprecipitation followed by massive sequencing (ChIP-seq). We chose mice expressing endogenous APP to avoid possible effects of A-beta pathology on transcription factor binding. Since we were particularly interested in determining the effect of RXR binding in APOE4 and APOE3 expressing mice we chose 6 months old *APOE* targeted replacement mice (WT/E4). The result from Functional Annotation revealed that “neuronal differentiation” category was significantly enriched in samples from Bexarotene treated animals. RXR binding to select genes related to neuronal differentiation was validated using ChIP-QPCR in brain samples and cells.

To determine how Bexarotene affects mRNA levels and gene expression in brain, we applied mRNA-seq. GO categories “behavior” and “neuron differentiation”, were amongst the most significantly enriched. We validated the effects of Bexarotene on neuron differentiation/neurogenesis using QPCR in APOE4 mouse brain and in ES (embryonic stem) cells. We demonstrate that Bexarotene treatment increased neuronal lineage commitment in ES cells as well as their differentiation into mature neurons. Finally, we show that in response to Bexarotene there is enhanced maturation of postmitotic neurons in dentate gyrus of WT mice.

The study demonstrates the applicability of new and powerful sequencing technologies for research in Alzheimer's disease model mice and for addressing questions with significant therapeutic implications.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0562
STATIC MAGNETIC FIELD PROTECTS AGAINST BETA-AMYLOID-INDUCED NEURONAL APOPTOSIS

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Objectives
Weak static magnetic fields (SMF) in the order of milliTesla (mT) strength cause distinct physiological effects on the human and animal body, however little is known about the way in which magnetic fields affect neuronal survival. The objective of this study was to determine whether SMF exerts neuroprotective properties in primary neuronal cultures.
Methodology
Primary cultures were prepared from cortex and hippocampus of day 1 rat pups and grown in neurobasal medium for 6 days in the presence or absence of 5 mT SMF, achieved by positioning a neodymium magnetic disc beneath each well of the 24-well culture plate. On DIV6, etoposide (12 microM) or beta-amyloid 1-42 (25 microM) was added for 24 h, and the percentage of apoptotic cells was determined using Hoechst 33342. Levels of free Ca2+ were determined on DIV7 without neurotoxin addition using Indo1.
Results
Exposure to SMF reduced the percentage of etoposide-induced apoptosis in cortical cultures by 57.1 ± 6.3%. Similarly, the percentage of beta-amyloid-induced apoptosis in hippocampal cultures was reduced by 59.0 ± 4.0%. Kinetic analysis using indo-1 showed that resting levels of intracellular free Ca2+ in primary cortical cultures, as well as the expression of voltage gated calcium channel proteins, were higher in the presence of SMF.
Conclusions
Constant exposure to SMF conferred significant neuroprotection in both cortical and hippocampal primary neuronal cultures. The mechanism behind this effect may be linked to an increase in free Ca2+ leading to enhanced release of neurotrophic factors, and activation of additional anti-apoptotic mechanisms.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0896

PERIPHERAL LIRAGLUTIDE THERAPY COUNTERACTS ALZHEIMER'S DISEASE-RELATED BRAIN CHANGES

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1. Objectives: Amelioration of brain insulin resistance upon glucagon-like 1 peptide (GLP-1) analogue treatment suggests that these drugs could be relevant in type 2 diabetes (T2D)-associated cognitive dysfunction, and in neurodegenerative diseases that share several common features with T2D (*e.g.* Alzheimer disease (AD)). Herein, we hypothesized that chronic peripheral liraglutide administration has neuroprotective effects in a mouse model of AD.
2. Methods: 11 month-old triple transgenic AD (3xTgAD) mice were given liraglutide (0.2mg/kg, once/day, 28 days) subcutaneously. Wild-type age-matched female mice were used as controls. Spatial memory was analyzed by the Morris watermaze (MWM) test. Blood and brain cortical homogenates were collected for several analyses.
3. Results: Liraglutide significantly decreased blood and brain glucose levels, peripheral hyperinsulinemia and insulin resistance, and slightly decreased glycated hemoglobin (HbA1C) in 3xTgAD females. Liraglutide also partially restored protein kinase A activity (a downstream kinase of the GLP-1 receptor-mediated signaling), decreased C-reactive protein (inflammatory marker) and thiobarbituric acid reactive substances and 8-hydroxy-2'-deoxyguanosine (lipid and protein oxidation markers, respectively). Liraglutide's ability to improve spatial memory for 3xTgAD female mice was accompanied by a decrease in amyloid-beta peptide and tau protein phosphorylation levels.
4. Conclusions: Chronic treatment with liraglutide ameliorates cognitive function and AD pathological hallmarks in 3xTgAD female mice. The improvement of peripheral and brain insulin sensitivity and glucose homeostasis, and decreased oxidative stress and neuroinflammation underlie the benefits of liraglutide.

Supported by FEDER (Programa Operacional Factores de Competitividade – COMPETE) and Portuguese funds via FCT (projects: PTDC/SAU-TOX/117481/2010; Pest/SAU/LA0001/2011; fellowships: SFRH/BD/90036/2012, PTDC/SAU-TOX/117481/2010 and SFRH/BPD/84473/2012).

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1094
TARGETING THE RETINOID X RECEPTOR: A NEW WAY FOR INVESTIGATION AND THERAPY IN ALZHEIMER'S DISEASE
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OBJECTIVES: By regulating brain cholesterol homeostasis, the anticancer drug bexarotene – an agonist of RXR - has been shown to restore cognitive functions and decrease the amyloïd brain burden in animal models of Alzheimer's Disease (AD). Nevertheless, its exact mechanism of action remains elusive. The main objective of this study is to find out the potential of RXR stimulation at the BBB level in therapy of AD.
METHODS: Transcriptional, protein and functional analyses of receptors and transporters involved in cholesterol and Aß exchanges between the blood and the brain compartments were undertaken on a human *in vitro* BBB model (fig.1), following 24h of bexarotene treatment.

RESULTS: Our preliminary results show that nanomolars concentrations of bexarotene, non-toxic for the BBB, increase the expression of ABC transporters (ATP-binding cassette) as ABCA1 and ABCB1, respectively involved in the brain cholesterol homeostasis and Aß clearance across the BBB. These preliminary data are in accordance with an increase in cholesterol exchanges between the blood and brain compartments. Consequences of ABCB1 up-regulation on Aß transport are currently being characterized.
CONCLUSIONS: These preliminary data could explain in part the beneficial effect of bexarotene in AD's models, and reinforce the interest of RXR stimulation in AD therapy. Moreover, these results highlight the importance to consider the BBB in pathophysiology and therapeutic research in AD.

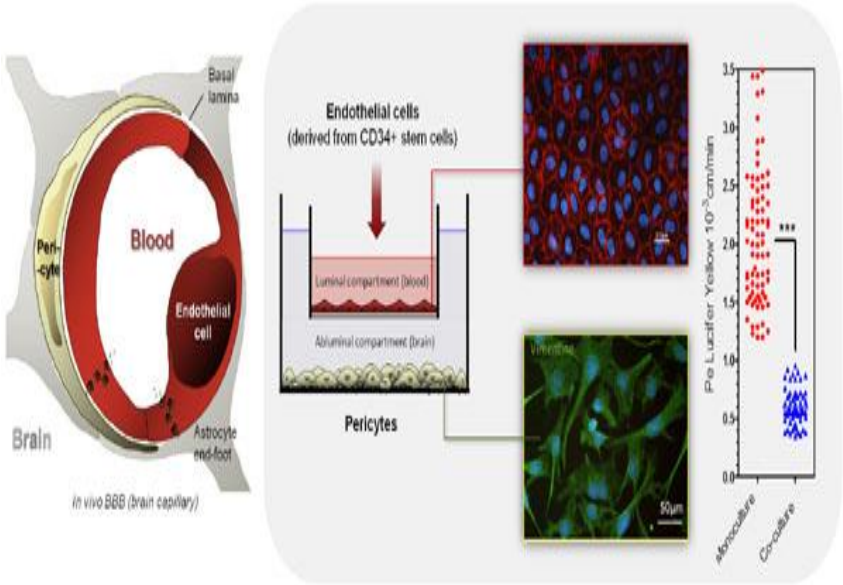

Fig 1. Human *in vitro* BBB model

Human cord blood CD34+ stem cells are differentiated into BBB endothelial cells by growing them on Matrigel-coated filters and cocultivating them with brain pericytes during 6 days (Cecchelli *et al.*, 2014).

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1175

PATTERNS OF LONGEVITY-ASSOCIATED SIRTUINS IN HUMAN DEVELOPING, DOWN SYNDROME AND AGING BRAIN

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Objectives: Aging is a risk factor for neurodegenerative diseases such as Alzheimer´s disease (AD). AD-related pathology appears early in Down syndrome (DS). Recent studies suggest commonalities between the developing and aging brain. The sirtuin protein family was shown to be associated with longevity.
Methods: To evaluate a relation of sirtuin expression levels with the progression of AD we examined the highly vulnerable entorhinal cortex and compared with less affected regions in 45 cases grouped according to Braak stages of neurofibrillary degeneration. We applied morphometric immunohistochemistry and immunoblotting in subcellular fractions. In addition, we evaluated the distribution of SIRT1 and 3 in normal and Down developing brains by immunohistochemistry.
Results: Our studies revealed a subcellular redistribution of SIRT1 in aging brains suggesting a stepwise loss of neuroprotection. SIRT1 and 3 expression decreases during the progression of AD which is contrary to the increase of SIRT5. This increase associates with its appearance in activated microglial cells. In developing brains SIRT1 and 3 show strong positivity in the germinal matrix. The cellular distribution differs between DS and normal development.
Conclusions: The distinct pattern of sirtuin distribution in the developing and aging brain suggests a complex pathogenetic scenario, which should be considered when aiming sirtuins as therapeutic targets.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1284

INVESTIGATING NOVEL TRANSLOCATOR PROTEIN (TSPO) LIGANDS IN VITRO AS A POTENTIAL THERAPEUTIC TREATMENT OF ALZHEIMER'S DISEASE

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Objectives Reduced neurosteroid levels have been reported in AD brain and thus increasing neurosteroid production may provide a novel therapeutic approach. Recently, the classic Translocator protein (TSPO) ligand, Ro5-4864, has been shown to increase neurosteroidogenesis and reduced cerebral beta amyloid deposition in an AD animal model. However, this ligand has limited clinical application due to low specificity and poor blood-brain barrier penetration. Here, we report three novel TSPO ligands : PBR175, PBR162, and CLINDE; that have favourable safety profiles on their effect on steroidogenesis and their ability to rescue against Aβ toxicity *in vitro*. **Methods** The three novel TSPO ligands based on substituted 2'-phenyl imidazopyridine structure and the acetamide side chain were shown to be non-toxic (10nM-100μM) by MTS and LDH release assay in M17 neuroblastoma and C6 glioma cell lines. Their neurosteroidogenic potency were assessed by measuring pregnenolone level by ELISA and neurosteroid levels were measured by LC-MS/MS in these cell lines. In addition, TSPO protein levels following TSPO ligands were measured by Western Blot. The ability to rescue against Aβ toxicity was also investigated by pre-treatment with the ligands following incubation with Aβ42 oligomers. **Results** These three novel TSPO ligands exerted a trend towards increasing levels of some (but not all) neurosteroid, with CLINDE being the most effective. However, they were not found to attenuate Aβ toxicity. **Conclusions** This study evaluates new generation TSPO imidazopyridine ligands as promising candidates that specifically target neurosteroid production for AD treatment.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1312
USING MICRO-IMMUNOELECTRODES TO STUDY MECHANISMS OF RAPID AMYLOID-BETA CLEARANCE IN MICE

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OBJECTIVES: Human studies strongly suggest that a key factor leading to Aβ accumulation in the brain is a defect in clearing the peptide. Aβ clearance from the brain occurs through multiple pathways, some of which may be fast-acting. The goal of this study was to use micro-immunoelectrodes (MIEs) to study the rapid removal of Aβ from the ISF.
METHODS: MIEs detect rapid changes in Abeta using an electrochemical approach (Prabhulkar et al., 2012). MIEs were implanted in the hippocampus of APP/PS1 mice, and baseline Aβ levels were measured. Mice were injected with a gamma secretase inhibitor (GSI) to block Aβ production, and the rate of Aβ clearance (elimination half-life) from the ISF was calculated. A second group of mice were pre-treated with a p-glycoprotein (Pgp) inhibitor to block active transport of Aβ across the blood-brain barrier. Clearance rates with and without PGP inhibitor treatment were compared to determine the relative impact of this clearance mechanism on Aβ halflife.
RESULTS: Baseline levels of Aβ40 before treatment are relatively stable and ISF levels begin to decline within 4 minutes of GSI injection. The elimination half-life of Aβ40 was calculated to be 38 minutes. Pre-treatment with a Pgp inhibitor resulted in a significant prolongation of Aβ40 half-life.
CONCLUSIONS: Inhibiting active transport slows clearance of Aβ, but does not completely block it, indicating multiple mechanisms are likely involved in Aβ clearance from the ISF.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1373

YOUNG CIRCULATORY FACTORS REGENERATE ALZHEIMER-LIKE DISEASE IN MICE

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Alzheimer's disease (AD) pathology starts long before clinical symptoms manifest. It is likely that the earliest cognitive deficits are the result of high Aβ levels causing disrupted synaptic transmission leading to neuronal network dysfunction and depletion of calcium-dependent proteins, such as calbindin. Currently there is no therapy to treat, delay or prevent Alzheimer's disease. Recently we showed that rejuvenating the systemic environment of aged mice reverses age-related changes in synaptic plasticity and cognitive deficits. To investigate the possible therapeutic effects of young blood in the context of AD we used heterogenetic parabiosis, in which we joined young animals together with the Thy1-hAPPLond/Swe ($hAPP^{L/S}$) mouse model of AD. This resulted in a markedly increased expression of neuronal activity-related markers in the dentate gyrus of old heterogenetic APP parabionts restoring expression levels close to those observed in age-matched wild-type parabionts from isochronic pairs. Microarray analysis revealed that rejuvenating the systemic environment may restore several systems in the hippocampi of $hAPP^{L/S}$ mice, including calcium homeostasis. Together these transcriptional changes are indicative of a regenerated neuronal circuit, less neuronal cell death and improved cognition. Repeated intravenous injections of young plasma led to improvements in the memory of $hAPP^{L/S}$ mice and restored levels of neuronal activity related markers. Our data indicate that degenerative changes associated with AD pathology can be reversed by rejuvenation of the systemic environment, which opens the possibility for cognitive improvements in humans with AD.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1502

TUDCA ATTENUATES ABETA LOAD IN APP/PS1 MICE BEFORE AND AFTER THE ONSET OF AMYLOID PATHOLOGY BY MODULATING ABETA CLEARANCE

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Objectives:
Our aim was to explore the neuroprotective mechanisms by which tauroursodeoxycholic acid (TUDCA) modulates amyloid-beta (Abeta) load in Alzheimer's disease (AD).

Methods:
We used 2 month-old APP/PS1 mice fed a diet containing 0.4% TUDCA, or no bile acid, for 6 months. Moreover, 7 month-old APP/PS1 mice were injected 500 mg/kg bw TUDCA i.p., or vehicle, every 3 days for 3 months. Wild-type littermates were used as controls. Brains were processed for Abeta immunohistochemistry; total proteins were prepared from dissected hippocampus and frontal cortex for Western blot (WB) and ELISA. Abeta clearance was evaluated using differentiated N2a cells treated with TUDCA before or after Abeta42 incubation, followed by ELISA and WB of cell lysates and supernatants. Finally, we analyzed the effect of TUDCA in intracellular Abeta levels, after wash-out procedures.

Results:
TUDCA significantly reduced amyloid plaque burden of APP/PS1 mice comparing to untreated controls. Importantly, this effect was observed in animals treated before amyloid deposition started, and in animals treated after the onset of amyloid pathology. Similar results were obtained by ELISA and WB analysis. *In vitro*, TUDCA treatment of differentiated N2a cells decreased both cell-associated and extracellular Abeta levels. Finally, we demonstrated that TUDCA still decreased Abeta intracellular levels after washing out Abeta from the culture medium. Therefore, TUDCA may influence not only the uptake of Abeta, but also Abeta intracellular degradation and/or exocytosis.

Conclusions:
Our data suggest TUDCA is a potential therapeutic alternative for prevention and treatment of AD-associated Abeta pathology, possibly by modulating intraneuronal amyloid clearance.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1647

UNFOLDED PROTEIN RESPONSE (UPR) REGULATES THE ACCUMULATION OF AMYLOID BETA DEPOSITS IN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE

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Introduction: Alterations of the protein homeostasis network have been extensively reported in Alzheimer's disease, in particular endoplasmic reticulum (ER) stress and the activation of UPR stress sensor IRE1 and its downstream target, the transcription factor XBP1. Importantly, a polymorphism in the XBP1 promoter was recently linked to AD, consistent with a global gene expression profile analysis that revealed XBP1 regulates a cluster of AD-related genes. Here we investigated the contribution of IRE1 to AD using a conditional knockout model in the context of AD and measured the impact on amyloid-beta aggregation/deposition.

Methods: We generated a brain specific knockout mouse for IRE1 and crossed it with an AD transgenic model termed 5xFAD. In order to evaluate the amyloid beta deposition, half of the brains of 6-month-old animals were processed for Abeta histological and biochemical analysis.

Results: We observed a reduced load of amyloid plaques in the brains of AD mice deficient for IRE1 measured by ThS staining and 4G8 specific antibody against amyloid beta. We also found that brain specific ablation of IRE1 significantly decreased soluble and insoluble forms of $Abeta_{42}$, as quantified by ELISA assay.

Discussion: IRE1 could modulate the processes affecting AD progression, offering new potential strategies for ameliorating AD pathology.

Support from FONDECYT 3140466 (CDA), fellowship (AF), CONICYT PhD fellowship (VHC), Alzheimer Association, FONDECYT no. 1140549, CONICYT USA2013-0003, Millennium Institute No. P09-015-F, Muscular Dystrophy Association, FONDEF D11I1007, Ring initiative ACT1109.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1659

DBS VS RADIO-FREQUENCY ABLATIVE PROCEDURES : A COST EFFECTIVE ALTERNATIVE FOR MOVEMENT DISORDER FOR DEVELOPING COURTRIES

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Aim : To Study and to compare the outcomes following DBS and Pallidotomy/subthalamotomy procedures for movement disorders.

Material and Methods : radiofrequency ablative neurosurgery and DBS done for PD and dystonia at single center KIMS hospital, Hyderabad, India, presented here. UPDRS and UDRS scores used for preop and follow up clinical assessment. DBS done as standard protocols. Functional neurosurgery done by using sterotaxy and brainlab protocol for nucleus localization and lesion is created by radiofrequency probe which leads to thermocoagulation of the structure. Intraop stimulation of the target and neurological assessments were done by neurologist.

Results : At KIMS last 3 years there were 10 DBS procedures done One for Dystonia and 9 for parkinson's disease. 9 Ablative procedures done 5 for dystonia and 4 for parkinson's disease. Improvement noted in all the cardinal symptoms of the disease and functional status of patient on evaluation there was approximately more than 50% improvement in UPDRS and UDRS scales. Both the groups done well at the end of 1 year followup.

Discussion : Subthalamotomy and pallidotomy are seems tobe clinically effective procedures for patients having movement disorder. There were sustained clinical benefits by both the procedures. DBS is very costly and not available in most of centers in India.

For ablative neurosurgy cost of the procedure was significantly less [six times] than DBS. Ablative neurosurgery is affordable option in selected patient may return to the stage in Indian population but require further experience.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1705

DIFFERENTIAL CONTRIBUTION OF CB1 AND CB2 CANNABINOID RECEPTORS TO THE PROGRESSION OF THE ALZHEIMER-LIKE PATHOLOGY IN APP/PS1 MICE

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Objectives: Growing evidence indicates that the pharmacological stimulation of the endogenous cannabinoid system represents a promising therapy to curb several neurodegenerative processes associated to Alzheimer's disease (AD). The aim of our study was to compare the specific contribution of CB_1 and CB_2 receptors, the main targets of the cannabinoid-based therapies, to the progression of AD-like pathology in an animal model.
Methods: Two new mouse strains were generated by crossing APP/PS1 mice, a transgenic model of AD, with CB_1 and CB_2 knockout mice. The cognitive performance as well as the cortical Abeta burden and gliosis were evaluated at different ages (3 and 6 months) in both colonies.
Results: The neonatal viability of APP/PS1/Cnr1$^{-/-}$ was drastically reduced and the few mutants that were born died before 2 months of age. APP/PS1/Cnr1$^{+/-}$ were viable but exhibited an accelerated cognitive impairment from 3 months of age. APP/PS1/Cnr2$^{-/-}$ developed normally and exhibited similar cognitive performance than CB_2 sufficient transgenic mice. However, higher Abeta burden and gliosis were observed in the cortex of APP/PS1/Cnr2$^{-/-}$.
Conclusions: CB_1 receptor plays a prominent role in the progression of AD pathology since its deficiency compromises the viability of APP/PS1 mice and accelerates their cognitive impairment. In contrast, CB_2 receptor deficiency does not alter APP/PS1 mice cognitive performance but exacerbates the Abeta-related pathology. In conclusion, a potential cannabinoid-based therapy against AD should target both CB_1 and CB_2 receptors in parallel in order to take advantage of the differential neuroprotective properties of these endogenous cannabinoid system components.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1914

RXR AGONIST CNX-013-B2 HAS A POTENTIAL TO REDUCE Aβ DEPOSITION, INCREASES CLEARANCE, ALONG WITH REDUCED NEURONAL DEATH IN BOTH N2A CELLS AND ASTROCYTES.

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Objectives: Alzheimer's disease (AD) is a neurodegenerative process involving amyloid-β (Aβ) peptide deposition, neuroinflammation, and progressive memory loss. Aβ accumulation leads to the deposition into plaques and is thought to initiate the pathologic cascade leading to neuronal death. Also cholesterol transport protein Apolipoprotein E along with ATP-binding cassette transporter A1 (ABCA1) play an important role in the clearance of Aβ from the brain. It is known that RXR/LXR functions as a sensor of cellular cholesterol concentration and mediates cholesterol efflux by inducing transcription of key cholesterol shuffling vehicles, ABCA1 and ApoE. In this study, we report impact of a potent and selective RXR agonist CNX-013-B2 on Aβ deposition, clearance, neuroinflammation/stress and neuroprotection in both neuronal cells and astrocytes.

Methods: N2a cells and mouse astrocytes were used to study the impact on the expression of markers of different mechanisms that regulate Aβ deposition, clearance, neuroinflammation/stress and neuroprotection after treating the cells with high cholesterol.

Results: Treatment with CNX-013-B2, a selective small molecule rexinoid with an EC_{50} of 48 nM towards human RXRa resulted in a significant increase in cholesterol efflux along with 3-5 fold increase in both ABCA1 and ABCG1 gene expression. CNX-013-B2 increases mitochondrial biogenesis and activity along with increased expression of NMDR, PGC1a and FOXO1 which are known as neuroprotective markers. CNX-013-B2 reduces ROS levels, MCP1 and IL1b expression along with decrease in caspase3 levels.

Conclusions: RXR activation with CNX-013-B2 can reduce Aβ deposition, neuroinflammation/stress, increase clearance and can be a neuroprotective agent.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-2201

REPEATED PERIPHERAL DELIVERY OF ADULT ISCHEMIC-TOLERANT MESENCHYMAL STEM CELLS LOWERS CEREBRAL ABETA AMYLOIDOSIS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disorder that represents one of today's major healthcare challenges. AD is neuropathologically characterized by the deposition of amyloid-beta (Abeta) peptides into extracellular senile plaques. The application of adult ischemic-tolerant mesenchymal stem cells (itMSC) for the treatment of AD is still not fully exploited. Therein we have evaluated the impact of repeated intravenous itMSC injections in a mouse model of AD.

Aged amyloid-depositing APPPS1 mice received a weekly intravenous itMSC injection for a total of 10 weeks. One injection consisted of a tail vein injection of 0.5 M itMSC in 150 ul of Lactated Ringers Solution (LRS). Repeated injections of LRS in aged-matched APPPS1 mice were performed as controls. One week after the last injection, APPPS1 animals were subjected to neuropathological examination. Biodistribution of itMSC in brains and peripheral organs was performed in different mice at several time-points after the intravenous delivery. The impact of itMSC on recovering brain dysfunction is being investigated using neuroimaging in living APPPS1 animals.

Our pre-clinical results indicate that repeated intravenous itMSC delivery in a mouse model of AD leads to significant reduction of cerebral Abeta amyloid plaques. The beneficial effect on plaques is accompanied by a significant diminution of neuroinflammation markers without appearance of cerebral amyloid angiopathy (CAA) or microhemorrhages. As revealed in the current study, the combination of safety and efficacy to remove amyloid plaques offered by itMSC, together with successfully completed safety phase I clinical trials, may put itMSC as a perspective candidate for treating AD.

05a. Drug Development & Clinical Trials: active vaccination

ADPD5-1808

RESULTS FROM A PHASE II STUDY TO ASSESS THE CLINICAL AND IMMUNOLOGICAL ACTIVITY, SAFETY AND TOLERABILITY OF AFFITOPE® AD02 IN PATIENTS WITH EARLY ALZHEIMER'S DISEASE

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Objectives: A multicenter, parallel group 18-month study was performed in both late MCI and mild AD subjects to assess the safety and efficacy of AFFITOPE® AD02, which is an *amyloid-beta (Aβ)-targeting vaccine*. AD04, an adjuvant control possessesing immune enhancing properties was originally designated as a placebo but appears to have disease-modifying activity.
Methods: The study enrolled 332 patients, randomly assigned to 5 groups: two formulations of AD04 (formulation 1 and 2), 25ug AD02 (in two different formulations) and 75ug AD02. Patients received 6 subcutaneous injections with final assessments at week 78. The efficacy analysis compared treatment groups on an Adapted ADAS-cog scale, Adapted ADCS-ADL and a Composite Scale.
Results: Dropout rates were similar across groups and exceptionally low for an 18 month study at 19.4% indicating good tolerance. A statistically significant difference in favor of AD04 (formulation 2) was found on the Composite Scale ($p=0.038$). Formulation 2 of AD04 showed statistically significant effects on several clinical outcomes: Adapted ADAS-cog, Adapted ADL, Composite, ADAS-cog, CDR-sb, and QOL-AD Caregiver as well as two biomarker outcomes: Right and Total Hippocampal volume (all $p<0.05$). 48% of patients in this group had no decline in the composite outcome over 18 months.
Conclusions: Primary efficacy outcomes in this phase 2 trial were significant in favor of AD04 over AD02. Exploratory analyses indicate potential treatment effects with the formulation 2 of AD04, supporting further studies of AD04. Clinical outcomes and biomarker evidence converged in favor of formulation 2 of AD04, consistent with a disease modifying effect.

05b. Drug Development & Clinical Trials: antibody-based immunotherapy

ADPD5-0625

QUALITY OF LIFE IN ALZHEIMER'S DISEASE TRIALS: PRELIMINARY RESULTS AND FUTURE DIRECTIONS

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Objectives: There is limited understanding in the ability of quality of life (QOL) scores to measure clinical impact of treatment effects in trials for disease-modifying agents of Alzheimer's disease (AD). This pre-specified secondary analysis explores the effects of solanezumab, a novel therapeutic, on QOL in patients with mild AD dementia.
Methods: Participants with mild AD dementia were drawn from two Phase 3 randomized clinical trials (EXPEDITION/2) that compared solanezumab with placebo. Patients and their caregivers completed the Quality of Life in AD (QOL-AD) scale (range 13-52) in addition to other outcome measures. Mixed-model repeated measures were used to estimate the change in QOL-AD over 18 months and compare the total QOL-AD scores for each treatment group.
Results: At baseline, QOL-AD mean scores were higher/better when self-reported compared with proxy-reported in both the placebo (37.88±6.00 versus 35.18±6.12) and solanezumab groups (37.73±6.10 versus 35.18±6.19). While there was numerically less decline in solanezumab-treated patients compared with placebo, there were no significant differences between the solanezumab and placebo groups for the change in self-reported total score (p=0.080) or the proxy-reported scores (p=0.434). Change in the self-report total scores declined less than the proxy-reported scores in the solanezumab group (-0.89 versus -1.95).
Conclusions: Although previous secondary analyses have demonstrated a significant benefit of solanezumab on cognition and function, the impact of treatment on QOL was limited. Trial duration, sample size and multiple non-treatment factors may have contributed to these findings. Self-reported and proxy-reported QOL measures may behave differently for patients with mild AD dementia.

05b. Drug Development & Clinical Trials: antibody-based immunotherapy

ADPD5-1209

PLANNING TRIALS WITH PRODROMAL AND MILD AD POPULATIONS: BASELINE AND PROGRESSION DATA FROM ADNI AND THE BAN2401 PHASE 2 STUDY

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OBJECTIVES: Compare pre-dementia Prodromal AD and mild AD dementia subpopulations with regard to baseline characteristics and 12-month progression.

METHODS: Baseline clinical scores were summarized for Prodromal AD and mild AD subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI) (data collection and sharing funded by ADNI NIH Grant U01AG024904) and Study BAN2401-G000-201 (Study 201). Progression was assessed with 12-month follow-up ADNI data. Overlapping coefficients were calculated to quantify the degree of overlap between the prodromal AD and mild AD subpopulations.

RESULTS: Considerable overlap exists between Prodromal AD and mild AD baseline clinical scores in both ADNI and Study 201 (to date). For Study 201, the baseline clinical overlap between Prodromal AD and mild AD subjects ranged from 68% to 76%. Within ADNI, the baseline clinical overlap between the two subpopulations ranged from 43% to 60%, and persisted over 12 months of follow-up.

CONCLUSIONS: Baseline data from ADNI and Study 201 suggest there is no clear boundary between Prodromal AD and mild AD dementia based on clinical scores. Longitudinal ADNI data indicates that the lack of clear clinical score boundaries between the subpopulations persists over time, indicating similarity in disease progression. Prodromal AD and mild AD dementia subpopulations represent distinct clinical stages on the AD continuum that share common AD pathology. However, there exists a wide range of severity within each subpopulation, resulting in considerable overlap in baseline characteristics between them on common clinical tools. Collectively, these findings support the use of these two subpopulations as a single 'Early AD' population.

05b. Drug Development & Clinical Trials: antibody-based immunotherapy

ADPD5-1699

EFFECTS OF HUMAN BRAIN PROTEIN EXTRACTS IMMUNODEPLETED WITH AN ANTIBODY AGAINST OLIGOMERIC AMYLOID BETA 1-42 INJECTED INTO THE ZEBRAFISH

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Alzheimer's disease (AD) is one of the most common diseases in elderly and will become even more prevalent with the constant increase in life expectancy. This will lead to increased suffering for patients and families, as well as elevated costs for society. However, there is currently no treatment for AD.

In this project we were interested in testing whether immunodepletion with ALZ-201, a monoclonal antibody 100% specific for amyloid-beta 1-42 ($A\beta_{1-42)}$ oligomers, removes toxic Aβ species from human brain protein extracts. Brain protein extracts from both controls and patients diagnosed with AD were immunodepleted with ALZ-201 (a generous gift from Alzinova AB). Then, the toxicity of the remaining brain extracts were tested in a zebrafish model for Aβ-mediated memory impairment and neurotoxicity.

Our preliminary results show that zebrafish embryos injected into the brain ventricle with brain extracts from control patients did learn. On the other hand, animals injected with brain extracts from AD patients failed to learn. However, when the AD brain extracts were immunodepleted with ALZ-201 prior to brain injection, embryos were able to learn. This data indicates that $A\beta_{1-42}$ oligomers are the most toxic form of Aβ in the AD brains, since ALZ-201 does not bind to monomeric or fibrillary $A\beta_{1-42.}$ In conclusion, our data supports that immunotherapy in the future could serve as preventive treatments for AD.

05c. Drug Development & Clinical Trials: beta-secretase inhibitors

ADPD5-0576

A HIGHLY SPECIFIC ASSAY FOR BACE1: CORRELATION OF BACE1 PROTEIN LEVELS WITH AGE AND TOTAL TAU IN CEREBROSPINAL FLUID

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Objectives For all new drugs in clinical development, dose-finding is ultimately important. BACE1 is currently a prime drug target for Alzheimer's disease (AD) and even though explorative studies suggest that small changes in BACE1 protein levels are associated with pathological processes, so far only activity-based assays are widely available. A specific robust immuno-assay that determines the individual BACE1-levels and that is flexible enough to be combined with additional immuno-assays for target-engagement is not yet available. Therefore we present in this study the adaptation, according to the CSLI recommendations, of a previously reported research assay. As a first step towards clinical applications we also report age-dependent reference levels of BACE1 in CSF according to well-established guidelines.
Methods A research prototype ELISA was developed using monoclonal biotinylated antibodies, peroxidase conjugated streptavidin as reporter and recombinant human BACE1 as calibrator. CSF samples were picked from unselected diagnostic groups.
Results The analytical characteristics according to CSLI guidelines were: LOD 0,57 ng/ml; intra-assay and inter-assay variability 3% resp 12%, and cross-reactivity with human BACE2 was less than 5%. In more than 100 unselected CSF samples, a correlation between BACE1-levels and age was observed as well as a correlation with CSF tau.
Conclusion To our knowledge, this BACE1 assay is the only assay described today based on well-defined monoclonal antibodies, capable of measuring BACE1 levels in a wide range of CSF samples. The high specificity of this ELISA could complement the various activity-based BACE1 assays, enforcing BACE1 as a potential theranostic marker in clinical trials.

05d. Drug Development & Clinical Trials: gamma-secretase modulators

ADPD5-0249

ACTIVE SITES OF GAMMA-SECRETASE AND SPP ARE DISTINGUISHABLE WITH PHOTOREACTIVE PROBES

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Objectives
GSMs have been at the frontline of AD research since it was discovered that they can selectively reduce Aβ42 without affecting other Aβ species and Notch. While GSMs are therapeutically promising, little is known about their interactions with proteins other than gamma-secretase. Signal peptide peptidase (SPP), like gamma-secretase, is a multi-span transmembrane aspartyl protease that catalyzes RIP. We studied the effect of GSIs/GSMs on the active sites of gamma-secretase and SPP to determine whether SPP interacts with these compounds and how this interaction may impact SPP activity.
Methods
Active site-directed, photoreactive probes were employed to investigate differences in the active sites of gamma-secretase and SPP. Moreover, conformational changes in the active sites of both enzymes were studied upon GSI/GSM treatment.
Results
Comparison of the photolabeling profiles of SPP and gamma-secretase demonstrated that the active sites of these proteins are similar, yet some differences are apparent in specific active site sub-pockets. Furthermore, while GSIs block labeling of gamma-secretase and have no effect on or enhance SPP labeling, the opposite is true of GSMs, which block SPP labeling and have little effect on gamma-secretase labeling.
Conclusions
The structural similarity between the active sites of SPP and gamma-secretase indicated that SPP activity may be affected by GSIs/GSMs. In fact, photophore walking experiments suggest that GSIs enhance and GSMs reduce SPP activity. Reduction in SPP activity might result in undesirable side-effects for AD patients. Alternatively, the ability to target SPP with GSIs/GSMs may be therapeutically useful for SPP-dependent diseases, such as viral infections.

05d. Drug Development & Clinical Trials: gamma-secretase modulators

ADPD5-0485

GAMMA-SECRETASE MODULATORS WITH IMPROVED DRUG-LIKE PROPERTIES FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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The therapeutic rationale of gamma-secretase modulators (GSMs) for Alzheimer's disease (AD) is based on genetic evidence of more than 160 distinct mutations in presenilin (PS) 1 and 2 leading to early onset familial AD. Some of these PS mutations decrease the processivity of the enzyme complex whereas GSMs increase it. This suggests that GSMs could potentially offset some of the effects of PS mutations thereby addressing the root cause of early onset AD. Unfortunately, the field has generated few, if any, molecules with good drug-like properties to enable proof-of-mechanism studies for GSMs. In our efforts to identify non-carboxylic acid GSMs (non-CA-GSMs), we designed, synthesized and screened over 50 different novel scaffolds (>1300 compounds). The current lead molecules demonstrate much improved drug-like properties, efficacy in rodent models and good in vivo-in vitro correlations. These new molecules, as opposed to CA-GSMs, increase the production of both Abeta38 and Abeta37. Here we report the screening strategy, properties, in vivo profile and the PKPD relationships of the current leads. We also show that changing the ratio of Abeta37/Abeta42 greatly reduces the aggregation properties of Abeta42 in vitro, a phenomenon that could further impact disease progression. In conclusion, these new GSM scaffolds combine good drug-like properties with in vivo efficacy to deliver GSMs that may soon be suitable for proof-of-mechanism studies in humans.

05d. Drug Development & Clinical Trials: gamma-secretase modulators

ADPD5-2197

INHIBITION OF DE NOVO PLAQUE FORMATION UPON CHRONIC GAMMA SECRETASE MODULATOR TREATMENT

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Objectives

γ-Secretase modulators (GSMs) are promising therapeutic agents by reducing generation of the aggregation prone Aβ42 species without blocking general γ-secretase activity. We now aimed to investigate the effects of a novel GSM [8-(4-Fluoro-phenyl)-[1,2,4]triazolo[1,5-a]pyridin-2-yl]-[1-(3-methyl-[1,2,4]thiadiazol-5-yl)-piperidin-4-yl]-amine (RO5506284) displaying high potency in vitro and in vivo on amyloid plaque burden and used longitudinal Aβ-microPET to trace individual animals.

Methods

Female transgenic APP-Swe mice aged 12 months were assigned to vehicle (N=12) and treatment groups (N=12), which received daily RO5506284 (30 mg/kg) treatment for six months. A total of 131 Aβ-PET recordings were acquired at 3 time points, whereupon histological analyses of Aβ were performed. Fibrillary β-amyloid plaques were stained with the fluorescent dye methoxy-X04, plaqueload, plaque density and histogram of the size of the plaques were determined.

Results

Plaque load in the TG-GSM mice was reduced by 42% relative to the TG-VEH group ($p<0.05$), while plaque density was 48% lower in the TG-GSM group compared to TG-VEH mice ($p<0.05$). Furthermore, histogram plotting of plaque size revealed differing distributions between groups of TG mice, showing fewer ($p<0.001$) small plaques (size<800 μm²) in TG-GSM animals. Individual plaque load assessed by PET remained nearly constant in the TG-GSM group during six months of RO5506284 treatment, whereas it increased progressively in the TG-VEH group.

Conclusions

We found clear attenuation of de novo amyloidogenesis upon prolonged treatment with a potent GSM in an AD mouse model. However GSM treatment was less effective in inhibiting the growth of pre-existing plaques.

05e. Drug Development & Clinical Trials: tau modifyers

ADPD5-1489

DEHYDROEVODIAMINE-DERIVATIVE AMELIORATES THE MEMORY IMPARIMENT AND PATHOLOGY OF ALZHEIMER'S DISEASE IN 5XFAD MICE

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Objectives: Dehydroevodiamine(DHED), derived from *Evodia rutaecarpa*, has been known to improve memory impairments and decrease Alzheimer's disease(AD)-related pathology including amyloid plaque and hyperphosphorylated tau. However, it showed low water solubility in human gastrointestinal tract. Carboxy-DHED(cx-DHED), derivative of DHED, has high solubility in water so it is expected that cx-DHED has better therapeutic effects on AD with its increased bioavailability. In the present study, we investgated whether cx- DHED could improve memory deficits and modulate posttranslational modification of tau in 5xFAD, AD model mice.
Methods: Cx-DHED or donepezil (1mg/kg/day) was administered to 4month–old mice intraperitoneally for 2 months. To evaluate the cognitive impairment, the Y-maze and passive avoidance tests were performed. At the end of the behavior experiments, brains were collected and analysed histology and molecular mechanism.
Results: Cx-DHED treated–Tg mice exhibited significantly higher spontanuous alteration in the Y maze test than did the Tg-vehicle mice. In the passive avoidance test, the latency time was significantly increased by cx-DHED treatment compared to vehicle treatment. We also found that AD-related pathologies were reduced in the brains of Cx-DHED treated–Tg mice.
Conclusions: Cx-DHED, derivative of DHED with a high water solubility, significantly rescued memory deficits and ameliorated pathology in 5xFAD, AD model mice. These results suggest that Cx-DHED also has therapeutic effects on AD and it might be a potential candidate for AD treatment.

05f. Drug Development & Clinical Trials: amyloid clearance

ADPD5-0597

MOLECULAR EVOLUTION OF COMPOUNDS WITH POTENTIALLY THERAPEUTIC EFFECTS AGAINST PROTEIN MISFOLDING DISEASES

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It has now been widely recognized that many age-associated and very serious human diseases, such as Alzheimer's disease, Parkinson's disease, certain forms of cancer etc., are initiated by a common mechanism: the misfolding of specific proteins. Pharmacological chaperones, i.e. compounds with the ability to specifically bind to these proteins and rescue their misfolding, are valuable potential therapeutic agents for this type of disorders. In this work, we describe the application of molecular evolution approaches for the discovery of pharmacological chaperones for misfolding-prone proteins (MisPs) associated with protein misfolding diseases (PMDs). First, *Escherichia coli* cells are genetically engineered so as to biosynthesize very large libraries of test compounds exhibiting high levels of structural diversity. Then, the same cells are modified further so that the rare clones producing molecules with the ability to correct the folding of the target MisPs can be rapidly identified by high-throughput genetic screening. Lead compounds identified in this manner are subsequently subjected to more detailed evaluation by biochemical and biophysical methods of protein analysis, and their ability to inhibit MisP-induced pathogenicity is tested using appropriate human cell assays or *in vivo* models of the PMD of interest. We will describe our efforts to identify such "pharmacological chaperones" against the misfolding of the amyloid β (Aβ) peptide and of certain carcinogenic misfolded variants of human p53, with the aim of developing potentially therapeutic compounds against Alzheimer's disease and certain forms of cancer, respectively.

05f. Drug Development & Clinical Trials: amyloid clearance

ADPD5-1428

THE EFFECT OF APPLE AND HAWTHORN IN CHANGING THE STRUCTURE OF AMYLOID BETA OLIGOMERS AND THEIR MEMBRANE ATTACHMENT

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It has been elucidated that accumulation of amyloid beta oligomers (named ADDLs) in CNS is strongly the cause of AD. As a result, compounds capable of changing the structure and relative constituents of these ADDLs would be potential AD therapeutics. Nowadays natural compounds are of specific interest in AD treatment that some have been found to change the oligomer structure and cytotoxicity.

In this study we used the whole extract of two natural compounds (apple as MPG and hawthorn as CD) on preformed ADDLs to see their effects on the structure of the ADDLs. We also investigated the change in ability of the ADDLs in synaptosome binding after the addition of the compounds.

ADDLs were made according to Klein's Lab. protocol and were incubated with different concentrations of compounds (0.5, 1, 2, and 3 mg/ml in F12) in 4°C and 37°C for 6, 24, and 48 hours.

It was found that compounds were able to change the structure and MW distribution of ADDLs. According to western blot results when assayed with conformation specific antibody (NU2), in comparison with control ADDLs, CD showed a downward where MPG resulted in an upward shift in MW, the later caused a ladder-like distribution of SDS-stable ADDLs' subspecies. In synaptosome binding assay, CD showed a dramatic reduction in bADDLs attachment but not the MPG.

These results indicated that the whole extract of apple and hawthorn are capable of altering the oligomerization state of ADDLs where the later protected the synaptotoxicity effects of them.

05f. Drug Development & Clinical Trials: amyloid clearance

ADPD5-1789

ANALYSES OF TRAMIPROSATE PHASE 3 TRIALS SHOW IMPROVEMENT IN COGNITION AND FUNCTION IN APOE4 POSITIVE ALZHEIMER'S DISEASE SUBJECTS REACHING 4 POINT IMPROVEMENT FROM PLACEBO ON ADAS-COG IN HOMOZYGOUS APOE4 SUBJECTS, AND SUPPORT DEVELOPMENT OF ALZ-801, A NOVEL PRODRUG OF TRAMIPROSATE WITH OPTIMIZED DRUG PROPERTIES

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ALZ-801 is a novel, orally available small molecule prodrug of tramiprosate with improved pharmaceutical properties. ALZ-801 has an improved oral absorption profile, gastrointestinal tolerability and pharmacokinetic properties. Compared with oral tramiprosate in a single dose Phase 1 study, ALZ-801 pharmacokinetic variability was reduced by ~50% with an extended terminal t1/2 of 14.9 hours, allowing once-daily dosing. Oral tramiprosate was advanced to a 2,000 patient Phase 3 program which completed in 2007 with inconclusive results due to Alzheimer's disease misdiagnosis, which reached ~30% of the subjects. Post-hoc analyses of subjects with at least one e4 allele of apolipoprotein E gene (ApoE4 positive) in the North American Phase 3 study (n=599), showed that tramiprosate produced a clinically meaningful improvement in cognition (ADAS-cog) and function (CDR-SB) through 18 months, on top of treatment with acetylcholinesterase inhibitors and/or memantine (tramiprosate 150 mg BID, n=183; ADAS-cog: slope vs. placebo p

05f. Drug Development & Clinical Trials: amyloid clearance

ADPD5-2094

OPTIMIZATION OF POTENTIAL D-ENANTIOMERIC PEPTIDES BY CHEMICIAL MODIFICATIONS FOR THE TREATMENT OF ALZHEIMER´S DISEASE

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1. Objectives

Alzheimer's disease (AD) is a neurodegenerative disorder, which is the most common cause of dementia. However, there is no drug available for causal treatment of AD. We developed D-enantiomeric peptides which target the cytotoxic amyloid beta (Abeta) oligomer species and optimized them by chemical modifications according to their ability to remove Abeta oligomers *in vitro*.

2. Methods

To investigate the efficacy of the chemically modulated D-enantiomeric peptides, several *in vitro* methods were used. The affinities of the potential drugs to various Abeta species were determined using surface plasmon resonance spectroscopy (SPR). Furthermore, the potency to remove cytotoxic Abeta oligomers was examined as well as the cytotoxicity of the Abeta-ligand-complex to neuronal cells. Finally, the efficacy of the optimized D-enantiomeric peptide was tested *in vivo* using a transgenic AD mouse model.

3. Results

By adding several chemical modifications, the affinities of the D-enantiomeric peptides to Abeta oligomers were improved. Additionally, an increased potency of cytotoxic Abeta oligomer removal was shown, resulting in decreased cytotoxicity of the Abeta-ligand-complex to neuronal cells. *In vivo* experiments showed that the treatment of transgenic AD mice with the optimized peptide leads to enhanced cognition in the animal model.

4. Conclusions

By introducing several chemical modifications to Abeta binding D-enantiomeric peptides, the *in vitro* efficacy of cytotoxic Abeta oligomer removal was successfully optimized. *In vivo* studies of transgenic AD mice yielded reduced cognitive impairments after the treatment.

05f. Drug Development & Clinical Trials: amyloid clearance

ADPD5-2113

PHARMACOKINETIC AND PHARMACODYNAMIC (PK/PD) ASSESSMENT AND COVARIATE ANALYSIS OF BIIB037 IN A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PHASE 1B STUDY IN PATIENTS WITH PRODROMAL OR MILD ALZHEIMER'S DISEASE

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Background: BIIB037, a fully human IgG monoclonal antibody that binds preferentially to aggregated forms of Aβ, is being investigated in a Phase 1b study as a disease-modifying treatment in patients with prodromal or mild AD.

Objectives: To assess the pharmacokinetic and pharmacodynamic (PK/PD) relationships and the possible covariate effects for amyloid removal following 6 months of treatment of BIIB037 in a Ph1b study.

Methods: In a staggered, parallel-group design, the treatment doses were: 1, 3, up to 10 mg/kg, and placebo. Sparse samples in the multiple ascending dose study and intensive samples from an earlier single ascending dose study were combined to construct a Population PK model. Cumulative AUC up to month 6 was estimated for each individual and used as the exposure variable for the assessment of PK/PD relationship. The relationship between BIIB037 exposure, SUVR change from baseline, and other covariates were evaluated with the Analysis of Covariance (ANCOVA) method.

Results: Cumulative BIIB037 exposures and baseline SUVR levels were found to be correlated with SUVR change from baseline. APOE4 carrier status (carriers vs non-carriers), stage of AD (mild vs prodromal), sex and age were not found to be correlated with change in SUVR.

Conclusions: BIIB037 treatment for 6 months resulted in a dose dependent removal of brain amyloid in AD patients. In addition, there was a significant correlation between BIIB037 exposures and degree of change in SUVR. The BIIB037-dependent change in SUVR was not significantly different in APOE4 carriers versus non-carriers or in prodromal versus mild AD patients.

05f. Drug Development & Clinical Trials: amyloid clearance

ADPD5-2136

CHARACTERIZATION OF A NOVEL D-ENANTIOMERIC PEPTIDE THAT IS ABLE TO ELIMINATE ABETA OLIGOMERS

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Objective:

Although Alzheimer's disease (AD) is the most prominent neurodegenerative disease and the sixth-leading cause of death, until now there is no causal therapy available. The accumulation of amyloid-beta (Abeta) aggregates is a key feature of AD. Especially small neurotoxic oligomers of Abeta are thought to be responsible for the development and progression of the disease and their elimination will therefore be a promising objective for therapy.

Methods:

We screened for new d-enantiomeric peptide derivatives of the compound D3 with enhanced specific binding to Abeta42 monomers and oligomers and characterized them for their Abeta42 oligomer elimination potential. Various biophysical and biochemical methods were used, like SPR, BLI, ThT assay, MTT assay, TEM (transmission electron microscopy) to characterize their potentials.

Results:

One of the newly identified peptides binds to Abeta42 monomers and oligomers with submicromolar affinity. Furthermore it was shown that it efficiently eliminates toxic Abeta42 oligomers *in vitro*. It inhibits Abeta42 self-aggregation to fibrils and reduces Abeta toxicity *in vitro*.

Conclusions:

By further modification of D3, it was possible to identify a new compound, which proved to eliminate toxic Abeta42 oligomeric species highly effective. *In vivo* results will show, whether the new compound's *in vitro* properties can translate into enhanced therapeutic potential in AD animal models.

05f. Drug Development & Clinical Trials: amyloid clearance

ADPD5-2276

STUDY OF ELND005 IN AGITATION AND AGGRESSION OF ALZHEIMER'S DISEASE (HARMONY-AD): DIAGNOSTIC CRITERIA AND PATIENT CHARACTERISTICS

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Objectives: Agitation and Aggression are among the most disruptive neuropsychiatric symptoms (NPS) in AD. ELND005 (Scyllo-inositol) is being evaluated in an ongoing global clinical trial of Agitation and Aggression in AD (Study AG201; NCT01735630).
Methods: The study is a 12-week, double-blinded, placebo-controlled 2 arm study. Patients with AD are eligible if screening and baseline NPI- Agitation/Aggression score is ≥ 4 (at least moderate severity and frequency). Primary outcome measure is summed Agitation and Aggression scores from the expanded NPI (NPI-C), which separates Agitation and Aggression into distinct domains (De Medeiros et al., 2010).
Results: Baseline data from the first 260 AD patients was analyzed: mean age 75 years, 53% female, and 89% Caucasian. Patients mean baseline MMSE was 14.8 (range 0-28), NPI-total was 48.6 (range 4-144), NPI Agitation/Aggression score was 7.3 (range 4-12), and NPI-C Agitation and Aggression summed score was 18.8 (range 2-56). Baseline mean scores of depression, anxiety, apathy, irritability, and aberrant motor behavior were > 4. Distribution of NPI-C scores amongst mild, moderate and severe AD was similar.
Conclusions: The inclusion criteria in this study, as applied by clinical investigators, include AD patients who exhibit at least moderate levels of Agitation and Aggression requiring pharmacologic intervention. These patients also have high prevalence of anxiety, depression, apathy, and aberrant motor behaviors. Patient characteristics of the HARMONY-AD study are similar to the citalopram study (CitAD, Porsteinsson et al., 2014), and consistent with the recently published provisional criteria for Agitation and Aggression in AD (Cummings et al., 2014).

05f. Drug Development & Clinical Trials: amyloid clearance

ADPD5-2284

DEVELOPMENT OF "COGNITIVE CLARITY": A UNIQUE DIETARY SUPPLEMENT FOR REDUCTION OF BRAIN "PLAQUES AND TANGLES" AND ENHANCEMENT OF MEMORY, FOCUS AND CONCENTRATION

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Beta-amyloid and tau are key proteins identified in the aging and Alzheimer's brain that are linked to memory loss and cognitive decline. There is currently no pharmaceutical drug that has been approved that can reduce and remove both beta-amyloid protein-containing "plaques" and tau protein-containing "tangles" in the brain. Cognitive Clarity may be the first nutraceutical product with the potential to reduce both brain "plaques and tangles", two pathological hallmarks of brain aging, memory loss and cognitive decline. Cognitive Clarity is a specific polyphenol-enriched combination of 1) a proprietary extract derived from the Amazon woody vine *Uncaria tomentosa* (cat's claw), and 2) a specific oolong tea extract identified after screening for direct activity against "plaque and tangles". A number of *in vitro* studies including Thioflavin T fluorometry, Congo red binding, Congo red and Thioflavin S staining, circular dichroism spectroscopy, and negative stain electron microscopy all demonstrated that Cognitive Clarity (and its major components) are effective reducers/disaggregators of beta-amyloid and tau protein "plaques and tangles". Transgenic "amyloid plaque" mouse model studies demonstrate that Cognitive Clarity's main plant extract ingredients cause a reduction and clearance of brain beta-amyloid protein and plaques, leading to marked improvement in memory (as assessed by water maze testing and probe trials). A randomized, double-blind, placebo-controlled human trial is currently underway to confirm the effects of Cognitive Clarity on improving memory, concentration and focus in subjects with age-associated memory impairment.

Funded by ProteoTech Inc.

05g. Drug Development & Clinical Trials: immunomodulators

ADPD5-0559

IDENTIFICATION OF A NOVEL PHARMACOLOGICAL INHIBITOR OF NEUROINFLAMMATION BY PHENOTYPIC SCREENING EFFECTIVE IN ALZHEIMER MODELS

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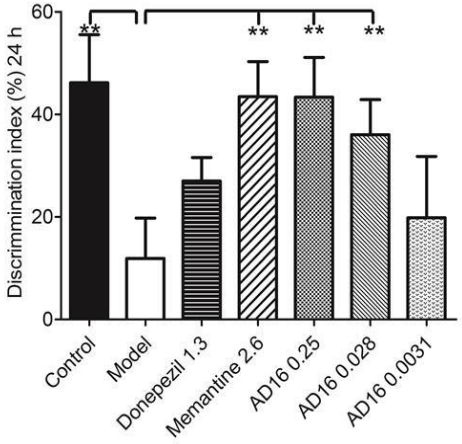
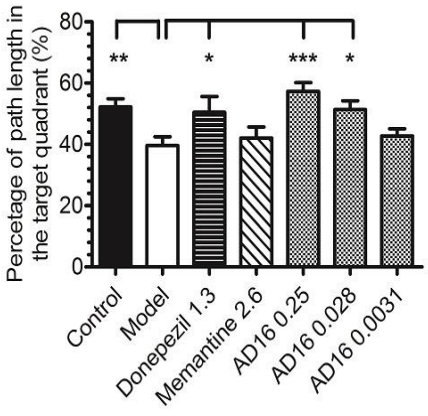
Objective: Phenotypic screening is making its renaissance in drug discovery as an alternative to target-focused approaches. Alzheimer's disease is a complex neurodegenerative disorder characterized by progressive impairment of memory and cognition. Since unclear aetiology of Alzheimer's disease and absence of validated therapeutic protein targets, it makes anti-AD drug discovery challenging in target-based approaches.

Methods: We employed microglia-based phenotypic screening to identify small molecules that can modulate the phenotype of detrimental neuroinflammation in Alzheimer brain.

Results: A novel pharmacological inhibitor (AD16, 0.25 mg/kg, oral) of neuroinflammation was discovered and showed comparable effect of cognitive impairment relief as donepezil (1.3 mg/kg, oral) and memantine (2.6 mg/kg, oral) did in both β amyloid-induced and *APP/PS1* double transgenic Alzheimer murine models. It exhibited desired safety properties and pharmacokinetic profiles of excellent blood-brain barrier penetration ability and acceptable half-life.

Conclusion: Our studies not only provide a potential drug candidate for further anti-AD drug development but also strongly support the concept that inhibition of neuroinflammation might be alternative strategy for combating AD.





Druglikeness	Lipinski's Rule of Five	None violation
	Clog*P*	2.163
Pharmacodynamic properties	Activity *in vitro*	LPS-stimulated microglia IL-1β inhibition IC_{50} = 3.4 nM
	Activity *in vivo*	Therapeutic effect was comparable to that of donepezil and memantine in 2 AD models
Pharmacokinetic properties	BIoavailability in rat	74.9%
	Half-life in rat	4.32 h
	BBB penetration	AUC(Brain/Plasma) = 0.21
	Metabolism stability	76% remains in 4 h in human liver microsome
Safety	*h*ERG	IC_{50} > 100 μM
	Acute toxicity	Maximum tolerance dose > 2000 mg/kg

05g. Drug Development & Clinical Trials: immunomodulators

ADPD5-1805

MICROVESICLES FROM MESENCHYMAL STEM CELLS INDUCE AN ANTI-INFLAMMATORY PHENOTYPE OF MICROGLIA EXPOSED TO HUMAN ABETA 1-42

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Aim: Based on the evidence that microvesicles (MVs) shed from Bone -Marrow Mesenchymal Stem Cells (BM-MSCs) can play an immunomodulatory role, protecting the injured tissue, we aimed at assessing the possible anti-inflammatory effects of BM-MSCs-deriving MVs in microglia cultures exposed to hAbeta 1-42.

Methods: MVs were isolated by ultracentrifugation from mouse BM-MSCs and then characterized by FACS analysis. MVs were then incubated with primary microglia in the presence of human Abeta 1-42 peptide. The expression of different inflammatory or anti-inflammatory markers was investigated by ELISA, FACS, RT-PCR and immunocytochemistry.

Results: Microglial cells assume an amoeboid phenotype, changing its morphology and releasing anti-inflammatory cytokines, such as IL10, without significantly affecting the release of the pro-inflammatory cytokines TNFa and IL6. Also, MVs lead to the decrease in the expression of markers like MHC II, which is associated with a pro-inflammatory phenotype.

Conclusions: BM-MSC MVs down-regulate the inflammatory processes induced by exposure of microglia to h 1-42 Abeta peptide *in vitro*. The involved molecular mechanisms are under investigation.

05g. Drug Development & Clinical Trials: immunomodulators

ADPD5-2324

A NEW ANTI-INFLAMMATORY DRUG WITH NEUROPROTECTIVE ACTIVITY IN THE OLIGOMERIC Ab25-35 PEPTIDE-INDUCED ALZHEIMER'S DISEASE MOUSE MODEL

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Objectives: Epidemiological and clinical studies have established the role of pro-inflammatory cytokines in the pathophysiology of Alzheimer's disease (AD). Anti-inflammatory agents like anti-TNF biologics have emerged as therapeutic alternatives for AD patients. AMYLZEN® is a nonapeptide analog of thymulin with potent analgesic and broad anti-inflammatory action that crosses the BBB. AMYLZEN® is safe and well tolerated in humans. We set out to evaluate the pharmacological activity of AMYLZEN® in a preclinical non-transgenic mouse model of AD.
Methods: has been tested in a validated mouse model where the intracerebral injection of Ab25-35-peptide (ABP) induces neuroinflammation, neurotoxicity and behavioral impairment. Cytokine levels, lipid peroxidation and formation of b-amyloid plaques were measured in hippocampus of ABP-injected mice 5 to 7 days post treatment. Two functional tests were used to evaluate spatial working (spontaneous alternation performance in the Y maze) and contextual long-term memory (passive avoidance test).
Results: Coinjection of AMYLZEN® at a low dose (5 ug/kg, intracerebral) with ABP completely blocked the overproduction of TNFa and IL-1b. Oxidative stress and formation of neurotoxic b-amyloid plaques were significantly reduced. AMYLZEN® had a protective effect on ABP-induced learning deficits. Systemic treatment with AMYLZEN® (1 to 10 mg/kg, intraperitoneal during 7 days) had protective effects similar to Donepezil (0.5 mg/kg, intraperitoneal), one of the few approved symptomatic treatment.
Conclusions: The encouraging neuroprotective activity demonstrated in a validated preclinical mouse model together with the excellent pharmacological, safety and toxicological profile of AMYLZEN® favorably position this compound as a new candidate for clinical investigation in AD patients.

05h. Drug Development & Clinical Trials: hormones

ADPD5-2168

ALLOPREGNANOLONE: A REGENERATIVE THERAPEUTIC FOR MILD COGNITIVE IMPAIRMENT AND EARLY ALZHEIMER'S DISEASE

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Objectives:
Regenerative therapeutics hold the promise of self-renewal and repair. While ageing and age-associated neurodegenerative diseases are marked by decline in these functions, capacity for regeneration is retained. Discovery research demonstrated that the neurosteroid, allopregnanolone (ALLO), induced neurogenesis in brain and restored cognitive function while simultaneously reducing AD pathology burden. Objectives of this program of research is to develop the neurosteroid, allopregnanolone (ALLO), as regenerative therapeutic for Alzheimer's disease.
Methods: Phase 1b clinical trial to establish safety and maximally tolerated dose.
Results: Outcomes of discovery and translational research led to an NIA funded Phase 1b clinical trial of Allo in persons with MCI and early Alzheimer's. Primary safety objectives are to determine maximally tolerated dose and incidence and severity of treatment emergent adverse events. Exploratory safety and feasibility analyses of cognitive function and MRI-based biomarkers relevant to regeneration will be conducted. Trial outcomes will provide: 1) an estimated safe and well-tolerated dose of Allo; 2) parameter estimates for cognitive efficacy to advance to a Phase 2 proof of concept trial of Allo; and 3) parameter estimates for MRI-based biomarkers.
Conclusion: Allopregnanolone as a regenerative therapeutic is based on a strong foundation of well defined mechanistic targets for neurogenesis and disease modification, dosing requirements, optimal treatment regimen, route of administration and the appropriate formulation necessary to advance to Phase2 proof of concept clinical studies to determine efficacy of allopregnanolone as a regenerative and disease modifying therapeutic for Alzheimer's disease.
Research supported by NIA U01 AG031115 and UF1 AG046148 to RDB.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-0231

NEUROPROTECTIVE EFFECTS OF HARMINE ALKALOIDS ON SODIUM NITRITE-INDUCED HYPOXIA AND ETHANOL-INDUCED NEURODEGENERATION IN ANIMAL MODELS RELEVANT TO ALZHEIMER'S DISEASE

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Background: Cognitive impairment is a multidimensional and complicated concept that subsumes the attention, concentration, learning, memory, problem-solving ability, visuospatial abilities, mental flexibility, psychomotor efficiency and manual dexterity.
Objective: To study the effects of harmine alkaloids from the seeds of Peganum harmala on cognitive deficit mice.
Methods: Harmine alkaloids were screened for Sodium nitrite induced hypoxia and Ethanol induced neurodegeneration using behavioral models such as rotarod, Passive shock avoidance paradigm, special water bottle case model, elevated plus maze and Morris water maze. HM (5, 2.5 and 1.25 mg/kg p.o.) were administered to the mice. Acquisition, retention, Transfer latency (TLT), Time spent in target quadrant (TSTQ), Step down latency (SDL) and Escape latency (ELT) and biochemical parameters such as AChE, MAOA, TBARS, epinephrine, 5 HT were determined. DNA fragmentation studies were conducted which were compared with donepezil.
Results: HA (5, 2.5 and 1.25 mg kg(-1) p.o.) significantly ($p < 0.001$) protected the Sodium nitrite induced memory impairment by decreasing the time require to find the water bottle in special water bottle case model. HA improved acquisition and retention memory significantly ($p < 0.001$) by decreasing the Transfer Latency Time (TLT), escape latency time, TSTQ and increased the Step Down Latency (SDL. HA inhibited Acetylcholinestrase ($p < 0.01$) activity, increased GSH,decreased TBARS and inhibited MAOA activity. Reduced the metabolism of epinephrine, 5-HT. HA (5 mg kg(-1)) protected the DNA fragmentation of frontotemporal cortex from hypoxic effects.
Conclusion: Harmine alkaloids are beneficial in the management of Neurodegenerative disorders particularly for Alzheimer's disease.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-1465

DEVELOPMENT OF NOVEL CURCUMINOIDS FOR THE TREATMENT OF ALZHEIMER'S DISEASE AND OTHER NEUROCOGNITIVE DISORDERS

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Curcumin, a *bis*-phenolic compound isolated from the rhizomes of *Curcuma longa* is known for its potent antiretroviral, anticancer, anti-diabetic, anti-inflammatory, and memory-improving properties. However, in spite of its impressive biological activity, curcumin is not used in therapeutics because of its poor oral absorption, short plasma half-life and facile conversion into its glucuronide and/or hydrogenated metabolites that are devoid of the biological activity. We have systematically and covalently altered the chemical structure of curcumin to furnish a focused library of curcumin-inspired compounds referred to as curcuminoids. Initially, we replaced the 1,3-diketone moiety in curcumin with a monoketone moiety. We then synthesized conformationally-restricted analogs of curcumin by the addition of a carbocyclic or heterocyclic ring. We also synthesized lipophilic analogs of curcumin via the replacement of one of the phenolic moieties in curcumin with strategically placed fluoro-substituents. The effect of the parent compound curcumin and the synthetic curcuminoids was analyzed on neuroprotective genes, AKT1, MAP Kinase, and Brain-derived Neurotrophic Factor (BDNF). The data shows that the pretreatment of one of our synthetic analogs resulted in the upregulation of mRNA as well as the protein levels of all three genes investigated. The most dramatic impact was observed on with BDNF. These studies indicate a novel application for these analogs in the treatment of neurocognitive disorders, such as the Alzheimer’s disease. Our recent findings on the plausible mechanism(s) of action of these analogs will also be presented.

05j. Drug Development & Clinical Trials: neurotransmitter modulators

ADPD5-0657

MECHANISTIC CHARACTERIZATION OF S 38093, A NOVEL INVERSE AGONIST AT HISTAMINE H3 RECEPTORS

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Histaminergic H3 inverse agonists, by stimulating central histamine release, represent attractive drug candidates to treat cognitive disorders. The present studies aimed to describe the mechanistic profile of S 38093 a novel H3 receptors inverse agonist.
S 38093 displays a moderate affinity for rat, mouse and human H3 receptors (Ki = 8.8, 1.56 and 1.2 µM, respectively) with no affinity for other histaminergic receptors.
In cellular models, the compound was able to antagonize mice H3 receptors (KB = 0.65 µM) and to suppress cAMP decrease induced by an H3 agonist via human H3 receptors (KB = 0.11 µM). The antagonism properties of the compound were confirmed by electrophysiological studies on rat hippocampal slices (from 0.1 µM). In cells expressing a high H3R density, S 38093 behaved as a moderate inverse agonist at rat and human H3 receptors (EC50 = 9 and 1.7 µM, respectively).
S 38093 was rapidly absorbed in mouse and rat (Tmax= 0.25-0.5h), slowly in monkey (2h), with a bioavailability ranging from 20 to 60% and t1/2 ranging from 1.5h to 7.4h. The compound was widely distributed with a moderate volume of distribution and low protein binding. The brain distribution of S 38093 was rapid and high.
In mice, S 38093 significantly increased *ex vivo* N-tele-Methylhistamine cerebral levels from 3 mg/kg p.o. and antagonized R-alpha-Methylhistamine-induced dipsogenia from 10 mg/kg i.p..
Taken together, these data suggest that S 38093, a novel H3 inverse agonist, is a good candidate for further *in vivo* evaluations, in particular in animal models of cognition.

05j. Drug Development & Clinical Trials: neurotransmitter modulators

ADPD5-0706

IN VIVO PHARMACOLOGICAL PROFILE OF S 38093, A NOVEL INVERSE AGONIST AT HISTAMINE H3 RECEPTORS

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S 38093, a novel inverse agonist at histamine H3 receptors, was tested in a series of neurochemical and behavioral paradigms designed to evaluate its procognitive and arousal properties.

In intracerebral microdialysis studies performed in rats, S 38093 dose-dependently increased histamine extracellular levels in the prefrontal cortex and facilitated cholinergic transmission both in prefrontal cortex and hippocampus of rats after acute and chronic administration (10 mg/kg i.p.).

Oral administration of S 38093 at 0.1 mg/kg significantly improved spatial working memory in rats in the Morris water maze test. The compound also displayed cognition enhancing properties in the two-trial object recognition task in rats, in a natural forgetting paradigm at 0.3 and 1 mg/kg p.o. and in a scopolamine-induced memory deficit situation at 3 mg/kg p.o.. The property of S 38093 to promote episodic memory was confirmed in a social recognition test in rats at 0.3 and 1 mg/kg i.p..

Arousal properties of S 38093 were assessed in freely moving rats by using electroencephalographic recordings: at 3 and 10 mg/kg i.p., S 38093 significantly reduced slow wave sleep delta power and induced at the highest dose a delay in sleep latency. S 38093 at 10 mg/kg p.o. also decreased the barbital-induced sleeping time in rats.

Taken together these data indicate that S 38093, a novel H3 inverse agonist, displays cognition enhancing at low doses and arousal properties at higher doses.

S 38093 is currently in clinical trial (Phase II) for the symptomatic treatment of mild to moderate Alzheimer's disease.

05j. Drug Development & Clinical Trials: neurotransmitter modulators

ADPD5-0897

PROTECTION OF RADIAL GLIAL-LIKE CELLS IN THE HIPPOCAMPUS OF APP/PS1 MICE: A NOVEL MECHANISM OF MEMANTINE IN THE TREATMENT OF ALZHEIMER'S DISEASE

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The failure of adult neurogenesis in the hippocampal dentate gyrus (DG) is closely correlated with memory decline in Alzheimer's disease (AD). Radial glial-like cells (RGLs) localized to the adult DG generate intermediate progenitor cells and immature neurons and thus contribute to adult hippocampus neurogenesis. Memantine (MEM) has been indicated to dramatically increase hippocampal neurogenesis by promoting the proliferation of RGLs. In this study, MEM significantly improved the nest building scores of APPswe/PS1dE9 transgenic (APP/PS1) mice at 9 and 13 months of age .We further found that MEM could enhance hippocampal neurogenesis and increase the number of RGLs in the DG subgranular zone (DG-SGZ) of both APP/PS1 and WT littermates at 9 and 13 months of age compared to saline-treated age-matched APP/PS1 and WT mice. Moreover, MEM decreased amyloidogenesis in 13-month-old APP/PS1 mice and protected cultured radial glia cells (RGCs, L2.3 cells) from apoptosis induced by the β amyloid peptide (Aβ). Additionally, MEM inhibited microglial activation in a vertical process in DG-SGZ and retinas of APP/PS1 mice and induced the reduction of RGLs and Müller cells. Reelin is involved in the proliferation of RGLs in the hippocampus, which was typically upregulated in the hippocampus of APP/PS1 mice by MEM and thought to be an active signaling pathway associated with the MEM-induced increase in RGLs. Our data suggest MEM can enhance hippocampal neurogenesis in APP/PS1 mice by protection of RGLs within the adult DG-SGZ through inhibition of microglial over-activation and enhancing reelin expression.

05j. Drug Development & Clinical Trials: neurotransmitter modulators

ADPD5-1218

THE 5-HT6 RECEPTOR ANTAGONIST IDALOPIRDINE FACILITATES NEURONAL OSCILLATIONS AND NEUROTRANSMISSION IN BRAIN REGIONS THAT MEDIATE COGNITION.

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Objectives
The 5-HT_6 receptor is a promising target for cognitive disorders. Idalopirdine is a selective 5-HT_6 receptor antagonist in development for the symptomatic treatment of Alzheimer's Disease (AD) as adjunctive therapy to acetylcholinesterase inhibitors. We investigated the effects of idalopirdine on neuronal oscillations and neurotransmission in the rat hippocampus and cortex and studied the neuronal substrates for these effects.
Methods
In situ hybridization was used to study cellular expression and co-localisation of 5-HT_6 receptor mRNA with a range of neuronal and interneuronal markers. Acetylcholine and glutamate levels were measured in dorsal hippocampus and frontal cortex of freely-moving male Sprague Dawley (SD) rats by microdialysis. Local field potentials were recorded in anesthetized SD rats with tungsten electrodes during electrical stimulation in the nucleus pontis oralis. Recordings were Fast Fourier transformed to yield the power of oscillatory activity.
Results
The 5-HT_6 receptor was located postsynaptically, co-localising mainly with glutamatergic and less frequently with GABA-ergic markers. In the frontal cortex, idalopirdine increased extracellular levels of glutamate and transiently increased gamma oscillations. Furthermore, idalopirdine potentiated and prolonged the effects of donepezil on acetylcholine efflux, theta- and gamma oscillations in the dorsal hippocampus and frontal cortex.
Conclusion
Idalopirdine potentiates the effects of donepezil on acetylcholine efflux and neuronal oscillations and facilitates glutamatergic signaling in areas critical to memory and executive function such as the hippocampus and frontal cortex. These effects are likely to be mediated via glutamatergic and GABA-ergic systems, in which the 5-HT_6 receptor was demonstrated to be expressed.

05k. Drug Development & Clinical Trials: GPCR ligands

ADPD5-0810

DONECOPRIDE: A NOVEL MULTI TARGET-DIRECTED LIGAND AGAINST AD

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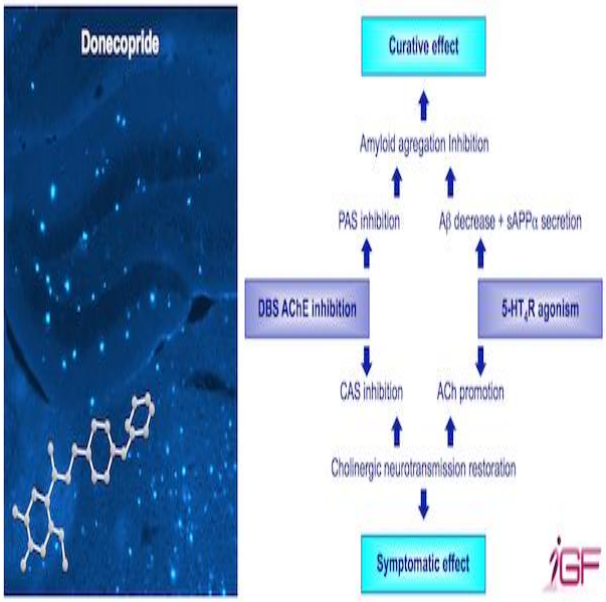
Objectives: Complex pathologies such as Alzheimer's disease (AD) would benefit from a combination of actions targeting, in the same time, several molecular causes implied in the pathogenesis. Our aim was to design a multi target-directed ligand (MTDL) gathering two properties: acetylcholinesterase (AChE) inhibition and 5-HT_4 receptor activation. AChE inhibition is the action mechanism of donepezil, the current available drug for AD. Activation of 5-HT_4 receptors promotes the non-amyloidogenic cleavage of the amyloid precursor protein (APP) and the release the neuroprotective soluble APPα fragment. Moreover, we have demonstrated that chronic administration of 5-HT_4R agonists delays amyloid pathology and prevents cognitive deficits in a mouse model of AD.
Methods: Combining a dual-binding site pharmacophore of AChE inhibitors with a pharmacophore of 5-HT_4R ligands, we isolated a candidate compound and performed pharmacomodulation of this hit to optimize its properties.
Results: We selected donecopride as a druggable lead able to inhibit AChE (IC_{50} = 16 nM) and to induce sAPPα release (EC_{50} = 11.3nM) upon 5-HT_4R activation (K_i = 10.4 nM; 48.3% of serotonin response). *In vivo* properties of this new compound in the 5XFAD mouse model of AD (acute and chronic administration) will be presented.
Conclusions: Donecopride is an innovative MTDL for AD, which combine symptomatic properties (restoration of cholinergic transmission) with disease-modifying actions (sAPPα release, decrease of Aβ accumulation and aggregation).



05k. Drug Development & Clinical Trials: GPCR ligands

ADPD5-1875

DISCOVERY OF DONECOPRIDE A NOVEL MULTI-TARGET DIRECTED LIGANDS FOR ALZHEIMER'S DISEASE

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Objectives

Targeting more than one molecular cause implied in the pathogenesis of Alzheimer's disease (AD) with a sole drug is considered a promising challenge, because it may address the numerous failures that recently occurred during clinical trials that were conducted in this area. In this aim we decided to develop such peiotropic compound associating acetylcholinesterase inhibition and 5-HT_4R activation which could lead to symptomatic and disease modifying properties.

Methods

Starting from an *in silico* high throughput screening and a rational drug design strategy we have identified that RS67,333, a reference 5-HT_4R partial agonist, possess moderate AChE inhibition properties. Pharmacomodulation of this hit and rapid evaluation of *in vitro* biological activities against both targets as well as drugability led to the identification of a novel family of pleiotropic ligands.

Results

These efforts allowed us to select donecopride[1] as a valuable dual (h)5-HT_4R partial agonist (Ki = 10.4 nM)/(h)AChEI (IC_{50} = 16 nM) that further promotes sAPPα release (EC_{50} = 11.3nM). Donecopride could improve memory performances at 0.3 and 1 mg/kg on the object recognition test. In vivo results obtained in the 5XFAD mouse model of AD will be for the first time presented.

Conclusion

On the basis of these in vitro and in vivo activities, donecopride seems to be a promising drug candidate for AD treatment.

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ADPD5-0259

EVALUATION OF NOVEL POLYAMINE AND MEMANTINE-DERIVATIVE COMPOUNDS TARGETING NMDA RECEPTORS FOR ALZHEIMER'S DISEASE TREATMENT

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Objectives: To evaluate novel compounds as potential treatment for Alzheimer's disease targeting *N*-methyl-D-aspartate receptor (NMDAR). These ionotropic glutamate receptors are highly permeable to Ca^{2+}, thus are implicated with excitotoxicity in neurodegenerative disorders.
Methods: The *Xenopus laevis* oocyte expression system was employed in this study where cRNAs for NMDAR subunits were injected into the oocytes and responses to NMDA/glycine and channel blockers were recorded using two-electrode voltage clamp. Three novel polyamine compounds (CR) and eight memantine-derivatives (MAB) incorporated with antioxidant groups (alpha-tocopherol, lipoic acid, melatonin and methoxyphenol) were tested on GluN1/2A NMDAR subunits at -60 mV and compared with Mg^{2+} and memantine.
Results: All spermine-derived CR compounds proved to be highly effective in blocking the NMDAR channel; CR18, CR8 and CR24 recorded IC_{50}s of 0.692, 2.214 and 2.352 µM respectively, all significantly lower than Mg^{2+} (IC_{50} = 10.08 µM). MAB compounds were much less potent; MAB22, MAB30, MAB14 and MAB7 recorded the lowest IC_{50}s (26.90, 38.19, 43.09 and 55.92 µM respectively) versus memantine (IC_{50} = 3.178 µM) while the rest were all higher than 100 µM. This suggests the loss of memantine functionality due to attachment of the antioxidant structure to its amine group. All compounds were also found to act through a voltage-dependent manner assessed via the Woodhull model.
Conclusions: Our data suggests that the CR compounds particularly CR18 (spermine polyamine incorporated with alpha-tocopherol) has the potential for development of Alzheimer's disease treatment which not only can inhibit excitotoxicity, but also suppresses oxidative stress and beta-amyloid plaques.

05l. Drug Development & Clinical Trials: nicotinic & ionotropic ligands

ADPD5-2154

S44858, A POSITIVE ALLOSTERIC MODULATOR OF AMPA RECEPTORS AND NR2B SELECTIVE NON-COMPETITIVE NMDA ANTAGONIST: IN VITRO AND IN VIVO CHARACTERIZATION.

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S44858 presents unique and innovative mechanism of action, as it both potentiates glutamate AMPA receptors and blocks glutamate NMDA receptors. The aim of the present work was to characterize its mechanism of action and pharmacological properties.

In vitro, S44858 did not present affinity towards glutamate binding sites on AMPA, kainate and NMDA receptors nor towards glycine and PCP binding sites. A low affinity for the ifenprodil binding site was detected. On oocytes injected with rat cortex or human hippocampal mRNA, S44858 increased AMPA responses, did not affect kainate responses and decreased NMDA responses. S44858 displayed selectivity toward NR1/NR2B subunits both on murine and human NMDA receptors. The inhibition was neither competitive nor voltage-dependent. S44858 protected cultured rat brain neurons towards glutamate and NMDA toxicity and increased AMPA-mediated BDNF expression.

In vivo, S44858 (30 mg/kg ip) both increased the synaptic response and its Long-Term Potentiation in the dentate gyrus of the hippocampus on anaesthetized rats. S44858 (0.3-1 mg/kg po) improved episodic-like memory in a novel object recognition test in rats. No effect on general behaviour, body temperature, spontaneous locomotor activity or occurrence of epileptic seizures was noticed after acute administration in mice and rats (10-100 mg/kg po).

Taken together, these results indicate that S44858 is neuroprotective against glutamate excitotoxicity, it enhances synaptic plasticity and cognition in the absence of side effects in rodents after acute administration. S44858 may therefore be of interest for the treatment of various CNS diseases in which dysfunctions of glutamatergic pathways have been implicated.

05n. Drug Development & Clinical Trials: mitochondrial drugs

ADPD5-0587

THE AMYLOID BINDING ALCOHOL DEHYDROGENASE ENZYME AS A THERAPEUTIC TARGET FOR TREATING ALZHEIMER'S DISEASE

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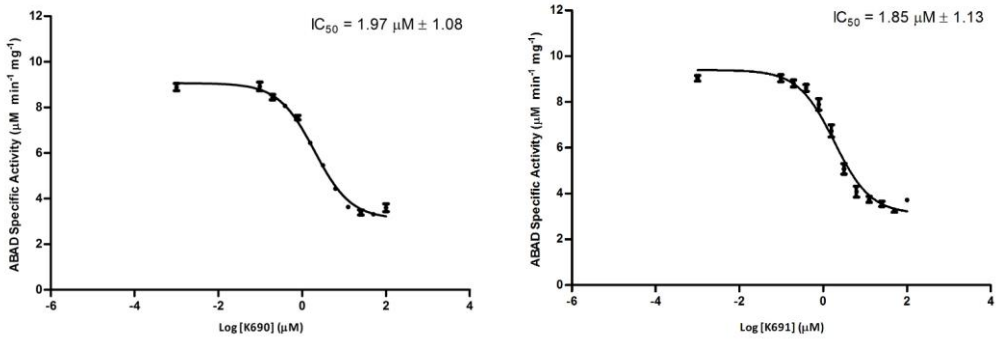
Objectives – The aim of the project is to develop novel therapeutic agents against Alzheimer's disease by targeting a mitochondrial enzyme termed the Amyloid Binding Alcohol Dehydrogenase (ABAD).

Methods – Two therapeutic approaches appear to hold merit in treating Alzheimer's disease, firstly to inhibit the interaction between the ABAD enzyme and amyloid beta peptide oligomers and secondly, to inhibit the ABAD enzyme directly. Initially, we are pursuing the second approach.

Results – We have developed a novel series of compounds, derived from the immunosuppressant Frentizole molecule, and have shown two of them, compounds K690 and K691, to be relatively potent inhibitors of the ABAD enzyme with IC_{50} values in the low micro-molar range. We have also characterised the mechanism of inhibition utilised by these two inhibitors using an enzyme kinetics based approach.

Conclusions – We have shown that through a few small modifications the parent, non-inhibiting, Frentizole molecule can be turned into a relatively potent inhibitor of the ABAD enzyme, offering a novel scaffold for the development of subsequent generations of compounds with improved pharmacological properties.



05n. Drug Development & Clinical Trials: mitochondrial drugs

ADPD5-1664

ABAD MODULATORS TARGETED TO AD MITOCHONDIAL DYSFUNCTION – DESIGN, SYNTHESIS AND IN VITRO SCREENING

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Although the aetiology of AD is still unknown, the build-up of amyloid β-peptide (Aβ) is considered to play a central role in the pathogenesis of the disease. It is well established that the intracellular accumulation of Aβ is associated with AD and increasing evidence suggests that mitochondria may be an important target for intracellular Aβ to exert its neurotoxic effects.

Amyloid-binding alcohol dehydrogenase (ABAD) is to date the most characterized Aβ-binding intracellular protein. Direct interaction of this mitochondrial enzyme with Aβ was confirmed by many different methods. Aβ binding to ABAD triggers a series of events leading to mitochondrial dysfunction characteristic for AD. Thus this interaction may represent a target for treatment strategy against AD.[1-4]

The benzothiazolyl analogues related to known immunosuppressant frentizole were synthesized and *in vitro* evaluated for their capability to inhibit ABAD. Several prepared compounds showed ability to inhibit ABAD *in vitro* on low μM or nM scale or conversely activation of the enzyme.[5] These promising compounds are going to be further tested on inhibition/influence of Aβ-ABAD interaction.

This work was supported by project OP VaVpI (no. CZ.1.05/3.1.00/10.0213).

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05p. Drug Development & Clinical Trials: cell-based therapies

ADPD5-0223

PLURIPOTENT STEM CELL-DERIVED BASAL FOREBRAIN CHOLINERGIC NEURONS AMELIORATE THE COGNITIVE SYMPTOMS ASSOCIATED WITH ALZHEIMER'S DISEASE IN MOUSE MODELS

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The early and substantial degeneration of basal forebrain cholinergic neurons (BFCNs) in Alzheimer's disease (AD) patients is the key factor in cognitive decits associated with the disease, implying that BFCNs hold potentials in developing stem cell-based therapy for AD. However, the mechanisms underlying BFCN generation *in vivo* are not well characterized and there is therefore a lack of knowledge on how to direct BFCNs generation from pluripotent stem cells (PSCs) *in vitro*. To date, the direct differentiation of BFCNs from mouse PSCs has not been achieved, and it was still unknown if PSC-derived BFCNs can restore cholinergic function and alleviate cognitive deficits in AD model mice. Here, we show that cholinergic neurons with a basal forebrain regional identity can be efficiently derived from both mouse and human pluripotent stem cells in a serum-free system. The PSC-derived cholinergic neurons exhibit BFCN-like features, including gene expression and acetylcholine secreting, and possess abilities to fire action potentials and form synapse *in vitro*. After transplanted into the basal forebrain of AD model mice, the PSC-derived basal forebrain progenitors predominantly differentiate into mature cholinergic neurons that functionally integrate into the endogenous basal forebrain cholinergic projection system. The AD model mice grafted with both mouse and human BFCNs exhibit significant improvements in the spatial learning and memory abilities. These results suggest that PSC-derived BFCNs may contribute to ameliorate the cognitive decline of AD mice, and may be a promising tool for the development of stem cell-based therapies for the treatment of AD.

05p. Drug Development & Clinical Trials: cell-based therapies

ADPD5-1417

EXAMINING THE ROLE OF CXCR4 IN THE DIFFERENTIAL MIGRATION OF ESC-DERIVED AND FETAL-DERIVED HUMAN NEURAL STEM CELLS.

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Objectives

Many studies have begun to examine neural stem cell (NSC) transplantation in animal models of neurodegeneration. Yet, disorders such as Alzheimer's Disease (AD) involve multiple brain regions and thus successful use of NSCs may depend on the ability of these cells to migrate large distances. Our lab has found that fetal-derived NSCs (f-NSCs) migrate far greater distances than pluripotent -derived NSCs (p-NSCs). However, p-NSCs offer several advantages including scalability and potential use of a patient's own iPS cells. To determine why these two NSC populations exhibit such different migration we examined a gene expression database and found that f-NSCs typically express higher levels of CXCR4 than p-NSCs. As CXCR4 is implicated in cell migration, we hypothesized that overexpression could be used to increase the migratory capacity of p-NSCs.

Methods

H9-derived NSCs were modified to stably overexpress CXCR4 via lentiviral transduction. In vitro chemotaxis in response to CXCL4 was then examined. Cells were also transplanted into [M1] xenotransplantation-compatible AD transgenic mice (Rag-5xFAD) generated in our lab to assess the effects of CXCR4 on migration in vivo.

Results

Migration assays revealed that CXCR4-p-NSCs migrate greater distances than control p-NSCs. CXCR4-p-NSCs also engraft well in Rag-5xFAD and ongoing analysis will determine the effect of CXCR4 overexpression on migration in vivo.

Conclusions

CXCR4 appears to play an important role in the differences between p-NSC and f-NSC migration. Increasing CXCR4 expression may also provide a novel approach to bolster the migratory capacity of p-NSCs and their potential application for neurodegenerative disorders such as AD.

ADPD5-0282

REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION ENHANCED ATTENTION AND PSYCHOMOTOR SPEED IN PATIENTS WITH EARLY ALZHEIMER'S DISEASE

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Objectives
Repetitive transcranial magnetic stimulation (rTMS) is a promising tool to study and modulate brain plasticity. The aim of this study was to investigate the effects of high-frequency rTMS over the right inferior frontal gyrus (IFG) and the right superior temporal gyrus (STG) on cognitive functions in patients with mild cognitive impairment (MCI) and early stages of Alzheimer's disease (AD).
Methods
Twenty patients (11 women, 9 men; age 73.0 ± 6.9 years; 12 mild AD, 8 MCI) participated in the placebo-controlled study. Each patient received 3 sessions of 10 Hz rTMS (2250 stimuli per session at 90% of resting motor threshold) applied over the IFG, STG and vertex (a control stimulation site) in a randomized order. The Trail making test (TMT) and the Stroop test (ST) were used to evaluate attention prior to and immediately after the each session.
Results
Significant improvement in the Word part of the ST was found after the stimulation over IFG and STG (Wilcoxon paired test; p=0.023 and 0.033, respectively), while the IFG stimulation induced additional enhancement in the TMT A and B (Wilcoxon paired test; p = 0.002 and 0.005, respectively). When controlling for placebo effects, the improvement in the ST (Word part) remained significant after both IFG and STG stimulation.
Conclusions
rTMS of the right IFG and the right STG enhanced psychomotor speed and attention in patients with MCI and early AD and may thus have a therapeutic potential that should be subjected to the further research.

05r. Drug Development & Clinical Trials: transcranial magnetic stimulation

ADPD5-2024

A NEUROREHABILITATION CLINIC FOR PATIENTS WITH ALZHEIMER'S DISEASE: AN ITALIAN INITIATIVE FOR A NEW THERAPEUTIC PARADIGM

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Objectives.To date, there are only few promising data (Lefaucher et al., 2014) supporting a beneficial effect of the application of repetitive transcranial magnetic stimulation (rTMS) on patients with Alzheimer's disease (AD), especially in an integrative approach with cognitive rehabilitation (shortly: "neurorehabilitation"). Thus, the level of evidence is currently insufficient to warrant any recommendation for a therapeutic use. The main issues are: limitation of placebo-controlled studies and lack of harmonized procedures (stimulation parameters, methodology, patient selection), as well as small sample and effect sizes. Aims of the present project are to implement harmonized procedures for neurorehabilitation and to define an optimal protocol at two Italian centres of excellence for AD care.
Methods.Firstly, the project will develop harmonized procedures for neurorehabilitation and clinical outcome evaluation, qualifying both centres. Secondly, 200 AD patients will be enrolled and the efficacy of high-frequency rTMS or computer-assisted cognitive rehabilitation or a combination of the two will be assessed. Finally, to enable the replication of our results, the protocol agreed upon at the two centres will be disseminated.
Results.Exclusion/inclusion criteria, a sufficiently large sample size and common procedures for neurorehabilitation and clinical outcomes evaluation have been defined. Moreover, common methods have been detailed: the project will be longitudinal, randomized, placebo-controlled, single blinded, with a multiple-baseline and adaptive design.
Conclusions.Standardized procedures for the first multi-centric study on "neurorehabilitation" for AD have been defined by two Italian clinical centres. This innovative project will contribute to the field in the attempt to surmount its current controversial issues.

05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-0573

NOVEL DRUG CANDIDATE FOR THE TREATMENT OF ALZHEIMER'S DISEASE RESTORED DISINHIBITED BEHAVIOR AND IMPROVED LEARNING AND MEMORY DEFICITS IN 5XFAD-MICE

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Objectives: Design and in-vitro and in-vivo evaluation of a multi-functional drug candidate, SSA37, for the treatment of Alzhiemer's disease (AD) based on a naturally occurring molecule.
Methods: Synthesis; pharmacological evaluation at G-Protein-Coupled-Receptors; evaluation of antioxidant and neuroprotective activity; in-vivo studies in transgenic mice (5xFAD).
Results: We found that SSA37 is a most potent P2Y1-R agonist (EC50 2.6nM) and shows no activity at P2Y11-R. SSA37 inhibited Fenton reaction (IC50 37microM) acting mainly as Fe(II)-chelator, and inhibited reactive oxidative species production in PC12 cells (IC50 40nM), possibly through P2Y12-R. SSA37 potently rescued cortical neurons from cell death caused by FeSO4 (EC50 40nM), and from Abeta42 toxicity. Co-application of 5microM SSA-37 with 50microM Abeta42 for 48h to a primary neuron culture resulted in almost 80% protection (IC50 0.5microM). Next, we treated 6 weeks old 5xFAD mice with SSA37A by daily injection (IP, 1 mg/kg) for 7.5 weeks (male n=8-9 and female n=6-8). We demonstrated that SSA37 treatment markedly restored disinhibitied behavior in the elevated plus maze both in male and female mice, as indicated by a 85% decrease in time spent in the open arms ($p<0.05$). SSA37-treated female mice also showed improvement in learning and memory, represented by a 32% reduction in the number of days required for learning in the water T maze assay ($p=0.07$).
Conclusions: SSA37 is a biocompatible multifunctional drug candidate, which showed high efficacy using behavioral and biochemical parameters in models of AD. We believe that this compound may delay or slow down the progression of AD in patients.

05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-0694

DESIGN AND SYNTHESIS OF MULTITARGET-DIRECTED DIALLYL DISULFIDE DERIVATIVES: A BIOCHEMICAL, MOLECULAR MODELLING AND BEHAVIOURAL STUDY

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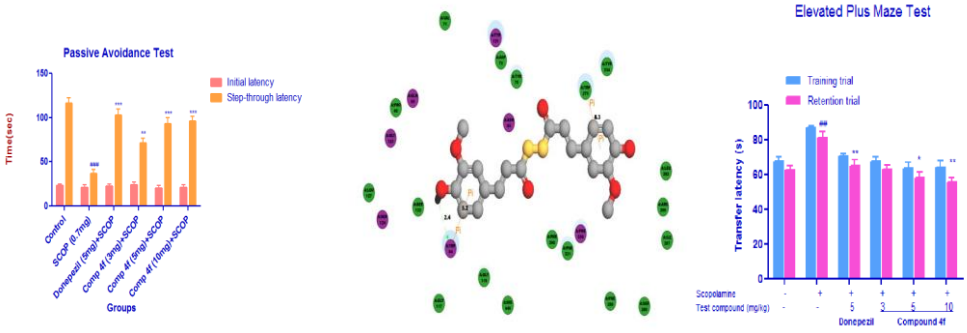
Objective: Designing drugs with a specific multi-target profile is a promising approach against multifactorial illnesses as Alzheimer's disease. Diallyl disulfide (DADS), an active principle of garlic, has been reported to prevent APP processing in AD. But its use is restricted due to its volatile and unstable nature. Based on the above understanding, novel DADS derivatives with greater stability were synthesized and tested to assess their potential as anti-Alzheimer's agents.
Methods: Diallyl Disulphide derivatives were synthesized by a novel scheme. *In vitro* Aβ anti-aggregation studies were carried out using ThT -florescence assay. The oxygen radical absorbance capacity assay using ORAC-FL was performed to determine antioxidant activity of compounds. *In vivo* assessment of cognitive deficits, associated to Alzheimer's disease, induced by scopolamine was performed on male wistar rats. Molecular docking studies with targets such as $A\beta_{1\text{-}40}$ and Acetylcholinesterase (AChE) were carried out using Discovery studio 2.1
Results: Biochemical evaluation of synthesized DADS derivatives indicated that most of the target compounds exhibit significant inhibition of self-induced and Cu^{2+}-induced β-amyloid (Aβ) aggregation, acted as potential antioxidants and AChE inhibitor. Molecular docking studies and ADMET analysis have further confirmed their activities and drug like properties. Furthermore*, in vivo* behavioural studies with best active derivative **compound 4f** showed attenuation of scopolamine-induced amnesia in a dose-dependent manner, as revealed by the elevated plus maze and passive avoidance test.
Conclusion: Taken together, our data indicate that DADS derivatives emerges as an interesting anti-Alzheimer's lead compound with potent anti- Aβ aggregatory, antioxidant, metal chelating and cognition enhancing effects.



05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-0921

MULTITARGET DRUG DESIGN STRATEGY: QUINONE-TACRINE HYBRIDS DESIGNED TO BLOCK AMYLOID-BETA AGGREGATION AND TO EXERT ANTICHOLINESTERASE AND ANTIOXIDANT EFFECTS

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We report the identification of multitarget anti-Alzheimer compounds designed by combining a naphthoquinone function and a tacrine fragment. *In vitro*, **12**-**29** displayed excellent acetylcholinesterase (AChE) inhibitory potencies and interesting capabilities to block amyloid-*β* (A*β*) aggregation. The X-ray analysis of AChE-**20** complex allowed rationalizing the outstanding activity data (IC_{50} = 0.72 nM). Selected compounds **16** and **20** showed negligible toxicity in immortalized mouse cortical neurons Neuro2A and primary rat cerebellar granule neurons.

However, only **16** was less hepatotoxic than tacrine in HepG2 cells. In T67 cells, **16** and **20** showed antioxidant activity, following NQO1 induction. Furthermore in Neuro2A, they were able to completely revert the decrease in viability induced by A*β*. Importantly, they crossed the blood-brain barrier, as demonstrated in *ex vivo* experiments with rats. When *ex vivo* results were combined with *in vitro* studies, **16** and **20** emerged to be promising multitarget lead candidates worthy of further pursuit.

05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-0922

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL TACRINE DERIVATIVES AND TACRINE-COUMARIN HYBRIDS AS CHOLINESTERASE INHIBITORS

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A series of novel tacrine derivatives and tacrine-coumarin heterodimers were designed, synthesized, and biologically evaluated for their potential inhibitory effect on both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Of these compounds, tacrine-coumarin heterodimer 7c and tacrine derivative 6b were found to be the most potent inhibitors of human AChE (hAChE), demonstrating IC50 values of 0.0154 and 0.0263 μM. Ligands 6b, 6c, and 7c exhibited the highest levels of inhibitory activity against human BuChE (hBuChE), demonstrating IC50 values that range from 0.228 to 0.328 μM. Docking studies were performed in order to predict the binding modes of compounds 6b and 7c with hAChE/hBuChE.

05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-2061

CHLOROQUINE AND CHLOROQUINE RELATED COMPOUNDS AS A MODEL FOR THE DESIGN OF ANTI-ALZHEIMER COMPOUNDS

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Objectives.
The amyloid precursor protein (APP) plays a central role in Alzheimer's disease (AD). Preventing deregulated APP processing by inhibiting amyloidogenic processing of carboxy-terminal fragments (APP-CTFs), and reducing the toxic effect of amyloid beta (Aβ) peptides remains an effective therapeutic strategy. Chloroquine was shown to modulate processing of APP-CTFs and production of Aβ peptide. Our objective was to evaluate the effect of piperazine-containing compounds derived from chloroquine on APP metabolism.
Methods.
Chloroquine or related heterocycles were functionalized with piperazine-containing amino side chain to improve the ability to accumulate into acidic cell's compartments. All compounds were tested for their potential interference with APP processing in SY5Y-APP^{wt} cell lines. Cytotoxicity, APP fragments and Aβ levels were determined. Acute administration (po) was performed on naive mice.
Results.
Compounds which retained alkaline properties and high affinity for acidic cell compartments were the most effective. The present study demonstrates that (1) the amino side chain of chloroquine can be efficiently substituted by a bis(alkylamino)piperazine chain, (2) the quinoline nucleus can be replaced by a benzyle or a benzimidazole moiety, and (3) pharmacomodulation of the chemical structure allows the redirection of APP metabolism toward a decrease in Aβ peptide release, and increased stability of APP-CTFs and amyloid intracellular fragment.
Conclusions.
Benzimidazole derivatives increases APP-CTFs *in vivo* and shows promising activity by the oral route. This family of compounds retains a lysosomotropic activity which inhibits lysosome-related Aβ production, and is likely to be beneficial for therapeutic applications in AD.

05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-2176

NEW MULTITARGET INHIBITORS OF THE ACETYLCHOLINESTERASE FOR THE TREATMENT OF ALZHEIMER DISEASE.

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Objectives.
Alzheimer's disease (AD) is by far the most prevalent cause of dementia. The complexity of this disease weight on the strategic choice for the discovery of new drug candidate. The development of multi-target compounds is one of this possible strategy. In this way, we have developed new family of multitarget compounds. These compounds are characterized by a combination between N,N'-disubstitued piperazine (MSBD), discovered in our lab to be active on the amyloid precursor protein metabolism (APP), and acetylcholinesterase inhibitors (AChEI).
Methods.
Among available AChEI we chose tacrine and rivastigmine because their structure are the most prone to respect the SAR established in the MSBD family. Target compounds were synthesized and evaluated for both AChE inhibition and *in vitro* modulation of APP metabolism (SY5Y cells).
Results.
This novel family of mixed compounds modulates APP metabolism by selectively reducing the secretion of Aβ peptides and by increasing several APP metabolites including the gene regulatory fragment of APP (AICD). Moreover, these multitarget compounds displayed improved affinity with respect to known AChEI.
Conclusions.
We discovered a series of multitarget compounds displaying high AChE inhibition along with a good ability to modulate APP metabolism. These results underline the interest to develop multitarget compound for the treatment of AD.

05v. Drug Development & Clinical Trials: structure-activity relationships

ADPD5-1326
THE STRUCTURE FUNCTION RELATIONSHIP OF SCYLLO-INOSITOL AND ALZHEIMER'S DISEASE

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Objective: Determine the mechanism of action of *scyllo*-inositol that leads to improvement in cognitive function in a mouse model of Alzheimer's disease.
Methods: To better understand the structure-function relationships of amyloid-beta peptide assembly in the presence of *scyllo*-inositol, we utilized a reliable and high-throughput method for screening *scyllo*-inositol derivatives, a novel electrospray ionization mediated high throughput mass spectrometry-based platform to address the role of various small molecules in modulating the dynamic assembly of amyloid-beta peptide and atomic force microscopy. The data from these in vitro studies, lead us to investigate whether the same was recapitulated *in vivo*, using the TgCRND8 mouse model of Alzheimer's disease. Biochemical and pathological examination of disease-bearing mice after 30 days of treatment with *scyllo*-inositol were analyzed.
Results: We found that two *scyllo*-inositol molecules bound to one amyloid-beta peptide, that the hydrophobic faces and hydrogen-bonding hydroxyl groups were necessary for interaction and inhibition of peptide assembly. We further demonstrate *in vivo* that alterations to the structure of *scyllo*-inositol greatly diminishes the beneficial effects of treatment. This latter effect was not dependent on the effective drug dose within the CNS.
Conclusions: *scyllo*-inositol is the most effective compound so far identified for the amelioration of cogntive deficits in mouse models of Alzheimer's disease.

05x. Drug Development & Clinical Trials: non-pharmacological interventions

ADPD5-0260

APPROACHES TO IMPROVING RATER ACCURACY IN EARLY/PRODROMAL ALZHEIMER´S DISEASE CLINICAL TRIALS

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A total of 698 raters from 23 countries conducting assessments in 3 Early/Prodromal AD studies were included in this survey. Data regarding professional background, experience with the study populations, and familiarity with the study-specific rating scales were gathered as part of formalized rater qualification programs for each study. Once trained and approved, raters submitted in-study assessments for central review, which were then evaluated for administration and scoring errors. Raters were divided into two groups based on type of assessments performed (Psychometric versus Global role). Means and standard deviations of years of experience by dementia indication and rating scale experience were calculated. Pooled results of central rating review findings were examined to determine trends in rater performance over time as a measure of the effectiveness of an enhanced rater training program.

Raters reported different levels of indication experience depending upon the type of assessments they conducted in the trial, with psychometric raters reporting relatively less Early AD study scale experience. In-study rater performance, as measured by the number of errors found per visit in an enhanced training program including central rating review, improved over time.

Raters' reported experience both with the Early/Prodromal population and the scales most sensitive to change in Early/Prodromal AD calls into question the assumption that these qualifications are functionally equivalent across types of clinical trials. Augmenting traditional rater training programs is one strategy to improve the accuracy of raters at all levels of experience with the assessment of emergent symptoms of the early phases of the disease.

05x. Drug Development & Clinical Trials: non-pharmacological interventions

ADPD5-0806

BENEFICIAL EFFECTS OF HYPERBARIC OXYGEN THERAPY ON THE PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE MOUSE MODELS

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Background. Hypoxia is intimately entwined in the pathogenesis of Alzheimer's disease(AD). Reduced cerebral perfusion starting early in the disease can lead to cerebral hypoxia, which contributes to the accumulation of A-Beta, hyperphosphorylation of tau and degeneration of neurons. Hyperbaric Oxygen Therapy (HBOT), the medical administration of 100% oxygen at environmental pressures greater than 1 atmosphere absolute (ATA), has recently been used successfully in the treatment of other hypoxia-related neurological conditions such as strokes and traumatic brain injury.
Objective. Elucidating the mitigating effects of HBOT on the early and late stages of AD pathology in the 3xTg and 5XFAD mouse models.
Methods. 17month-old 3xTg mice were exposed to HBOT at 1 ATA for 60 minutes daily for 14 days. In addition, 4 month-old 5xFAD and wt mice were exposed to HBOT at 1 ATA for 60 minutes daily, 5 days a week for 1 month (20 treatments). Subsequently to HBOT in both sets, a behavioral test battery including nest building test, rotarod, y-maze, open field and object recognition test, was performed.
Results. HBOT improved the performance of 3xTg treated mice in the Y-maze, open field test and object recognition test. HBOT-treated 5xFAD mice travelled more distance and in higher velocity than controlsin the open field test, and similarly to 3xTg, showed improved performance in the Y-maze.
Conclusions. HBOT ameliorated cognitive deficits of old 3xTg mice andpromoted improvement in performancein cognitive tasks in 5xFAD mice.These findings suggest that HBOT may present a novel therapeutic intervention of AD.

05x. Drug Development & Clinical Trials: non-pharmacological interventions

ADPD5-0990

LOW DOSE WHOLE BRAIN IRRADIATION (LDWBI) AS A POTENTIAL TREATMENT FOR ALZHEIMER'S DISEASE (AD)

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Objective:
To determine if LDWBI can retard the memory disturbance progression in a genetically altered AD mouse
Methods:
16 month B6.Cg-Tg (APPswePSEN1dE9) 85Dbo/J mice were given LDWBI (5 x 200 cGy (n=19)) and compared to untreated animals (n=14). Neurocognitive testing utilized the Morris Water Maze with a Noldus Etho Vision video tracking system. Testing was completed pre treatment and then 8 weeks after treatment at which time the animals were then sacrificed.
Mice were trained with 3 trials/day (90 second maximum) with a 30 minute intra-trial interval for 5 consecutive days.
At sacrifice amyloid count , volume , synaptophysin along with other stains were completed
Results:
Latency period for controls pre treatment were 58 secs (SD +/- 23s) and 48 secs (SD +/- 15s) for 'to be treated' animals (p=.39). At 8 weeks post treatment the control animals latency period was 60 secs (SD +/- 15s) and for the treated animals it was 31secs(SD+/- 17s) (p=0.03)
The treatment group located the platform significantly faster than the control animals on the final day of testing. This finding was not attributed to baseline learning the groups did not differ in latency to find the platform pre treatment
Treatment animals had statistically lower amyloid plaque numbers and trended towards significance in volume of residual plaques
Conclusions:
This data confirms our previous hemi brain irradiation data but documents improved memory performance when LDWBI is utilized. Based on this data we have been granted FDA approval to initiate a Ph I human clinical trial using LDWBI

05y. Drug Development & Clinical Trials: other

ADPD5-0350

VARIOUS OUTCOMES OF CHOLINESTERASE INHIBITOR TREATMENT INFLUENCE SURVIVAL OF PATIENTS WITH ALZHEIMER'S DISEASE

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Objectives: Various outcomes of cholinesterase inhibitor (ChEI) therapy have been observed in Alzheimer's disease (AD). It is not clear whether the duration of treatment, type of ChEI, or dose affect mortality. We aimed to investigate the association between ChEI therapy and patient survival.
Methods: The Swedish Alzheimer Treatment Study (SATS) is a prospective, observational, multicentre study to evaluate long-term treatment with ChEIs in clinical practice. This study included 1021 outpatients with a clinical diagnosis of mild-to-moderate AD (Mini-Mental State Examination score, 10–26) at the start of ChEI treatment (shortly after diagnosis). The date of death of participants was recorded.
Results: After up to 16 years of follow-up, 841 (82%) of the patients in the SATS had died. The mean ± standard deviation time from diagnosis of AD to death was 6.0 ± 2.9 years, and differed between individuals with varying durations of ChEI treatment in the study, from 7.2 ± 2.5 years (3-year completers) to 4.9 ± 2.9 years (<1 year) (P<0.001). Patients who received a higher mean dose of ChEIs during the study had a longer lifespan than those who received a lower dose (6.4 ± 2.9 vs 5.5 ± 2.8 years; P<0.001). The median cutoff values were donepezil 6.9 mg, rivastigmine 6.0 mg, and galantamine 15.0 mg. No difference in mortality between the types of ChEIs was found after adjusting for sex, age, and disease severity.
Conclusions: Longer survival can be expected for AD patients who receive and tolerate higher ChEI doses and a longer duration of treatment.

05y. Drug Development & Clinical Trials: other

ADPD5-0727

USE OF A TELEPHONE INTERVIEW METHODOLOGY TO IDENTIFY SUBJECTS WITH COGNITIVE IMPAIRMENT AMONG THOSE REPORTING SUBJECTIVE MEMORY COMPLAINTS

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Objective: The treatment of AD with disease-modifying drugs is believed to require intervention at the earliest possible stage of the illness. These studies were conducted to evaluate the utility of a telephone screening methodology to identify subjects with cognitive impairment from among individuals reporting subjective memory complaints in primary care networks.

Methods: Two non-interventional, observational studies were completed in the UK (1 site) and US (3 sites). In these studies, participants with subjective memory complaints were identified through the CHARIOT registry (UK), or by self-report, caregivers/informants, or PCPs (US). Participants were administered the Alzheimer's Disease-8 (AD8) questionnaire and Rey Auditory Verbal Learning Test (RAVLT) through a telephone contact center.

Results: A total of 18/82 (22%) UK subjects and 54/191 (28%) US subjects who completed telephone assessments were determined to have abnormal cognition. Data suggested that informants were more accurate than patients in identifying cognitive dysfunction. 10/18 (56% UK) and 54/54 (100% US) individuals indicated they would consider enrollment into a hypothetical clinical trial for AD. With various recruitment approaches ranging from targeted clinical screening to mass mailings, the study identified ~1 individual with abnormal cognition per week per network. No one method appeared superior in identifying prospective treatment study subjects.

Conclusions: Compared with or combined with traditional patient recruitment tools such as print or web-based advertisements, telephone based cognitive assessment of individuals with subjective memory complaints is a relatively efficient means of identifying potential early-stage AD subjects for recruitment into clinical trials. Recruiting strategies should be tailored to each site.

05y. Drug Development & Clinical Trials: other

ADPD5-1345

DISCOVERY OF NOVEL MULTIFUNCTIONAL DRUG CANDIDATES FOR TREATMENT OF ALZHEIMER'S DISEASE FROM A HIGH-THROUGHPUT COMPOUND SCREEN

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Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder and the most frequent cause of dementia. To date, there are few approved drugs for AD, which show little or no effect on disease progression. Impaired intracellular calcium homeostasis is believed to occur early in the cascade of events leading to AD. Here we examined the possibility of normalizing the disrupted calcium homeostasis in the endoplasmic reticulum (ER) store as an innovative approach for AD drug discovery. High-throughput screening of a small-molecule compound library led to the identification of tetrahydrocarbazoles, a novel multifactorial class of compounds that can normalize the impaired ER calcium homeostasis. We found that the tetrahydrocarbazole lead structure, firstly, dampens the enhanced calcium release from ER in HEK293 cells expressing familial Alzheimer's disease (FAD)-linked presenilin 1 mutations. Secondly, the lead structure also improves mitochondrial function, measured by increased mitochondrial membrane potential. Thirdly, the same lead structure also attenuates the production of amyloid-beta (Aβ) peptides by decreasing the cleavage of Amyloid Precursor Protein (APP) by β-secretase, without notably affecting α- and γ-secretase cleavage activities. Considering the multiple modes of action of tetrahydrocarbazoles in addressing three key pathological aspects of AD, these compounds hold promise for development of a potentially effective AD drug candidate.

05y. Drug Development & Clinical Trials: other

ADPD5-1729

THE EARLY EFFICACY OF BRIGHT LIGHT THERAPY FOR DEMENTIA OF THE ALZHEIMER TYPE; A PRELIMINARY STUDY

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(Introduction)

It is expected that bright light therapy for the patient with dementia may improve sleep disturbance and night wondering. This non-pharmacological therapy is in the process of comprehending recently. However, in according to the recommended protocol, the patient must be forward dazzling light box, during fixed time every day, in several weeks, so it is actually difficult to carry out precisely. Nevertheless, the some cases respond by intervention of 1 to2 weeks. We investigate to eliminate the declined level of cognitive function of dementia of the Alzheimer type (AD) to respond in a short term.

(Method)

The hospitalized AD with sleep disturbance and/or night wondering were assigned and treated 5000lux bright light therapy every from 9 to 10 o'clock in two weeks. The effect was assessed by sleep-awareness chart and Neuro-psychiatric inventry.

(Result)

Six AD cases were treated. Three cases showed improvement for sleep disturbance and reduction of care burden within from 7 to 10 days although the therapy has not taken sufficient time because of incorporative attitude.

(Discussion)

The effective cases of AD were shorter morbidity period or at from mild to moderate dementia state. As melatonin neuronal system conducting circadian rhythm reduces as aging or deterioration for AD, it was expected that the sleep disturbance in early and middle stage of AD can be responded by blight light therapy. As this presentation was a preliminary data, more objects would be needed.

05y. Drug Development & Clinical Trials: other

ADPD5-1773

EFFECTS OF ADD-ON MEMANTINE ON DAILY FUNCTIONING IN PATIENTS WITH MODERATE TO SEVERE ALZHEIMER'S DISEASE RECEIVING STABLE DONEPEZIL TREATMENT

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Objectives: Previous post hoc analyses of the total ADCS-ADL_{19} score, an assessment of daily functioning, suggested that add-on memantine treatment in moderate to severe Alzheimer's disease (AD) may result in significant improvement versus placebo in patients with ≥24 months of stable donepezil pretreatment. This analysis examined the effects of add-on memantine on individual ADCS-ADL_{19} items.
Methods: Patient-level data (ITT population) were pooled from two randomized, placebo-controlled, 24-week trials of add-on memantine in individuals with moderate to severe AD (MMSE range: 3-16) receiving stable treatment with donepezil (MEM-MD-02; n=384) or any cholinesterase inhibitor (MEM-MD-50, donepezil subset only; n=444). Placebo/donepezil and memantine/donepezil groups were compared based on the covariate duration of donepezil monotherapy, using a mixed model (main effects: α=0.05, two-sided; interaction effects: α=0.10, two-sided).
Results: Overall, duration of donepezil pretreatment (mean ± SD) was 20.8 ± 17.7 months. The memantine/donepezil group significantly outperformed the placebo/donepezil group on the items of grooming, conversing, and finding belongings in patients pretreated with donepezil for ≥12 months and on the items of toileting, bathing, watching TV, and turning the light off in those pretreated with donepezil for ≥24 months. The placebo/donepezil group was superior on the items of walking and travelling outside the house in patients receiving donepezil pretreatment for 0-12 months.
Conclusions: In patients with moderate to severe AD, adding memantine to stable donepezil treatment of ≥12 months may be associated with improvements in both basic and instrumental daily functions.

05y. Drug Development & Clinical Trials: other

ADPD5-1814

METHODOLOGICAL ASPECTS OF A PHASE II STUDY TO ASSESS THE CLINICAL AND IMMUNOLOGICAL ACTIVITY, SAFETY AND TOLERABILITY OF AFFITOPE® AD02 IN PATIENTS WITH EARLY ALZHEIMER'S DISEASE

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Objective: AD02 and AD04 were evaluated in a multicenter, parallel group study in early AD, including 332 prodromal and mild AD patients randomized to 5 groups. The statistical methodology used in this study was specialized for a mixed stage early AD population.

Methods: Partial least squares models were fit to historical data to identify an optimally weighted cognitive (adapted ADAS-cog), functional (adapted ADCS-ADL), and cognitive/functional composite. The Adapted ADAS-cog scale combined ADAS-cog items, Neuropsychological test items and CogState items, the Adapted ADCS-ADL included a subset of the ADCS-ADL items, and the composite combined these. The positive signal in one of the placebo groups resulted in the need for accurate historical estimates of placebo decline. Pooled ADNI mild and ADCS study data was used for this.

Results: Heavily weighted cognitive items were Word Recall, Word Recognition, and Orientation. Delayed Word Recall and Digit Cancellation were among those excluded due to ceiling or floor effects. Heavily weighted ADL items were telephone use, traveling, preparing a meal/snack, selecting clothing, shopping and using appliances. Excluded items were primarily basic ADLs such as eating, walking, toileting and bathing. Adapted scales showed improved sensitivity over traditional scales. Historical analyses supported a strong treatment effect for AD04.

Conclusions: To our knowledge, this is the first completed early AD study combining prodromal MCI and mild AD subjects. It is also the first to prospectively use optimized composites as primary endpoints and to demonstrate their superior power in early disease. Historical analyses added to interpretability of the results.

05y. Drug Development & Clinical Trials: other

ADPD5-1992

D-PEPTIDES DEVELOPED TO BE THERAPEUTICALLY ACTIVE AGAINST BETA-AMYLOID OLIGOMERS SHOW PROMISING PHARMACOKINETIC PROPERTIES

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Objectives: To date, no treatment exists to cure Alzheimer's disease (AD). Aggregation of beta-amyloid (Abeta) plays an important role in the pathology of AD. Currently, Abeta oligomers are thought to be the most toxic species. D3, a D-enantiomeric peptide (D-peptide), was developed that specifically eliminates Abeta oligomers in vitro and has been shown to improve cognition and reduce plaque load and inflammation in transgenic Alzheimer's mice. D-peptides have several advantages over L-peptides since they are less immunogenic and more protease-resistant. Therefore, they are thought to remain longer in the body providing more time to be therapeutically active. Here, we show pharmacokinetic studies of some derivatives of D3 in mice and estimate their plasma protein binding.
Methods: Radioactively labelled peptide was administered via several administration routes and organs were harvested at different time points post injection. The amount of radioactive D-peptide in the organ homogenate was measured by liquid scintillation counting. Furthermore, binding to plasma proteins as well as brain membranes was determined, also using radioactively labelled peptide as indicator.
Results: Results show that all D-peptides indeed reach the brain where they may exhibit their therapeutic activity. Furthermore, the peptides show small elimination constants and long half-lives of more than a day in plasma as well as a high bioavailability after i.p., s.c. or p.o. administration.
Conclusions: Promising pharmacokinetic properties confirm that D-peptides may be very potent AD-therapeutic agents on their way to clinical studies.

05y. Drug Development & Clinical Trials: other

ADPD5-2208

ANTI-INFLAMMATORY EFFECTS OF ARN14494, A NEW SERINE PALMITOYLTRANSFERASE INHIBITOR, IN AN IN VITRO MODEL OF ALZHEIMER'S DISEASE.

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Objectives: Alzheimer's disease (AD) is a neurological disorder that present a progressive loss of memory, neuronal death, inflammation and a debilitation of intellectual capacity. Neuronal death in the brain play a key role in AD progression and is directly linked to neuroinflammation. Recent evidences has suggested a link between ceramides and AD showing that these sphingolipids are increased in AD patients. Activation of serine palmitoyltransferase (SPT), increases ceramide levels. The aim of this work is the development of a new SPT inhibitor, ARN14494, that have shown promise in vitro by attenuating glial activation. **Methods:** Studies of Ceramide measurement by LC/MS analyses, Serine palmitoyltransferase Assay, Quantitative reverse transcription-polymerase chain reaction (PCR), Enzyme-linked immunosorbent assay (ELISA), Western Blot, Nitrite measurement, SiRNA transfection were performed in Neuronal cell line (N2a, CCF-STTG1) and Primary astrocytes culture. **Results:** Results demonstrated that ARN14494 treatment exerted potent anti-inflammatory action by inhibiting the production of Nitric Oxide, Inteleukin-1β (IL1β) and tumor necrosis factor alpha (TNFα). Moreover, significantly suppressed the expression of cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS) at protein and mRNA levels. In addition reduce ceramide levels and SPT activity, by using mouse astrocytes cultures stimulated with beta amyloid 1-42. **Conclusions:** These results suggest that ARN14494 can reduce neuroinflammation and the regulation of neuroinflammatory processes might be a practical strategy for the treatment of AD.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0415

VISUAL RATING OF POSTERIOR ATROPHY AS A MARKER OF PROGRESSION TO DEMENTIA IN MILD COGNITIVE IMPAIRMENT PATIENTS: A PRELIMINARY STUDY

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Objectives
We evaluated whether posterior atrophy (PA) could be a useful marker of progression to dementia in patients with mild cognitive impairment.
Methods
We retrospectively identified 148 patients with mild cognitive impairment who underwent a coronal MRI and were followed up for a mean of 21.17 months (range, 11 to 36 months) in Clinical Neuroscience Center of Seoul National University Bundang Hospital. Medial temporal atrophy (MTA) and PA were rated visually using a 5-point and 4-point rating scale, respectively. Cox regression analysis was used with follow-up time as time variable and conversion to dementia as status variable.
Results
At follow-up, 47 (30.5% of 148) patients fulfilled criteria for dementia. MTA and PA were significantly associated with dementia at follow-up with a hazard ratio (HR) of 3.194 ($p < 0.001$) and 2.583 ($p = 0.002$) for the presence of atrophy, respectively. Including both MTA and PA ratings as covariates, MTA remained independently predictive of progression (HR, 2.634, $p = 0.004$) and PA also remained statistically significant (HR, 1.922, $p = 0.044$). The predictive accuracy of MTA and PA was independent of age and *APOE* **ε**4 allele.
Conclusions
Visual assessment of PA is a useful predictor of progression to dementia, together with MTA, in mild cognitive impairment patients.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0437
AUTOMATIC PREDICTION OF RAPID COGNITIVE AND FUNCTIONAL DECLINE IN AN ELDERLY POPULATION

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Objectives: Identifying subjects with rapid cognitive or functional decline (RCD or RFD) in MCI and dementia patients as well as in elderly subjects is a relevant task in care planning and management. We present an approach to predict cognitive and functional decline from structural imaging and baseline clinical assessment.
Methods: 647 healthy (217), AD (114) and MCI (316) subjects recruited in the ADNI I/II/GO studies (www.adni-info.org) that had MR imaging, MMSE and FAQ scores at baseline, month 12 and 24 were included. Subjects were classified as RCD and RFD if the rate of decline was >= 8 MMSE points or 10 FAQ points over 2 years respectively. Using baseline MRI, volumes were automatically extracted with LEAP for the hippocampus, amygdala, temporal horn and the lateral ventricle. Individual volumes were aggregated into a weighted atrophy index (WAI), combined with baseline MMSE or FAQ score, and used as 2D-features for Gaussian mixture population estimation. The difference between progressing and non-progressing (cognitively or functionally) populations was modelled as a sigmoid function in order to obtain a class-likelihood index.
Results: Class-likelihood indices derived from both WAI-MMSE and WAI-FAQ were used to predict RCD and RFD subjects. Prediction accuracies of 75% and 80% were respectively obtained. This compares to 68% (WAI) and 72% (MMSE) for RCD, 69% (WAI) and 74% (FAQ) for RFD, when using individual measurements alone.
Conclusion: Class-likelihood indices derived from structural imaging and baseline clinical assessment (MMSE/FAQ) give a good indication as to whether a subject is likely to be an RCD/RFD.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0469

MAGNETISATION TRANSFER CONTRAST IMAGING REVEALS WHITE MATTER PATHOLOGY IN THE APP/PS1 MOUSE MODEL OF AMYLOIDOSIS.

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Objectives: While no definitive cure for Alzheimer's disease exists yet, current treatments would benefit greatly from an early diagnosis. We have previously shown that Magnetisation Transfer Contrast imaging (MTC) is able to detect amyloid β plaques in old APP/PS1 mice [1]. In the current study we investigated if MTC is also able to visualize early amyloid-induced pathological changes.

Materials and methods: In a cross-sectional study, we used wild type (n=61) and APP/PS1 mice (n=76) [2] of 2, 4, 6, 8 and 24 months of age. Mice were imaged at the respective ages and sacrificed thereafter for histological analysis. MTC was acquired using a 7T Pharmascan MRI system (Brucker, Germany) using a Turbo Spin Echo sequence with an off-resonance pulse at -16875 Hz.

Results: We first validated our previous results using 24 month old mice as, compared to our previous study, we acquired the MTC images using a different sequence and a lower field strength MRI scanner. We observed an increased MTC ratio in grey matter regions of APP/PS1 mice, validating our previous results. Next we observed that in young mice, MTC is unable to consistently visualize amyloid β-induced grey matter pathology. Instead, we consistently observed decreased MTC ratios in the splenium of 4, 6 and 8 month old APP/PS1 mice. Histology is currently ongoing and will allow to correlate our MTC values to various histological markers.

Conclusion: MTC is able to visualize early pathological changes in the splenium of APP/PS1 mice.

[1] DOI:10.1016/j.neuroimage.2013.10.056
[2] DOI:10.1038/sj.embor.7400784

06a. Imaging & Biomarkers: structural MRI

ADPD5-0515

DEVELOPMENT OF VOLUMETRIC PROGRAM FOR FAST EVALUATION OF REGIONAL BRAIN ATROPHY

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Introduction: Brain atrophy is one of the reliable markers for the evaluation of neurodegenerative change of brain and severity of dementia. FreeSurfer and other surface morphology based volumetric programs took much time, require high performance computer system.

Aims: We developed a volumetric analysis program that can measure 26 regional volumes of brain with high feasibility for health professionals to use in clinical practice.

Methods: Based on SPM8 and MATLAB, we made a tool box to measure the 26 regional brain volumes in both hemispheres with segmented masks. The regions were frontal (anterior, antero-medial, dorso-lateral, dorso-medial, inferior, orbital), temporal (anterior, lateral, medial), parietal (medial, lateral), occipital and central. We also calculated ventricle volume, brain volume and total intracranial volume (TIV). We validate the program with T1 high resolution 3D MRI data from 25 AD and 36 normal control subjects.

Results: The average running time for single data analysis was 5 minutes and 36 seconds. Test-retest reliability of this program was perfect, ICC=0.999. AD patients showed decreased brain volume and increased ventricle volume compared to normal controls (p

Discussions: This automatic brain volumetric program was reliable and feasible for the researchers in clinical practice who require fast and reliable results. We will obtain more brain volume data of normal controls with different age and sex to complete normative data set.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0525

DISTRIBUTION ANALYSIS OF CEREBRAL MICROBLEEDS IN ALZHEIMER'S DISEASE AND STROKE WITH SUSCEPTIBILITY WEIGHTED MR IMAGING

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Cerebral microbleeds (CMBs) is an important finding in cerebral amyloid angiopathy and hypertensive arteriopathy. A few studies showed that these two disorders have different patterns in CMBs distribution on the brain. The aim of this study is determining different patterns in CMBs distribution on the brain in AD and stroke by susceptibility weighted imaging (SWI) which is known to be a sensitive magnetic resonance technique in detecting microbleeds.

Seventy-one patients presenting at our neurology department were included and 1.5 Tesla SWI was used to image. Thirty AD patients and 21 patients who had recent ischemic stroke. Remnant 20 patients was classified with healthy control with subjective memory complaint.

The Microbleed Anatomical Rating Scale (MARS) was used to localize each CMB (lobar versus basal ganglia/thalamus (deep), and infratentorial). Incidence, and numbers of microbleeds in each anatomical division were counted and statistically compared each other.

Total CMBs were revealed a preference for the lobar and basal ganglia/thalamus (deep) regions. There were significance that CMBs in patients with AD had higher incidence of CMBs in lobar region and showed predominant distribution of CMBs in lobar brain area than infratentorial region significantly. In stroke patients, had higher incidence of CMBs in basal ganglia/thalamus (deep) region and showed significant predominance in basal ganglia/thalamus (deep) region than infratentorial region. And there were statistical significance for predominant distribution of CMBs in ganglia/thalamus (deep) region in stroke patients compared with AD and controls. But there is no statistically significant difference of distribution predominance between AD and controls.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0664

DIFFUSION TENSOR IMAGE FOR EARLY DIAGNOSTIC TOOL FOR SUBJECTIVE COGNITIVE DECLINE AND RELATIONSHIP OF BASELINE MEMORY SCORE

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Background and purpose Recent study showed subjective cognitive decline(SCD) may be the first symptomatic stage AD. The SCD is very heterogeneous state and identification who will converte to dementia is very important. We investigated microstructural changes of brain using DTI in SCD patients and compared DTI parameters between SMI Patients with normal memory scores and with abnormal memory scores in MMSE. **Methods** We enrolled 60 subjects who visited the neurology department of Seoul St. Mary's hospital from Jan, 2009 to May, 2013. We defined SCD who have self-reported memory impairment, but with normal cognitive performance in neuropsychological test.There were 20 normal people, 40 SMI patients and among SCD patients, 15 patients have abnormal recall scores in MMSE. We measured the FA and MD of bilateral entorhinal cortex, hippocampal head and body by using the program Volume-One and dTVII. **Results** There were significant differences of MD values of left entorhinal cortex and FA value of bilateral hippocampal head between normal and SCD groups. And there were significant differences of FA values of bilateral hippocampal head between SCD Patients with normal recall scores and with abnormal recall scores. **Conclusion** This study showed that early microstructural changesoccur in SCD patients and low baseline recall scores in MMSE are related to DTI value change in hippocampus head. This result suggest the possibility DTI can be used as a useful tool to detect microscopic brain changes of SCD patients with low baseline recall scores in MMSE.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0711
CORTICAL THICKNESS IN INDIVIDUALS WITH SUBJECTIVE MEMORY IMPAIRMENT: MEMORY CLINIC VERSUS HEALTH PROMOTION CENTER BASED POPULATION

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Background
Subjective memory impairments (SMI) without objective memory deficits are quite common among older adults, but their clinical impact remains unclear. We hypothesized that health-seeking behavior may predict the causes or prognosis of SMI. We aimed to determine whether there is difference in cortical thickness between SMI who visited a memory clinic for concern of their memory problem and those who visited health promotion center for disease-preventive medical check-up.
Methods
We consecutively recruited 387 SMI individuals (cSMI), who had no objective cognitive impairments, in memory clinic from September 2008 to June 2012. We also recruited 2155 individuals who had no dementia in health promotion center during the same period. According to the question 'Do you have memory complaints?', 771 individuals were classified into SMI (hpSMI) and 1384 individuals were classified into normal control (NC).
All participants underwent magnetic resonance imaging (MRI), including three-dimensional volume images. Automated surface-based analyses of the MRI data were used to measure cortical thickness. Analysis of covariance analysis was performed after controlling for age, sex and intracranial volume.
Results
Relative to NC, cSMI showed cortical thinning predominantly in the left frontal, temporal, bilateral parietal and occipital regions. Relative to hpSMI, cSMI showed cortical thinning predominantly in the left temporal, bilateral parietal and occipital regions. However, there were no differences in the mean cortical thickness between NC and hpSMI groups.
Conclusions
Our findings revealed that cSMI, but not hpSMI, had cortical thinning compared to NC, suggesting that health seeking behavior reflects early marker for neurodegeneration.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0784
STRUCTURAL CORRELATES OF CEREBROSPINAL FLUID BIOMARKERS IN COGNITIVELY NORMAL SUBJECTS
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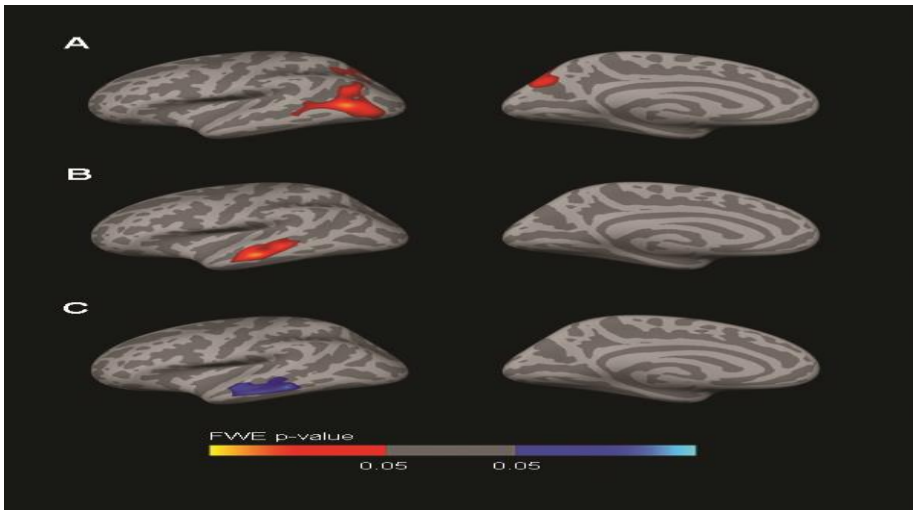
Objectives: To study the relationship between cerebrospinal fluid (CSF) biomarkers of Alzheimer's disease (AD) and their interactions on brain structure in a cohort of cognitively normal subjects (CN).
Methods: Eighty CN subjects underwent structural 3T magnetic resonance imaging and lumbar puncture. We measured Abeta42, total tau, phospho-tau (p-tau) and the marker of inflammation YKL-40 in CSF by enzyme-linked immunosorbent assay. Cortical thickness (CTh) was measured by Freesurfer. Subjects were classified as Abeta42+ (below 550pg/ml) or Abeta42- (above 550pg/ml). CTh difference maps were derived from group, correlation and interaction (by Abeta42 status) analyses.
Results: 9/80 (11.25%) participants were Abeta42+. There were no differences in age or gender between subgroups. The Abeta42+ group showed cortical thickening in AD-related areas compared to Abeta42- subjects (Fig 1A). The interaction analysis showed that the relationship of CTh with YKL-40 is modified by Abeta42 status (Fig 1B). In the stratified correlation analyses YKL-40–dependent thinning was found in Abeta42+ subjects only (Fig 1C). Similar results were obtained with p-tau, but these did not survive family-wise error correction.
Conclusions: Our results support the importance of considering interactions between biomarkers in preclinical AD. According to our results, there is a cortical thickening in relation to decreasing CSF Abeta42 in CN subjects. Atrophy would develop in the presence of a synergistic effect of p-tau and YKL-40-associated inflammation.



06a. Imaging & Biomarkers: structural MRI

ADPD5-0801

PHARMACOLOGICAL EFFECTS OF DONEPEZIL AS A DISEASE MODIFIER

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Objectives: Donepezil is an acetylcholinesterase inhibitor used to treat Alzheimer's disease (AD) and has recently been proven to have a neuroprotective effect. In this study, we used a Voxel-Based Specific Regional Analysis System for AD (VSRAD) to analyze hippocampal volume and to assess the pharmacological effects of donepezil as a disease modifier.
Methods: 185 patients with AD underwent MRI, 120 (43 men and 77 women, 77.8±7.1 years) without treatment of donepezil and 65 (29 men and 36 women, 78.4±6.0 years) with treatment of donepezil. VSRAD was compared between both groups and against a database from 80 normal subjects. The Z-score was used to assess the degree of hippocampal atrophy.
Results: No siignificant difference between the groups was found for age, sex, or Z-scores, but a significant difference was found for mean Mini-Mental State Examination (MMSE) scores (p=0.02, Student's t-test). In single regression analysis, there was no significant association between Z-score and the MMSE score in the treated group (p=0.494), whereas there was significant association between the Z-score and the MMSE score in the untreated group (p=0.001**).** This implies that the MMSE score becomes lower when the Z-score is higher in untreated patients, whereas there is no significant trend in the treated group.
Conclusion: Donepezil affects the relationship between hippocampal volume and cognitive function. Donepezil, therefore, has a pharmacologic effect as a disease modifier, affecting the disease pathogenesis. This effect may be caused by the involvement of donepezil in hippocampal neurogenesis and its interaction with the sigma-1 receptor for neurite outgrowth.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0898

WHAT DO WE ASSESS USING MEMORY TESTS? A VOLUMETRIC MRI STUDY OF THE FCSRT AND DMS48

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We explored the neuro-anatomical correlates of two memory tests used to assess verbal and visual anterograde memory in order to determine whether they relied on the medial temporal lobe (MTL), and could be predictive of Alzheimer's disease (AD).
We analysed data from a cohort of 140 right-handed patients with mild cognitive impairment (MCI) participating in the longitudinal multicentric clinical research study BALTAZAR. Verbal memory was assessed using the Free and Cued Selective Reminding Tests (FCSRT) and visual recognition memory using a computerized forced-choice task, the DMS-48, among a larger cognitive baseline. Performances in verbal and visual recognition memory using the two tests were correlated to local gray matter atrophy via structural MRI using Voxel Based Morphometry.
Our results confirmed the involvement of the MTL prominently on the left for the FCSRT and on the right for the DMS-48 in the whole group of MCI patients. Interestingly, MTL remained implied only in the subgroup of patients who had deficient scores on the cued recall or recognition, but not on the free recall phase of the FCSRT. For the DMS-48, MTL remained implied only for the group of patients whose performances degraded between Set 1 and Set 2.
The FCSRT and the DMS-48 are able to detect specific features of memory loss due to MTL damage, under the condition that the cued recall or delayed recognition scores are deficient. These results have significant clinical implications with regards to the clinical diagnosis of the underlying etiologies of MCI, such as Alzheimer's disease.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0926

MYELIN BASIC PROTEIN AND ALZHEIMER'S DISEASE IN DOWN SYNDROME

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Objectives: Virtually all adults with Down syndrome (DS) over the age of 40 years have Alzheimer Disease (AD) neuropathology. We previously showed that white matter (WM) integrity was reduced in clinically demented adults with DS by magnetic resonance imaging and measures of fractional anisotropy. We hypothesized that losses in WM integrity in our cohort was associated with reduced myelin basic protein (MBP) in the frontal cortex.

Methods: To test this hypothesis, we used a separate set of autopsy cases and immunohistochemistry with a rat monoclonal antibody to MBP. MBP levels were compared in the frontal cortex from cases with DS (n=16, 8-25 years), DS with AD (n=41, 50-55 years), sporadic AD (>70 years, n=13, 71-85 years), and age-matched controls (n=35, 15-90 years). Image J was used to quantify the % area occupied by positive immunolabeling for MBP.

Results: An unexpected positive linear relationship between MBP and age in non-DS samples was observed with sporadic AD cases having the highest percent immunolabeling for MBP. Interestingly, there is an exponential increase in MBP immunolabeling and age in DS samples with a rapid rise occurring at approximately 30 years of age.

Conclusions: We had predicted the MBP levels would drop with age and the presence of AD neuropathology in DS. Higher MBP immunolabeling in AD and in DS with AD may reflect increased expression of MBP as a compensatory response. However, higher MBP levels in DS with AD may also be due to reduced degradation and aggregation, and/or accumulation of posttranslationally modified MBP.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0965

IMPROVED AD DISEASE CLASSIFICATION USING STRUCTURAL VOLUMES OF THE WHOLE BRAIN

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Objectives

We automatically segment 366 T1-weighted MR brain scans from the ADNI1 database into numerous structures. We investigate the potential of structural volumes to differentiate between AD, MCI and healthy control subjects (N=84/179/103). Comparing to an established technique, we analyse the diagnostic value of individual structures and their joint potential.

Methods

We calculate whole-brain segmentations using MALP-EM, which infers 134 structural labels from manually annotated images. For comparison, we employed volumes of 6 structures calculated with an established technique, LEAP. MALP-EM compensates for incorrectly aligned reference segmentations using intensity information and parcellates the whole brain. LEAP improves the alignment itself in predefined regions and yields single structure segmentations.

Results

Hippocampus and temporal horn perform similar for both methods (cf. Table 1). Amygdala segmentations calculated with LEAP have higher predictive value. Combining all available volumes in a single classifier substantially improves the results for MALP-EM.

MALP-EM	AD/HC	HC/MCI	AD/MCI
All 134 structures	**85.6%**	**71.9%**	**69.1%**
Hippocampus R/L	76.4%/79.9%	70.9%/66.5%	64.4%/59.4%
Amygdala R/L	74.8%/75.0%	58.0%/61.5%	63.7%/64.6%
Temporal Horn R/L	68.0%/66.1%	57.7%/55.7%	64.5%/62.2%
+ 128 other structures			

LEAP	AD/HC	HC/MCI	AD/MCI
All 6 structures	80.7%	70.2%	66.2%
Hippocampus R/L	74.4%/77.7%	71.0%/68.5%	63.3%/63.2%
Amygdala R/L	79.3%/78.3%	63.6%/66.7%	66.2%/61.8%
Temporal Horn R/L	64.0%/66.5%	56.9%/58.7%	63.6%/64.8%

Table 1: Classification accuracy using cross validation.

Conclusions

MALP-EM allows the analysis of many distinct structures and their combination improves classification results. Combining LEAP (improved alignment) and MALP-EM (intensity-based refinement) can potentially further increase the diagnostic value.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1053

SUBCORTICAL SHAPE ALTERATIONS, HIPPOCAMPAL SUBFIELD ATROPHY AND CORTICAL THINNING IN ALZHEIMER'S DISEASE PATIENTS AT PREDEMENTIA STAGE

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Objectives

Potential treatments and interventions for Alzheimer's disease (AD) may be most effective in prodromal stages, referred to as mild cognitive impairment (MCI). However, there is heterogeneity among MCI, with only some patients progressing to AD. Thus, the establishment of biomarkers that accurately identify future converter to AD is crucial.

Methods

We applied recently developed analyses techniques to investigate differences in subcortical shape and volume changes (thalamus, striatum) in patients with stable MCI (MCIsbl, n=23), future converter at baseline (MCIcbl, n=10) and at time of conversion (MCIctc, n=10) compared to age/gender matched cognitively normal subjects (CNS). Improved analyses techniques were used to further confirm cortical thinning and of the volume of the hippocampus and its subfields as key magnetic resonance imaging-based markers for AD.

Results

Apart from striatal volume reductions ($p<.05$), MCIsbl revealed no morphometric alterations in any of the investigated structures. In contrast, we identified shape alterations using FDR at $q<.10$ in striatal (caudate head and body, ventral lateral putamen) and thalamic regions (anterior and medial dorsal thalamus) paralleling AD like patterns of morphometric changes (cortical thinning in medial temporal regions using FDR at $q<.10$; hippocampal total and subfield atrophy $p<.05$) in MCIcbl and MCIctc.

Conclusions

Based on the ability to distinguish MCIcbl and MCIctc from CNS, our results evidence the value of early thalamic and striatal shape alterations to act as potential markers for the early detection of AD. Our results further confirm the key role of cortical thinning and hippocampal atrophy in the early detection of AD.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1102

PRACTICAL CUT-OFFS FOR VISUAL RATING SCALES OF MEDIAL TEMPORAL, FRONTAL, AND POSTERIOR ATROPHY IN ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT

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Objectives. Atrophy in medial temporal, frontal, and posterior cortex can be measured with visual rating scales such as MTA, GCA-F, and PA, respectively. However, practical cut-offs are urgently needed especially now that different presentations of Alzheimer's disease (AD) are included in the revised diagnostic criteria. Our aim was to generate a list of practical cut-offs for MTA, GCA-F, and PA, both for AD diagnosis and MCI prognosis, and to evaluate the influence of key demographic and clinical factors.
Methods. AddNeuroMed and ADNI cohorts were combined, comprising 1147 participants (345 controls, 480 MCI patients, 322 AD patients). MTA, GCA-F, and PA scales were applied and a broad spectrum of cut-offs was evaluated.
Results. MTA showed better diagnostic and predictive performance than GCA-F and PA. Age, ApoE ε4 status, and age at disease onset influenced all three scales. For each of the age ranges 45-64, 65-74, 75-84, and 85-94 years, the following cut-offs should be used: MTA (≥1.5, ≥1.5, ≥2, ≥2.5), GCA-F (≥1, ≥1, ≥1, ≥1), PA (≥1, ≥1, ≥1, ≥1), with an adjustment for early-onset ApoE ε4 non-carriers AD patients: MTA (≥2, ≥2, ≥3, ≥3), GCA-F (≥1, ≥1, ≥2, ≥2).
Conclusions. If successfully validated in clinical settings, the list of practical cut-offs proposed here might be beneficial for clinical practice. This will also foster research on atrophy subtypes, increasing our understanding of different presentations of AD, improve diagnosis and prognosis and aid clinical trials.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1106

CLINICALLY DIFFERENT AD SUBTYPES BASED ON VISUAL RATING SCALES OF MEDIAL TEMPORAL, FRONTAL, AND POSTERIOR ATROPHY

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Alzheimer's disease (AD) is clinically heterogeneous. Studying AD subtypes based on structural atrophy patterns could help understanding of the different presentations of the disease. Here we aimed to characterize AD subtypes based on visual rating scales of medial temporal (MTA), frontal (FA) and posterior (PA) atrophy.

Regional visual rating scales were applied to 322 AD patients (AddNeuroMed and ADNI). Eight AD subtypes were defined: no atrophy, atrophy exclusively in one region: MTA, PA, FA, and multi-region atrophy: MTA+PA, MTA+FA, PA+FA, MTA+PA+FA. Orthogonal projection to latent structures (OPLS) was utilized to characterize subtypes based on demographic and clinical aspects, cognitive performance and CSF biomarkers. Subtypes were also quantitatively validated with analyses of cortical thickness and subcortical volumes.

OPLS models based on demographic and clinical variables were able to discriminate the typical MTA AD group from other AD groups including frontal atrophy: MTA+FA (Q^2_Y=0.156), PA+FA (Q^2_Y=0.139) and MTA+PA+FA (Q^2_Y=0.102). Demographic and clinical variables could discriminate patients with and without frontal atrophy (Q^2_Y=0.118), with the frontal atrophy group older, demonstrating later disease onset and showing higher CDR sum of boxes memory scores. CSF measures were able to discriminate atypical AD subtypes (no medial temporal atrophy) with and without frontal atrophy, where subjects with frontal atrophy had lower level of Amyloid-beta.

MTA, FA and PA visual rating scales helped to discriminate clinically different AD subtypes. This could be useful to improve diagnostic and predictive methods and aid population selection and enrichment for clinical trials, and, as a central goal, improve clinical care.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1156

A LONGITUDINAL MRI/MRS STUDY IN DIFFERENT TRANSGENIC MODELS OF ALZHEIMER'S DISEASE

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Objectives Understanding the metabolic and structural changes during Alzheimer's disease (AD) is a task of major relevance since it would allow identifying clinical biomarkers suitable for an early diagnosis. Within the IMI-PharmaCog European Consortium (Grant Agreement n°115009, www.alzheimer-europe.org), we performed a longitudinal MRI and MRS analysis in two different transgenic mice.

Methods With a 7T Bruker Biospec high-res (146x117x146 µm voxel) in vivo MRI and MRS (PRESS sequence TR/TE=2500/10ms) in two single voxels (~4mm^3) positioned in the dorsal hippocampus and striatum were acquired. We followed from 4 to 24 months double (TASTPM) and triple (APP/PS2/Tau) transgenic mice.

Results Already at 4 months TASTPM showed a pronounced atrophy (-20%) not age-related whereas in APP/PS2/Tau hippocampal volume progressively decreased with age (-32%). Also the striatal area was affected by a progressive age-dependent atrophy in both Tg (APP/PS2/Tau -30%; TASTPM -27%). We observed: a progressive reduction of Glutamate (Glu) (TASTPM -20%; APP/PS2/Tau -10%) in the hippocampus; a reduction of N-acetylaspartate (NAA) at 4 months in TASTPM (-16%) although not progressive; a progressive increase of Myo-Inositol (mIns) starting from 12 months reaching a 2 fold increase in elderly TASTPM mice. Also in the striatum of double Tg mice a progressive reduction of Glu reaching about ~40% was detected.

Conclusions Changes observed in these transgenic mice are partially reminiscent of the cerebral alterations in familial AD. This non-invasive analysis could be adopted in preclinical investigation of therapeutic approaches: we are now addressing whether these alterations are modified as a result of amyloid lowering treatment.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1342

COGNITIVELY NORMAL SUBJECTS WITH LOW BETA-AMYLOID LEVELS IN CEREBRAL SPINAL FLUID HAVE FASTER AGE-RELATED HIPPOCAMPAL ATROPHY

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Objectives.
Several studies have shown that the volume of the hippocampus (HC) is reduced with age. However the underlying causes for this are not fully understood.
We investigated the association between levels of β-amyloid (Aβ42) and phosphorylated tau (P-tau) in the cerebral spinal fluid (CSF) and age-related hippocampal volume decline in 252 cognitive normal participants (65-87 years) from the prospective Swedish BioFinder study.

Methods.
Volumetric hippocampal data were acquired from structural 3D magnetic resonance images using the machine learning algorithm 'AdaBoost' (www.loni.usc.edu) with a training set produced by 'Harmonized hippocampus protocol' initiative (www.hippocampal-protocol.net). CSF Aβ42 and P-tau were quantified using Innotest ELISA.

Results.
A linear regression analysis including CSF measures and age as dependent variables revealed that only age (Beta=-0.29) was associated with right hippocampal volumes, while age (Beta = -0.35, $p<0.001$) and levels of Aβ42 (Beta = 0.12, $p=0.04$) were associated with left hippocampal volume.
The correlation between age and HC volume in whole cohort was $r= -0.47$ (left HC) and $r= -0.38$ (right HC). In 76 participants with pathologic levels of CSF Aβ42, we found an increased age-associated HC atrophy (left HC, $r=-0.60$; right HC, $r= -0.44$ right HC) compared to those with normal levels of Aβ42 (Left HC, r=-0.39; right HC, -0.35).

Conclusions.
These results reveals that the levels of CSF Aβ42, but not P-tau, have a modest association with HC volume in cognitively healthy subjects. Increased amyloid burden in the brain may enhance age-related HC atrophy particularly on the left side.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1383

ANALYSIS OF CALLOSAL ATROPHY IN ALZHEIMER'S BRAIN MR IMAGES USING NON-LINEAR DIFFUSION FILTER AND EDGE BASED LEVEL SET METHOD

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Objective: Alzheimer's Disease (AD) is a complex neurodegenerative disorder that results in the atrophy of gray and white matter structures and whole brain shrinkage. In this work, callosal reduction in sagittal view MR images is analyzed using anisotropic diffusion filter based level set method. Anisotropic diffusion filtering is a non-linear diffusion process, which performs region specific smoothing on images while preserving edges.
Methods: Images for this study are obtained from OASIS, a public domain database. Non-linear anisotropic diffusion filtering is used to enhance the intensity variations and feature boundaries. Level set method without re-initialization is used to segment corpus callosum from the sagittal view MR images. The penalty term added in the level set evolution overcomes reinitialization problem.
Results: It is found that the level set method is able to segment Corpus Callosum (CC) in both the normal and Alzheimer conditions. The edges obtained using non-linear anisotropic diffusion filtering is found to be distinct with less discontinuity. Consequently, edges of CC are preserved. The geometric features such as area, minor axis and convex area are found to be highly significant ($P < 0.001$) in differentiating the pathology. The morphometry of CC is apparently affected in AD subjects.
Conclusions: In this work, an attempt has been made to segment CC using anisotropic diffusion based level set method. The features extracted from the segmented CC are able to differentiate normal from Alzheimer conditions. Thus atrophy analysis of CC could be useful in the prognosis and diagnosis of AD.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1395

FORMULATION OF MINKOWSKI BASED RATIO METRIC INDEX IN ALZHEIMER'S MR BRAIN IMAGES USING LOCALIZED REGION BASED LEVEL SET

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Objectives: Alzheimer's disease (AD) is a chronic brain disorder characterized by behavioral change and most common cause of dementia. The analysis of the hippocampus and ventricle in structural MR brain images helps in diagnosis of Alzheimer's disease.In this work, an attempt is made to analyze hippocampus and ventricle based on localized region based level set method and Minkowski based ratio metric index for severity detection.
Methods: The normal and abnormal images considered in this work are obtained from MIRIAD database. The T1 weighted sagittal images (N=120) are considered. The hippocampus and the ventricles are segmented using multi-object localized region based level set. This method formulates the curve evolution with multiple signed distance functions using advance and retreat components. The analysis of the segmented hippocampus and ventricle are carried using Minkowski functionals. The prominent Minkowski feature, hippocampus to ventricle ratio is correlated with the Mini-Mental State Examination (MMSE) score. The longitudinal analysis of the proposed index is also carried out and the results are analyzed.
Results: Results show that multi-object localized region based level set is able to delineate the boundary of the hippocampus and ventricle. It is observed that the Minkowski area of segmented regions helps in better discrimination of normal and pathology conditions. The correlation of ratio metric index with MMSE score is found to be 0.83, 0.44, 0.91 and 0.94 for normal, mild, moderate and severe respectively.
Conclusion: The longitudinal analysis shows that index could be used as a biomarker in Alzheimer like disorders.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1430

THE BRAIN ATROPHY PATTERN ASSOCIATED WITH EPISODIC MEMORY PERFORMANCE IN HEALTHY ELDERLY DIFFERS BETWEEN CASES WITH OR WITHOUT BETA-AMYLOID PATHOLOGY

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Objectives

We aimed to study the brain atrophy pattern associated with episodic memory decline in healthy elderly individuals with or without brain beta-amyloid (Aβ) pathology.

Methods

In the prospective Swedish BioFinder study we compared the association between memory performance, hippocampal volume and cortical thickness in cognitively healthy elderly (65-87 years) with (N=76) or without (N=176) pathologic levels of cerebrospinal fluid (CSF) Aβ42. CSF Aβ42 was measured using Innotest ELISA and memory performance using ADAS-cog item 3 (wordlist delayed recall). Structural 3D magnetic resonance images were analyzed using "AdaBoost" (www.loni.usc.edu) with a training set produced by "Harmonized hippocampus protocol" initiative (www.hippocampal-protocol.net) and Freesufer 5.3 (http://freesurfer.net).

Results

In participants with normal levels of Aβ42 we found a strong correlation between memory performances and decreased hippocampal volumes and cortical thickness in several regions of the medial temporal lobe (including entorhinal cortex). Interestingly, hippocampal volumes and left temporal cortical structures were not significantly correlated with memory performance in participants with low levels of Aβ42 in the CSF. Instead we found strong correlations between episodic memory decline and the left and right superior frontal gyrus as well as the right precentral gyrus.

Conclusion

Episodic memory performance in cognitively healthy elderly with Aβ pathology is more strongly associated with frontal areas compared to cases without Aβ pathology where memory performance is more strongly associated with hippocampal volumes.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1543

PATHOLOGIC VALIDATION OF THE EADC-ADNI HARMONIZED HIPPOCAMPAL PROTOCOL

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[4]Neurology, LENITEM S. Giovanni di Dio-Fatebenefratelli, Brescia, Italy
[5]Neurology, Alava University Hospital, Victoria-Gastaiz, Spain
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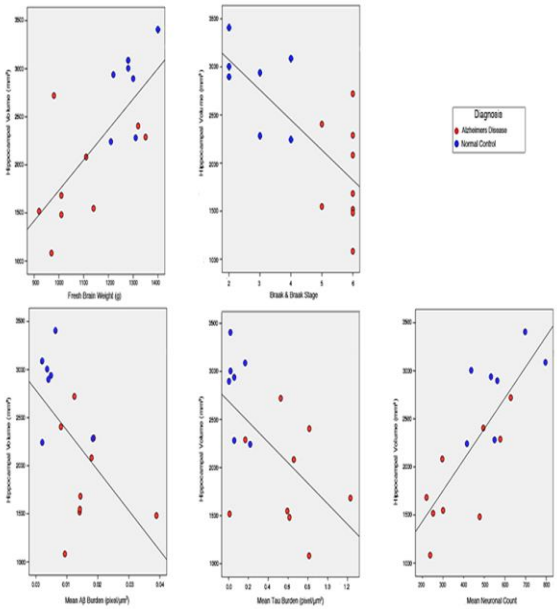
[7]Medicine, UCLA, Los Angeles, USA
[8]Pathology, NA, Besancon, France
[9]Radiology, Mayo Clinic, Rochester, USA

Background: Hippocampal atrophy is the most established structural imaging biomarker for Alzheimer's disease (AD) to date. European AD Consortium and ADNI investigators recently developed a Harmonized Protocol for Hippocampal Segmentation (EADC-ADNI HarP). EADC-ADNI HarP has not been pathologically validated.
Methods: The temporal lobes of 9 AD and 7 cognitively normal subjects (NC) were scanned post-mortem on a 7T Bruker Biospec MRI scanner. Pathologic diagnosis of AD was based on Braak and Braak and CERAD criteria. Hippocampal volumes were obtained with the EADC-ADNI HarP. 6µm-thick hippocampal slices were stained for amyloid beta (Aβ1-40), tau and cresyl violet. The demarcations of each hippocampal subfield were manually drawn with Aperio ImageScope® CS on the digitally scanned stained tissue. Subfield margins were identified based on cytoarchitectonic features. Neuronal counts, Aβ and tau burden for each hippocampal subfield were obtained.
Results: Mann-Whitney comparison of medians showed significant differences between the two groups for total hippocampal tau and Aβ burden and trend difference for neuronal count. Significant median differences were seen in all subfields for tau, in the subiculum, CA1, CA3 and CA4 for Aβ, and in the CA1 and subiculum for neuronal count. We found significant correlations between hippocampal volume and fresh brain weight, Braak and Braak staging, tau, Aβ burden and neuronal counts. Subfield-wise significant association were found for Aβ and neuronal count in CA1 and subiculum, and for tau in CA2-4.
Conclusions: The observed associations provide pathologic validation for the EADC-ADNI HarP and pathologic confirmation of hippocampal morphometry as a valid AD biomarker.


Diagnosis
Alzheimers Disease
Normal Control
Fresh Brain Weight (g)
Braak & Braak Stage
Mean Aβ Burden (pixel/μm²)
Mean Tau Burden (pixel/μm²)
Mean Neuronal Count

06a. Imaging & Biomarkers: structural MRI

ADPD5-1626

WHITE MATTER CHANGES MEASURED BY DIFFUSION TENSOR IMAGING IN AMNESTIC MILD COGNITIVE IMPAIRMENT WITH RETRIEVAL DEFICIT: A PRELIMINARY STUDY

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Introduction: Encoding failure with poor recognition is characteristic of memory impairment seen in AD patients. We think that the pattern of WM alterations in aMCI patients with retrieval deficit (aMCI-R) is somewhat different from that of AD. Methods: We selected subjects diagnosed with aMCI-R (n=7). The control subjects were recruited from general population (n=7). MRI was performed on a 3.0-T MR system equipped with an 8-channel head coil. For the DTI data, a single shot spin-echo EPI sequence was used. FA and trace maps for each subject were obtained with DTIstudioV2 software. Further imaging processing was performed with the statistical parametric mapping-version 5 (SPM5) program. We compared the DTI indices of aMCI-R with that of normal control. Results: FA in aMCI-R was reduced in WM of the bilateral cingulate and the middle frontal gyri, and the right superior frontal gyrus (p

06a. Imaging & Biomarkers: structural MRI

ADPD5-1703

STRUCTURAL CHANGES IN MIDDLE-AGED ADULTS WITH SUBJECTIVE MEMORY COMPLAINTS

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[2]Institut de la Mémoire et de la Maladie d'Alzheimer (IM2A) & Institut du Cerveau et de la Moelle épinière (ICM), Sorbonne Universités Université Pierre et Marie Curie, Paris, France
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Objectives. Aim of the present study is to compare whole brain gray matter volumes between cognitive intact middle-aged individuals with subjective memory complaints (SMC+) and without (SMC-).
Methods. 134 middle-aged cognitive intact individuals (49.2±5.6 years, MMSE 29.1) underwent clinical, neuropsychological evaluation and T1-weighted volumetric MRI. Subjects were considered SMC+ if they believed to have some memory disturbances (yes/no question). MRI images were pre-processed using DARTEL tool in SPM8 to perform Voxel-Based Morphometry analysis. Whole brain gray matter volumes differences between groups were computed using an analysis of variance model with age, gender, education and depressive symptoms as covariates, after correction for total intracranial volume. Comparison of clinical data between groups were performed using independent sample t-test or chi-square test when appropriate.
Results. SMC+ (N=78) compared to SMC- (N=56) were more female (68% vs 37% $p<0.001$), had lower education (10.0±4.0 vs 12.3±3.9 $p=0.001$) and showed more depressive symptoms at Brief Symptoms Inventory (6.3±4.6 vs 3.9±3.2 $p=0.001$). No significant differences in vascular and genetic (Apolipoprotein E4 allele) risk factors and neuropsychological performance were found between groups. SMC+ show significant lower gray matter volumes than SMC- in caudate nuclei and right superior medial frontal gyrus (FWE correction, $p<0.05$). SMC- did not show any voxel with significantly reduced grey matter density.
Conclusions. This is the first study aimed to assess cortical changes in middle-aged individuals complaining about memory problems. Follow-up studies should clarify whether structural differences found between groups are normal inter-individual genetically-based features or pathological abnormalities predisposing to the development of Alzheimer's disease.

06a. Imaging & Biomarkers: structural MRI

ADPD5-2010

APOE-BY-SEX INTERACTIONS ON BRAIN METABOLISM AND STRUCTURE

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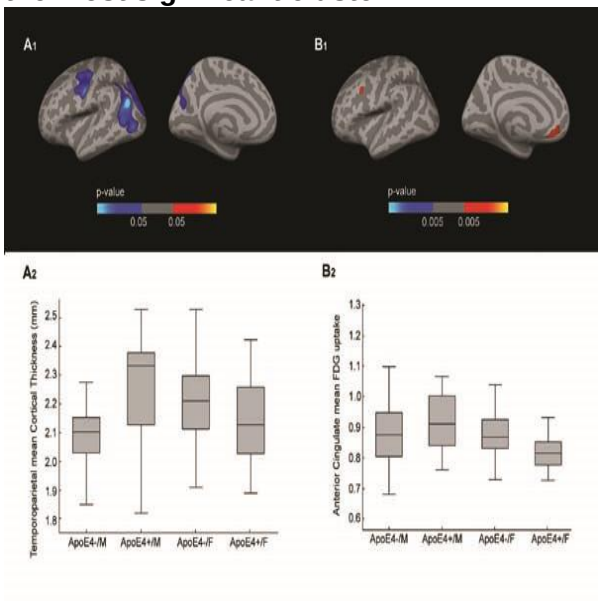
Objective: To assess the APOE4-by-sex interaction on brain structure and metabolism in healthy controls (HC).

Methods: Cross-sectional study. HC from the Alzheimer´s Disease Neuroimaging Initiative (ADNI) underwent structural 3T-MRI (n=146) and/or a FDG PET (n=242) and lumbar puncture. Cerebrospinal fluid (CSF) beta-amyloid-1-42 (AB1-42) and phospho-tau (p-tau) levels were measured by Luminex. Cortical thickness (CTh) was measured by Freesurfer and FDG was analyzed by SPM8. CTh and FDG difference maps were derived from interaction and group analyses. Clusters from the interaction analysis were isolated to analyze the directionality of the interaction in an ANCOVA.

Results: There was a significant APOE4-by-sex interaction for brain structure and metabolism. ApoE4 carriers had lower CSF AB1-42 and higher CSF p-tau values with respect non-carriers. Hypometabolism was found only in ApoE4-carrier females. CTh was increased in ApoE4-carrier males and decreased in ApoE4-carrier females (Figure). The stratified group analyses by gender confirmed these results.

Interpretation: The impact of APOE4 on brain structure and metabolism is modified by gender. Females APOE4 carreriers show greater hypometabolism and atrophy than males. This APOE4-by-sex interaction should be considered in current clinical trials in pre-clinical AD in which APOE4 status is a selection criteria**.**

Figure. Clusters with APOE4-by-sex interaction on CTh (A1) and metabolism (B1). Box-and-whisker plots illustrate individual CTh (A2) or FDG-uptake (B2) values in the most significant cluster.



06a. Imaging & Biomarkers: structural MRI

ADPD5-2181

ALTERATIONS IN REGIONAL BRAIN VOLUMES IN CARRIERS OF THE C677T MUTATION IN MTHFR IN YOUNG AND ELDERLY POPULATIONS

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1. Objectives

We previously reported that the C677T variant (MAF=0.245) in the methylene-tetrahydrofolate reductase (*MTHFR*) gene was associated with smaller regional brain volumes in two independent elderly cohorts. This variant causes a reduction in the function of the MTHFR enzyme. Here, we tested if the effects of C677T on brain volumes could be consistently detected across the life span.

2. Methods

At each voxel in the brain, we used tensor-based morphometry to examine whether regional volume differences were associated with carrying the risk allele, and compared findings between two large cohorts of elderly (N=738, average age 75.52 years) and young (N=578, average age 23.80 years) individuals.

3. Results

After FDR correction for multiple comparisons, young carriers of the risk variant showed small reductions in localized brain regions and also showed slightly larger volumes in the medial occipital lobes. Elderly subjects showed extended areas of brain atrophy, especially in the thalamus, frontal lobes, temporal lobes, and cingulate gyrus. These effects were twice as pronounced in the elderly. Each risk allele was associated with a 1-2 % change in local brain volumes in the young sample, and with a 2-4% variation in elderly individuals.

4. Conclusions

These findings suggest that carriers of the C677T variant have differences in brain structure, which may vary over the lifespan. Previous reports have shown that the effects of this variant could be modulated by dietary folate, which may influence the onset or rate of progression of brain aging and neurodegeneration in carriers of this polymorphism.

06a. Imaging & Biomarkers: structural MRI

ADPD5-2184

CEREBRAL ANATOMICAL ALTERATIONS IN HEALTHY MIDDLE-AGED APOE4 CARRIERS

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[2]Radiology, Hospital Clinic i Provincial Barcelona., Barcelona, Spain
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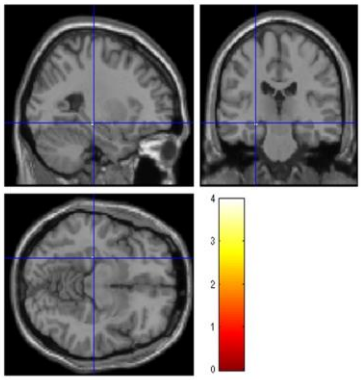
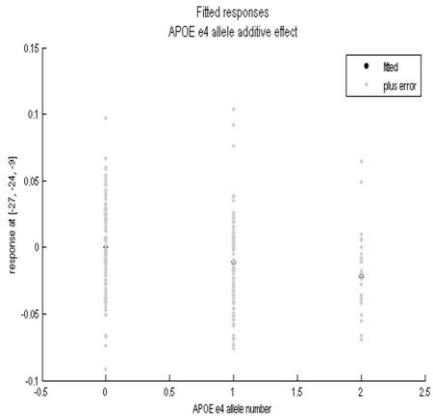
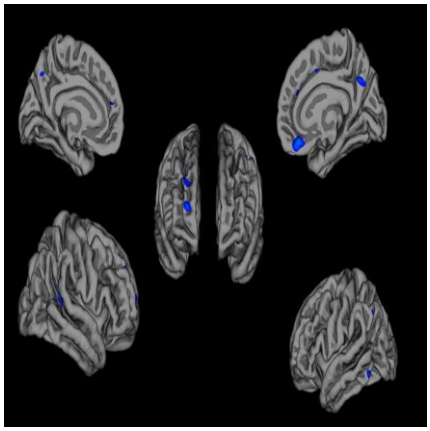
Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Spain
[4]Clinical Research, BarcelonaBeta Brain Research Center, Barcelona, Spain

APOE e4 carriers, specially the homozygous ones, are at increased risk for developing AD. Healthy late-middle aged APOE4 carriers show alterations such as decreased glucose metabolism, reductions in cortical thickness and alterations in task-related and default mode network activity in brain regions known to be affected by AD. Conversely, there is controversy on whether, at these ages, asymptomatic APOE4 carriers display reduced hippocampal volumes, even though this is a characteristic feature by the time AD symptoms occur. In this work, we aimed at determining APOE4-associated structural changes in cognitively healthy late-middle age subjects in the whole brain and, specifically, in the hippocampus.
Within the ALFA project, 238 subjects aged 45-65 were recruited (107 noncarriers, 100 heterozygous and 31 homozygous). Cerebral 3D-T1 weighted MRIs were submitted to VBM-SPM and Freesurfer analyses ($p<0.001$) of APOE4 additive effects after correcting for intracranial volume, age and sex. Additionally, the hippocampal formation was further segmented into 8 subfields which were subsequently analyzed ($p<0.05$).
The VBM analysis revealed a cluster of APOE4-associated volumetric decrease in the right hippocampus. No reductions could be detected either in the volumes of the whole hippocampus or in the hippocampal subfields in either hemisphere. No significant changes in cortical volume could be detected in the VBM-SPM analysis. However, cortical thickness was bilaterally reduced in the precuneus, among other brain regions, in a dose dependent manner.
Our results indicate that cognitively healthy late-middle aged APOE4 carriers show subtle cerebral morphological alterations in key regions in the development of AD pathology.




Fitted responses
APOE e4 allele additive effect
fitted
plus error
response at [-27, -24, -9]
APOE e4 allele number
0.15
0.1
0.05
0
-0.05
-0.1
-0.5
0
0.5
1
1.5
2
2.5



06a. Imaging & Biomarkers: structural MRI

ADPD5-2204

LONGITUDINAL CORTICAL THICKNESS CHANGES ASSOCIATED TO AMYLOID-BETA DEPOSITION IN COGNITIVELY HEALTHY SUBJECTS

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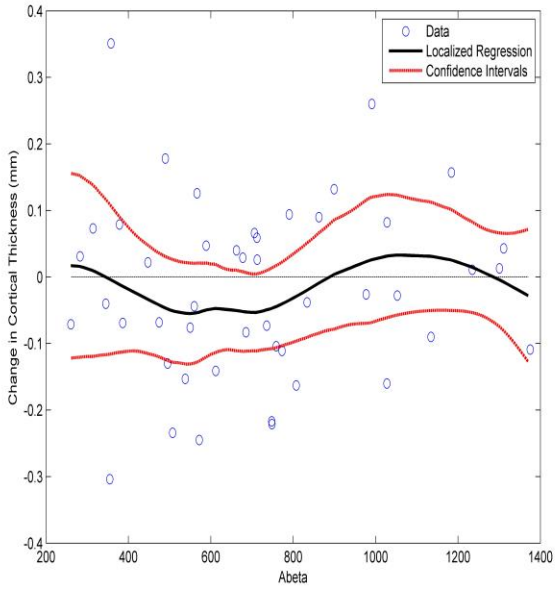
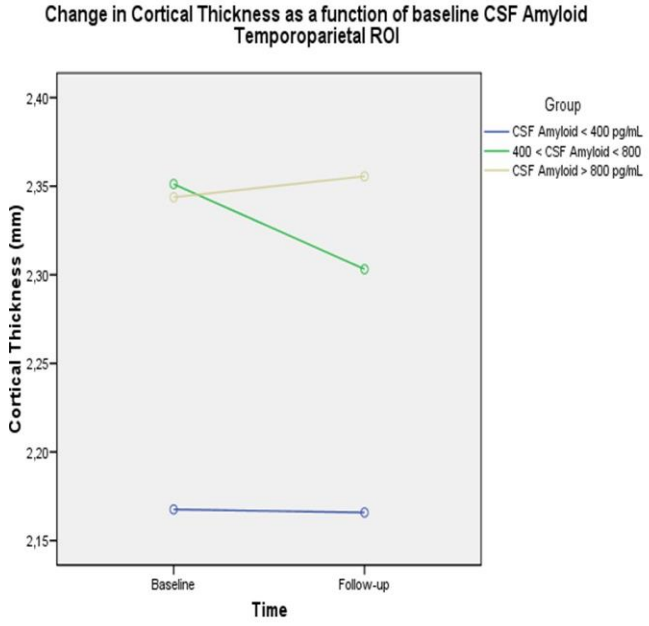
The characteristic progressive memory impairment in AD is related to a pattern of mesial temporal and temporoparietal atrophy. However, Amyloid-β (Aβ) deposition begins many years before symptoms appear. Indeed, several recent studies have shown that Aβ is associated with changes in cortical thickness prior to the development of cognitive impairment. In this regard, increases in cortical thickness have been reported in temporopariteral regions which might undergo neuronal hypertrophy and/or inflammation. In this work, we report the longitudinal changes in cortical thickness of cognitively healthy subjects associated to baseline CSF Aβ.

Forty-seven healthy subjects underwent MRI and lumbar puncture. Two years later, MRI was repeated on the same individuals. Freesurfer was used to calculate longitudinal cortical thickness changes. In a temporopariteral ROI that showed cortical thickening, we performed a nonparametric regression against CSF Aβ. Based on this, subjects were divided into three groups according to their baseline CSF Aβ values (over 800 pg/mL, between 400 and 800 pg/mL, and under 400 pg/mL) and, then, longitudinal cortical thickness changes were compared between them.

Subjects with higher CSF amyloid concentrations showed a moderate cortical thickening in this ROI that reversed in subjects with transitional amyloid values. The lower CSF Aβ group showed significantly lower baseline thickness (-0.215 mm; p=0.020) but minor longitudinal changes. Only the middle group showed a significant variation over time (0.025 mm/year; p = 0.049).

Our results support that Aβ deposition is associated with a subtle yet complex pattern of cerebral structural changes prior to the development of cognitive impairment.


Data
Localized Regression
Confidence Intervals
Change in Cortical Thickness (mm)
Abeta
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
200
400
600
800
1000
1200
1400


Change in Cortical Thickness as a function of baseline CSF Amyloid
Temporoparietal ROI
Group
CSF Amyloid < 400 pg/mL
400 < CSF Amyloid < 800
CSF Amyloid > 800 pg/mL
Cortical Thickness (mm)
2,40
2,35
2,30
2,25
2,20
2,15
Baseline
Follow-up
Time

06a. Imaging & Biomarkers: structural MRI

ADPD5-2281

BETWEEN COHORT TRANSFERABILITY OF ALZHEIMER'S DISEASE CLINICAL TRIAL ENRICHMENT WITH AMYLOID IMAGING, HIPPOCAMPAL VOLUME AND APOE-4 GENOTYPE

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Background: Biomarkers and genetics are proposed to enrich populations in MCI / AD clinical trials. Amyloid burden and structural neurodegeneration have both been qualified by the EMA as enrichment markers. The transferability of these biomarkers between cohorts has not yet been well characterized. Here, the performance of these biomarkers are assessed individually and in combination on the ADNI I/II MCI cohorts and compared to enrichment with ApoE-4 status.
Methods: Included are the 260 MCI subjects from ADNI I/II for which an Amyloid marker and clinical follow-up with MMSE/CDR-SB after 24 months is available. We calculated required sample sizes per arm and trial cost for a hypothetical 2-year trial based on the 24 month change in MMSE/CDR-SB [1]. Hippocampal volume was extracted using LEAP [1]. Compared where enrichment performance of the three markers individually and combined. Marked-benchmarked values were used to estimate trial costs [1].
Results: Presented are sample sizes and trial cost ($m) for different enrichment strategies and different clinical endpoints in both cohorts.
Conclusions: Sample size and trial cost can be significantly different across cohorts and clinical endpoints. Individual and combined enrichment cannot just help to reduce required sample sizes and trial cost, but can also help to significantly reduce this variability across cohorts and clinical endpoints.

References:
[1] Yu. Neurobiology of aging, 2014
[2] Wolz.NeuroImage , 2010

		Sample Size		Trial Cost	
		MMSE	CDR-SB	MMSE	CDR-SB
Unenriched	ADNI I	1199	418	109	38
	ADNI II	459	841	42	77
Amyloid	ADNI I	704	284	81	33
	ADNI II	281	303	34	37
HCV	ADNI I	558	239	60	26
	ADNI II	324	445	34	46
Apoe-4	ADNI I	704	284	81	26
	ADNI II	279	317	34	28
Amyloid & HCV & Apoe-4	ADNI I	350	211	54	27
	ADNI II	195	180	24	22

06a. Imaging & Biomarkers: structural MRI

ADPD5-2292
NEURO-IMAGE GUIDED INVESTIGATION OF EARLY WHITE MATTER ALTERATIONS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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The corpus callosum (CC) plays an important role in interhemispheric transfer of cognitive information and has been shown to be susceptible to Alzheimer's disease (AD). There is an emergent need for new in vivo non-invasive studies to monitor CC changes longitudinally in order to clarify how and when the integrity of CC alters in AD. The aim of this study was to probe *in vivo* T_2 relaxation time changes longitudinally in the CC (Fig.1) of the Tg2576 (Tg) and wild-type (WT) mice with age and to investigate potential biological mechanisms contributing alterations in the CC. The major finding of our longitudinal study was a significant prolongation of the T_2 in the CC, reflecting significant microstructural changes in Tg mice compared to WT mice at and above 10 months of age ($P < 0.0031$) (Fig. 2A). These changes may be related with activated glial cells, increased vacuole formation, increased demyelination and Aβ plaque load (Fig. 2B,C &D). To our knowledge, this is the first longitudinal *in vivo* T_2 study assessing microstructural changes in the CC of the Tg2576 mice. Our results suggest that inflammatory pathology accompanied by demyelination may lead to prolonged T_2 which can be mark as an early event during AD progression in this animal model.

Acknowledgment: This work was partially supported by grants from Alzheimer Forschung Initiative e.V. (AFI, Grant Nr 13810), Stichting Alzheimer Onderzoek (SAO-FRA, Grant Nr 13026). Firat Kara is a Postdoctoral Fellow of the Research Foundation-Flanders (FWO) from University of Antwerp.

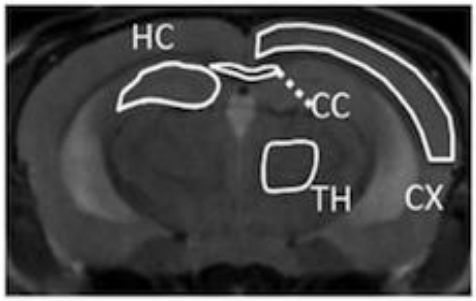

Fig. 1. T2-weighted image of a mouse brain. Corpus callosum (CC), hippocampus (HC), cortex (CX) and thalamus (TH).

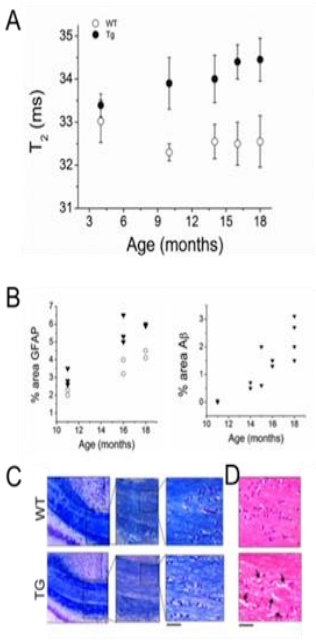

Fig. 2. (A) Age-dependent *in vivo* T_2 changes of the CC region of the wild-type (WT) and Tg2576 (TG) mice. Values are expressed as mean T_2 in ms ± SD (error bars) (95% C.I.). Two tailed student t- test show significant difference between wild type and Tg2576 mice at the age of 10 months ($P < 0.00313$), 14 months ($P < 0.00063$), 16 months ($P < 0.00063$) and 18 months ($P < 0.00063$). No significant difference was observed between WT and TG mice at the age of 4 months ($P > 0.05$). (B) Quantitaive analysis of GFAP stained area and Aβ load in the CC with age in Tg2576 (▼) and control mice (○). (C) Histological section of the brain of a WT and a Tg mouse, stained with the Klüver-Barrer method, which stains the myelin in blue, and standard H&E stain (D). Demyelination and vacuolation in the CC region of Tg mouse (C and D, lower layer) is more prominent compared to the WT mouse (C, D upper layer) as can be clearly seen (arrows) in the magnified subsampled areas. Scale bars: 500 μm.

06b. Imaging & Biomarkers: functional MRI

ADPD5-0302

RESTING STATE FUNCTIONAL CONNECTIVITY CHANGES ASSOCIATED WITH VISUO-SPATIAL COGNITIVE DEFICITS IN PATIENTS WITH MILD ALZHEIMER'S DISEASE

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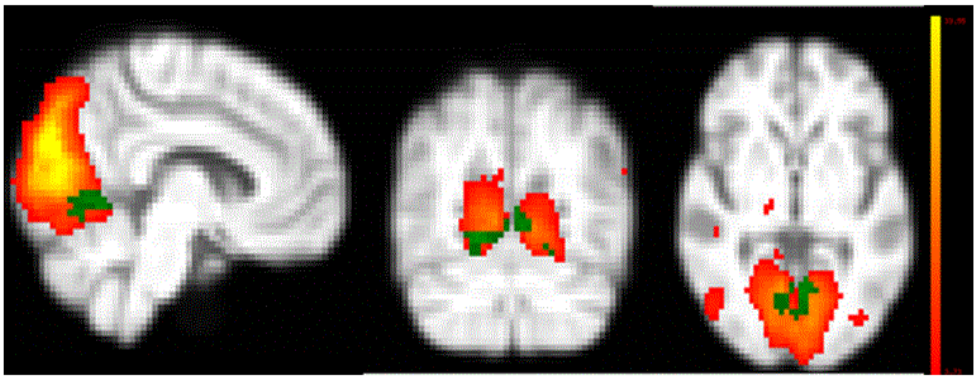
Objectives: Alzheimer's disease (AD) is a neurodegenerative disorder characterised by disconnection within and between various brain networks along with diffuse neuropsychological impairment. Pathology in the visual association areas has been documented in pre-clinical AD and therefore we aimed at examining the relationship between brain connectivity and visuo-spatial (V-S) cognitive deficits in AD.

Methodology:
Patients with AD (N=23; females=8) diagnosed according to NIAAA criteria and of mild illness severity (CDR=1) were recruited after obtaining informed consent. The study was approved by institutional ethics committee of NIMHANS. Tests for V-S working memory, episodic memory and construction were administered as part of comprehensive neuropsychological assessment using NIMHANS Neuropsychological Battery for Elderly. Resting state fMRI (rsfMRI) and high resolution structural T1 images were acquired as per the standard protocols. Based on the severity of V-S impairment, the sample was sub-divided into those with severe (N=12;F=4) and mild (N=11;F=4) deficits. Between-group differences in resting state functional connectivity (rsFC) were examined by performing independent component analysis (ICA) and dual regression analysis in FSL, correcting for age, gender, age of illness onset, and total brain volume.

Results:
Patients with AD having severe V-S deficits exhibited significantly increased rsFC in medial occipital area of the visual network and right paracingulate region of executive network compared to patients with mild V-S deficits.

Conclusion:
Increased rsFC in visual and executive networks in patients with more severe V-S deficits might be considered as a functional neuroimaging biomarker reflecting an attempt to compensate for the progressive visuo-spatial neurocognitive impairment in

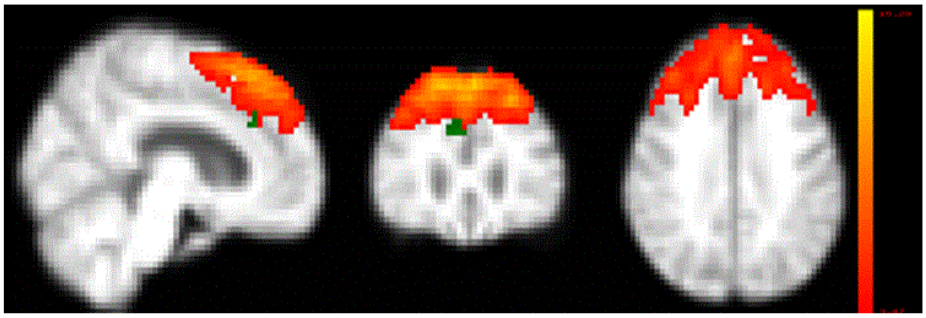
early AD.



Group resting state visual network is represented by voxels in red-yellow (threshold between 3.73 – 33.55 respectively) Green voxels shows clusters of significantly increased functional connectivity in patient with severe visuo-spatial cognitive deficits compared to patients with mild visuo-spatial cognitive deficits ($p<0.05$, family wise error (FWE) corrected). Results are corrected for Gender, age of illness onset and total brain volume. Images are laid over the group mean structural image.

Sl. No.	Voxels (N)	P value	MNI co-ordinates			Location (reported from probabilistic Harvard cortical and subcortical atlas)
			X	Y	Z	
1.	30	0.016	35	19	20	Left Lingual Gyrus
2.	23	0.03	29	18	41	Left Precuneous Cortex
3.	18	0.032	30	17	31	Left Supracalcarine Cortex

Regions showing increased resting state functional connectivity in the visual network among Alzheimer's patients with ***severe*** Visuo-spatial cognitive deficits compared to AD patients with ***mild*** visuo-spatial cognitive deficits.
p-values are after family wise error (FWE) correction,
MNI: Montreal Neurolgical Institute.



Group resting state executive network is represented by voxels in red-yellow (threshold between 3.47 – 15.29 respectively) Green voxels shows clusters of significantly increased functional connectivity in patient with ***severe*** visuo-spatial cognitive deficits compared to patients with ***mild*** visuo-spatial cognitive deficits ($p<0.05$, family wise error (FWE) corrected). Results are corrected for Gender, age of illness onset and total brain volume. Images are laid over the group mean structural image.

Sl. No.	Voxels (N)	P value	MNI co-ordinates			Location (reported from probabilistic Harvard cortical and subcortical atlas)
			X	Y	Z	
1	6	0.03	28	53	34	Right Paracingulate Gyrus

Regions showing increased resting state functional connectivity in the executive network among Alzheimer's patients with ***severe*** Visuo-spatial cognitive deficits compared to AD patients with ***mild*** visuo-spatial cognitive deficits.
p-values are after family wise error (FWE) correction,
MNI: Montreal Neurolgical Institute.

Demographic table

	AD Patients with SEVERE V-S deficits (N=12)	AD Patients with MILD V-S deficits (N=11)	P value
Gender (female : male)	4:8	4:7	
Age in years	70.4 ± 2.7	69.8 ± 3.9	0.8
Age of illness onset	62.1 ± 2.2	63.7 ± 1.8	0.72
Number of formal years of education	8.2 ± 1.6	11.4 ± 1.2	0.53

Neuro-cognitive performance:

NIMHANS – Neuropsychology Battery for Elderly (N-NBE)	AD Patients with SEVERE V-S deficits (N=12)	AD Patients with MILD V-S deficits (N=11)	P value (Mann–Whitney U-test)
Auditory verbal learning task			
Immediate recall trial 1	2 ± 1.5	2.1 ±1.4	0.7
Immediate recall trial 2	2.8 ± 1.4	2.4 ± 1.5	0.4
Immediate recall trial 3	4 ± 1.2	3.9 ± 1.6	0.5
Delayed recall (Free)	1 ± 0.9	0.8 ±0.7	0.2
Logical memory			
Immediate recall	3 ± 2	3.4 ± 2.1	0.7
Delayed recall	1 ± 1.6	0.8 ± 1.1	0.7
Visuo-spatial			
copy	15.3 ± 4.5	23.7 ± 2	<0.01
Immediate recall	5.9 ± 3.5	16.9 ± 4.1	<0.01
Delayed recall	1 ± 2	3.3 ± 2.1	0.02
Digit span forward	3.8 ± 1	4.2 ± 1.1	0.5
Digit span backward	1.8 ± 1	2.4 ± 1	0.3
Spatial span forward	3.1 ± 0.4	4.6 ± 0.9	<0.01
Spatial span backward	1.5 ± 0.8	3.1 ± 0.8	<0.01
VFT - fruits	5.9 ± 2.6	7 ± 2.9	0.2
VFT - animals	6.8 ± 2.1	7.6 ± 3	0.3
VFT - vegetables	6.1 ± 3	6.6 ± 3.6	0.7

06b. Imaging & Biomarkers: functional MRI

ADPD5-0429

A NOVEL COGNITIVE FMRI TASK TO ASSESS BRAIN MECHANISMS UNDERLYING VISUAL PROCESSING AND ATTENTION IN EARLY ALZHEIMER'S DISEASE

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Objectives
Visual processing deficits can be found in early Alzheimer's disease (AD). We aimed at developing a visual cognitive task to study brain mechanisms underlying visual processing in early AD using functional MRI (fMRI). To achieve our goal we developed a novel visual object matching task using conventional and unconventional (spatially rotated) views of object pairs. Here we present the fMRI results of a pilot study performed in healthy subjects (HS).

Methods
Twenty-two right-handed HS (11 men; age 25.3±2.8 years) performed a visual cognitive task in a 1.5T MR scanner. fMRI data were processed using SPM8 and the standard pipeline. The significance level was set to $p<0.05$ FWE corrected.

Results
At the group level, we found major activation of bilateral temporo-occipital areas, precuneus and inferior frontal gyri during the conventional conditions as compared to the control task. The unconventional conditions revealed additional activation of the posterior parietal areas including the superior parietal lobule and intraparietal sulcus as compared to the conventional conditions.

Conclusions
In the visual object matching task with conventional views of objects we observed major engagement of the ventral visual pathway. The task with spatially unconventional views of objects revealed additional recruitment of the dorsal visual pathway and the dorsal attentional network which is involved in the top-down attentional control of visual processing. Our fMRI paradigm shows promise for investigating the functional integrity of the ventral and dorsal visual pathways and for assessing task-dependent attentional modulation of visual processing in early AD.

06b. Imaging & Biomarkers: functional MRI

ADPD5-0502

COMPARISON OF AMNESTIC MILD COGNITIVE IMPAIRMENT WITH AND WITHOUT PARKINSON'S DISEASE: A RESTING STATE FMRI STUDY

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Backgrounds: Amnestic mild cognitive impairment (AMCI) is regarded as an early clinical stage of dementia, especially Alzheimer's disease (AD). Memory impairment is also common in Parkinson's disease (PD) patients with cognitive impairment (PDMCI). We aimed to find the changes in functional connectivity in patients with AMCI with PD (AMCI-PD+) and those without PD (AMCI-PD-) using resting-state functional MRI (rs-fMRI).

Methods: A total of 65 patients with AMCI (AMCI-PD-, n = 33, AMCI-PD+, n = 32) and 29 subjects with normal cognition (NC) underwent rs-fMRI. We analyzed the resting-state functional connectivity from seed region of interest in precuneus, posterior cingulate cortex (PCC), substantia innominata (SI), and caudate in patients with AMCI-PD- and AMCI-PD+ relative to NC group.

Results: Seed-based analyses with precuneus and PCC seeds showed that, patients with AMCI-PD+ had a decreased functional connectivity mainly in the posterior cortical regions including bilateral occipital, lateral parietal, and temporal lobes, while patients with AMCI-PD- had an increased connectivity in the medial temporal, frontal, and parietal lobes, insular cortex, and thalamus. Both AMCI groups exhibited an increased connectivity from the SI in widespread cortical regions including the frontal, temporal, and parietal lobes. Seed-based analyses with caudate seed showed that patients with AMCI-PD+ had a decreased cortical and cerebellar functional connectivity; however, no areas of decreased cortical functional connectivity were observed in the AMCI-PD- group.

Conclusion: The pattern of changes in cortical functional connectivity from the PCC/precuneous and striato-cortical connectivity differ between AMCI-PD- and AMCI-PD+, which may reflect different neuropathological basis of memory dysfunction.

06b. Imaging & Biomarkers: functional MRI

ADPD5-0575

COURSE PREDICTION FOR ALZHEIMER DISEASE BY DIFFUSION TENSOR PARAMETERS OF UNCINATE FASCICLES.

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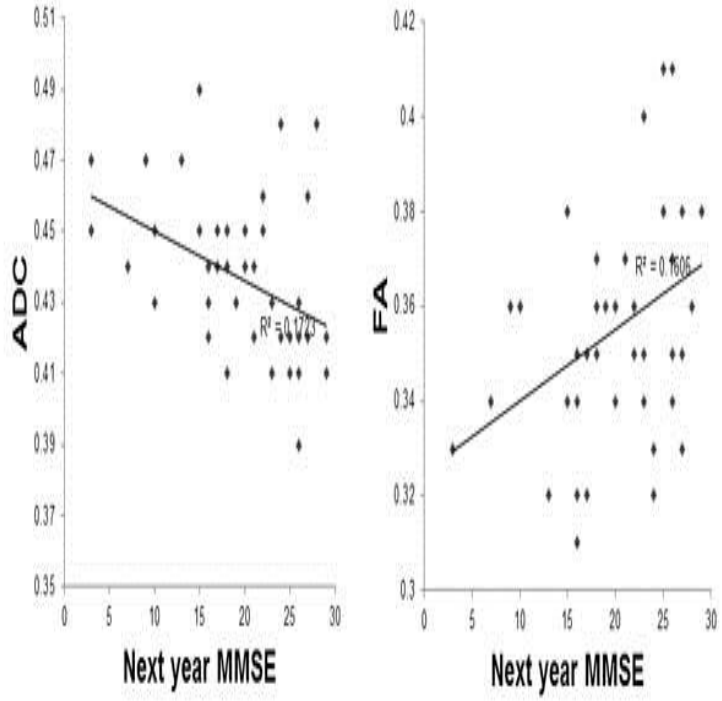
Purpose: The purpose of the current study is to access the feasibility of diffusion tensor parameters for predicting the prognosis of Alzheimer disease (AD). We made analysis for correlation between diffusion tensor parameters and the MMSE.
Subject and Methods: Subjects were 29 AD cases in which annual follow up of diffusion tensor study and clinical study including MMSE score were made for more than two years. Diffusion tensor image were acquired and tractographies were made to measure apparent diffusional coefficient (ADC) and fractional anisotropy (FA) of uncinate fascicles. We evaluated following points. 1) FA, ADC vs MMSE of the same year, 2) FA, ADC vs MMSE of the next year, 3) ΔFA, ΔADC vs ΔMMSE of the same interval, and 4) ΔFA, ΔADC vs ΔMMSE of the next interval.
Results: 1) ADC and FA of showed statistically significant correlation with the MMSE of the same year. 2) ADC and FA showed correlation with the MMSE of the next year (Figure). 3),4)ΔFA and ΔADC did not show correlation with changes in MMSE of the same interval nor the next interval.
Conclusion: ADC and FA of uncinate fascicles can be used in prediction for the severity of Alzheimer disease.



06b. Imaging & Biomarkers: functional MRI

ADPD5-1324

DIFFERENTIAL FUNCTIONAL DISRUPTION BETWEEN VENTRAL AND DORSAL POSTERIOR CINGULATE CORTEX IN MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE

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The posterior cingulate cortex (PCC) is a major hub connecting sub-systems of the default mode network which are functionally impaired in early Alzheimer's disease (AD). The PCC can be subdivided into ventral (vPCC) and dorsal (dPCC) regions. We aimed at identifying relationships between cognitive impairment and ventral and dorsal PCC functional connectivity (FC) disruptions in amnestic Mild Cognitive Impairment (aMCI) and AD patients.

Forty-one healthy old controls (HC), 26 aMCI and 25 AD patients underwent neuropsychological tests and resting-state functional MRI. FC maps from vPCC and dPCC were obtained. In HC, the maps were compared to each other and correlation analyses were carried out with neuropsychological performances to identify ventral and dorsal networks. In patients, connectivity disruptions were investigated by comparing with HC networks.

In HC, the ventral network implied the hippocampus and temporo-parietal regions, whereas the dorsal network held the precuneus, supramarginal, anterior temporal and dorso-medial prefrontal regions. The ventral network FC correlated with autobiographical memory, while the dorsal network FC correlated with visual mental imagery. In aMCI, the dorsal network FC was preserved, while the ventral network FC was altered in the bilateral hippocampus. In AD, ventral network FC disruptions spread into the left supramarginal region and the dorsal network FC was also affected in the right inferior middle temporal region.

In aMCI, only the vPCC network FC may be disturbed, which could be related to episodic memory impairment. In AD, FC disruptions in both ventral and dorsal networks could be related to additional cognitive disruptions arising lately.

06b. Imaging & Biomarkers: functional MRI

ADPD5-1332

SELF-REPORTED PHYSICAL EXERCISE AND DEFAULT MODE NETWORK

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Introduction: The default mode network (DMN) is a set of brain regions that typically deactivate during performance of cognitive tasks. The DMN is detectable using task-free functional connectivity MRI and has been implicated in episodic memory processing. Some studies have shown that physically active people show better DMN connectivity when compared to sedentary. Objective: We aimed to investigate if self-reported physically active elderly present better DMN functional connectivity than sedentary elderly. Methods: 42 cognitive preserved elderly (mean age 68.4, SD=7.2, mean schooling years of 12.12, SD=6.8), with average MMSE score of 28.4 out of 30 (SD=0.9), were divided into two groups: 20 self-reported physically active (A) and 22 sedentary elderly (S). It was considered physically active those subjects who reported practicing physical exercise at least 3x per week for 30 minutes each session. Functional neuroimaging measures were collected in resting state, no task involved. *UF²C* software was used to perform FC analysis and the posterior cingulate cortex (0,-51,15) was settle as seed region. The resultant individual statistical maps were used as input of a second level analysis (group inference), by using *SPM8* (Statistical Parametric Mapping). Results: Two Sample *t*-test ($p<0.005$ cluster with at least five voxels) did not show any difference in DMN functional connectivity between groups. Conclusion: It was not possible to observe DMN functional connectivity difference between self-reported activity elderly, we may consider the fact self-report may not be the best way of assessing physical activity level, since it does not represents fitness level per se.

06b. Imaging & Biomarkers: functional MRI

ADPD5-1449

RESTING STATE FUNCTIONAL CONNECTIVITY OF SUBJECTS WITH AMNESTIC MILD COGNITIVE IMPAIRMENT AND PATHOLOGICAL CSF LEVELS OF AMYLOID BETA

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OBJECTIVE
CSF levels of β-amyloid (Aβ42) has emerged as a powerful predictor of subsequent development of AD in subjects with Mild Cognitive Impairment (MCI). In the present study we investigate the changes in interregional connectivity in MCI patents with pathologic CSF Aβ42 levels.
METHODS
We included 105 cognitively healthy controls with normal CSF Aβ42 levels (>550ng/l) and 55 patients with amnestic MCI with pathologic CSF Aβ42 levels (<550ng/l) from the Swedish BioFinder study. CSF Aβ42 was analysed with Innotest ELISA. The whole-brain functional connectivity was gauged by resting-state fMRI (Siemens Trio 3T, 6min gradient-echo EPI TR/TE=2000/30ms).
RESULTS
Relative controls, patients with aMCI exhibit reduced interregional connectivity (reduced correlation between regional BOLD time-series) in a very large network component, highly focused on a hub region involving thalamus, caudate and putamen, also including key regions, e.g. hippocampus (see Fig. 1 and 2). Several interconnected networks are affected: the basal ganglia, frontal executive, visual and default mode network.
CONCLUSIONS
These findings are in accordance with the hypothesis of cognitive decline following a global disconnection syndrome and failing hub connectivity. Furthermore, we aim to elucidate a progression line ranging from amyloid pathology in healthy elderly, subjective cognitive decline to aMCI and AD.

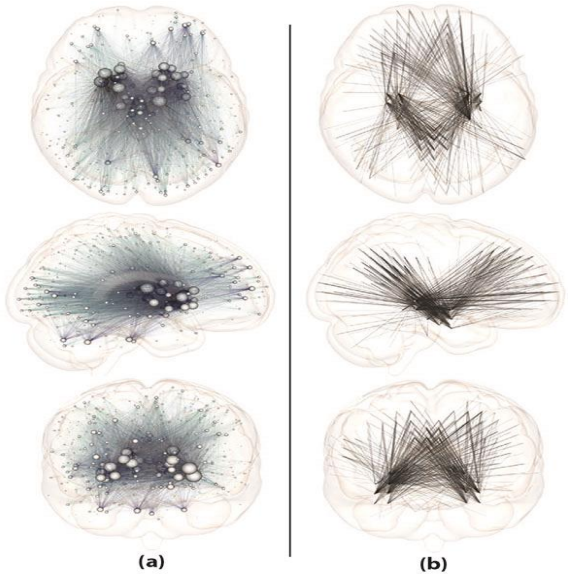

Figure 1. (a) Network component with reduced functional connectivity in aMCI relative controls (Sphere-size represents number of connected links (degree), links colored by end-point degree). (b) Part of high-resolution component involving hippocampus reveals links with default mode and executive networks.

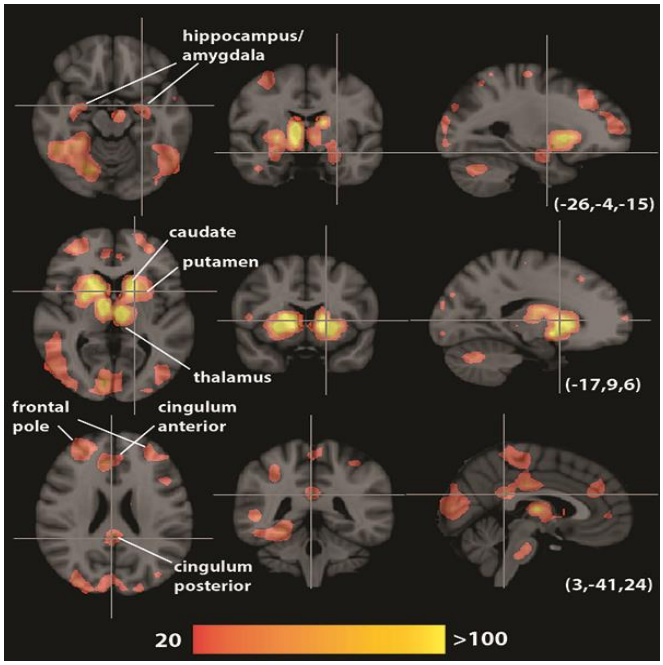

Figure 2. Representative slices of reduced connectivity network component degree.

06b. Imaging & Biomarkers: functional MRI

ADPD5-1807

DOMAIN-SPECIFIC PRECISION OF RECOGNITION MEMORY IN THE MEDIAL TEMPORAL LOBE IN YOUNG AND HEALTHY AGED INDIVIDUALS

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In the medial temporal lobe there are domain-specific pathways that support two different types of memory - memory of object/item (PRC-LEC) and spatial/context information (PHC-MEC). The dentate gyrus and CA3 of the hippocampus might be the regions where the information coming from these two domains is integrated. The aim of this study was to investigate these pathways during a memory task that poses high demands on the precision of recognition memory and pattern separation of similar appearing stimuli.

We used 3T functional magnetic resonance imaging (fMRI) to investigate the precision of recognition memory for object and scene stimuli. During the fMRI session, 44 young and 44 healthy elderly subjects discriminated original stimuli from similar appearing lures. The stimuli were quasi-realistic images of indoor objects and spatial scenes. Older individuals were specifically impaired in detecting lures compared to young individuals, but there was no dissociation between the accuracy to detect object and scene lures. FMRI data showed that there were reduced novelty responses within the hippocampus of elderly subjects for both types of stimuli. However, whereas novelty responses were evident for similar appearing scene lures, they were diminished for object lures.

Elderly subjects show a drop in precision of recognition memory in object and scene stimuli, which is accompanied by functional differences in the processing of object lures. This selective functional impairment is compatible with the possibility that object-processing pathways (PRC-LEC) are affected early by tau pathology.

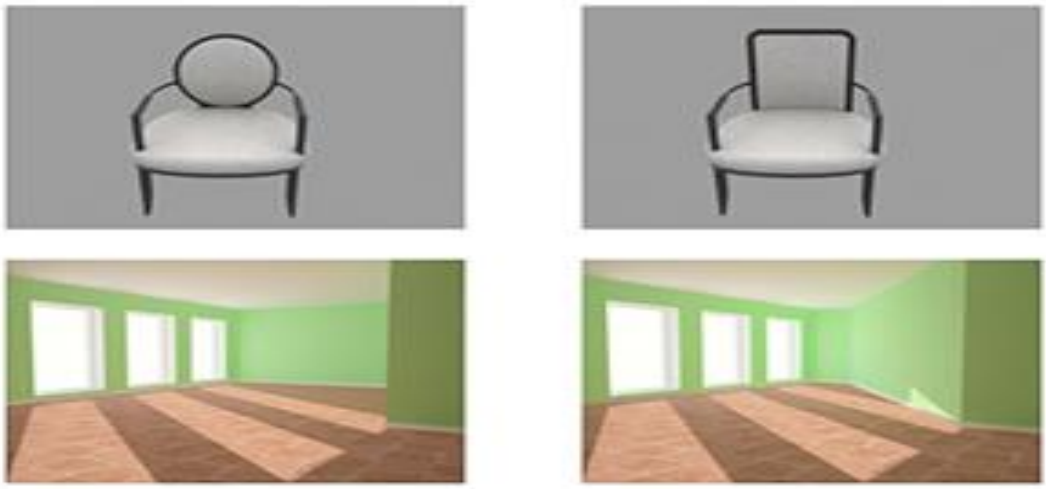

Figure 1: Original and similar appearing lure stimuli for the object and scene condition.

06b. Imaging & Biomarkers: functional MRI

ADPD5-2137
IN VIVO IMAGING TECHNIQUES FOR PRECLINICAL TESTS IN NEURODEGENERATIVE DISEASE

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In vivo imaging is wildly used to investigate disease mechanisms in both humans and animals since disease progression can be easily monitored repeatedly in the same subject. Specifically, MRI is a very powerful tool to acquire high resolution anatomical images and functional scans. Further, the introduction of smart MRI sensor can provide a way to detect molecular signalling in the brain. Here I present a novel dopamine mapping technique to possibly study Parkinson's disease using functional MRI. In addition, MRI technique is applied to study animal model of dementia.

Dopamine mapping was performed in the rat brain using dopamine sensitive MRI sensor. The sensor was slowly infused into the nucleus accumbens area and dopamine release was measured while the medial forebrain bundle was periodically stimulated. A series of T1 images were acquired in Bruker 9.4T magnet and processed to obtain dopamine concentration. The highest concentration was measured in the nucleus accumbens core. The spatial dopamine release was mapped in real-time. This result can be applied to study dopamine related disease such as Parkinson's disease, drug addiction, etc.

MRI technique was also applied to study Alzheimer's disease and vascular dementia in mice. Their brain was repeatedly scanned to obtain anatomical images, angiography and diffusion tensor imaging. Neuronal atrophy appeared in the hippocampus of 5XFAD mouse over 6 months. Bilateral common carotid artery stenosis in C57BL/6 mice also developed hippocampal damages 2~3 months after surgery. In conclusion, various MRI techniques are essential to study neurodegenerative disease in vivo.

06b. Imaging & Biomarkers: functional MRI

ADPD5-2139

DEFAULT MODE NETWORK FUNCTIONAL CONNECTIVITY IN THE EARLY AND LATE MILD COGNITIVE IMPAIRMENT: RESULTS FROM THE ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE

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Neuroimaging studies have identified a breakdown of functional connectivity in the default mode network (DMN) in individuals with Alzheimer's disease or mild cognitive impairment (MCI). However, no studies have investigated DMN connectivity at different stages of MCI, such as early MCI (EMCI) and late MCI (LMCI). Therefore, the aim of this study was to investigate the patterns of breakdown of DMN connectivity occurs using a quantitative index of functional connectivity among cognitively normal (CN), EMCI, and LMCI subjects. Resting state functional magnetic resonance images and neuropsychological test scores from 130 subjects (CN = 43, EMCI = 47, LMCI = 40) were obtained from the Alzheimer's Disease Neuroimaging Initiative. Functional connectivity in the DMN of each subject was computed using independent components analysis. DMN connectivity maps were compared among groups. Functional connectivity in the precuneus, right medial frontal, right parahippocampal, and right middle temporal gyrus was decreased in the EMCI and LMCI compared with CN subjects. When the two MCI groups were directly compared, LMCI subjects exhibited decreased functional connectivity in the precuneus, right medical frontal, left parahippocampal, left middle temporal, and left superior frontal gyrus. Conversely, LMCI presented increased connectivity in the right superior temporal gyrus and left superior parietal lobule. Mean DMN *z*-scores correlated well with measures of cognitive performance. These findings indicate that DMN connectivity is in a continuum going from CN to LMCI. Even in the EMCI state, functional connectivity in the precuneus and medial prefrontal gyrus is decreased.

06b. Imaging & Biomarkers: functional MRI

ADPD5-2143

AMYLOID DEPOSITION IN MILD COGNITIVE IMPAIREMENT IS ASSOCIATED WITH INCREASED HIPPOCAMPAL ACTIVITY, ATROPHY AND CLINICAL PROGRESSION

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Cross-sectional functional magnetic resonance imaging (fMRI) studies using a memory task in mild cognitive impairment (MCI) patients have produced discordant results, with s studies reporting increased hippocampal activity -consistent with findings in genetic at-risk populations- and other studies reporting decreased hippocampal activity, relative to normal controls. However, previous MCI studies have not included markers of amyloid-β (Aβ), which may be particularly important in prediction of progression along the Alzheimer's disease continuum. Here, we examine the contribution of Aβ deposition to cross-sectional and longitudinal measures of hippocampal fMRI activity, hippocampal volume, global cognition and clinical progression over 36 months in thirty-three patients with MCI (Figure 1). Aβ-status was examined with positron emission tomography imaging using Pittsburg compound-B, hippocampal fMRI imaging activity was assessed using an associative face-name memory task, and hippocampal volume was quantified with structural MRI. Finally, global cognition was assessed using the Mini Mental State Exam (MMSE) and clinical progression using Clinical Dementia Rating (CDR) sum of boxes. At baseline, Aβ+MCI patients showed increased hippocampal activation, smaller hippocampal volumes, and a trend towards lower MMSE and higher CDR scores compared to Aβ-MCI patient. Longitudinally, Aβ+MCI patients continued to show high levels of hippocampal activity, despite increasing rates of hippocampal atrophy, decline on the MMSE and faster progression on the CDR. When entered simultaneously into the same linear mixed model, Aβ status, hippocampal activation, and hippocampal volume independently predicted clinical progression. These results indicate that Aβ+MCI patients are more likely on a path towards Alzheimer's disease dementia than Aβ-MCI patients.

06b. Imaging & Biomarkers: functional MRI

ADPD5-2235

APATHY IS ASSOCIATED WITH INCREASED SEGREGATION OF BRAIN NETWORKS IN ALZHEIMER'S DISEASE

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Objectives: The development of apathy in the course of Alzheimer's disease (AD) increases the risk of disease progression. Using connectivity measures based on graph theory, we investigated changes in brain networks associated with apathy in healthy older individuals and those at different stages of AD (subjective memory complaints (smc), mild cognitive impairment (MCI) and AD).
Methods: Resting state MRI scans from 142 individuals (healthy=31, SMC=24, MCI=61, AD=26) (data from the Alzheimer's Disease Neuroimaging Database (ADNI); see adni.loni.usc.edu) were assessed. Following standard preprocessing steps, data was additionally processed to remove volumes with excessive motion and signal variation to reduce motion related effects. Graph theoretical measures of modularity and global efficiency were assessed over a range of thresholds in those with and without apathy across all subjects. We further assessed whether differences in network measures between the groups were influenced by age, gender, diagnostic category or apolipoprotein E status (ApoE) using analysis of covariance.
Results: Modularity, measuring the extent of segregation in brain networks, differed significantly across those with and without apathy. This effect was independent of the changes associated with diagnostic category, age, gender and ApoE status. Further, global efficiency, a measure of functional integration of brain networks, did not differ significantly between the groups.
Conclusions: Apathy is associated with increased segregation of brain networks suggesting that apathy appear to be related to disconnectivity between brain regions. Such alterations in brain networks may underlie the development of apathy in AD

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-0768

UPREGULATION OF MGLUR5 AT EARLY AGE IN MOUSE MODEL FOR ALZHEIMER'S DISEASE VISUALISED WITH [11C]ABP688 PET.

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Objectives: Amyloid-beta (Aβ) oligomers act as co-receptors for the metabotropic glutamate 5 receptor (mGluR5); forming a complex that mediates increases of intracellular calcium in neurons, and leads to dendritic spine loss. Aβ also upregulates astrocytic mGluR5 receptor expression; and mGluR5 antagonism reverses learning, memory and synapse density deficits. In a transgenic mouse model (tg-ArcSwe) for Alzheimer's disease, with enhanced intraneuronal Aβ aggregation starting before 4 months of age, we investigated mGluR5 characteristics using the [^{11}C]ABP688 PET tracer for mGluR5.

Methods: Mice expressing the Arctic (E693G) and Swedish (KM670/671NL) APP mutations (tg-ArcSwe), and C57/Bl6 control littermates of different ages were PET-scanned in a Triumph trimodality PET/SPECT/CT scanner using mGluR5 ligand [^{11}C]ABP688. Mice were sacrificed after scanning, followed by brain extraction. Soluble Aβ protofibrils and mGluR5 concentrations were determined in brain tissue using ELISA, and the amount of mGluR5 protein in brain was also assessed using Western immunoblotting.

Results: PET data and *ex vivo* measurements indicated a tendency of higher mGluR5 concentrations in 4-month-old tg-ArcSwe compared with control mice and this observation was supported by ELISA and Western blot analyses. The mGluR5 ELISA also revealed increased mGluR5 at 8 months of age in tg-ArcSwe mice compared with controls. At these ages, tg-ArcSwe mice also had increased levels of soluble Aβ protofibrils.

Conclusions: Upregulation of mGluR5 occurs before formation of insoluble Aβ plaques. Aβ oligomers can possibly cause toxic effects through the mGluR5 receptor, and [^{11}C]ABP688 could be an early biomarker for Alzheimer's disease.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-0802
THE MRC UK DEMENTIAS PLATFORM: ACCELERATING DEMENTIA RESEARCH
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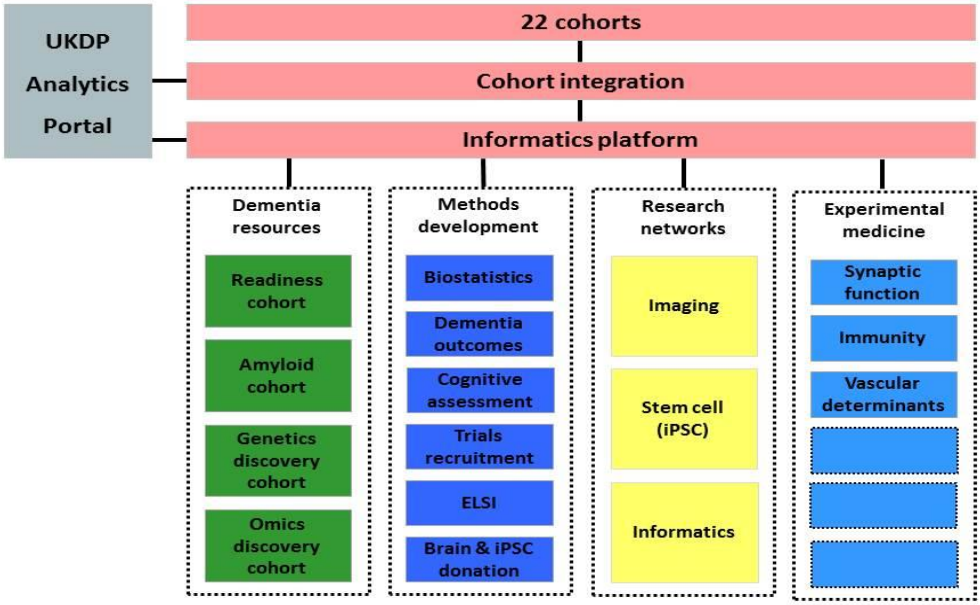
Objective:
UKDP is a £53M dementia dedicated 'big-data' platform created to provide closer synergy between epidemiology and experimental medicine (EM) through the re-purposing of epidemiologic cohorts for trials readiness.
Methods:
Data from 2M intensively phenotyped participants from 22 cohorts will enable the rapid testing of complex hypotheses across multiple independent datasets. Population and familial disease cohorts will enable unique and common mechanisms across the dementias to be identified. Data access will be through a single informatics portal (figure).
Results:
Integration of the research environment, linking strategic resource cohorts and methods development programs to specialist PET/MR, informatics and iPSC networks will support an in-depth EM programme of discovery studies and early phase trials. Initial EM foci include innate and acquired immunity, synaptic function, and vascular risk factors with other programmes to be developed.
Readiness cohorts will include 10,000 UK Biobank participants with 2 year repeat 3T brain MRI, cognitive phenotyping and bio-sampling; providing a step-change in the size and phenotypic detail of trials ready cohorts.
UKDP is a PPP bringing together a broad range of academic and industry expertise. Linking molecular pathology at the cellular level with biological systems impact and in-depth phenotyping of outcomes, will powerfully enable new therapeutics development.
Conclusions:
UKDP brings many opportunities for accelerating dementia research and the development of new treatments and welcomes collaborative proposals.



06c. Imaging & Biomarkers: PET - amyloid

ADPD5-0824

THE ROLE OF CEREBRAL HYPOPERFUSION FOR BRAIN BETA-AMYLOID DEPOSITION IN PATIENTS WITHOUT MEMORY IMPAIRMENT, AN 18F-FLUTEMETAMOL STUDY.

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Objectives

Chronic brain hypoperfusion has been suggested to play a causative role in the development of beta-amyloid deposition in Alzheimer's disease.

We hypothesize that cerebral hypoperfusion due to a unilateral internal carotid artery (ICA) occlusion may increase beta-amyloid deposition in the affected regions.

Methods

We plan to include 10 non-demented patients with unilateral ICA occlusion, or other asymmetric vascular pathology on an atherosclerotic basis and 5 patients with occlusion due to ICA arterial dissection. Major brain infarction on the affected side is an exclusion criterion. The patients will undergo clinical assessment, neuropsychological testing, brain MRI including: structural MRI sequences, MR-angiography, MR-perfusion, and 18F-Flutemetamol-PET. Based on MR perfusion sequences cortical ROIs will be evaluated for 18F-Flutemetamol binding. A contralateral, normally perfused, region will be used as an internal control for amyloid deposition.

Results

So far 7 patients have been recruited (4 right ICA, 2 left ICA, and one with bilateral occlusions and a 50% left middle cerebral artery-stenosis). Age ranges from 51-77 years, MMSE 27-30. Atherosclerotic cardiovascular disease, smoking and hypertension are the causes for vessel occlusion in the patients recruited so far. In one patient radiation due to ear-nose-and throat carcinoma is a probable contributor to vessel occlusion.

Conclusions

We have designed a small scale study to address the hypothesis that cerebral hypoperfusion may cause beta-amyloid deposition. Recruitment is currently ongoing. MRI and PET data will be presented at the meeting.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-0934

AMYLOID PLAQUES DETECTION BY MRI WITH GADOLINIUM-VHH ANTIBODY CONJUGATES

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Objective: Few methods have been developed to detect amyloid plaques by MRI but they always required an invasive process to open the blood brain barrier (BBB). Camelids heavy chain only antibody fragments (VHH), because of their small size and their basic isoelectric point, can cross the BBB. Hence, in order to detect amyloid plaques by MRI, we developed anti-Abeta VHHs. Alexa-Fluor488 or Gadolinium (Gd) were conjugated to VHHs and the resulting contrast agents were evaluated by multi-photon imaging or MRI.
Methods: Experiments were performed on PS2APP mice with VHH conjugates. *In vivo* multi-photon experiments were realized after intravenous injections of VHH-AlexaFluor488. MRI acquisition were recorded following three different protocols 1) *in vitro* incubation of the brain with VHH-DOTA/Gd overnight 2) *in vivo* injection followed by post-mortem imaging, 3) Gd-staining, a reference technique to reveal amyloid plaques by soaking the brain in a Gd-solution.
Results: Live multi-photon imaging showed gradual extravasation of the fluorescent VHHs from blood vessels and penetration in brain parenchyma with an exquisite tropism for amyloid plaques. MR images obtained after *in vitro* incubation or *in vivo* administration with VHH-DOTA/Gd showed numerous hypointense spots in the cortex. Several hypointense spots were colocalised with amyloid plaques revealed by the reference technique of Gd-staining.
Conclusion: VHHs cross the BBB, label amyloid plaques *in vivo* and can be detected by MRI following conjugation with a contrast agent. VHHs thus appear as promising tools with translational value for *in vivo* detection of amyloid deposits by MRI.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1004

11C-PIB PET IMAGING IN EARLY ONSET ALZHEIMER'S DISEASE IN THE ABSENCE OF KNOWN MUTATIONS

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Background: Early-onset Alzheimer's disease (EOAD) has a different pathologic burden and clinical features compared with late-onset Alzheimer's disease (LOAD). We examined the effects of age-at-onset on the burden and distribution of β-amyloid in patients with EOAD in whom well-characterized mutations associated with Alzheimer's disease (AD) were absent. **Methods:** We genotyped *ApoE, APP, PSEN1* and *PSEN2* in 23 patients with AD; after application of exclusion criteria, our study contained 9 patients with EOAD (age < 65), 11 with LOAD (age > 70), and 8 normal controls (NCs), all of whom had undergone ^{11}C- labeled Pittsburgh compound B (PiB)-positron emission tomography (PET) imaging. Statistical parametric mapping (SPM) analysis was used to compare distributions of PiB deposition in three groups. **Results:** Patients with EOAD exhibited a similar topographical distribution of PiB deposition compared with patients with LOAD; however, patients with EOAD exhibited higher z-scores and larger cluster sizes. Furthermore, patients with EOAD retained higher levels of PiB in the bilateral thalamus and in some parts of the globus pallidus, compared with patients with LOAD ($p < 0.05$, FDR). **Conclusion:** Distribution of amyloid deposition in EOAD outside the context of genetic mutations differs topographically from that in LOAD, a finding that is consistent with a previous study on familial EOAD.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1034

A BIOMARKER PANEL TOWARDS THE EARLY DIAGNOSIS, PREDICTION AND MONITORING OF ALZHEIMER'S DISEASE: AUSTRALIAN IMAGING BIOMARKERS AND LIFESTYLE STUDY OF AGING COHORT

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Objectives: To study Alzheimer's disease (AD) related changes in the peripheral body fluids such as blood plasma.
Methods: Mesoscale Discovery platform was utilised to measure protein biomarkers in plasma of Australian Imaging Biomarkers lifestyle (AIBL) study of aging cohort. The biomarkers tested belonged to endocrine, vascular injury, inflammatory, coagulation cascade, apoptotic and lipoprotein pathways consisting of a 4-plex, 5-plex and a 6-plex. Generalized Linear model (GLM) was used to assess the potential of these proteins as biomarkers for AD.
Results: GLM results show that biomarkers such as Thrombopoietin (TPO), Fatty acid Binding protein 3 (FABP3), Pancreatic polypeptide Y (PPY), chemokine 309 (I309), soluble vascular cell adhesion molecule (sVCAM), beta 2 microglobulin (B2M), adiponectin and apolipoprotein J differ significantly between healthy controls (HC) and AD patients ($p<0.005$). Biomarkers such as FABP3, sVCAM, B2M, adiponectin and apoJ specifically differentiate between HC and Mild cognitively impaired (MCI) participants ($p<0.05$), however only I309 was able to differentiate between MCI and AD patients ($p=0.001$). We also classified participants based on their progression towards the severity of the disease into non-transition and transitional categories and found that markers such as TPO, FABP3, PPY, I309, sVCAM and adiponectin significantly differentiate between non-transition and transitional categories ($p<0.005$). These biomarkers also showed a significant correlation with the brain amyloid beta deposition obtained from brain imaging using Positron Emission tomography. **Conclusion:** The findings from this study suggest that these set of biomarkers could be used to assess cognitive decline and monitor changes in periphery during the progression of the AD.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1135

CATIONIZATION OF AN ABETA PROTOFIBRIL SELECTIVE ANTIBODY FRAGMENT INCREASES BRAIN UPTAKE IN APP TRANSGENIC MICE

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Objectives: Antibodies and antibody fragments are, because of high selectivity for their targets, considered as potential therapeutics and biomarkers for several neurological disorders including Alzheimer's disease. However, due to their large molecular size, antibodies/fragments do not easily penetrate the blood-brain-barrier. The aim of the present study was to improve the brain uptake of an Abeta protofibril selective $F(ab')_2$ fragment ($F(ab')_2$-158) via adsorptive-mediated transcytosis to be used as a radioligand.
Methods: $F(ab')_2$-158 fragment was cationized to different extents with the polyamine putrescine and its specific and unspecific binding was studied with ELISA. Next, cationized $F(ab')_2$-158 was labelled with ^{125}I and its brain distribution and pharmacokinetics was studied in wild type and APP transgenic mice (tg-ArcSwe) with single-photon emission computed tomography (SPECT) imaging.
Results: Cationization did not alter the *in vitro* binding to amyloid-beta protofibrils, but increased the unspecific binding somewhat. *Ex vivo* experiments revealed a doubling of brain concentrations compared to unmodified $F(ab')_2$-158 and *in vivo* SPECT imaging showed that the cationized $F(ab')_2$-158, but not the unmodified $F(ab')_2$-158 could be visualized in the brain.
Conclusions: To conclude, cationization is a means to increase brain concentrations of antibodies or antibody fragments and may enable their use as imaging biomarkers in the brain.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1146

TRANSFERRIN RECEPTOR MEDIATED TRANSCYTOSIS OF AN ANTIBODY BASED PET RADIOLIGAND FOR IN VIVO QUANTIFICATION OF SOLUBLE ABETA AGGREGATES

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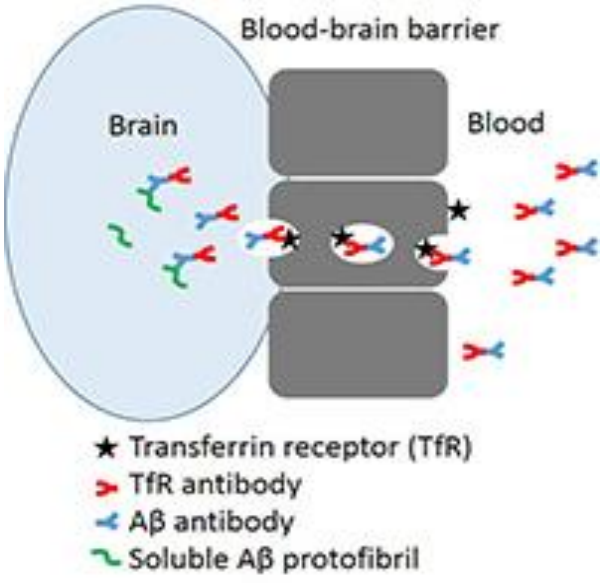
Objectives: Positron emission tomography (PET) [^{11}C]PIB imaging detects amyloid plaques formed early in the Alzheimer's disease (AD) pathogenesis. However, plaque pathology does not correlate well with disease progression or outcome of therapeutic interventions. This project aims to develop a PET radioligand based on an antibody (mAb158) that binds selectively to soluble Abeta protofibrils, which may correlate better with disease severity than amyloid plaques. Brain uptake of the antibody was increased with transferrin receptor (TfR) mediated transcytosis (Figure).

Methods: A F(ab')$_2$ fragment of mAb158 was chemically fused with a TfR antibody (8D3) and the fusion protein was analyzed with ELISA for retained binding to Abeta and TfR. Brain uptake of ^{125}I radiolabelled fusion protein was studied *ex vivo* in wild type and APP transgenic (tg-ArcSwe) mice and correlated to brain levels of Abeta protofibrils. Brain distribution of ^{124}I labelled fusion protein was studied with PET.

Results: The fusion protein had retained binding properties and displayed a near ten-fold higher brain uptake compared to F(ab')$_2$-158 three days post injection. Brain retention increased with age, correlating closely with brain levels of soluble Abeta protofibrils and a clear difference between tg-ArcSwe and wt mice was seen with PET.

Conclusions: This fusion protein has the potential to become an important tool for *in vivo* PET-imaging of soluble Abeta aggregates in AD patients.



06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1309

PRECLINICAL EVALUATION OF FUNCTIONALIZED [18F]NANOLIPOSOMES FOR BETA-AMYLOID IMAGING IN ALZHEIMER'S DISEASE

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Objectives

Our aim was to evaluate novel functionalized ^{18}F-labelled nanoliposomes (NLs) as PET imaging agents. The NLs were tailored for Alzheimer's disease treatment by functionalization with phosphatidic acid alone (PA-^{18}F-NL) or with an additional peptide derived from Apolipoprotein-E receptor-binding domain (PA-mApoE-^{18}F-NL).

Methods

Pharmacokinetic properties of ^{18}F-NLs were studied in FVB/N mice with dynamic 60 min PET/CT scans and the results were verified with ex vivo tissue counting at 15, 60 and 90 min after ^{18}F-NL injection. Brain uptake and distribution was evaluated from coronal cryosections at 15 and 60 min using digital autoradiography. ^{18}F-NL binding to beta-amyloid deposits was studied in transgenic APP23 mice at 60 min with methods mentioned above.

Results

PA-^{18}F-NL and PA-mApoE-^{18}F-NL had different distribution in mice; 90 min after PA-^{18}F-NL injection high ^{18}F-radioactivity was seen in blood, spleen and liver. Additional mApoE functionalization resulted in 7-fold decrease in mean blood ^{18}F-radioactivity and in 2-, 4- and 70-fold increase in mean ^{18}F-radioactivity in spleen, liver and lungs. Total brain uptake at 90 min was low with PA-^{18}F-NL and PA-mApoE-^{18}F-NL and brain-to-blood -ratios were 0.02 and 0.05, respectively. High ^{18}F-radioactivity "hot spots" were seen in APP23 brain tissue autoradiographs, however, no co-localization with thioflavin stained beta-amyloid deposits was observed.

Conclusions

PA-^{18}F-NLs and PA-mApoE-^{18}F-NLs had different biodistribution in mice. Brain uptake of both functionalized ^{18}F-NLs was low but additional mApoE functionalization increased the brain-to-blood –ratio. Specific binding to beta-amyloid was not detected at 90 min.

The research received funding from the European Community's 7th framework Programme (FP7/2007-2013, nº212043).

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1314

REGIONAL CEREBRAL BLOOD FLOW MEASURED BY EARLY [11C]-PIB-UPTAKE DECLINES WITH AGE IN SUBJECTS WITH ELEVATED CEREBRAL BETA-AMYLOID-DEPOSITION

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Objective:

Alterations in cerebral glucose metabolism and blood flow were consistently described in individuals at risk of Alzheimer's disease (AD), e.g.: Apo-E-epsilon-4-allele carriers, family history of AD or amnestic MCI. Early regional [11C]-PiB-uptake (0-6 minutes; ePiB) estimates regional cerebral blood flow (rCBF) while late [11C]-PiB-uptake measures cerebral beta-amyloid deposition. We made use of both signals in a dynamic PET study in elderly subjects without cognitive impairment (HCS) and patients with mild cognitive impairment (MCI). **Methods:**

Dynamic 11-C-PiB-PET early and late frame measurements in MCI (n=23) HCS (n=85) in six bilateral volumes of interest: posterior cingulate (PCC), hippocampus (Hip.), temporoparietal region (TR), superior parietal (SPG), parahippocampal (Parahip.) and inferior frontal gyrus (IFG).

Results:

13 MCI and 11 HCS were classified PiB-positive. Subjects with MCI showed lower ePiB signal as compared to HCS in the following regions: left PCC (p<0.001), left IFG (p<0.001), left (p<0.001) and right Hip ($p=0.004$). Effects were strongest in MCI above the age of 70 years with high cortical PiB binding.

We observed no difference in rCBF in the total population between PiB-positive and PiB-negative subjects. There was a moderate negative correlation of ePiB with age in bilateral SPG (p=0.004), bilateral TR (p=0.007), right IFG (p=0.003), right PCC (p=0.004) and left Parahip. (p=0.001) in PiB positive subjects but not in the PiB-negative group.

Conclusions:

rCBF apparently declines with age in the presence of AD pathology and contributes to cognitive impairment. On the other hand, normal CBF in amyloid negative subjects may be a characteristic feature of healthy aging.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1390

BRAIN AMYLOID LOAD IS ASSOCIATED WITH IMPAIRED EXECUTIVE FUNCTIONING IN ELDERLY INDIVIDUALS AT-RISK TO DEVELOP DEMENTIA

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Background: FINGER study is a randomized 2-year multidomain lifestyle intervention study in subjects at increased risk of cognitive decline.[1,2] A sub-group of the study population participate in a PET study to investigate brain metabolism and amyloid deposition at baseline and at the end of intervention. At present, data from the baseline [11C]PIB scans is available and the association of PIB data to baseline characteristics and cognition are reported.
Methods: 48 elderly subjects underwent a [11C]PIB PET scan, brain MRI and neuropsychological examination. Subjects were divided into two groups (PIB+ and PIB-) based on a visual PET scan analysis. Hippocampal atrophy and brain vascular changes were visually graded according to Scheltens and Fazekas scores. Between-groups differences in the cognitive function were analyzed.
Results: Twenty subjects (42%) were PIB positive. PIB- group performed better in executive functions than PIB+ group (Z-score difference p = 0.02). PIB+ group showed a trend to higher amount of white matter lesions and hippocampal atrophy (Fazekas score 2-3: 50% in PIB+ vs. 29% in PIB-; Scheltens score 1-3: 40% right, 45% left in PIB+ vs. 29% and 21% in PIB-).
Conclusions: The high percentage of PIB positive subjects provides evidence of a successful recruitment process of the at-risk population in the FINGER trial. The results suggest an association between early brain amyloid accumulation and decline in executive functions, as well as a trend of increased vascular changes and hippocampal atrophy in amyloid positive subjects.

1.Kivipelto et al. Alzheimers Dement.2013;9(6):657-665.
2.Ngandu et al. Int J Environ Res.2014;10;11(9):9345-9360

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1687

LONGITUDINAL ASSESSMENT OF ABETA ACCUMULATION IN NON-DEMENTED INDIVIDUALS: A 18F-FLUTEMETAMOL STUDY

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Objectives: We investigated if longitudinal Aβ imaging with ^{18}F-flutemetamol (Flute) PET can show change over time in Aβ burden as previously shown with ^{11}C-PiB in non-demented participants in the AIBL study .
Methods: Flute-PET was performed in 187 non-demented AIBL volunteers who had not previously had Aβ imaging, 50 with Mild Cognitive Impairment (MCI; 74.5±5.7 yo) and 137 healthy controls (HC; 74.5±5.6 yo). To date, a repeat scan at 18 months has been obtained in 76 participants. SUVR was calculated using the pons as reference region. Change was expressed as the yearly SUVR %change from baseline.
Results: At baseline, 54% of MCI and 23% of HC showed high Flute retention. At 18-month follow-up, increases in Flute SUVR were observed in 38/68 (56%) of HC and 6/7 (86%) of MCI. The increase for the whole cohort (accumulators & non-accumulators) was 1.8% (P=0.06). The prevalence of Aβ accumulators was similar in those HC who presented with either high or low Aβ burden at baseline (60% and 56%, respectively). The average change for Aβ accumulators was 2.6% per year, the same as we have reported for PiB accumulators. SUVR %change was the same for HC and MCI. Finally, despite presenting with an almost 40% greater prevalence of positive scans, the differences between HC accumulators with and without memory complaints were not significant.
Conclusions: With a relatively small sample size, there is a clear trend for an increase in Flute binding over time, similar to that observed with PiB in another AIBL cohort. Follow-up data collection continues.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1698
IN VIVO PET IMAGING OF ABETA PLAQUES USING 89ZR LABELED BAPINEUZUMAB IN THE 5XFAD MOUSE MODEL

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Background: Recent advances in PET imaging tracers have provided means to diagnose AD patients, but still have drawbacks such as a short half-life, and misdiagnosis. Our results show that it might be feasible to use antibodies as a diagnostic tool.
Methods: Bapineuzumab (hu-IgG1) or isotype controls were labeled with ^{89}Zr. 100 µg antibody (3.7 MBq) was injected intraperitoneally in 5xFAD or wild type (WT) littermate controls. Mice were subsequently scanned for 0.5 hour using a microPET over a period of up to 10 days. Activity in brain was measured using a gammacounter, and autoradiography of brain slices was performed for validation of the PET imaging data.
Results: 1 day after injection a specific binding could already be detected in 5xFAD mice injected with bapineuzumab. The signals remained stable over a period of 10 days. No difference between 5xFAD and WT animals was found in the isotype control group. While difference between 5xFAD and WT animals could readily be detected at 6 months of age, this difference increased at 9 months of age.
Conclusion: This study shows the first proof of principle that anti-Aβ antibodies accumulate in the brain in vivo, and this within one day after injection using PET. The longer half-life of ^{89}Zr compared to other isotopes also allows this method to be used in more clinics around the world for diagnosis of AD. Furthermore, this new technique holds great promise for the further study of AD, by using for instance antibodies directed against different forms of Aβ.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1780

[18F]FLORBETABEN-PET IMAGING IN CLINICAL ROUTINE CASES – BENEFIT OF EARLY ACQUISITION

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Objectives

In the last few years [^{18}F]-labelled amyloid PET radiotracers have been developed and recently received clinical approval. We now report on our experience with a heterogenous patient cohort undergoing [^{18}F]Florbetaben (FBB) PET. A major goal was to investigate the benefit of additionally performed early post injection acquisition of the FBB-PET.

Methods

All subjects for amyloid imaging were recruited in a clinical setting. Aside from undergoing a detailed neuropsychiatric test battery, collection of biomarkers (MRI, CSF, FDG-PET) was performed as well. Furthermore 300 MBq [^{18}F]Florbetaben were injected i.v. and PET scans were routinely acquired 90 min p.i., and an early acquisition starting just after injection was performed in a subset of patients.

Results

A total of 89 patients (mean age 70.1 ± 9.5y) were scanned. In a subset of 40 patients an early acquisition of the FBB-PET could be performed, in 35/40 patients the individual FDG-PET was available. In 12/35 cases the regularly acquired amyloid PET was read negative. When the early acquired FBB-PET was assessed in 3D-SSP and compared to a normative database, the visual interpretation resembled the interpretation of the individual FDG-PETs, irrespective of the amyloid-status of the late FBB-scans.

Conclusions

We report on the clinical use of Florbetaben-PET in a non-selected patient cohort. Early [^{18}F]Florbetaben acquisitions closely resembled FDG-PET characteristics. These findings need to be further investigated in a larger patient cohort but could potentially substitute an additional FDG-PET scan in the future.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1991

RECERCALIA STUDY: COGNITIVE COMPOSITES AND PIB-PET AMYLOID BIOMARKERS AND PRODROMAL ALZHEIMER'S DISEASE

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Objectives: To assess the usefulness of cognitive function composites (CFC) to discriminate between healthy aging, non-Alzheimer's Mild Cognitive Impairment (MCI), and prodromal Alzheimer's disease (AD).

Methods: A sample of 59 subjects, > 64 years old, recruited from Recercalia project, divided in 39 healthy controls (HC) (20 men; 19 women) and 20 amnestic mild cognitive impairment (aMCI)(12 man; 8 woman) with storage memory impairment, and without comorbidities that could explain their cognitive impairment. All subjects received an extensive neuropsychological assessment, including CFC sensitive to: language, memory, praxis, visual gnosis and executive functions. A PET-PIB scanner, was administered at baseline, and subjects were divided in PiB+ or PiB- with a cutoff value of PIB=1.5, that is, HC+(n= 2), HC- (n=37), aMCI+ (n=12), aMCI- (n=8). The HC+ group was excluded from the analysis due to the sample size.

Results: Verbal delayed recall on memory, executive and language CFCs showed as the highest values to discriminate between, HC-, aMCI- and aMCI+ groups. Verbal learning and recognition on memory CFC did significantly discriminate between HC and aMCI whole groups, but not between aMCI+ or aMCI- groups. Although some differences could be appreciated between all three groups on visual gnosis and praxis CFCs , they were not enough discriminative.

Conclusions: A brief neuropsychological battery comprising tests sensitive to fronto-temporal functions, such as memory, language and executive functions may be useful to discriminate between healthy aging, non-AD MCI and prodomal AD, as PET-PiB measurements and applied CFCs showed.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-2110
METABOLOMIC CORRELATES OF AMYLOID-BETA RELATED COGNITIVE DECLINE IN NON-DEMENTED ELDERLY SUBJECTS
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Background: The current study aims to investigate whether information embedded in the peripheral metabolome may be used for inferences on Amyloid-beta (A-beta) deposition associated cognitive decline in non-demented elderly subjects.
Methods: 115 non-demented elderly subjects were randomly split into a discovery (n=58) and replication sample (n=57). Regional extent of A-beta-deposition was measured non-invasively by Pittsburgh Compound-B amyloid imaging (PiB-PET). Blood was drawn for metabolomic assessment (Biocrates Absolute-IDQ P180 Assay). Learning and memory performance over time was assessed by performing neuropsychological testing at two time points. After preprocessing and quality control, metabolites were administered to a multivariate analysis, including independent component analysis (ICA) based linear model building in the discovery sample and validation of predictive precision in the replication sample.
Results: 139 Metabolites representing 33 amino acids, biogenic amines and hexoses, 11 Acylcarnitines, 11 Lysophophosphatidylcholines, 70 Phosphatidylcholines, 14 Sphingomyelins could be quantified at high quality and attributed to 15 independent components by using ICA-decomposition. By applying a stepwise-regression linear fit algorithm (MatLab V8.4, statistics toolbox V9.1), a linear model for cognitive decline in association with high A-beta-plaque density could be identified for the discovery sample and replicated with >75% predictive accuracy in the second sample (coefficients of determination: r2 sample#1=0.57; r2 sample#2=0.50). Major predictive weights resulted for measures derived from the Phosphatidylcholine group.
Discussion: Our data indicate that A-beta associated cognitive decline in the elderly is associated with specific patterns of peripheral metabolomic measures, which may be used for stratification of prospective A-beta-targeted early interventional clinical trials.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-2118
AMYLOID PET-POSITIVE, POSTERIOR VARIANT ALZHEIMER'S DISEASE
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Objectives:The causes of posterior cortical atrophy (PCA) are diverse. A portion of PCA patients have underlying Alzheimer's pathology. Since 2012, an amyloid tracer has been first utilized in USA to clarify Alzheimer's diagnosis in patients with atypical presentation. In this study, patients with PCA according to the diagnostic criteria are investigated based on the positive findings of the amyloid PET. **Methods**: The medical records of PCA patients followed at a specialty clinic from January 1st 2011 to December 31st 2014 were collected and reviewed. Five PCA patients were diagnosed with probable Alzheimer's disease based on the amyloid PET study. Clinical features, neuroimaging findings and their impression with the PET study would be reported. **Results**: One man (age 64) and four women (age 54, 58, 60, 77) all took about two years before the diagnosis of PCA was made. They previously had been wrongly diagnosed with either a refraction disorder or anxiety, despite progressive visuospatial symptoms. The 77-year-old woman even became "blind" and displayed visual hallucinations. One man and three women exhibited marked cortical atrophy of bilateral parietal lobes in brain MRI studies. One woman had reduced metabolism of parieto-occipital regions shown by the FDG PET. Interestingly, the amyloid PET study demonstrated cortical deposition of neuritic plaques prominently in both frontal and temporal lobes. All patients received standard treatments. The fact that the amyloid PET would provide a certain diagnosis satisfied all patients and their family members. **Conclusions**: The amyloid PET can definitely enhance clinicians' ability to treat patients properly.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-2310

Comparison of clinical diagnosis and biomarker constellation in AD in a clinical setting

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Objectives: The diagnosis of Alzheimer's disease (AD) can be made with reasonable accuracy using neuropsychological assessment and MRI. CSF and PET biomarkers have been incorporated in diagnostic criteria for AD. The biomarker results are thought to confirm or exclude the diagnosis of AD.
Methods: A cross-sectional observational study of 54 (mean age 72.1 ± 7.8y, 23 women) patients. Without knowledge of biomarker results 40 cases were diagnosed as AD, 14 cases as non AD. As biomarkers tau and Aβ 1-42 concentrations in the CSF and amyloid-targeting PET using either Pittsburgh Compound-B ([11C]PIB, n=25) or Florbetaben ([18F]FBB, n=29) were used.
Results: Four biomarker constellations were observed: 1. Completely congruent biomarkers (pathological/non-pathological), 2. at least one pathological degeneration and one pathological Aß marker, 3. at least one pathological Aß (CSF and/or PET), but normal degeneration marker (MTA and CSF), 4. pathological degeneration (MTA and/or tau), but no pathological Aß marker (CSF and PET). The percentage of inconsistent findings was high. In only one third of cases AD diagnosis was fully confirmed. In a minority of cases it seemed justified to switch the clinical diagnosis due to biomarker interpretation.
Conclusions: Biomarkers are no simple support or confirmation of the diagnosis of AD. Interpretation of AD biomarkers is complicated by multiple biomarker constellations. They may reflect different aspects of the pathological process. The design of this study does not allow to address the question of diagnostic accuracy since no gold standard in terms of neuropathological verification is available.

06f. Imaging & Biomarkers: PET - glucose

ADPD5-0472

18F-FDG PET IN EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE

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Investigation of the possibilities of functional neuroimagingin early diagnostics of cognitive impairment is one of the most actualdirections in neurology.
The aim was to investigate the cerebral metabolism inpatients with amnestic MCI type (aMCI) and Alzheimer's disease (AD) using18F-FDG PET.
The study involved 18 persons aged 64 to 76 years withaMCI, and 9 persons with non-amnestic MCI matched by age as a control group (6persons without cognitive decline). Evaluation of cognitive status wasconducted using a set of validated neuropsychological tests.
Analysis of the results showed that aMCI group wascharacterized by the formation of zones of bilateral hypometabolism in thefrontal, parietal and temporal regions, in cingulate cortex and hippocampuswith prevalence in the dominant hemisphere. The most expressed decrease inmetabolism was determined in the hippocampal and parietal-temporal regions.With progression joined violations in the orbitofrontal cortex. However, one ofthe earliest domain involved in the pathological process in patients with aMCIwas the posterior cingulate cortex. When comparing with the results ofneuropsychological assessment it was revealed a relationship between theseverity of hypometabolism in hippocampal, parietal-temporal regions, posteriorcingulate cortex and the results of verbal memory tests. These results are consistentwith the identified pattern of metabolic disorders and literature data on thepathological changes in the brain (including amyloid deposits) in AD.
Thus, the use of18F-FDG PET in clinical practice can significantly improve early diagnosticaccuracy of AD.

06f. Imaging & Biomarkers: PET - glucose

ADPD5-0731

CEREBRAL CORRELATES OF COGNITIVE RESERVE IN PRODROMAL AND MILD ALZHEIMER´S DISEASE. A FDG- PET STUDY.

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Objectives: It is generally assumed that cognitive reserve can compensate for cerebral changes in prodromal Alzheimer´s disease (AD) and mild AD. Therefore we compared cerebral changes between patients with a low vs. high cognitive reserve who consulted our memory clinic.

Methods: Up to now 22 patients with prodromal AD (mean age: 72.30; SD: 6.85), 17 patients with mild AD (mean age: 74.06; SD: 5.51) and 8 cognitive normal controls (mean age: 67.38; SD: 6.48) were included. Years of school education were used as a proxy for cognitive reserve and the Mini Mental State Examination (MMSE) was applied. ^{18}F-fluoro-deoxy-glucose positron emission tomography (FDG-PET) scans were analyzed by using SPM8. In the low vs. high cognitive reserve comparison MMSE scores were entered as a covariate.

Results: When contrasted with the controls the prodromal AD patients presented a significantly reduced glucose uptake in the posterior cingulate and the middle frontal gyrus. Within the prodromal AD group, patients with a high cognitive reserve showed a significantly lower glucose uptake in the right frontal and inferior parietal lobe, the posterior cingulum and the middle occipital gyrus than those with a low reserve. Similar differences were found between mild AD patients with a high vs. a low cognitive reserve.

Conclusions: The results of our ongoing study support the hypothesis that cognitive reserve can compensate brain changes in both prodromal and mild AD. This mechanism should be considered in clinical practise especially when establishing clinical diagnosis in ambiguous cases.

06f. Imaging & Biomarkers: PET - glucose

ADPD5-0860

BILINGUALISM AS A CONTRIBUTOR TO COGNITIVE RESERVE? EVIDENCE FROM CEREBRAL GLUCOSE METABOLISM IN MILD COGNITIVE IMPAIRMENT AND ALZHEIMER`S DISEASE

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Background
Growing epidemiological evidence suggests that bilingualism could be one factor contributing to 'cognitive reserve' (CR) (e.g. Bialystok et al., 2014). Therefore we analysed differences in cerebral glucose metabolism between bilinguals and monolinguals with mild cognitive impairment (MCI) and Alzheimer´s disease (AD) using [^{18}F]fluorodeoxyglucose (FDG) positron emission tomography (PET) under a resting condition.
Methods
30 patients (73.1 ± 7.4) with a diagnosis of MCI or probable AD received a physical examination, neuropsychological assessment, blood tests and a FDG-PET scan. 16 patients were classified as lifelong bilinguals following the criterion of Bialystok et al. (2007); groups were matched for age, gender and MMSE scores. SPM 8 was used for analyses utilising the whole brain as a reference region for intensity normalization and years of education as covariate.
Results
Compared to the monolingual patients, bilinguals with MCI and AD showed a substantially greater impairment of glucose uptake in frontotemporal and parietal regions (including Brodmann areas 9, 47, 40 and 21) and in the left cerebellum.
Conclusions
According to our findings bilingualism appears to increase the CR since the bilingual patients presented more severe brain changes than the monolinguals. The latter did not only comprise Brodmann areas relevant to speech and language but also structures typically involved in AD such as the temporal and the parietal cortex.

06f. Imaging & Biomarkers: PET - glucose

ADPD5-0862

THEORY OF MIND - CEREBRAL CORRELATES IN PRODROMAL AND MILD ALZHEIMER'S DISEASE A FDG- PET STUDY

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Objectives: Theory of Mind (ToM), the attribution of mental states to oneself and others interferes with cognitive decline, however, neuroimaging studies with regard to ToM are scarce. Therefore we presented first- and second-order-false-belief-tasks based on the concept of the bands-aid-task (Carlson & Moses, 2001) to patients with prodromal and mild AD of our memory clinic and compared their 18F-fluoro-deoxy-glucose positron emission tomography (FDG-PET) findings.
Methods: Up to now 27 AD patients and 8 cognitive normal controls were included. The patient group was splitted according to their results in the ToM-tasks. 11 patients with a deficit of ToM (mean age: 74.5; SD: 5.3), 16 patients with preserved ToM ability (mean age: 72.3; SD: 5.6) and 8 cognitive normal controls (mean age: 67.38; SD: 6.48) were compared with each other. FDG-PET scans were analyzed by using SPM8. In the ToM comparison MMSE scores were entered as a covariate.
Results: When contrasted with the controls the patients with prodromal and mild AD showed a bilaterally reduced glucose uptake in the superior and the right middle frontal gyrus and the precuneus. Patients with a preserved ToM presented a significantly higher glucose uptake in the cerebellum, the right superior temporal and the left cingulate gyrus than those with an absent ToM-ability.
Conclusions: The results of our ongoing study demonstrate that ToM in patients with prodromal and mild AD corresponds to cerebellar, temporal and cingulate brain regions. From a clinical perspective these deficits should be considered in the evaluation of patients with prodromal and mild AD.

06f. Imaging & Biomarkers: PET - glucose

ADPD5-1539

A NEW LOOK AT FDG PET LONGITUDINAL ANALYSES IN ALZHEIMER'S STUDIES USING A FREESURFER NATIVE SPACE METHOD

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Objectives: We compare results of standard uptake value ratio (SUVR) analyses in the Alzheimer's Disease population using a native space compared to an SPM template method. While an SPM approach has shown metabolic differences between normal and AD subjects, the smoothing and deformable registration used for alignment may sacrifice information otherwise retained in a native space approach.
Methods: Freesurfer was used to obtain an ROI parcellation on T1 MRI data from 458 subjects (175 Normal, 92 EMCI, 153 LMCI, 39AD). FDG-PET data from two time points approximately 24 months apart were registered to the MRI data in T1 native space, and SUVR's were computed for 110 subregions. Eight reference regions including whole cerebellum and brainstem were evaluated. SUVR's were also computed in template space using SPM and a 'Meta-ROI' composite approach. Effect sizes between AD and Normal groups were evaluated using Cohen's d. Longitudinal change in SUVR was also compared.
Results: The native space approach showed largest differences between AD and normals in regions traditionally seen to be hypometabolic, including the precuneus and cingulate. Unique to the native space approach is that hippocampal regions also showed a very large effect size. Effects were largest using the whole cerebellum reference region. In a longitudinal analysis, the composite measure showed similar effect size but less variability than the template-based approach.
Conclusions: A native space approach shows promise in providing improved regional differentiation between normal and AD groups, and possibly reduced sample size requirements in clinical trials.

06f. Imaging & Biomarkers: PET - glucose

ADPD5-1804

EVALUATION OF THE CLINICAL VALUE OF FDG-PET IN MEMORY ASSESSMENT IN A TERTIARY CARE CENTER

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Objective: to investigate the usage and clinical value of FDG-PET in assessment of mild cognitive decline in a memory clinic. **Methods**: Patients were referred for cognitive symptoms and had undergone routine memory assessment including neurological examination, blood analysis (including APOE genotyping), CT or MRI imaging, CSF biomarker analysis and neuropsychological testing. When the diagnosis was uncertain PET-FDG was used. Regional SUVs and z-scores were extracted from the PET-FDG images. **Results**: Two hundred twenty patients, investigated in-between November 2012 and May 2014 were included. All patients had seen a physician prior to PET-investigation, and most (174 patients), had been followed-up after PET-FDG imaging. Patients were generally young (mean age=67.5 yrs.; SD=8.7) and high-functioning (mean MMSE=24.6; SD=5.3). The most common diagnoses before PET-investigation were: MCI (28%), non-AD dementia (18%) and AD (10%). In 43% of patients the diagnosis was changed after the PET-FDG scan (34% in MCI, 53% in non-AD dementia and 6% in AD). In MCI and non-AD dementia the diagnosis was predominately (62% and 65% respectively) changed to AD. The final diagnoses were: MCI 40%, non-AD dementia 21% and AD 28%. **Conclusions**: We have yet to demonstrate causality between FDG-PET investigation and change in diagnoses, however, z-scores are still to be analysed. Nonetheless, there is a large increase in AD diagnoses following PET-FDG investigation, many of which had previously been considered MCI patients. Furthermore, the majority of non-AD dementia patients received a changed diagnosis following PET-FDG evaluation, indicating the usefulness of PET-FDG in differential diagnosis of dementia.

06g. Imaging & Biomarkers: PET - receptors

ADPD5-0447

COMPARISON OF AGONIST AND ANTAGONIST 5-HT1A RECEPTOR-BINDING SITES IN ALZHEIMER'S DISEASE: A POSTMORTEM STUDY WITH PET RADIOTRACERS

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Objectives. PET imaging studies using 5-HT_{1A} receptor radiotracers show a decreased density of this receptor in hippocampi of patients with Alzheimer's disease (AD) at advanced stages. However, current 5-HT_{1A} receptor radiotracers used in neuroimaging are antagonists, thought to bind to 5-HT_{1A} receptors in different functional states (i.e., both the one which displays high affinity for agonists and is thought to mediate receptor activation, as well as the state which has low affinity for agonists). Comparing the PET imaging obtained using an agonist radiotracer, which binds selectively to functional receptors, with the PET imaging obtained using an antagonist radiotracer would therefore provide original information on 5-HT_{1A} receptor impairment during AD.

Methods. Quantitative autoradiography using ^{18}F-F13640 and ^{18}F-MPPF, a 5-HT_{1A} agonist and antagonist, respectively, was measured in hippocampi of patients with AD (n=26, at different Braak's stages) and control subjects (n=10). The specific binding of both radiotracers was determined by addition of WAY-100635 and the agonist binding of ^{18}F-F13640 was revealed by addition of Gpp(NH)p.

Results. The autoradiography distribution of both 5-HT_{1A} PET radiotracers varied across hippocampus regions. The highest binding density was in the pyramidal layer of CA1. The incubation with Gpp(NH)p, a non-hydrolysable analogue of GTP, reduced significantly ^{18}F-F13640 binding, confirming its specific binding to G-coupled receptors. ^{18}F-F13640 binding compared to ^{18}F-MPPF binding revealed specific modifications of the density of functional 5-HT_{1A} correlated to the Braak's stages.

Conclusions. These in vitro results support testing the concept of functional imaging using agonist radiotracers in future clinical studies.

06g. Imaging & Biomarkers: PET - receptors

ADPD5-1090

ALTERATIONS OF CB1 EXPRESSION IN ALZHEIMER'S DISEASE – A LONGITUDINAL [18F]FMPEP-D2 PET STUDY WITH APP/PS1-21 MOUSE MODEL

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Objectives

Decreased cannabinoid receptor 1 (CB_1) activity has been found in *postmortem* brains of the patients of Alzheimer's disease (AD). We aimed to study changes in CB_1 expression in a transgenic mouse model of AD with [^{18}F]FMPEP-*d2*, an inverse agonist of CB_1, and longitudinal PET imaging.

Methods

In vivo binding of [^{18}F]FMPEP-*d2* was studied using the Inveon Multimodality PET/CT scanner with 30 min static PET scans 90 min after tracer injection. Transgenic (Tg) APP/PS1-21 (n= 8) and wild type control mice (WT) (n= 8) were imaged at 6, 9, and 12 months of age. [^{18}F]FMPEP-*d2* binding in the brains of Tg (n= 4) and WT (n= 4) mice was also evaluated with *ex vivo* autoradiography. Radiometabolites of [^{18}F]FMPEP-*d2* were analyzed from plasma and brain homogenates.

Results

[^{18}F]FMPEP-*d2* demonstrated high binding to the CB_1-rich areas in mouse brain. The binding was significantly ($p<0.05$) lower in Tg mice at 9 months of age than in WT mice at 7 months of age. Metabolite analyses from plasma showed three radiometabolites, of which two were more polar than the parent compound. One more polar metabolite was found in the brain tissue.

Conclusions

Our preliminary studies with Tg mice demonstrated decreased CB_1 expression in transgenic animals; however additional longitudinal studies are needed with transgenic and age-matched wild-type animals in order to further confirm these results. This study was funded by the European Community's 7th framework programme (FP7/2007-2013) under grant agreement no. HEALTH-F2-2011-278850 (INMiND).

06j. Imaging & Biomarkers: PET - other

ADPD5-0341

CHROMOSOMAL DNA DAMAGE MEASURED USING THE CYTOKINESIS-BLOCK MICRONUCLEUS CYTOME ASSAY IS SIGNIFICANTLY ASSOCIATED WITH COGNITIVE IMPAIRMENT IN SOUTH AUSTRALIANS

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Objectives
Loss of genome integrity may be associated with increased risk for neurodegenerative disease. The aim of this study was to investigate whether mild cognitive impairment (MCI) or Alzheimer's disease (AD) individuals have increased DNA damage relative to age and gender matched controls using the cytokinesis block micronucleus cytome (CBMN-Cyt) assay.
Methods
DNA damage was measured as micronuclei (MNi), nucleoplasmic bridges (NPB), and nuclear buds (NBUD) in once divided lymphocytes that are recognised by their binucleate appearance. The assay was performed on blood samples from 80 participants consisting of (i) MCI cases (N=20) and age and gender matched controls (N=20), and (ii) AD cases (N=20) and age and gender matched controls (N=20).
Results
There was a significant increase in MCI NBUD frequency (P= 0.006) relative to controls, which was also observed in male (P=0.03) and female (P=0.04) subgroups. For AD cases, there were no significant differences in assay biomarkers relative to controls. There was a significant negative correlation between Mini Mental State Examination (MMSE) and (i) MNi in all controls, (R=-0.3, P= 0.04), and AD cases (R=-0.4, P=0.03), (ii) NPB in all controls, (R=-0.4, P=0.006) and AD cases (R=-0.5, P=0.01), and (iii) NBUD in MCI cases (R=-0.5, P=0.007) and AD cases (R=-0.7, P=0.0002).
Conclusion
The results suggest that an increase in lymphocyte CBMN-Cyt DNA damage biomarkers maybe associated with cognitive decline.

06j. Imaging & Biomarkers: PET - other

ADPD5-1244

PET IMAGING AND EX VIVO BRAIN AUTORADIOGRAPHY IN APP/PS1-21 MICE USING THE NOVEL INFLAMMATION TRACER [18F]DPA-714

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Objective
We aimed to characterize the novel TSPO binding PET tracer [^{18}F]DPA-714 for *in vivo* and *ex vivo* detection of neuroinflammation in a mouse model Alzheimer´s disease (AD).
Methods
We used the APP/PS1-21 mouse model of AD. For 60 minutes *in vivo* PET-CT scans, groups of 6 mice were imaged at the ages of (1mo, 3mo, 6mo, 9mo and 12mo) with [^{18}F]DPA-714. For the *ex vivo* brain autoradiographic study, immediately after the *in vivo* scans, the mice were killed, the brain dissected, frozen and sliced (20 µm) with a cryostat, and then apposed to an imaging plate. Immunohistochemical and histological staining for amyloid and microglial activation were performed.
Results
In vivo PET results showed increase [^{18}F]DPA-714 uptake initially in cortex (3mo) and later in thalamus and striatum (6mo) of AD mice compared to the 1 month old mice used as baseline. Wild type mice (WT) were used as controls.
The *ex vivo* results demonstrated increasing cortex to cerebellum ratios from 3 months of age until 12 months. Cortex to thalamus and striatum ratios peaked at 3 months, and later slightly declined as mice aged and amyloid plaques and inflammation started to increase in the thalamus and striatum.
Conclusions
This preclinical study demonstrates the validity of the novel ^{18}F tracer [^{18}F]DPA-714 for the detection of neuroinflammation in a mouse model of AD, and could serve as an alternative to the widely used ^{11}C tracer [^{11}C]PK11195. Funding by: HEALTH-F2-2011-2788850 (INMiND)

06j. Imaging & Biomarkers: PET - other

ADPD5-1737

CAN RESULTS OF NEUROINFLAMMATION USING NOVEL TSPO TRACERS IN TSPO HAB/MAB SUBGROUP BE TRANSLATED TO THE ENTIRE AD COHORT

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Objectives: Neuroinflammation plays a significant role in Alzheimer's disease (AD). Novel TSPO PET imaging allows *in vivo* quantification of neuroinflammation, which is dependent on TSPO genotype, with 40-50% increase in BP in high affinity (HAB) compared to mixed affinity (MAB) binders; while low affinity binders (LAB) are unsuitable for evaluation. To translate the findings of neuroinflammation studies to the entire population, it is crucial to establish the influence of TSPO genotypes on AD course. Here we investigated whether different TSPO genotypes have influences on their cognitive function and amyloid load at baseline, and disease progression during follow up.
Methods: We evaluated 798 subjects (225 normal, 388 MCI and 185 AD) from ADNI cohort, who underwent TSPO genotyping (SNP rs6971), MRI, amyloid PET and neuropsychometric assessments with longitudinal follow-up.
Results: The prevalence of genotypes (Thr147Ala, Thr147Thr and Ala147Ala) was similar among AD, MCI and control groups; including sex, age, amyloid status, MMSE, ADAS 11, ADAS MOD and GDS. During the follow up, mean change of neuropsychometric test scores were similar in different genetic groups. The covariates showed that diagnostic group (normal, MCI, AD), ApoE4 status, and gender all had a significant effect on ADAS MOD decline but not age or TSPO genotype.
C**onclusion:** Our findings suggest that the efficacy of intervention studies and observational studies with an anti-glial agent in a TSPO genetic subgroup of high or medium affinity binders could, therefore, be translated to the entire AD and MCI population.

06j. Imaging & Biomarkers: PET - other

ADPD5-1806

EARLY ASTROCYTOSIS IN AUTOSOMAL-DOMINANT AND SPORADIC AD: A LONGITUDINAL MULTITRACER PET IMAGING STUDY

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Objectives: Early astrocytosis was reported from cross-sectional multitracer PET imaging in autosomal-dominant AD (ADAD) and sporadic-AD (sAD)[1]. Here we sought to validate the cross-sectional findings by a longitudinal follow-up investigation in a larger cohort, and investigate regional and temporal distributions of brain astrocytosis, amyloid deposition and glucose metabolism.
Methods: 52 baseline participants (26 followed-up at 2.8±0.6 years) included ADAD-carriers (n=11), non-carriers (n=16), sporadic-MCI (sMCI) (n=17) and sAD (n=8). Participants underwent baseline/follow-up PET-scans with ^{11}C-Deuterium-L-Deprenyl (^{11}C-DED), ^{11}C-PIB, and ^{18}F-FDG; and neuropsychological testing. Linear mixed models (LMMs) were applied to longitudinal PET data versus estimated years to symptoms onset (EYO).
Results: Increased ^{11}C-DED binding and ^{11}C-PIB retention were first observed in ADAD-carriers around EYO ≈ -20 years, and subsequently showed opposite time-courses. ^{11}C-DED binding in ADAD-carriers declined steadily, while ^{11}C-PIB retention showed increase rates, steepest in anterior/posterior cingulate and putamen (≈0.034 SUVR/year). LMMs of ADAD-carriers demonstrated both higher ^{11}C-PIB and lower ^{11}C-DED in symptomatic ADAD-carriers versus sMCI. Presymptomatic ADAD-carriers (EYO ≈ -12 years) showed incipient hypometabolism. ^{18}F-FDG decline rates were largest in caudate and parietal cortices (-0.022, -0.016 SUVR/year respectively) of ADAD-carriers, and in sMCI until 5-years after diagnosis. Hypometabolism was more pronounced in symptomatic ADAD versus either sMCI/sAD.
Conclusions: The longitudinal investigation demonstrated that PET data was reproducible after mean follow-up of 2.8-years, statistically strengthening cross-sectional findings. Early and declining astrocytosis, increasing amyloid-plaque deposition and decreasing glucose metabolism characterized AD evolution. The observed type of early astrocytosis might have downstream pathological consequences, potentially identifying a new therapeutic target.

[1]A.Nordberg(2014),Neurodegener.Dis;13:160-162.

06l. Imaging & Biomarkers: PET-CT

ADPD5-1788

FDG-PET CT BRAIN CHANGES IN MCI PARTICIPANTS CHARACTERISED BY COGNITIVE DOMAIN

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FDG-PET CT Brain Changes in MCI Participants Characterised by Cognitive Domain

Objectives

To compare FDG-PET CT brain findings in mild cognitive impairment (MCI) participants according to their cognitive domain classification.

Methods

Participants were recruited as a sub-study within a larger cohort. 36 patients underwent neuropsychological evaluation and PET images were acquired by 'Discovery ST' PET-CT equipment. Spatially normalised images were smoothed with an isotropic Gaussian kernel of 8 mm using the Statistical Parametric Mapping programme implemented in Matlab 7.8.

Groups were compared on a voxel-by- voxel basis using a two-sample t test (SPM8). Significant differences between groups (i.e. normal v deficit) were examined at $p<0.001$ for voxel height, uncorrected with a minimum cluster extent of 20 voxels. Deficits in all domains (memory, executive function, language and visuo-spatial processing) were tested in this manner.

Results

Mean age of participants was 73.4 years (sd7.7), 47% were female. Mean Addenbrooke's Cognitive Examination (ACE) score was 84.33 (sd7.0). Table 1 shows the areas of reduced activation on FDG-PET CT associated with specific cognitive deficits.

Conclusions

Results suggest fairly robust relationships between domains of cognitive impairment and specific regional changes. It would be of interest to continue this work in a larger cohort of participants.

Table 1 Summary of Findings from FDG-PET Analyses in MCI Participants

Normal/Impaired	Deficit	Areas of Reduced Activation
12/24	Memory	Occipital lobe; Sub-lobar, right lentiform nucleus and right thalamus
21/15	Executive function	Frontal lobe; Temporal lobe; Occipital lobe; Sub-lobar, R/L lentiform nuclei
29/7	Language	Frontal lobe
29/7	Visuo-spatial	Extensive multi-lobe deficits

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-0295

201TlDDC-SPECT IMAGING OF ALTERATIONS IN CNS K+-METABOLISM IN NEURODEGENERATIVE DISEASES

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Neuronal activity and membrane potential are coupled to transmembrane potassium (K^+)-turnover rates and intra- to extracellular K^+-gradients. We have recently shown, using histochemical methods in rodents, that the K^+-probe thallium (Tl^+) or the lipophilic chelate complex thallium diethyldithiocarbamate (TlDDC), respectively, can be used for mapping acute (stimulus-dependent) and chronic (disease related) changes in neuronal activity as well as a breakdown of neuronal K^+-gradients.

During development of cortical β-amyloidosis in the transgenic 5xFAD mouse model single cell resolution mapping of neuronal thallium uptake revealed that electrical activity of pyramidal cells breaks down throughout infragranular cortical layer V long before cell death occurs (Lison et al., 2014). We here show, in rodent models of neurodegenerative diseases, that pathological alterations in CNS K^+-metabolism can be imaged in vivo using ^{201}TlDDC single-photon emission computed tomography (SPECT).

In still ongoing preclinical studies validating the use of ^{201}TlDDC we found that ^{201}Tl-uptake and -redistribution are severely altered in focal cerebral ischemia and that ^{201}TlDDC-SPECT can be used to dynamically image the progressive breakdown of K^+-gradients as an early indicator of neuronal damage. Preliminary data indicate that uptake and redistribution patterns are altered in mouse models of dementia.

The close coupling of changes in K^+-metabolism and chronic up- or down-regulation of neuronal activity as well as cell death suggests the use of ^{201}TlDDC as a tracer for diagnosing and monitoring neurodegenerative diseases in preclinical and clinical studies.

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-0630

VISUAL RATING OF SPECT PERFUSION BRAINS USING 3D NEUROGAM FOR VERY EARLY ALZHEIMER DISEASE

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Objectives
To develop simple regional semi-quantification of 3D brain perfusion using SPECT and use for of very early Alzheimer disease (AD) diagnostics.
Methods
We transformed SPECT examinations into 3D brains using NeuroGam^{TM}Segami software in 35 cognitively normal controls (NC) (Mini-Mental State Examination (MMSE) 29±1 points) and 33 patients with mild cognitive impairment (MCI) and mild dementia due to Alzheimer disease (AD) (MMSE 26±3 points). Each lobe convexity, cingulum, mediotemporal and frontobasal regions were rated 0,1 or 2 in both hemispheres and combined into a single bilateral score ranging from 0 (normal) to 3 (pathological on both sides).
Results
Since AD patients with MCI and dementia did not differ in age, gender, education, MMSE, any brain perfusion score, we merged them into one AD patient group. In comparison with the NC group, these patients had significantly higher scores of perfusion defects only in temporal and parietal convexity separately on each side and also bilaterally. All other regions including mediotemporal ones were similarly perfused in the patients and in the controls.
Conclusions
Visual rating of 3D Neurogam brain SPECT is a simple and convenient tool for routine clinical practice. Very early AD patients had already incipient perfusion alterations in temporo-parietal regions on both sides. Surprisingly, mild mediotemporal changes were common even in normal seniors and were indistinguishable from those found in AD patients. This unusual pattern for early diagnosis needs further exploration.
Supported by grant IGA NT 13183, PRVOUK 34/LF3 and DRO (PCP, 00023752).

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-1837

DIFFERENCES IN THE EFFECTS OF VARIOUS DEMENTIA THERAPEUTIC DRUGS - COMPARISON USING ADAS-JCOG AND SPECT –

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[Purpose]
A comparative study was conducted based on changes in Alzheimer's Disease Assessment Scale (ADAS) and SPECT imaging in order to assess differences in the effects of various dementia drugs.
[Methods]
1. The subjects consisted of 274 cases of Alzheimer's disease (AD) being treated in out-patient. The subjects were comprised of 142 cases using Donepezil (Group D), 33 using Rivastigmine (Group R), 32 using Galantamine (Group G) and 14 using Memantine (Group M), and 53 as concomitant drug therapy. Mini Mental State Examinations (MMSE) and ADAS were performed prior to administration and six months after administration of each drugs, followed by a comparative study of changes in scores among each of the drug groups.
2. 226 of the above cases able to undergo SPECT imaging were similarly categorized into groups. 99mTc-ECD SPECT imaging was performed , and changes in cerebral blood flow (CBF) were compared using Statistical Parametric Mapping (SPM-8).
[Results]
1. Groups D and G demonstrated significant improvement with respect to "word recall", and with "following commands" in Group R among ADAS subscales ($p<0.05$).
2. Those sites where CBF increased consisted of the anterior cingulate in Group D, a wide range of the parietal lobe in Group G, the orbital gyrus, straight gyrus, and a portion of the thalamus in Group R, and the occipital lobe, in Group M.
[Discussion]
It`s interesting to note that ADAS subscale showed improvements in that differed among using drugs, and that there was a correlation with sites of improved cerebral blood flow.

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-0489
IMAGING IN ALZHEIMER DISEASE (AD) TRIALS: IMPACT ON PATIENT RECRUITMENT, RETENTION AND LOGISTICS

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Background: MRI and PET is now extensively utilized in AD trials for patient eligibility, efficacy assessment efficacy, and safety evaluations.
Methods: Standardization of MRI and PET methodology across the sites is essential to acquire consistent data. This requires prospective site qualification, evaluation of phantom data, training and continuous monitoring. standardization is important for estimation of brain volumes and PET data.
Results and discussion:
Patient Eligibility: Many neurological diseases like vascular dementia, multiple sclerosis, vascular pathology, neoplasms have similar presentation as AD or could confound the assessment of drug therapy. Inclusion of wrong patients has ethical and legal issues and data could be excluded from the analysis. Eligibility evaluation and optimization of eligibility read process will be discussed.
Evaluation of Amyloid Related Imaging Abnormalities (ARIA): ARIA were observed in amyloid-β trials. MRI findings of ARIA include vasogenic edema (ARIA E), micro and macro hemorrhages (ARIA H) and superficial siderosis. FDA had mandated frequent MRIs in all AD trials putting burden on sites and patients. Our experience in ARIA evaluations in large phase III study at >350 sites will be presented.
Efficacy evaluation: MRI is utilized to evaluate various volumes of brain. FDG PET has been used to measure metabolic activity of brain. We will share our experience about site and central independent reads.
Conclusion: MRI and PET are used for patient eligibility, efficacy and safety assessments. Imaging must be prospectively planned including standardizing imaging methodologies, site selection process and selecting eligibility and efficacy criteria. Training should be transparently conducted and documented.

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1005

THE EFFECT OF NEURODEGENERATION ON THE ANATOMICO-FUNCTIONAL CIRCUITRY OF THE MEMORY NETWORK

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1. Objectives

The objective of the study was to identify the critical alterations in the anatomico-functional circuitry of the memory network that mediate the transition from healthy ageing to Alzheimer's disease (AD). Specifically, we aimed to quantify pathological changes in white and grey matter integrity and relate them to the delineated functional connectivity patterns and measures of cognitive deterioration.

2. Methods

We used diffusion-weighted, structural, and functional magnetic resonance imaging to examine structural and functional changes in three groups of individuals: healthy older adults, adults with mild cognitive impairment (MCI), and patients in early stages of AD. We delineated the functional connectivity of the underlying memory network, modeled the relevant white matter pathways, and measured cortical and subcortical volume of the network's main nodes. We compared the delineated anatomico-structural changes across the three groups of participants.

3. Results

MCI and AD patients showed alterations in the functional connectivity of the memory network, especially in the connections to and from the medial temporal lobe structures, but also between parietal and prefrontal cortices. These changes were significantly more pronounced in AD patients and correlated with measures of cognitive ability. The functional alterations in the memory circuitry correlated with large-scale decreases in cortical volume, hippocampal atrophy, and white matter integrity.

4. Conclusions

Examination of neurodegenerative changes in both the structural integrity and functional connectivity of known networks in patient groups will help identify early signs of AD-related neuronal degeneration and successfully predict later development of dementia in healthy participants.

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1328

BRAIN STRUCTURAL CHANGES IN THE COURSE OF ALZHEIMER'S DISEASE

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Objectives. To investigate gray (GM) and white matter (WM) structural changes in Alzheimer's disease (AD) patients and the relationship between biological measures at baseline and their brain structural progressive changes over time.

Methods. T1-weighted and diffusion tensor (DT) MRI from 14 patients with probable AD were obtained at baseline and after a mean follow-up of 16 months. At baseline, CSF samples from patients and MRI from 37 controls were also acquired. Regional GM volume loss and DT MRI metrics from the interhemispheric and major long-association WM tracts were obtained. MRI metrics were compared between patients and controls, and changes over time were evaluated in patients.

Results. Compared to controls, patients showed GM atrophy in the medial temporal and inferior parietal regions, as well as WM damage of the corpus callosum (CC), bilateral cingulum, inferior (ILF) and superior longitudinal fasciculus (SLF), left corticospinal tract and uncinate fasciculus. At follow-up, additional damage was observed in CC, bilateral SLF, right cingulum, and left ILF. No further GM damage was detected. Patients with higher CSF total-tau levels presented greater WM damage over time in the right cingulum.

Conclusions. Although AD patients already presented GM volume loss and reduced WM integrity at baseline, considerable WM changes over time were detected. Interestingly, only WM changes over time in regions typically hit by the disease were related to the baseline high levels of CSF total-tau, suggesting the tau crucial role in predicting WM damage progression once the disease onsets.

Funding. Italian Ministry of Health (#GR-2010-2303035).

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1378

THE EFFECT OF GREY MATTER ATROPHY ON FUNCTIONAL ANALYSIS OF THE DEFAULT MODE NETWORK IN ALZHEIMER'S DISEASE AND AMNESTIC MILD COGNITIVE IMPAIRMENT

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Background: It is not completely known how grey matter (GM) atrophy influences the patterns of functional connectivity and activity analyses in Alzheimer's disease (AD) and amnestic Mild Cognitive Impairment (aMCI), especially in the Default Mode Network (DMN). We aimed to compare DMN's functional connectivity (FC) as well as amplitude of low frequency fluctuations (ALFF) measures among mild AD, aMCI and healthy elderly subjects considering GM values.

Methods: We evaluated 72 participants: 29 mild AD, 14 aMCI, and 29 controls in a 3T MRI. DMN was divided in 3 subparts: Frontal, Parietal and medial Temporal. We evaluated anatomical (regional and global cortical thickness and volume) and functional (FC and ALFF) aspects.

Results: AD patients were significantly more atrophic than aMCI and controls considering total GM volume and frontal and temporal DMN. Before atrophy correction, AD patients had lower ALFF values only in DMN's Parietal subpart. Also, AD showed lower FC in frontal and parietal DMN. Regarding aMCI, they were more atrophic than controls only in temporal DMN. Before atrophy correction, they presented higher ALFF values than AD and controls in Frontal DMN; no differences were found in FC in comparison with the other groups.

After GM correction, aMCI patients had lower ALFF value than controls in the temporal DMN. AD DMN's Frontal FC was no longer significantly lower than controls, while all other analysis did not change.

Conclusions: Atrophy did not account as much for the DMN FC analyses, but influenced, at some extent, ALFF measures in AD spectrum.

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1409

IRON AND PLAQUES: CORRELATING PIB-PET TO QUANTITATIVE SUSCEPTIBILITY MAPPING MRI IN MILD COGNITIVE IMPAIRMENT

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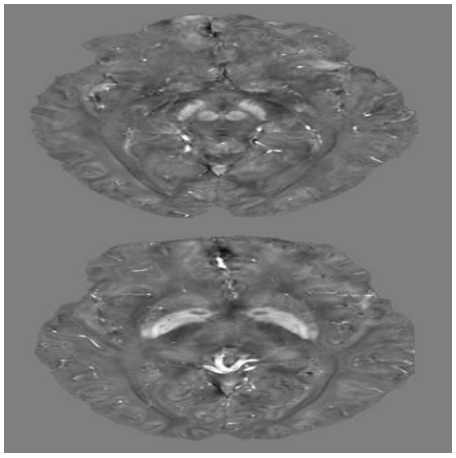
Introduction: Postmortem neuropathology studies and in vivo MRI studies have demonstrated altered iron levels in both cortical and deep grey matter structures of late stage Alzheimer's patients. Since Abeta plaques start to form long before clinical symptoms occur, sensitive non-invasive in vivo techniques to detect these changes are required for early intervention. Quantitative Susceptibility Mapping (QSM) allows quantitative in vivo mapping of iron levels in the brain using MRI phase data and is more specific than other, e.g. relaxation based, iron measures. By acquiring QSM maps at 7T and Carbon-11 based Pittsburgh compound-B Positron Emission Tomography (PiB-PET) images in patients with mild cognitive impairment (MCI) and age matched controls, we want to confirm the AD biomarker potential of iron levels as detected by QSM.

Methods: 40 elderly study participants aged between 62 and 89 years (22 cognitively normal, 18 MCI), without evidence for significant medical illness, were recruited at our hospital. QSM maps were reconstructed using the phase images of a 3D multi-echo GRE sequence, acquired on a 7T MR scanner (figure shows a healthy control). PiB-PET images were acquired to determine regional Aβ load. Automated atlas based image segmentation and co-registration will be performed to select the regions of interest. Preliminary results will be presented on region based QSM iron levels and the correlation with Abeta load in MCI and controls.



06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1416

HIPPOCAMPUS AND BRAINSTEM OF COGNITIVELY-NORMAL ELDERLY INDIVIDUALS SHOW AMYLOID-BETA-ASSOCIATED TISSUE ALTERATIONS AS INDICATED BY FLUID-ATTENUATED INVERSION RECOVERY (FLAIR) MRI AT 7 TESLA AND 11C-PIB PET

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Introduction: Cerebral accumulation of amyloid-beta (abeta) is a pathological hallmark of Alzheimer's disease (AD), and begins prior to the clinical onset of AD. Abeta deposits can be assessed in vivo using Pittsburgh compound-B positron emission tomography (PiB PET), showing significant amyloidosis of the brain in up to 30 % of healthy elderly individuals. To study the effects of abeta on brain tissue integrity, the individual abeta load was correlated with regional intensities of fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) in asymptomatic elderly subjects.
Methods: All participants (n=14; age=69±8; female/male=6/8) underwent neuropsychological assessment, PiB PET and MRI at high field strength of 7 Tesla including FLAIR and MPRAGE sequences. Volume-normalized signal intensities of PiB PET and FLAIR were calculated based on automated subcortical segmentation of PIB PET, FLAIR and structural MRI volumes. Regional associations between PiB PET and FLAIR signal intensities were investigated using spearman's rank correlation, followed by Holm-Bonferroni correction for multiple testing.
Results: All participants showed normal cognitive performance, and structural analysis indicated no significant brain atrophy. Three anatomical subregions demonstrated significant statistical dependence between volume-normalized PiB PET and FLAIR intensities: hippocampus (right: rho=0.86; left: rho=0.84), brain-stem (rho=0.85), left basal ganglia vessel region (rho=0.82).
Conclusions: We found significant associations between PiB PET and FLAIR MRI signals in the hippocampus and brainstem, suggesting abeta-associated brain tissue abnormalities such as edema in cognitively normal elderly individuals. Further studies are warranted to understand the significance of these results regarding preclinical detection and pathophysiology of neurodegenerative diseases such as AD.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-0669

QUANTITATIVE EEG IN PATIENTS WITH ALZHEIMER ´S DISEASE, MILD COGNITIVE IMPAIRMENT AND HEALTHY CONTROLS BEFORE AND AFTER SHORT AND LONGTERM TREATMENT

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Objectives. Patients with mild cognitive impairment (amnestic and vascular type) and Alzheimer·s disease (AD) are characterized by changes in spectral power and EEG-coherence, which could be visualized more with specific tests of fatigue.
The aim was to evaluate spectral power and EEG-coherence in MCI, AD patients and healthy elderly controls, to find their reactivity before and after standartized tests on fatigue before and after 3 months and after 2.9 years of treatment with memantine, galantamine and to determine correlations between EEG-changes, cognitive and neuropsychiatric impairments.
Materials and methods. 46 patients with AD, 36 patients with amnestic MCI, 42 patients with vascular MCI, 40 age-matched controls were examined using EEG -recordings, neuropsychological investigations and neuropsychiatric inventory (NPI).
Results. There was found significant increase of slow-wave activity in frontal,central and temporal regions and decreased alpha rhythm in the same regions in both MCI groups vs controls (<0.05), in AD vs controls , which become significantly stronger after tests on fatigue. Coherence in the same regions was significantly lower in both groups of MCI vs controls(p<0.05), AD vs MCI, AD vs controls.
We found significant positive dynamic in EEG-changes on short and longterm treatment after tests on fatigue (p<0.05).
Conclusions. EEG changes before and after tests on fatigue , cognitive and neuropsychiatric impairments have positive dynamic on short and longterm treatment. Significant positive dynamic in spectral power and EEG-coherence was visualized especially after standartized probe on fatigue, which could serve a specific indicator of the dynamic and efficacy of treatment patients with AD and both MCI types.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-1366

VIRTUAL ELECTRO-PHYSIOLOGICAL MARKER REFLECTING CHOLINERGIC ACTIVITY IN THE BRAIN : PROPERTIES IN VARIOUS DEMENTIAS AND PROGRESSION OF AD

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Objectives

Demonstrate and profile the properties of an EEG based AchIndex in an elderly population of both healthy controls and patients suffering from different stages and types of dementia.

Methods

An electroencephalography (EEG) based acetylcholine (Ach) index reflecting cholinergic activity in the brain was developed using data from a scopolamine challenge study. A data-driven approach incorporating statistical pattern recognition methods and a genetic algorithm was used to select the relevant features from the EEG. To demonstrate the applicability of the AchIndex its behaviour was comprehensively examined using EEG recordings from an elderly population of both healthy controls and patients suffering from various causes of cognitive decline (N=1054).

Results

The AchIndex showed a strong reduction in the severe stages of AD dementia ($p<0.001$), in which lower cholinergic activity was expected. A high correlation was also demonstrated between the Ach Index and cognitive function as estimated by MMSE ($r=0.52$, $p<0.001$) and DSST ($r=0.57$, $p<0.001$). The index was reduced in patients with MCI and prodromal AD ($p<0.001$), indicating decreased cholinergic activity and contradicting previous post-mortem findings.

Conclusion

The AchIndex provides a physiological means of virtually monitoring cholinergic activity. This has great potential for aiding diagnosis and patient stratification, for monitoring disease progression and treatment response, and for assisting further research into dementia.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-1472

EEG SOURCE RECONSTRUCTION AND ASSOCIATIVE MEMORY TESTS FOR THE DETECTION OF ASYMPTOMATIC ALZHEIMER'S DISEASE

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The ability to differentiate between those people who will go on to develop Alzheimer's disease (AD) from normal ageing adults, at the earliest possible time point, is of primary importance. Episodic memory problems are a central feature of Alzheimer's disease. However, in the asymptomatic stage of AD, no explicit difference in memory performance can be observed, as it has been shown that compensatory activity within the episodic memory network, especially in the pre-frontal cortex, is initially successful in overcoming the pathological changes in medial temporal lobe areas. Recent advances in EEG techniques, including source reconstruction procedures, make it a good candidate for providing a simple biomarker for detecting these compensatory changes in neural activity. Furthermore, as task difficulty increases in certain types of memory test, it can be assumed that the compensatory activity will increase or breakdown. Our laboratory is designing a series of associative memory tasks that are completed whilst EEG recordings are taken. Advanced analysis including source reconstruction should theoretical allow for the detection of compensatory processes and be sufficient to differentiate between normal and pathological ageing.

Results will be presented from a healthy young adult sample to illustrate how this technique could be applied to detection of AD at the asymptomatic stage and how advances in EEG analysis could prove to be an important and cost-effective biomarker.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-1521
THE CORRELATION OF QUANTITATIVE EEG AND MEDIAL TEMPORAL LOBE ATROPHY IN ALZHEIMER'S DISEASE
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Backgrounds: The electroencephalogram (EEG) abnormalities in Alzheimer's disease (AD) have been widely reported, and medial temporal lobe atrophy (MTLA) is one of the hallmarks in early stage of AD. We aimed to assess the relationship between EEG abnormalities and MTLA and its clinical validity. **Materials and Methods:** A total of 18 patients with AD were recruited (the mean age: 77.83 years). Baseline EEGs were analyzed with quantitative spectral analysis. MTLA was assessed by a T1-axial visual rating scale (VRS). **Results:** In relative power spectrum analysis according to the right MTLA severity, the power of theta waves in C4, T4, F4, F8, and T5 increased significantly and the power of beta waves in T6, C4, T4, F8, T5, P3, T3, and F7 decreased significantly in severe atrophy group. In relative power spectrum analysis according to the left MTLA severity, the power of theta waves in T3 increased significantly and that of beta waves in P4, T6, C4, F4, F8, T5, P3, C3, T3, F3, and F7 decreased significantly in severe atrophy group. **Conclusions:** The severe MTLA group, regardless of laterality, showed more severe quantitative EEG alterations. These results suggest that quantitative EEG abnormalities are correlated with the MTLA, which may play an important role in AD process.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-2013

GENDER DEPENDENT DIFFERENCES IN QUANTITATIVE NEURO-ELECTRIC ACTIVITY PATTERNS IN HEALTHY AND MILDLY COGNITIVELY IMPAIRED SUBJECTS: A RETROSPECTIVE ANALYSIS

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Objective. Mild cognitive impairment (MCI) is regarded as a transitional stage in the development of Alzheimer disease. MCI can be recognized by changes in EEG. The present retrospective analysis of several clinical studies was undertaken in order to sort out if there are gender dependent quantitative differences in brain regions in healthy subjects in comparison to cognitively mildly impaired subjects older than 40 years.
Methods. EEG recordings were taken using the CATEEM®. Spectral power - averaged during recordings of 5 minutes duration – was compared during relaxed states “eyes open” as well as during different cognitive tests. Data from 90 male and female subjects were grouped and statistically compared for 102 parameters by Wilkinson- Mann-Whitney non-parametric statistical tests and fed into linear discriminant analysis.
Results. Recording in the relaxed state revealed increases in spectral power in the left occipital region in both genders, but decreases in spectral alpha2 and beta power in the left frontal and temporal lobe only in male MCI subjects in comparison to healthy controls. During performance of the d2-test only in males significantly less left frontal delta and theta spectral power was observed. During performance of a calculation test or the memory test only in male subjects (MCI) significantly higher spectral power was documented in the left occipital region but decreases in the temporal lobe on both genders. Gender dependent differences were confirmed by discriminant analysis.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-2089

CALPAIN INHIBITORS INCREASE THE RELEASE OF ACETYLCHOLINE AND RAPID EYE MOVEMENT (REM) SLEEP

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Calpains are a family of calcium-dependent cysteine-proteases that proteolyze a wide variety of cytoskeletal, membrane-associated, and regulatory proteins and are activated in pathologic processes in the CNS including neurodegenerative diseases like Alzheimer´s disease (AD). Today, inhibitors of acetylcholine esterase (AChEI) represent the AD standard treatment. Such compounds increase the extracellular levels of acetylcholine thereby stimulating cholinergic receptors.

Objectives: We have synthesized a series of calpain inhibitors which besides their neuroprotective effects increase extracellular ACh levels. We investigated whether calpain inhibitors similar to the effects of AChEIs increased extracellular ACh and thereby stimulate REM sleep in polysomnographic studies suggesting its use as translational biomarker for clinical studies.

Methods: In rats we investigated the effects of the prototypic calpain-inhibitor A-705253 (K_i=56 nM) on acetylcholine release in the frontal cortex and hippocampus by *in vivo* microdialysis. In a rat polysomnographic study we also analyzed the sleep EEG.

Results: A-705253 induced a dose-related increase of acetylcholine levels in both brain regions suggesting potential pro-cognitive effects. A-705253 also increased REM parameters without affecting total sleep time.

Conclusion: The calpain inhibitor-induced increase of extracellular acetylcholine translates into increased REM sleep. Such effects have been observed in healthy volunteers and AD patients after treatment with AChEIs. This suggests the usefulness of polysomnographic studies as translational biomarker for the clinical development of calpain inhibitors.

Disclosures: All authors are employees of AbbVie. The design, study conduct, and financial support for this research was provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-2147

LONG-TERM EEG IS NECESSARY FOR DETECTING EPILEPTIC EEG-SIGNS IN PATIENTS WITH DEMENTIA

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Objectives
Major neurocognitive disorders represent a serious socio-medical and economical issue. An elevated risk of seizures has been shown in conditions with dementia, especially in Alzheimer's disease (AD). Several studies highlighted the major impact of seizures and epileptic activity on the progression of cognitive decline. Electroencephalography (EEG) is the tool for detecting dementia-related epileptic signs and clarifying unnoticed seizures.
In the framework of the Alzheimer-Epilepsy Project of the National Brain Research Program of Hungary, we compared the sensitivity in detecting interictal and ictal epileptic EEG signs of 'routinely' applied 30 minutes EEGs and 24 hours' EEG recordings in patients with dementia.
Methods
We performed 24 hours portable EEGs in 5 AD patients clinically suffering from epilepsy. We segmented the recordings into 30 minute-long epochs. We calculated the proportion of epochs with no ictal or interictal epilepsy pattern in the whole recording. The sleep-related distribution of the epochs containing epilepsy signs as well as the epochs' relation with REM or non-REM sleep was analyzed and calculated.
Results
60 percent of the 30 minute-long epochs did not contain any epileptiform activity. The occurrence of epochs containing interictal or ictal EEG patterns was higher during non-REM sleep.
Conclusions
Interictal and ictal EEG patterns are missed in 60 percent of 30 minute-long 'routine' EEGs. Long-term EEG is significantly more sensitive in capturing epileptiform activity in AD patients with epilepsy. Sleep EEG is especially useful, significantly increasing the chance of detecting epilepsy.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-2234

QUANTITATIVE EEG APPLYING THE STATISTICAL RECOGNITION PATTERN METHOD: A USEFUL TOOL IN THE DEMENTIA DIAGNOSTIC WORK-UP.

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Background: The aim of the study was to examine the discriminatory power of the quantitative EEG (qEEG) applying the statistical pattern recognition (SPR) method to separate Alzheimer's disease (AD) patients from elderly persons without dementia and from other dementia patients.

Methods: Participants were recruited from six Nordic memory clinics, 372 unselected patient, mean age 71.7 (s.d. 8.6), 54 percent women and 146 healthy old people, mean age 66.5 (s.d. 7.7), 60 percent women. After a standardized and comprehensive assessment clinical diagnoses were made according to international accepted criteria by at least two clinicians. EEG was recorded in a standardized way and analyzed independently of the clinical diagnoses using the SPR method

Results: In receiver operating characteristic (ROC) analyses the qEEG separated AD from healthy old person with an area under the curve (AUC) of 0.90, representing sensitivity (SS) of 84 percent and specificity (SP) of 81 percent. The qEEG further separated patients with Lewy body dementia or Parkinson's disease dementia from AD with AUC of 0.9, SS 85 percent and SP 87 percent.

Conclusion: The qEEG using the SPR method could be a useful tool in the dementia diagnostic work-up.

06q. Imaging & Biomarkers: MR spectroscopy

ADPD5-0474

1H-MRS CHANGES IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT

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1H-MRS allows to reveal quantitative changes in brainmetabolites which reflects the violation of functional activity ofstrategically important areas preceding the development of structural damage.
The purpose of research was to investigate thecerebral metabolism in patients with mild cognitive impairment (MCI) using forfinding a reliable marker of the pathological process at early stages of thedisease.
The study involved 38 persons aged 62 to 74 years(mean age 68,6 ± 6,3 years) with MCI of alzheimer's and vascular etiology, and20 healthy volunteers as a control group. Region of interest was cingulategyrus.
Was found that Alzheimer's group characterized by the increaseof inositol, choline, lactate, as well as reduction of N-acetylaspartate,glutamate/glutamine and creatine, more expressed in the posterior part of thecingulate gyrus. Diagnostically significant was the increase in theratio Ins/Cr over 0,76 ± 0,31 and Cho/Cr over 1,47 ± 0,69.
In vascular MCI were identified an increase of choline,lactate and decreased N-acetylaspartate and creatine in all parts of thecingulate gyrus, in the absence of significant changes in inositol andglutamate / glutamine. Diagnostically significant was the increase in theratio Cho/Cr over 1,39 ± 0,36 at normal ratio of Ins/Cr.
Thus, the use of 1H-MRS may be effective in clinicalpractice for early diagnostic of cognitive impairment of neurodegenerative andvascular etiology.

06q. Imaging & Biomarkers: MR spectroscopy

ADPD5-0777

MAGNETIC RESONANCE SPECTROSCOPY AND Aβ42 DEPOSITION IN PRECLINICAL ALZHEIMER'S DISEASE, SUBJECTIVE AND OBJECTIVE COGNITIVE IMPAIRMENT.

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Objective: A potential biomarker for predicting onset and progression of Alzheimer's disease is proton magnetic resonance spectroscopy (MRS). We aimed to determine the correlation of MRS brain metabolite concentrations with Aβ deposition measured by positron emission tomography (PET), cerebrospinal fluid (CSF) markers and ApoE in cognitively healthy elderly participants and individuals with mild cognitive complaints.

Methods: Study participants (N_{CTL}=215, N_{MCI}=285) underwent MRS and CSF collection; ^{18}F-flutemetamol PET scans and ApoE status were available for a subset of patients. MRS was collected at 3 Tesla from a 2x2x2cm^3 voxel placed bilaterally in precuneus/posterior cingulate. Concentrations of N-acetyl-aspartate (NAA), myo-inositol (mI) and choline (Cho) were evaluated relative creatine (Cre). Study sample was divided into groups based on CSF Aβ42 levels (Aβ42 ≤ 530 ng/L was considered pathological). Subjects with mild cognitive complaints were also divided based on the nature of the cognitive impairment (subjective or objective).

Results: In controls with asymptomatic amyloidosis (CSF Aβ42 ≤ 530 ng/L), we found that tracer uptake in the precuneus was associated negatively with NAA/Cre (Spearman r_s = -0.42, p<0.001) and positively with Ins/Cre (r_s = 0.57, p<0.001), controlling for age, gender and spectral signal to noise ratio (SNR). In biomarker-positive controls and biomarker-negative MCI there were significant negative associations between CSF Tau and NAA/Cre. Healthy ApoE ε4 carriers had higher myo-inositol levels than non-carriers; interaction between age and carrier status significantly modulated Ins/Cre concentrations.

Conclusions: In preclinical AD, several changes in the MRS neurochemical profile are associated with known pathological processes such as Aβ42 deposition.

06r. Imaging & Biomarkers: other

ADPD5-0343

RED BLOOD CELL FATTY ACIDS ARE SIGNIFICANTLY ALTERED IN SOUTH AUSTRALIAN MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE COMPARED TO CONTROLS

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Objectives
This study aimed to determine the red blood cell (RBC) fatty acid profile of newly diagnosed individuals with mild cognitive impairment (MCI) or Alzheimer's disease (AD) relative to each other and to age and gender matched controls.
Methods
Fatty acids were measured using the one step extraction and transesterification procedure
Results
There was a significant increase in palmitic acid ($p<0.0001$) for both MCI and AD cohorts. Further MCI saturated fats were significantly elevated including stearic acid ($p=0.0002$), arachidic acid ($p=0.0003$), behenic acid ($p=0.0003$), tricosanoic acid ($p=0.003$) and lignoceric acid ($p=0.0009$). The monounsaturated fatty acid elaidic acid was significantly elevated in both MCI and AD ($p=0.002$). N6 polyunsaturated fatty acids (n-6 PUFA) were significantly reduced in MCI including linolelaidic acid ($p=0.001$), γ-linolenic acid ($p=0.03$), eicosatrienoic acid ($p=0.01$), and arachidonic acid ($p<0.0001$). The n-3 PUFA Linolenic acid and docosahexaenoic acid were both significantly reduced in MCI and AD ($p<0.0001$). For MCI the area under the ROC curve (AUC) was >0.80 for eight fatty acids and for AD the highest AUC was 0.79 for elaidic acid and 0.75 for palmitic acid. Cross-correlation analysis between the Mini-mental state examination (MMSE) score and the RBC fatty acid analysis showed a positive correlation between MMSE score and nervonic acid in MCI ($r=0.54$, $p=0.01$) and a negative correlation with γ Linolenic acid in AD ($r=-0.43$, $p=0.05$).
Conclusion
Significant differences in RBC fatty acid profiles of MCI and AD individuals may prove to be useful potential biomarkers reflecting increased risk for dementia.

06r. Imaging & Biomarkers: other

ADPD5-0392

CYP2D6 GENE AND PLASMA CONCENTRATION OF S-DONEPEZIL AS BIOMARKERS TO PREDICT AND EVALUATE CLINICAL OUTCOME OF DONEPEZIL IN CHINESE ALZHEIMER'S DISEASE PATIENTS

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Donepezil (DNP) has been approved for the treatment for mild-to-moderate Alzheimer's disease (AD), but the therepeutic response rate varies from 20 to 60%. It lacks convenient and effective blood biomarkers to evaluate clinical outcome of DNP in early stage of medication. Our study is the first explored the target enzymes which is dominant in the metabolism of DNP *in vitro*, by clarifying the metabolism mechanism of DNP especially the effects of different types of cytochrome P450 enzymes on metabolism of two DNP chiral enantiomers .We identified CYP2D6 is the predominant P450 enzyme which has largest Clint in DNP liver metabolism , and CYP2D6 gene polymorphisms which have high variation rate in Chinese play different roles in metabolism of *S*-DNP and *R*-DNP. Secondly, we confirmed the *in vitro* experimental results n 64 Chinese AD patients, by separation and quantitation of pharmaco-effective *S*-DNP and genotyped CYP2D6 enzyme types (CYP2D6*1, CYP2D6*1*10, CYP2D6*10) ,in relation to clinical outcome of DNP. There is statistical correlations among effective *S*-DNP Cp/dose (steady-state plasma concentration/dose), CYP2D6 alleles (CYP2D6*1, CYP2D6*1*10, CYP2D6*10) and DNP clinical outcome. Conversely, there is no statistical difference in racemic DNP Cp/dose between responders and nonresponders, also no statistical difference n racemic DNP Cp/dose among the three CYP2D6 alleles .
Our results suggested that plasma concentration of *S*-DNP and CYP 2D6 gene (CYP2D6*1,CYP2D6*1*10 and CYP2D6*10) which exists the high mutation frequencies in Chinese, are potential blood biomarkers in early stage to predict and evaluate DNP clinical outcome in Chinese AD patients.

06r. Imaging & Biomarkers: other

ADPD5-0508
PREPARATION AND STABILITY TESTING OF A QUALITY CONTROL MATERIAL FOR NEUROCHEMICAL DEMENTIA DIAGNOSTICS ASSAYS

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Objectives: (a) to prepare and test samples of different matrices to be used as quality control (QC) material for the Neurochemical Dementia Diagnostics (NDD); (b) to test short- and long-term stability of the NDD biomarkers (Amyloid β (Aβ) peptides and Tau/pTau proteins) in different matrices; (c) to test if antibacterial stabilizing component improves the stability of the samples and (d) to provide results enabling preparation of samples for an external quality control scheme (EQCS).
Methods: Large volumes of samples were prepared of: (a) human pooled cerebrospinal fluid (CSF), (b) artificial Aβ peptides dissolved in pre-diluted human plasma, and (c) Aβ peptides diluted in BSA/PBS. In addition to native material, samples containing antibacterial stabilizer were also tested. For a short term stability, the samples were stored for 1-14 days: (a) at room temperature, (b) refrigerated, and (c) under -20°C, following deep-freezing (isochronous method). All aliquots of a given matrix were then thawed and analyzed on one ELISA plate per biomarker to avoid the influence of inter-assay variability.
Results: In most cases, all the samples were be short-term stabile under all three conditions, with somehow better stability of the samples stored at -20°C. Addition of a stabilizer did not influence the stability of the biomarkers in any of the matrices.
Conclusions: Our results show that it is plausible to use a non-tailored, assay manufacturer-independent QC samples for controlling quality of the NDD assays performance. Currently, a project is running to test different matrices for their utility in an inter-center EQCS.

06r. Imaging & Biomarkers: other

ADPD5-0509

AMYLOID BETA 42/40 CSF CONCENTRATION RATIO WITH TWO NOVEL ASSAYS FOR AN EARLY DIAGNOSIS OF ALZHEIMER'S DISEASE

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Objectives: (a) to technically validate two novel assays (IBL International GmbH, Hamburg, Germany) specifically measuring Amyloid β (Aβ) 1-42 and Aβ1-40 in human cerebrospinal fluid (CSF); (b) to compare the diagnostic utility of Aβ42/40 Ratio and Aβ1-42 CSF concentration.
Methods: The novel Aβ1-40 and Aβ1-42 ELISA assays have been analytically validated considering their specificities, linearity, precision, repeatability of the standard curves, and recovery. In the clinical study, the hypothesis was tested if CSF Aβ42/40 concentration ratio gives better separation of AD and controls compared to the CSF Aβ42 concentration alone.
Results: The assays characterized with only marginal cross-reactivity (Aβ42 vs. Aβ40); recoveries were in the range of 85–100% for Aβ1-40, and 92–104% for Aβ1-42. For Aβ1-40, the intra-assay, the inter-assay, and the inter-lot imprecision were 2.1%, 4.4%, and 5.4%. For Aβ1-42, the numbers were 3.1%, 6.2%, and 6.9%, respectively. The imprecision of the optical densities of the standards (ten repetitions) was equal or less than 5% for all standards. Aβ1-42 showed the sensitivity and the specificity of 69.3 %, and 88.9%, respectively, Aβ42/40 ratio showed even better sensitivity and specificity of 93.3 %, and 100 %, respectively. The area under the ROC curve for Aβ42/40 (0.974) was highly significantly larger compared to the AUC of the Aβ1-42 concentration ROC curve (0.827, $p<0.0001$).
Conclusions: (a) the novel Aβ1-40 and Aβ1-42 ELISA assays characterize with very good analytical performance; (b) we reconfirm that the CSF Aβ42/40 concentration ratio shows significantly better diagnostic performance compared to the CSF Aβ1-42 concentration alone.

06r. Imaging & Biomarkers: other

ADPD5-0590

SEARCHING FOR A MOLECULAR SIGNATURE OF ALZHEIMER'S DISEASE IN DOWN SYNDROME PLASMA

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Individuals with Down syndrome (DS) are at increased risk of developing Alzheimer's disease (AD) compared to the normal population. As a consequence of chromosome 21 trisomy, they progressively develop amyloid plaques, neurofibrillary tangles and CNS inflammation. Although the pathology of AD and DS is multifactorial, in both disorders there is a progressive degeneration of forebrain cholinergic neurons, which depend on nerve growth factor (NGF) to maintain a healthy phenotype. Notably, the proper maturation of the NGF precursor and the degradation of mature NGF are compromised in AD and DS brains.

Diagnosing AD in DS is challenging due to the underlying intellectual disability and cognitive decline associated with aging. Therefore, identifying novel biomarkers of an evolving pathology is a major unmet objective, with significant clinical impact.Toward this aim, we have obtained plasma samples from DS cases comprising the following subgroups: 1) young DS subjects (20-34 years), 2) older DS subjects without dementia and 3) DS with dementia (35-60 years); including age-matched controls. Amyloid-β peptides were quantified using a multiplex array (MesoScale Discovery, USA). $A\beta_{38}$ was detectable in only 40% of samples, with no apparent differences between control and DS subjects. Conversely, $A\beta_{40}$ was significantly elevated in DS, with the highest levels detected in plasma from adult DS subjects with and without dementia. Levels of $A\beta_{42}$ were higher in DS compared to controls but significantly elevated only in cases with dementia. Our long-term objective is to monitor these markers longitudinally, obtaining plasma from the same patients 9-12 months after the first blood draw.

06r. Imaging & Biomarkers: other

ADPD5-0666

IMPACT OF FREQUENT CSF SAMPLING ON CSF ABETA LEVELS: SYSTEMATIC APPROACH TO ELUCIDATE INFLUENCING FACTORS

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Objectives
Cerebrospinal fluid (CSF) Aβ peptides are core biomarkers for Alzheimer's disease and have been proposed as pharmacodynamic markers in amyloid-lowering therapies. This is challenging, as frequent sampling with catheterization technique results in increased CSF Aβ levels compared to baseline. We assessed the impact of sampling frequency, schedule and sample volume on CSF Aβ levels using continuous CSF sampling over 36 hours.
Methods
Healthy participants (55 to 85 years of age) were randomized into 1 of 3 cohorts (n=6/cohort). All 3 cohorts were subjected to high-frequency CSF sampling: Cohorts 1 and 3 sampling started immediately post-catheterization; Cohort 2 sampling started 6 hours post-catheterization. Cohort 3 received ibuprofen (800 mg) before lumbar puncture. In an additional cohort 4 (n=6) an optimized sampling scheme, based on interim data review from cohorts 1-3, was applied. CSF Aß1-37, Aß1-38, Aß1-40 and Aß1-42 levels were assessed.
Results
Increases and fluctuations in mean CSF Aß1-40 levels were noted in cohorts 1-3 at times of high frequency sampling. Inter-participant variability was noted in all cohorts regarding the increase in CSF Aß levels. The optimized sampling scheme in cohort 4 (lower frequency and volume) resulted in low fluctuation of CSF Aß1-40 on group and individual level.
Conclusions
Increases and fluctuation of CSF Aß levels are primarily dependent on sampling frequency and not the catheterization procedure. Optimized sampling scheme and volume results in reduced to no relevant increase of CSF Aß levels, which will improve the predictive value when using this technique as a pharmacodynamic marker for amyloid-lowering therapies.

06r. Imaging & Biomarkers: other

ADPD5-0842
DEVELOPMENT OF NEW MASS SPECTROMETRY BASED ASSAYS FOR ANALYSIS OF HUMAN CEREBROSPINAL FLUID

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Introduction:
Mass spectrometry (MS) is well suited for biomarker analysis, diagnosis, prognosis, and theragnosis of many neurological diseases, including Alzheimer's disease (AD). The MS based assays are flexible and can easily be adjusted.

Objective:
The objective was to develop MS based assays to monitor cerebrospinal fluid (CSF) proteins using relatively little CSF. The assays should be robust, sensitive, and fast enough to allow analysis of larger study sets.

Methods:
Three different panels were developed. Panel 1 consisted of six high-to-medium abundant proteins previously implicated in AD. Panel 2 contained seven proteins involved in synaptic functioning. Panel 3 was a single protein panel for investigation of potential differences in processing pattern of amyloid precursor protein.
A set of 120 CSF samples (40 controls, 40 mild cognitive impairment, and 40 AD) were selected from the Amsterdam Dementia cohort. Trypsinated CSF was analyzed using microflow liquid chromatography coupled to a high resolution mass spectrometer (Q Exactive).

Results:
The overall CV was <3% for all three panels. Of the proteins investigated neurosecretory protein VGF showed decreased levels in AD, while the remainder showed no or little difference between controls and AD. Interestingly several proteins showed increased levels in the MCI group; the reason for this is presently unclear and requires further investigation.
For complex samples high resolution instrumentation is advantageous because of the superior ability to handle signal interferences.

06r. Imaging & Biomarkers: other

ADPD5-0843

AUTOMATION OF COMMERCIAL AMYLOID-BETA AND TAU ELISAS WILL LOWER INTER-LAB VARIABILITY

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Background:
Measuring Amyloid-beta 1-42, Amyloid-beta 1-40 and Total Tau in CSF becomes more and more accepted as diagnostic tools in the clinical work up of Alzheimer's disease patients. Yet, available Quality assessment schemes show a high variability between labs lowering the trust in these biomarkers. One reason amongst others for these high variations might be linked to lab-specific differences in working up manual assays (e.g. sample predilution, operator qualification). This source of variation can possibly be reduced using fully automated systems.
Methods:
IBL International deliberately developed its Amyloid-beta (1-42), Amyloid-beta (1-40) and Total Tau ELISAs with easy-to-use protocols and ready-to-use reagents allowing adaption on any type of open ELISA processors. The performance of our assays was done with IBL International's quality control sample panel, that is composed of pooled CSF samples spiked with recombinant peptide spanning the entire range of the assays. Performance criteria were inter-, intra- assay and intra-platform variability.
Results:
The reagent composition of IBL International's Amyloid-beta and Total Tau assays and the manual protocols are highly compatible to all used open ELISA processors, as there were Dynex DSX, Siemens BEP2000 and the Tecan EVOLyzer. All automated procedures highly correlated with the manual procedures and stabilized the already very good low manual variability.
Conclusion:
Automation of IBL International's Amyloid-beta and Total Tau ELISAs is easily achieved on different automation platforms thus biomarker measurements in different labs will become less error prone and will have reduced variability and therefore become more trustworthy.

06r. Imaging & Biomarkers: other

ADPD5-0859
IDENTIFICATION OF SYNAPTIC PROTEINS DETECTABLE IN THE CEREBROSPINAL FLUID IN THE SEARCH FOR BIOMARKERS OF PRECLINICAL ALZHEIMER'S DISEASE

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Objectives: To identify and quantify synaptic protein levels in cerebrospinal fluid (CSF) in the search for biomarkers of synaptic loss at the preclinical stage of AD. **Methods:** The CSF proteome was constructed by performing a proteomic study of the CSF of 50 cognitively healthy controls and 10 age-matched AD patients (mean age=64 years). Samples were pooled, immunodepleted, digested, and analysed by liquid chromatography mass-spectrometry. The synaptic proteome was constructed by retrieving proteins from a systematic literature search of proteomic studies of mouse, rat or human synaptic fractions (n=16) and proteins annotated with a synaptic function in the Gene Ontology, Kegg and SynSysNet databases. **Results:** In total, CSF proteomic profiling identified 2,482 proteins (1,826 from control pools and 1,705 from the AD pool). Cross-referencing the CSF (n=2,482) and synaptic proteomes (n=3,359), resulted in 817 CSF proteins functionally and/or topographically related to the synapse. Proteins considered to be non-specific to the synapse (expressed at ≥moderate/medium levels in glia or neuronal soma and ≤moderate/medium levels in the neuropil of the human cortex according to the 'Human Protein Atlas' database) were subsequently removed leaving 192 proteins (8% of the CSF proteome). Quantification of these proteins in CSF from cognitively healthy controls, age-matched AD and preclinical AD cases using targeted mass spectrometry is currently underway. **Conclusions:** By performing a detailed characterization of the CSF and synaptic proteomes, we have identified 192 synaptic proteins detectable in the CSF, which, if confirmed as biomarkers of synaptic loss, could be invaluable for the diagnosis of preclinical AD.

06r. Imaging & Biomarkers: other

ADPD5-0886

PLASMA AND CSF PROTEINS JOINTLY PREDICT MID-TERM PROGRESSION FROM MCI TO AD

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Objective

To predict progression from Mild Cognitive Impairment (MCI) to Alzheimer's Disease (AD) by combining information from diverse groups of variables.

Methods

We combined variables from the AD Neuroimaging Initiative (ADNI) database including clinical, cognitive, proteomic, genomic, and imaging variables as well as demographics to build models that predict progression to AD 12-72 months from baseline assessments (n=94 MCI subjects). To that end, we performed joint variable selection and classification and compared performances of predictive models using variables from each data type independently and from the fully merged dataset.

Results

Signatures predicting mid-term progression (within 24 and 36 months) proved to be more robust in terms of sensitivity, specificity and stability than signatures for faster or slower progression. The best predictive model for progression from MCI to AD within 36 months (30 with stable MCI and 39 progressors to AD) involved a combination of 6 proteins measured in plasma and CSF (80% classification accuracy, 88% sensitivity, 70% specificity). These identified proteins are linked to AD-related biological processes. Interestingly, including imaging or cognitive variables in the model did not improve classification accuracy.

Conclusions

There is a clear need for developing better tests to identify individuals with early AD and a high risk to progress to AD in order to support clinical trials design and development of novel therapies. Our results suggest that combining different types of molecular biomarkers which represent different aspects of AD pathology can improve prediction of progression to AD.

06r. Imaging & Biomarkers: other

ADPD5-1037

RATIONAL DESIGN OF ENVIRONMENT SENSITIVE FLUOROPHORES FOR DETECTION OF AGGREGATED AMYOID PROTEIN BIOMARKERS IN NEUROLOGICAL DISEASES

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Objectives

We describe the development of a class of environment-sensitive amino-aryl nitrile fluorophores that show an enhancement of fluorescence upon binding amyloid aggregates and the capability to discriminate amyloid-beta, prion, and alpha-synuclein species by their emission wavelengths. Utilizing this property, we seek to further investigate and characterize the amyloid basis of neural pathologies including Alzheimer's Disease (AD) and prion disease.

Methods

Fluorophore design was guided by a modified Lippert equation for solvatochromism where we model the relationship between probe fluorescence emission wavelength and the amyloid binding pocket microenvironment. We tuned fluorophores to improve both the sensitivity to different amyloid species as well as their biocompatibility. *Ex vivo* histological staining of neuronal amyloid-beta deposits from AD mice models and human AD cases, as well as prion plaques in innoculated mice models, were performed to evaluate our design features.

Results

By modifying the fluorophore core of the molecules, we developed a series of compounds with enhanced discrimination capabilities towards differentiating between amyloid-beta and prion plaques in hippocampal and cortical tissue. We have also improved biocompatibility by red-shifting emission towards the near-infrared and increasing their stability in biological environments. Finally, we generated a library of characteristic fingerprints for several amyloids associated with neurological disorders by their unique emission signatures.

Conclusions

We show the potential application of a new class of biocompatible amyloid-binding fluorophores with discriminatory properties as a diagnostic tool for the detection and identification of neurodegenerative amyloid biomarkers.

06r. Imaging & Biomarkers: other

ADPD5-1105

CHANGES OF PLASMA AMYLOID BETA 40/42 RATIOS IN KOREAN SPORADIC EARLY-ONSET ALZHEIMER'S DISEASE PATIENTS

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Objectives: The levels of plasma Aβ have been studied as a marker of Alzheimer's disease (AD), however the results were inconsistent. Recently accumulating evidences support the heterogeneity of AD. It is possible that the inconsistency is from the inhomogeneity of the subjects at least partly. We evaluate the validity of plasma levels of Aβ40, Aβ42, and their combined ratio in relation to AD including early onset AD without known AD mutations.
Methods: This study was conducted as a part of the Center for Dementia of South Korea study. We have consequently enrolled the clinical probable early-onset AD patients (EOAD) with positive AD biomarkers from February 2013 to June 2014. Plasma levels of Aβ40 and Aβ42 were measured using sandwich enzyme-linked immunosorbent assay.
Results: The plasma levels of Aβ40 and combined ratio of Aβ40/Aβ42 were significantly higher in sporadic EOAD patients compared with controls. According to the presence of APOE*ε4* allele the levels of Aβ40, Aβ42, and Aβ40/Aβ42 ratio were not altered. The integrity of blood-brain barrier (BBB) was disturbed in sporadic EOAD groups revealing higher cerebrospinal/plasma albumin ratio. Although there was a tendency of inverse correlation between Aβ40 level and BBB integrity, it did not reach statistic significance. On multivariate analysis considering various covariates, higher plasma Aβ40 levels and Aβ40/Aβ42 ratio were still found to be highly valuable in discriminating AD from controls.
Conclusions: We found that the plasma levels of Aβ40 and Aβ40/Aβ42 ratio have a high validity in discrimination of sporadic EOAD from age-matched controls.

06r. Imaging & Biomarkers: other

ADPD5-1148

GLYCOSYLATION OF HUMAN PLASMA CLUSTERIN PROVIDES NOVEL BIOMARKERS OF ALZHEIMER'S DISEASE

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Objective

Expression of Clusterin has been shown to be significantly elevated in AD brain and several molecular forms of clusterin have been reported to correlate with AD progression. Clusterin contains six N-linked glycosylation sites, three in the α subunit (α^{64}N, α^{81}N and α^{123}N), and three in the β subunit (β^{64}N, β^{127}N, and β^{147}N) and because glycosylation plays an important role in the physiological functions of clusterin, we hypothesised that the detailed glyco-peptide profiling of plasma clusterin might reveal disease-relevant isoforms and enable a better understanding of clusterin structure-function relationships associated with AD.

Methods

Following immuno-precipitation and SDS-PAGE, the characterisation of glycosylated peptides from human plasma clusterin was performed by LC/MS/MS using an Orbitrap Fusion Tribrid mass spectrometer.

Results

Having comprehensively described 42 distinct clusterin glyco-peptides, we further hypothesised that the overall glycopeptide profile may be useful as a diagnostic tool, especially since the difference in glycosylation patterns associated with distinct glycosylation sites may prove relevant to disease status. To explore this possibility, we compared the clusterin glycoform distribution in plasma obtained from a set of low atrophy (n=13) and high atrophy (n=14) subjects attending the memory clinic.

Conclusions

We will present the results of this study and also describe the development of a targeted glyco-peptide Selective Reaction Monitoring (Glyco-SRM) assay to measure selected glycans specifically associated with β^{64}N. The Glyco-SRM assay now facilitates high throughput testing of β^{64}N Clusterin glycoforms within larger cohorts of clinical specimens.

06r. Imaging & Biomarkers: other

ADPD5-1164

HIPPOCAMPAL SUBFIELDS REPRODUCIBILITY: A EUROPEAN MULTI-SITE 3T STUDY

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Objectives: Hippocampal subfields are differentially affected in Alzheimer's Disease. However, their volume reproducibility has never been investigated. We report the evaluation of the across-session test-retest reproducibility of the hippocampal subfields segmentations derived from FreeSurfer.

Methods: Five healthy local volunteers (55-90 ys) were enrolled in 13 3T MRI sites (Siemens, GE, Philips) across Europe and were scanned in two sessions a week apart. Hippocampal subfields segmentation was performed using the longitudinal pipeline of Freesurfer. For each site and ROI, volumes reliability was computed evaluating test-retest absolute differences relative to the mean (absolute error) and intraclass correlation coefficient (ICC).

Results: Analysis was focused on: Cornu Ammonis (CA) 1, CA2-3, CA4-dentate gyrus (DG), subiculum, presubiculum, fimbria and hippocampal fissure. Absolute errors across MRI sites were comparable to those found for total hippocampus and in the range 1.5–5.0% for CA1, CA2-3, CA4-DG, subiculum, presubiculum and 15-25% for fimbria and hippocampal fissure. The ICC results were in line with the absolute error analysis (from 0.94 to 0.99 except for fimbria and hippocampal fissure where was in the range 0.79-0.95).

Conclusions: Despite the differences of the 13 MRI scanner configurations we found consistent hippocampal subfields reproducibility across sites. Test-retest reproducibility was lower in smaller relative to higher volumes. The poor reproducibility of fimbria and hippocampal fissure should be considered in studies involving subfield volumes to differentiate MCI from AD or to predict the conversion from MCI to AD. Pharmacog is funded by the EU-FP7 for the Innovative Medicine Initiative (grant n°115009).

06r. Imaging & Biomarkers: other

ADPD5-1260

A NOVEL IMMUNOASSAY FOR LONGITUDINAL MEASUREMENTS OF AMYLOID-BETA OLIGOMERS IN HUMAN CSF WITH POTENTIAL TO ASSESS THERAPEUTIC RESPONSE TO IMMUNOTHERAPY

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Objectives: Develop an ELISA to: 1) quantify amyloid beta (Abeta) oligomers in CSF of Alzheimer dementia (AD) and cognitively normal age-matched controls (CN), 2) examine longitudinal analyte stability and 3) correlate levels with MMSE.

Methods: In the ELISA, a mAb that preferentially binds multimeric Abeta species was used for capture (1g5 mAb, Aliquot LLC.). For detection, an Abeta N-terminal mAb, biotiniylated-82E1 (Covance) was used. The Meso Scale Discovery platform was used to establish the ELISA, synthetic Abeta protofibrils were used to generate the standard curve. Both inter- and intra- assay variability was <10%. Commercial longitudinal CSF samples (PrecisionMed Inc.) from the same subjects were tested.

Results: The assay detected Abeta oligomers in both AD and CN CSF, with AD demonstrating significantly higher levels compared with CN (p=0.0025). In this sample set of 10 AD patients, longitudinal Abeta oligomer and MMSE levels were relatively stable over 1 year. AD patients with lower average MMSE scores had higher average CSF Abeta oligomer levels.

Conclusions: Quantification of Abeta oligomers in CSF was achieved. Endogenous Abeta oligomers in CSF exhibited relative stability over the course of one year which suggests potential utility for assessing pharmacodynamic effects of treatments that target oligomers. There appears to be a moderate correlation (r^2=0.4149, p=0.0001) between MMSE scores and CSF Abeta oligomer levels in this subset of AD patient CSF samples that warrants testing of additional longitudinal CSF samples collected over a longer period of time.

06r. Imaging & Biomarkers: other

ADPD5-1297

DECREASED LEVELS OF CGMP IN CSF ARE ASSOCIATED WITH COGNITIVE DECLINE AND AMYLOID PATHOLOGY IN ALZHEIMER'S DISEASE

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Objectives: Levels or the cyclic nucleotides cGMP or cAMP that play important roles in memory processes are not characterized in Alzheimer´s disease (AD). The aim of this study was to analyze the levels of these nucleotides in cerebrospinal fluid (CSF) samples from patients diagnosed with clinical and prodromal stages of AD and study the expression level in the brain of the enzymes that hydrolized them (phosphodiesterases: PDEs).
Methods: For cGMP and cAMP CSF analysis the cohort (n=79) included cognitively normal participants (SCI), individuals with mild cognitive impairment stable or AD converters (sMCI and cMCI) and mild AD patients. A high throughput LC-MS/MS method was used. Interactions between CSF cGMP or cAMP with MMSE score, CSF Aβ(1-42), and CSF p-tau were analyzed. For PDE expression analysis, brains of AD patients *vs* controls (n=7 and n=8) were used.
Results: cGMP, and not cAMP levels, were significantly lower in the CSF of patients diagnosed with mild-AD when compared to non-demented controls. cGMP reduction in CSF compartment showed a strong association to MMSE-diagnosed clinical dementia and to CSF biomarker Aβ42 within AD patients. Significant increase in PDE5 expression was detected in temporal-cortex of AD patients compared to that of age-matched healthy control subjects. No changes in the expression of others PDEs were detected.
Conclusions: These results support the potential involvement of cGMP in the pathological and clinical development of AD. The cGMP reduction in early stages of AD might participate in the aggravation of amyloid pathology and cognitive decline.

06r. Imaging & Biomarkers: other

ADPD5-1444
EVALUATION OF RETINAL CHANGES IN MILD COGNITIVE IMPAIRMENT, ALZHEIMER DEMENTIA, NON-ALZHEIMER DEMENTIA AND PD

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Background
Retinal nerve fiber layer (RNFL) thickness has been reported as a biomarker of Alzheimer's (AD) and PD. Previous studies had lacked detailed clinical characterization of the patients with MRI volumetric analysis and neuropsychology evaluation to corroborate diagnosis. In this study we evaluate if retinal biomarkers relate to the degree of cognitive impairment in amnestic MCI, AD dementia, non-AD dementias, PD and differentiate them from age and sex matched controls in a well characterized cohort.
Methods
20 patients each of amnestic MCI, AD dementia, non-AD dementia and PD diagnosed after comprehensive neurological, neuropsychology evaluation and MRI volumetry were studied with 40 age and sex matched controls.Iin vivo cross-sectional RNFL thickness, macular volume and ganglion cell layer thickness (GCL) were measured by Optical coherence tomography (OCT) using Zeiss Cirrus 4000 spectral domain machine. Analysis of variance models were used to compare groups on MRI measures, cognitive test results and OCT measures. Associations between OCT measures and other measures were performed using mixed effect models.
Results
None of the groups differed from controls on RNFL thickness, macular volume and GCL thickness. Mean and Std Dev of Optic Disc average RNFL thickness OD: Controls (85.1±8.1), MCI (90.1±6.8), AD (88.5±8.0) Non-AD (89.4±15.5), PD(90.9±10.8). OS: Controls (86.1±8.8), MCI (89.6±7.0),AD(89.1±9.2),non-AD dementia(90.1±8.6), PD(87.9±14.0). No correlation between cognitive scores and OCT measures were noted.
Conclusions
OCT analysis of retinal biomarkers did not differentiate between the amnestic MCI, AD dementia, PD and non-AD dementia from age and sex matched controls in a well characterized patient cohort.

06r. Imaging & Biomarkers: other

ADPD5-1579
SPEECH DISORDERS IN ALZHEIMER´S DISEASE
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Aims
Aim of the study is to evaluate the oral-motor speech disorders in Alzheimer´s disease as compared to the speech disorders occurring in the physiological process of aging.
Materials and methods
We examined 22 patients with Alzheimer´s disease (AD) at the age of 68 to 90 years. In all subjects with AD were diagnosed the initial stage of the disease with preserved speech processing and communication ability. The control group (C) consisted of 22 people. The test has 5 parts: dialogic speech, descriptive speech, naming, repeating simple & complex sentences, and repeating monosyllabic (easy) & polysyllabic (difficult)) words & nonsense words, and speaking automated verbal sequences. All studies were recorded in order to supplement the quantitative information on a sheet of observation, and a detailed analysis of the structure of speech.
Results
Statistically significant differences between AD and C groups in the number of correct and incorrect answers were found. The greatest difference was found between AD and C groups in the descriptive speech. During the repetition of monosyllabic and polysyllabic words in AD occurred significantly more often the articulation errors on repeated speech productions of the same utterance.
Conclusion
The results confirmed the presence of oral-motor speech disorders in Alzheimer´s disease. It was stated articulation errors, periphrases and jams. These errors occur in the structure of lexical-semantic and phonological system of the language. No abnormalities were noted in the area of grammar.

06r. Imaging & Biomarkers: other

ADPD5-1675

THE LYMPRO® ASSAY: A BIOMARKER FOR ALZHEIMER'S DISEASE USING BLOOD SAMPLES FROM CLINICALLY DIAGNOSED ALZHEIMER'S DISEASE AND COGNITIVELY INTACT SUBJECTS

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Objective A blood biomarker would be advantageous for early identification of Alzheimer's disease (AD). Multiple reports have identified Cell Cycle Dysregulation as a key pathology in AD. Furthermore, it appears likely that this dysfunction is systemic, affecting peripheral blood lymphocytes as well as neurons. This study measures lymphocyte proliferation in response to a mitogenic stimulus to quantify the extent to which lymphocytes have entered the cell division cycle. It potentially represents a blood-based biomarker for AD.

Methods Whole blood samples obtained from 72 subjects (36 AD and 36 age matched controls) were shipped to a reference laboratory (Becton Dickinson) for flow cytometry analysis. Statistical analysis methods included uni- and multivariate receiver-operating curve (ROC) and logistic regression analysis, and calculation of diagnostic performance parameters.

Results Interim analysis demonstrated significant areas under the ROC curve for univariate measures including CD19, CD3+4, CD3+8, CD14, and CD45. These markers were also highly correlated to MMSE scores. On univariate analysis, sensitivity of 66.7% at 83.3% specificity was achieved by the CD3+8 marker. Multivariate model building yielded maximal AUC of 0.871 (odds ratio=24.0) and sensitivity and specificity of 80% and 85.7% at the optimal cutpoint.

Conclusions In this interim analysis, the LymPro assay provides multiple univariate indicators and multivariate combinations, which discriminate AD from controls. The assay appears to be a robust and reproducible measure of a key pathology of AD and the findings corroborate previously published LymPro findings. Final data from the current study will be completed by the time this abstract is presented.

06r. Imaging & Biomarkers: other

ADPD5-1697

PLASMA IL-6 AND TRAIL IDENTIFIED AS BIOMARKERS OF ALZHEIMER'S DISEASE

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Objectives
Several biomarkers including inflammatory biomarkers of Alzheimer's disease (AD) were reported inconsistently. We aim to identify biomarkers of AD to improve diagnostic accuracy at mild stage.
Methods
Patients with age older than 50 years and being diagnosed as AD fulfilled revised NINCDS criteria of year 2007 were included to the disease group. For subjects enrolled in normal control group, no memory problem was complained, and the score of AD8 below 2 or Montreal Cognitive Assessment (MoCA) score above 26. We evaluated the relationship between potential blood and CSF inflammatory biomarkers, cognitive status, and disease severity. Inflammatory biomarkers including IL-6 and TRAIL levels were measured.
Results
We enrolled 45 subjects in the disease group and 40 subjects in normal control group. The majority of subjects in the disease group were mild AD. Elevated levels of plasma IL-6 and decreased levels of plasma TRAIL in the disease group were noted compared to control group. In small number of subjects (n=6), plasma levels of IL-6 and TRAIL were highly correlated with each of their CSF levels ($p<0.05$ and $p<0.01$, respectively). Plasma levels of IL-6 and TRAIL were significantly correlated with each of their CSF levels.
Conclusions
Plasma IL-6, and TRAIL are identified as biomarkers of AD at early stage. Highly correlated plasma and CSF biomarkers levels found in IL-6 and TRAIL might implicate in the strong connection between peripheral and central inflammatory cytokines.

06r. Imaging & Biomarkers: other

ADPD5-1722

CSF BIOMARKER VARIABILITY IN THE ALZHEIMER´S ASSOCIATION QUALITY CONTROL PROGRAM

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Background

The Alzheimer's Association quality control (QC) program for biomarkers in cerebrospinal fluid (CSF) enables participating labs to monitor their performance of measuring tau and Beta-Amyloid proteins using enzyme-linked immunosorbent assays (ELISA). The goal is to reduce site-to-site variation, harmonize levels and pave the way for uniform cut-off levels.

Methods

The Alzheimer's Association QC program consist of three rounds each year and at each round, three samples of pooled CSF prepared at the Clinical Neurochemistry Laboratory at the University of Gothenburg, Sweden, are analyzed byparticipating laboratories for tau and Beta-Amyloid proteins by ELISA based assays.

Results

The coefficients of variation (CV) between the laboratories using Fujirebio ELISA kits have so far been around 15-29% for Beta-Amyloid1-42, 12-28% for Total-tau, and 12-28% for Phospho-tau, while the between laboratories CV in the newly introduced ELISAs with ready to use calibrators this far is 6-11% for Beta-Amyloid 1-42, 6-13% for Total-tau and 7-17% for Phospho-tau.

For the Meso Scale Discovery new validated V-Plex kit results of the latest round the between laboratories CV% for Beta-Amyloidx-42, Beta-Amyloidx-40 and Beta-Amyloidx-38 were between 7-20%.

Conclusions

The overall variability of CSF biomarker measurements are still too high (target variability may be below 10-15%) to allow assignment of universal cut-off values for a specific intended use. But with the introduction of validated and ready to use kits it looks like steps in the right direction is made and with introduction of new assays in coming rounds exiting efforts from the industry is made.

06r. Imaging & Biomarkers: other

ADPD5-1799
PLASMA CHOLINE ACETYLTRANSFERASE IN PATIENTS WITH COGNITIVE IMPAIRMENT AND AD
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Objectives: Decrease in cholinergic neurotransmission is observed in AD and cholinesterase inhibitors (ChEI) treatment can only delay its progression. The acetylcholine synthesizing enzyme, choline acetyltransferase (ChAT) is recently shown to exist in CSF and plasma. We have hypothesized that it regulates the extracellular ACh levels, which may in turn have an impact on the patients' prognosis and cognitive performance. The aim of this study was to measure activity and protein levels of ChAT in plasma, and to investigate the influence of different ChEI treatment on levels of plasma ChAT in AD patients.
Methods: Plasma samples were taken from ChEI-naïve subjects with AD (32), MCI (30) and SCI (22). Plasma samples at baseline, 3 and 12 monhts were also taken from AD patients treated with rivastigmine (11) or tacrine (17).
Results: The plasma ChAT activity and protein concentration were higher in AD patients compared to MCI and SCI patients (*p<0.0001*). Plasma ChAT activity correlated with the patients' cognitive test scores in MMSE after 12 months of treatment with tacrine. In the rivastigmine group, changes in the plasma ChAT protein showed 146% significant increase in patients with less annual cognitive decline.
Conclusions: This study shows for the first time that plasma ChAT differs between AD, MCI and SCI patients, which might allow to distinguish patients in different stages of dementia. The correlation between plasma ChAT and MMSE scores, suggests ChAT as a potential plasma biomarker for monitoring treatment effect.

06r. Imaging & Biomarkers: other

ADPD5-2243

BINDING IN VISUAL WORKING MEMORY: A COGNITIVE BIOMARKER FOR EARLY ALZHEIMER'S DISEASE?

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Introduction:
Binding single features to coherent objects in visual working memory (VWM) is impaired in early Alzheimer's dementia (AD) and asymptomatic individuals with familial AD, suggesting this may be an important cognitive biomarker for early detection and monitoring of individuals at risk of AD. We investigated healthy elderly people with impaired binding, comparing them to elderly and young individuals with intact binding.

Methods:
We tested 39 healthy elderly (69.2±4.9y) and 19 young (24.4±2.4y) participants on an object-location VWM task and performed structural brain MRI on them. On each trial, participants had to remember the identity and location of 1 or 3 objects. We analyzed memory for object identity and localization as well as identity-location binding performance. A median split was performed on the elderly according to binding ability. Imaging data were analyzed using voxel-based morphometry (VBM).

Results:
There was an age-related impairment in memory for object identity and localization. The low binding group was significantly impaired in object localization memory for 3 items compared to the high binding group. Crucially, their deficit in location memory could be fully explained by decreased binding performance. VBM showed structural alterations associated with a binding deficit in medial prefrontal cortex and left anterior cingulate cortex.

Conclusions:
Binding ability varied in elderly individuals who performed alike on all other measures. Its potential as a screening tool in AD will be evaluated in follow-up studies examining cognitive decline in individuals with impaired versus intact binding ability.

06r. Imaging & Biomarkers: other

ADPD5-2255

PARKINSON'S DISEASE BIOMARKER DISCOVERY USING SOMASCAN PROTEOMIC TECHNOLOGY

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Objectives: Discovery of a blood-based biomarker that detects pre-clinical neurodegenerative disease could greatly accelerate the development of targeted therapeutics aimed at prevention. Such an approach is proving to be promising for Alzheimer's and may also be successful for Parkinson's disease (PD). Mapping Proteomics to Parkinson's Disease (MAP2PD) is a multi-phase programme funded by Parkinson's UK, aimed at the discovery and validation of a protein signature in the blood to detect and track progression in early PD.

Methods: In the discovery phase, we used an apatmer-based technology (SomaLogic ,Inc.), that quantified 1129 plasma proteins from 161 PD subjects (disease duration < 3 yrs) and 66 controls, age- and gender-matched. In addition to a case-control design, an endophenotype approach was used to define PD subgroups by cognitive and motor severity. Both regression and machine-learning models were then employed to analyse proteins associated with PD outcomes.

Results: A total of 55 proteins were differentially expressed in PD versus controls at $p < 0.05$. Multivariate analyses revealed a subset of 22 proteins that differentiated PD from controls with an area under the curve of 0.91, after controlling for covariates. Some of the proteins identified showed clinical and biological significance, which will be explored further in larger samples.

Conclusions: The discovery phase revealed a preliminary but promising set of biomarkers that will be tested for replication in larger samples in Phase 2, with subsequent biological validation. These data point to the potential for blood-based biomarkers to be used for early diagnosis of PD.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-0277

HERITABILITY OF AGE RELATED COGNITION

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The role of genetic and environmental factors in specific cognitive abilities in the elderly is poorly understood. Here we estimated their contributions to variation in the cognitive domains memory, executive function and fine motor skills and tested the genetic correlations between these domains.

The study was conducted in the Austrian Stroke Prevention Study (ASPS), a population-based cohort study (n=479, mean age=64.6 years, 54.9% women) and in the ASPS-Family including relatives of the ASPS participants (n=376, 177 families, mean age=63.6 years, 60.1% women). The neuropsychological test battery included Bäumler's Lern-und Gedächtnistest, Trail Making B, Digit Span Backward, the Wisconsin Card Sorting and the Perdue Pegboard Test. Heritability was estimated by variance component analyses using SOLAR and by SNP genotypes using GCTA. Bivariate heritability was computed using SOLAR. We adjusted for age, gender, education and APOE.

Heritability of memory, executive function and fine motor skills using family structure were 60, 41 and 59%, and 11, 23 and 29% using SNP genotypes. Genetic and environmental correlations between memory and executive function were 53% and 20%, between memory and fine motor skills 41% and 14%, respectively. We found no genetic correlation between executive function and fine motor skills, the environmental correlation was 37%.

There is a substantial heritability of memory and fine motor skills and a moderate of executive functions. Half of the heritability of executive function and fine motor skills can be explained by common SNP. Our results support shared genetic factors for executive function and memory as well as fine motor skills.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-0296

MEMORY IMPAIRMENT CAUSED BY HIPPOCAMPAL ATROPHY BUT NOT BY CEREBRAL SMALL- VESSEL DISEASE IN HEALTHY ELDERLY PEOPLE

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Background: To estimate association hippocampal atrophy and memory impairment in normal elderly people, we investigated psychological tests and measured hippocampal volume of community-dwelling elderly people. Method: We examined 213 of healthy elderly community-dwelling subjects(age>or=58years,97men, 116women, mean age 68.6years)with no dementia or no psychiatry diseases. Memory impairment was assessed by Rivermead Behavioral Memory Test [RBMT]. Executive function was assessed by modified Stroop test, [MST] and apathy was assessed by apathy scale. A brain MRI examination was performed on a 1.5-Tscanner (Achieva; Philips) using theT1- and T2 - weighted images (WI), fluid-attenuated inversion recovery , T2*- WI. We used voxel-based specific regional analysis system for Alzheimer's disease (VSRAD). We used the Z-score which indicates severity of atrophy obtained from the averaged in the target volume of interest. Results: We operationally defined a total of individuals with Standard profile score (SPS)of RBMT less than 17 as having a memory impairment. In a multivariate analysis, there was a significant relationship between memory impairment defined by SPS and Z score(OR3.843, 95%CI 1.678 - 8.803, p=0.001). Impaired executive function defined by MST was related to existence of silent brain infarction (OR2.89,95%CI 1.019 - 8.214, p=0.046) and apathy defined by Apathy scale was related to existence of deep white matter lesions(OR2.077,95%CI 0.025 - 4.209, p=0.043)in a multivariate analysis. Conclusions: Memory impairment in healthy elderly people was caused by hippocampal atrophy but not by cerebral vascular diseases.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-0390

CHARACTERISTIC FEATURES OF COGNITIVE, AFFECTIVE, AND DAILY LIVING FUNCTIONS OF LATE-ELDERLY DEMENTIA

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Objectives: The world is rapidly aging, and is facing an increase of late-elderly dementia patients. **Methods**: We examined 1,554 dementia patients in our clinic, dividing into 3 subgroups according to the age; young- (≤64 years old [y.o.]), middle- (65-74 y.o.), and late-elderly (75 y.o.≤), and investigated the cognitive, affective, and activities of daily living functions (ADL), especially in late-elderly dementia comparing with young- (presenile) and middle-elderly dementia. **Results**: Among 1,554 dementia patients, Alzheimer's disease (AD) dominated 62%, followed by mild cognitive impairment due to AD (12%), vascular dementia (9%), but AD was age-dependently increased up to 69% in late-elderly dementia. Total scores of 4 cognitive tests were significantly worse with aging, with specific subscales of orientation, recall, visual retention, word fluency, etc. On the other hand, total scores of the affective tests showed only an increase in apathy scale in the late-elderly group. Each group showed depressive/depression in 63.2-55.2 %, and apathy in 44.2-54.8%. Furthermore, ADL especially in the instrumental ADL items deteriorated significantly in the late-elderly group, which statistically correlated with mini-mental state examination (MMSE) score. **Conclusions**: These results demonstrates that late-elderly dementia group is characterized as significant cognitive declines, increasing apathy, more than half in depressive/depression and apathy, and instrumental ADL decrease. The cognitive decline may be related to such an affective and ADL declines.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-0786

TIME DELAY FROM FIRST MEMORY COMPLAINTS TO DIAGNOSIS OF AD. DATA FROM CZECH BRAIN AGING STUDY

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Objectives: Czech Republic has 10.5 million inhabitants; average catchment area of 1500-1800 inhabitants per GP (15-20 dementia patients per GP), only 2-4 patients are actually referred to specialists who can prescribe ChEI. At the end of 2012, we have conducted educational campaign via media and lectures aiming on GPs and public about the importance of early diagnosis of Alzheimer´s disease (AD). We report the effect on early detection of patients with dementia and at risk of AD.
Methods: Subjects were examined at the Memory Center ICRC, St. Anne´s Teaching Hospital Brno. They underwent physical, neurological evaluation, neuropsychological testing covering: memory, attention/processing speed, language, executive and visuospatial functions. MRI scans were performed for vascular changes and hippocampal volume assessment.
Results: 348 subjects with memory complaints were examined, during the year 2013. 148 subjects had no objective cognitive deficit, 88 subjects were diagnosed with mild cognitive impairment (MCI), 100 were newly diagnosed with dementia (21 in moderate and 9 in severe stage of dementia). 54 of the dementia patients expressed long term memory complaints to their doctor and were neglected. 230 of the non-demented subjects were included in the Czech Brain Aging Study database. Current data about conversion rates after 1 year follow up will be presented.
Conclusion: Big proportion of patients with dementia and at risk of AD is not recognized by GPs or other physicians. Educational campaigns aiming on GPs and public help to bring subjects at risk earlier to specialized care.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-0881

NEUROLOGICAL SOFT SIGNS IN HEALTHY AGING, MILD COGNITIVE IMPAIRMENT, AND MILD ALZHEIMER´S DISEASE

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Objectives: Neurological soft signs (NSS), i.e. minor motor and sensory changes, are a common feature in severe psychiatric disorders. Nevertheless, they have rarely been investigated in healthy aging or in patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD).
Methods: NSS were examined using an abbreviated version of the Heidelberg NSS Scale in 226 'old' participants born from 1930 to 1932 (63 with MCI, 15 with AD, 148 healthy controls) and 231 healthy 'young' participants (born from 1950 to 1952) of the population-based Interdisciplinary Longitudinal Study of Ageing (ILSE). Subjects were examined at three examination waves (t1:1993/94; t2:1997/98; t3:2005/07). Years of school education were used as a proxy for cognitive reserve.
Results: NSS scores were significantly ($p<0.001$) higher in the AD patients (74.7±1.0) than in the healthy controls (73.9±1.0) while the MCI patients (74.2±1.0) took an intermediate position. This result was confirmed after years of school education were entered as a covariate which were inversely correlated ($r = - 0.27$; $p<0.001$) with NSS. Neither a history of major depression, ApoE and COMT genetic polymorphisms nor sex was significantly associated with NSS. Comparison of NSS scores between 'old' (2.8 ± 1.8) and 'young' (2.5 ± 2.0) controls yielded only minor, non-significant differences.
Conclusions: Our results demonstrate that NSS are frequently found even in mild AD. NSS refer to educational levels rather than sex, age, depression or important genetic polymorphisms. Since NSS primarily involve motor coordination it appears plausible that the respective deficits can be partly compensated for by cognitive reserve.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-1642

ASSOCIATION OF TELOMERE LENGTH ALTERATIONS WITH INCREASED RISK OF AMNESTIC MILD COGNITIVE IMPAIRMENT

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Objective: To investigate the associations of peripheral blood telomere length with incident amnestic mild cognitive impairment (aMCI)**,** a putative precursor of AD, among Mayo Clinic Study of Aging participants. **Methods:** Using a nested case-control design, we matched 137 incident aMCI cases (mean age 81.1 years, [range 70.9-90.8]; 49.6% men) by age and sex to 137 cognitively normal controls. We measured peripheral blood telomere length (T/S ratio) in DNA from blood drawn at baseline using quantitative PCR. We investigated the association of peripheral blood telomere length with incident aMCI. **Results:** We observed a non-linear association of telomere length with aMCI ($p = 0.04$), with the lowest risk in the middle quintile. Compared to the middle T/S quintile (Q3), the risk of aMCI was elevated for subjects with the shortest (Q1: HR, 2.85, 95% Confidence interval [CI] 0.98, 8.25; $p = 0.05$) and the longest telomere lengths (Q5: HR, 5.58, 95%CI, 2.21, 14.11; $p = 0.0003$). **Conclusion:** In our elderly cohort, both short and long telomeres were associated with an increased risk of aMCI. The findings also suggest that both long and short telomeres are involved in the pathogenesis of aMCI and may be markers of increased risk of aMCI.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-1997

THE ASSOCIATION BETWEEN AMYLOID PATHOLOGY TO CSF BIOMARKERS OF CONCOMITANT PATHOLOGY IN COGNITIVELY HEALTHY ELDERLY

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Objectives: The objective of this study was to examine if amyloid pathology, measured by CSF Aβ42 < 550pg/mL, predicts concomitant pathology in cognitively healthy elderly from a Swedish population based sample. **Methods:** A total of 129 dementia free elderly participants from the Gerontological and Geriatrics Population Studies took part in a lumbar puncture in 2009/2010 and underwent a neuropsychiatric and neuropsychological examination. CSF levels of Aβ42, total tau, phospho-tau(p181), neurogranin and YKL-40 were assessed. Dementia was diagnosed in accordance with DSM-III criteria. The group was divided in two groups based on their CSF Aβ42 levels (cut off 550pg/mL) where levels below 550pg/mL are considered to reflect brain amyloid pathology. **Results**: Healthy elderly with CSF Aβ42 levels below 550pg/ mL (n=46) displayed significantly increased levels of total –tau (p<0,001), p-tau(p<0,001), neurogranin (p=0,011), and YKL40(p=0,044). **Conclusion**: This study indicates that amyloid pathology in cognitively healthy elderly individuals can predict neuronal damage (t-tau and neurogranin), tangle pathology (p-tau) and microglial activation (YKL-40).

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-2077

FIVE YEARS EVOLUTION OF MMSE SCORE AT DIAGNOSIS OF ALZHEIMER'S DISEASE: DATA FROM THE FRENCH ALZHEIMER DATABASE

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Objectives: To describe the 5 years evolution of the MMSE score at diagnosis of Alzheimer disease (AD) in French memory centers.
Methods: The French Alzheimer Database (BNA) collects the activity of 415 French memory centers. We included in the study patients newly diagnosed as AD between 2009 and 2013 within participating centers. The considered MMSE score was at diagnosis; in the absence of MMSE at diagnosis we used the closest MMSE within the limit of +/-1 year. MMSE scores at diagnosis were compared by variance analysis. Multivariate linear regression analysis was conducted to explore the association between mean MMSE score and year of diagnosis taking into account other factors.
Results: 65,270 AD patients were included in the analysis. Women accounted for 70.1% and the mean age at diagnosis was 81 years old (sd 7.3). The mean MMSE score at diagnosis was 20 for subjects diagnosed in 2009 and 17.6 for patients diagnosed in 2013. The decrease is linear. After taking into account age, sex, type of center (care vs care and research centers), and patients' education a significant decrease of MMSE score with time is still observed.
Conclusions: These results indicate that the level of MMSE at diagnosis of AD decreased between 2009 and 2013 in France. In the context of an observed decrease of the incidence and prevalence in some countries, these results would suggest that the diagnosis is performed at a more advanced stage than before.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-2296

PREVALENCE OF AMYLOID POSITIVITY IN HEALTHY ELDERLY PARTICIPANTS OF A METHODOLOGY STUDY EVALUATING REMOTE TABLET-BASED COGNITIVE LEARNING

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Objective
Subtle changes in Divided Attention (the ability to cope with distractions) have been reported in early Alzheimer's disease (AD).
As accumulation of amyloid in the brain, is both a biomarker and a risk factor for progression toward development of AD cognitive symptoms, it is hypothesized that amyloid carriers may have higher cognitive vulnerability and will thus benefit to a lesser extent from the learning associated with 28 days of dual-task cognitive training than non-carriers.
The primary study objective is to compare the effect of dual-task training on cognitive measures between amyloid carrier and non-carriers.
Method
A9001489 is a randomized double-blind, sponsor-open, parallel group, placebo controlled trial with repeated self-administration of Akili Interactive's EVO dual-task assessment in healthy elderly subjects. It is estimated that a total of approximately 100 subjects will be randomized in the study, at a ratio of no greater than 3:2 in each of the two amyloid groups (non-carrier:carrier).
Results
While A9001489 study is ongoing, a preliminary analysis of screening data revealed a lower rate of amyloid carrier status than expected in subjects of 60-80 years of age, thus challenging the feasibility of study conduct. These demographic and amyloid data are presented, along with mitigation strategies to enable study conduct.
Conclusion
Amyloid prevalence in the healthy elderly population needs to be carefully considered when planning a preclinical or early AD study. Selection criteria should be adjusted accordingly to allow study execution.

07b. Epidemiology, Risk Factors, Genetics & Epigenetics: environmental

ADPD5-0398

'ALZHEIMEROGENIC' PRODUCTS IN THE HUMAN CHEMICAL EXPOSOME? TRIAZINES HERBICIDES INDUCE AMYLOID-B42 PRODUCTION

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Objective

Aftins (**A**myloid-β **F**orty-**T**wo **In**ducers) is a family of molecules triggering robust up-regulation of extracellular/secreted Aβ-42 (1,2), similar to what is described in late onset Alzheimer's disease (AD). Our objective was to see whether such Aβ-42 inducers are found among products of common exposure ('human chemical exposome' (HCE)).

Methods

Cultures of cell lines, primary neurons and iPSCs derived neurons, ELISA, IP-MS.

Results

We screened 3500+ HCE products and detected a few Aβ-42 inducers, including some triazine herbicides. Among 37 commercial triazines, 6 triggered Aβ-42 production in various cell lines, primary neuron cultures and neurons derived from human iPSCs. Neurons derived from IPScs from AD patients (APP-K724N mutation) showed increased Aβ-42 production which was further enhanced by triazines or aftin-5. These results, initially obtained by ELISA, were confirmed by immunoprecipitation/mass spectrometry identification of the complete range of secreted Aβ fragments. To identify the molecular targets of triazines, we developed an affinity chromatography matrix of agarose-immobilized triazines.

Conclusion

(i) like Aftins, some triazines trigger a shift in the γ-secretase cleavage site preference at APP, leading to a relative increase in the production of longer, more aggregation-prone amyloids, **(ii)** the HCE contains products able to induce production of Aβ-42, **(iii)** these molecules may constitute new pharmacological tools to develop chemically-induced animal models of AD, **(iv)** such potentially 'Alzheimerogenic' products may contribute to the development of late onset AD, **(v)** identification of environmental Aβ-42 inducers may lead to a novel prevention approach to AD.

(1) FASEB J. 26, 5115.
(2) J. Alzheimer's Disease 35, 107.

07b. Epidemiology, Risk Factors, Genetics & Epigenetics: environmental

ADPD5-0852

ACCESSIBILITY OF THE DOMINANT AND NONDOMINANT LANGUAGE IN VERBAL FLUENCY AND PICTURE NAMING IN BILINGUALS WITH MCI AND AD

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Background
Bilingualism leads to enhanced performance in a variety of executive function tasks (e.g. Bialystok et al., 2008). Neuropsychological differences between monolinguals and bilinguals with mild cognitive impairment (MCI) and Alzheimer`s disease (AD) as well as MCI- and AD- related effects on bilinguals' dominant and nondominant language have only rarely been analysed.
Methods
Neuropsychological performance was investigated in 41 lifelong bilinguals (mean age: 73.6 ± 11.5), and 45 monolinguals (mean age: 78.1 ± 10.9) subjects matched for age, education, sex, and severity of cognitive deficits. According to the results of a thorough psychiatric examination 22 and 47 subjects were diagnosed with MCI or AD, respectively, while 17 showed no cognitive deficits. Language dominance in bilinguals was determined using the objective method following Gollan et al. (2011).
Results
Both mono- and bilingual patients with MCI and with AD scored significantly lower than the healthy controls in nearly all neuropsychological domains tested. When compared between the dominant and the non- dominant language, bilingual patients with MCI had more severe deficits in their dominant than in their non- dominant language while a reversed pattern of deficits applied for the bilingual patients with AD.
Conclusions
Our findings demonstrate that bilinguals with MCI or AD do not differ from monolinguals in neuropsychological performance. The dominant language appears to be first compromised in bilingual patients while severe deficits of the nondominant language develop later in the course with manifestation of AD. This finding confirms previous studies describing language related deficits early in the course of AD.

07b. Epidemiology, Risk Factors, Genetics & Epigenetics: environmental

ADPD5-2056

MATERNAL EXPOSURE TO DIESEL EXHAUST NANOPARTICLES ENHANCES THE RISK OF ALZHEIMER'S DISEASE BY PROMOTE BETA-AMYLOID FIBRILLATION IN OFFSPRING

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Objective: Alzheimer's disease (AD) is one of amyloid diseases; the pathogenesis of AD is associated with β-amyloid (Aβ) fibrillation. Nanoparticles are being explored for their role in diagnosing, preventing, treating or even causing amyloid diseases. But the relations between nanoparticles and Aβ aren't proved at the present. We have already reported that Diesel exhaust particles (DEP; one of nanoparticles) exposure to animals exert severe influences on brains. In this study, we observed Aβ fibril formation in adult marine brain of maternal DEPs exposure.
Methods: Pregnant ICR mice were exposed to DEP and delivered of babies in clean air. At 12 and 40 weeks, brains were obtained from mice born from DEP-exposed and control mothers and examined by light and electron microscopy, compared between with and without DEP exposure. The storage of Aβ, which is characteristic of neurodegenerative diseases, was detected with immunohistochemical staining.
Results: All mice with DEP exposure showed swelling of astrocytes' endfoot, endothelial cell apoptosis, and diffuse obstruction of capillaries. Specifically, mice at 40 weeks have the neurofibrillary tangles, and showed positive for components of Aβ.
Conclusions: These findings suggest that maternal DEP exposure might effect fetal brain development, carry atrophy and promote Aβ fibrillation in the offspring. The damages of fatal brain make influences reach not only in infants and youths but also in adults, even if they live in clean environment after birth, the risk that suffers from Alzheimer's diseases etc. must be higher. The interaction between Aβ and nanoparticles may contribute to AD etiology.

07c. Epidemiology, Risk Factors, Genetics & Epigenetics: metabolic

ADPD5-0243

ALZHEIMER-ASSOCIATED A BETA OLIGOMERS IMPACT THE CENTRAL NERVOUS SYSTEM TO INDUCE PERIPHERAL METABOLIX DEREGULATION

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Alzheimer's disease (AD) is increasingly associated with peripheral metabolic disorders. Clinical/epidemiological data indicate increased risk of diabetes in AD patients. Here, we show that intracerebroventricular infusion of Ab oligomers (AβOs) in mice triggered peripheral glucose intolerance, a phenomenon further verified in a triple-transgenic mouse model of AD. AβOs failed to induce glucose intolerance when injected systemically, suggesting that brain regions involved in control of peripheral metabolism were targeted by AβOs. Accordingly, we show that AβOs bind to hypothalamic neurons and induce oxidative stress. AbOs further activated pro-inflammatory IKKβ/NF-κB signaling and instigated endoplasmic reticulum (ER) stress in the hypothalamus of mice and macaques given AbO infusions. AbOs failed to trigger peripheral glucose intolerance in tumor necrosis factor-a (TNF-a) receptor 1 knockout mice. Pharmacological inhibition of brain ER stress prevented glucose intolerance and the increase in sympathetic tonus induced by AbOs, indicating that oligomers act via a central route to affect peripheral glucose homeostasis. Results reveal a novel pathogenic action of AbOs in the brain, and indicate shared molecular mechanisms between hypothalamic dysfunction in metabolic disorders and AD.

07c. Epidemiology, Risk Factors, Genetics & Epigenetics: metabolic

ADPD5-0749
DEMENTIA AND ADIPOKINES

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The aim of the study was to investigate the role of adipokines (hormones secreted by adipose tissue) in the development of Alzheimer disease and of other forms of dementia.
Material: The investigated group consisted of 327 individuals 120 men and 207 women, aged 72.7 ± 8.7 years, 68 of them diagnosed as probable Alzheimer disease (AD), 33 as dementia of vascular origin (VaD), 47 as mixed dementia (MD), 83 as mild cognitive impairment (MCI) and 96 persons without dementia which served as control group. Dementia was diagnosed using DSM IV criteria and the type of dementia on NINCDS-ADRDA and NINDS-AIREN scales and Hachinski score.
Methods: In blood serum the levels of adipokines: adiponectin, leptin and resistin were determined using ELISA methods. Fasting glucose and 2-hour post-load glucose levels were determined using enzymatic method. Insulin concentration was assayed using ELISA method and HOMA-IR (Homeostatic Model Assessment Index) illustrating insulin resistance was calculated.
Results: As compared with controls significantly higher adiponectin levels were stated in MD, higher resistin concentration in MD and VaD and tendency to lower leptin levels in MD group. Impaired glucose tolerance was observed in MD and VaD. In the whole group of dementia negative correlation of adiponectin and positive correlation of leptin and markers of glucose metabolism disturbances and obesity were observed.
It can be concluded that adipokines play an important role in those forms of dementia which are connected with vascular pathology and glucose intolerance.

07c. Epidemiology, Risk Factors, Genetics & Epigenetics: metabolic

ADPD5-0863

RESISTANCE TO DIET-INDUCED OBESITY IS ASSOCIATED WITH INTACT COGNITION IN AGING LOU/C/JALL RATS.

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Objectives: Type 2 diabetes and obesity increase the risk of Alzheimer's disease (AD) and may favor memory deficits. Here, we investigated the effect of diet-induced obesity on cognition using the LOU/C/Jall (LOU) rat. This strain is considered a model of healthy aging due to its increased longevity, maintenance of low body fat mass throughout life, low incidence of age-related diseases and an absence of cognitive deficits.
Methods: We challenged 6- and 24-month-old LOU rats with a long-term high-fat high-glucose (HF/HG) diet (16 weeks, 60% calorie intake from high fat chow + 10% glucose in water). Body composition (echoMRI), recognition and spatial memory (novel object recognition and Morris Water Maze tasks) were assessed before and after the diet. BW, food/water consumption were measured regularly.
Results: Young and old rats on the 16-week diet had similar BW then controls. Interestingly, they reduced considerably HF intake to compensate for their elevated consumption of glucose water. Nevertheless, their glycemia remained low and their cognition was not affected. RNA-Seq analysis is currently performed to compare hippocampus gene expression signatures and unravel other characteristics of the LOU rat genome.
Conclusions: Taken together, our results suggest that the LOU rat strain is not only characterized by the maintenance of memory functions despite advanced age but is resistant to both age- and diet-induced obesity. Identification of molecular targets linked to this phenotype should help developing strategies to preserve or enhance cognitive abilities in aging and delay memory impairments associated with AD.

07c. Epidemiology, Risk Factors, Genetics & Epigenetics: metabolic

ADPD5-1742

POSSIBLE ROLE OF INSULIN DEGRADING ENZYME (IDE) IN RELATIONSHIP BETWEEN DIABETES MELLITUS AND ALZHEIMER'S DISEASE

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Several studies have shown that Diabetes Mellitus (DM) increases the risk of Alzheimer's Disease (AD). IDE is an enzyme cleaves small polypeptides including insulin, IGFs, amylin and amyloid beta. However up to date little is known about how DM affects IDE.

We performed *in vitro* experiments using rat primary neuronal cultures incubated with high glucose, lack of insulin or both conditions. *In vivo* experiments were performed using cortex of experimentally induced diabetic rats by injecting streptozotocin (STZ). IDE protein and mRNA levels were examined by western blot and RT-PCR respectively. In neurons from rat primary culture, IDE protein levels decreased in the lack of insulin group (18.78 %) and lack of insulin/high glucose group (29.11 %) compared to control group whereas IDE levels of high glucose group did not change. mRNA level of IDE decreased in lack of insulin group (16.28 %) and lack of insulin/high glucose groups (26.41 %) compared to control group. In neurons from diabetic rats, IDE protein levels decreased in diabetic group (41.22 %) compared to control group.

It is reported that in AD, IDE is reduced both in protein levels and activity. Our results suggest that DM lowers the levels of IDE. Thus, this could be one of the mechanisms by which DM confers high risk of AD development.

07c. Epidemiology, Risk Factors, Genetics & Epigenetics: metabolic

ADPD5-2155

RISK FACTORS FOR COGNITIVE IMPAIRMENT IN PATIENTS WITH TYPE 2 DIABETES MELLITUS (DM2)

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Objectives:
Patients with DM2 may have an increased risk for Alzheimer's disease and vascular dementia. With our study we explored risk factors for cognitive impairment in Slovenian patients with DM2
Methods: The 427 patients were included (190 women). We evaluated cognitive impairment with Slovenian version of Clock Drawing Test (CDT). Patients who scored 3 out of 4 points or less were considered cognitive impaired. In logistic regression predictive model we included age, gender, duration of the DM2 and the years of education.
Results: Almost half of the patients (44%) scored less than 4 points. Cognitive impaired patients had significantly lower CDT score (1.97; SD 0.97 points vs. 4 points), were more common men, older (67.3; SD 10.2 years vs. 62.8; SD 12.4 years) with longer duration of DM 2 (13.1; SD 9.7 years vs. 10.9; SD 7.1 years) and less educated (10.9; SD 2.5 years vs. 11.7; SD 2.4 years). All variables were statistically significant in the logistic model. The highest prediction value had low education, followed by gender, age and DM2 duration.
Conclusion: Cognitive impairment is common among patients with DM2 in our group. We should be particularly alert to the men who are older than 63 years, with only primary education and suffer from the DM2 for more than 11 years.

07d. Epidemiology, Risk Factors, Genetics & Epigenetics: cardiovascular

ADPD5-0397

IMPACT OF PHYSICAL ACTIVITY ON PROGRESSION OF ALZHEIMER'S DISEASE: A CREDOS STUDY

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Objectives: There is a little information on the association of physical activity with progression of Alzheimer's disease (AD). The objective of this study is to evaluate an impact of physical activity on progression of AD.

Methods: A total of 343 AD patients enrolled from 31 memory clinics using the standard assessment and diagnostic processes were followed for progression of dementia for mean (SD) 14.9 (5.7) months. Demographic and vascular risk factors were evaluated and physical activity was investigated using Physical Activity Questionnaire of the Clinical Research Center for Dementia of South Korea (CREDOS) at baseline in all patients.

Results: In a univariate mixed model, an increment of Clinical Dementia Rating Scale-Sum of Boxes (CDR-SB) score was associated with older age (p=0.009), lower Mini-Mental State Examination (MMSE) score (p<0.001), lower Body Mass Index (BMI) (p=0.03), and less physical activity (p=0.001) at baseline. In a multivariate mixed model adjusted for age, BMI, and MMSE, AD patients who had done physical activity of moderate intensity such as quick walking and stretching over 90 minutes a week had a less increment of CDR-SB (Estimate=-1.42, 95% CI= -2.12 ~ -0.72, p=0.001) compared with those without physical activity. Changes of CDS-SB in AD patients with physical activity less 90 minutes a week or in those with physical activity of severe intensity such as running and climbing did not differ significantly with a change of CDR-SB in those without physical activity.

Conclusions: Physical activity of moderate intensity over 90 minutes a week may delay progression of AD.

07d. Epidemiology, Risk Factors, Genetics & Epigenetics: cardiovascular

ADPD5-0639

DIFFERENTIAL IMPACT OF CEREBRAL WHITE MATTER LESIONS AND LIFESTYLE-RELATED DISEASES ON THE PROGRESSION OF COGNITIVE DECLINE

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[Objectives] It has been suggested that cerebral white matter lesions and lifestyle-related diseases have interactive associations with cognitive decline, but there have been few reports based on longitudinal observation. In the current study we investigated the effects of white matter lesions and lifestyle-related diseases on the progression of cognitive decline in Alzheimer's disease and amnestic mild cognitive impairment subjects.

[Methods] A total of 80 subjects were enrolled in this study. All subjects underwent baseline brain MRI and neuropsychological assessments, and follow-up neuropsychological assessments were performed within 1 to 2 years. We performed Fisher exact test to examine the association between white matter lesions and lifestyle-related diseases and the multivariate regression analysis to examine the associations among white matter lesions, lifestyle-related diseases, and cognitive performance assessed prospectively.

[Results] Fisher exact test revealed a relationship between deep white matter hyperintensities (DWMH) and hypertension(p=.04), shown by a significantly higher prevalence of hypertension in subjects whose Fazekas rating were graded2. The multivariate regression analysis, making changes of cognitive scores from baseline as dependent variables, indicated a significant association of DWMH with lower scores on the MMSE (p=.03), and that of periventricular hyperintensities with lower scores on the initial letter fluency (p=.03). Meanwhile DWMH and existence of diabetes explained lower scores on the digit symbol (p=.02, .01).

[Conclusions] The present study suggested an involvement of hypertension with changes of some cognitive functions indicated by significant interactions between, whereas diabetes may influence the process by other mechanism different from hypertension.

07d. Epidemiology, Risk Factors, Genetics & Epigenetics: cardiovascular

ADPD5-0645

EFFECT OF POLYUNSATURATED FATTY ACIDS ON THE EVOLUTION OF COGNITIVE ABILITY IN ELDERLY PATIENTS WITH ALZHEIMER'S DISEASE PUFA, EPA

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[Background] As the disease modifying therapy is not yet available for Alzheimer's disease (AD), the management of modifiable vascular risk factors (VRFs) including lipid metabolism is now considered to be the best strategy to minimize the impact of AD lesions, especially in elderly subjects. **[Objective]** To elucidate the effect of polyunsaturated fatty acids (PUFAs) on the cognitive ability, we investigated the relationship between the plasm PUFA profile and neuropsychological performance in elderly AD patients. **[Methods]** Present study was based on 133 elderly patients (51 men and 82 women) with probable AD, and their mean age was 78.6 years. Mini-mental State Exam (MMSE) and clock drawing test were used for neuropsychological evaluation. Blood samples were obtained for the measurement of PUFA profiles .Neuropsychological evaluation was repeated with one-year interval in 49 subjects, who were classified into two categories; stable group in which the MMSE score was unchanged or improved, and deteriorating group in which the MMSE score worsened. A receiver operating characteristic (ROC) curve was used to evaluate the relationship between the EPA/AA ratio and the evolution of cognitive ability. **[Results]** Total MMSE score correlated positively with the eicosapentaenoic acid (EPA)/ arachidonic acid (AA) ratio, and negatively with AA concentration. In the ROC curve analysis, the threshold EPA/AA ratio was estimated as 0.67 for the stable MMSE score with 66% sensitivity and 70% specificity [odds ratio (OR) = 4.43] . **[Conclusion]** The EPA/AA ratio can be regarded as a predictive marker for the preservation of cognitive ability in elderly AD patients.

07d. Epidemiology, Risk Factors, Genetics & Epigenetics: cardiovascular

ADPD5-2320

ESTIMATING THE RISK FOR CONVERSION FROM MILD COGNITIVE IMPAIRMENT TO ALZHEIMER'S DISEASE IN AN ELDERLY ARAB COMMUNITY

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Objectives: Vascular risk factors and lack of formal education may increase the risk of Alzheimer's disease (AD). We aimed to determine the contribution of vascular risk factors and education to the risk of Mild Cognitive Impairment (MCI) and AD and estimate the risk for conversion from MCI to AD.

Methods: This door-to-door survey was performed by an Arab-speaking team in Wadi Ara villages in Israel. All consenting residents aged> 65 years were interviewed for medical history and underwent neurological and cognitive examinations. Individuals were cognitively classified as normal (CN), MCI, AD, vascular dementia (VD) or unclassifiable. MCI patients were re-examined at least one year later to determine conversion to AD. The contributions of age, gender, school years and vascular risk factors to the probability of conversion were estimated using logistic regression models.

Results: Of the 906 participants, 297 (33%) had MCI and 95 (10%) had AD. The risk of AD vs. CN was significantly associated with age (p

Conclusions: In this population, age, female gender, lack of formal education and hypertension are risk factors for both AD and MCI. Conversion risk from MCI to AD could be estimated using a formula as a function of age, time interval between examinations and hypertension.

07e. Epidemiology, Risk Factors, Genetics & Epigenetics: infectious

ADPD5-0627

CYTOMEGALOVIRUS INFECTION DOES NOT INCREASE THE RISK OF ALZHEIMER'S DISEASE

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1. Objectives
In recent years accumulating evidence has come to suggest a relationship between herpes simplex virus infection and the development of Alzheimer's Disease (AD). Another human herpes virus, Cytomegalovirus (CMV), has also during the last year been found to associate with an increased risk of AD in three epidemiological studies. The aim of this study was to confirm these observations using a nested case control material with very high diagnostic quality and long follow-up time.
2. Methods
The study included 360 AD cases and 360 closely age- and sex-matched dementia free controls. AD cases was diagnosed at the University Hospital Memory Clinic in Umeå, Sweden, and validated before inclusion in the study. Plasma samples taken on average 9.6 years before diagnosis was taken from the Medical Biobank in Umeå, for both AD cases and controls. Anti-CMV IgG and IgM antibodies were analysed using ELISA.
3. Results
There were 312/360 (86.7%) anti-CMV IgG positive persons among the AD cases and 318/360 (88.3%) in the control group. Presence of anti-CMV IgG antibodies did not increase the risk for AD (Odds Ratio 0.857, 95% Confidence Interval 0.549 – 1.338, p=0.497).
4. Conclusions
The present data does not support either a direct or an indirect relationship between CMV infection and the development of AD. Previously published findings could possibly explained by confounding factors or immune alterations in AD affecting CMV infection, rather than any relationship to the contrary.

07e. Epidemiology, Risk Factors, Genetics & Epigenetics: infectious

ADPD5-1421

INFLAMMASOME GENE REGULATION FOLLOWING CHLAMYDIA PNEUMONIAE INFECTION OF THP-1 MONOCYTES HAS IMPLICATIONS FOR LATE-ONSET ALZHEIMER DISEASE

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Objectives: Neuroinflammation is often a major component of neurological diseases, including Alzheimer disease. Research focusing on the cause of late-onset Alzheimer disease suggests that chronic infection(s) may be key initiators or contributors to the pathogenesis. Our laboratory has shown that *Chlamydia pneumoniae* infects many different cell types *in vitro*, can survive intracellularly for long periods of time, and is detectable in Alzheimer disease brain tissues. In this study, we sought to determine how infection may influence gene regulation, including activation of inflammasomes, that may result in the neuroinflammation observed in Alzheimer disease.
Methods: THP-1 monocytes were infected with either AR-39 or CWL-029 respiratory strains of *Chlamydia pneumoniae* for 24, 48, or 72 hours and subsequently analyzed using commercial human inflammasome real time PCR Arrays. These arrays consist of 84 primers for gene transcripts known to be involved with inflammasome pathways.
Results: When compared with uninfected monocytes, *Chlamydia pneumoniae* infected monocytes revealed 21 of 84 total gene transcripts that were significantly up-regulated at least 4 fold or greater. CCL2 and IL-1beta gene transcripts showed up-regulation at 24, 48, and 72 hours. Interestingly, the AIM2 inflammasome gene transcript was significantly up-regulated in all infected cells.
Conclusions: Our data suggest that upon infection of THP-1 monocytes with *Chlamydia pneumoniae*, significant changes occur in the regulation of gene transcripts that correlate to the inflammatory signature observed in sporadic late-onset Alzheimer disease. Thus, gene regulation changes due to infection could lead to the neuroinflammation observed in Alzheimer disease.

07f. Epidemiology, Risk Factors, Genetics & Epigenetics: inflammation

ADPD5-0402

RELATIONSHIP OF NEUROINFLAMMATION ACCORDING TO AGING PROCESS AND PATHOGENESIS OF ALZHEIMER'S DISEASE

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Background: Microglia are involved in immune surveillance in intact brains and become activated in response to inflammation and neurodegeneration. Microglia have different functions, neuroprotective or neurotoxic, according to aging in patients with PD. The clinical effect of microglia in patients with Alzheimer's disease (AD) is poorly defined. This prospective study was conducted to investigate the clinical effects of microglia according to the aging process in newly diagnosed AD.
Methods: We examined 532 patients with newly diagnosed AD and 119 healthy controls, and the differences in hs-CRP between these groups were investigated. The patients with AD was classified into 3 subgroups according to age of newly diagnosed AD to investigate the relationship between hs-CRP and the aging process in newly diagnosed AD.
Results: There were significantly higher serum high-sensitivity C-reactive protein (hs-CRP), levels in patients with AD compared with healthy controls. A post-hoc analysis of the 3 AD subgroups showed no significant differences in serum hs-CRP level between each group.
Conclusion: We assumed that neuroinflammation play a role in the pathogenesis of AD, but found no clinical evidence that microglia senescence underlies the microglia switch from neuroprotective in young brains to neurotoxic in aged brains. To clarify the role of microglia and aging in the pathogenesis of AD, future longitudinal studies involving a large cohort are required.

07f. Epidemiology, Risk Factors, Genetics & Epigenetics: inflammation

ADPD5-0745

DEMENTIA, VITAMIN D AND INDICES OF EXISTING INFLAMMATION

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The aim of the study was to investigate the importance of vitamin D and inflammatory markers in the development of Alzheimer disease and other forms of dementia.

Material: The investigated group consisted of 327 individuals, 120 men and 207 women, aged 72.7 ± 8.7 years, 68 of them diagnosed as probable Alzheimer disease (AD), 33 as dementia of vascular origin (VaD), 47 as mixed dementia (MD), 83 as mild cognitive impairment (MCI) and 96 persons without dementia which served as control group. Dementia was diagnosed using DSM IV criteria and the type of dementia on NINCDS-ADRDA and NINDS-AIREN scales and Hachinski score.

Methods: In blood serum the levels of vitamin 25(OH)D, high sensitivity C-reactive protein (hsCRP) and high sensitivity interleukin 6 (IL-6) were assayed using ELISA methods. HDL cholesterol was determined after removing apoB containing lipoproteins by enzymatic method.

Results: Significantly higher IL-6 levels were stated in MD and VaD groups as compared to controls. In MD group a tendency to higher prevalence of serum vitamin 25(OH)D deficiency was observed. In the whole group of dementia negative correlation between 25(OH)D and IL-6 and hsCRP was observed. The positive correlation of 25(OH)D with HDL-cholesterol (anti-inflammatory marker) was also stated.

It can be concluded that vitamin D deficiency and inflammation could play an important role in dementia development, particularly in those forms of dementia which are connected with vascular pathology. The inverse association of 25(OH)D with IL-6 and hsCRP could suggest a potential anti-inflammatory role for vitamin D in dementia.

07f. Epidemiology, Risk Factors, Genetics & Epigenetics: inflammation

ADPD5-1291

DIET, ELEVATED CHRONIC INFLAMMATION AND COGNITIVE DECLINE: DATA FROM THE AUSTRALIAN IMAGING, BIOMARKERS AND LIFESTYLE STUDY OF AGEING

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Identification of lifestyle and dietary modifications which prevent or delay cognitive decline and Alzheimer's disease (AD) would confer significant socioeconomic benefit. Yet, there is a relative lack of large-scale longitudinal investigations of lifestyle-related factors impacting cognitive decline and AD-related pathology.
Inflammation is heavily implicated in AD pathology, with evidence suggesting it is both a reaction to the disease process and a contributor to neuronal damage. The relationship between diet and inflammation however, remains poorly understood, and a paucity of information exists which characterises the relationship between diet, inflammatory biomarkers and AD risk.
We report on data collected from 527 participants of the Australian Imaging, Biomarkers and Lifestyle (AIBL) Study of Ageing who were classified as healthy controls at baseline. Food Frequency Questionnaire data was used to construct an inflammatory dietary index (IDI) which categorises individuals' nutritional intake on a continuum from maximally anti-inflammatory to maximally pro-inflammatory. The IDI was subsequently analysed in conjunction with blood-based biomarkers of inflammation and comprehensive longitudinal neuropsychological assessment data.
Analysis over 54 months revealed that as individuals consume less anti-inflammatory dietary components (indicated by a higher IDI), levels of numerous inflammatory biomarkers increase. Further, multinomial logistic regressions used to calculate odds ratios revealed that high IDI was associated with increased likelihood ($OR=3.08$; $p<0.05$) of transitioning from healthy control classification at baseline to mild cognitive impairment or AD status after 54 months.
Taken together, our results suggest an interplay between diet and elevated chronic inflammation which may contribute to increased likelihood of cognitive decline and AD.

07f. Epidemiology, Risk Factors, Genetics & Epigenetics: inflammation

ADPD5-1347

CD33-MEDIATED MICROGLIAL DEGRADATIVE PATHWAYS IN ALZHEIMER'S DISEASE

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Objectives: To define the cellular signature of CD33 activity in microglia. CD33 is a sialic acid-binding immunoglobulin-like lectin that regulates innate immunity. We have previously shown that CD33 activity in microglia promotes amyloid-beta (Aβ) pathology by inhibiting Aβ uptake and clearance (Griciuc et al., Neuron, 2013). Microglia can assume activation states in a spectrum from M1 (pro-inflammatory) to M2 (pro-phagocytic).
Methods: We assessed expression levels of M1-markers F4/80 and iNOS in 7-month-old WT, *APP/PS1*, *APP/PS1/CD33*$^{-/-}$ and *CD33*$^{-/-}$ brains by immunohistochemistry and western blotting. We isolated microglia from 14-month-old WT, *5xFAD*, *5xFAD/CD33*$^{-/-}$ and *CD33*$^{-/-}$ mice. Mouse forebrains were dissociated, and the cell suspension was labeled with CD11b/CD45 antibodies and underwent FACS. RNA extracted from microglia was used to determine M1/M2 marker abundance by RT-PCR.
Results: *CD33* knock-out led to reduced numbers of F4/80-positive microglia and to decreased iNOS protein levels in the *APP/PS1* brain. FACS experiments showed that the *5xFAD* brain exhibited a 1.9-fold increase in microglial numbers. *CD33* knock-out had no effect on CD11b/CD45-positive microglial numbers in both WT and *5xFAD* mice. Remarkably, *CD33* knock-out in a *5xFAD* background led to decreased levels of M1-markers, but increased M2-marker expression in adult microglia. *CD33* knock-out in a WT background did not alter M1/M2 marker levels.
Conclusions: Collectively, these observations raise the possibility that *CD33* knock-out results in skewing of microglia from M1 towards M2-activated state in brains that exhibit Aβ pathology. These findings suggest that therapeutic inhibition of CD33 may be considered as a means for curbing neuroinflammation in Alzheimer's disease.

07f. Epidemiology, Risk Factors, Genetics & Epigenetics: inflammation

ADPD5-1441

AN IN VITRO MICROGLIA-LIKE MODEL FOR EXPLORING GENETIC-DRIVEN INNATE IMMUNE SYSTEM DYSFUNCTION IN NEURODEGENERATIVE DISEASES.

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In order to understand the functional consequences of disease-associated common genetic variation, we must be able to analyze the outcomes in the correct cell type. In terms of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and multiple sclerosis an increasing number of microglia-related genes have been associated with disease. However, these analyses cannot be performed at the scale required using primary human microglia as there is limited access to these resident innate immune cells of the central nervous system. We have validated an *in vitro* differentiation protocol using polarizing cytokines for converting monocytes to microglia-like (MDMi) cells, leveraging the plasticity of immune cells, using a recently defined unique microglia signature. Specifically, we see an increase in mRNA expression of PROS1, GAS6, GPR34, CD39, C1QA and TGFBR1 (all $p<0.001$) in MDMi cells compared to monocytes, confirming this *in vitro* system as a valid model for human microglia. We also observe an increase in TREM2 surface protein expression in the MDMi cells relative to monocytes ($p=0.001$). We are using this *in vitro* system to examine genotype-induced phenotypes. We have confirmed that the Alzheimer's disease-associated SNP, rs3865444, leads to differential CD33 protein surface expression ($p=0.001$) and uptake ability ($p=0.05$) in MDMi cells. Using monocytes isolated from 96 genotyped subjects from the PhenoGenetic cohort, we are assessing the functional consequences of 94 known neurodegenerative disease susceptibility loci in MDMi cells using the Fluidigm platform.

07h. Epidemiology, Risk Factors, Genetics & Epigenetics: drug-induced

ADPD5-0916

THE IMPACT OF PSYCHOTROPIC DRUGS ON THE RISK OF FALLS, ACUTE HOSPITALISATIONS AND MORTALITY AMONG PERSONS WITH DEMENTIA: A NATIONWIDE CASE-CONTROL STUDY

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Objectives: To investigate whether psychotropics (i.e. antipsychotics, anxiolytics, hypnotics/sedatives and antidepressants) increase the risk of falls, acute hospitalisations and mortality in a large population of dementia patients.
Methods: We performed a nationwide matched (age, sex and case event day) case-control study between January 1st and December 31st 2011 based on several Swedish registers (n=45 914 dementia patients aged ≥65 years). We analysed the impact of use of psychotropics on the risk of falls, acute hospitalisations and mortality by multivariate conditional logistic regression adjusted for education, number of inpatient days, Charlson comorbidity index and number of other drugs.
Results: There was no association between use of psychotropics and falls. However, there was an increased risk of acute hospitalisations associated with use of anxiolytics (adjusted OR: 1.20; 95% CI: 1.10-1.31) and hypnotics/sedatives (adjusted OR: 1.15; 95% CI: 1.06-1.25), but a negative association for antidepressants (adjusted OR: 0.90; 95% CI: 0.83-0.97). Mortality was increased among those who used antipsychotics (adjusted OR: 1.50; 95% CI: 1.41-1.60), antidepressants (adjusted OR: 1.14; 95% CI: 1.08-1.21) and anxiolytics (adjusted OR: 1.33; 95% CI: 1.25-1.41). Additionally, a dose-response relationship was found between number of psychotropics and risk of acute hospitalisations (4 psychotropics vs 0: adjusted OR: 1.36; 95% CI: 1.14-1.62) and risk of death (4 psychotropics vs 0: adjusted OR: 1.99; 95% CI: 1.76-2.25).
Conclusions: Psychotropic drugs have an impact on the risk of acute hospitalisations and death in a dose-response manner among older dementia patients. Our findings emphasise the importance of reducing perfunctory psychotropic prescribing in dementia.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-0361

A NOVEL TMP21 SNP AND ITS ASSOCIATION WITH ALZHEIMER'S DISEASE

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Objectives

TMP21, a type I transmembrane protein of the p24 protein family is a trafficking protein. Recent studies suggest that TMP21 is a selective modulator of γ-secretase and its dysregulation may play a pivotal role in Alzheimer's disease (AD) pathogenesis. However, the genetic association between *Tmp21* and AD remains elusive. This study aims to identify the genetic link between TMP21 gene and AD and to further investigate the role of TMP21 in Alzheimer's pathogenesis

Methods

Genomic DNA was extracted from sporadic AD patients and healthy controls for targeted sequencing. Single nucleotide polymorphism (SNP) detection was performed. RT-PCR, Western blot analysis and luciferase assay were used to further define the effect of the identified SNP on TMP21 gene expression.

Results

In this study, we first identified that a novel T>C SNP located in intron 4 of *Tmp21* and is associated with AD patients by screening AD patients and controls. The SNP did not affect the splicing site recognition, but it significantly increased TMP21expression at both mRNA and protein levels. Furthermore, we found that this SNP significantly increased the splicing efficiency of *Tmp21* pre-mRNA, leading to the elevation of mature mRNA. However, the stability of *Tmp21* pre-mRNA and transcription activity of *Tmp21* was not affected.

Conclusions

Our study identified an AD-associated *Tmp21* SNP, and the results suggest that dysregulation of TMP21 may contribute to AD pathogenesis and that TMP21 may be a potential target for AD treatment.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-0534

THREE GENETIC LOCI ASSOCIATED WITH DISEASE PROGRESSION MAY CONTRIBUTE TO DONEPEZIL NON-RESPONSE IN MILD COGNITIVE IMPAIRMENT

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Objectives: To evaluate the association between genetics and donepezil responsiveness in mild cognitively impairment (MCI).
Methods: We conducted a candidate-gene secondary analysis of the vitamin E/donepezil trial using the genotyping data performed with the Illumina Human610-Quad Bead-Chip. We used linear regression models, adjusting for gender and APOE4 genotype, to test the influence of 24 SNPs - previously identified for their association with disease/endophenotypes or donepezil pharmacogenetics in Alzheimer's Disease (AD) - on changes in mini-mental state examination (MMSE) and Clinical Dementia Rating sum of boxes (CDRSB) scores at the end of the trial.
Results: We found a nominal association between three genetic loci associated with AD/MCI endophenotypes and non-response to donepezil when examining changes in MMSE and CDRSB after 3 years of therapy. The association between genotype and MMSE decline remained significant for the K-variant of BCHE (rs1803274; p=0.00062/corrected P=0.0149) after Bonferroni correction.The association between genotype and CRDRSB worsening remained significant for *ACOT11* (rs12752888; p=0.0032/corrected P=0.038) and *UBR5* (rs7840202; p=0.004/corrected P=0.043), after correction.
Conclusion: Our findings suggest that MCI subjects carrying genetic polymorphisms associated with AD are at risk of developing resistance to donepezil. This study represents a first effort at studying the pharmacogenetics of donepezil in MCI and calls for leveraging existing data sets to boost the statistical power and to replicate our findings. This study also reinforces the importance of examining donepezil pharmacogenetics and AD biological pathways as it can lead to the development of novel pharmacological compounds for the treatment of MCI/AD.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1253

ROLE OF BUTYRYLCHOLINESTERASE-K GENOTYPE IN ALZHEIMER'S DISEASE AND LEWY BODY DEMENTIA

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Objective: A commonly known polymorphism of butyrylcholinesterase gene, the K-variant (BCHE-K) is found to be associated with reduced BuChE activity. Previous studies from our group suggest a possible interaction between APOE4 and BCHE-K. Our objective was to study the associations of BCHE-K and APOE4 with diagnosis and rate of cognitive decline in AD and DLB.

Method: Genomic DNA from 370 subjects (108 AD, 176 DLB and 86 Controls) from two studies in Norway, namely DemVest and TronderBrain were genotyped for BCHE-K and APOE4. The DemVest subjects were also followed annually for up to 5 years, including cognitive assessment with MMSE, and annual decline on MMSE was calculated.

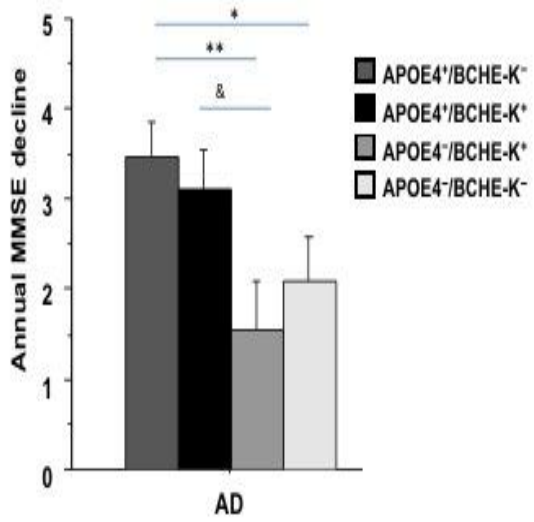
Results: The controls had higher BCHE-K frequency, than DLB ($p=0.01$) and AD ($p<0.06$) (Table-1). The observed BCHE-K allele frequency in controls was even higher (51%) than the expected frequency (24%) calculated according to previous literature. Interestingly, in AD, $APOE4^-/BCHE\text{-}K^+$ patients had significantly slower annual MMSE decline than $APOE4^+/BCHE\text{-}K^-$ group ($p<0.01$) and $APOE4^+/BCHE\text{-}K^+$ group ($p<0.05$)(Figure-1). This pattern was observed in patients with mild AD, but not in moderate and severe AD.

Conclusion: These findings suggest that BCHE-K allele alone in absence of APOE4 may have a protective role, but when occurring along with APOE4, its protective contribution may be suppressed, most likely by the over-expression of APOE protein during the early stage of AD.

Table-1. Demographics of the study cohorts

DIAGNOSIS	DLB	AD	CONTROLS
No. of subjects	176	108	86
Females (%)	44.5	71.3	48.8
Age at inclusion (years)	76.3±7.6	74.5±7.9	73.9±5.9
MMSE	20.6±6.6	24±2.1	NA
APOE4 carriers (%)	53.1	63.9	25
BCHE-K carriers (%)	34.7	38	51.2

Figure-1. Annual cognitive decline in AD



07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1394

THE GENETIC ARCHITECTURE OF ALZHEIMER DISEASE IN THE MID-WESTERN U.S. AMISH

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Objectives: There is a strong genetic influence on late onset Alzheimer disease (LOAD). Large international efforts have identified numerous susceptibility loci for LOAD and yet the majority of the genetic risk remains unidentified. We are examining LOAD in the Amish (an isolated religious group of Swiss-German origin) of Indiana and Ohio because of their cultural and genetic isolation from the general population and their more homogeneous life style.

Methods: Individuals scoring below 87 on the 3MS were given a detailed neuropsychological battery; those scoring ≥87 were considered cognitively normal. We performed genome-wide SNP linkage and association studies on 921 individuals (109 with LOAD) using the Modified Quasi-Likelihood Score (MQLS) test and Merlin, respectively. We determined the overall burden of known LOAD loci by calculating a genetic risk score (GRS). Whole exome sequencing (WES) was performed on 166 cases and controls.

Results: Affected individuals have a significantly higher GRS score than unaffected individuals (P= 1 x 10-6); but a much lower burden than an equivalent general population dataset of affected individuals (P= 1.6 x 10-7). Linkage analysis identified four likely loci that do not overlap with the known loci. WES identified a number of novel rare variants that are nominally associated with LOAD, but none reach Bonferroni-corrected significance.

Conclusions: While the Amish clearly carry some of the same risk loci as the general population, the GRS, linkage, and sequencing data strongly indicate that there are different loci that also play a significant role in LOAD in the Amish.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1445

WHOLE-EXOME SEQUENCING IN EARLY-ONSET ALZHEIMER DISEASE CASES IDENTIFIES SEVERAL NOVEL CANDIDATE GENES

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Objectives
Mutations in *APP*, *PSEN1* and *PSEN2* lead to early-onset Alzheimer disease (EOAD). These mutations account for ~11% of EOAD overall, leaving the majority of genetic risk for the most severe form of Alzheimer disease unexplained.
Methods
We performed Whole-Exome Sequencing (WES) in 50 Caucasian EOAD cases previously screened negative for *APP*, *PSEN1*, and *PSEN2* to search for rare variants contributing to risk for EOAD. Variant filtering for functional, damaging rare variants (MAF<0.1%) was performed. Genes with shared (2+ cases with the same variant), damaging variants were examined for interactions with known EOAD genes (*APP, PSEN1, PSEN2, SORL1, GRN*, *MAPT*) and *APOE* using STRINGdb.
Results
176 genes had rare functional variants shared in two or more cases. 46 of these genes were prioritized for their damaging potential, defined by their shared rare variants having a Combined Annotation Dependent Depletion (CADD) score in the top 10% of all variants. Gene network analysis of these 46 genes with known EOAD genes and *APOE* identified five top candidate genes: *HSPG2* (interacts with *GRN*, *APOE*, and *APP*), *CLSTN1* (interacts with *PSEN1* and *APP*), *DOCK3* (interacts with *PSEN1* and *PSEN2*), and the *APOE* interactors *SAR1B* and *STAT1*. 5 cases have a variant in *HSPG2*, a gene potentially involved in amyloidogenesis and tau aggregation in AD, while 4 cases have a variant in *DOCK3*, a gene expressed exclusively in the central nervous system and associated with neurofibrillary tangles in AD brains.
Conclusions
WES of EOAD cases identified several genes with potential roles in AD pathogenesis.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1662

EXOME SEQUENCING IN LATE ONSET FAMILIES IDENTIFIES ADDITIONAL CANDIDATE GENES FOR ALZHEIMER'S DISEASE SUSCEPTIBILITY

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Using exome-sequencing in fourteen late-onset Alzheimer's disease (AD) families, we found that *PLD3* variant V232M segregated with disease status in two families, and confirmed its association with AD risk. However, on average eight variants per family segregated with disease status.

We followed up 400 AD cases and 1080 controls for all variants (in genes including *LRP4, CALCR, DMRT2, KIF1A, ZNF341, LRP4, PRKD2, EPHA2)* that segregated with disease status in the sequenced families. The most interesting genes were re-sequenced in an additional 800 cases and 400 controls. In order to analyze the association of the selected genes with disease risk we performed gene-based tests stratifying by minor allele frequency (MAF), and analyzing variants that were unique to cases or controls.

Analyses on variants with a MAF<1% had power to find gene-wide significant results. In addition we found analyses focused on variants unique to cases or controls lead to greater effect sizes (odds ratio; OR) but lower p-values due to the very low frequency of these variants. We hypothesize that this strategy may be used to prioritize genes for follow-up. From all the genes that were selected for the replication study, one showed a significant gene-based association (variants MAF<1%) with AD risk after correction for multiple testing, and a strong association when only "unique-variants" were included (OR=1.97, p=2.51x10^{-4})

Our analyses indicate that additional genes harboring low-frequency risk variants exist, and that family-based studies could help identify such genes and variants. Extensive additional sequencing and functional analyses to validate our findings are underway.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1822

COMT VAL158MET POLYMORPHISM IS ASSOCIATED WITH RATE OF COGNITIVE DECLINE IN ALZHEIMER'S DISEASE

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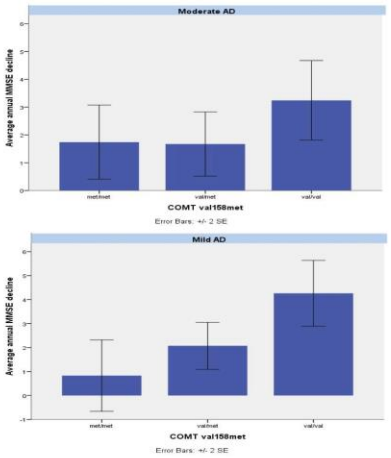
On the basis of prior evidence linking the COMT val158met polymorphism with cognition, mediated via prefrontal cortical dopamine, we hypothesised that the high activity val/val genotype would be associated with faster average annual decline in MMSE than the met/met or val/met genotypes. DNA from 237 people who were part of existing cohorts with clinically or pathologically diagnosed Alzheimer's disease were genotyped for the COMT val158met polymorphism. Average annual decline in MMSE was calculated based on at least two assessments in patients with an MMSE greater than 9 at first assessment to avoid floor effects. The sample was stratified by severity and mean annual MMSE decline was compared across the three COMT genotypes (val/val, val/met and met/met). One-way ANOVA showed that mild AD carriers of the val/val genotype had a significantly higher rate or annual MMSE decline than met/met carriers (4.2 points per year compared with 0.8 points per year; $p<0.01$). There was no difference across genotypes in the moderate AD group. This study presents the first evidence that the *COMT* val/val genotype is associated with more rapid cognitive decline in AD, suggesting reduced DA in the PFC may be an important target for treatment of cognitive decline among individuals with this genotype.



07k. Epidemiology, Risk Factors, Genetics & Epigenetics: disease-causing mutations

ADPD5-0851

FUNCTIONAL STUDIES OF AN ISOGENIC MODEL FOR FAMILIAL ALZHEIMER'S DISEASE WITH PRESENILIN-1 MUTATIONS

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Objectives: Most cases of autosomal-dominant familial Alzheimer's disease (FAD) are due to mutations in the presenilin-1 (PSEN1) gene. PSEN1 mutations increase the proportion of the aggregation-prone Abeta42 peptides, and might impair crucial cellular processes such as calcium homeostasis. However, previous studies were frequently limited by the use of overexpression models that did not accurately reflect the heterozygous expression of PSEN mutations in FAD patients.
Methods: Specific mutations were incorporated into the endogenous PSEN1 gene in mouse embryonic stem cells by dual recombinase-mediated cassette exchange (dRMCE). dRMCE takes advantage of conditional mouse alleles containing recombinase recognition sites that allow to efficiently re-engineer the PSEN1 genomic locus.
Results: Mutations were successfully introduced into the conditional PSEN1 allele with a frequency of approximately 30%. RT-PCR and Western blotting verified that the re-engineered PSEN gene locus was fully functional and expressed at normal endogenous levels as compared to the unmodified wild type allele. To study the functional interaction between PSEN1 alleles with regard to endoproteolysis, we introduced the D385N mutation targeting an aspartate residue critical for enzymatic activity. Western blot analysis demonstrated that the catalytically inactive mutant accumulated as a full-length protein despite the presence of a second catalytically competent wild type allele.
Conclusions: Our genome editing approach provides an FAD model that accounts for the heterozygous expression of PSEN mutants and is suitable for stringently controlled biochemical experiments. Studies of the D385N mutation indicated that PSEN1 endoproteolysis did not occur in trans arguing against functional interaction of mutant and wild type PSEN alleles.

07k. Epidemiology, Risk Factors, Genetics & Epigenetics: disease-causing mutations

ADPD5-1041

NOVEL AND KNOWN PSEN1 MUTATIONS IN KOREAN EOAD PATIENTS: AN UPDATE

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Alzheimer's disease (AD) is the most common form of senile dementia, which occurs mostly in the elderly population. AD, under 65 years of age (early onset AD) represents a minority of all AD cases, but its genetic background is well understood. Three genes have been discovered, which are causative genes for EOAD: APP, PSEN1 and PSEN2. Up to 2010, Korean patients with EOAD were poorly studied, since only 4 mutations were discovered in PSEN1 gene. In 2011, we discovered a novel PSEN1 mutation, H163P in an EOAD patientwithout family history of dementia, and this study was published inNeuroscience Letters in 2012.

In our studies we screened several AD and dementia patients. We performed PCR – based mutation detection methods, such as SSCP and heteroduplex analysis. To validate the presence and absence of variants, all PCR products were sequenced.
As results, we reported novel and known mutations in PSEN1. Two known pathogenic mutations have been discovered in PSEN1, T116I and L226F, but both of them were reported in Asia for the first time. Patient with T116I had strong family history of dementia, but L226F seemed to be a *de novo* mutation. Our most recent data is a novel PSEN1 mutation, L232P, which might be involved in AD progression. Further analysis will be needed for this mutation.
Our findings supported that novel pathogenic variants might be possible in the PSEN1 gene. Genetic testing of EOAD patients could improve the disease diagnosis.

07k. Epidemiology, Risk Factors, Genetics & Epigenetics: disease-causing mutations

ADPD5-1078

A 30-GENE RESEQUENCING ASSAY FOR PRECLINICAL DETECTION AND ELUCIDATION OF AETIOLOGICAL HETEROGENEITY OF ALZHEIMER'S DISEASE

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Background

Two outstanding challenges in the effort to bridge the translational gap in Alzheimer's disease (AD) research are preclinical detection and recognition of aetiological heterogeneity. Molecular genetic screening has the potential to address both challenges.

Methods

We developed an assay based on advanced PCR amplicon multiplexing (Multiplicom) and massive parallel sequencing (MiSeq, Illumina) targeting 30 validated genes involved in AD, frontotemporal lobar degeneration, Lewy body disorders, amyotrophic lateral sclerosis and Creutzfeldt-Jakob disease including 480 amplicons across 14 multiplexes. We screened 749 Belgian Alzheimer patients using this assay. Data processing was performed with rsBWA, GATK and GenomeComb.

Results

The assay achieved an average coverage of >600x, and for 94% of samples >80% was covered >50x. We identified ≥ 1 rare variants in 19% of patients, of whom 7% carried ≥ 2 variants. These include 5 predicted damaging *APP* mutations located outside the Abeta domain, 4 previously unreported *PSEN1* and *PSEN2* mutations and 3 carriers of a *PSEN2* mutation resulting in increased $Abeta_{42/40}$, but also numerous pathogenic *GRN* mutations, a compound heterozygous *PINK1* mutation, 27 *LRRK2* mutations of which 7 probable and 13 possible pathogenic, 3.5% heterozygous variations in recessive Parkinson genes and a pathogenic *CSF1R* mutation.

Interpretation

These data exemplify the relevance of genetic screening across the clinical boundaries of AD. This cost-effective neurodegeneration gene assay has an immediate clinical application in genetic diagnostics, and can be applied for genetic pre-selection in basic research (e.g. for whole genome sequencing studies) as well as clinical research to streamline biomarker and drug trials.

07k. Epidemiology, Risk Factors, Genetics & Epigenetics: disease-causing mutations

ADPD5-1482

NOVEL AND KNOWN MUTATIONS IN SORL1, PSEN1, AND PSEN2 GENES ARE FOUND IN MULTIPLEX ALZHEIMER'S DISEASE FAMILIES WITH VARYING AGE OF ONSET AND PATHOLOGICAL PRESENTATIONS

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Objectives
To identify causative AD-related mutations in 50 Caucasian multiplex families with at least one individual with early-onset Alzheimer's disease (EOAD).
Methods
Variants in the *SORL1, PSEN1, and PSEN2* genes were identified using an established whole-exome sequencing (WES) pipeline. Clinical characteristics of affected members were obtained via review of medical and research records.
Results
A novel *SORL1* mutation that is predicted to be damaging (T588I) was found in all 4 affected individuals in one family (age of onset [AOO] range 59-82; three with APOE genotype 3/4, one with 3/3). A second family carried a previously reported AD *SORL1* mutation (T749M), with 3 affected individuals with the mutation (AOO55-84, all APOE 3/3), and 1 affected individual without the mutation (AOO76, APOE 3/4).
A known *PSEN1* AD mutation (A79V) was identified in two families, with AOO54 years (APOE 3/4) and 56 years (APOE 3/4). A rare *PSEN1* variant (R269G) predicted to be damaging was identified in a third family (AOO50 years, APOE 3/3).
A single individual (AOO48, APOE 3/3) represented a compound heterozygote for 2 variants predicted to be damaging in *PSEN2* (R71W, M174V).
A large range of AOO was seen within the families, with some members classified as EOAD and other as LOAD. On autopsy, Lewy bodies were seen in an EOAD individual carrying *SORL1* T749M without clinical Parkinsonism.
Conclusions
Mutations were found in 6/50 families. The presence of an APOE-4 allele may account for disease status in one affected non-carrier (T749M). No prominent atypical clinical features were identified.

07l. Epidemiology, Risk Factors, Genetics & Epigenetics: whole genome sequencing

ADPD5-0885

RARE GENETIC VARIANTS IN PLD3 DO NOT INCREASE RISK FOR EARLY-ONSET ALZHEIMER IN A FLANDERS-BELGIAN COHORT

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Objectives:
We analyzed next-generation sequencing (NGS) data obtained in early-onset or autosomal dominant Alzheimer disease (AD) patients for mutations in known causal and risk genes for AD. We identified a missense mutation in *PLD3*, a gene which was previously implicated in familial late-onset AD. To follow-up on this finding we screened an extended early-onset AD cohort for additional genetic variants in *PLD3*.
Methods:
We obtained whole genome sequencing data of 22 unrelated early-onset AD patients and whole exome sequencing of probands of 5 autosomal dominant AD families. Sanger sequencing was used to screen *PLD3* in an additional 261 early-onset AD patients and 319 control individuals. We included in the data analysis NGS data of 56 unrelated non-AD individuals. Gene-based rare variant burden analysis was used to calculate association with early-onset AD. Bioinformatics' prediction, cDNA sequencing and cloning were used to characterize a newly identified splicing variant in *PLD3*.
Results:
We detected rare genetic variants (N = 5) that were previously identified as well as novel variants (N = 8). However the variant frequency was comparable between patients (3.13%) and control individuals (3.13%). Besides missense mutations, we identified one novel splicing variant that leads to either exon 10 skipping or intron 10 retention.
Conclusions:
We did not identify a genetic association with rare variants in *PLD3* suggesting that this gene is unlikely contributing to disease etiology in early-onset AD patients.

07l. Epidemiology, Risk Factors, Genetics & Epigenetics: whole genome sequencing

ADPD5-1380

NOVEL FINDINGS FROM WHOLE-GENOME SEQUENCE ANALYSIS OF THE NIMH AD INITIATIVE STUDY FAMILIES

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Objectives: Over 200 rare and highly penetrant pathogenic variants in APP, PSEN1 and PSEN2 cause a subset of early-onset familial Alzheimer's disease (AD), while APOE-e4 and close to 60 other genomic loci are associated with late-onset forms of AD. In this study we performed a comprehensive and systematic analysis of whole genome sequencing data from 1440 subjects from 452 multiplex NIMH AD families.
Methods: WGS data was generated using the Illumina HiSeq 2500 platform. The genotypes were called using Freebayes; ultimately, a single large annotated GEMINI database was created for the purpose of downstream analysis. We used a multi-pronged approach to identify both, rare, highly penetrant functional variants, as well as, more frequently occurring functional variants acting as genetic risk-factors for AD.
Results: In addition to confirming previously known variants in the NIMH families, our analyses revealed several novel functional variants, comprised of both single nucleotide variants (SNVs) and insertion-deletion (Indels). Furthermore, our analyses have revealed several SVs showing association with AD risk.
Conclusions: Our WGS analysis approach yielded several novel genetic functional variants influencing risk for AD. These variants are predicted to significantly alter normal gene function, and associated molecular pathways. To our knowledge, this is the first WGS study reporting novel functional genetic variants influencing risk for AD.

07m. Epidemiology, Risk Factors, Genetics & Epigenetics: micro RNA

ADPD5-0866
ROLE OF ABCA1 AND MICRORNAS IN ABETA METABOLISM
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1. Objectives
Dysregulation of Abeta metabolism is critical for Alzheimer's disease (AD). Several key proteins implicated in AD pathogenesis, such as APP, BACE1, and PSEN1, are membrane proteins and their trafficking and proteolytic activity are altered by cholesterol dysregulation. Due to its critical role in maintaining cholesterol homeostasis, ATP-binding cassette transporter A1 (ABCA1) has been considered as a promising therapeutic target. However, whether ABCA1 expression is regulated at the posttranscriptional level is largely unknown. Identification of a novel pathway that regulates ABCA1 expression may provide new strategy for regulating cholesterol metabolism and Abeta levels.
2. Methods
We used molecular and cellular biology methods, genetically modified microRNA mouse models, and an APP/PS1 transgenic mouse model to determine the role of microRNAs in regulating ABCA1 and Abeta levels.
3. Results
We identified several microRNAs that strongly downregulate ABCA1 and impair cholesterol metabolism in neuronal cells. In addition, these microRNAs impaired Abeta clearance and increased Abeta production.
4. Conclusions
Our data suggest that inhibition of microRNA function using antisense oligonucleotide may provide novel therapeutic strategy for treating AD.

07m. Epidemiology, Risk Factors, Genetics & Epigenetics: micro RNA

ADPD5-2031

AMYLOID PRECURSOR PROTEIN REGULATES THE PROLIFERATION AND DIFFERENTIATION OF NEURAL PROGENITORS BY ANTAGONISING MIR-574-5P IN THE DEVELOPING CEREBRAL CORTEX

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Amyloid precursor protein (APP) is a type I transmembrane glycoprotein proteolytically processed to release amyloid beta, a pathological hallmark of Alzheimer's disease (AD). APP is expressed throughout the developing and mature brain; however, the primary function of this protein is unknown. We previously demonstrated that APP deficiency enhances neurogenesis in the developing cerebral cortex, but the cellular and molecular mechanisms underlying this process are not known. Here, we showed that APP regulates the expression of microRNAs (miRNAs) in the cortical brain, and specifically miR-574-5p is repressed by APP. We also showed that the overexpression of miR-574-5p promotes the differentiation of neural progenitor cells (NPCs) into neurons but reduces the neural progenitor pool. In contrast, the reduced expression of miR-574-5p in NPCs inhibits neurogenesis and stimulates proliferation *in vitro* and *in vivo*. We further demonstrated that the inhibition of miR-574-5p in APP-knockout cells rescued the phenotypes associated with APP deficiency to enhance proliferation, inhibiting neurogenesis and migration *in vitro* and *in vivo*. Taken together, these results reveal a novel mechanism in which APP regulates the proliferation and differentiation of NPCs through miRNA-mediated post-transcriptional regulation.

07o. Epidemiology, Risk Factors, Genetics & Epigenetics: histone modification, DNA methylation

ADPD5-1533

DIFFERENTIAL DNA METHYLATION PATTERN OF LAYER OF CORTICAL PYRAMIDAL NEURONS BETWEEN LATE ONSET ALZHEIMER`S DISEASE AND CONTROLS

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Background: DNA methylation has been found altered in Alzheimer Disease (AD). At present, there are no DNA methylation studies in AD controlling the heterogeneity of the cellular composition between brain samples, which is a recently known confounding factor in epigenetic studies.

Objective: To perform DNA methylation analysis controlling the cellular heterogeneity in of Late Onset Alzheimer's Disease (LOAD) brain samples.

Methods: Analysis was performed in 36 human brains from the *Biobanco Navarrabiomed*, Navarra, Spain (19 LOAD cases and 17 controls), all patients were clinical and pathological diagnosis of LOAD. Using laser guided micro-dissection samples form pyramidal cortical layers Genome-wide methylation analysis was performed on the Infinium 450k platform. Analysis was done by using *limma* and *minfi* R bioconductor packages.

Results: We found highly significant differences on the DNA methylation pattern in several genes between cases and controls. These genes include Homeobox A3, a sequence-specific transcription factor rich in interaction networks; Endothelin Converting Enzyme-Like 1, related to a recently associated pathway with AD; Dendritic Cell Associated Lectin1; and MYOM2, which has recently been shown to be differentially methylated in an AD model. **Conclusions:** The differences in the methylation pattern observed in the present samples intended to control for cellular heterogeneity suggests that epigenetic changes are important in the pathophysiology of AD. These observations need to be corroborated by bisulfite sequencing analysis. Acknowledgements. This work was funded by grants from Colciencias (Contract # 401-2011).

07o. Epidemiology, Risk Factors, Genetics & Epigenetics: histone modification, DNA methylation

ADPD5-2057

CONCERTED EPIGENETIC ALTERATIONS IN ALZHEIMER AND LEWY BODY DISEASES SPECTRUM

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Emerging evidence suggest that epigenetics plays an important role in neurodegenerative diseases, including Alzheimer's disease, Down's syndrome, dementia of Lewy bodies and Parkinson's disease. To face this challenging issue, we carried out a whole genome bisulfite sequencing on prefrontal cortex examining their DNA methylation profiles at base-pair resolution. We identified differentially methylated regions (DMR) and further analyzed the meaning of such alterations as well as the level of overlap between different diseases. Interestingly, we observed a protruding overlap of DNA methylation alterations, suggesting that these diseases might be just a consequence of alternative manifestations of similar phenomena. These data might constitute an important resource for future studies representing the most complete catalogue of DNA methylation differences between neurodegenerative diseases done so far.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0683

CLINICAL SIGNIFICANCE BETWEEN TWO SUBGROUPS OF AMNESTIC MILD COGNITIVE IMPAIRMENT, ENCODING FAILURE AND RETRIEVAL FAILURE

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Object : We tried to estabilish whether a clinical significance exists between two subgroups of aMCI, encoding failure(aMCI-E) and retrieval failure(aMCI-R).
Methods : We retrospectively reviewed medical records of 96 subjects with aMCI who visited in Neurocognitive behavior center of Seoul University Bundang Hospital. They were divided to two subgrups, encoding failure and retrieval failure, by results of Seoul Verbal Learning Test (SVLT). We investigated basic characteristics including age, education, previous medical history and Apo E genotype. We also reviewed initial and follow up neuropsychological test to ascertain whether patients progressed or not.
Results : Among the 96 subjects, 65 (67.7%) subjects were classified into aMCI-R and 31 (32.3%) into aMCI-E. The baseline characteristics of two groups including previous medical history and Apo E genotype did not have any differences. There were no differences between two subgroups of aMCI on the initial neuropsychological test except immediate recall and recognition index of SVLT. On follow up neuropsychological test, KMMSE(p=0.0020) and CDR-SOB (p=0.005) scored lower in aMCI-E. The 14 subjects (45.2%) of aMCI-E progressed to dementia whereas 12 subjects (18.5%) of aMCI-R. The relative risk of progression was 2.97. Adjusted odds ratio of aMCI-E by a logistic regression was 5.688 (95% CI 1.905 to 16.982).
Conclusion : Our results showed that subjects with aMCI-E progress more likely than aMCI-R. This may suggest aMCI-E is independent prognostic factor for progression and clinicians might be better to consider more active therapeutic intervention to subjects with aMCI-E.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0811

NEUROCOGNITIVE ASSESSMENT IN MILD COGNITIVE IMPAIRMENT: TRADITIONAL METHODS AND LATENT PROFILE ANALYSIS

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Background: Cognitive profiles can help delineate who is specifically at risk of conversion to dementia in mild cognitive impairment (MCI).
Objectives: To categorise patients by MCI subtype using a standard approach, to create latent profiles and to assess progression over time.
Methods: MCI participants (n=139) and controls (n=98) were recruited. Participants had a full neurocognitive assessment. Results were analysed 1) according to number of cognitive domains affected and 2) using latent profile analysis (LPA)
Results: Most MCI participants were in the amnestic multidomain (AMD) subgroup (46.8%); they were most at risk of conversion to dementia. LPA revealed a 3 profile solution. Profile 3 was the largest group (40.3%); the most neurocognitively impaired and most closely represented the AMD group. In both analyses age had a significant impact on risk of conversion to dementia.
Conclusions: Older MCI patients with multiple cognitive deficits are most at risk of conversion to dementia

Table 1 Classification of MCI Participants According to the Methods of Petersen and Morris (2005)

Group Categorisation by Petersen Criteria	Number of MCI patients (%)	Of the total MCI patients in each category, breakdown in each profile (from LPA)		
		Profile 1 Amnestic Single Domain N=51 (36.7%)	Profile 2 Cognitively Normal N=32 (23.0%)	Profile 3 Amnestic Multidomain N=56 (40.3%)
No impairment of clinical significance	25 (18.0%)	11 (44.0%)	14 (56.0%)	0 (0.0%)
Amnestic single domain	13 (9.4%)	12 (92.3%)	1 (7.7%)	0 (0.0%)
Amnestic multidomain	65 (46.8%)	17 (26.2%)	1 (1.5%)	47 (72.3%)
Nonamnestic single domain	26 (18.7%)	8 (30.8%)	15 (57.7%)	3 (11.5%)
Nonamnestic multidomain	10 (7.2%)	3 (30.0%)	1 (10.0%)	6 (60.0%)

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-1018

THE PROFILE OF CSF BIOMARKERS AND ITS ASSOCIATION WITH APOE GENOTYPE IN PATIENTS WITH ALZHEIMER'S DISEASE

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Objectives. There are still no published data on CSF and serum biomarkers of AD in Lithuanian population. Thus the aim of our study was to evaluate levels of main CSF biomarkers and its' relationship with age of onset, initial MMSE score, and to compare the differences in APOE ε4+ and APOE ε4- groups.
Methods. Patients diagnosed with early to moderate AD (n=30) were observed from 6 to 24 months. Cognitive decline was assessed by MMSE and Blessed dementia scale. Aβ42, t-tau, p-tau CSF concentrations and Aβ40 serum level were measured. Patients were grouped according APOEε4 allele, severity of dementia, age of AD onset.
Results. Main group consisted of AD patients (n=25, mean age 68.96 yrs, mean MMSE 20.44 pts), the rest of patients (n=5, mean age 61.80 yrs, mean MMSE 22 pts) during period of observation showed signs of another cognitive disease. Levels of CSF biomarkers differed significantly among groups of AD *vs* another dementia. Aβ42, but not tau nor serum Aβ40, differed in groups APOEε4+ *vs* APOEε4-. P-tau concentration was higher in group of moderate AD. No significant data estimated according to sex and age of AD onset.
Conclusions. AD patients have reduced CSF Aβ42 and increased t-tau and p-tau levels. Carriers of at least one APOEε4 allele have lower Aβ42 concentrations. Higher p-tau concentration is associated with deeper cognitive disturbance at the onset of disease. No significant differences in serum Aβ40 levels were found among groups. More extended research is needed to confirm these findings.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-1112
ALZHEIMER'S DISEASE AND THE TASTY SERIES
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Understanding the mechanisms leading to the development of neuropathology in non-demented individuals will provide answers to the question as to why some individuals remain dementia-free even in the presence of large amounts of brain lesions. Additionally, by investigating plaques and tangles in this manner, we may begin to elucidate the pathways leading to their occurrence and develop therapies to reduce or abate their development.
At the University of Tampere we have, and are in the process of collecting a second larger cohort of brains (amongst a cardiovascular disease-based designed series) from individuals most closely representing the general population. Altogether 1600 cases with data on cardiovascular disease measurements, brain regions (frontal cortex, insula-putamen, hippocampus, substantia nigra, pons and the cerebellar cortex), blood, cerebrospinal fluid (CSF) samples, medical histories (from available hospital records) and surveys on lifestyle factors (answered by family members of the deceased) will provide a plethora of data in which to determine factors that confer risks as well as benefits in the prevalence of neuropathology.
Utilising genetics, proteomics, biomarker analyses and many other powerful tools, this study of non-demented individuals is an internationally unique opportunity to study these mechanisms. Please feel free to contact me for collaborative opportunities at eloise.mikkonen@uta.fi!

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-1133

INVESTIGATING THE SYNERGISTIC RELATIONSHIP BETWEEN SLEEP QUALITY, EXERCISE AND LEVELS OF BRAIN BETA-AMYLOID

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There is currently no effective treatment for Alzheimer's disease (AD), thus, attention has turned to preventative measures, such as lifestyle modifications, that have the potential to delay AD onset. Recently, higher levels of physical activity and good sleep quality have been separately linked to lower levels of brain beta-amyloid (Aβ). Furthermore, previous work suggests that active individuals are more likely to experience good sleep quality. However, to our knowledge, no previous research has investigated whether a synergistic link between sleep and exercise exists, and the effect of such a link on AD-related neuroimaging biomarkers. Thus, we aim to investigate whether the association between higher levels of physical activity and lower brain Aβ remains salient in individuals experiencing poor quality sleep. Similarly, we also aim to investigate whether the relationship between good sleep quality and lower brain Aβ is salient in sedentary individuals. Using data from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of Ageing, we investigated the interaction between sleep and exercise and its relationship with brain Aβ burden, measured by Pittsburgh Compound B positron emission tomography. The results from our analysis indicate that a synergistic relationship between exercise and sleep exists, and is related lower brain amyloid burden. Our results provide support for the hypothesis that both sleep quality and exercise levels are important in maintaining cognitive health, and through interpretation of AD-related neuroimaging results, may indicate reduced risk or delay in onset of AD.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-1785

REAL LIFE MOVING ABILITY CORRESPONDS TO SPATIAL NAVIGATION IMPAIRMENT IN EARLY ALZHEIMER'S DISEASE

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Objectives: Spatial navigation (SN) impairment is present already in early stages of Alzheimer's disease (AD) – in patients with amnestic mild cognitive impairment (aMCI). Particulary in patients with AD dementia it interferes with their activities of daily living. The aim of our study was to assess to what extent SN impairment interferes with real life moving ability in patients with AD dementia and aMCI.
Methods: Subjects with aMCI (n=35), mild AD dementia (n=8), and age, gender and education matched controls (n=15) underwent neurological and neuropsychological examination and SN testing (body-centered [egocentric] and world-centered [allocentric] SN) in the real-space human analogue of the Morris Watter Maze. A life-space questionnaire with five levels of difficulty was used to evaluate subjects' real life moving abilities due to SN impairment.
Results: The control group outperformed AD dementia (ps<.001) and aMCI (ps≤.002) groups in egocentric and allocentric SN. The AD dementia (p=.009) and aMCI (p=.01) groups had a lower total score on a life-space questionnaire than the control group. In the egocentric navigation task the higher error score mildly correlated with lower score on level IV (visiting places outside the neighborhood) (r=-0.20; p=.027) and level V (visiting places outside the town) (r=-0.30; p=.001). In the allocentric navigation task the higher error score mildly correlated with lower score on level V (visiting places outside the town) (r=-0.25; p=.005).
Conclusions: Moving ability in real life is affected in patients with mild AD dementia and aMCI. The level of real life moving ability may reflect severity of SN impairment.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-2079

CONVERSION OF MILD COGNITIVE IMPAIRMENT PATIENTS TO ALZHEIMER'S DISEASE IN CLINICAL SETTINGS

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Objectives: To describe the positive predictive value of Mild Cognitive Impairment (MCI) and factors associated with progression in daily practice.
Methods: A retrospective cohort study was conducted from the French National Alzheimer Database. Among 446 439 patients cared in the participating centres between January 2009 and January 2014, 45 386 (10.2%) were classified as MCI and 23 676 had a follow-up visit at least one time. Annual conversion rate was used to describe the progression of MCI patients to AD. Hazard ratios of AD were estimated using Cox regression model.
Results: Annual conversion rate (ACR) was 13.7 % persons-year (py) with higher rate for amnestic MCI (aMCI) (18.2%py) than for non-amnestic MCI (naMCI) (9.5%py). Separate regression models were performed for each MCI subtypes. Higher education, older age and lower Mini Mental State Examination score were associated with an increased risk of conversion for both subtypes. Male gender (adjusted Hazard Ratio [95% confidence interval]: 0.86 [0.78-0.95]) and anxiolytics (0.78 [0.66-0.91]) were protective factors for aMCI whereas antidepressant drugs (1.16 [1.04-1.29]) were associated with an increased risk. For naMCI prescription of antipsychotics (0.56 [0.33-0.95]) and having a full reimbursement for severe disease (0.66 [0.56-0.78]) were protective for progression.
Conclusions: In real life the positive predictive value of MCI diagnosis is in line with previous specific clinical studies and external validity of the concept strengthened. Distinguishing between aMCI and naMCI is particularly relevant.

08a. Animal Models: transgenic mice

ADPD5-0353

NEPRILYSIN DEFICIENCY INFLUENCES THE NEUROPATHOLOGICAL AND BEHAVIORAL PHENOTYPE OF 5XFAD MICE

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OBJECTIVE: By numerous *in vitro*, *in vivo* and reverse genetics studies, the metalloprotease neprilysin (NEP) has been shown to be the most potent Aβ-degrading enzyme (ADE) in the brain. 5XFAD mice represent a highly valuable AD mouse model showing accelerated plaque pathology and behavioral deficits. This project aimed on evaluating the effect of NEP deficiency on the 5XFAD mouse model.
METHODS: Expression levels of NEP and other ADEs were determined by qRT-PCR analysis. Spatial working memory of hemizygous 5XFAD/NEP$^{+/-}$ mice was evaluated by performing Y- and Cross Maze tasks. Amyloid-pathology and inflammatory processes as well as soluble Aβ levels in the brain were studied by immunohistochemistry and ELISA, respectively.
RESULTS: We found that 5XFAD mice per se show decreased NEP expression levels compared to WT mice, which is even aggravated upon hemizygous NEP depletion. 5XFAD/NEP$^{+/-}$ mice demonstrate impairments in spatial working memory and increased astrocytosis. In addition, NEP deficiency in aged mice led to an overall increased level of soluble Aβ42 as well as region-specific increases in extracellular Aβ deposition. Surprisingly, in young mice, a more abundant cortical Aβ plaque pathology could be observed in 5XFAD compared to 5XFAD/NEP$^{+/-}$ mice. Additionally, young 5XFAD/NEP$^{+/-}$ as well as NEP$^{-/-}$ mice show elevated levels of endothelin-converting enzyme 1 (ECE1), leading to the assumption of a mutual regulation of ECE1 and NEP at young ages.
CONCLUSION: We show that NEP deficiency aggravates the behavioral and neuropathological phenotype of 5XFAD mice and provide *in vivo* evidence that NEP mainly affects soluble Aβ species.

08a. Animal Models: transgenic mice

ADPD5-0407

PHYSICAL ACTIVITY AMELIORATES NEURON LOSS AND MEMORY DEFICITS IN TG4-42 MICE

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Objectives: N-truncated Aß$_{4-42}$ is one of the most abundant Aß peptide species in the brain of sporadic AD patients. We have recently developed a transgenic mouse model (Tg4-42) expressing Aβ$_{4-42}$ without mutations under the control of the neuron-specific Thy1-promotor, which develops age-dependent deficits in spatial reference memory accompanied by robust CA1 pyramidal neuron loss. The impact of enhanced physical activity on hippocampal neuron loss and behavioral deficits was assessed using an enriched environment paradigm.

Methods: Tg4-42 mice were housed under standard conditions or in enriched cages equipped with various objects and running wheels. The number of CA1 pyramidal and dentate gyrus (DG) granule cell layer neurons was assessed using design-based stereological methods. In addition, spatial reference memory using the Morris Water Maze task, as well as neurogenesis in the subgranular zone (SGZ) were analyzed.

Results: In addition to CA1 neuron loss, homozygous Tg4-42 at 6 months showed a ~ 45% decrease in SGZ neurogenesis, accompanied by a ~20% neuron loss in DG. Housing under enrichment conditions maintained both neurogenesis and granule cell neuron loss and led to a partial rescue of CA1 neuron loss and spatial reference memory deficits.

Conclusion: We provide evidence that enhanced physical activity due to environmental enrichment leads to an amelioration of neuronal loss and behavioral deficits in a transgenic mouse model reflecting the sporadic form of AD. This might indicate the relevance of increased physical activity as a potential strategy in the prevention of dementia.

08a. Animal Models: transgenic mice

ADPD5-0604
APP-CTFBETA IS ELEVATED IN A LINE OF APP TRANSGENIC MICE WITH COGNITIVE IMPAIRMENT AND SEIZURES, BUT NO DETECTABLE BETA-AMYLOID OLIGOMERS

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Objectives: Premature death and seizures have been observed in many lines of APP transgenic (TgAPP) mice. Recently, seizures in a line of TgAPP mice were shown to be associated with the overexpression of APP rather than the overproduction of Abeta. Here, we examined a line of TgAPP mice, previously shown to exhibit premature death, cognitive impairment, and seizures – but no amyloid plaques – to determine whether total Abeta or Abeta oligomers were associated with these phenotypes.
Methods: Cognitive performance was assessed using a Corner Index Test as a measure of neophobia (Hsiao et al., Neuron, 1995). Mice were sacrificed and brains lysates were prepared and analyzed by Western blot.
Results: We found no Abeta oligomers in these mice. However, we did find that those animals with cognitive impairment and/or seizures had significantly more APP-CTFbeta than unimpaired/non-epileptic littermates.
Conclusions: Our findings support the hypothesis that seizures and premature death in some lines of TgAPP mice reflect the effects of APP or APP fragments (e.g., CTFbeta) other than Abeta.

08a. Animal Models: transgenic mice

ADPD5-0992

SEX DIFFERENCES IN THE SEARCH STRATEGIES EMPLOYED BY TGCRND8 MICE DURING MORRIS WATER MAZE (MWM) PERFORMANCE

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Objectives

To determine if there is a sex-specific impairment in searching strategies employed by the TgCRND8 mouse model of Alzheimer's disease

Methods

Navigation strategies were analyzed using a novel algorithm, MWM Visual, developed by our team for the efficient classification of spatial, systematic and repetitive looping search patterns in the MWM.

Results

TgCRND8 mice are impaired in MWM compared to NonTg littermates. This is due to a marked difference in searching strategies, notably a phenotypic tendency towards repetitive looping at the expense of spatial learning. Unlike NonTg mice, TgCRND8 mice are unable to learn a primarily spatial strategy over the test days. We show that females are more impaired than males at six months of age. Male TgCRND8 mice show an initial impairment in spatial navigation compared to NonTg mice but are capable of adapting their learning strategy over time, adopting a predominant spatial navigation profile. Females exhibit more robust learning impairments than males. Female TgCRND8 mice are capable of spatial navigation but this strategy never predominates with females unable to overcome their phenotypic preference for repetitive looping. Sex differences are not due to the visual impairments inherent in this mixed C57BL/6 x C3H/HeN line but rather inconsistencies in adopting a more efficient searching strategy.

Conclusions

These results identify subtleties in learning and memory impairment in TgCRND8 mice that model potential sex differences associated with disease severity.

08a. Animal Models: transgenic mice

ADPD5-0999
MUTATION OF AN AMYLOID BETA PROTEIN BINDING SITE ON ENDOGENOUS MYELIN BASIC PROTEIN IN MICE
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Endogenous inhibitors of amyloid ß protein (Aß) assembly in brain may play an important role in suppressing amyloid formation and deposition in Alzheimer's disease and related disorders. Previously, we showed that myelin basic protein (MBP), a prominent component of the axonal myelin sheath, can strongly bind to Aß peptides and potently inhibit their assembly into fibrils. More recently, we identified residues K54, R55 and G56 in MBP as important in mediating its interaction with Aß. Here, we show that mutation of all three residues together completely disrupts MBP binding to Aß and blocks its inhibition of Aß fibrillar assembly in vitro. To investigate the in vivo consequences of this site on MBP we generated novel mice (MBP-KRG mice) where these three key residues in endogenous mouse MBP were mutated. MBP-KRG mice were fertile, viable and exhibited no overt behavioral abnormalities. Upon further examination by electron microscopy MBP-KRG mice appeared to exhibit a thicker myelin layer surrounding axons suggesting that this site on MBP may play a role in myelination. The MBP-KRG mice were bred with two lines of human AßPP transgenic mice: Tg-5xFAD mice that develop robust parenchymal amyloid plaque pathology and Tg-SwDI mice that develop extensive cerebral microvascular amyloid pathology. The bigenic MBP-KRG/Tg-5xFAD mice and MBP-KRG/Tg-SwDI are being evaluated to determine if mutation of this key site on MBP influences the onset and severity of amyloid pathologies.

08a. Animal Models: transgenic mice

ADPD5-1293
AGE-RELATED CHANGES IN SHORT-TERM AND LONG-TERM PLASTICITY IN TG2576 MICE
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Objectives: TG2576 mice are one of the best characterized models to study amyloid-dependent synaptic dysfunction related to Alzheimer's Disease (AD). Several studies using this model previously described an impairment of long-term potentiation (LTP) in different regions of the hippocampus, although in each study only a single form of LTP was investigated. Here, we describe a series of experiments to study short-term and long-term plasticity by using paired-pulse facilitation (PPF) and both early and late forms of LTP, respectively. Additionally, we examined these different forms of plasticity during aging in wild-type and TG2576 mice.
Methods: Hippocampal slices from 3, 10 and 15 month old wild-type and TG2576 mice were used to measure the slope of fEPSPs in the CA1 region. Validated protocols were applied for the induction of PPF, early-LTP and late-LTP and its interaction with pharmacological manipulations.
Results: Our preliminary results show that in hippocampal slices from 10 months old TG2576 mice, early-LTP is compromised in contrast to late-LTP. Moreover, pharmacological investigation regarding the involvement of NMDA receptors and L-type voltage-gated calcium channels on late-LTP suggests a differential mechanism of induction among groups.
Conclusions: Our results indicate that early-LTP and late-LTP are differentially affected regarding wild-type and TG2576 during age. Furthermore, these differences can be partially explained under the scope of NMDA receptors and L-type voltage-gated calcium channels as a molecular background. New experiments are currently ongoing to create a wide-ranging framework of synaptic plasticity in TG2576 mice during aging.

08a. Animal Models: transgenic mice

ADPD5-1391
IN VIVO DIFFUSION PROFILE OF TWO MOUSE MODELS OF ALZHEIMER'S DISEASE
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Objectives. Diffusion Tensor Imaging (DTI) is useful to detect alterations in mild cognitive impairment (Stahl et al., Radiology 2007) and Alzheimer's Disease (AD) (Liu et al, Neurobiol Aging 2011). Within the IMI-PharmaCog European Consortium (www.alzheimer-europe.org) we compared the diffusion profile of two mouse models of age-dependent amyloid deposition in order to detect markers of disease progression homologous to those found in human AD patients.
***Methods*.** Wild-type (WT), double (TASTPM) and triple (TauPS2APP) mice underwent DTI at 4, 8, 12, 18 and 24 months of age. Several regions of interest including corpus callosum, anterior commissure and hippocampus, were manually drawn and diffusion indices were extracted using FSL.
Results. Corpus callosum and anterior commissure of TASPM showed a decrease in anisotropy and axial diffusivity (index of axonal injury) concomitant to an increase of radial diffusivity (index of axonal demyelination). These alterations started at 12 months of age ($p < 0.001$). Greater hippocampal mean diffusivity was reported in older TauPS2APP mice relative to WT.
***Conclusions*.** Age-related deficits in the corpus callosum of TASTPM mice and in the hippocampus of TauPS2APP seems to reflect the human AD pathology (Acosta-Cabronero et al., PLoS One 2012; Kantarci et al., Neurology 2005). Treatments using amyloid lowering agents are on-going to validate these promising murine biomarkers.

08a. Animal Models: transgenic mice

ADPD5-1726

BEHAVIORAL, BIOCHEMICAL AND IMAGING CHARACTERIZATION OF ALZHEIMER'S DISEASE MOUSE WITH APPSWEDI MUTATIONS AND NOS2 GENE KNOCK-OUT (CVN MOUSE)

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CVN mouse, with APPSweDI mutations NOS2 knockout background, exhibits wide range of AD hallmarks including increased insoluble Aβ and plaque formation, inflammation, tau phosphorylation, hippocampal neuronal death and behavioral deficits (Wilcock et al 2008). Validation data from Charles River CVN mouse in-house testing colony shows reproducible AD defects, and positions CVN mouse as the most complete AD model available.

Wild-type and CVN mice were studied starting at age of 3 months. Behavioral battery included Open field, Barnes maze, Radial arm water maze, and Contextual fear conditioning. At 3,6,9 and 12 months of age, tissue were collected for biochemical analysis. Immunohistochemical stainings for Aβ1-40 and microglia were performed, and neuronal number was evaluated by CFV histological staining. MRI volumetric as well as spectroscopic (1H-MRS) analyses were performed.

CVN mice exhibited significant age-dependent behavioral deficits in Barnes maze, Radial arm water maze and Contextual fear conditioning. Robust biochemical changes, including increased number of dense amyloid plaques in hippocampus, thalamus and cortex, and increased levels of insoluble Aβ subtypes were evident. Significant inflammatory response detected by Iba-1 and CD45 immunoreactive microglia was heavily condensed around the plaques in all brain regions studied. The number of viable neurons in hippocampus was significantly decreased in aged animals. In 1H-MRS several translational AD-related metabolic changes were detected.

The CVN mouse provides a more complete tool to study novel therapies targeted for treatment of AD. Several desired AD-related end-points are present in this mouse line making it a valuable model for drug development.

08a. Animal Models: transgenic mice

ADPD5-2140

MRNA PROFILING OF HIPPOCAMPAL NEURON LOSS IN THE TG4-42 MODEL EXPRESSING ABETA4-42

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Objectives: N-truncated Aβ4-42 has been identified as a particular abundant Aβ species in the hippocampus and cortex of AD patients. Tg4-42 mice exclusively express N-truncated $A\beta_{4\text{-}42}$ and develop intraneuronal Aβ aggregation and behavioral deficits albeit without plaque formation. Strikingly, Tg4-42 mice develop a massive CA1 neuron loss. The gene expression profiles of Tg4-42 mice were compared to the widely used plaque-developing 5XFAD mouse model using next-generation sequencing.
Methods: Next generation sequencing was used to identify differentially expressed genes (DEGs) in an unbiased manner. DEGs were subsequently verified by quantitative PCR. Furthermore, mice were assessed for their ability to learn in the Morris water maze.
Results: Aged Tg4-42 displayed memory deficits similar to 5XFAD mice. Nineteen DEGs were identified in presymptomatic young 5XFAD mice, while no DEGs could be isolated in young Tg4-42 mice. In the aged cohort, 131 DEGs were found in 5XFAD and 56 DEGs in Tg4-42 mice. Intriguingly, 36 DEGs were identified in both mouse models.
Conclusions: The comparison of the Tg4-42 mouse model with 5XFAD model, revealed a remarkable overlap in the molecular signature. The pool of genes that showed differential expression exclusively in Tg4-42 is likely associated to soluble Aβ as no extracellular plaques are found in this model. Many of the DEGs specific to the 5XFAD model belong to neuroinflammatory processes typically associated with plaques. The jointly differentially expressed genes might indicate common pathways that are involved in the comparable memory decline and neuron loss apparent at twelve months of age in both transgenic models.

08a. Animal Models: transgenic mice

ADPD5-2231

EARLY COGNITIVE DYSFUNCTION IN A HUMAN AMYLIN OVEREXPRESSING APP TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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Objectives
Recently, accumulating evidence points to a prominent role of type 2 diabetes (T2D) in age-related cognitive decline and neurodegenerative dementia including Alzheimer's disease (AD). Impaired glucose tolerance and insulin resistance have been associated with impaired cognitive performance both in human patients and in animal models of AD. To investigate the effects of amylin-induced impaired glucose tolerance on beta-amyloid-related pathology and cognitive dysfunction *in vivo*, we generated a novel mouse model of both T2D and AD-like neuropathology.
Methods
Human amylin (hIAPP) transgenic mice on an FVB background were crossbred with PSAPP bigenic mice overexpressing human APP carrying the Swedish mutation and the Presenilin1 delta exon 9 mutation. Metabolic parameters including fasting glucose, glucose tolerance and insulin secretion as well as spatial and object recognition memory were assessed in the four different genotypes PSAPP/hIAPP, PSAPP, hIAPP and WT at 4.5 months of age.
Results
Glucose tolerance was impaired both in the hIAPP single transgenic mice modeling T2D and in the PSAPP/hIAPP double transgenic mice modeling both T2D and AD-like pathologies. Interestingly, cognitive assessment on two hippocampus-dependent cognitive tasks revealed impaired memory performance only in the PSAPP/hIAPP double transgenic mice but not in the hIAPP nor in the PSAPP single transgenic mice.
Conclusions
The results of this study reveal that overexpression of human amylin exacerbates hippocampus-dependent cognitive dysfunction in the PSAPP transgenic mouse model of Alzheimer's disease and suggest that the PSAPP/hIAPP double transgenic mice may be a valuable model to study the role of amylin-related glucose intolerance in AD pathogenesis.

08a. Animal Models: transgenic mice

ADPD5-2246

DEVELOPMENT AND CHARACTERIZATION OF A NOVEL TRIPLE TRANSGENIC MOUSE MODEL FOR ALZHEIMER'S DISEASE

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Amyloid-plaques and neurofibrillary tangles composed of Tau are the defining neuropathological features of Alzheimer's disease (AD). Recently, many studies report that amyloid-beta trigger Tau pathology in experimental systems. But the other studies had shown that tau pathology could trigger Abeta aggregation. To answer this controversy, we have crossed 5XFAD mice co-expressing human mutant APP695 with the Swedish, Florida, and London mutations and human mutant presenilin-1 (PS1) with the M146L and L286V mutations with the MAPT mice over-expressing human mutant tau with the P301L mutation. Our triple transgenic 5XFAD/MAPT mice have been characterized at 3, 5 and 7 months of age. Extracellular amyloid deposits were not changed in the 5XFAD/MAPT compare with 5xFAD at all ages. However, hyper-phosphorylated and accumulated tau pathology is dramatically increased in the hippocampus at 5 month old mice. Also memory deficit is observed compare with age-matched MAPT mice. These data suggest that tau pathology being downstream of amyloid pathology in our model mice. 5XFAD/MAPT mouse model shows a fast development of disease and aspects of AD neuropathology. It will be useful model for Abeta and tau interact study, also for pre-clinical intervention trials.

08a. Animal Models: transgenic mice

ADPD5-2251

DISTINCT EARLY AND LATE BEHAVIORAL CHANGES IN MOUSE MODELS OF ABETA TOXICITY, AS MEASURED IN AN AUTOMATED HOME-CAGE SYSTEM

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Alzheimer's disease (AD) is characterized by progressive neuropathological changes and decline in cognitive function. Transgenic mice carrying AD-causing mutations in PS1 and APP have enhanced levels of toxic Aβ peptide species in the brain. These transgenic mice, as well as mice that are i.c.v. infused with toxic Aβ oligomers, show deficits in memory tasks, confirming the idea that Aβ oligomers play a critical role in AD. Strikingly, these memory deficits have been reported before the onset of pathological changes (e.g. plaques) and in the absence of neurodegeneration. To differentiate between the behavioral effects of toxic Aβ oligomers in the absence and presence of neuropathological changes, we longitudinally compared the behavioral profile of several mouse models of Aβ toxicity. Using an automated home-cage system and 24/7 video tracking and analysis we measured numerous spontaneous behavioral phenotypes including locomotor activity, feeding, sheltering and circadian rhythm. In addition, we measured cognitive performance in a novel automated 4-day discrimination and reversal learning task for cognitive flexibility in an automated home-cage. We observed highly significant behavioral changes in transgenic mice at 6 weeks of age, before neuropathology, whereas other behavioral features were only detected at 6-12 months of age, after onset of neuropathology. Moreover, we also detected highly significant age-dependent changes in wild type littermates in the majority of behavioral readouts. We conclude that longitudinal measurements are required to dissociate the effects of Aβ oligomer toxicity, neuropathological changes, and normal aging in mouse models of AD.

08b. Animal Models: transgenic rats

ADPD5-0610

HIPPOCAMPAL PROTEOME ANALYSES REVEAL STAGE-SPECIFIC CHANGES IN A RAT MODEL OF ALZHEIMER'S DISEASE.

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Alzheimer's disease (AD) is characterized by progressive cognitive impairment associated with the accumulation of amyloid beta-peptide and neuronal degeneration in several brain regions and more importantly in the hippocampus. To elucidate the molecular changes occurring as the disease is triggered and progresses, we used the McGill-R-Thy1-APP rat model of AD-like amyloid pathology at 2 time-points: at 3 months of age when the amyloid pathology is only present in the intraneuronal compartment but cognitive deficits are already present; and at 12 months of age when extracellular plaques are abundant and cognitive deficits have progressed. Quantitative proteomics analyses of hippocampal tissue were carried out using isobaric tagging for relative and absolute quantitation (iTRAQ) with liquid chromatography/mass spectrometry analyses in a Q-Exactive Orbitrap to identify genotype and time-dependent changes in protein expression. After correction for multiple testing, expression levels of 64 proteins were found to be considerably different in transgenic versus wild-type rats at 3 months of age, as were 86 proteins in the 12 month age group. Interestingly, the proteins affected at the pre-plaque stage vary widely from the proteins impacted at the post-plaque stage, with only 9 proteins common to the two time-points. This minimal overlap supports the hypothesis that distinctive processes are at work in the hippocampus during the progression of AD. Oxidative stress processes are prominent at early stages while disturbances in amino acid metabolism develop later on. Further exploration of these changing molecular profiles could lead to identification of novel therapeutic targets for early intervention in AD.

08b. Animal Models: transgenic rats

ADPD5-0972

NANOPARTICLE BLOCKADE OF TGF-BETA SIGNALING IN PERIPHERAL MACROPHAGES MITIGATES ALZHEIMER-LIKE PATHOLOGY IN TGF344-AD RATS

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Objectives: Transforming growth factor-beta (TGF-beta) is a critical immunoregulatory cytokine with increased abundance in AD patient brains. Our group has previously shown that genetic ablation of TGF-beta-Smad 2/3 signaling in peripheral macrophages causes brain recruitment of these cells and resolution of cerebral amyloidosis, which does not come at the cost of damaging neuroinflammation. Currently, we are exploring whether pharmacological blockade of TGF-beta signaling in peripheral macrophages using next-generation nanoparticle technology can re-balance inflammation and mitigate AD-like pathology in our pre-clinical TgF344-AD rat model. These rats manifest the full spectrum of age-dependent AD pathologies and cognitive disturbance. **Methods:** To specifically target peripheral macrophages, we developed PEG-PLGA nanoparticles encapsulating SB505124 (a small molecule TGF-beta-Smad 2/3 inhibitor) and the non-toxic fluorescent tracker, Coumarin-6 (designated nano-C6/SB). In vitro experiments were performed to test whether nano-C6/SB target peripheral murine macrophages, inhibit TGF-beta-Smad 2/3 signaling, and alter Abeta uptake by these cells. Following long-term peripheral treatment with nano-C6/SB, aged TgF344-AD rats were behaviorally tested and their brains were analyzed for AD-like pathology. **Results:** Nano-C6/SB directly target peripheral macrophages, effectively inhibit TGF-beta signaling, and increase macrophage Abeta uptake. Moreover, peripheral nano-C6/SB treatment of TgF344-AD rats promotes 1) brain infiltration of C6-positive mononuclear phagocytes that 2) localize to amyloid plaques, 3) attenuates cerebral amyloidosis and 4) tauopathy, and 5) partially remediates cognitive deficits. **Conclusions:** PEG-PLGA nanoparticles encapsulating small molecule TGF-beta-Smad 2/3 inhibitors hold pre-clinical promise to directly target peripheral macrophages for cerebral amyloid clearance.

08b. Animal Models: transgenic rats

ADPD5-1306

THALIDOMIDE RAPIDLY REVERSES SYNAPTIC PLASTICITY DISRUPTION IN A RAT MODEL OF ALZHEIMER'S DISEASE AMYLOIDOSIS

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Pro-inflammatory mechanisms may play a key role in AD pathogenesis. Previously we reported that transgenic rats overexpressing mutant human APP (McGill-R-Thy1-APP) develop a deficit in long-term potentiation (LTP) as early as 3-4 month of age, long before the extracellular deposition of neuritic plaques.

Objectives:

Here we investigated if the immunomodulatory agent thalidomide reversed the deficit in LTP, at an age when these transgenic rats show signs of pre-plaque inflammatory processes in the brain (Hanzel, 2014).

Methods:

In vivo electrophysiology was carried out on either urethane (1.5 g/kg, i.p.) anaesthetized or chronically implanted freely moving male rats. Electrically evoked field excitatory postsynaptic potentials were measured at CA3 to CA1 synapses in the dorsal hippocampus. Our standard 200 Hz high frequency stimulation (HFS) protocol was used to induce LTP.

Results:

LTP was completely inhibited in transgenic rats aged 4 month and over ($p>0.05$, compared with pre-HFS baseline). Repeated i.p. treatment with thalidomide (50 mg/rat/day for 4 consecutive days) reversed the LTP deficit in 6 month-old animals under anesthesia (137±15%, $n=5$, $p<0.05$). The same treatment regime also enabled LTP induction in freely moving animals of similar age ($n=6$, $p<0.05$). Because of the anti-TNFalpha action of thalidomide, we tested the effects of the anti-TNF antibody infliximab (5X50 µg over 3 days, i.c.v.). To our surprise there was no improvement of LTP ($n=4$, $p>0.05$).

Conclusions:

These results support the development of thalidomide-like agents as potential candidates for early AD treatment. However, the beneficial effect of thalidomide may not be related to its ability to reduce TNFalpha production.

08b. Animal Models: transgenic rats

ADPD5-1310

NON-FIBRILLAR ABETA MEDIATES EARLY SYNAPTIC PLASTICITY DISRUPTION IN A RAT MODEL OF ALZHEIMER'S DISEASE AMYLOIDOSISS

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Previously we reported that transgenic rats overexpressing mutant human APP (McGill-R-Thy1-APP) develop a deficit in long-term potentiation(LTP) as early as 3-4 month of age, long before the extracellular deposition of fibrillar Abeta in neuritic plaques.

Objectives:

Here, we applied an anti-Abeta antibody, a BACE1 inhibitor and a gamma-secretase inhibitor to investigate whether or not the LTP deficit in these transgenic rats is Abeta-dependent.

Methods:

In vivo electrophysiology was carried out on chronically implanted freely moving male rats. Electrically evoked field excitatory postsynaptic potentials were measured at CA3 to CA1 synapses in the dorsal hippocampus. A cannula was implanted in the lateral ventricle. Our standard 200 Hz high frequency stimulation (HFS) protocol was used to induce LTP.

Results:

LTP was completely inhibited in transgenic rats aged 4 month and over ($p>0.05$, compared with pre-HFS baseline). Repeated intracerebroventricular injection of the anti-Abeta antibody McSA1 (5X10 microg over 3 days) reversed the LTP deficit in 4 month-old animals (142.7±7.2%, n=5, $p<0.05$). Moreover, the BACE1 inhibitor LY2886721 (5X0.2 nmol over 3 days) reversed the LTP deficit in 4 month-old animals (140.3±16.1%, n=6, $p<0.05$). Similarly, the gamma-secretase inhibitor MRK-560 (5X0.19 nmol over 3 days) reversed the LTP deficit in 5 month-old animals (124.8±8.0%, n=7, $p<0.05$).

Conclusions:

These findings strongly indicate that the LTP deficit in this transgenic rat model is Abeta-dependent. Furthermore, the rapid reversal of the impaired LTP in the pre-plaque stage of amyloidosis provides encouragement for the development of therapeutic interventions early in AD pathogenesis.

08c. Animal Models: primate models

ADPD5-0910
TRANSLATIONAL APPROACH OF COGNITIVE EVALUATION IN THE PRIMATE MICROCEBUS MURINUS

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Objective: The CANTAB battery is a computer-based cognitive assessment using a touch screen. It allows to assess cognitive abilities in humans. Among the tests developed, the Pairwise Discrimination (PD) test was confirmed to be sensitive to prefrontal pathologies. We have adapted this test to the lemurian primate *Microcebus murinus*. Our purpose was to differentiate healthy ageing from ageing-associated disorders such as Alzheimer's Disease (AD).

Methods: The subject has to discriminate two visual stimuli. The acquisition phase (PD) assesses the capability to succeed in a simple associative learning using working memory. The reversal phase (PDR) assesses the cognitive flexibility by measuring the capability to reverse the stimuli. When a subject reaches the criterion in PD (80% of correct responses in two consecutive sessions), it is tested in the PDR.

Results: We trained 32 old mouse lemurs. Seventeen were successfully trained and 12 reached the criterion in PD acquisition and 10 in PDR. Old healthy animals needed 9 sessions for PD and 11.5 sessions for PDR, whereas for AD-like animals, *i.e.* animals with risk to develop amyloid deposits, 10.25 sessions were necessary for PD and 20 sessions for PDR.

Conclusion: By using a computerized and standardized battery, we were able to distinguish between healthy old and AD-like animals. These observations highlight the usefulness of CANTAB tests for studying cognitive dysfunction in *Microcebus murinus*. Other tests are now developed to complete our approach that may help in diagnosing age-related disorders.

08c. Animal Models: primate models

ADPD5-1059
GENE ANALYSIS OF THE APP GENE IN THE GREY MOUSE LEMUR, A NONHUMAN PRIMATE WITH ALZHEIMER'S DISEASE-LIKE (AD-LIKE) PATHOLOGY

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Objectives: The development of small nonhuman primate models for aging and neurodegenerative disease studies has considerable advantages in terms of costs, rapid colony growth and phylogenetic relationships to humans. The grey mouse lemur, *Microcebus murinus* (MIM), is a small primate with a short lifespan (6-8 years). Some of them develop the lesions of Alzheimer's disease (AD), such as amyloid deposits and/or cerebral atrophy. They are called 'AD-like'. Our objective is to look for APP mutations that might be linked to these lesions.
Methods: We selected 52 'AD-like' animals based on their genealogical tree showing a familial history of brain amyloid deposit and/or cerebral atrophy, and 41 wild-type (WT) as controls.
Results: The sequencing of the APP gene for the 93 animals led us to identify 86 single nucleotide variations (SNVs). Two of them are more frequently associated with the AD-like trait ($p<0,05$). By reconstruction of the LD-blocks for these 2 SNVs we found them on the block 10 (19 kbp), and on the block 15 (16 kbp) respectively. We found that the haplotypes 6 (H6) from the block 10 and the haplotype 2 (H2) from the block 15 are significantly associated to the AD-like phenotype ($p<0,002$). In addition, the genotype H1/H6 from the block 10 and the genotype H1/H2 from the block 15 were associated to AD-like animals ($p<0,002$).
Conclusion: Our results showed that some of the genetic variations observed in the APP gene are associated to the AD-like trait in our primate model MIM.

08d. Animal Models: drosophila

ADPD5-0571
EVALUATION OF ABETA43 TOXICITY IN VIVO USING DROSOPHILA MELANOGASTER
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OBJECTIVES: Amyloid plaques observed in Alzheimer's disease (AD) brains are composed of a heterogeneity of Aβ peptides. Next to the well-studied $A\beta_{42}$ and $A\beta_{40}$ species, respectively highly pathogenic and rather harmless, $A\beta_{43}$ peptides were lately hypothesized to be instrumental in AD pathogenesis. Indeed, $A\beta_{43}$ is frequently found in AD brains in both dense and diffuse amyloid plaques and recent *in vitro* studies have stressed the ability of $A\beta_{43}$ to induce toxic effects on neuronal cultures. However, whether $A\beta_{43}$ directly triggers neurotoxicity *in vivo* has not been evaluated so far.
METHODS: We generated a new transgenic *Drosophila* line of human $A\beta_{43}$. Since eye roughening is a commonly-used readout to evaluate the extent of protein toxicity including Aβ peptides, we constitutively overexpressed human $A\beta_{43}$ in the fly eye-compound using the GMR-Gal4 driver.
RESULTS: We found that the specific overexpression of human $A\beta_{43}$ in the *Drosophila* eye-compound caused ommatidial disorganization together with the development of a rough eye phenotype.
CONCLUSIONS: Our data suggest that human $A\beta_{43}$ is toxic *in vivo* and delineate its contribution to the pathological events leading to neurotoxicity in AD.

08d. Animal Models: drosophila

ADPD5-0637
ROLE OF SARAH/NEBULA IN AMYLOID-BETA42-INDUCED NEUROLOGICAL IMPAIRMENT IN DROSOPHILA

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The Down Syndrome Critical Region 1 (DSCR1) protein, an inhibitor of Ca^{2+}-dependent phosphatase calcineurin, has been shown to be elevated in the brains of Down syndrome (DS) or Alzheimer's disease (AD) patients. Because DSCR1 has been implicated in the neuropathology of DS, an increased level of DSCR1 is believed to be deleterious to neuronal health. On the other hand, because hyper-activated calcineurin has been implicated in neuronal damage, calcineurin inhibitors such as DSCR1 are expected to exert beneficial effects against AD neuropathology. However, the role of DSCR1 in the pathogenesis of AD is still unclear. Here, we investigated the role of *sarah* (*sra*)/nebula, a *Drosophila DSCR1* ortholog, in the amyloid-β42 (Aβ42)-induced neurological phenotypes in *Drosophila*. Overexpression of the *sra* using the *UAS-GAL4* system exacerbated the rough eye phenotype, and decreased the survival rates of the *Aβ42*-expressing flies without modulating the *Aβ42* expression. Similarly, treatment with chemical inhibitors of calcineurin such as FK506 and cyclosporine A, or knockdown of *calcineurin* expression by RNAi, worsened the rough eye phenotype induced by *Aβ42*. Furthermore, the *sra*-overexpressing flies displayed significantly decreased mitochondrial DNA content, as well as increased susceptibility to oxidative stress compared to the control flies. Taken together, our results demonstrating that overexpression of *sra* has an augmenting role on Aβ42 cytotoxicity in *Drosophila* suggest that the *DSCR1* up-regulation or *calcineurin* down-regulation in the brains of DS or AD patients may accelerate neuropathogenesis of AD in humans as well.

08d. Animal Models: drosophila

ADPD5-1483

ROLE OF GLUCOSE METABOLISM IN Aß PATHOLOGY IN DROSOPHILA MELANOGASTER

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Objectives

Reduced glucose metabolism in the brain is one of the main symptoms of Alzheimer Disease. This can precede the appearance of the clinical symptoms and is a strong predictors of who will go on to develop clinical AD. This suggests that glucose metabolism could play a role in the development of AD.
However, this relationship has not been extensively studied and a possible molecular mechanism is currently unknown.
The aim of this project was to establish whether modulation of glucose metabolism in neurons had an effect on Aß toxicity and how this was mediated.

Methods

Our laboratory has developed an adult-onset Drosophila model of AD which expresses a highly pathogenic form of human Aß (Arctic mutant) only in neurons of adult flies. These flies recapitulate some of the pathologies of AD such as defects in mitochondrial transport and synaptic dysfunction, leading to deficits in motor behaviour, thus making them a good model to investigate the role of Aß in neuronal dysfunction.
We altered glucose metabolism in this model by down-regulating and overexpressing a Glucose transporter (GluT1) to increase or decrease glucose transport in Aß expressing neurons.

Results

Overexpression of GluT1 improved several of the phenotypes of Aß expressing flies, including lifespan and motor ability, as well as a number of molecular phenotypes.

Conclusions

An increase in glucose metabolism can reduce the toxicity of Aß in Drosophila neurons. This suggests that targeting glucose metabolism could possibly provide a promising therapy for AD.

08e. Animal Models: zebra fish

ADPD5-1792

VALIDATION OF A NEW ZEBRAFISH MODEL FOR AMYLOID-BETA TOXICITY AS A FUTURE TOOL FOR DRUG DISCOVERY

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With our increased life expectancy, the number of people affected by AD and other neurodegenerative diseases will be substantially larger. There is a huge need to find novel treatments and disease preventions for AD. In this project we were interested in validating a new zebrafish model for Aβ-mediated memory disturbances as a tool for future AD drug discovery. We pretreated zebrafish embryos with the chemicals prior to the injection of Aβ into the brain ventricle. The next day, we tested learning as well as neuronal cell death levels in vivo. We tested a number of currently used AD medications as well as newly developed compounds, including nuclear receptor ligands. Our preliminary data show that both memantine and donepezil prevents against Aβ-mediated learning impairments in our model. This indicates that the zebrafish can serve as a tool to validate compounds that could be potential therapeutics for AD.

08f. Animal Models: pharmacological & lesion models

ADPD5-0693

AFTIN-5, A CHEMICAL INDUCER OF BETA-AMYLOID (1-42) PRODUCTION: TOWARDS A NOVEL ANIMAL MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Objectives: The Aβ1-42-inducing aftins constitute unique chemical tools for both fundamental investigations and translational studies in sporadic Alzheimer's disease (sAD). Following the demonstration that aftin-5 triggers Aβ1-42 production in cultured neurons and various cell lines, our objective is to develop a chemically-induced sAD animal model.

Methods: Aftin-5 passage through the blood-brain barrier (BBB) was assessed *in vitro* in a cell-based BBB model. Pharmacokinetic (PK) parameters were then evaluated after intraperitoneal and oral administration of aftin-5 in mice. Due to short aftin-5 brain elimination half-life, C57Bl/6J mice were exposed sub-cutaneously to aftin-5 or vehicle during 28 days through an Alzet pump. Blood and brain tissue were collected at various times. Aftin-5 levels were measured by LC-MS/MS and Aβ1-42 was quantified using MSD technology. Data was analysed by one-way ANOVA and results were considered significant if $p<0.05$.

Results: Analysis of *in vitro* BBB permeability data and *in vivo* PK parameters confirm the ability of aftin-5 to cross the BBB. Our findings reveal that continuous subcutaneous infusion of aftin-5 in mice triggers a dose-dependent increase in the brain levels of Aβ1-42 (mean fold change ±SEM): at 3mg/kg/d (4.24±0.46 ; $p<0.01$) and at 30mg/kg/d (5.74±1.08 ; $p<0.001$). The mechanisms underlying *in vivo* Aβ1-42 induction remain to be identified.

Conclusions: Brain translocation of aftin-5 through the BBB is associated with a significant increase in Aβ1-42 production. Cognitive tests, molecular imaging and biochemical analysis, currently in progress, will determine whether aftin-5 could be used as a new pharmacological tool to develop chemically-induced sAD animal models.

08g. Animal Models: natural & seminatural models

ADPD5-2109

STABILIZED LOW-N ABETA PEPTIDE OLIGOMERS INDUCE COGNITIVE DEFICITS ASSOCIATED WITH SYNAPTIC DYSFUNCTION IN RAT

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Objectives

With current treatments for Alzheimer's disease (AD) only providing temporary symptomatic benefits and an ageing population, disease modifying drugs are urgently required. This approach relies on improved understanding of the early pathophysiology of AD. A new hypothesis has emerged, in which early memory loss is considered a synapse failure caused by low-n Aβ oligomers (AβO).
Our aim was to investigate synaptic markers and cognitive function following Intracerebroventricular (ICV) infusion of AβO in the rat.

Methods

Adult (male and female) hooded Lister rats received ICV administration of vehicle or AβO (n=10/group). Animals were tested in the novel object recognition (NOR) paradigm, to assess recognition memory at different time points following AβO administration (days 4-70). Following behavioral experiments brains were removed and analyzed for synaptic and neuronal markers. Data from vehicle and AβO groups were analyzed in SPSS using a one-way ANOVA.

Results

We found robust long lasting deficits in NOR in the AβO treated animals. This deficit was found in both male and female animals and was present at day 4 and up to 70 days post-administration of AβO. Post-mortem analysis revealed significant deficits in synaptic (SNAP25 and PSD 95) but not neuronal (n-acetyl aspartate) markers in AβO treated animals.

Conclusions

Taken together the results suggest that acute ICV administration of AβO may be a useful model to study the early mechanisms involved in AD and may provide us with a platform for testing novel therapeutic approaches that target the early underlying synaptic pathology.

08g. Animal Models: natural & seminatural models

ADPD5-2290

THE CANINE MODEL OF ALZHEIMER'S DISEASE PROGRESSION: DISEASE PROGRESSION AMYLOID BIOMARKERS FOR EVALUATION OF NOVEL DISEASE MODIFYING THERAPEUTICS

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Objectives
The current study sought to establish cross-sectional and longitudinal evidence of age differences in CSF concentrations of amyloid-beta in Beagle dogs.
Methods
CSF samples from N =130 (age range: 2.0 – 17.3 years) dogs were collected by cysterna magna puncture under short-term anesthesia. To determine reliability, a second sample was collected from a subset of 21 dogs, at the same time of day, four days later. Dogs were divided into four age groups (young, n=17, 2.00–2.58 years; middle-age, n=21, 6.33–7.25 years; old, n=57, 8.00–11.92; and senior, n=35, 12.00–17.33 years). Approximately 1.5 years later, an additional sample was collected from a subset of dogs to evaluate longitudinal changes. Amyloid-beta isoforms were quantified by ELISA. Percent amyloid-beta42 served as the dependent variable for the age analysis conducted by ANOVA.
Results
A significant effect of age [$p<0.002$] on %amyloid-beta42 was found, which reflected significantly higher levels in middle aged dogs compared to all other age groups ($p<0.05$ in all cases). Also, levels in senior dogs were significantly ($p<0.05$) lower than in old dogs. Analysis of the longitudinal data is ongoing.
Conclusions
Prior to the decline in %amyloid-beta 42 previously reported in canine aging, there is a significant increase in %amyloid-beta 42 from young to middle age. This supports the hypothesis that a threshold concentration of amyloid-beta42 is achieved during middle-age prior to brain amyloid deposition. Overall, CSF amyloid-beta42 levels should serve as a reliable and translational biomarker for preclinical aged canine studies.

08h. Animal Models: other

ADPD5-0856

AGED DOGS ARE NOT SUITABLE ANIMAL MODELS FOR STUDY OF ALZHEIMER'S DISEASE

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Objectives

Aged dogs develop many features of human aging and Alzheimer's disease (AD) such as disorientation, confusion, anxiety, changes in sleep cycle, and neuronal loss. Many studies have reported the presence of amyloid plaques in various brain areas of dogs. Therefore, dogs are proposed as suitable models for AD. We aimed to characterize the neuropathological hallmarks in aged dog brains.

Material and methods

Brains from clinically tested dogs were extracted postmortem and fixed in paraformaldehyde. Cortex and hippocampus were stained by immunohistochemistry using antibodies recognizing tau (AT 8), beta amyloid (4G8), phosphor TDP-43 (11-9), FUS (anti-FUS), alpha synuclein (4D6), neurofilament (SMI 31) and p62 (3/p62).

Results

Our results show that the senile plaques in dogs were exclusively in diffuse forms, which is sign of normal aging. We did not find any neuritic plaques in the aged dog brains. Furthermore, only one case out of 40 examined aged dogs showed very few NFT's pathology in the hippocampus. Other pathological lesions typical for human's neurodegenerative disorders such as Lewy bodies, FUS aggregates, TDP-43 aggregates and neurofilaments aggregates were not observed in any of the dog brains examined.

Conclusions

These results suggest that the pathological lesions in aged dog brains are different than those observed in AD brains. Therefore, aged dogs are suitable for studying aging process, but cannot be considered as an appropriate model for the study of human neurodegenerative diseases such as AD. Acknowledgement This work was supported by research grant APVV-0206-11, APVV-0677-12.. ;

08h. Animal Models: other

ADPD5-1372
LYCOPENE ATTENUATES INSULIN RESISTANCE ASSOCIATED COGNITIVE DEFICITS IN RATS
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Objective: Alzheimer's is primarily a metabolic disease, which is characterized by glucose intolerance and central insulin resistance (IR). Excessive fructose consumption produces hippocampal insulin resistance. Lycopene is a natural polyphenol which has demonstrated neuroprotective effects in various preclinical and clinical studies. Thus, the present study was designed to elucidate the role of NF-κB pathway in neuroprotective effect of lycopene.
Methods: Six-week-old male Wistar rats were fed with 15% fructose in drinking water for 24 weeks. Body mass, food and water intake was measured regularly as well as plasma insulin, blood glucose, glycosylated heamoglobin, HOMA-IR, lipid levels and blood pressure were measured to ensure development of IR. Cognitive impairment was measured by Morris water maze test and elevated plus maze on 24th week. Lycopene treatment was initiated after 6th week and continued till 24th week.
Results: Insulin resistance was evident at 6th week and persisted till end of study (24th week) as demonstrated by significant increase in body weight, plasma insulin, blood glucose, glycosylated heamoglobin, HOMA-IR, blood pressure and a deranged lipid profile. Cognitive deficit was significantly evident at 24th week. Fructose-induced neuronal deficits were coupled with significant alterations in oxidative-nitrodative stress along with elevation of NF-κB and its downstream mediators as TNF-α, TGF β1, caspase-3 and IL 1β levels. Lycopene dose-dependently ameliorated emergence of insulin resistance induced memory impairment along with mitigation of cytokine levels.
Conclusion: These results provide a novel insight into the neuroprotective effects of lycopene and its possible therapeutic role in neuronal insulin resistance-induced memory impairment.

08h. Animal Models: other

ADPD5-1628

ANALYSES FOR LYSOPHOSPHATIDIC ACID RECEPTOR GENES IN MEDAKA TOWARD GENERATING FISH MODELS OF NEURODEGENERATIVE DISEASE

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Neurite branches are fundamental for neuronal functions, and their collapse or degeneration might lead to neurological dysfunction observed in neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. Lysophosphatidic acid (LPA), a signalling phospholipid, has been shown to be involved in neurite retraction and branching, tau phosphorylation, and amyloid beta formation. However, how abnormal LPA receptor-mediated signaling is related with the onset or progression of neurodegenerative diseases remains still unclear. To address this issue, we attempted to generate genetically-engineered medaka fish (*Oryzias latipes*) for LPA receptor genes. Medaka is an experimental fish that can be easily maintained, propagated, and analyzed, and whose genome has been completely sequenced. Because there was limited information available regarding medaka LPA receptors. we first examine the genomic structures, expression, and functions of six LPA receptor genes, *Lpar1–Lpar6*. Our analyses reveal that the genomic structures of *Lpar1* and *Lpar4* are different from those deduced from the database. Functional analyses using a heterologous expression system demonstrate that all medaka LPA receptors except for LPA_{5b} respond to LPA treatment with cytoskeletal changes. These findings provide useful information on the structure and function of medaka LPA receptor genes to generate genetically-engineered medaka using a Crisper/Cas system. Our future study will generate and analyze these medaka to explore the pathophysiological significance of LPA signaling in neurodegenerative diseases.

08h. Animal Models: other

ADPD5-1772
CHANGES IN BEHAVIOR AND BRAIN ELECTROPHYSIOLOGY IN A MOUSE MODEL OF DOWN SYNDROME

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Objective: Down syndrome is characterized by mental retardation, muscle hypotonia, and age-related Alzheimer-like disease neuropathology. The Ts65Dn mouse is the first trisomic Down syndrome model harboring an extra copy of a portion of the murine chromosome 16, including genes believed to cause many of the symptomatology of the disorder; these mice also show elevated expression of APP. The current investigation was focused on the relationship between behavioral deficits and changes in LTP.
Methods: PhenoCube® NeuroCube® and SmartCube® are high-throughput platforms that assess circadian, cognitive, motor behavior, anxiety, gait, and other domains using PGI's proprietary Computer Vision automated scoring system and machine learning algorithms to define phenotypic signatures. Other standard test follow published protocols. Extracellular fEPSPs were evoked in the CA1 region in hippocampal slices (13-14mo old mice). LTP was induced with tetanic stimulation (3 x 1 sec x 100 Hz trains; 5 min inter-train interval).
Results: We show that Ts65Dn mice are hyperactive especially during the night using our high throughput phenotypic platforms in addition to the expected learning deficits, reduced grip strength and weight. We also report deficient LTP following repeated tetanic stimulation (reduced by approximately 60% in Ts65Dn trisomic male mice compared to their disomic controls), suggesting impairment in the induction phase of LTP.
Conclusion: Cognitive changes are consistent with the observed decrease in LTP later in life. The observed functional changes suggest that these mice are a useful model for investigation of brain amyloid associated diseases occurring without formation of plaque pathology.

08h. Animal Models: other

ADPD5-2039

DEVELOPMENT AND CHARACTERIZATION OF AN AGED ONSET MODEL OF ALZHEIMER'S DISEASE IN DROSOPHILA MELANOGASTER

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Objectives: The biggest risk factor for developing Alzheimer's disease (AD) is age. Though many animal models of AD exist, very few are capable of analyzing the effect of older age on AD pathology. In an attempt to better model LOAD, we developed a novel “aged AD” model using Drosophila melanogaster.
Methods: In our model, we express low levels of the human AD proteins APP and BACE1 specifically in the fly's central nervous system by using the Gal4/UAS system and raising flies at 18°C. We assessed Aβ levels, neuroanatomy, climbing, learning, and memory in APP;BACE and control flies over the course of their lifespan.
Results: Expression of APP;BACE did not significantly alter lifespan. In flies expressing APP;BACE, significant increases in Ab levels were detected, compared to control flies, starting at day 30 and continuing until death. At the same age, degeneration of mushroom body axons, dendrites and cell bodies were also observed in these flies. Defects in climbing were observed in APP;BACE flies starting at day 42. Control and APP;BACE flies display normal learning across all ages, but day 80 APP;BACE flies have defective immediate recall memory.
Conclusion: This fly model of late onset AD displays numerous similarities to the disease course observed AD patients. The gradual accrual of gradual accrual of Aβ within the CNS leads to the onset of behavioral and neuropathological defects late in the life of these transgenic animals.

09a. Patient Care & Support: caregiver support

ADPD5-0527

DEMENTIA IN NATIVE POPULATIONS; MAKING THE CASE FOR CULTURALLY TAILORED DEMENTIA CARE WITH INDIGENOUS ELDERS AND FAMILIES

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An estimated 370 native peoples—whose ancestors first settled varied regions around the globe—reside in 70 Countries stretching across the vast expanse from the Arctic to the South Pacific Basin. Dementia rates for indigenous elders are presently unknown, but recent longevity improvements and greater prevalence for varying social and health disparities including diabetes and obesity place them at high risk for certain dementias such as Alzheimer's Disease. Objectives: Examine dementia and family care in native populations; identify culturally tailored health programs successful with native populations, and propose research and program approaches to promote culturally tailored psychosocial dementia care in native populations. Method: Using the case of indigenous populations in the U.S. (Native Hawaiians, Alaska Natives, and American Indians), we summarize results from a comprehensive literature review and study data on native eldercare conducted at the University of Hawaii. Results: We present summary data on dementia and psychosocial dementia care in native populations in the US, and provide examples of evidence-based culturally tailored programs that produce positive heath outcomes in breast cancer, obesity, and diabetes; and highlight potential practices for developing supportive family dementia care programs. Conclusion: Greater numbers of native elders are living longer. Dementia is associated with advanced age, and mounting evidence supports cultural variations in elder care preferences. As the family continues to be the major care provider, we argue for research and program development to increase family caregiver support by testing psychosocial dementia care approaches that incorporate cultural values and traditions prevalent in global indigenous elder populations.

09a. Patient Care & Support: caregiver support

ADPD5-1480

ALZHEIMER´S DISEASE: THE DEVELOPMENT OF A WEBSITE

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Objectives: The aim of this study is to present a website development containing information to patients with Alzheimer´s Disease (AD) and their caregivers focusing on the instructions of Speech and Language. **Methods:** The material production followed the stages of analysis and planning, modelling, implementation and evaluation. The website content will be evaluated by healthcare professionals, elderly and caregivers through an online questionnaire available on website. **Results:** Contents on the topic were designed and implemented, specifically on AD definition, what happens in the brain, the characteristic and disease´s steps, Speech and Language orientation, treatment, impacts on the communication and importance of the caregiver. Next step will be the evaluation of this website. This project was approved by Research Ethics Committee´s by number 20836813.0.0000.5417. **Conclusion:** It was sought to make available contents with simple, clear and objective language, providing knowledge to the general public who can have access to this material helping them to get a better understanding about AD.

09a. Patient Care & Support: caregiver support

ADPD5-1487

ALZHEIMER'S DISEASE: QUALITY OF LIFE FOR ELDERLY AND THEIR CAREGIVERS.

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Objective: To investigate the differences on perceptions of each quality of life dimension of Alzheimer's Disease (AD) patients and their caregivers. **Methods:** Approval number by the Research Ethics Committee´s is 11599412.1.0000.5417. Sample consisted of elderly and their caregivers. Scale for the Assessment of Quality of Life in AD was applied - QOL-AD. There are three versions: one for the elderly to evaluate their own quality of life (QVi), another for the caregiver to evaluate the elderly (QVci) and for the caregiver self-assess (QVc). **Results:** Sample consisted of 70 individuals, 35 elderly, 71.42% women and 28.57% men; 35 caregivers, 88.57% women and 11.42% men. Age average of the elderly was 78.31 years old, 17.14% were illiterate, 65.71% had not concluded elementary school, 14.28% had completed elementary school and 2, 85% had finished secondary school. Caregivers' age average was 51.54 years. QVi presented a greater perception on the family and worse on the willingness and ability to do leisure activities. QVci pointed to a better perception on the mood and family and worse on memory. QVc presented a greater perception concerning family and worse on capability to leisure activities. There was no statistically significant difference between QVi, age and education level of the elderly, and caregiver age. Statistically significant difference was found between QVc and QVci. **Conclusion:** Perception on the QVi and QVci were similar regarding the greatest and poorest perception. In this context it has been found the family item as the aspect with greater perception in the three versions applied.

09a. Patient Care & Support: caregiver support

ADPD5-1561

CAREGIVER BURDEN OF DEMENTIA: CORRELATION WITH STAGES OF DEMENTIA AND BEHAVIORAL AND PSYCHOLOGICAL SYMPTOMS OF DEMENTIA

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Objective- To evaluate caregiver burden (CB) of dementia alongwith the correlation between caregiver burden with stages of dementia and behavioral and psychological symptoms of dementia (BPSD).
Methods- Study population consisted of 20 consecutive patients of dementia and their caregivers. Dementia was diagnosed by DSM-IV criteria. Patients were assessed using structured proforma of cognitive clinic of Bangur Institute of Neurosciences. Staging was done by Clinical Dementia Rating (CDR) scale. Standard criteria were used to delineate types of dementias. BPSD were assessed with Neuropsychiatric Inventory (NPI). CB was evaluated with Zarit Burden Interview (BI).
Results- Of the 20 patients included in the study, 14 were male and 6 female. Average age of patients was 66.2 years. 12 patients had Alzheimer Disease, 3 had Mixed dementia, 3 Frontotemporal Dementia, 1 Vascular dementia, 1 Dementia with Lewy Bodies. Average BI scores in Global-CDR groups were 11.5 for CDR-0.5, 37.9 for CDR-1, 57 for CDR-2 and 54 for CDR-3. Total NPI scores were: 3.3 for little-or-no burden, 19.7 for mild-to-moderate burden, 24.9 for moderate-to-severe burden, 41.2 for severe burden, respectively. CDR- sum of boxes scores were: 3.3 for little-or-no burden, 6.7 for mild-to-moderate burden, 13 for moderate-to-severe burden, 13.4 for severe burden, respectively.
Conclusions- CB was more with increasing severity of dementia. However, moderate-to-severe and severe burden groups had similar severity of dementia. Behavioral and psychological symptoms of dementia have immense effect on the caregiver burden; especially in moderate to severe dementia it seems to have greater effect than the stage of dementia itself.

09a. Patient Care & Support: caregiver support

ADPD5-2095

CARE OF PATIENTS WITH AD, WHEN THERE IS NO FORMAL CAREGIVERS: CASE DESCRIPTION IN CALI, COLOMBIA

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Background: With the worsening of AD, the patient is becoming increasingly dependent and therefore requires a 24 hours day caregiver to support it in performing activities of daily living. In Colombia, traditionally family caregivers are in charge of this work. Currently, the Health System does not include the cost of institutional care or nursing homes for these patients.
Objective: To describe the motivations and difficulties identified by a group of family caregivers of patients with AD in Cali Colombia.
Methods: A qualitative study was conducted. Family caregivers of patients with AD in moderate and severe degree were invited to participate. After obtaining informed consent, an anthropologist conducted in-depth interviews.
Results: 5/12 caregivers contacted, agreed to participate. Four of the respondents are female. All are first-degree relatives with the patient to whom they care. The most important motivation to assume responsibility for the care incorporates affective and religious references. The difficulties identified are related to patient care management and daily living, out-of-pocket expenses to support it, and self-care. The process is made more difficult by the limited emotional and financial support of other family members, as well as insufficient information on the care received from the Health System. However, it is clearly a reluctance to institutionalize the patient or to surrogate care to non-family caregivers.
Conclusions: In countries like Colombia, where care of these patients is not institutionalized, the system must consider strategies to support family caregivers. This would enhance the health care of patients and avoid negative impacts on caregivers

09a. Patient Care & Support: caregiver support

ADPD5-2129

EFFICACY OF AN ITALIAN PSYCHOSOCIAL INTERVENTION FOR CAREGIVERS OF ALZHEIMER'S PATIENTS

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Objectives. To determine the impact of a psychosocial intervention for caregivers of Alzheimer disease (AD) patients: a) on caregivers' burden, mood, health, social support, service use, and relationships with others; b) on patients' troublesome behaviours.

Methods. 145 home-dwelling dyads (AD patients and their primary caregivers) were enrolled in this randomized controlled study. 75 caregivers (CG, Control Group) received the care/treatment usually given at our center. The remainders (IG, Intervention Group) underwent 6 sessions of individual/family counselling over 4 months, based on the strategies developed both in the REACH program (Wisniewski et al., 2003) and at the NYU-ADRC (Mittelman et al., 1999). Both groups were reassessed at 6 and 12 months. Between and within group analyses were performed using t-test, Repeated-Measures ANOVA, and ANCOVA.

Results. The two groups were comparable at baseline for age, sex, education and patients' disease severity, but not for severity of depressive symptoms. Compared to CG, IG had better health ($p = 0.03$) and lower Hamilton Depression Rating Scale scores at 6 months, even after controlling for depression severity at baseline ($p = 0.03$). However, between group differences disappeared at 12 months.

Within the IG, health and relationships with others improved from baseline to month 6, while emotional distress, depressive symptoms and social support, as well as patients' troublesome behaviours, significantly improved from baseline to month 12. Conversely, within the CG, service use increased from baseline to month 12 ($p<0.05$).

Conclusions. These data support the long-term efficacy of a psychosocial intervention for caregivers of AD patients.

09b. Patient Care & Support: mobile applications

ADPD5-1113

DRIVING PERFORMANCE IN INDIVIDUALS WITH MILD COGNITIVE IMPAIRMENT(MCI): THE ROLE OF DEPRESSIVE SYMPTOMS

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Objectives: Studies focusing on the general population indicate that depression affects driving competence. Goal of the present research was to explore in drivers with MCI the association between depressive symptoms and various indexes of driving performance by applying a driving simulator experiment. **Methods:** A CDR score of 0.5 was required for the diagnosis of MCI. Additional inclusion criteria were the presence of a valid driver's license and regular car driving. The collection of the data included: (a) detailed clinical medical/neurological assessment, (b) extensive neuropsychological assessment, and (c) a driving simulation experiment. **Results:** Depressive symptoms were associated with various driving indexes that include longitudinal driving control measures, namely average speed (r=.570, p=.004), average headway distance (r=-.569, p=.004) and headway distance variation (r=-.564, p=.004) as well as lateral driving control measures, such as lateral position variation (r=.723, p<.001) and average wheel position (r=-.434, p=.034). Moreover, significant associations were present with measures linked to the possibility of a road accident, such as actual number of crashes (r=.584, p=.003), hits of side bars (r=.425, p=.039) and number of speed limit violations (r=.499, p=.013). Notably, the link between depressive symptoms and various driving indexes was evident even after controlling for sleepiness and cognitive functioning. **Conclusion:** The findings suggest that depressive symptoms influence the driving capacity of patients with MCI. Thus, their presence in the specific clinical group should be systematically assessed in order to implement proper therapeutic interventions that might help to improve the driving fitness of certain individuals with a diagnosis of MCI.

09b. Patient Care & Support: mobile applications

ADPD5-1381

THE RELIABILITY OF IPAD COGNITIVE TESTING IN THE HOME ENVIRONMENT: POTENTIAL USE IN CLINICAL TRIAL RESEARCH

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Objectives: With the widespread use of mobile devices, can these technologies be reliably used in clinical trials? We sought to determine the reliability and validity of cognitive test data obtained at-home on an iPad in contrast to in-clinic assessments.

Methods: Forty-five clinically normal older subjects (CDR=0, age=72.9±7.3) were recruited from Massachusetts General Hospital. Subjects were required to make two in-clinic visits one week apart and perform five 30-minute alternate version assessments at-home on an iPad. A Reliability Analysis determined the consistency between the alternate iPad test versions. Correlational analyses examined the association between in-clinic vs. at-home assessments and between iPad vs. standardized paper and pencil tests.

Results: There was excellent reliability between alternate iPad versions (Cronbach alpha coefficient=0.906). Comparing subjects' single in-clinic score to their average at-home score, there was no significant difference in performance between test environments. The in-clinic and at-home scores were significantly correlated (r-squared=0.501, $p<0.001$) suggesting that the at-home tests could act as a proxy for supervised in-clinic assessments. There was also a significant correlation between standard paper and pencil tests and both in-clinic (r-squared=0.322, p=0.002) and at-home iPad assessments (r-squared=0.217, p=0.013).

Conclusions: The findings suggest that reliable cognitive data can be obtained from iPad assessments in the home environment without the assistance of a trained administrator. The at-home and in-clinic assessments were correlated with standard neuropsychological tests suggesting the validity of the at-home tests. IPad assessments performed in the home environment show promise for use in Alzheimer Disease prevention trials.

09f. Patient Care & Support: cognitive training

ADPD5-1223

THE USE OF VIRTUAL REALITY FOR COGNITIVE STIMULATION IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE

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Objective. Virtual reality (VR) technologies hold great opportunities for the development of effective cognitive trainings, because they can provide realistic, familiar but highly controllable environments. In the present studies, we tested the acceptability of two VR solutions to stimulate autobiographical memory and attention in elderly participants and patients with Mild Cognitive Impairment (MCI) and early to moderate Alzheimer's disease (AD).

Methods. *Study 1*. 13 healthy participants (>60years) were administered an autobiographical memory stimulation task in two conditions: a classical stimulation condition, in which photographs of environments were used as prompts, and a VR condition, in which the prompts consisted in VR environments. At the end of each condition, the acceptability and the quality of the experiences were assessed employing standard questionnaires. *Study 2*. 25 patients with MCI and 25 patients with AD (>60years) performed an attention task, in which they needed to identify target characters hidden in a group. In the VR condition, the task was performed in a VR setting, while in the classical stimulation condition the task had a paper-pencil format. The acceptability and the quality of the experiences were assessed employing standard questionnaires.

Results. The results of the two studies confirmed that the VR setting were considered acceptable, interesting and motivating by both healthy participants and patients with MCI and AD.

Conclusions. Our results suggest that VR solutions are useful motivational tools. As apathy (a disorder of motivation) is very common among these clinical populations, having motivating tools is a key challenge in designing effective rehabilitation programs

09f. Patient Care & Support: cognitive training

ADPD5-1682

PERFORMANCE IN MONTREAL COGNITIVE ASSESSMENT (MOCA) AND MINI-MENTAL STATE EXAMINATION (MMSE) OF ELDERLY SUBJECTS ON REGULAR MAH-JONG VERSUS PHYSICAL EXERCISE

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One of the many benefits that higher cognition brings is increased likelihood to achieve better economic outcomes even in older age. Although the prevention of dementia has emerged as a major health priority, there is paucity of potential preventive strategies. Identifying protective factor is essential to the formulation of effective interventions for dementia. Physical exercise has been proven to be a significant factor which can preserve cognitive function among elderly. Higher physical activity level is related to less impaired cognitive functions and less incidence of dementia. Mah-jong has been noted to produce consistent gains across all cognitive performance measures. Thus this cross sectional study explored the performance of elderly engaged in mah-jong versus physical exercise based on MoCA and MMSE. A total of 95 elderly were included: 31 played mah-jong, 30 engaged in physical exercise and 34 without any significant activity. One-Way ANOVA was done to assess whether there are significant differences among the study groups which resulted in the mah-jong group obtaining the highest mean score on MoCA and MMSE. Multiple comparison tests was done to identify which between physical exercise and mah-jong brought about better cognitive function which showed that despite both having a higher score compared to control group, there was no significant difference between physical exercise and mah-jong. This study therefore concludes that participation in mah-jong is associated with better MoCA and MMSE scores and can be a good treatment option for the prevention of cognitive decline especially for those with contraindication to physical activity.

09f. Patient Care & Support: cognitive training

ADPD5-1760

COGNITIVE REHABILITATION: A STATE OF THE ART FOR LONG TERM MANAGEMENT OF ALZHEIMER DISEASE FOR INDIAN POPULATION

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Introduction: cognitive rehabilitation for dementia patients in India is a new approach to long term management of cognitive deficits. Unfortunately, there is no documented work on the same from India. This approach is essentially a highly individualised, personalised one, acknowledging the heterogeneity of underlying impairments, literacy level, culture specificity and individual situations and responses. While the individual focus is paramount, various formats and modalities may be adopted in the process of working towards achievement of rehabilitation goals.

Aim: to report 3 case series of cognitive rehabilitation on patients suffering from mild to moderate Alzheimer's Disease.

Methodology: a highly extensive and individualized cognitive rehabilitation was carried out for 8-10 weeks on these patients which included compensatory techniques focussed on retraining A-B-C of dementia, i.e., Activities of Daily Living, Behaviour and cognition.

Results: there was an evidence of improvement of 1.0 standard deviation in all the patients in their A-B-C domains post rehabilitation which were documented by conducting detailed standardized neuropsychological testing, pre and post cognitive rehabilitation.

Conclusion: Cognitive rehabilitation with a specific focus on individual strengths and limitation implemented with environmental modification is a promising method for long term management of Alzheimer's disease patients which can aid in optimizing not only the quality of life of the patients but also their caregivers'. However, well-designed studies with ample sample are required to obtain more definitive evidence.

09f. Patient Care & Support: cognitive training

ADPD5-2066

DEVELOPMENT OF RE-CREATE (REHABILITATION OF COGNITION USING RESTORATIVE EXERCISES & ACTIVITIES TARGETED FOR ELDERLY): COGNITIVE REHABILITATION FOR LOW LITERATE INDIANS

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Objective: It is essential to use culturally appropriate, sensitive & specific assessment and interventions for true cognitive performance of individuals. However, studies have shown that several factors including education influence both the treatment and the management of dementia. Unfortunately, there is 37 million illiterate or low educated elderly Indians, thus, making management of dementia (understanding the concept of non pharmacological intervention) challenging. Hence, **Aim** was to develop & standardized a culture & education free cognitive rehabilitation intervention. **Methodology**: a culture specific picture based cognitive rehabilitation package was developed. It consisted of 112 category based pictures for 8 weeks which has been compiled for retraining attention, memory, abstract ability, perceptual tracking and verbal ability for dementia patients. It has been pilot try out on 20 healthy elderly between 60-85years of age with 0-15+ years of education**.** Later, it has been standardized on 50 healthy elderly matched on age, sex and education. **Results:** sample with 0-5 years of education (N=20) completed the whole intervention in 91.7±19.1 seconds; sample with 5-9 years of education (N=9) took 74.01±19.5 seconds; sample with 10-14 years of education (N=10) took 82.2±21.2 seconds while sample with 15+ years of education (N=11) took 77.5±21.8 seconds. Moreover, there was no significant difference between the education groups. **Conclusion:** RE-CREATE is found to be quite effectively working on dementia cases as it is free from any educational bias. Additionally, it helped us to extend non- pharmacological intervention to illiterate -low literate elderly to whom education was a barrier to their management.

09g. Patient Care & Support: art, music & life style

ADPD5-0490
LA TÊTE EN L'AIR - A GRAPHIC NOVEL AND ANIMATION FILM REPRESENTATION OF DEMENTIA AND ITS SYMPTOMS

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Objective: To review the many symptoms of demented patients with Alzheimer's Disease living in nursery homes as represented in the graphic novel and animation film La Tête En L'Air (Roca, P, Éditions Delcourt, 2013).
Methods: La Tête En L'Air is both a graphic novel and animation film in which the main character, diagnosed with Alzheimer's Dementia, is instituted in a nursing home. The main character and additional characters present with several symptoms of AD and other conditions. All of these symptoms are presented in graphic form from the standpoint of the main character/patient and additional characters, acting as either patients or caregivers, thus presenting a layman's interpretation of dementia symptoms.
Results: By analyzing the different symptoms graphically represented in both the graphic novel and animation film, one can have a portrayal of the semiological features and the impact of dementia from the perspective of the main character/patient's viewpoint, as well as by the other additional characters, acting either as patients or caregivers.
Conclusions: In La Tête En L'Air, both a graphic novel and an animation film, the main character, diagnosed with Alzheimer's Dementia, is instituted in a nursing home and throughout the story different symptoms of AD and how they impact on caregivers are graphically represented from a layman's perspective.

09i. Patient Care & Support: other

ADPD5-0834

PREVALENCE AN TREATMENT OF „BEHAVIOURAL AND PSYCHOLOGICAL SYMPTOMS OF DEMENTIA" (BPSD) IN PATIENTS WITH ALZHEIMER´S DISEASE

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Objectives: Alzheimer's disease (AD) is the most common cause of dementia among the elderly. Although cognitive deficits are the clinical hallmark of AD, various non-cognitive symptoms termed „behavioural and psychological symptoms of dementia" (BPSD) are common and can dominate disease presentation. BPSD have been observed in up to 60% - 98% of patients with dementia and include agitation, aggression, anxiety, delusions, sleep disturbances, and hallucinations among other symptoms.
Methods: We included 97 patients with AD within their regular visits at the outdoor memory clinic of the psychiatric department in Innsbruck between 2012 and 2014. All participants underwent a clinical examination, a neuropsychological testing, and patient's medication history was reviewed. Further, neuropsychiatric symptoms were assessed using the neuropsychiatric inventory (NPI). The association of neuropsychiatric symptoms, dementia severity and psychotropic medication was analysed by Pearson´s correlation.
Results: Clinically relevant neuropsychiatric symptoms (NPI score ≥ 4) were found in 76% of AD patients. The presents of neuropsychiatric symptoms positively correlated with dementia severity and more severe cognitive impairment. While the use of benzodiazepines (BZD) was associated with more sleep disturbances, the intake of neuroleptics was associated with more prevalent disruptive behaviours such as agitation and aggression. Further, we found a positive correlation between a higher NPI total score and the number of psychotropic medication.
Conclusion: Neuropsychiatric symptoms are very common in AD patients. Although good practice guidelines recommend a limited and caution use of BZD and neuroleptics in patients with AD, they are prescribed frequently to manage BPSD in AD patients.

09i. Patient Care & Support: other

ADPD5-1756

LINK BETWEEN THE NEUROPSYCHIATRIC SYMPTOMS OF ALZHEIMER'S DISEASE AND THE DEPRESSION OF ELDERLY CAREGIVERS

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Objective: To evaluate the link between the severity of the psychological and behavioural symptoms in Alzheimer's disease (AD) and the depression of the caregiver.
Methods: The sample of elderly caregivers of patients with AD (N=70) was investigated using Geriatric Depression Scale (GerDS). The behavioural disorders of the care-recipients and stress level of the caregiver were evaluated with Neuropsychiatric Inventory (NPI).
Results: Positive correlation between NPI disorders and stress subscales was significant, but did not reach strong level (Spearman's correlation coefficient 0.72, $p<0.05$). There was a positive link between GerDS score of the caregiver and the NPI disorders subscale score of the patient (Spearman's correlation coefficient 0.35, $p<0.05$). The similar link was revealed between the GerDS score and the NPI stress subscale score (Spearman's correlation coefficient 0.27, $p<0.05$). Caregivers with clinically significant depressive symptoms had higher NPI stress subscale score (Mann-Whitney test, $p=0.002$).
Conclusions: Caregiver's stress caused with behavioural and psychological symptoms of AD patient depends not only on severity of the disorders of the care-recipient, but on other factors, probably personal traits and affective status of the caregiver. More prominent neuropsychiatric symptoms of a care-recipient and caregiver's stress are positively linked with severity of depression among caregivers. The link between these measures may be bidirectional, but it is reasonable to suggest that stress is a risk factor for the depression. NPI can be used not only for evaluating a state of a patient with AD, but also for screening of stress in caregivers as a risk factor for depression.

10a. Other: cell, molecular & systems biology

ADPD5-1092
MODULATION OF PSA-NCAM TURNOVER BY NMDA AND INSULIN IS ASSOCIATED WITH EXPRESSION OF TAU, APP AND SNCA GENES.
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Objectives: Cellular interactions mediated by the neural cell adhesion molecule (NCAM) are critical in cell migration, differentiation and plasticity. Switching of the NCAM-interaction mode, from adhesion to signalling, is determined by NCAM carrying a particular post-translational modification, polysialic acid (PSA). Our objective was to determine the mechanisms for desialylation are also at play in mammalian cells and to measure if this would cause overexpression of key Alzheimer and Parkinson-related genes due to uncoupling of PSA from NCAM (causing increased NCAM interaction).
Methods: Cell culturing of human medulloblastoma and primary mouse cells using EndoN enzyme (that specifically cleaves PSA from NCAM) and exploiting the calcium dependence of polysialyltransferases allowed us to study the addition and removal of PSA to and from the cell surface, respectively.
Results: In TE671 cells, PSA-NCAM turnover requires internalization of the molecule into the cytosol. PSA-NCAM internalization was specifically triggered by nitric oxide and collagen in the extracellular matrix (ECM) and is blocked by insulin-like growth factor (IGF1) and insulin. Our results show that cultured-neurons treated with EndoN overexpress *Tau*, *App* and *SNCA*. Furthermore, addition of NMDA to the cultures resulted in a significant decrease in PSA-NCAM which was accompanied by overexpression of genes such as *Tau*, *App* and *SNCA*. However overexpression was prevented when NMDA culture medium was supplemented with insulin.
Conclusion: This data reveals an important role for PSA-NCAM in regulating the expression of genes associated with neurodegenerative diseases, and demonstrates a role for NMDA and insulin in the regulation of these genes.

10b. Other: disease mechanisms

ADPD5-0829

PATH INTEGRATION IS A BEHAVIORAL MARKER FOR ALZHEIMER DISEASE

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Background: Many people with mild Alzheimer's disease (AD) have navigational impairment. The gene dosage of AD may play a role in the incipient deficit of path integration, which is related to hippocampal function and is important for navigation. Based on the gene dosage theory, among the F2, F1 and F0, the probability of AD-to-be is the highest in F2 and the lowest in F0. F1: the family with only one AD patient; F2: the family with two or more AD patients; F0: the family without AD patient.

Methods: We used the Pointing Test to evaluate path integration. Each participant was instructed to point to the start at 5 stops along a campus stroll of 660 meters. The dependent variables were absolute angle deviation and the presence of angle deviation over 22.5 degrees.

Results: 47 AD, 36 F2, 30 F1 and 42 F0 completed the study. In the Pointing Test, the F2 showed less angle deviations than AD at Point 3 ($p=0.023$); so did F2 and F0 than AD at Point 4 ($p=0.002$) and at Point 5 ($p<0.001$). When considering angle deviation at 22.5 degrees as a cut-off, non-AD groups outperformed AD at Point 4 ($p=0.003$) and at Point 5 ($p=0.038$), indicating a potential risk for AD to be lost.

Discussion: Path integration is a discriminator for AD from non-AD but the effect of gene dosage was not shown. It is worthwhile to follow non-AD with more angle deviations up to determine the predictive value of path integration in AD.

10b. Other: disease mechanisms

ADPD5-0847

THE POSITIVITY EFFECT ON INTENSITY OF EXPERIENCED EMOTION AND MEMORY PERFORMANCE IN MILD COGNITIVE IMPAIRMENT AND DEMENTIA

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Objectives: In the course of a life a preference to positive stimuli is built whereas negative stimuli tend to be neglected. We investigated this “positivity effect” on memory performance in mild cognitive impairment (MCI) and dementia.
Methods: 81 patients (32 with MCI, 27 mild and 32 with moderate dementia) and 28 age-matched controls were presented 12 pictures showing positive, negative or neutral objects. At first, we investigated immediate and delayed (after 30´) recall and delayed recognition of the pictures. Moreover, the emotional valence of the pictures perceived and the emotions evoked in the subjects were evaluated.
Results: Irrespective of the condition, patients with mild and moderate dementia recalled fewer pictures than those with MCI or the healthy controls (main effects diagnosis $12.5<F<14.8$*). Across groups, the positive pictures were better memorized than the negative or neutral ones (main effects valence $9.3<F<18.2$*). In delayed recognition a significant interaction between “diagnosis” and “valence” arose. In all diagnostic groups pictures were experienced as expected. However, the positive pictures induced a higher arousal than the negative and neutral ones.
Conclusions: Our findings indicate that the positivity effect does not only apply to healthy elderly but also to patients with MCI or mild and moderate dementia. This effect does not refer to the compliance of the patients investigated since they perceived and experienced the pictures in the expected way. Since arousal levels were highest for positive pictures the positivity effect may rather correspond to a higher reactivity with greater arousal induced by the positive pictures.
*Note: *p*<0.001

10b. Other: disease mechanisms

ADPD5-0873

FUNCTIONAL EFFECT OF RARE CLU VARIANTS ASSOCIATED WITH ALZHEIMER DISEASE RISK.

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Objectives The role of rare variants in Alzheimer disease (AD) susceptibility was emphasized by our finding of genetic association with rare non-synonymous and small insertions/deletions (indels) in clusterin (*CLU*). Applying a multidisciplinary approach, we attempted to untangle the effect of these rare variants on the pleiotropic CLU protein.
Methods The effect of 11 coding *CLU* variants was determined on protein localization and secretion using immunocytochemistry, live cell imaging, ELISA's, Western blot and immune-detection. For variants located at the interface between alpha and beta protein domains, the effect on hetero-dimerization is assessed by yeast-two-hybrid experiments (Hybrigenics). The novel SwithSENSE technology (Dynamic Biosensors) is used to investigate the influence on Abeta40 interaction and conformational changes.
Results Three variants in the beta-chain (p.I303NfsX13, p.R338W and p.I360N) caused an alteration of the subcellular localization of CLU. While the relative amount of CLU-EGFP signal was reduced in Golgi, it was almost exclusively present in endoplasmic reticulum. Further, we observed a significantly decreased CLU secretion in cell medium compared to an increased presence of full length CLU protein in cell lysate for p.R338W and p.I360N variants.
Conclusion Our data suggest a reduced secretion of the CLU protein as one of the modes of action for the rare variants identified in the beta-chain. Additional research will address whether beta-chain variants lead to conformational changes like altered protein folding or interaction with the Abeta40 peptide.

10c. Other: preclinical research

ADPD5-0432
CHARACTERISTICS OF THE AIBL STUDY COHORT AT 36 AND 54 MONTHS
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Of 1112 subjects recruited (211 AD, 133 MCI, 768 HC), 824 (74%) returned at 36 months and 718 (65%) at 54 months. Nearly 60% of the MCI subjects at baseline progressed to dementia due to AD by 54 months, but under 4% of healthy controls developed MCI or AD by 54 months. Having AD or MCI at baseline was associated with increased death and drop out rates. The low level of progression to cognitive impairment among those who were healthy at baseline reflects a low average age (70) and good education and general health in this group.

10c. Other: preclinical research

ADPD5-0996
EVIDENCE FOR AFFECTIVE SYMPTOMS AS AN EARLY MANIFESTATION OF COGNITIVE CHANGE

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'Subjective Cognitive Impairment' (SCI) persons have the subjective belief that memory is impaired, but show normal performance on psychometric tests. In this study, we aim to assess the nature of cognitive and affective symptoms of SCI, and develop tools to identify early predictors of cognitive decline in the evolution towards MCI and AD. METHOD: 136 healthy subjects, aged ≥ 50 years, presenting at the NYU Alzheimer's Disease Center were evaluated through self-reported scales : A Visual Analog Scale (VAS), (Guillo Benarous, 2013), the Brief Questionnaire Regarding Severity of Memory & Emotional Problems (BQRS-M&E =S1), (Reisberg, 2013), and the Memory Complaint Questionnaire (MAC-Q). Alternating subjects were evaluated on time related scales: the ADNI Cognitive Change Index =S2 (Saykin, 2012), the Sahlgrenska Academy Self-Reported Cognitive Impairment Questionnaire (SASCI-Q = S5) or on a severity based questionnaire SEVCOG =S3 and an Emotional Questionnaire =S4; on depression and anxiety scales: Geriatric Depression Scale, Hamilton; and on psychometric measures: MMSE, Logic1, Digit Span F&B, TMT, DSST, Paired Associates Recall and the Boston Naming Test. The evaluations at baseline were studied.

Table1: Baseline Characteristics

N=136	AGE	EDUC	GENDER	VAS	S1	S2	S3	S4	S5
Native English speakers 86%	72.22y/o +/-8.40 median	16.78 +/- 2.30 median	Females 66%	2.90 +/-1.54 median	2.08 +/-0.77 median	1.73 +/-0.64 median	1.88 +/-0.55 median	1.69 +/-0.61 median	-5.14 +/- 7.84 median
Multicultural 14%	72 range 52-95	18 range 12-21	Males 33%	3 range 0-9	2 range 1- 4.6	1.55 range 1-3.65	1.80 range 1.05-3.3	1.50 range 1-3.83	-2.00 range -28-5.0

Table 2 : Comparison of affective and psychometric tests with demographic and subjective measures (P-values)

N=136	DEMOGRAPHICS AND SUBJECTIVE SCALES								
AFFECTIVE AND PSYCHOMETRIC TESTS	AGE	EDUC	GENDER	VAS	S1	S2	S3	S4	S5
GDSD	0.017	ns	ns	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001
HAMILTON	0.12	ns	ns	0.002	0.0013	ns	0.005	0.0066	ns
MAQ	ns	ns	ns	0.001	<0.0001	0.04	0.004	0.06	0.015
MMSE	0.1	ns	0.03	ns	ns	ns	ns	ns	ns
LOGIC1	ns	0.06	0.001	ns	ns	ns	ns	ns	ns
DSPF	ns	ns	ns	ns	ns	ns	ns	ns	ns
DSPB	ns	ns	ns	ns	ns	ns	ns	0.03	0.03
TMTA	0.02	ns	ns	ns	ns	ns	ns	ns	ns
TMTB	ns	ns	ns	ns	ns	ns	ns	ns	0.03
BOSTON	ns	ns	ns	0.09	ns	ns	ns	ns	ns
DSST	0.004	ns	ns	ns	ns	ns	ns	ns	ns
PAIR	ns	ns	0.07	ns	ns	ns	ns	ns	ns
PARD	ns	ns	0.1	ns	ns	ns	ns	ns	ns

RESULTS: Very few significant relationships are found between objective testing and subjective evaluations. We found a robust correlation between depression scales and all of the subjective self-reported scales, stronger with severity based self-reported scales. CONCLUSION: Affective symptoms might be early indicators of cognitive and associated physiologic brain changes and could point to prevention strategies.

10c. Other: preclinical research

ADPD5-1339

COGNITION IS ASSOCIATED WITH TIME TO CLINICAL ONSET, SPECIFIC GENES AND COGNITIVE RESERVE IN AUTOSOMAL DOMINANT ALZHEIMER'S DISEASE

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Objective. Cognitive decline is reflected by time to clinical onset (TCO) in familial Alzheimer's disease (fAD). However, it is not known how specific AD-causing mutations (APP vs. PSEN1), APOE and cognitive reserve (CR) influence the relationship between TCO and cognitive decline.

Methods. Cognitive functions covering five domains were assessed in 38 mutation carriers (n=24; APP_{SWE}, and APP_{ARC}, and n=14; $PSEN1_{M146V}$, $PSEN1_{H163Y}$ and $PSEN1_{I143T}$) and 43 non-carriers (n=22; APP-families and n=21; PSEN1-families). CR was estimated by years of formal education. Carriers and non-carriers were comparable in TCO (-7.3 vs. -7.4; carriers vs. non-carriers), proportion of at least one e4 allele (0.53 vs. 0.36; carriers vs. non-carriers), age, gender distribution and CR (10.9 vs. 10.6; carriers vs. non-carriers)(all p's>0.1).

Results. A 4-way ANOVA on episodic memory as dependent variable and with APOE, CR, mutation status and type of AD-gene (APP vs. PSEN1) as factors showed strongly significant main effects of CR (the higher the better), mutation status (in favor non-carriers) and type of AD-gene (in favor of PSEN1), but no significant effect of APOE. A similar pattern of significant effect main effects (CR, mutation status and AD-gene) was obtained for visuospatial and executive functions, but not for verbal and immediate memory. The interaction between CR and mutation status was significant in 3 out of 5 cognitive functions showing a strong impact of high CR for mutation carriers but not for non-carriers.

Conclusion. Cognitive reserve has a strong favorable influence on cognition for mutation carriers but not for non-carriers.

10c. Other: preclinical research

ADPD5-1688
ISORHYNCHOPHYLLINE IMPROVES LEARNING AND MEMORY IMPAIRMENTS INDUCED BY SCOPOLAMINE IN MICE

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Objectives: The present study aimed to investigate whether isorhynchophylline (IRN, Fig. 1), an alkaloid isolated from Chinese herb *Uncaria rhynchophylla*, could reverse scopolamine (SCOP)-induced learning and memory impairments in mice and to evaluate its action mechanisms.
Methods: The cognitive-enhancing effect of IRN on amnesic mice induced by SCOP was investigated by the Morris water maze test. To elucidate the underlying mechanisms of memory enhancing effects of IRN, the activity of acetylcholinesterase (AChE) and neuroinflammatory parameters were measured.
Results: The results showed that IRN significantly improved the learning and memory functions in SCOP-treated mice. Mechanistically, IRN significantly decreased the protein and mRNA levels of interleukin (IL)-1β and the activity of AChE, but markedly accentuated the protein and mRNA levels of IL-10 and the level of acetylcholine in the brain of the SCOP-treated mice. Moreover, IRN also significantly suppressed the production of prostaglandin E2 and mRNA expression of cyclooxygenase-2 while markedly enhanced the protein level of phosphorylation of ERK1/2 in the brain of the SCOP-treated mice.
Conclusions: These results amply demonstrated that IRN was able to improve the learning and memory impairments induced by SCOP in mice, and the underlying mechanisms involved the inhibition of AChE activity and the amelioration of the neuroinflammatory processes via protecting ERK activity in the mice with SCOP-induced cognitive impairments.

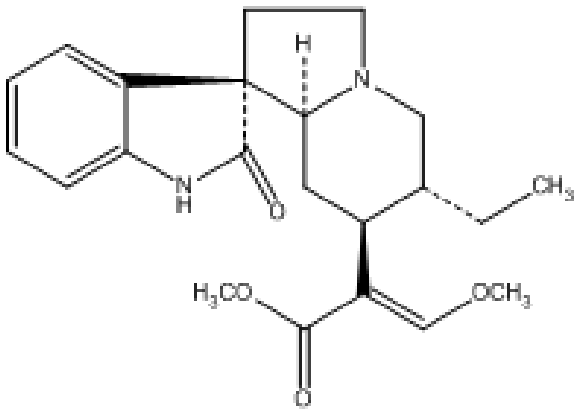

Fig. 1. Chemical structure of isorhynchophylline.

10c. Other: preclinical research

ADPD5-1966

PRECLINICAL PHARMACOKINETIC STUDIES OF TRITIUM LABELLED D-ENANTIOMERIC PEPTIDE D3 FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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Objectives: We are developing small D-enantiomeric peptides for elimination of toxic Aβ oligomers during causal treatment of Alzheimer´s disease (AD). D-enantiomeric peptides composed of D-amino acids have theoretical advantages over all L-amino acid peptides, such as resistance to proteases and reduced or non-existent immunogenicity. We previously developed D3, an all D-peptide, which specifically binds to Abeta, inhibits aggregation into toxic species and shows diagnostic and therapeutic potential in vitro and in vivo. Only little information is known about pharmacokinetic properties of D-peptides. Therefore, we conducted experiments with a tritium labelled D-peptide D3 (^{3}H-D3) in mice with different administration routes to study its distribution in plasma and organs, as well as its bioavailability by i.p. and p.o. administration. We also tested its metabolic stability in mouse brain, liver and kidney homogenate, plasma and liver microsomes.

Methods: Tritium labelled D3 was administered i.v., i.p., p.o. as bolus dose and through osmotic pump i.p. implantation. Plasma and organs were sampled at different time points after administration. Radioactivity in the plasma or organ homogenate was measured by liquid scintillation counting. Stability tests were performed through incubation of ^{3}H-peptides with plasma or organ homogenates, using thin layer chromatography combined with ^{3}H-autoradiography. PK parameters were calculated with non-compartmental analysis using WinNonlin.

Results: D3 showed resistance to proteases, long plasma half-life and adequate brain-plasma ratio, as well as high oral bioavailability.

Conclusions: Our results indicate that D3 and its derivatives may be promising drug candidates for AD treatment.

10c. Other: preclinical research

ADPD5-2130

AMYLOID-BETA-OLIGOMER INDUCED ALZHEIMER'S DISEASE MODELS FOR TARGET VALIDATION AND DRUG DEVELOPMENT

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Objectives

Amyloid-β peptide oligomers (AβO) are the likely initiator of synaptic failure associated with early Alzheimer's dementia (AD). SynAging has successfully industrialized the preparation of stabilized AβO with the aim to offer preclinical models facilitating drug development for AD.

Methods

In vitro, rodent primary neurons challenged with AβO preparations resulted in well characterized and highly reproducible synaptic degeneration and neuronal death facilitating target validation, hit selection and lead optimization. Recently, microfluidic devices, separating the dendritic and soma from the axonal compartment have been established, enabling compound's mode of action investigations.
In vivo testing of compounds is performed in rodents injected with minute amounts of AβO ICV. Recently, intra-peritoneal injection of AβO in mice resulted in memory loss in the novel object recognition (NOR). Indeed, Y-maze, Morris Water Maze and NOR assays are fully validated and used in drug development.

Results

Reproducibility of AβO neurotoxicity in AD assays has been verified e.g. in Y-maze assay. While normal mice exhibited an alternation behavior of 65.8±2.4%, the AβO-injected mice showed an average of 53.9±1.5% of alternation behavior over many months. Humanin is used as positive control and customer compounds where successful in full protection of mice from AβO induced cognitive decline.

Conclusion

AβO induced AD models are thought to:

- Have better translatability than transgenic models
- Induce AD symptoms in rodents of all ages and large group sizes
- Show high convergence of *in vitro* and *in vivo* results
- Be faster, more reproducible and versatile than transgenic models

10d. Other: diagnostics

ADPD5-0232

THE VALIDITY AND RELIABILITY OF THE MINI-MENTAL STATE EXAMINATION-2 FOR DETECTING MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE IN A KOREAN POPULATION

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The Mini-Mental State Examination, 2nd edition (MMSE-2) is developed as a reliable cognitive screening measure to provide finer discrimination than the MMSE in recent years. The goal of this study was to examine the validity and reliability of the MMSE-2 for assessing the patients with mild cognitive impairment (MCI) and Alzheimer's disease (AD) in a Korean population. Two hundred and twenty six patients with MCI, ninety seven patients with AD, and 91 healthy older adults were recruited. All participants accepted examination by the MMSE-2, the MMSE, and other detailed neuropsychological assessments. The MMSE-2 performed well in discriminating participants across Clinical Dementia Rating (CDR) stages and CDR-Sum of Boxes (CDR-SOB) and showed excellent internal consistency, high test-retest reliability, and high interrater reliability and good concurrent validity with the MMSE and other detailed neuropsychological assessments. Also, the MMSE-2 was performed into two factors in normal cognitive aging and the patients with AD, but the MMSE-2 was factored into three factors in the patients with MCI. The sensitivity and specificity of three versions of the MMSE-2 were relatively high in discriminating normal cognitive aging and the patients with MCI and AD. The MMSE-2 is a valid and reliable cognitive screening instrument for assessing the cognitive impairment in a Korean population, but its ability to detect the patients with MCI from normal cognitive aging may not be as highly sensitive as expected.

10d. Other: diagnostics

ADPD5-0238
DISCRIMINATION ABILITY OF SHORT TEST OF MENTAL STATUS (STMS) COMPARED TO MMSE IN THE SPECTRUM OF NORMAL COGNITION-MCI-AD: THE TURKISH STANDARDIZATION STUDY

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Identification of mild cognitive impairment and its discrimination from normal cognition and Alzheimer's disease is a crucial process in clinic. The Mini Mental State Examination (MMSE) developed by Folstein is the most commonly used bedside mental examination test. In spite of its widespread use, on the other hand, the discriminative ability of MMSE in the spectrum of controls, mild cognitive impairment (MCI) and Alzheimer's disease (AD) is below the required level. Therefore, the lack of an ideal cognitive screening test, which is easy to apply and able to differentiate normal cognition from mild cognitive impairment, is a well-acknowledged fact. This prompted Emre Kökmen to develop STMS (Short Test of Mental Status) in Mayo clinic (1987). As a time-saving, economic and practical test, STMS can be more advantegous in differentiating normal people from MCI and, MCI from AD, compared to MMSE. The aim of the present study was to create standardization of the STMS in the general Turkish aging population and to find its disciminative ability in the spectrum of normal cognition, MCI, and AD. According to our results, STMS was found to be significantly better then MMSE both in discrimination of normal cognition from MCI and MCI from AD, with cut-off scores high in both sensitivity and specifity.

10d. Other: diagnostics

ADPD5-0289

ECOLOGICAL ASSESSMENT OF AUTONOMY IN INSTRUMENTAL ACTIVITIES OF DAILY LIVING USING AN AUTOMATIC VIDEO MONITORING SYSTEM

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Objectives: To investigate the use of a video monitoring system for automatic event recognition for the assessment of autonomy in Instrumental Activities of Daily Living (IADL) in dementia patients.

Methods: Three groups of participants (healthy control, Mild Cognitive Impairment and Alzheimer's disease) had to carry out a standardized scenario consisting of directed tasks (single and dual task) and IADLs such as preparing pillbox. During this time they were recorded by 2D video cameras capturing all their activities. The performance quality of each participant was manually annotated and assessed based on the amount of successfully carried out activities. Recorded data was processed by a platform of video signal analysis in order to extract kinematic parameters detecting activities undertaken by the participant. We developed a classifier based on the extracted video features for diagnostic prediction and further autonomy performance prediction.

Results: Overall activities were correctly automatically detected. The most accurate detected activities were: using the phone with 91% accuracy and preparing pillbox with 88% accuracy. The diagnostic group classifier based on the automatically extracted video features obtained accuracy of 71.79 % when combining directed tasks and IADLs. Autonomy group classifier obtained an accuracy of 84.61% when combining directed tasks and IADLs.

Conclusions: The results suggest that it is possible to assess autonomy with the help of an automatic video monitoring system (AVMS) and that the use of such technologies could provide clinicians with diagnostic relevant information to improve autonomy assessment in real time and decrease observer biases.

10d. Other: diagnostics

ADPD5-0368

SERUM MIR-206 AND MIR-132 AS POTENTIAL CIRCULATING BIOMARKERS FOR MILD COGNITIVE IMPAIRMENT

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Abstract

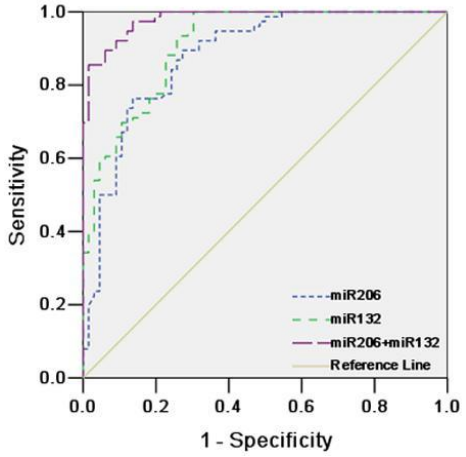
Objectives: Circulating microRNAs (miRNAs) are reportedly potential diagnostic biomarkers for many diseases due to their stable, content-rich, easy quantitative detection and non-invasive advantages. This study investigated the potential role of circulating miRNAs as diagnostic biomarkers for mild cognitive impairment (MCI).

Methods: We collected 66 patients with MCI and 76 normal controls from our previous cross-sectional cohort study. Seven miRNAs (miR-206, miR-132, miR-193b, miR-130b, miR-20a, miR-296, and miR-329) related to Alzheimer's disease (AD) were detected using a quantitative real-time PCR (qRT-PCR) method. Each miRNA's diagnostic performance was evaluated by receiver operating characteristic (ROC) curves and the areas under curves (AUC) analysis.

Results: The miR-206 and miR-132 levels in MCI patients' serum were significantly elevated compared to normal controls. Combining miR-206 and miR-132 had the highest AUC of 0.981, followed by miR-206 (AUC = 0.880) and miR-132 (AUC = 0.912). Importantly, miR-206 and miR-132 were respectively correlated with the Montreal Cognitive Assessment (MoCA) score in MCI patients.

Conclusions: The results revealed that circulating miR-206 and miR-132 as novel miRNAs over expressed in MCI and suggested that serum levels of miR-206 and miR-132 were potential predictive biomarkers for MCI.

Figure. 1. **Receiver-operator characteristic (ROC) curve analyses.** ROC curves of miR-206, miR-132, and combined miRNAs were established to distinguish MCI from normal controls.



10d. Other: diagnostics

ADPD5-0376

ANTIBODIES AGAINST SYNTHETIC FRAGMENTS OF ABETA RECEPTORS AS NOVEL BIOMARKERS IN BLOOD SERA OF PATIENTS WITH ALZHEIMER'S DISEASE

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Pathogenic Abeta binds to many receptors on the cell surface leading to cell death. Degradated proteins appear in blood sera and must be eliminated by antibodies. We detected the level of antibodies against fragments of these proteins in sera of patients with Alzheimer's disease (AD) and control donors.

We have formed a pannel of several fragments from extracellular regions of different Abeta receptors such as alpha7-type of acetylcholine receptor (AChR), prion protein, neurotrophin receptor p75 and receptor for advanced glication end products (RAGE). As control we used peptide fragments from tumor assosiated proteins such as survivin and nucleophosmin. To investigate the level of antibodies towards these peptide fragments, more than 30 patients with AD and more than 10 control individuals were taken.

The ELISA revealed that the level of antibodies against one peptide from AChR and one fragment from p75 receptor differs in the control group and in patients with AD. Moreover, the level of antibodies against the AChR peptide was statistically lower in the group of patients than in the blood sera of control individuals, whereas the level of antibodies against the p75 peptide on the contrary was higher in patients' sera. The antibody titers against other fragments were shown to have almost no difference between control sera and blood samples from AD patients.

Thus, detection of the level of antibodies against two fragments from AChR and p75 in blood samples of patients with AD is a novel approach for easy diagnostics of AD.

Supported by grant RFBR 14-04-31232.

10d. Other: diagnostics

ADPD5-0385

CORRELATION OF ABETA OLIGOMER LEVELS IN MATCHED CSF AND SERUM SAMPLES.

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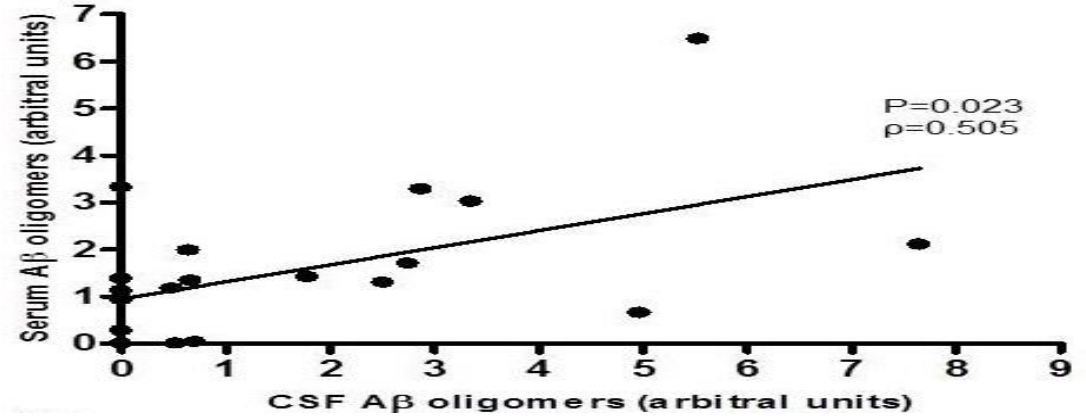
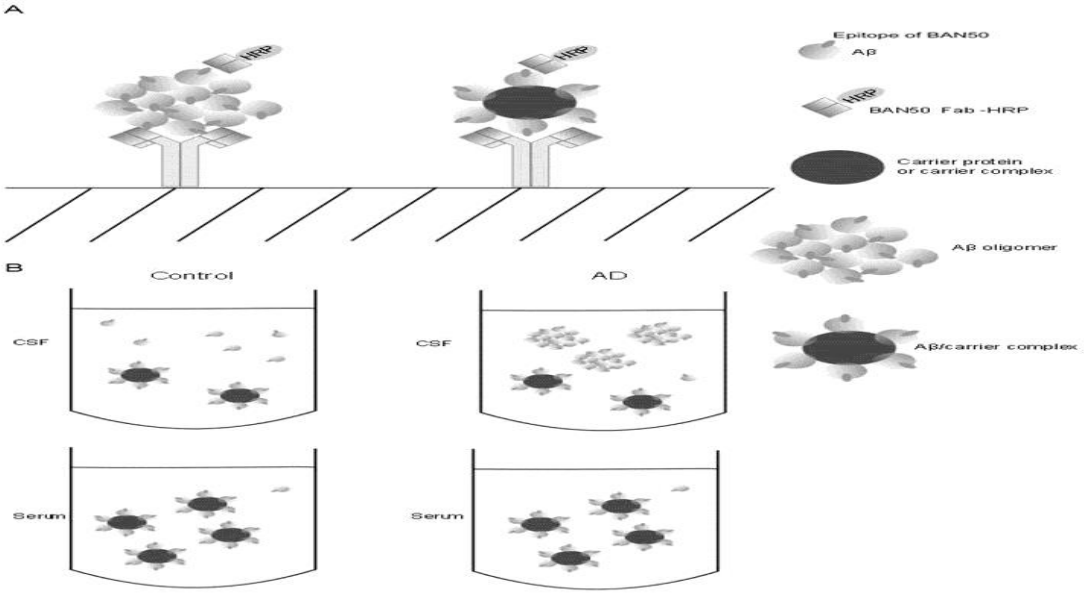
Objectives. We recently developed an ELISA for amyloid-beta (Abeta) oligomers in CSF, in which the same Abeta monoclonal antibody, BAN50, was used for both capture and detection for selective detection of oligomers. However, the invasive CSF sampling procedure, limits its use in routine clinical practice. In this study, firstly we determined whether the BAN50 SAS-ELISA can detect Abeta oligomers in serum. Secondly, we examined the possible relationship between the levels of Abeta oligomers in matched samples of CSF and serum collected at the same time.
Methods. Twenty subjects with no dementia who were required to give CSF and serum at the same time were enrolled. We measured Abeta oligomers by the BAN50 SAS-ELISA and Abeta1-40/1-42 by commercially available kits in each sample.
Results. Our ELISA could detect signals in 60% of serum samples and in 80% of CSF samples obtained from the subjects. Heterophilic antibodies that are a primary confounding factor in this type of ELISA did not affect the signals obtained. Although the levels of serum Abeta oligomers were unexpectedly high, suggesting the possible detection of non-pathological Abeta complexes associated with serum carrier proteins, they did show a significant positive correlation with the levels obtained from matched CSF samples (Figure).



Coclusions. This correlation between CSF and serum Abeta oligomer levels implies that the levels of serum Abeta oligomers measured with our ELISA might be useful as a marker for AD that reflects an intact system of Abeta transport across the blood brain

barrier (Figure).



10d. Other: diagnostics

ADPD5-0420

TECHNICAL VALIDATION OF A FULLY AUTOMATED ROCHE ELECSYS IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF AMYLOID-BETA1-42 IN HUMAN CEREBROSPINAL FLUID

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Objectives:

Cerebrospinal fluid (CSF) amyloid-beta1-42 peptide (Abeta42) is a well-established biomarker for Alzheimer's Disease. Several immunoassays for Abeta42 are on the market as research use only (RUO) assays in the US. Their technical qualification as in vitro diagnostics (IVD) worldwide is hindered by imprecision, lot-to-lot variability, and lack of standardization to either a reference method or material. The fully automated Roche Elecsys Abeta42 immunoassay has been developed to overcome these technical hurdles.

Methods:

Technical validation of the assay was performed according to CLSI guidelines. Imprecision was assessed by CLSI EP05 over 21 days with two runs per day. Lot-to-lot variability was evaluated based on a comparison of 90 native CSF samples from individual patients measured with three assay lots. The assay was standardized based on the mass spectrometry-based reference method from Leinenbach et al. 2014 to ensure that assay values are traceable to SI units.

Results:

The measuring range of the assay is 200-1700 pg/mL with a limit of quantification (LoQ) < 6 pg/mL. Imprecision studies yielded within-run coefficients of variation (CVs) of 0.4-1.5%, between-day CVs of 1.2-2.0%, and intermediate CVs of 1.9-4.0%. Lot-to-lot comparison of three lots showed excellent correlation, with a coefficient of r>0.99 (Pearson).

Conclusion:

The Roche Elecsys Abeta42 immunoassay is a technically validated assay for the quantitative determination of Abeta42 in human cerebrospinal fluid. The technical performance of the assay shows high lot-to-lot comparability, high precision, and traceability of the results to an IFCC-endorsed reference method.

10d. Other: diagnostics

ADPD5-0425

SEQUENCE OF COGNITIVE DECLINE IN ALZHEIMER'S DISEASE (AD) PATIENTS – RESULTS FROM AN OBSERVATIONAL STUDY

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Introduction: Understanding the pattern of decline in cognitive functioning in Alzheimer's disease (AD) could assist primary care physicians in explaining AD progression to patients and caregivers. The objective of this analysis is to determine if there is an identifiable order in which cognitive abilities are lost within the progression of AD.
Methods: GERAS is an 18-month observational study on AD. Patients diagnosed with probable AD and Mini Mental State Examination (MMSE) ≤26 were enrolled. Proportional odds logistic regression model was applied to model MMSE subscales of orientation, registration, attention and concentration (spelling and counting), word recall, language and copying. The model converted ordinal scores of each subscale to corresponding probabilities of cognitive impairment at each MMSE total score level where the probabilities were estimated based on start of and complete cognitive impairment.
Results: 1495 patients were analyzed. Figure 1 shows the probability estimates of start of and complete impairment for each subscale. The first aspect of cognition to become impaired is word recall, followed by orientation in time. The last abilities to fully deteriorate are orientation in place, language, and registration.
Conclusions: The process of cognitive decline was visualized by means of probability estimates of key aspects of cognition. This might be useful to set expectations on disease progression for patients/caregivers.

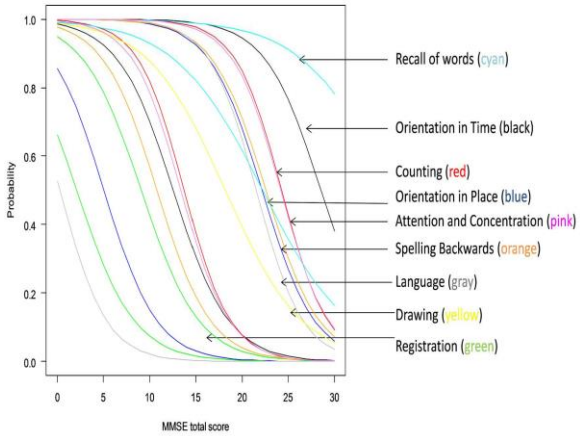

Figure 1 Leftmost curve for each color denotes the probability of complete impairment (individual subscore=0). Rightmost curve denotes the probability of start of cognitive impairment (first error on each individual subscore).

10d. Other: diagnostics

ADPD5-0439

SIMPLE BAJA SCORING OF THE CLOCK DRAWING TEST FOR MILD ALZHEIMER DISEASE

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Objectives
Our goal was to develop a simple, but unambiguous scoring of the clock drawing test (CDT) that is able to reliably distinguish serious from minor errors and to assess its diagnostic potential for mild dementia due to Alzheimer disease (ADD).
Methods
We administered the Mini-Mental State Examination (MMSE) and CDT with 11:10 to 30 ADD patients (MMSE 24±4 points) and to 30 matched normal seniors (NOS) (MMSE 29±1). We prepared our own Baja scoring system ranging from 0 to 9.5 points. It contains 9 items to evaluate separately quality of a watch face, its hands and the required time. We compared it with a 5-point scoring of CDT in the Addenbrook´s cognitive assessment (ACE).
Results
Both groups did not differ in CDT scores according to ACE rules (p=0.8). Patients even with mild ADD made significantly more mistakes and thus had higher scores than the controls (average ± SD: 7±2 vs 8±1 points, p=0.0023). We found an optimal Baja cut-off for CDT ≤ 7.5 points associated with sensitivity (Se) 60 %, specificity (Sp) 77 % and area under curve (AUC) of receiver operating characteristic 0.71. Similar variables for MMSE are ≤ 27 points, Se and Sp equally 93 %, AUC 0.86.
Conclusion
Our newly developed scoring Baja is a brief and easy instrument to screen mild ADD. MMSE is better, but requires longer time for screening.
The study was supported by grant IGA No. NT 13183 and PRVOUK 34/LF3.

10d. Other: diagnostics

ADPD5-0589

THE EFFECT OF NEURODEGENERATION ON VISUOMOTOR INTEGRATION IN ALZHEIMER AND PARKINSON

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Objectives:
Alzheimer's disease (AD) and Parkinson's disease (PD) both affect visuomotor integration areas (frontal-parietal brain regions). Eye - and hand responses to visual stimuli are a good model to objectively quantify the efficiency of visuomotor integration. The goal of this study is to assess eye - and hand responses across a series of visuomotor tasks to investigate disease specific effects of AD and PD on visuomotor integration.
Methods:
39 AD patients, 59 PD patients and 54 healthy controls performed a series of three visually guided reaching tasks, two counterpointing tasks and one memory guided reaching task. Subjects performed these tasks on a touch screen while their eye movements were recorded using an eye tracking system and their hand movements were recorded using a motion detection system. Outcome parameters describing task performance, response latencies to visual stimuli and task execution speed were calculated and compared between groups.
Results:
AD patients had delayed hand latencies and increased task execution times compared to controls in two of three visually guided reaching tasks and in the memory guided reaching task. PD patients performed worse than controls in one counterpointing task and displayed increased task execution times in two visually guided tasks.
Conclusions:
Parameters describing performance, latencies and execution times across a series of eye-hand coordination tasks provide a quantitative description of disease specific deficits in visuomotor integration. A combination of eye - hand coordinated tasks may serve as an additional tool to diagnose AD and PD and to monitor disease progression.

10d. Other: diagnostics

ADPD5-0717

ANALYTICAL PERFORMANCE EVALUATION OF A FULLY AUTOMATED CHEMILUMINESCENT HUMAN TAU ASSAY ON THE LUMIPULSE G1200 SYSTEM

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Objectives: Cerebrospinal fluid (CSF) β-amyloid$_{(1-42)}$ peptide and tau protein are important biomarkers supporting Alzheimer's disease diagnosis and disease state. Today there is a growing need for reliable and automated tests to quantify both biomarkers. Analytical performance was evaluated for a novel total tau assay for the LUMIPULSE G1200 instrument, a fully automated chemiluminescent enzyme immunoassay system.
Methods: The LUMIPULSE G1200 system uses single, ready-to-use cartridges composed of three wells and has a throughput of 120 tests/hour. Sequential immunoreaction steps are carried out at pre-determined intervals while the cartridge is transported through the system. Quantitative results are available within approximately 25 minutes. The total tau assay was developed using established monoclonal antibodies to human tau and analytical performance parameters e.g. limit of detection (LoD), limit of quantitation (LoQ), linear measuring interval, reproducibility and correlation with INNOTEST hTAU Ag were evaluated according to well-defined protocols.
Results: Within-run reproducibility is <5% for CSF and control samples. Based on 20 measurements of the blank, the LoD is <37 pg/mL. LoQ with 10% coefficient of variation is 66.5 pg/mL. The linear measuring interval, allowing a deviation of 10% is between 190 and 1100 pg/mL. Analysis of CSF tau showed a correlation coefficient of >0.95 compared to INNOTEST hTAU Ag assay.
Conclusions: The fully automated LUMIPULSE G1200 instrument rapidly quantifies biomarkers for neurodegeneration in biological samples in a highly standardized manner. The Lumipulse human tau assay quantifies tau in CSF samples, displays good precision and correlates well with the established INNOTEST hTAU Ag assay.

10d. Other: diagnostics

ADPD5-0721

ANALYTICAL PERFORMANCE EVALUATION OF A FULLY AUTOMATED CHEMILUMINESCENT BETA-AMYLOID(1-42) ASSAY ON THE LUMIPULSE G1200 SYSTEM

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Objectives: Cerebrospinal fluid (CSF) β-amyloid$_{(1-42)}$ peptide (Aβ$_{1-42}$) and total tau protein are important biomarkers supporting Alzheimer's Disease (AD) diagnosis and disease state. Today there is a growing need for reliable and automated tests to quantify both biomarkers. Analytical performance was evaluated for a novel Aβ$_{1-42}$ assay on the LUMIPULSE G1200 instrument, a fully automated chemiluminescent enzyme immunoassay system.

Methods: The LUMIPULSE G1200 system uses single, ready-to-use cartridges composed of three wells and has a throughput of 120 tests/hour. Sequential immunoreaction steps are carried out at pre-determined intervals while the cartridge is transported through the system. Quantitative results are available within approximately 25 minutes. The Aβ$_{1-42}$ assay was developed using established monoclonal anti-Aβ antibodies and analytical performance parameters e.g. limit of detection (LoD), limit of quantitation (LoQ), linear measuring interval, precision and correlation with INNOTEST β-AMYLOID$_{(1-42)}$ were evaluated according to well-defined protocols.

Results: Within-run reproducibility was <5% for controls and CSF samples. Based on 20 measurements of the blank, the LoD was determined to be <25 pg/mL. LoQ with a coefficient of variation of 10% was <30 pg/mL. The assay was found to be proportionally linear over the clinically relevant range and correlated well (r>0,90) with the reference device INNOTEST β-AMYLOID$_{(1-42)}$.

Conclusion: The fully automated LUMIPULSE G1200 instrument has the potential to rapidly quantify biomarkers for neurodegeneration in biological samples in a highly reproducible manner. The Lumipulse Aβ$_{1-42}$ assay fulfils current needs for quantification of AD biomarkers: superior sensitivity and precision and good correlation with the established INNOTEST β-AMYLOID$_{(1-42)}$ assay.

10d. Other: diagnostics

ADPD5-0762
AUTOMATIC SPEECH ANALYSIS FOR THE ASSESSMENT OF PRE-DEMENTED AND ALZHEIMER PATIENTS

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Background: Various types of dementia and MCI are manifested as irregularities in human speech and language, which have proven to be strong predictors for the disease presence and progression. Therefore, automatic speech analysis is expected to be an useful tool in providing indicators for assessment and detection of early stage Alzheimer's disease (AD) and MCI.
Method: 15 Healthy elderly subjects (HC), 23 MCI patients and 26 AD patients were recorded while performing several short vocal cognitive tasks, including verbal fluency, picture description and counting down, during a regular consultation.
Voice recordings were processed in two steps: first vocal markers were extracted using speech signal processing techniques; second, vocal markers were tested to assess their 'power' to distinguish between HC, MCI and AD. The second step included training automatic classifiers for detecting MCI and AD, based on machine learning methods, and testing the detection accuracy.
Results: classification accuracy of automatic audio analyses were as follows: between HC and MCI: 79 ± 5%, between HC and AD: 87 ± 3%, and between MCI and AD: 80 ± 5%.
Conclusions: Decline in cognitive functioning affects speech production in different ways. Preliminary analysis indicates the potential value of vocal cognitive tasks for accurate automatic differentiation between HC, MCI and AD. This can provide the clinician with meaningful information for assessment and early diagnosis purposes, based on non-invasive, simple and low-cost method. Investigations of new and improved vocal tasks, signal processing tools and pattern recognition tools, are planned.

10d. Other: diagnostics

ADPD5-0902

LOW CSF AMYLOID-BETA LEVELS CORRELATE WITH POOR PERFORMANCE IN CERAD-NB SUBTESTS MEASURING DELAYED MEMORY IN WOMEN

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The relationship between The Consortium to Establish a Registry for Alzheimer's Disease Neuropsychological Battery (CERAD-NB) and CSF biomarkers for Alzheimer's disease (AD) have been evaluated only in few studies. Our aim was to study the correlation of pathological changes in the CSF biomarkers with various CERAD-NB subtests.
This study consisted of 80 subjects (53.8 % women, 46.2 % men) with mean age of 70,4 years. 65 had undergone an assessment of cognitive status with CERAD-NB and a CSF biomarker analysis due to a suspected memory disorder and 15 were controls with no memory complaint. A total of 23 subjects were diagnosed with AD, 19 with Mild Cognitive Impairment (MCI) and 38 with another memory problem.
We found a significant correlation between CSF amyloid-beta ($Abeta_{1-42}$) and several subtests measuring delayed recall in women. Word list recall correlated with all markers: $Abeta_{1-42}$ (r=0,323, p=0,035), tau (r=-0,304, p=0,050) and hyperphosphorylated tau (p-tau) (r=-0,331, p=0,046). No such correlations were found in men. Moreover, within the group diagnosed with MCI $Abeta_{1-42}$ correlated with a memory compound score measuring delayed recall (r=0,568, p=0,022).
CSF biomarkers, particularly $Abeta_{1-42}$, correlate with delayed memory score in CERAD-NB and women may have more actual AD pathology at the time of the investigations than men who may be likely to have other reasons behind the memory deficit.

10d. Other: diagnostics

ADPD5-0912

THE TRANSFER OF CUT-OFF VALUES FOR A CSF AD BIOMARKER FROM AN ESTABLISHED ASSAY TO A NEW ONE

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Objectives
Numerous technologies are available to quantify CSF biomarkers for Alzheimer's disease diagnosis, resulting in different biomarker concentrations and cut-off values for a specific intended use. If a laboratory wants to use a new generation assay, cut-off values need to be re-established. It is a common practice to test available samples 'side-by-side' with the old and new assay and to transfer the cut-off by means of a linear regression formula. We evaluate the effect of this transfer method on the biomarker's performance and introduce an alternative, Bayesian methodology.
Methods
We simulated two sets of data that mimic the structure of CSF $A\beta_{1-42}$ values: a 'cut-off' dataset used to derive the cut-off for the current assay and a 'transfer' dataset containing biomarker values measured with the current and new assay, without the availability of diagnostic information. The cut-off for the new assay was transferred with the linear regression approach. Additionally, we applied a Bayesian method, which consists of using the 'cut-off' data as prior information for estimation of the biomarker's distributions in the 'side-by-side' dataset.
Results
We show that the Bayesian method results in less variable cut-off values and associated sensitivities and specificities. We show the effect of increasing sample sizes of the 'cut-off' and 'transfer' dataset on the precision of the new cut-off.
Conclusions
The Bayesian transfer method results in a more precise cut-off than the linear regression approach. Hence, the Bayesian approach is preferred for a cut-off transfer and will result in a faster integration of the new assay.

10d. Other: diagnostics

ADPD5-1003
FALSE RECOGNITION MEMORY IN REY AUDITORY-VERBAL LEARNING TEST FOR MILD COGNITIVE IMPAIRMENT

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Objectives: Patients with Alzheimer's disease (AD) and with Mild Cognitive Impairment (MCI) often show false recognition errors on neuropsychological assessment of memory. We retrospectively examined the qualitative difference in false recognition memory in Rey auditory-verbal learning test (RAVLT) for MCI patients.
Methods: Forty-two out patients (male=17, female=21; mean age=72.7 (SD=6.9); Clinical Dementia Rating Scale=0.5) were recruited from Memory Clinic at Keio University Hospital. We classified MCI patients into those who progressed to AD (MCI-p, N=19) and those who did not (MCI-np, N=23) according to the CDR after mean 38.8 (SD=10.4) months.
Results: There was a tendency for MCI-p group to produce more false recognition errors than for MCI-np group ($p = .054$). Moreover, MCI-p group showed significantly more false recognition errors phonetically close to target words than MCI-np group [MCI-p: 0.42 (SD 0.69); MCI-np: 0.09 (SD 0.29), ($p < .05$)]. Regarding false recognition errors semantically close to or non-related target words, both MCI-p and MCI-np groups showed equivalent false recognition errors.
Conclusions: MCI patients tend to produce false recognition errors in the auditory-verbal learning task, and it was revealed that phonetically close words to target words might intrude into auditory-verbal memory for MCI patients who progressed to AD in 3 years. It implies that the qualitative aspect of false recognition memory is important for the prediction of the progression to AD for MCI.

10d. Other: diagnostics

ADPD5-1033
DIAGNOSTIC VALUE OF CSF NON-PHOSPHORYLATED TAU IN NEURODEGENERATIVE DEMENTIA

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Objective: Our present objective is testing if the CSF concentrations of CSF non-Phosphorylated Tau (non-P-Tau) can be useful to detect Alzheimer's disease (AD) from different others neurodegenerative diseases. This study is supplementary to another presented work during this event on the analytical validation of CSF non-P-Tau determination with a novel ELISA (AJ Roboscreen, Leipzig, Germany).
Methods: Non-P-Tau concentration was measured in CSF from patients with AD (n=61) from patients with frontotemporal dementia (FTD; n=33), from patients with Lewy body dementia (LBD; n=30). Patients with neuropsychiatric disorders without alteration in the CSF biomarkers (n=19) were chosen as controls. 'Classical CSF Alzheimer biomarkers', Aβ1-42 and/or Aβ42/40 Ratio, Tau, and pTau181, were available for each patient tested. The diagnostic accuracy and receiver operating characteristic (ROC) of the non-P-Tau were evaluated in this study.
Results: Patients with AD presented significantly higher non-P-Tau levels than either controls ($p<0.001$) or all the other neurodegenerative non-Alzheimer patients ($p<0.001$). Interestingly, the sensitivity and the specificity of the discrimination of AD patients from FTD patients were 87% and 86% , respectively, resulting in the area under the ROC curve of 0.918.
Conclusions: our results suggest for the first time, that CSF non-P-Tau could be taken account as a reliable candidate biomarker of Alzheimer's Disease at the dementia stage.

10d. Other: diagnostics

ADPD5-1035

CLOCK DRAWING TEST IN SCREENING FOR ALZHEIMER'S DEMENTIA AND MILD COGNITIVE IMPAIRMENT IN CLINICAL PRACTICE

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Objectives: The Clock Drawing Test (CDT) is widely used for brief evaluation of cognitive impairment in elderly. The aim of the study is (1) to describe the diagnostic accuracy and inter-rater agreement of subjective CDT ratings by different specialists and (2) to compare it with complex rating performed by a neuropsychologist in patients with amnestic mild cognitive impairment (aMCI) and Alzheimer's dementia (AD).

Methods: Three cognitive neurologists, three neuropsychologists and six medical residents with no experience in cognitive neurology rated 200 CDTs (50 mild AD, 50 aMCI, 50 healthy elderly and 50 patients with subjective memory complaints) using a binary (yes - no) classification to evaluate impairment in the CDT. An experienced neuropsychologist rated the CDTs using a 17-point scoring system. All raters were blinded to the diagnosis.

Results: When using the binary classification, neuropsychologists had highest sensitivity (89%) in differentiating mild AD patients, followed by neurologic residents (80%) and cognitive neurologists (79%). When differentiating aMCI patients, the sensitivity was 84% for neuropsychologists, 64% for cognitive neurologists and 62% for residents. All groups of raters showed moderate agreement (kappa = 0.4 - 0.6). The sensitivity using the 17-point scoring system was 92% in mild AD patients and 69% in aMCI patients.

Conclusions: A binary classification of CDT shows high sensitivity for mild AD even in non-experienced raters. However, neuropsychologists outperformed residents and neurologists in differentiating aMCI patients from cognitively healthy elderly. The diagnostic accuracy was not substantially improved by using a complex scoring system.

10d. Other: diagnostics

ADPD5-1062
COGNITIVE IMPAIRMENT RELATED WITH DEPRESSION PRESENTING AS "FRIDGE SIGN"

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Objectives
The 'Fridge sign', defined as the behavior of putting wrong things in the refrigerator, is considered as a warning sign indicating dementia in public. Here we demonstrate its value in cognitive assessment by examining initial neuropsychological data between the 'Fridge sign' group and the mild cognitive impairment (MCI) or suspected early stage Alzheimer's disease (AD) group with later AD progression.
Methods
We reviewed the medical charts of 23 patients showing 'Fridge sign' and 10 AD patients whose initial status was MCI or early stage of AD (CDR 0.5). We analyzed the demographic data, initial neuropsychological test and diagnosis of the two groups.
Results
The diagnosis and neuropsychological test of 'Fridge sign' group significantly differed from initial profile of AD. 78% of patients were confirmed as subjective or objective cognitive complaints related to depression (13 patients (56.5%) as cognitive impairment related with depression (CIRD), 5 patients (21.7%) as subjective memory impairment with depression). 3 patients (13.0%) were MCI and 2 patients (8.7%) were AD. On initial neuropsychological test, the scores of 'Fridge sign' group were higher in most items, however, the digit span forward, phonemic word fluency and word stroop test scores did not differ from AD progression group.
Conclusion
In contrast to common belief, the 'Fridge sign' group keeps favorable outcome in many domains except attention and frontal lobe function compared to AD group. It is probable that depression may be associated with attention deficit and frontal lobe dysfunction, thus causing the 'Fridge sign'. Further longitudinal study is needed.

10d. Other: diagnostics

ADPD5-1065

POST-LUMBAR PUNCTURE HEADACHE EXPERIENCE IN THE STUDY FOR ALZHEIMER'S DISEASE

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Objectives
Headache worsened by standing and improved after lying down is a frequent complication of lumbar puncture (LP). We performed LP in our study for searching biomarker of Alzheimer's disease (AD) to the target of AD patient and normal control. We analyzed the incidence and the risk factor for post-lumbar puncture headache (PLPH).
Methods
We retrospectively collected data from 47 participants who underwent LP. Twenty AD patients and twenty-seven normal control aged 51-88 underwent LP and drainage of cerebrospinal fluid with Quincke 20G needle. Data included needle characteristics, CSF pressure assessed by LP, degree of small vessel disease and hippocampal atrophy on MRI, clinical scale for cognition and basic demographic factors including age, sex and BMI. One to two weeks after the procedure, we checked PLPH in participants.
Results
PLPH was reported in 23 (48.94%) subjects, among which noted severe in 15 (65.22%) subjects. The incidence of PLPH was significantly high in younger normal control subjects. Risk factors for PLPH were examined using logistic regression. Only young-age is associated with increased risk for PLPH (p value=0.047).
Three of young-aged subjects who were excluded from this study had lumbar puncture performed with atraumatic spinal needle, and none of them suffered from headache.
Conclusions
Young age is a strong risk factor for PLPH. But it may be possible to reduce PLPH by using atraumatic spinal needles. Further study is needed.

10d. Other: diagnostics

ADPD5-1080
SOLUBLE FULL-LENGTH AND HETEROMERS OF AMYLOID PRECURSOR PROTEIN IN THE CEREBROSPINAL FLUID

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Objective: The aim of this study is to assess the presence of soluble full-length amyloid precursor protein (sAPPf) in cerebrospinal fluid (CSF), the oligomerization state of sAPP and how heteromers affect with the quantitation of sAPPα and sAPPβ by ELISA.
Methods: sAPPf and sAPP heteromers were characterized in CSF and brain extracts by co-immunoprecipitation, ultracentrifugation in sucrose density gradients and native gel electrophoresis. Alzheimer's disease and non-disease CSF were assayed for sAPPα and sAPPβ by ELISA following immunoprecipitation with different anti-APP antibodies.
Results: sAPPf co-exists in CSF with sAPPα and sAPPβ, and all forms are capable of assembling into heteromers. These CSF heteromers differ from APP membrane-dimers in brain extracts. ELISA determination of the levels of sAPPα and sAPPβ in CSF samples was affected by previous immunoprecipitation of the CSF by APP C-terminal or sAPPβ antibodies.
Conclusions: Quantitation of CSF sAPPα and sAPPβ in CSF by ELISA is affected by presence of sAPPf and assembly of all sAPP species into heteromers. This should be taken into consideration when exploring their potential role as CSF biomarkers.

10d. Other: diagnostics

ADPD5-1103

BLOOD METABOLITES AS DIADNOSTIC MARKERS FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurodegenerative disease, and represents the most prevalent cause of dementia. Specific biomarkers for diagnose of AD at the early stage are essential to prevent and treat the disease. To find such biomarkers, we analyzed 579 plasma metabolites of age-matched healthy controls (n=60, 68.2 year old), mild cognitive impairment (MCI) patients (n=35, 75.2 year old), and AD patients (n=31, 73.5 year old) by capillary electrophoresis/mass spectrometry. The average plasma levels of 136 metabolites, which were 20% of total 579 detectable metabolites, were relatively low in all plasma samples and exhibited a high measurement deviation. Such 20% metabolites were excluded from the statistic analysis. Of the remaining 463 metabolites, a significant part of the metabolites was not detected in the samples. This gave a high individual deviation as biomarkers. Therefore, 70 metabolites detectable in more than 80% of all participants were selected and further subjected to t-test statistical analysis. Our results revealed that plasma levels of 6 and 7 metabolites significantly changed in MCI and AD, respectively, compared with the controls. Receiver Operating Characteristic (ROC) analyses of these metabolites enabled us to diagnose MCI and AD with high accuracy. The ROC Area Under the Curve (AUC) values for MCI and AD were 0.930 and 0.927, respectively. This study was supported a research program by the National Institute of Biomedical Innovation (NIBIO).

10d. Other: diagnostics

ADPD5-1142
CSF SPHINGOLIPIDS AND PHOSPHOLIPIDS CHARACTERIZATION IN DEMENTIA PATIENTS

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Objectives: Cerebrospinal fluid (CSF) biomarkers have been recently described for the improvement of AD diagnosis, even though, to date, diagnosis remains mainly based on neuropsychological tests. The objective of the present effort is to transfer MALDI profiling technology to the study of lipid profile in CSF samples, to assess a MALDI specific CSF lipid profile and identify new biomarkers.
Methods: Total lipids from CSF samples (AD n=10; *idiopathic* normal-pressure hydrocephalus (iNPH)=10; controls=10) were extracted according to Bligh and Dyer modified method and analyzed by MALDI. Profiles were analyzed by ClinProTools software. For sphingolipids and phospholipids characterization, lipid extracts were separated by HPTLC and directly analyzed by MALDI (Torretta E. et al, Electrophoresis 2014).
Furthermore, for a direct MALDI CSF lipid analysis, crude CSFs diluted in distilled water (1:2) were analyzed by MALDI and peaks searched against NIST database.
Results: Profiling preliminary results of lipid extracts from CSFs of 10 controls, 10 iNPH and 10 AD indicated a characteristic quantitative lipid CSF profile able to differentiate controls and iNPH vs AD.
Sphingolipids and phospholipids semiquantitative pattern provides a characteristic pattern in AD vs iNPH and controls.
Furthermore qualitative lipid characterization can be obtained by analyzing directly the crude CSF by MALDI in patients vs control samples for the identification of new lipid targets.
Conclusions: MALDI profiling of CSF lipid extracts contributed to the detection of specific lipid profile and possible biomarkers for an early AD diagnosis. HPTLC-MALDI and direct lipid analysis offered a hint for the identification of new lipid markers.

10d. Other: diagnostics

ADPD5-1143

CSF AND SERUM MALDI PROFILING FOR THE IDENTIFICATION OF NOVEL BIOMARKERS IN DEMENTIA PATIENTS

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Objectives: In elderly, the increase of dementia incidence and the lack of effective therapies have stimulated the search for early markers for discrimination and prevention of this pathology. In this contest we profiled the CSF protein content in old patients with cognitive impairment affected by Alzheimer's disease (AD) and idiopathic Normal Pressure Hydrocephalus (iNPH) compared to controls.

Methods: 10 CSF from AD, 10 from iNPH patients and 12 from controls were profiled by MALDI-MS for the detection of small proteins discriminating the two forms of dementia from healthy subjects. Statistics (Wilcoxon test $p<0.01$, PCA analysis and ROC AUC>0.800) and classification models were performed by ClinProTools Software. For the identification of putative biomarkers (peaks discriminating the different classes), an in-gel approach (SDS-PAGE separation) coupled to MALDI-MS was adopted.

Results: MALDI Profiling allowed to discriminate iNPH (resulting similar to controls) from AD patients through the presence of 28 differentially changed peaks in the acquisition range of 4-34 kDa. Among them, 6 were selected (on the base of the corresponding p-values, ROCs and box-plots) and combined to build models to classify a blind set (n=50) of CSFs.

SDS-PAGE coupled to MALDI-MS allowed peak identification corresponding to a protein involved in lipid transport.

Conclusions: CSF MALDI profiling can be adopted to identify protein differences in various types of dementia in order to obtain a specific pattern to support clinical and biochemical diagnosis. This approach can also provide new putative biomarkers to couple to the existing molecules, whose expression is currently evaluated in clinical practice.

10d. Other: diagnostics

ADPD5-1251

COMBINING COHORTS OF PATIENTS WITH MILD COGNITIVE IMPAIRMENT TO PREDICT PROGRESSION TO ALZHEIMER'S DISEASE

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Objectives: It is important to detect which patients with mild cognitive impairment (MCI) have a high risk for dementia in order to identify those who could benefit from treatment and preventive measures. We evaluate the feasibility of combining MCI cohorts and test how well our prediction model performs when different cohorts are used as training and test sets. This is a vital issue when evaluating clinical utility.

Methods: We combined data from four large cohorts of MCI patients: ADNI, AddNeuroMed, DESCRIPA and Kuopio MCI to predict progression to Alzheimer's Disease. All patients had age and gender corrected MRI data, MMSE and the APOE genotype available and additionally some results on neuropsychological tests and CSF. Altogether 875 patients were included in the analysis. We used the Disease State Index to classify patients as stable or progressive MCI.

Results: We tested each cohort and their combination using 10-fold cross-validation and compared the result to inter-cohort bootstrapping analysis done by testing each cohort separately with a model built from the other three cohorts. The cohorts differed in several aspects, including age, years of education and MMSE score. The AddNeuroMed study had a fixed follow-up time of 1 year, while the other three cohorts had mean follow-up times of over 2 years. Despite the differences between the cohorts, the intra-cohort prediction efficiency of the combined cohort (AUC=0.76) was close to the average of the individual cohorts.

Conclusions: It is feasible to combine different cohorts but for accurate predictions they need to be sufficiently similar.

10d. Other: diagnostics

ADPD5-1265

WHAT IS THE WORSE IN HEALTHY ELDERLY INDIVIDUALS: HAVING BRAIN AMYLOID OR REFERRING TO A MEMORY CLINIC WITH A SUBJECTIVE-COGNITIVE-DECLINE?

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Objectives. Both healthy elderly individuals with amyloid deposition in the brain or with a subjective cognitive decline (SCD) have higher risks of developing Alzheimer's disease (AD). The objective was to identify which condition in cognitively normal (CN) individuals is the most associated with an AD-like pattern of neurodegeneration.

Methods. We compared structural-MRI and FDG-PET data in i) a group of 15 CN individuals with SCD recruited from a memory clinic to 45 matched controls without SCD recruited from the general population, and ii) a group of 10 CN individuals with amyloid-positive Florbetapir-PET scan to 29 matched controls with amyloid-negative scan.

Results. Regarding structural-MRI, highly significant gray matter atrophy in the hippocampal region was found in the SCD compared to the controls with no SCD. This result was not related to amyloid deposition since both groups did not differ in the proportion of amyloid-positive individuals. By contrast, the amyloid-positive individuals did not show any sign of brain atrophy relative to the amyloid-negative controls. Regarding FDG-PET, no significant hypometabolism was found either in the SCD or in the amyloid-positive groups.

Conclusions. Our findings showed that only CN individuals with SCD who refer to a memory clinic, but not CN individuals with amyloid deposition in the brain, have greater AD-like hippocampal atrophy. Thus, even if they are still asymptomatic, the short-term prognosis in individuals with SCD could be worse than that of individuals having amyloid deposition in the brain. These results emphasize the critical interest for SCD in preclinical AD.

10d. Other: diagnostics

ADPD5-1322
AUTOMATIC EVALUATION OF PARKINSON'S DISEASE TREMOR WITH SUPPORT VECTOR MACHINES

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OBJECTIVE: In this study, we propose a system to automatically assess the Hoehn Yahr Score (HYS) of patients with Parkinson's disease. Our main objective is determine the HYS from 0 to 4 with a low-cost accelerometer for assisting the Parkinson's Disease clinical assessment.
MATERIAL: The resting tremor data of subjects are gathered with the accelerometer of the Nintendo Wii (Wiimote). Instead of determining the amplitude and frequency of the tremor data, we extract various features like maximum, minimum, average and standard deviation values with a windowing technique from the acceleration values. The clinical disability of the PD was graded by the Hoehn and Yahr staging (HYS) and tremor was recorded twice from the more affected side in each patient and from the dominant extremity in each control for a 60 seconds period.. The HYS are learned using Support Vector Machines (SVM) which is a popular machine learning technique. The system is evaluated and tested on a dataset containing 55 subjects where 20 of them were healthy (HYS 0) and 35 of them were patients (HYS:1-4).
RESULTS: Leave-one-out technique is used for training the SVM and the system has average 0.89 accuracy rate (Range: 81-100% changing according to grading by HYS) which is promising for the proposed system.
CONCLUSION: Resting tremor related features were much studied by using different accelerometer methods, but a Wiimote accelerometer with a machine learning system was not assessed previously. The subjects was automatically diagnosed as having PD or normal in the present study.

10d. Other: diagnostics

ADPD5-1549
EVENT-RELATED POTENTIALS IN DIAGNOSIS AND ASSESSMENT OF TREATMENT OF ALZHEIMER'S DISEASE

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To improve the efficiency of the patient in the treatment of Alzheimer's disease are used acetylcholinesterase inhibitors, e.g. donepezil, which leads to increase the amount of acetylcholine in the cortex of the brain and enhances cognitive processes, including memory and thinking efficiency, especially in the initial stage of the disease, delaying the moment of the failure. The aim of this study was to evaluate the usefulness of event-related potentials system for monitoring patients with Alzheimer's disease treated with oral inhibitors of acetylcholinesterase. Material consisted of event-related potentials findings of 30-patient during routine treatment of Alzheimer's disease using inhibitors of acetylcholinesterase, madein the EEG Laboratory of our Neurological Clinic. Tests were performed before inclusion and during therapy. The control group consisted of the 30 healthy subjects of the similar age. The results revealed that event-related potentials in patients with Alzheimer's disease had in some cases prolongation of P300 wave in comparison with healthy controls. Patients treated with acetylcholinesterase inhibitors showed a marked improvement in cognitive functioning, that seen with the Mini Mental State test. In the course of acetylcholinesterase inhibitors treatment in some cases there was a shortening of P300 wave latency. The obtained results allow to hope that finally there is the possibility of supplementing subjective psychological tests for early diagnosis and monitoring of Alzheimer's disease progression and treatment with new, this time objective kind of electroneurophysiological tests.

10d. Other: diagnostics

ADPD5-1564
BRAIN-ENRICHED MICRORNAS CIRCULATING IN PLASMA AS BIOMARKERS FOR MILD COGNITIVE IMPAIRMENT, ALZHEIMER'S AND PARKINSON'S DISEASES

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Objectives: There is a great need in an accurate and minimally invasive assay for early detection and differentiation of neurodegenerative diseases. We will present analysis of plasma levels of brain-enriched microRNA biomarker pairs comprised of (i) microRNA enriched in brain regions affected by the pathology (hippocampus in AD, midbrain or frontal cortex in PD) and present in neurites and synapses, and (ii) microRNA enriched in brain regions/cells not involved in the pathology.
Methods: Plasma levels of pre-selected microRNAs are measured by individual RT-qPCR; statistical analysis is performed as described in Sheinerman, Aging 2013, 5:12. Clinical data and plasma samples of PD patients and healthy controls used in the present work include those obtained from the BioFIND Study.
Results: Independent cohorts of plasma samples from PD, MCI, and AD patients and healthy controls have been analyzed. Correlation between levels of microRNAs in plasma was considered in selection of effective microRNA biomarker pairs. Differentiation of MCI and AD from PD and of MCI and PD from control by selected microRNA pairs with up to 95% to 100% accuracy has been achieved.
Conclusions: The analysis of brain-enriched circulating cell-free microRNAs as biomarkers for detection and differentiation of neurodegenerative diseases demonstrates validity of this approach, which could also be complementary to neuroimaging and CSF biomarkers.
This work is supported in part by grants from the NIH/NIA and The Michael Fox Foundation for Parkinson's Research. BioFIND is funded by The Michael Fox Foundation for Parkinson's Research and the National Institute Neurological Disorders and Stroke.

10d. Other: diagnostics

ADPD5-1708

MAGNETIC-NANOPARTICLE-BASED ULTRA-HIGH-SENSITIVITY QUANTITATIVE DETECTIONS OF PLASMA A-BEAT-40, A-BETA-42, AND TAU PROTEIN FOR DIFFERENTIATING MILD COGNITION IMPAIRMENT FROM EARLY-STAGE ALZHEIMER'S DISEASE

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Magnetic nanoparticles biofunctionalized with antibodies against A-beta-40, A-beta-42, and tau protein, which are promising biomarkers related to Alzheimer's disease (AD), were synthesized. We characterized the size distribution, saturated magnetizations, and stability of the magnetic nanoparticles conjugated with antibodies. In combination with immunomagnetic reduction technology, it is demonstrated such biofunctionalized magnetic nanoparticles are able to label A-beta-40/-42 and tau protein specifically. The ultra-low-detection limits of assaying A-beta-40/-42 and tau protein *in vitro* using the magnetic nanoparticles via immunomagnetic reduction are determined to a concentration of ~10 pg/mL. Further, immunomagnetic reduction signals of A-beta-40/-42 and tau protein in human plasma from normal samples and patients with mild cognition impairment due to AD and early-stage AD were analyzed, and the results showed a significant difference between these three groups. These results show the feasibility of using antibody-functionalized magnetic nanoparticles as reagents for assaying low-concentration A-beta-40/-42 and tau protein through immunomagnetic reduction, and also provide a promising new method for early diagnosis of mild cognition impairment due to AD and early-stage Alzheimer's disease from human blood plasma.

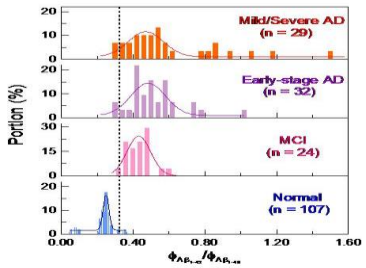

Fig. 1. Statistic results of the ratio of plasma A-beta-42 to A-beta-40 for normal controls and patients.

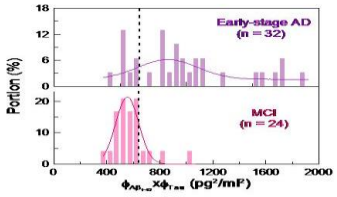

Fig. 2. Statistic results of the products of plasma A-beta-42 and tau protein for patients with mild cognition impairment due to AD and early-stage AD.

10d. Other: diagnostics

ADPD5-1719

INTEREST OF CSF HYPOCRETIN-1 LEVEL IN AN EARLY STAGE OF ALZHEIMER DISEASE.

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Objective: To study cerebrospinal fluid (CSF) Alzheimer' disease (AD) biomarkers and hypocretin-1 levels relationships in patients with cognitive troubles including patients in an early stage of AD patients and hypocretin-deficient narcolepsy-cataplexy (NC) diagnostic accuracy.
Methods: Ninety-one cognitive patients (37 AD, 16 mild cognitive impairment (MCI due to AD), 38 others dementia) and 15 old patients with NC were recruited. Diagnoses were performed blinded with CSF results. CSF Ab_{42}, total Tau, Phospho-Tau_{181}, hypocretin-1 were measured.
Results: A higher hypocretin-1 levels were found in MCI due to AD patients compared to other dementias. CSF hypocretin-1 was significantly and independently associated with AD/MCI due to AD with an OR of 2.70 after full-adjustment, greater than $A\beta_{42}$. A positive correlation was reported between $A\beta_{42}$ and hypocretin-1 levels ($r=0.43$, $p=0.001$) in the AD/MCI due to AD group only. No association was found between sleep problems and any of CSF biomarkers. None of patients with NC achieved pathological cut-offs of $A\beta_{42}$, with respectively one and four patients with NC above Tau and P-Tau cut-offs, without any correlation between hypocretin-1 and other biomarkers.
Conclusions: Our results suggest a pathophysiological relationship between $A\beta_{42}$ and hypocretin-1 in AD process especially in an early stage of the disease. Further longitudinal studies are required to validate these biomarker interactions, and to precise the cause-effect relationship and the role of wake/sleep behaviour in the regulation of the amyloid plaque formation.

10d. Other: diagnostics

ADPD5-1813
SACCADIC EYE MOVEMENTS IN ALZHEIMER´S DISEASE
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Introduction: There are abnormalities in eye movements in individuals with Alzheimer´s disease,
which are related to oculomotor frontal-subcortical circuit dysfunctions.
Objectives and aims: The aim of the study is to compare the parameters of saccadic eye movements in individuals with Alzheimer´s disease with those in older adults without dementia.
Methods: 31 individuals with mild and intermediate Alzheimer´s dementia (AD) (MMSE> 13) (26
women, mean age 76.8 ± 6.41 and 5 men, mean age 79.1 ± 5.21) and 30 individuals without
symptoms of dementia (matched for age) were examined.
The parameters of saccadic eye movements were measured with the use of Saccadometer Advanced.
Two experiments were performed: Latency Trials (LAT) and Reflexive with Gap (RXG). Saccadic
latency [ms], promptness [Hz], duration [ms], amplitude [deg], peak velocity [deg/s] and the number of executed saccades were measured.
Results: Statistically significant differences in the number of saccades (p = 0.000024), latency (p = 0.039), promptess (p=0,01), duration (p = 0.000035) between patients with AD and control group.
Conclusions: It was found that the level of oculomotor efficiency in mild and intermediate Alzheimer´s disease is significantly lower in relation to older people without dementia.

10d. Other: diagnostics

ADPD5-1817

INFORMANTS ARE BETTER IN DISTINGUISHING BETWEEN SMC AND MCI IN MCNAIR FREQUENCY OF FORGETTING QUESTIONNAIRE. FINDINGS FROM CZECH BRAIN AGING STUDY.

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Objectives: Subjective memory complaints (SMC) and Mild cognitive impairment (MCI) as pre-dementia stages of cognitive decline could be diagnosed and differentiate by using clinical examination, neuropsychological cognitive evaluation and subjective memory questionnaires. McNair Frequency of Forgetting Questionnaire (MFFQ) is one of the instruments, which are used in this field. MFFQ consists of two identical lists of possible memory failures, first for participants and second for informants.
We tried to find out which part of MFFQ is more sensitive for distinguishing between SMC and MCI.
Methods: Subjects, participants of Czech Brain Aging Study (CBAS), were examined at the Memory Center ICRC, St. Anne´s University Hospital Brno. They underwent clinical examination, neuropsychological testing and self-assessment, including MFFQ. Results acquired from both parts of MFFQ were compared to neuropsychological conslusions.
Results: 151 subjects, with memory complaints were examined, during the year 2013 and 2014. Difference in MFFQ scores between subjects and informants was 9,25 points and was stastically significant. Informants discriminated good between SMC and MCI (stastically significant difference in MFFQ scores), while subjects themselves not.
Conslusion: Subjects showed overestimation in self-assessment of memory impairment and their evaluation was not able to discriminate between SMC and MCI. In opposite, informants distinguished these stages successfully.

10d. Other: diagnostics

ADPD5-1952

RELATIONSHIP BETWEEN EEG POWER AND EARLY DIAGNOSIS AND COGNITIVE FUNCTION OF ALZHEIMER'S DISEASE AND VASCULAR DEMENTIA

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Objective: To explore the relationship between electroencephalography (EEG) power and early diagnosis and cognitive function of Alzheimer's disease (AD) and vascular dementia (VaD) in a Han Chinese.
Methods: 30 AD patients, 30 VaD patients and 30 healthy controls were recruited in the present study. All participants were measured and evaluated using quantitative EEG and visual EEG. We recorded all four wave frequencies including δ (0.8-4.0Hz), θ (4.0-7.8Hz), α (7.8-12.8Hz) and β (13.0-20.0Hz) from different brain regions, and calculated the value of δ+θ/α+β as the final evaluation index. The differences in δ+θ/α+β ratio and EEG scores were analyzed among three groups.
Results: The value of δ+θ/α+β and EEG scores were significantly increased in AD patients group compared with healthy controls in the measured brain regions ($P < 0.05$). Additionally, the similar consistent results were also observed in VaD patients compared with healthy controls, except for right temporal lobe and right central regions ($P < 0.05$). Importantly, δ+θ/α+β ratios in left hemisphere were significantly higher to those in opposite regions of right hemisphere in VaD group compared with AD or control group. Moreover, we found that MMSE scores were negatively related to δ+θ/α+β ratio and EEG scores in AD or VaD groups (all $P < 0.05$).
Conclusions: The present study suggested that quantitative EEG may contribute to early diagnosis for AD or VaD, and it may be an effective method for distinguish AD and VaD.
Keywords: Electroencephalography; Wave frequency; Alzheimer’s disease; Vascular dementia

10d. Other: diagnostics

ADPD5-1969
CATEGORIZATION PROCESSES IN MILD AND MODERATE STAGES OF ALZHEIMER DISEASE

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The concept that the progression of Alzheimer's disease (AD) leads to a sequence of cognitive losses corresponding to the inverse order of the normal sequence of ontogenetic cognitive acquisition is called retrogenesis. In this way, specific developmental markers would contribute to identify early changes in cognitive losses. Semantic memory processes, one of the first systems impaired in AD, develop progressively in typical development. Children become more able to associate semantic related stimuli in more complex, taxonomic, ways. Objective: In this study, we investigated if AD-related deterioration of semantic memory involves a decrease in categorization skills with progression of the disease, according to the retrogenesis hypothesis. Methods: We compared the performance of AD patients at mild and moderate stages, and of groups of 7, 10 and 14-year-old children in tasks of free association of semantically related stimuli. Results: ANOVAS showed a decrease in taxonomic associations and an increase in diffuse associations between mild and moderate stages, corresponding to the inverse order shown by children groups. At the moderate AD stage, the pattern was similar to that of 7-year-old children. Conclusions: These results corroborate the hypothesis of an involution of the processes of semantic processes in the course of the disease. The identification of the early cognitive signs of the progression of Alzheimer's disease (AD) may contribute to early interventions.

10d. Other: diagnostics

ADPD5-1988

THE MEMORY BINDING TEST: A PROMISING TOOL FOR EARLY DETECTION OF MEMORY IMPAIRMENT.

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Objective: To describe normative data of a Spanish version of the Memory Binding Test (MBT), a novel test that has been developed with the aim to detect pre-symptomatic memory impairment suggestive of Alzheimer's disease. ***Method:*** Participants were 472 healthy and cognitively unimpaired subjects, 45 to 65 years old. Raw scores of the variables of the test were transformed to scaled scores (SS) on which multivariate regression analysis was applied adjusting by age, gender and education level. A standard linear regression was employed to derive the scaled score adjusted (SSA). Sociodemographic corrections were applied when needed and an adjustment table was constructed. ***Results:*** The performance of the subjects was heterogeneously influenced by sociodemographic factors. Mainly, age influenced negatively the free recall. Education had an overall effect in the performance of the test, resulting in lower performance with lower education level. Women outperformed men especially in the initial learning, influencing the results in some subsequent variables. The only variables unaffected by sociodemographic factors were those related to semantic proactive interference (SPI) and to the retention of learned material. Our results shown that, a certain level of vulnerability to SPI is within the psychometrically normal range, independently of any sociodemographic variable. Regarding retention, close to 100% of the learnt material was maintained across the delay interval. ***Conclusion:*** This study describes normative data for the MBT providing the necessary adjustments for sociodemographic characteristics. This data may prove to be useful for detecting asymptomatic at risk candidates for secondary prevention studies of AD.

10d. Other: diagnostics

ADPD5-2042
SFIDA – PLATFORM TECHNOLOGY FOR EARLY DIAGNOSTICS OF PROTEIN MISFOLDING DISEASES

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Protein misfolding is a common hallmark of many neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and prion diseases. Mounting evidence argues that especially smaller aggregates, so called oligomers, are the pathological culprits in these proteinopathies. Therefore these oligomers might be the most direct and reliable biomarker of the respective disorder. To quantify oligomers in body fluids like blood plasma and cerebrospinal fluid (CSF), however, two major technical challenges have to be addressed: i) the extremely low concentration of oligomers and ii) the ubiquitous presence of an excess of monomers.
We have developed a method designated sFIDA (surface-based fluorescence intensity distribution analysis) for detecting single oligomers of amyloid beta, alpha-synuclein and prion protein, respectively. In sFIDA, protein aggregates are fixed on a capture-coated glass surface and loaded with at least two antibodies carrying two different fluorescence dyes. Single protein aggregates are detected by high-resolution imaging like laser scanning and total internal reflection fluorescence microscopy. The use of the same epitope for capturing and probing renders the assay insensitive against monomeric proteins. By sFIDA analysis of CSF samples, a small cohort of AD-affected patients could be distinguished from an age-matched control group.
Here we present recent advancements of the sFIDA technology including application of standard molecules for calibration and quantification, assay automatization as well as novel data obtained on patient samples. The sFIDA assay will be employed for biomarker-based monitoring of therapeutic success in drug trials and further adapted for differential diagnostics of other protein misfolding diseases.

10d. Other: diagnostics

ADPD5-2099

TARGETING THE GENERATION OF MONOCLONAL ANTIBODIES TO PROTEIN POST TRANSLATIONAL MODIFICATIONS IN THE DIAGNOSIS OF ALZHEIMER DISEASE

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I It has become increasingly clear that post translational modifications of two key proteins, beta amyloid and tau, play a major role in the neurodegeneration process in Alzheimer disease. The IBR Monoclonal Antibody Facility has for many years focused on the generation of mAbs to these two proteins for diagnostic and therapeutic intervention in this disease. In the past, focus has been placed on the production of mAbs to either linear or conformational epitopes of these proteins. It has become increasingly clear that the post translational modifications of these proteins play a key role in the modulating the progression of disease and should be our immunogenic targets. Hybridoma clones have been generated to several pyroglutamate modified sites on beta amyloid and also to several phosphorylation modified sites on tau. Several strategies for the generation of these hybridomas were employed. One highly successful approach was the process of inducing tolerance in newborn mice to the non-modified immunogen followed by immunizatioin of these mice as weanlings with the modified form of the same protein. These monoclonal antibodies which will be discussed in our presentation have the ability to detect diagnostic biomarkers as well as the potential to serve as therapeutic tools.

10d. Other: diagnostics

ADPD5-2101

ALZHEIMER'S DISEASE OUTPATIENT REFERRALS TO A DEMENTIA CENTRE: DIAGNOSTIC CHALLENGES

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Objectives: For the last several years, since partial reimbursement for acetylcholinesterase inhibitors and memantine has been introduced in Bulgaria for AD cases, referrals from general practitioners and neurologists to university hospital based dementia centers have increased. According to the National health insurance fund, only patients with probable AD according to NINDS-ADRDA criteria are eligible for reimbursement. Nevertheless, patients with significant other disorders which could cause cognitive impairment, who do not conform to the criteria, are often sent for administration of treatment with a diagnosis of AD. The objective of our study is to reevaluate cases of outpatients who have been referred with a diagnosis of AD and assessed at the dementia center of First neurology clinic, Sveta Marina university hospital in Varna, Bulgaria, for a one-year period, and point out the main diagnostic challenges faced by referring physicians.
Methods: Retrospective review of medical records.
Results: A total of 150 records were identified. Repeat visits were not counted. Eighty-six cases (57.3%) were compliant with NINCDS-ADRDA criteria for probable AD, and 52 (34.7%) for possible. Twelve patients were classified as not having AD, cognitive decline being due to vascular or psychiatric disorders.
Discussion: Even if AD is the most prevalent among dementing disorders, it may be a difficult diagnosis to establish, especially in cases with comorbidities. Further training on AD diagnosis, differential diagnosis and respective criteria could be useful for physicians who manage AD outpatients in our region, in order to improve diagnostic accuracy.

10d. Other: diagnostics

ADPD5-2117

A COMPARATIVE ANALYSIS OF A NOVEL PSYCHOMETRIC TOOL FOR ALZHEIMER'S DEMENTIA WITH THE MODIFIED ADAS-COG

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Objectives:

To examine the accuracy of the Montreal Cognitive Assessment (MoCA) in evaluating Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI).

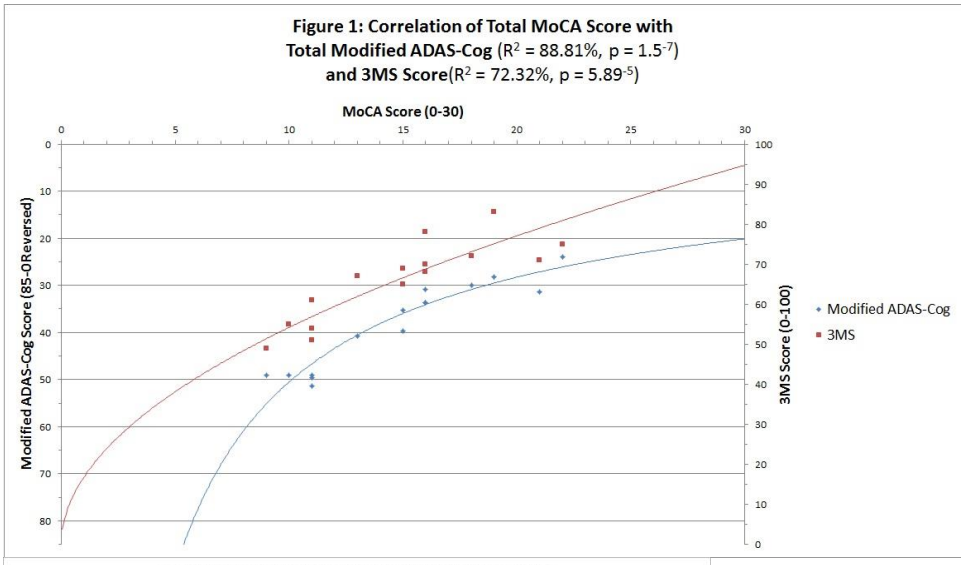
Methods:

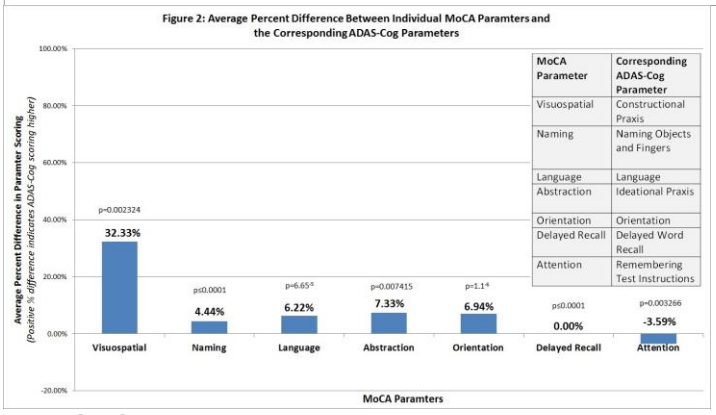
A sample of 15 patients in a dementia facility in Texas, were selected based on a clinical diagnosis of AD or MCI. Patients averaged 83.3 (SD=4.8) years of age.

A Modified Mini-Mental (3-MS) score of ≤ 85 and ≥ 48 was used as an inclusion guideline. MoCA was then administered, followed by Modified ADAS-cog. The sample had a mean 3-MS score of 65.9 (SD=10.0), MoCA score of 14.9 (SD=4.0), and ADAS-Cog score of 38.4 (SD=9.2).

The individual parameters of the MoCA and ADAS-cog were converted into a percentage, and corresponding parameters were compared.

Results:





Conclusion:

The use of formal mental status testing by primary care practitioners for diagnosing dementia was estimated to be only 58%, with the main reported barrier being a lack of time.

The MoCA correlated significantly with both the 3-MS and ADAS-Cog ($p<0.001$), and all individual parameters had a statistically significant correlation with the ADAS-Cog parameters ($p\leq0.01$).
The relative ease of MoCA administration, may allow it to serve as an adequate assessment tool of dementia for primary care practitioners.

10d. Other: diagnostics

ADPD5-2134

ILLITERACY AND LOW SCHOOLING: IMPACT ON COGNITIVE TEST SCORES

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Schooling significantly impacts cognitive performance and is usually regarded as a proxy measure of cognitive reserve. The effect of illiteracy and minimum schooling on cognition is poorly understood. Objectives: To investigate the impact of illiteracy and low schooling on a set of cognitive tests commonly used in dementia diagnosis.
Methods: 164 non-demented community-dwelling women - 60 were illiterate, 52 had 1-2 years of schooling and 52 had 3-4 years of schooling - were assessed in an outpatient geriatric facility with the Mini Mental State Examination (MMSE), Brief Cognitive Screening Battery (BCSB) – episodic memory test of 10 pictures of common objects (Incidental, Immediate and Delayed Recall), Verbal Fluency animal category (VF), Clock Drawing Test (CDT), Digit Span Forward and Backward (DSF, DSB), Raven´s Coloured Progressive Matrices (CPM), Wisconsin Card Sorting Test (WCST).
Results: The groups were equivalent as to age, number of diseases, medications taken daily, depression and anxiety symptoms (Table 1). There were schooling effects in all tests with the exception of the BCSB memory test. For FV, CDT, CPM, DSB and number of categories completed in the WCST, illiterates and those with 1-2 years of schooling had equivalent scores but lower than those with 3-4 years. For the MMSE, DSF and the other WCST parameters, minimum schooling generated higher scores (Table 2).
Conclusions: Minimum schooling generates a significant increase in cognitive test scores. The BCSB memory test does not seem to be influenced by schooling and could be recommended for cognitive screening among seniors with heterogeneous educational backgrounds.

Table 1 – Characteristics of the sample stratified in levels of schooling (N = 164)

Variables	Illiterates		1-2 years		3-4 years		
	Median	25/75 percentile	Median	25/75 percentile	Median	25/75 percentile	p value*
Age	70	(67-73)	69	(68-72,5)	70	(66,5-73)	0,940
Number of diseases	3	(3-4)	3	(2-4)	3	(2-4)	0,145
Number of medications	4	(2,5-5,5)	4	(3-5)	4	(3-5)	0,453
MMSE	21	(20-23)	22	(22-24)	25	(23-26)	<0,001[a]
GDS	2	(2-1)	3	(2-3)	2	(0,5-3)	0, 023
GAI	7,5	(6-8)	7	(6-8)	7	(6-8)	0,162

Note: *Kruskal Wallis test, MMSE=Mini Mental State Examination, GDS=Geriatric Depression Scale, GAI=Geriatric Anxiety Inventory, a = illiterate ≠ 1-2 years ≠ 3-4 years.

Table 2 – Cognitive performance stratified in levels of schooling (n= 164)

Tests	Illiterate		1- 2 years		3-4 years		
	Median	25/75 percentile	Median	25/75 percentile	Median	25/75 percentile	p value*
Memory for 10 pictures							
Incidental recall	6	(5/6)	6	(6/7)	6	(5/7)	0,051
Immediate recall	7	(7/7)	7,5	(7/8)	8	(7/8)	0,674
Delayed recall	8	(7/9)	8	(8/8,5)	8	(8/9)	0,213
Verbal Fluency	11	(10/12)	11	(10/12)	13	(11/14,5)	<0,001[a]
Clock Drawing Test	3	(1/4)	3	(2/4)	4	(3/5)	<0,001[a]
Digit Span Forward	6	(5/6)	6	(5,5/7)	6	(6/7)	<0,001[b]
Digit Span Backward	3	(2/3)	3	(2/3)	4	(3/4)	<0,001[a]
CPM	20	(18/21)	21	(19,5/22)	24,5	(22/27)	<0,001[a]
WCST							
Total correct	52	(45/57)	63	(57,5/67,5)	68	(63/74)	<0,001[c]
Perseverative errors	41	(36/51)	37	(32/42)	33	(28,5/35)	<0,001[c]
Number of categories	1	(0/1)	1	(1/2)	2	(1/2)	<0,001[a]
Trials for the 1st category	46,5	(39/128)	32,5	(29/41,5)	29	(22,5/34)	<0,001[c]
Failure to maintain the setting	3	(2/4)	2	(2/3)	2	(2/3)	<0,001[b]

Note: *Kruskal Wallis test, CPM=Coloured Progressive Matrices, WCST=Wisconsin Card Sorting Test, a=illiterates and 1-2 years ≠ 3-4 years; b=illiterates ≠ 1-2 years and 3-4 years; c = illiterates ≠ 1-2 years ≠ 3-4 years.

10d. Other: diagnostics

ADPD5-2316

The impact of genetic risk loci in Parkinson's disease on age at onset

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Genome-wide association studies (GWAS) in Parkinson's disease (PD) have identified 27 independent loci associated with disease risk. The aim of the current study was to assess whether the polymorphisms associated with PD risk also explain age at onset (AAO) variability in PD patients. To this end, we genotyped 24 independent single nucleotide polymorphisms (SNPs) in a sample of 1,526 well-characterized Danish patients with idiopathic PD and tested for association with AAO. Statistical analyses were based on single and multi locus models. The latter entailed constructing a weighted genetic risk score (wGRS) for each individual. The most significant single locus finding was observed with SNP rs12726330 (located in the GBA [glucosidase, beta, acid] locus). Carriers of the minor allele of this SNP on average showed a reduction in onset age of 3.5 years per risk allele (beta = -3.536, p = 3.2 x10-5). In addition, carriers of the risk allele of SNP rs34311866 (located in the TMEM175/GAK locus) also showed a significantly earlier PD onset age (beta = -1.199, p = 3.8 x10-3). No other nominal AAO associations were observed in the single locus analyses. Combining all tested risk alleles in the wGRS showed significant association with reduced AAO (p = 1.9x10-4), although the variance in AAO explained by the wGRS was small (0.8%). Overall, our study shows that genetic variation in two PD risk loci, i.e. GBA and TMEM175/GAK, also significantly alter AAO in PD, while, in this sample, the cumulative impact of the currently known risk loci only make a minor contribution to AAO variability.

10e. Other: imaging

ADPD5-0317

PREPARATION OF DNA APTAMERS THAT SPECIFICALLY RECOGNIZE HIGHER-ORDER ABETA STRUCTURES

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Objectives: Alzheimer's disease (AD) is the most common neurodegenerative disorder worldwide, characterized by the hallmark accumulation of neurotoxic oligomers and fibrils of the amyloid-beta peptide (Abeta). While it is accepted that oligomers are more neurotoxic than fibrils, *typical immunohistochemcial methods used to characterize Abeta oligomers are not entirely reliable*. The development of more accurate, reliable reagents for detecting and identifying Abeta oligomers is critical to the study of the pathogenesis of AD.
Methods: Following literature precedence, we have prepared two Abeta fibrils that contain structural motifs that may be present in Abeta oligomers: in-register parallel or antiparallel beta-sheets. We will use these structures as templates for the development of DNA aptamers, single-stranded oligonucleotides that can selectively recognize structural variants of proteins. We will use solid state NMR to decipher the structural basis for the binding of the aptamers to the two types of fibrils.
Results: Two distinct quaternary structures of Abeta have been produced and validated using biophysical techniques. These structures have been used as templates for DNA aptamer development. Sensitivity and selectivity data will be presented.
Conclusions: We have used stable, structurally defined, Abeta fibrils to begin developing a toolkit of DNA aptamers that bind selectively to different classes of Abeta assemblies. This toolkit may be used to probe the quaternary structure of Abeta oligomers in humans with AD and transgenic mice that model AD. Structure-specific aptamers may provide mechanistic insights into the neurotoxicity of Abeta oligomers, aiding the development of diagnostic tools and therapeutic reagents.

10e. Other: imaging

ADPD5-1168

GENE DOSE EFFECTS OF APOE4 ON SUBCORTICAL VOLUMES IN MCI PATIENTS

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Objectives. To investigate the presence of a specific ApoE4 dose effect on subcortical volumes in MCI patients as a whole and grouped by their CSF Abeta level.
Methods. 141 MCI patients were enrolled in WP5 of PharmaCOG (E-ADNI) and underwent CSF and blood collection and high resolution 3T MRI. Subcortical volumes were computed using Freesurfer. ANCOVA with post hoc Bonferroni's test was performed to investigate biological and imaging differences among ApoE genotype groups in MCI patients as a whole (All) and grouped in Abeta+ (550 pg/mL).
Results.

		ApoE4 dose (numbers of allele)			p-value		
		0	1	2	0vs1	0vs2	1vs2
All	n	76	53	12			
	CSF Abeta level (pg/mL)	852±292	522±163	456±98	.000	.000	
	Thalamus (mm^3)	6084±637	5879±612	5641±451	.047	.047	
	Putamen (mm^3)	4816±690	4558±619	4351±491	.022	.006	
	Hippocampus (mm^3)	3518±697	3340±568	2761±416	.053	.000	.003
	Caudate (mm^3)	3477±530	3340±568	2761±416		.006	.049
	Pallidum (mm^3)	1513±206	1481±158	1401±163		.031	
	Amygdala (mm^3)	1381±328	1351±240	1144±183		.017	.046
Abeta+	n	14	31	11			
	CSF Abeta level (pg/mL)	436±75	411±98	439±83			
	Hippocampus (mm^3)	3620±594	3258±566	2740±430	.003	.000	.009
	Amygdala (mm^3)	1422±264	1329±240	1157±187	.019	.002	.077
Abeta-	n	62	22	1			
	CSF Abeta level (pg/mL)	940±240	675±97	633	.000		

Conclusions. A specific ApoE4 dose effect is associated with decreased of CSF Abeta level and of several subcortical volumes in MCI patients. ApoE4 genotype and abnormal CSF Abeta level seems to act synergistically to increase volume loss in the hippocampus and amygdala.

10f. Other: clinical trials

ADPD5-0365

DIFFERENCES OF DEPRESSION SYMPTOMS IN PATIENTS WITH EARLY-ONSET ALZHEIMER'S DISEASE BY WHITE MATTER HYPERINTENSITIES

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Depression which is frequently combined with dementia shows higher incidence in early-onset Alzheimer's disease (EOAD) than late-onset AD. It has hypothesized that vascular component or small vessel disease influence depression. However, the influence on EOAD remains uncertain. We investigated the differences in incidence and characteristics of depression by white matter hyperintensities (WMHs) in EOAD patients.

We enrolled 412 EOAD patients. The 15-item Korean version Geriatric Depression Scale (GDS-15) was administered to 412 subjects. We subdivided into mild WMHs (353, 85.7%) and moderate WMHs (59, 14.3%) groups by visual rating scale. Factor analysis was used to assess GDS-15 factor structure. We compared the incidences of individual GDS-15 items between two groups.

Mean age, scores of K-MMSE, CDR were 58.4 years(SD 5.1), 18.8(SD 5.0), and 0.9(SD 0.5). There was no statistical differences in mean scores of GDS-15 (mild 6.1±4.4 vs moderate 7.1±4.7;P=0.75) and incidence of depression by cut-off value (mild 38.4% vs moderate 48.3%,P=0.326) between 2 groups. Three factors were generated in EOAD, which were hopelessness/negative thoughts (items 6,8,12,14,15), unhappiness/unsatisfaction (items 1,3,5,7,11), and monotony/lack of energy (items 2,4,9,10,13). Regardless of WMHs, items 2,10,13 were reported most frequently. Moderate WMHs demonstrated more empty feeling (item 3,P=0.019), a preference to stay at home (item 9,P=0.042), and loss of energy (item 13,P=0.049).

WMHs severity itself did not influence incidence of depression in EOAD and frequent depressive symptoms were similar. However, moderate WMHs showed trends complaining more depressive symptoms. In particular, based on factor analysis, symptoms related to monotony/lack of energy were significantly increased.

10f. Other: clinical trials

ADPD5-0417

REAL-WORLD EVALUATION OF COMPLIANCE AND PREFERENCE (RECAP) IN ALZHEIMER'S DISEASE: ANALYSIS OF PROSPECTIVE DATA FROM SOUTH KOREA

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Objectives: To examine caregiver preference and treatment compliance in Korean patients with mild-to-moderate AD under “real-world” settings.
Methods: This was a 24-week prospective, non-interventional, multi-center study. Eligible patients were grouped into two treatment cohorts based on the baseline AD therapy: oral (donepezil, galantamine, memantine, rivastigmine capsule) and transdermal (rivastigmine patch). Primary effectiveness variables were caregiver preference and patient compliance for AD treatment. Secondary variables included physician’s preference for treatment and adverse events.
Results: A total of 398 patients (65.8% women; mean age 76 years) were enrolled in the study, of which 192(48.2%) were in the transdermal therapy cohort. For patients who were exposed to both oral and transdermal therapies, results showed a significant preference by caregivers for transdermal therapy (65.9%) at week 24. Patient compliance (range: 0-10) to treatment was good and similar in both groups at week 24 (mean scores: oral therapy, 8.8; transdermal therapy, 8.9). Of the 15 physicians who participated in the study, eight physicians preferred transdermal therapy at week 24. A total of 133 patients (33.4%) reported at least one adverse event during the study period: 29.1% in the oral cohort and 38.0% in the transdermal cohort. Nausea (1.9%) and depression (1.9%) were most frequent adverse events in the oral cohort and pruritus (9.9%), rash (4.7%), and dizziness (3.6%) were most frequent in the transdermal cohort.
Conclusions: The RECAP study in patients with mild-to-moderate AD showed a caregiver and physician preference and a good patient compliance for transdermal therapy.

10f. Other: clinical trials

ADPD5-1422

INVESTIGATING THE VALUE OF REPEATED MEASURES ANALYSIS IN EVALUATION OF TREATMENT EFFECTS IN SMALLER STUDIES IN ALZHEIMER'S DISEASE

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1. Objectives:
To examine the usefulness of Repeated Measures Analysis in the evaluation of data from small studies in Alzheimer's disease.

2. Methods
Literature reviews as well as modelling were performed using cognitive data from a double-blind, placebo controlled Phase II study of a putative neuroprotective agent, ACI-91 in 62 patients with mild to moderate probable Alzheimer's disease.
Primary analysis of cognitive/clinical variables used an ANCOVA model at end-point (52 weeks) with log change from baseline as explanatory variable and Treatment as fixed effect, log Baseline as covariate, and Treatment by log Baseline as interaction term. Additionally change from baseline at weeks 12,24,36 and 52 was assessed using a repeated measures mixed model ANCOVA, with Treatment as fixed effect, Baseline as covariate, Time as repeated measure effect and Patient as random effect.

3. Results
Where consistent effects were seem over time, repeated measures analysis highlighted the differences observed. For example, for ADAS-cog-12 in the per protocol population the overall LS mean difference (95% CI) was 3.72 (0.61, 6.83) (p value 0.020) incorporating results at weeks 12,24,36 and 52, compared with an LS mean difference (95% CI) of 3.79 (-1.53, 9.11) for the ANCOVA 52 week analysis (p=0.122).

4. Conclusions
In certain situations the repeated measures analysis can be used to 'add value' to the ANCOVA endpoint analysis. With appropriate caution, this can be used to demonstrate consistency of the treatment effect over time, increasing confidence in the result, increasing the power and thereby significance of the outcome (smaller p value).

10f. Other: clinical trials

ADPD5-1427

A SENSOR-BASED SYSTEM TO SUPPORT DIAGNOSIS AND ASSESSMENT OF PEOPLE WITH DEMENTIA

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The objective of this research is the development of a complete system providing personal health services to people with dementia, as well as medical professionals and caregivers, by using a multitude of sensors, for context-aware, multi-parametric monitoring of lifestyle, ambient environment, and health parameters.

The system consists of three parts:

1. a group of wearable (physiological, motion) and ambient (audio, video, motion, electric) sensors to record the patients' activities
2. Semantic Interpretation of the recorded data and
3. end-user applications which allow the clinicians to manipulate the sensors and view the results.

The evaluation of the system is conducted in two phases. The first phase involves short-term tests (between 1 to 1 1/2 hours) in Alzheimer' s day care center. 90 participants will be monitored in lab conditions and the goals of this pilot are (a) to provide a brief overview of the participants' health status (cognition, behaviours and function), (c) to assess the system's wearable and ambient technology in aspects such as suitability, accuracy, security, fault tolerance, operability and attractiveness and (c) to correlate the system results with the typical clinical assessment tools. The second phase will be based on the findings of the first phase and will include long-term tests (2-6 months) involving 4 participants in their homes.

Acknowledgment

This work has been supported by the EU FP7 project Dem@Care: Dementia Ambient Care – Multi-Sensing Monitoring for Intelligent Remote Management and Decision Support under contract No. 288199.

10f. Other: clinical trials

ADPD5-1523

THE EFFICACY OF CENTRAL REVIEW MODALITIES IN THE QUALITY CONTROL OF ADAS-COG FOR AD CLINICAL TRIALS

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Objectives: Despite the urgent need to find therapies for Alzheimer's disease (AD), the success rate of AD trials has been very limited. One likely contributor to this variability in endpoint measurement within AD trials. It has been reported that the ADAS-Cog, the most widely used endpoint in AD trials, is prone to high error rates that may contribute to unwanted variance (e.g., Shafer et al., 2011). The recognition of these high error rates has led to the use of centralized oversight methodologies to identify and correct scoring errors. The goal of this study was to compare two such approaches: Review of audio recordings of assessments and review of paper source documents alone.
Methods: Aggregated data from 3 double-blind, placebo-controlled AD clinical trials were reviewed. Each trial included assessments using the ADAS-Cog, reviewed via either audio recordings (N = 3,148) or worksheet review alone (N = 2,578). The percentage and rate of errors identified by the two modalities were compared.
Results: There were a higher percentage of assessments that were identified as having at least one error by audio recording review (52%) than by worksheet alone (14%). In addition, the overall rate of errors identified by review of audio recordings was approximately five times higher than those identified by worksheet alone.
Conclusions: Central review of audio recordings of ADAS-Cog administrations is far more effective in identifying scoring errors than review of worksheets alone. The findings are discussed in the context of additional approaches to improving measurement reliability in AD clinical trials.

10f. Other: clinical trials

ADPD5-1524

DISCORDANCE BETWEEN OBJECTIVE AND INFORMANT RATED MEASURES OF COGNITION: RESULTS FROM THE PREDICTORS STUDY

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Background/Objectives: The literature has identified informant report as a valid assessment of patient symptomatology in AD. Despite this, discrepancies in specific areas suggest that informants may be biased by a variety of factors. Little is known about the characteristics of informants that may over or under-report symptoms when compared to objective measures of patient cognitive performance. Our aims were to examine the validity of informant report in the context of a longitudinally dataset and to identify characteristics of informants that predict discordance with objective assessment.
Methods: The sample included participants from the PREDICTORS study, which examined biological/lifestyle markers of AD progression. Informant insight was determined by comparing informant ratings and patient neuropsychological testing results (assessed via bivariate correlation and ANOVAs). Linear regression identified variables associated with informant ratings independent of patient cognition, including: caregiver demographics, time spent with patient, burden, caregiving satisfaction, caregiver-rated patient depression, and presence of cognitive complaint.
Results: Informant-rated cognitive impairment moderately correlated with objective measures ($r = 0.25$, $p < .05$). Notably, 20% of informants denied memory deficit in a population with known AD. Higher informant-rated impairment was associated with higher burden, lower caregiver satisfaction, and higher caregiver-rated patient depression. These variables were more strongly associated with informant ratings than patient cognitive performance.
Conclusions: These results challenge the notion of informant report as a gold score in clinical trials for AD, and underscore the importance of examining the integrity of informant ratings and the characteristics of informants prior to accepting their reports at face value.

10f. Other: clinical trials

ADPD5-1529
METHODOLOGICAL PROCEDURES IN CLINICAL REHABILITATION OF ALZHEIMER'S DISEASE PATIENTS

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Introduction: longitudinal studies of cognitive aging enable detection of the initial stages of Alzheimer's disease (AD), in which intact cognitive abilities and potentialities are being found, thus supporting the development of methodology for non-pharmacological intervention in the early stages.

Objective: Present a clinical rehabilitation program focusing on family guidance and introducing compensatory strategies, thus enabling maintenance of functionality, diminished psychiatric symptoms, and enhanced quality of life for patients and caregivers.

Methodology: 12 patients (6 female), average age 75.42 (06.22), and 9:58 (5.6) years of schooling. Fulfilled NINCDS / ADRDA criteria and were taking maximum anticholinesterase dose. Cognitive profile was evaluated using MMSE, ADAS-Cog, Wechsler Memory (Information and Orientation), Personal Data and Temporal Orientation. Caregivers responded to the NPI and Functional Activities Questionnaire (FAQ). After Initial testing (T1), retesting after 8 months of rehabilitation program (T2). Comprised two sessions per week and Family Guidance every fortnight. Techniques used: Reduced clues, spaced learning, reminiscence therapy, and reality orientation, guided by errorless learning. Patients filled out Personal Data (PD) and Temporal Orientation (TO) every day. **Results**: MMSE (T1: 23.25 (1.82)/ T2: 23.42(2.81); ADAS-Cog (T1: 17.11 (6.73)/ T2: 2.21 (8.59), WMS Information (T1: 4.33(1.89)/T2: 4,58(1,73); WMS Orientation (T1: 3,75(1,29)/T2: 3,67(0,89); DP-StreetT1: 3,00(0,00)/T2: 2.92 (0.29); District T1: 3,00(0,00)/T2: 3,00(0,00);TelephoneT1: 2.75(062)/T2: 2.75 (0.62), NPI-(T1: 23.42(23.38)/T2: 19.83(17.73); FAQ-(T1: 10.67(7.24)/ T2: 13.92(6.92).

Conclusion: These results show that external supports adapted to patients conditions may help maintain functionality for longer periods when introduced in the initial phase of the disease.

Support: FAPESP / AFIP

10f. Other: clinical trials

ADPD5-2302
ARE PEOPLE WITH DEMENTIA AND THEIR CARERS HAPPY FOR RESEARCHERS TO ACCESS ELECTRONIC HEALTH RECORDS TO SCREEN FOR RESEARCH STUDIES?

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Identifying suitable patients for clinical studies in dementia is a pressing issue for researchers. Creating registers of people who have given consent to be contacted and for their data to be used to screen them against study criteria has the potential to increase speed of cohort creation, reduce costs and increase equality of access to clinical research for people with dementia.

Objectives

To investigate whether people with dementia and their carers are willing to give consent for researchers to access their Electronic Health Records (EHR) for the purpose of screening them to assess their suitability to be contacted about specific studies.

Methods

A survey was conducted of the first 310 people registered on the new national dementia consent for approach register in the UK.

Results

124 people (40%) responded to the survey. 115 (93%) responded to the question about EHR data linkage. 67% said they would consent for researchers to access their EHR, 23% were unsure and 10% said they would not provide consent. Of the 'not sure' respondents the primary reason given was that more information was required to make a decision.

Conclusions

The majority of people joining the consent for approach register will consent to researchers accessing their EHR to screen them for studies. Provision of clear information about the use of data will increase consent. Developing links between the UK dementia consent-for-approach register and EHRs will be acceptable to a large majority of potential registrants and increase the value of the register to researchers and patients.

10g. Other: alternative hypotheses

ADPD5-0535

IMPROVEMENT OF INTESTINAL MICROFLORA BY FEEDING OF PREBIOTICS DELAYS ONSET OF LEARNING AND MEMORY DISORDER IN SENESCENCE-ACCELERATED MODEL MICE (SAM)P8

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Objectives:

Senescence-accelerated mice (SAM) P8 is a model of deflect in learning and memory, emotional disorder and age-related impairment, and resemble to symptoms in human senescence. Gastrointestinal microflora (GIM) changes and harmful microbiota markedly increases in accordance with the host aging. Consecutive ingestion of prebiotics affects antioxidant potentials, anti-inflammation via improvement of GIM. In this study we investigated that daily feeding of prebiotics affects the progressing of senescence and delays the onset of learning and memory disorder via the improvement of GIM using SAMP8.

Method:

We raised 45 male SAMP8 (4 weeks old) and 10 male SAMR1 as normal senescence mice individually until 42 weeks old. SAMP8 were fed 2 kinds of diet; control (AIN93) and replaced sucrose by 5% fructooligosaccharide (FOS). The assessment for the ability of learning and memory was performed using passive avoidance test (PA test). GIM were measured by molecular biological method. Inflammatory cytokines in sera and amyloid-beta concentration in brain were measured using kit, respectively.

Result:

GIM was significantly different and *Bifidobacterium* genus was significantly increased by the feeding of FOS ($p<0.05$). The assessment by PA test was significantly better in FOS group than that in control group ($p<0.05$). Serum TNF alpha, IL-6 and amyloid-beta in brain were significantly lower in FOS group than those in control group ($p<0.05$).

Conclusion:

These results demonstrate that daily intake of prebiotics delays the onset of senescence related symptoms via the improvement of GIM. These findings can contribute to support the treatment and care for senile patients.

10g. Other: alternative hypotheses

ADPD5-1951
MODULATION OF SYNAPTIC COMPLEXITY AND METABOLIC ACTIVITY PREDICTS COGNITIVE DECLINE OR DELAY TO DEMENTIA: A MATHEMATICAL APPROACH

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Objectives: Aging associated cognitive decline often results in some kind of irreversible dementia. The goal of this work is to generate a simple mathematical description of the process of cognitive decline to dementia. The synthesis and compactness of such a formulation could help to refine and focus models and analyses that need to take into account the multitude of factors associated with normal and pathological cognitive aging.
Methods: We formulate the energetic balance of the functional network to describe the cognition variations as functions of metabolic activity and the new defined 'synaptic complexity', which characterizes the connections between several functional networks.
Results: The model predicts that the neuronal network is exposed to an irreversible fall into poor cognition state due to losses of metabolic activity or synaptic complexity. However, this fall will be delayed if the combination of both variables remains high enough. Even after the irreversible fall occurs a little improvement of cognition could be reached by raising the metabolic activity of the brain.
Conclusions: This is a first catastrophe theory approach to cognitive decline. The cognitive decline appears as irreversible, as a consequence of the involved energy and the loss of synaptic complexity. Two important things emerge from the model. First, any kind of treatment must be applied as soon as possible to avoid the irreversible fall. Second, a combined treatment improving synaptic complexity and metabolic activity must be taken into account in dementia therapy.

10h. Other: other

ADPD5-0297
RELATIONSHIP BETWEEN HALLUCINATIONS AND DEPRESSION IN ALZHEIMER'S DISEASE
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Objectives: We investigated links between hallucinations and depression in patients with Alzheimer's Disease (AD).
Method: Thirty participants with probable AD and 32 healthy-older-adults voluntary participated in this study. We assessed the Launay–Slade Hallucination Scale (LSHS, Launay & Slade, 1981), evaluating auditory hallucinations (e.g., "I have been troubled by hearing voices in my head") and visual hallucinations (e.g., "Sometimes, I have seen objects or animals even though there was nothing there"). Participants were also administered The Hospital Anxiety and Depression Scale (HADS, Zigmond & Snaith, 1983), a widely used screening instrument for anxiety and depression in patients with AD. Participants were also administered neuropsychological battery, tapping episodic memory and executive functions.
Results: Hallucinatory experiences were significantly higher in AD participants compared to control participants. AD-related hallucinatory experiences were significantly correlated with depression.
Conclusion: Our outcomes suggest significant relationship between hallucinations and depression in AD, a relationship that is likely to be mediated by affective disturbances.
Keywords: Alzheimer's disease; hallucinations; inhibition

10h. Other: other

ADPD5-0301

NEURO-COGNITIVE-PSYCHOLOGICAL CHARACTERISTICS OF WANDERING IN PATIENTS WITH DRUG-NAÏVE ALZHEIMER'S DISEASE

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Background: Wandering represents one of a major problem occurring in patients with Alzheimer's disease (AD). To find the disproportionate neuropsychological deficit and behavioral psychological symptoms in dementia(BPSD) of AD patients with wandering compared to AD patients without wandering, this study examined the set of neuropsychological tests and caregiver-administered neuropsychiatric inventory (CGA-NPI). **Methods:** Psychotropic-naïve(drug-naïve) probable AD patients with wandering(64) and without wondering(278) were assessed with the Seoul Neuropsychological Screening Battery, which included measures of memory, intelligence, and executive functioning. **Results:** Patients with wandering had lower scores in the Rey-Osterrieth Complex Figure copy, Fist-edge-palm, Alternating hand movement tests compared to patients without wandering. The degree of wandering in AD patients was significantly related with CGA-NPI subdomains of aggression, disinhibition, depression, and delusions. **Conclusions:** This study showed that 1) AD patients with wandering have disproportionately cognitive deficit suggesting frontal and right parietal dysfunctions, 2) wanderings are related with specific BPSD. Considering these results, AD patients with wandering may have specific neuronal anatomic substrates related with pathology of Alzheimer.

10h. Other: other

ADPD5-0613

A 2.8 YEAR ANALYSIS OF THE PROGRESSION OF AMNESTIC MILD COGNITIVE IMPAIRMENT: CLINICAL RESEARCH CENTER FOR DEMENTIA DATABASE

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Background & Objectives
Amnestic mild cognitive impairment (a-MCI) is a risk factor of Alzheimer's dementia (AD). Our objectives are to determine which type of a-MCI is more significant in predicting AD conversion, as well as the other important predictors of AD conversion in a-MCI patients.
Methods
We recruited 183 a-MCI patients from the Clinical Research Center for Dementia (CRCD) database, who underwent at least three times annual detailed neuropsychological tests. All patients underwent blood tests and brain MRI, as well as several questionnaires. We defined verbal a-MCI as patients whose verbal memory is impaired but visual memory is intact; and vice versa for visual a-MCI. Additionally, we defined both a-MCI as patients who have deficits in both verbal and visual memory.
Results
We enrolled 43 verbal a-MCI, 44 visual a-MCI, and 96 both a-MCI patients. The mean age was 70 years and the mean MMSE score was 24 $\pm$ 1. More male patients were classified as verbal a-MCI and more female patients as visual a-MCI. Among the 183 a-MCI patients, 60 a-MCI patients (14 verbal a-MCI, 7 visual a-MCI, and 39 both a-MCI) converted to AD and 99 patients remained a-MCI during a 2.8 year follow up period. Twenty-five of the 45 converted a-MCI patients had at least one Apo e 4 allele.
Conclusion
Within 3 years, more than 30% of a-MCI patients converted to AD: most frequently seen in both a-MCI, then verbal a-MCI, and least in visual a-MCI.

10h. Other: other

ADPD5-1228

MASS SPECTROMETRIC MEASUREMENT OF AMYLOID BETA IN CSF: A CANDIDATE REFERENCE MEASUREMENT PROCEDURE

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Objectives
There is a major need in the Alzheimer's disease (AD) community for an assay that with a high sensitivity, specificity and reproducibly can measure biomarkers with an excellent stability over time. The assays used today are antibody-based and struggling with lot-to-lot variability and matrix effects that influence analyte concentrations depending on sample handling. To overcome the variability and the possible matrix effects that appear with antibody-based amyloid β (Aβ) assays, we have developed an antibody-independent mass spectrometry-based candidate reference measurement procedure (RMP) for $A\beta_{42}$.
Methods
The method uses solid-phase-extraction (SPE) in a 96-well format and differently isotope-labeled $A\beta_{42}$ peptides for calibration in human CSF and as internal standard. Extracted samples were injected on a reversed phase column and analyzed on a high resolution quadrupole-Orbitrap hybrid mass spectrometer.
Results
The candidate RMP method is validated for CSF $A\beta_{42}$ measurements from 150 to 4000 pg/mL with a recovery within 100±15%, intra- and inter-assay imprecision of 5% and 6.4% respectively and an expanded uncertainty of 15.7%.
Conclusion
The candidate RMP will help set the value of CSF $A\beta_{42}$ in a certified reference material, which could be used to harmonize $A\beta_{42}$ assays and facilitate the introduction of cut-off concentrations in clinical practice.

10h. Other: other

ADPD5-1597

A NORWEGIAN PILOT STUDY OF THE TYM TEST (TEST YOUR MEMORY)

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Background
The elderly population is growing and we need short cognitive instruments to screen for dementia. The widely used MMSE has some limitations and better instruments should be aspired for. The TYM was designed to take minimal operator time to administer, test a reasonable range of cognitive functions, and be sensitive to mild AD.
Aim
The aim of this pilot study was to investigate the sensitivity and specificity of the Norwegian version of TYM (TYM-N), and compared it with MMSE.
Method
Patients (n=16) who were referred to dementia assessment at the neurology department and old age psychiatry department at Haugesund hospital filled out the TYM test, in addition to MMSE. The test results were compared with the final diagnosis based on ICD-10.
Results
Mean age 66.5 y (range 52-89), mean completion time 13.3 minutes (range 6-30), mean score 32.2 point (14-47). Using 42 points as cut off, TYM-N had a specificity of 56 % for AD (64 % positive predictive value). For all cases of dementia the specificity was 100 % (100 % positive predictive value). Sensitivity was 100 %. Interrater reliability was also 100 %.
Conclusion
We found high sensitivity and specificity for detecting dementia or pseudodementia, but lower specificity for AD than in previous studies. The study sample was small, which limits the generalizability of results. TYM may, however, be a good alternative to MMSE as it has the advantage of measuring more cognitive domains, takes short time to administer, and is not influenced by hearing loss.

01b. Protein Misfolding & Aggregation: Abeta

ADPD5-1874

THE LOAD AND DISTRIBUTION OF AMYLOID-BETA PATHOLOGY IN SUBTYPES OF LEWY BODY DISEASE.

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Objectives: Lewy body disease (LBD) is the umbrella term for Dementia with Lewy bodies (DLB), Parkinson's disease (PD) and other related disorders. These movement disorders differ in clinical presentation, but pathologically share the same hallmarks, Lewy body pathology. Concomitant pathology is very common in LBD. The aim of the present study was to determine the load and burden of amyloid-beta (Abeta) plaques and neurofibrillary tangles (NFT) pathology in cortical and subcortical areas in subtypes of Lewy body disease.
Methods: Donors with Lewy body pathology at post-mortem evaluation collected by the Netherlands Brain Bank in the period 1999-2013 were included (n=410). Patients were retrospectively diagnosed according to the current diagnostic criteria. PD(D) patients were categorized further into tremor dominance, no-tremor dominance, early-onset and late-onset subtypes. The prevalence, distribution and burden of Abeta pathology was determined in 9 brain regions using morphometric analysis (n=120). In addition, Thal phases were used to evaluate NFT pathology.
Results: DLB patients had higher Thal phases and higher mean Abeta burden in all investigated areas than PD(D) patients. In contrast, PD, PDD and the other PD subtypes did not differ in mean Abeta burden or Thal phases. Longer disease duration and delay of dementia onset was associated with less Abeta pathology in cortical regions in LBD.
Conclusions: This study suggests that Aβ pathology contributes to differences between DLB and PD(D). Abeta pathology may be involved in the timing of dementia with respect to parkinsonian symptoms and progression of the disease.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0258

MINOR SALIVARY GLAND BIOPSY AS A EARLY PATHOLOGICALLY DIAGNOSTIC TOOL FOR SPORADIC PARKINSON'S DISEASE

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Objective: To confirm the presence of Lewy pathology in the minor salivary glands of Par kinson's disease (PD) patients and evaluate the diagnostic value of minor salivary gland s biopsy for the detection of PD.

Methods: Thirteen PD cases and 13 age-matched controls were recruited. PD patients were evaluated with the Hoehn and Yahr s tage and UPDRS III. We performed immunohistochemical staining for Lewy-type alpha-synucleinopathy in minor salivary glands using antibodies against α-synuclein. All the subjects underwent the 11C-CFT DAT-PET scan.

Results: Abnormal accumulation of α-SYN can be found aroud the gland cells in 9 out of 13 PD patients, but none of the contr ol subjects. We found that the α-SYN inclusions were located in the periacinar space. The 11C-CFT uptake in the caudate, anterior and posterior putamen was reduced at different leve ls in 12 the PD patients, but decreased in only one of the controls. When we compared with the DAT-PET results, the sensitivity, specificity, positive predictive value and negative predictive v alue of our biopsy results were 75%, 100%, 100% and 25% respectively. There was no s ignificant difference between the α-SYN inclusions positive group and α-SYN inclusions negative group when compared mean age, mean age of PD onset, mea n disease duration, mean Hoehn & Yahr stage and mean UPDRS-III total score.

Conclusions: These results suggest that minor salivary glands biopsy may be a feasible biomarker of PD, and it may help us improve the clinical diagnostic accuracy of this dise ase.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0275

MODULATION OF ENDOGENOUS ALPHA-SYNUCLEIN BY SMALL MOLECULES

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Objective: To investigate effects of the small molecules affecting alpha-synuclein aggregation *in vitro* on the oligomerization of the endogenous alpha-synuclein in mice.

Methods: Two compounds, FN075 and MS382, known to template and inhibit aggregation of alpha-synuclein *in vitro,* respectively, were stereotaxically injected into the striatum or the substantia nigra of C57B1/6 mice. In order to evaluate the effect of these compounds, 3 behavioral tests (adhesive removal test, cylinder test, and pole test) sensitive to impairment in the nigrostriatal system were performed in mice that had received the compounds. Cell counts of TH-positive neurons in the substantia nigra were performed in mice injected with the compounds into the striatum at 6 months and in mice with the nigral injection at 3 months postinjection.

Results: No acute toxicity of the injected compounds was detected. Behavioral tests revealed impairment in sensorimotor functions without significant reduction in the number of TH-positive neurons in the substantia nigra after the striatal injection of FN075 compared to MS382 and the control mice. In contrast, injection of FN075 into the substantia nigra demonstrated a significant reduction of TH-positive neurons already at 3 months. TH-negative inclusion-like structures were found in dopamine neurons in mice injected with FN075.

Conclusions: Most animal models for Parkinson's disease involving alpha-synuclein are focused on alpha-synuclein overexpression or administration of exogenous alpha-synuclein while here is demonstrated that the use of small molecules that can affect the aggregation of endogenous alpha-synuclein may provide a powerful animal model for Parkinson's disease to elucidate the aggregation of alpha-synuclein.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0278
IMPAIRMENT OF THE UNFOLDED PROTEIN RESPONSE ACCELERATES PROGRESSION OF PD

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Accumulation of α-synuclein relies both on the rate of synthesis and degradation of the protein. The unfolded protein response (UPR) is signaling system the serves keep protein homeostasis in balance. In old α-syn overexpressing transgenic animals, we observed that expression of the UPR chaperone BiP was lowered compared to control animals.

We speculated that if the protein homeostasis is not maintained by the UPR system, then this might allow an acceleration of accumulation and aggregation of toxic misfolded proteins. To address this we crossed our α-syn overexpression model with mice that had an impaired UPR system (the T51A eif2alpha heterozygous mice), and found that mice with an impaired UPR system displayed much faster progression of PD symptoms than did the littermate controls in behavioral tests. These mice also had increased levels of phosphorylated and total α-syn compared to control mice. In cell culture, we observed that if we instead increased phosphorylation of eif2alpha by treating the cells with the clinically approved phosphatase inhibitor Guanabenz, then we could lower the expression of α-syn. Furthermore, treating α-syn overexpressing animals with guanabenz also resulted in a behavioral improvement, suggesting a beneficial effect of guanabenz of restoring the UPR system. In summary, we demonstrate that impairment of the UPR system leads to accumulation and aggregation of α-synuclein and that this in turn accelerates disease progression. These results suggest that modulating UPR activity could be a novel avenue for treatment for PD patients.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0314

STRUCTURE ACTIVITY RELATIONSHIP OF PHENOLIC ACID INHIBITORS OF ALPHA-SYNUCLEIN FIBRIL FORMATION AND TOXICITY

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Introduction

The aggregation of alpha-synuclein is considered the key pathogenic event in many neurological disorders such as Parkinson's disease (PD), dementia with Lewy bodies and multiple system atrophy, giving rise to a whole category of neurodegenerative diseases known as synucleinopathies. Although the molecular basis of alpha-synuclein toxicity has not been precisely elucidated, a great deal of effort has been put into identifying compounds that could inhibit or even reverse the aggregation process.

Aim

The aim of the present study was to assess the anti-aggregating effect of gallic acid (GA) (3,4,5-trihydroxybenzoic acid), a benzoic acid derivative that belongs to a group of phenolic compounds known as phenolic acids.

Methods

An array of biophysical and biochemical techniques and a cell-viability assay was used.

Results

GA was shown not only to inhibit alpha-synuclein fibrillation and toxicity but also to disaggregate preformed alpha-synuclein amyloid fibrils. Interestingly, GA was found to bind to soluble, non-toxic oligomers with no β-sheet content, and to stabilize their structure. The binding of GA to the oligomers may represent a potential mechanism of action. Additionally, by using structure activity relationship data obtained from fourteen structurally similar benzoic acid derivatives, it was determined that the inhibition of alpha-synuclein fibrillation by GA is related to the number of hydroxyl moieties and their position on the phenyl ring.

Conclusion

GA may represent the starting point for designing new molecules that could be used for the treatment of PD and related disorders

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0514
ALPHA-SYNUCLEIN AMINO TERMINUS REGULATERS MITOCHONDRIAL MEMBRANE PERMEABILITY
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Objectives:
A functional characterization of the alpha-Syn N-terminal domain and investigation of its effect on mitochondrial membrane permeability were undertaken in this study.
Methods:
A functional characterization of the alpha-Syn N-terminal domain and investigation of its effect on mitochondrial membrane permeability were undertaken in this study.
Results:
A decrease in cell viability was observed in cells transfected with alpha-Syn/N but not alpha-Syn/delN. In addition, an alpha-Syn/N-induced increase in the level of intracellular reactive oxygen species, alteration in mitochondrial morphology, and decrease in mitochondrial membrane potential were accompanied by the activation of mitochondrial permeability transition pores (mPTP). These changes were also associated with a decline in mitochondrial cardiolipin content and interaction with the voltage-dependent anion channel and adenine nucleotide translocator in the mitochondrial membrane. The activation of mPTPs and reduction in cell viability were partially reversed by bongkrekic acid, an inhibitor of adenine nucleotide translocator (ANT), suggesting that the interaction between alpha-Syn and ANT promoted mPTP activation and was toxic to cells.
Conclusions:
These results suggest that the N terminus of alpha-Syn is essential for the regulation of mitochondrial membrane permeability and is a likely factor in the neurodegeneration associated with PD.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0675

METALLOTHIONEIN: NEUROPROTECTION AGAINST COPPER AND POTENTIAL BIOMARKER FOR NEURODEGENERATIVE ALPHA-SYNUCLEINOPATHIES

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Objectives: Intracellular aggregates of alpha-synuclein are the major pathological hallmark of Parkinson's disease (PD) and dementia with Lewy bodies (DLB) and are linked to neurotoxicity. Multiple triggers of alpha-synuclein aggregation have been implicated, including raised copper. The potential protective role of the endogenous copper- and zinc-binding proteins, metallothioneins (MT), was investigated in relation to copper-induced alpha-synuclein aggregation. As endogenous MT induction has been observed in alpha-synuclein disease[1], current studies are exploring MT levels and distribution across different brain regions in DLB tissue compared to normal controls and also examine copper-MT in plasma/urine samples from PD and unaffected cases as a potential biomarker. **Methods:** Up-regulation of endogenous MT expression was induced in SHSY-5Y cells by the synthetic glucocorticoid analogue, dexamethasone, and beta-thujaplicin. After treatment with dexamethasone/beta-thujaplicin to induce endogenous MT expression, immunofluorescence confocal microscopy was used to quantify protein aggregates in cells with and without 100μM copper treatment. **Results:** Dexamethasone treatment resulted in a significant (*p*, 0.01), dose-dependent up-regulation of MT expression and a significant reduction in copper-dependent alpha-synuclein intracellular aggregates (*p*, 0.01). Ubiquitous (MT-2) and brain-specific (MT-3) isoforms were investigated by transient transfection of the GFP-fusion proteins, resulting in equal alpha-synuclein aggregate suppression by each isoform. **Conclusions:** MTs show neuroprotective capability against copper-induced alpha-synuclein aggregation as up-regulation or over-expression yielded reduced alpha-synuclein aggregation and may be a useful disease biomarker.

1. Pountney, DL, Dickson, TC, Power, JH, Vickers, JC, West, AJ, Gai, WP (2011) Association of metallothionein-III with oligodendroglial cytoplasmic inclusions in multiple system atrophy. Neurotox Res. **19**: 115-22.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0776

EPITOPE MAPPING OF AGGREGATED FORMS OF ALPHA-SYNUCLEIN

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Objectives: Aggregated alpha-synuclein is the main component of Lewy bodies, intraneuronal deposits observed in Parkinson's disease and dementia with Lewy bodies. However, it is not completely elucidated which parts of the protein are involved in the aggregation process. The objective of the study was to map epitopes of alpha-synuclein aggregates *in vitro* and *in vivo*.

Methods: Polyclonal chicken antibodies were raised against short linear peptides spanning the alpha-synuclein molecule. Monomeric alpha-synuclein was expressed recombinantly and oligomers and fibrils were generated. An indirect ELISA was performed to identify surface exposed epitopes of the *in vitro* generated alpha-synuclein aggregates. The antibodies were used for immunohistochemical analysis of brain tissue from transgenic mice overexpressing human alpha-synuclein and from Parkinson's disease patients to determine exposed epitopes.

Results: In addition to the C-terminal part, two epitopes in the N-terminal and one in the hydrophobic mid-region of alpha-synuclein were found to be exposed in the *in vitro* generated oligomeric and fibrillar alpha-synuclein. Similarly, the antibodies specific for those epitopes labelled neuropil threads and neuronal cell bodies in brain from transgenic mice and Lewy bodies in Parkinson's disease patients' brains.

Conclusions: The regions exposed in the *in vitro* generated oligomer and fibrils are similar to those exposed in Lewy bodies *in vivo*. These findings can help to improve the detection of various alpha-synuclein species in biological tissues and fluids for diagnostic purposes.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0780

ALPHA-SYNUCLEIN: CAUSE OR CONSEQUENCE OF CYTOPATHOLOGICAL LESIONS OBSERVED IN VITRO MODELS OF PARKINSON'S DISEASE.

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Parkinson's disease (PD) is characterized by loss of dopaminergic neurons in substantia nigra pars compacta and intraneuronal protein aggregates (Lewy bodies (LB)). Mitochondrial involvement has been postulated based on observations with mitochondrial toxins causing Parkinson's like syndromes. α-synuclein (α-syn) mutations, major LB component, cause autosomal dominant familial PD. Additionally, α-syn-null mice demonstrate increased resistance to MPTP, whereas transgenic α-syn animals treated with MPTP present morphological abnormal mitochondria. Studies have demonstrated that extracellular LB and nigral aggregates immunoreactive to a-syn were often surrounded by activated microglia or inflammatory mediators. Altogether, these observation raise the question of the α-syn role in PD. To investigate this point, we used rat primary mesencephalic neurons enriched or not with microglial cells and injured with mitochondrial toxins, or with α-syn enriched oligomer preparations. Tyrosine hydroxylase (TH) neuron survival, a-syn protein and ATP pool levels were assessed. First, we showed that respiratory chain impairment significantly increased α-syn levels and aggregation. Second, consequently to respiratory impairment, some ATP pool disturbance appeared and was correlated with α-syn level increase. In parallel, we showed on microglia / mesencephalic neuronal culture that α-syn oligomeric form preparation induced significant TH neuronal death dose and time dependent. Interestingly, no toxicity was observed when a-syn was applied on cultures deprived of microglial cells. Additionally, α-syn preparation without any oligomeric fractions did not display any toxicity. In conclusion, we showed that a massive oxidative stress induced α-syn aggregation and that oligomeric fraction of this protein induced a large TH neuronal death involving microglial cells.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0878

INCREASE OF CSF TOTAL, BUT NOT OLIGOMERIC OR PHOSPHORYLATED ALPHA-SYNUCLEIN IN PATIENTS DIAGNOSED WITH PROBABLE ALZHEIMER'S DISEASE

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Objectives: Alpha-synuclein (α-syn) species in cerebrospinal fluid (CSF) are candidate biomarkers for PD, where total α-syn levels are reduced, as opposite to the increase of oligomeric and phosphorylated forms. In this study we investigate the levels of different α-syn species in AD.

Methods: Using our developed ELISA assays, we studied the levels of α-syn species, total (t-α-syn), oligomeric (o-α-syn) and phosphorylated at serine 129 (p-S129-α-syn) in a well characterized cohort of AD patients (n=225) showing at baseline a typical AD profile for the three classical CSF biomarkers - amyloid beta peptide 42 (Aβ42), total tau (t-tau) and phosphorylated tau (p-tau).

Results: The AD patients were compared with cognitively intact patients diagnosed with other neurological disease (n=68) and having a normal CSF profile for the three biomarkers. T-α-syn CSF levels were significantly increased in the AD group ($p < 0.001$ for t-tau; $r = 0.30$, $p < 0.001$ for p-tau) and negatively with the baseline MMSE score ($r = -0.21$, $p < 0.01$).

Conclusion: The increased levels of CSF t-α-syn in AD and the unchanged levels of the other α-syn species suggest a different role of α-syn in AD.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0905
FAMILIAL PD MUTATIONS RAISE THE SUSCEPTIBILITY TO PRION-LIKE AGGREGATION BY DESTABILIZATION OF THE ALPHA-SYNUCLEIN NATIVE TETRAMER

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Pathogenic aggregation of α-synuclein (αS) is implicated in familial and sporadic PD and other human synucleinopathies. Our lab has provided several lines of evidence that αS normally exists in neurons principally as a helically-folded tetramer which resists aggregation. We hypothesize that destabilization events which lead to an equilibrium shift toward unfolded monomeric αS precede the initiation of pathologic aggregation, analogous to what occurs in transthyretin-based amyloidosis. Our new experiments suggest that tetrameric αS from varying sources is unable to trigger pathological fibril formation by itself and is also resistant to prion-like misfolded protein propagation seeded by fibrillar αS from either recombinant or human tissue. This resistance is in stark contrast to αS monomer, which was susceptible to recombinant or human fibrillar material. Further, recent work in our lab has shown that single or compound fPD mutations destabilize the native tetramer, leading to increased relative amounts of the aggregation-prone monomer (Dettmer et al, this meeting). In accord, we tested the impact of fPD mutants on susceptibility to spontaneous or stimulated prion-like aggregation using biochemical approaches. We observed that this shift in the tetramer:monomer equilibrium leads to enhanced propensity towards the ß-sheet-rich aggregation pathway in human neuronal cells. Collectively, our data suggest that the helical, tetrameric form of αS appears devoid of a pathogenic function. Therefore, stabilizing αS in this physiological state represents a new therapeutic strategy aimed at a very early event in idiopathic PD and certain other synucleinopathies: the conversion of physiological oligomers into aggregation-prone monomers.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0913

DEVELOPMENT AND CHARACTERIZATION OF NOVEL CONFORMATION-SPECIFIC MONOCLONAL ANTIBODIES DIRECTED AGAINST ALPHA-SYNUCLEIN PATHOLOGY

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Objectives: To develop and characterize novel conformation-specific monoclonal antibodies directed against α-synuclein pathology.
Methods: Monoclonal antibodies (mAbs) were generated by fusion of Sp2/O myeloma cells and splenocytes from Balb/c mice immunized with α-syn. Positive hybridomas were screened by ELISA and purified by protein G affinity chromatography. mAbs were characterized by inhibition ELISA, dot blot and immunohistochemical analysis
Results: The antibodies described herein recognize specifically a conformational epitope present only in a-syn aggregates, given that they did not recognize the fibrils generated from other proteins of the synuclein family, namely b- and γ-syn, nor cross-reacted with fibrils generated from other amyloidogenic proteins including b-amyloid (Ab), tau protein, islet amyloid polypeptide (IAPP) and ABri. By employing a library of overlapping linear peptides spanning the entire sequence of a-syn, it was shown that the aforementioned antibodies do not recognize peptides with linear sequence of a-syn confirming further that they only detect the aggregated forms of the protein. In extensive immunohistochemical studies of PD, DLB and MSA cases, these antibodies detected a variety of histopathological features including LBs, LNs, GCIs and extracellular accumulations of α-syn, while they did not stain the brain tissue of the age-matched controls. Furthermore, employing one of our conformation-specific antibodies in a sandwich based ELISA, we observed an increase in levels of a-syn oligomers in brain lysates from DLB compared to Alzheimer's disease (AD) and control samples.
Conclusion: The conformation-specific antibodies portrayed herein represent a useful tool for research, biomarker development, diagnosis and even therapy for PD and related pathologies.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-0931
UNFOLDING THE SECRETS OF ALPHA-SYNUCLEIN – STRUCTURE, FORMATION AND PATHOPHYSIOLOGICAL EFFECTS
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PD is a neurodegenerative disorder characterized by the accumulation of α-synuclein (asyn) into Lewy body inclusions and the loss of dopaminergic neurons in the substantia nigra. asyn is a 14 kDa natively unfolded protein present in high amounts in presynaptic terminals. Its function is not known but it is suggested to control neurotransmitter release through its effects on the SNARE complex. It is increasingly accepted that it is the asyn oligomers, and not the fibrils, that are responsible for the cytotoxic effect. However, structural information about both fibrils and oligomers remains scarce due to their size and tendencies to aggregate.
Our aim is to probe the structure and the formation of both fibrils and oligomers using peptide antibodies covering the complete sequence of asyn. The setup in a unique manner enables the details of both fibrils and oligomers to be evaluated. We can today show that the fibrils in a significant manner differs from the oligomeric assemblies. Apart from pinpointing these differences it is our goal to define the features of oligomers found *in vivo* in PD patients.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-1008
NEUROTOXIC EFFECTS OF ALPHA-SYNUCLEIN VARIANTS ENGINEERED TO ABOLISH NATIVE MULTIMERS

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There is disagreement on what the major physiological structure of alpha-synuclein (aS) is in healthy, intact cells. Our work suggests that aS exists principally as a helically folded tetramer of ~60 kDa and related multimers. We hypothesize that if native multimers are triggered by various genetic or environmental factors to destabilize, unfolded monomers will accumulate in neurons, resulting in toxic aggregates characteristic of Parkinson's disease. To assess the determinants of stability of aS multimers in intact cells, we introduced strategic point mutations in the canonical aS repeat motifs (KTKEGV). We essentially abolished physiological aS multimers by certain (but not all) in-register repeat substitutions, as assessed by intact-cell methods: crosslinking and YFP complementation (see abstract by Dettmer *et al*.). We then searched for evidence of neurotoxicity from those aS variants that shifted aS equilibrium from multimers to monomers in cells. Trypan blue staining of M17D human neuroblastoma cells transfected with untagged aS showed multimer-abrogating variants (KLKEGV, KTKKGV, KTKEIV, KTKEGW) to be more neurotoxic: viability was significantly reduced versus cells expressing wild-type aS or multimer-permissive variants (GTKEGV, KTEEGV, KTKEGR). Strikingly, multimer-abrogating variants yielded SDS-stable assemblies suggestive of beta-sheet-rich aggregates, as shown by immunoblotting and immunocytochemistry. Further, we searched for differences in apoptosis-driven neurotoxicity and detected substantially greater levels of the apoptotic marker, cleaved PARP, for all multimer-abrogating variants, similar to the effect of the control pro-apoptotic protein Bax. In support of our overall model of PD pathogenesis, these data suggest strong neurotoxic effects from abolishing stable aS multimers in intact neural cells.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-1011
NEUROPROTECTANT TREHALOSE LEADS TO INHIBITION OF AUTOPHAGIC FLUX AND INCREASES TRANSMISSION OF ALPHA-SYNUCLEIN

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Abnormal accumulation of α-synuclein and formation of intraneuronal inclusions known as Lewy bodies are major pathological features of PD. Abnormal proteins and damaged cellular components accumulated in cells are major targets of quality control system, such as autophagy/lysosome pathway or proteasomal pathway, to maintain cellular homeostasis. In our recent study, we have shown that autophagic failure leads to the acceleration of α-synuclein aggregation and increase of α-synuclein exocytosis and transmission. To further demonstrate the relation between the activity of autophagy/lysosome system and transmission of α-synuclein, we have utilized trehalose, a novel mTOR-independent autophagy enhancer which is shown to be neuroprotective. Interestingly, trehalose-exposed cells were not only inducing the autophagosome formation but also interrupted autophagic flux, causing accumulation of autophagosomes and disruption of substrate clearance through lysosomal fusion. Trehalose-treated cells showed accumulation of intracellular α-synuclein aggregates and α-synuclein secretion was significantly elevated. These results suggest that in contrary to what is known to date, an autophagy inducing agent trehalose actually causes inhibition of autophagic flux and impairment of autophagy/lysosomal pathways, leading to accumulation /secretion of toxic substrates that may not be beneficial to cells.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-1024

DEVELOPMENT OF NOVEL ENZYME-LINKED IMMUNOSORBENT ASSAYS FOR DETECTING ALPHA-SYNUCLEIN SPECIES IN BIOLOGICAL SAMPLES

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Objectives: To develop novel immunoassays capable of detecting the pathogenic forms of alpha-synuclein (α-syn) that might likely serve as diagnostic tools for PD and related disorders.

Methods: We developed, thoroughly validated and optimized novel enzyme-linked immunoadsorbent assays (ELISAs) capable of specifically quantifying different species of α-syn namely total (t-α-syn), oligomeric (o-α-syn) and phosphorylated at serine 129 (p-S129-α-syn) in biological samples. Next, we examined whether our assays could effectively differentiate PD patients from control subjects based on CSF α-syn levels.

Results: Interestingly, our novel ELISA assays highlighted the importance of CSF o-α-syn for diagnosis and early detection of PD. Moreover, combining the measurements of different CSF α-syn species, we observed marked differential CSF patterns between PD and control subjects

Conclusion: Our assays can be promising tools for exploring the potential use of α-syn in diverse biological samples

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-1201
CHARACTERIZATION OF VARIOUS STRUCTURES OF ALPHA SYNUCLEIN PROBED BY HIGHLY EFFICIENT HYDROGEN EXCHANGE MASS SPECTROMETRY
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Alpha Synuclein (aSyn) is the major component of intracellular inclusions responsible for several neurodegenerative disorders including Parkinson's disease. The physiological structure of aSyn has been controversial from natively unfolded structure(s) to a stable helical tetrameric structure. aSyn has also been known to exist as membrane bound forms, dimer/oligomers, and amorphous/amyloid aggregates. Here we examined structures of aSyn in the presence of various additions including trichloroacetic acid. The structures were analyzed by deuterium hydrogen exchange mass spectrometer with highly efficient online fungal protease xiii HPLC system. About 110 peptides covering whole 140 residues was successfully produced. C-terminal domain of aSyn shows high exchange rate, indicating that it constitutes random disordered structure. In contract, N-terminal and NAC domains have slower exchange rate depending on the concentration. It seems to form molten globule-like structure at low concentration, and highly structural forms at high. This result suggests that aSyn could potentially form such highly organized structure(s) as a helical tetramer.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-1270

IMPACT OF THE GOLGI-LOCALIZED, GAMMA EAR-CONTAINING, ARF-BINDING (GGA) PROTEIN FAMILY ON ALPHA SYNUCLEIN OLIGOMERIZATION AND SECRETION

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Aggregation of alpha-synuclein (a-syn) and resulting cytotoxicity is a hallmark of sporadic and familial Parkinson's disease (PD) as well as dementia with Lewy bodies. Rising evidence points to oligomeric and pre-fibrillar forms as the pathogenic species, and oligomer secretion seems to be a crucial event in the spreading and progression of PD pathology. Recent studies implicate that dysfunction in endolysosomal/autophagosomal pathways increase secretion of a-syn via endosomal pathways. Furthermore, mutation in the retromer complex protein VPS35, which is involved in retrograde transport from endosomes to Golgi, was suggested to cause familial PD. GGA proteins (Golgi-localized, Gamma-adaptin ear homology, ARF-binding) regulate vesicular traffic between the trans-Golgi-network (TGN) and endosomes. Several studies reported that GGA proteins might work as an antagonist for retromer complex mediated transport. The aim of this study was to investigate the role of the GGA proteins in the a-syn oligomerization and/or secretion process. Using a novel protein fragment complementation assay where a-syn is fused to non-bioluminescent amino-or carboxy-terminus fragments of humanized Gaussia Luciferase we demonstrate here that GGA proteins alter a-syn oligomers secretion and a-syn oligomer mediated toxicity. Specifically, we determine that GGA3 modifies extracellular a-syn species in an exosome-independent manner. Our data suggest that GGA3 drives a-syn oligomers in endosomal compartments and thus facilitates a-syn oligomer secretion. Preventing the early events in a-syn oligomer release may be a novel approach to halt disease spreading in PD and other synucleinopathies.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-1303

REAL-TIME ANALYSIS OF CELL-TO-CELL TRANSMISSION OF ALPHA-SYNUCLEIN AGGREGATES IN VITRO AND IN VIVO

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Cell-to-cell transmission of protein aggregates is the underlying mechanism for pathological spreading in the brains of progressive neurodegenerative diseases. Under the basis that cell-to-cell transmission of aggregates is associated with the progression of PD, understanding the mechanism of transcellular transmission of aggregates would be a promising strategy to slow down the progression of disease. The greatest impediment to this goal is the lack of experimental systems in which both protein transfer and co-aggregation can be accurately and quantitatively analyzed. In this study, we describe a system in which cell-to-cell alpha-synuclein transmission in mammalian cells and *C. elegans* can be assessed in real-time with high accuracy using a novel dual-cell bimolecular fluorescence complementation (BiFC) technique. Using these models, we demonstrated in live cells and animals that the transferred alpha-synuclein co-aggregated with the endogenous protein, and especially in animals, transcellular transmission of aggregates is associated with nerve degeneration, behavioral deficits, and reduced life span. These models represent *in vitro* and *in vivo* systems by which genetic and chemical modifiers of protein aggregate transmission can be screened and analyzed.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-1446

REGULATION OF AMYLOID FORMATION BY A HAIRPIN MOTIF IN ALPHA-SYNUCLEIN

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Objectives: The beta1-beta2 region of alpha-synuclein (amino acid residues 35 to 60) contains most of the reported disease-related point mutations. Here we investigate the impact of hairpin conformations in the beta1-beta2 region on the aggregation of alpha-synuclein and other amyloidogenic proteins.
Methods: Protein aggregation and toxicity are monitored under conditions which stabilize hairpin conformations in the beta1-beta2 region. Stabilization is achieved either by engineered beta-hairpin-binding proteins (beta-wrapins) or by introduction of an intramolecular disulfide bond.
Results: Sequestration of a beta-hairpin in the beta1-beta2 region by beta-wrapins inhibits alpha-synuclein aggregation and toxicity at substoichiometric concentrations, indicating impeded nucleation of aggregation. Promotion of intramolecular beta1-beta2 contacts by introduction of a disulfide bond results in an aggregation-incompetent alpha-synuclein variant that potently inhibits aggregation and toxicity of wild-type alpha-synuclein, Abeta, as well as islet amyloid polypeptide.
Conclusions: The beta1-beta2 region is a critical regulator of alpha-synuclein aggregation. Hairpin conformers interfere with aggregation nucleation of alpha-synuclein as well as of other disease-related amyloidogenic proteins.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-1913
ALPHA-SYNUCLEIN IS PRESENT AS A MONOMER IN CSF
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Recent studies have suggested that α-synuclein (α-syn)(molecular weight: 14 kDa) exists predominantly as a tetramer (~58 kDa) in solution. However, a subsequent report has shown that brain- and RBC derived α-syn is present as a natively unfolded monomer. Therefore, the native structure of α-syn remains controversial.

To determine a structure of the α-syn in the biological fluid, we analyzed cerebrospinal fluid (CSF) fractionated using ultrafiltration (UF) and size-exclusion chromatography (SEC) , as well as Western blot (WB) and ELISA. We also expressed A140C α-syn variant, which forms dimers, and wild-type α-syn in Escherichia coli, which were then analyzed after fractionation. Recombinant α-syn proteins were subsequently compared with native α-syn in CSF.

When α-syn was evaluated with SEC columns, α-syn in CSF migrated as 50- to 60-kDa proteins; however, in denaturing gels, they migrated as 15-kDa proteins. The similar result was obtained in recombinant wild-type α-syn. The disulfide-linked dimer (A140C) and oligomer migrated slower than either the unfolded recombinant α-syn or the native α-syn in SEC. We also confirmed by UF in the presence of 8M urea. The recombinant α-syn proteins or CSF in these conditions were fractionated by centrifugal UF membrane devices with different molecular weight cut-offs: 30 and 100kDa. Neither the unfolded recombinant α-syn nor the CSF α-syn was passed through the 30KDa membrane of both normal and 8M Urea conditions.

These results suggest that α-syn detected in about 60kDa fraction in the SEC was a monomeric protein, implying that α-syn in CSF exists as a monomer, not a tetramer.

ADPD5-2009

GINSENOSIDE RB1 INHIBITS FIBRILLATION AND TOXICITY OF ALPHA-SYNUCLEIN AND DISAGGREGATES PREFORMED FIBRILS

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Introduction

Compelling evidence indicates that alpha-synuclein aggregation plays a central role in the pathogenesis of Parkinson's disease (PD) and other synucleinopathies. Identification of compounds that inhibit or reverse the aggregation process may thus represent a viable therapeutic strategy against PD and related disorders. Ginseng is a well-known medicinal plant that has been used in East Asia for more than two thousand years to treat several conditions. It is now understood that the pharmacological properties of ginseng can be attributed to its biologically active components, the ginsenosides, which in turn have been shown to have neuroprotective properties.

Aim

To determine for the first time, the potential of the most frequently used and studied ginsenosides, namely Rg1, Rg3 and Rb1, as anti-amyloidogenic agents.

Methods

The effect of Rg1, Rg3 and Rb1 on alpha-synuclein aggregation and toxicity was determined by an array of biophysical, biochemical and cell-culture-based techniques.

Results

Among the screened ginsenosides, only Rb1 was shown to be a potent inhibitor of alpha-synuclein fibrillation and toxicity. Additionally, Rb1 exhibited a strong ability to disaggregate preformed fibrils and to inhibit the seeded polymerization of alpha-synuclein. Interestingly, Rb1 was found to stabilize soluble non-toxic oligomers with no β-sheet content, that were susceptible to proteinase K digestion, and the binding of Rb1 to those oligomers may represent a potential mechanism of action.

Conclusion

Thus, Rb1 could represent the starting point for designing new molecules that could be utilized as drugs for the treatment of PD and related disorders.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-2026

INHIBITION OF THE DEUBIQUITINASE UCHL-1 RESULTS IN ALPHA-SYNUCLEIN CLEARANCE AND MICROTUBULE STABILIZATION IN OLIGODENDROCYTES: IMPLICATIONS FOR MULTIPLE SYSTEM ATROPHY

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Objectives
UCH-L1 is a deubiquitinating enzyme (DUB) belonging to the subfamiliy of ubiquitin-carboxy-terminal hydrolases. UCH-L1 transfers ubiquitin to alpha-synuclein (a-syn) and its overexpression inhibits microtubule formation. In neurons it is required for maintaining the structure and function of synapses, and colocalizes with Lewy bodies in Parkinson's disease. We investigated whether UCH-L1 is a constituent of oligodendrocytes, and is present in glial cell inclusions (GCIs) of patients with multiple system atrophy, which is considered a primary oligodendrogliopathy.
Methods
The presence of UCHL1 was determined in primary cultures of rat brain oligodendrocytes by immunoblot procedure, indirect immunofluorescence and RT-PCR, and in MSA brain sections by immunohistochemistry. To investigate whether UCHL-1 is involved in protein aggregate formation, a specific UCHL-1 inhibitor, namely LDN-57444 (LDN) was used and an oligodendroglial cell line stably expressing a-syn and GFP-LC3 (OLN-GFP-LC3), to monitor autophagy and the autophagic flux.
Results
The data show that UCHL-1 is expressed in oligodendrocytes and colocalizes with a-syn in GCIs of MSA brain sections. After treatment with LDN, small a-syn aggregates, which are constitutively present in OLN-GFP-LC3 cells, are removed. LDN induces the autophagic pathway as indicated by an increase in free GFP and GFP-positive autophagic vesicles. Furthermore, the microtubule network is altered, microtubules appear elongated and more bundled, and an increase in tubulin detyrosination is observed.
Conclusions
UCHL-1 inhibition in oligodendroglial cells results in microtubule reorganization, induction of autophagy and the clearance of a-syn aggregates. UCHL-1 is present in GCIs of MSA brains which further points to a role in aggregate formation during pathogenesis.

01c. Protein Misfolding & Aggregation: alpha-synuclein

ADPD5-2085

SMALL MOLECULE-MEDIATED STABILIZATION OF VESICLE-ASSOCIATED HELICAL ALPHA-SYNUCLEIN INHIBITS PATHOGENIC MISFOLDING AND AGGREGATION

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Alpha-synuclein (aSyn) is an abundant presynaptic protein that is important for regulation of synaptic vesicle trafficking and misfolds into insoluble protein deposits in the course of Parkinson's disease. Native brain aSyn exists in equilibrium between a disordered soluble state and an alpha-helical vesicle-bound conformation.

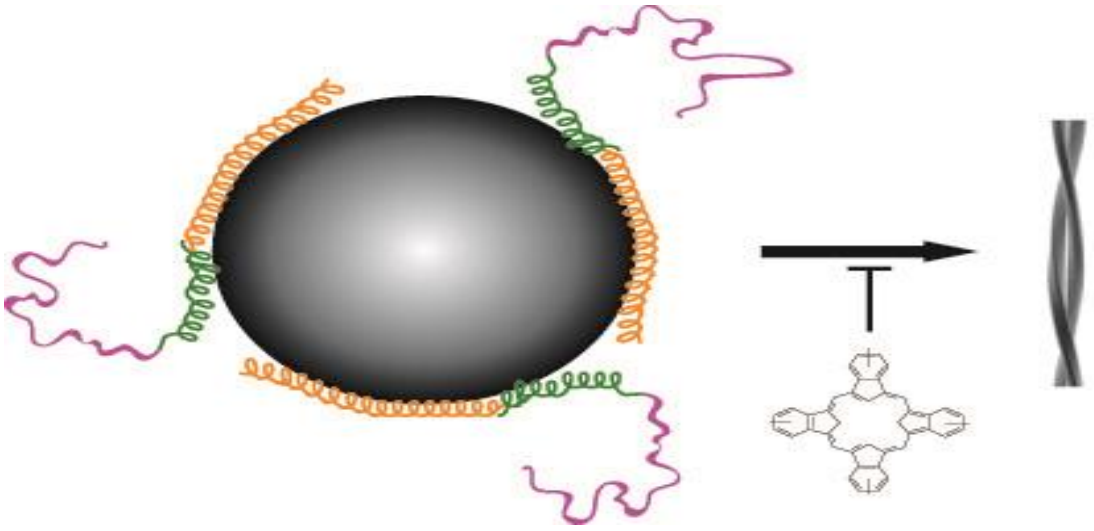
Objectives. To find small molecules that stabilize the helical structure of vesicle-bound aSyn.

Methods. Aggregation of soluble and vesicle-bound aSyn in the absence and presence of different small molecules was monitored using an integrated approach based on circular dichroism, fluorescence spectroscopy and electron microscopy. NMR spectroscopy in combination with site-directed mutagenesis and cell toxicity measurements were employed to obtain insight into the molecular mechanism of inhibition of the aggregation of vesicle-bound aSyn.

Results. Several small molecules, which delay aggregation of aSyn in solution, including the Parkinson's disease drug selegiline, fail to interfere with misfolding of vesicle-bound aSyn. In contrast, the porphyrin phtalocyanine tetrasulfonate directly binds to vesicle-bound aSyn, stabilizes its helical conformation and thereby delays pathogenic misfolding and aggregation [1].

Conclusions. Our results enable a better understanding of the pathogenic aggregation of aSyn and show that stabilization of helical, vesicle-bound aSyn might enable specific targeting of Parkinson's disease and related synucleinopathies.

[1] Fonseca-Ornelas L et al. Nat Commun. 2014 Dec 19;5:5857. doi: 10.1038/ncomms6857



ADPD5-2148

OLIGOMERIC ALPHA-SYNYCLEIN-MEDIATED SYNAPSIN LOWERING IMPAIRS MEMORY

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Objectives:
We recently reported that soluble monomeric intracellular αSyn might modulate Alzheimer's disease (AD) pathophysiology in absence of αSyn cytopathology. In the present studies, we hypothesized that oligomeric αSyn species were the likely effector of the changes described and sought to determine the causality and effect relationship of these events.
Methods:
To address this hypothesis, we used biochemical and imaging analyses of human and transgenic mouse brain tissues combined with a custom-made enzyme-linked immunosorbent assay (ELISA) designed to detect various oligomeric forms of αSyn. Mouse behavior was also used as a functional outcome for our *in vivo* genetic manipulations. Finally, we performed *in vitro* cell delivery of isolated αSyn species to identify the effector(s) involved in primary neurons.
Results:
We found an abnormal accumulation of oligomeric αSyn species in AD brains by ELISA, size-exclusion chromatography and non-denaturing/denaturing immunoblotting techniques. However, only a subset of these assemblies was elevated intracellularly. Importantly, the abundance of low molecular weight αSyn oligomers in human brain tissue correlated with cognitive impairment. Further, the overexpression of *SNCA* in an AD mouse model exacerbated amyloid-beta-induced cognitive deficits and triggered a selective decrease in synapsins, reminiscent of the reduction noted in humans. Following isolation of various monomeric or oligomeric αSyn assemblies from transgenic mice, we found that *in vitro* delivery of oligomeric αSyn but not monomeric αSyn was causing a lowering in synapsin expression.
Conclusions:
Our results demonstrate that endogenous αSyn oligomers can impair memory by selectively lowering synapsin expression.

01g. Protein Misfolding & Aggregation: parkin

ADPD5-1213
EXTRACELLULAR ALPHA-SYNUCLEIN-EVOKED PARKIN DEREGULATION PROMOTES TAU PHOSPHORYLATION AND DOPAMINERGIC PC12 CELLS DEATH.

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Objectives: alpha-Synuclein (ASN), the major component of Lewy bodies and Parkin, a multifunctional E3 ubiquitin ligase, play an important role in pathogenesis of PD. Parkin dysfunction leads to the accumulation of misfolded, aggregated proteins and Tau phosphorylation that results in a progressive neurodegeneration and cell death. In these studies, we examined the effect of extracellular ASN on expression and post-translational modifications of Parkin in rat pheochromocytoma (PC12) dopaminergic cells. Moreover, Tau modification in ASN treated cells was analyzed.
Methods: The experiments were performed using spectrophotometrical, spectrofluorometrical and immunochemical methods and real time PCR analysis.
Results: Exogenously added ASN led to free radicals generation and activation of stress response genes in PC12 cells. ASN-dependent nitrosative stress induced Parkin S-nitrosylation, its inactivation and loss of function. Moreover, ASN evoked significant decrease in Parkin immunoreactivity without changes in mRNA level. Then we analyzed Tau modification in PC12 cells with impaired Parkin E3 ligase function evoked by extracellular ASN. In these cells we observed significant increase in Tau phosphorylation on Ser396. In addition, nitrosative stress caused Tau translocation and nuclear accumulation. ASN-evoked Tau changes led to increase in the amount of free tubulin and concomitantly reduced the amount of polymerized tubulin leading to cells death.
Conclusions: We suggest that ASN-induced cellular stress affects Parkin function and impairs its E3 ligase activity, leading to Tau-dependent a microtubule destabilization and cells death. These findings may thus provide a molecular link between ASN liberation and Parkin dysfunction in sporadic PD.

Supported by NCN grant: 2012/05/B/NZ3/02047

02c. Cell, Molecular & Systems Biology: alpha-synuclein

ADPD5-0254
MUTATED ALPHA-SYNUCLEIN SPECIES ENHANCE AMYLOID-BETA PRODUCTION IN SH-SY5YS

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Objectives:
To determine the effect of wildtype and mutant alpha-synuclein over-expression on the amyloidogenic processing of the amyloid precursor protein (APP) in human SH-SY5Y cells.
Methods:
Stable transgenic lines of SH-SY5Y were generated that over-express wildtype or mutant human alpha-synuclein. The mutants either mimicked point mutations identified in familial early-onset Parkinson's disease (A30P, E46K, A53T), or were truncations of the N-terminus, C-terminus, or 'NAC' region of alpha-synuclein. Secretion of amyloid-beta-40 and amyloid-beta-42 peptides by the cells was quantified with an MSD sandwich immunoassay. Levels of APP amyloidogenic processing were also determined indirectly through a luciferase reporter assay, which detects the liberation of Gal4-tagged APP intracellular domain (AICD) through the action of endogenous beta- and gamma-secretases. A similar luciferase-reporter assay was performed that specifically detects gamma-secretase activity alone. The major enzyme responsible for beta-secretase cleavage of APP is BACE1, and its expression was investigated through Western blotting, and BACE1 promoter-luciferase reporter constructs.

Results:
Overexpressed wildtype alpha-synuclein does not produce a statistically significant effect on APP amyloidogenic processing. However, amyloidogenic processing is significantly enhanced when disease-associated point mutations are present. These mutant alpha-synuclein cells also have elevated BACE1 protein levels, which could account for the increased beta-cleavage of APP. Gamma-secretase activity was apparently unaltered.

Conclusions:
Mutant forms of alpha-synuclein increase the amyloidogenic processing of APP in human neuroblastoma cells. The mechanism for this requires further elucidation, but may involve the increased activity of BACE1.

02c. Cell, Molecular & Systems Biology: alpha-synuclein

ADPD5-0268

A STUDY OF A-SYNUCLEIN OLIGOMERS IN HUMAN RED BLOOD CELLS AS A POTENTIAL BIOMARKER FOR PARKINSON'S DISEASE

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Objective We evaluated the value of α-syn oligomers and the ratio of α-syn oligomers /total protein in human red blood cells (RBCs) as a potential biomarker for PD.

Methods Patients with PD and MSA were recruited according to the United Kingdom Brain Bank (UKBB) Criteria and Quinn Criteria.The total levels of protein in the RBCs were quantified by BCA Protein Assay Kitset. For further detection, α-syn oligomers were tested with a special ELISA.

Results α-syn oligomers were detectable in RBCs of PD, MSA and age-matched control group. The levels of RBCs α-syn oligomers and the ratio of RBCs α-syn oligomers / total protein in PD patients (3954.9 ± 2463.3ng/ml and 29.0± 19.8ng/mg) and in MSA patients(3843.7±1887.2ng/ml and 22.3±13.8ng/mg)were higher than those in control subjects(2716.9± 890.2 ng/ml and 15.4± 7.4 ng/mg.p<0.001). Age, duration, the Hoehn and Yahr stage,age at onset,UPDRS motot scale and motor progression weren't correlated with α-syn oligomers levels in RBCs. In PD group, area under ROC curve of the levels of a-synuclein oligomers was 0.65(p=0.039) and a cut-off value was 3802.5mg/ml with a sensitivity of 41% and a speci-city of 31%. While the area of the a-syn oligomers levels/protein was 0.76(p=0.033) and the cut-off value was 15.03ng/mg with a sensitivity of 79% and a speci-city of 44%.

Conclusions Our observations offer new opportunities for developing diagnostic tools for PD. Elevated Levels of a-syn oligomers and the ratio of α-syn oligomers/total protein in RBCs make them potential biomarkers for PD, especially the latter.

02c. Cell, Molecular & Systems Biology: alpha-synuclein

ADPD5-0448

EMERGING ROLE OF MANNOSE 6-PHOSPHATE RECEPTOR IN ALPHA-SYNUCLEIN ACCUMULATION AND DEPOSIT

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Parkinson's disease (PD) is characterized by intracellular protein deposits known as Lewy bodies, mainly containing α-synuclein protein (α-syn). Deregulation of α-syn and impaired neuronal trafficking, are largely considered as one of the early key factors contributing to PD (Ebrahimi-Fakhari et al., 2012). The primary lysosomal protease involved in α-syn degradation is Cathepsin-D (CD), a lysosomal aspartic endopeptidase synthesized in rough endoplasmic reticulum as pro-Cathepsin-D (proCD) (Cullen et al., 2010). The transport of proCD to endosomes is mediated by the cation independent Mannose 6-Phosphate Receptor300 (MPR300). Upon final delivery to lysosomes proCD is cleaved, at the low lysosomal pH, to form the mature CD (Bonifacino et al., 2003). We looked at the α-syn trafficking from mice and human models of PD, by employing confocal cell microscopy technologies and biochemical assays, and we found that α-syn overexpression induces the missorting of MPR300, leading to its increased degradation and secretion outside neurons. Such missorting seems to be related to an alteration in the endosome-lysosomal pathway and it is partially counteracted by lysosome's inhibitors. In turn, improper sorting of MPR300 affects CD maturation, leading to the increase of the CD precursor form and to the decrease of CD mature fragment. All these events result in the further accumulation and secretion of α-syn and consequently to neuronal degeneration.

Our results advice about a new possible mechanism influencing a-syn degradation and accumulation and may suggest MPR300 as new molecular target to address PD in humans.

02c. Cell, Molecular & Systems Biology: alpha-synuclein

ADPD5-0672

THREE-DIMENSIONAL STUDY OF CORTICAL LEWY BODIES AND SENILE PLAQUES IN DLB

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Triple labeling of brainstem Lewy bodies and neurites has demonstrated a shared three-layered structure with ubiquitin (Ub) at the core, neurofilament (NF) at the periphery with a-synuclein (aSyn) in between (Brain Pathol 2008:18:415-422). To examine whether similar structure is shared with aSyn deposits (Lewy bodies, Lewy neurites and those in senile plaques) in the cerebral cortex, thick floating sections were containing abundant aSyn deposits were obtained from four cases of dementia with Lewy body. Three-dimensional observation of cortical Lewy body on confocal demonstrated similar three-layered structure. Although colocalisation of aSyn and NF was extremely rare on axons, NF-positive segments were sometimes in continuity with aSyn-positive segments or Lewy bodies or with Ub-positive swelling in the senile plaques. Such axonal connections with aSyn and Ub were more abundant in the senile plaques, which provides structural and functional link of these proteins in the cortex of DLB.

02c. Cell, Molecular & Systems Biology: alpha-synuclein

ADPD5-0790

MUTATIONAL ANALYSIS OF THE FERRIREDUCTASE ACTIVITY OF ALPHA SYNUCLEIN

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Alpha synuclein (a-syn) is the predominant protein found within Lewy bodies which are characteristic of Parkinson's Disease (PD) and other synucleinopathies. It has recently been discovered that a-syn has ferrireductase activity. This is extremely important due to the large number of processes in which iron plays a role within the brain. The objectives of this study were to further investigate the ferrireductase activity of a-syn and to link this activity to structure.

Fe(II) production from a-syn was measured using an *in vitro* ferrozine assay. This was performed using both recombinant protein and cell lysates of SHSY5Y cells over-expressing wild type (WT) and mutant forms of a-syn. Recombinant protein was purified using ammonium sulphate precipitation followed by anion exchange chromatography. Circular dichroism was used to assess the conformational state of a-syn and respective mutants in the presence/absence of NADH.

PD mutations of a-syn result in the reduction of K_m for NADH and for Fe(III) resulting in ferrireductases which are less efficient Fe(II) producers compared to the wild type. Metal binding/truncation mutants showed variable effects in terms of activity. Loss of the C-terminal section did not seem to affect activity unless in conjunction with the N-terminal section.

In conclusion, this study has produced an in-depth analysis of the ferrireductase activity of a-syn and has shown that disease mutations of a-syn show altered ferrireductase activity compare to the WT enzyme.

02c. Cell, Molecular & Systems Biology: alpha-synuclein

ADPD5-1038

APOMORPHINE ATTENUATES THE A53T-INDUCED MICROGLIAL REACTIVITY

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INTRODUCTION - Parkinson's disease (PD) is an age-related motor disorder characterized by a progressive loss of dopaminergic neurons and the presence of proteinaceous insoluble inclusions called Lewy bodies containing high concentration of α-synuclein (α-syn). It has been described that α-syn released is phagocyted by microglia, the resident immune macrophages. Microglia can exhibit a pro-inflammatory phenotype. Microglial overactivation can give rise to the progressive neurodegenerative processes related to PD.

OBJECTIVES - Given the role of neuroinflammation in the development of PD, further studies are necessary to understand the precise role of α-synuclein in the microglial reactivity. We have focused attention on the role of microglia in the neurodegenerative PD process, but also on the role of dopamine receptors as potential regulators of activated microglial cells.

METHODS - Primary microglia were stimulated with synuclein preparations and apomorphine, a non-selective dopamine agonist. The effects of these treatments were established by immunocytochemistry, real-time PCR, western-blots and ELISA assays.

RESULTS - Microglial cultures were exposed to synuclein preparations. Synuclein peptides induced pro-inflammatory states, which appear to be peptide-dependent. The strongest response was observed with the A53T mutant. An apomorphine co-treatment reduced this pro-inflammatory state. Thereafter, we have dissected the A53T-induced microglial reactivity and highlighted the recruitment of MAP kinases and transcription factors.

CONCLUSIONS - These results support the hypothesis that activation of microglia in PD may be due to the presence of synuclein peptides. Furthermore, we have shown for the first time that the microgliosis intensity during PD could be diminished with an apomorphine treatment.

02c. Cell, Molecular & Systems Biology: alpha-synuclein

ADPD5-1187

MODELLING ALPHA-SYNUCLEIN PATHOLOGY - TRANSFER FROM IN VITRO STUDIES TO COMPLEX CELLULAR MODELS

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Objective: In several neurodegenerative diseases, pathological modes of action of alpha-synuclein (asyn) comprise a complex interplay of factors influencing oligomer formation. In this study, cellular models applying bimolecular fluorescence complementation (BiFC) were developed to investigate the physiological relevance of *in vitro* findings on asyn aggregation on the cellular level.
Methods: Single particle fluorescence techniques were applied to monitor oligomer formation of fluorescently labeled human asyn as well as interactions with small unilamellar vesicles of different lipid compositions. qRT-QuIC was used to analyze the aggregation characteristics of wild-type asyn as well as the pathological mutants A30P, E46K, and A53T. Lentiviral transduction of asyn-hemiVenus constructs inducible by a Gal4-UAS system was performed on LUHMES and H4 cells. asyn aggregation and subcellular localization were analyzed by fluorescence microscopy and sucrose gradient centrifugation.
Results: Exposure to physiological concentrations of Fe^{3+} induced the rapid formation of specific asyn oligomers exhibiting a pathological gain of function in binding to neutral lipid surfaces. Additionally, aggregation was altered in the pathological asyn mutants A30P, E46K, and A53T. In both H4 and LUHMES cells, transduction with asyn-hemiVenus constructs yielded a model of inducible asyn pathology. In accordance with our *in vitro* findings, fluorescence intensity was augmented by aggregation inducers and decreased upon exposition to known inhibitors of asyn oligomer formation (baicalein, anle138b/c) in the BiFC assay.
Conclusions: Inducible asyn cellular models are suitable both for evaluating the pathophysiological relevance of factors modifying asyn oligomer formation *in vitro* and for validating inhibitors of asyn aggregation identified by high-throughput screening assays.

02c. Cell, Molecular & Systems Biology: alpha-synuclein

ADPD5-1641

LYSOSOMAL DEGRADATION OF ALPHA-SYNUCLEIN: ANALYSIS IN VIVO

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Misfolded and aggregated structures of alpha-Synuclein are the main components found in Lewy bodies and are characteristic pathological features in parkinsonian brains. Therefore, down-regulating of alpha-Synuclein levels by enhancing its degradation represents an interesting therapeutic target. Recently, alpha-Synuclein has been demonstrated to be a substrate of the chaperone-mediated autophagy (CMA), a pathway in which proteins are directly translocated into the lysosome lumen via the lysosomal membrane receptor LAMP2A. Mutation of the CMA-motif (delta-CMA) of alpha-Synuclein slows down its turnover in inducible cells overexpressing a-Synuclein. A53T pathogenic mutation of alpha-Synuclein has been shown to act as blockers of LAMP2A and its overexpression increases toxicity in cells. The impact of these mutations *in vivo* regarding CMA remains unexplored. In this study, two conditional transgenic mouse lines expressing C-terminal truncated (deltaCT, 1-120) alpha-Synuclein (construct 1: A53T-deltaCT, construct 2: A53T-deltaCMA-deltaCT) were generated. Expression of alpha-Synuclein was regulated by the expression of tetracyclin-regulated transactivator (tTA) driven by the Pcp2 promoter. We found that alpha-Synuclein was highly expressed in presynaptic areas, cell bodies and neurites of Purkinje cells (PCs). Despite this overexpression, there was no loss of PCs or obvious pathology observed in cerebella of transgenic lines up to 24 months compared to control lines. Interestingly, transgenic mice showed impairments in motor behavioral tests (pole-climbing test and open-field). However, the fact that both lines showed similar motor behavior and alpha-Synuclein protein steady-state levels suggesting that mutation of the alpha-Synuclein CMA motif did not play a significant pathological role in our *in vivo* model.

02c. Cell, Molecular & Systems Biology: alpha-synuclein

ADPD5-1832

ALPHA-SYNUCLEIN REDUCES NMDA RECEPTOR-MEDIATED INWARD CURRENT BY PROMOTING RECEPTOR INTERNALIZATION IN NEURONS

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Objective: To explore the effect of alpha-synuclein on NMDA receptor (NMDAR)-mediated inward current in neurons. **Methods:** NMDAR-mediated inward current was recorded in primary cultures of rat brain neurons by whole cell patch clamp recording method. The internalization of NMDAR from the surface membrane of the neurons was measured by Western blotting using an antibody specific for NMDAR NR1 subunits. **Results:** Recombinant alpha-synuclein added to the culture medium entered into the neurons, which was detected in the cytoplasm within 30 min. Patch clamp recording revealed that the NMDA-induced inward current (that could be blocked by NMDAR antagonist MK801) was dramatically reduced in alpha-synuclein-treated neurons in a manner dependent on the alpha-synuclein concentration. Alpha-synuclein treatment also induced internalization of NMDAR from the neuron surface as was revealed by decrease in NR1 subunits in the membrane fraction. **Conclusion:** Alpha-synuclein can reduce the NMDAR-mediated inward current by promoting the internalization of NMDAR.

02c. Cell, Molecular & Systems Biology: alpha-synuclein

ADPD5-2278

HUMAN ALPHA-SYNUCLEIN 5' UNTRANSLATED REGION CONTAINS AN INTERNAL RIBOSOME ENTRY SEGMENT

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Genetic and biochemical studies have established a central role for alpha-synuclein (asyn) accumulation in the pathogenesis of Parkinson disease. Uncovering and subsequently interfering with physiological mechanisms that control asyn expression is one approach to limit disease progression. Several physiological and pathophysiological conditions that lead to lower levels of the predominant cap-dependent translation in cells such as oxidative stress, hypoxia and nutrient deprivation often drive an alternative mode of translation which is dependent on Internal Ribosome Entry Site (IRES) sequences in the 5´UTR of specific mRNAs. IRESes are hairpin structures that attract eukaryotic ribosomal translation initiation complexes promoting translation initiation independent of the presence of the commonly utilized 5'-m7G cap structure.
Methods: In order to investigate whether the 5'UTR of asyn mRNA has IRES activity, the bicistronic pRF reporter construct was used. Relative luciferase expression was determined after transfection of the pRF reporter into HEK293 cells. Protein and mRNA levels of asyn were determined by Western blot and RT-PCR respectively, in lysates or RNA from various cell lines treated with rapamycin or oxidative compounds.
Results: We show that asyn 5'UTR exhibits IRES activity and the middle part of asyn 5'UTR is probably critical for this activity. Moreover, under specific stress conditions, the asyn IRES activity is induced to promote asyn accumulation and asyn can be translated in a cap-independent manner in the presence of rapamycin.
Conclusions: Asyn can be translated in a cap-independent manner and this mechanism might contribute to asyn accumulation observed in pathological conditions, such as Parkinson disease.

02f. Cell, Molecular & Systems Biology: parkin

ADPD5-0988
ACTIVATION OF THE E3 UBIQUITIN LIGASE PARKIN
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OBJETIVES
The PINK1/Parkin-directed mitochondrial quality control pathway ensures the selective degradation of damaged mitochondrial proteins and of whole organelles. Loss of either gene function abrogates this protective pathway and results in early-onset PD. Upon mitochondrial stress, PINK1-dependent phosphorylation activates the E3 ubiquitin ligase Parkin from its 'closed', auto-inhibited structure to enable its translocation to and enzymatic functions at mitochondria. While the molecular mechanisms remain unknown, several therapeutic opportunities may exist along a sequential activation process that eventually results in the 'open', active Parkin conformation.
METHODS
We combined structural computational with functional cell-biological and biochemical methods to discover novel genetic and chemical modulators of Parkin activity.
RESULTS
We provide an all atom resolution model of Parkin and the first molecular dynamics simulation for its activation upon PINK1-dependent phosphorylation. We could show a sequential release of Parkin's intertwined domains towards its opening conformations that could enable binding of a charged E2 co-enzyme and ubiquitin transfer. Using virtual high throughput screening and cell-based high content imaging, we identified small molecule activators of Parkin and genetic regulators of its enzymatic functions.
CONCLUSIONS
With our structure-function approach, we provide the basis for a dissection of Parkin's regulation and a targeted drug design to develop small molecule activators of this broadly neuroprotective E3 ubiquitin ligase.

02f. Cell, Molecular & Systems Biology: parkin

ADPD5-1479

A SPECIFIC SUBSET OF E2 UBIQUITIN-CONJUGATING ENZYMES REGULATE PARKIN ACTIVATION AND MITOPHAGY DIFFERENTLY.

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Objectives
PINK1, a mitochondrial kinase and Parkin an E3 ubiquitin ligase functionally cooperate to label damaged mitochondria for degradation via the proteasomal and autophagic/lysosomal pathway. Although several other key proteins have been identified, the regulation(s) and the exact molecular mechanisms remain elusive. We set out to identify the specific E2 co-factors of Parkin required for mitophagy.
Methods
Using siRNA against 11 of the 35 human E2 enzymes we have screened for effects on mitochondrial translocation of Parkin using High Content Imaging and validated effects using Western blots of mitochondrial substrates and Ubiquitin charging of Parkin.
Results
We have identified several E2 enzymes (UBE2D, L3 and N) that are necessary for Parkin translocation and subsequent degradation of mitochondrial proteins in a cooperative and additive fashion. UBE2D and L3 are able to charge Parkin with ubiquitin. UBE2D, L3 and N are being discharged in response to CCCP. We found that knockdown of UBE2R1 enhances clustering of Parkin and mitochondria.
Conclusions
Parkin uses several different E2 enzymes for the ubiquitination of mitochondrial substrates upon depolarization of the mitochondrial membrane potential. UBE2D and L3 are necessary for the activation of Parkin, whereas UBE2N might be necessary to build K63-linked ubiquitin chains that are required for an efficient clustering of the mitochondria.

02f. Cell, Molecular & Systems Biology: parkin

ADPD5-1535
STRUCTURAL AND CELLULAR STUDIES WITH CDNF
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Neurotrophic Factors (NTFs) are a heterogeneous group of soluble polypeptides that promote survival, differentiation, maintenance and regeneration in the central and peripheral nervous system, acting via specific receptors. Traditionally, there are three families of NTFs: neurotrophins, GLFs (GDNF ligands factors) and neuropoietic cytokines. Another family was recently described and named CDNF/MANF. It comprises NTFs that are not structurally related to the classical families, suggesting a completely novel mechanism of action and therapeutic potential for the treatment of neurodegenerative diseases.
CDNF (Cerebral Dopamine Neurotrophic Factor) was first identified by bioinformatics and then biochemically characterized. This protein is able to protect and restore the function of dopaminergic neurons in the rat Parkinson's disease model, it has potential as a therapeutic protein or a basis for the development of drugs for the treatment of PD. We performed MTT assay to identify the domain necessary to the neuroprotective activity of CDNF in N2A differentiated cells. N-terminal domain appears to be responsible for the protection observed to date for this neurotrophic factor.
Its structure and molecular dynamics in solution suggests sites to interact with molecular targets. We design two mutants in hydrophobic aminoacids based on our NMR results to identify the site interaction of CDNF. However, these mutants were not well structured when visualized by 2D–[15N, 1H] HSQC.
Based on these results we assume that the N-terminal of CDNF is necessary to protect dopaminergic neurons to injuries like 6OHDA and alpha-synuclein.

ADPD5-2080

UNRAVELLING THE DJ-1 INTERACTOME IN CONTROL AND OXIDATIVE STRESS CONDITIONS

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Mutations in DJ-1 (*PARK7*) cause autosomal recessive Parkinson's (Bonifati et al., 2003). DJ-1 is known to protect cells from oxidative stress. However, exactly how DJ-1 functions as an "upstream" oxidative stress sensor and how it exerts its role in many other important cellular processes relevant to Parkinson's, is still to be elucidated.
In the present study we used affinity purification and mass spectrometry to gain insight into DJ-1 interacting proteins in normal and disease-relevant conditions. Proteins interacting with endogenous DJ-1 in HEK293T cells were pulled down using Dynabeads and subsequently identified by liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS). In parallel, DJ-1 protein partners were pulled down using the GFP Trap technique in HEK293T cells transfected with tagged WT DJ-1 constructs (Repici et al., 2013). Pull-down experiments were performed in normal and oxidative stress conditions. Fragment ion spectra generated by LC-MS/MS were searched using the MASCOT search tool against an updated copy of the UniProt protein database using appropriate parameters. Promising interaction partners were validated by co-immunoprecipitation and co-localization experiments.
We were able to detect 166 candidate proteins interacting with endogenous DJ-1 and 155 proteins specifically interacting with WT DJ-1 overexpressed in HEK293T cells, and not with the control. Candidate proteins identified in both experimental conditions fall into 6 functional categories: transcription regulation/splicing, translational control, mitochondrial proteins, chaperones, cytoskeletal proteins, and proteins involved in endolysosomal trafficking. Our results highlight an approach for detailed investigation of DJ-1 function in Parkinson's and of molecular mechanisms relevant to neurodegeneration.

ADPD5-0351

DROSOPHILA LRRK REGULATES THE DYNAMICS OF DENDRITIC GOLGI OUTPOSTS IN NEURONS

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Construction of dendritic arbor in neurons requires dynamic movements of Golgi outposts, the prominent component in the dendritic secretory pathway. Golgi outposts move in both anterograde and retrograde directions (toward dendritic distal ends and cell bodies, respectively), although the majority of them remain stationary. It is not clear how the dynamic profile of Golgi outposts is regulated in dendrites. Here, we show that Leucine-rich repeat kinase (Lrrk), the Drosophila homolog of Parkinson's disease (PD)-associated LRRK2, regulates dynamic behaviors of Golgi outposts in dendrites. Live imaging shows that Lrrk colocalizes with the pool of stationary Golgi outposts in dendrites and Lrrk-absent Golgi outposts are dynamic. Lrrk regulates directionality and movement of dynamic Golgi outposts: Lrrk loss-of-function mutation enhances anterograde movement and Lrrk overexpression promotes retrograde movements. Regulation of Golgi outpost dynamics is consistent with the activity of dendrite arborization, with an increase in dendrite complexity in Lrrk mutants and a reduction in Lrrk overexpression. Genetic and immunoprecipitation assays suggest that Lrrk interacts with the golgin Lava lamp (Lva) through coiled-coil Lva3 and Lva5, the domains that interact with the dynein/dynactin complex. The kinase activity of Lrrk is also crucial in these processes as kinase-dead mutant exhibits dominant-negative effects on Golgi outpost dynamics and dendritic arborization. Notably, overexpression of the LRRK2 PD mutation G2019S enhances retrograde movement of Golgi outposts, consistent with that G2019S has severe suppressive effect on dendrite arborization and implying that regulation of Golgi outpost dynamics might be altered in the most common PD-associated LRRK2 mutation G2019S.

ADPD5-0408

ABERRANT LRRK2 MODIFIES EXCITATORY INPUTS TO DORSAL STRIATAL MEDIUM SPINY NEURONS AT EARLY POSTNATAL AGES.

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Objectives: Mutations in the gene encoding leucine-rich repeat kinase 2 (LRRK2) strongly predispose humans to an autosomal dominant form of Parkinson's Disease (PD). LRRK2 is enriched in medium spiny neurons (MSNs) of the dorsal striatum, the principal target of dopaminergic neurons that degenerate in PD, but paradoxically, LRRK2 expression in striatum peaks during synaptogenesis in early postnatal life. We hypothesize that LRRK2 influences the formation of striatal input-output synaptic circuits such that PD-causing LRRK2 mutations lead to aberrant striatal synaptic development. Methods: We evaluated synaptic physiology, anatomy, and molecular organization in dorsal striatum of mice expressing an endogenous G2019S mutation (the most prevalent mutation seen in PD patients and known to cause an increase in kinase activity), mice lacking LRRK2, and mice expressing an endogenous D2017A mutation rendering LRRK2 kinase-dead. Results: At postnatal day 21, MSNs from mice lacking LRRK2 or expressing the G2019S mutant form of LRRK2 display a significant increase in the frequency of spontaneous excitatory postsynaptic currents in comparison with control mice (wild type and D2017A mice); additionally, they have an increased density of excitatory postsynaptic markers in dorsal striatum relative to the controls. Conclusions: These data suggest that excitatory synapse development is abnormally accelerated in the absence of LRRK2 or when LRRK2 activity is too high. These experiments further characterize the impact of LRRK2 activity on the generation and function of striatal circuits and synapses and reveal early dysfunction that may predispose the system to degeneration later in life.

ADPD5-0431

FUNCTIONAL PROPERTIES OF LRRK2 MUTATIONS IN TAIWANESE PARKINSON'S DISEASE

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Background: LRRK2 (leucine-rich repeat kinase 2) is a large protein encoding multiple functional domains. Mutations within different LRRK2 domains have been considered to be involved in the development of Parkinson's disease (PD) by different mechanisms. Our previous study found three *LRRK2* mutations, R767H, S885N and R1441H, in the Taiwanese patients with PD.
Methods: We evaluated functional properties of these mutations by overexpressing them in human embryonic kidney HEK-293 and neuroblastoma SK-N-SH cells. The common G2019S mutation in the kinase domain was included for comparison.
Results: In 293 cells, overexpressed R1441H but not R767H, S885N or G2019 increased GTP binding affinity to prolong the active state. Overexpressed R1441H and G2019S LRRK2 generated aggregates in 293 cells. In SK-N-SH cells, the α-synuclein was immunoreactive to wild type and mutated R767H, S885N, R1441H and G2019 LRRK2 proteins. Part of the perinuclear aggregates formed by R1441H and G2019S were colocalized with α- synuclein. Additionally, reduced interaction between LRRK2 with ARHGEF7, a putative guanine nucleotide exchange factor for LRRK2, was demonstrated by S885N, R1441H and G 2019S mutations, while this interaction is well preserved in R767H mutation.
Conclusions: Although being a pro-aggregation mutant, the non-aggregated R1441H protein may compromise its GTP hydrolysis by increasing its affinity for GTP.

ADPD5-0640

THE PARKINSON DISEASE-ASSOCIATED GENES LRRK2 AND RAB7L1 DEFINE AN EVOLUTIONARILY-CONSERVED REGULATORY MODULE ESSENTIAL FOR LYSOSOMAL INTEGRITY

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Objectives: Mutations in leucine-rich repeat kinase 2 (LRRK2) are the major genetic cause of familial PD. We previously identified RAB7-like variant 1 (RAB7L1) as an interactor of LRRK2: RAB7L1 modulates neurite morphology, intracellular trafficking and PD risk in coordination with LRRK2. But the detailed physiological roles as well as genetic relationships between LRRK2 and RAB7L1 are still unclear.
Methods: We examined the nematode *C. elegans* mutants deficient in orthologues of LRRK2 or RAB7L1, and explored the phenotypic associations.
Results: LRRK2 and RAB7L1 deficiency in *C. elegans* caused similar defects in neurite morphology and lysosomal integrity. Genetic epistasis analyses demonstrated that LRRK2 functions downstream of RAB7L1 in a linear pathway. We further identified a retromer and a lysosomal adaptor protein complex that link RAB7L1/LRRK2 dysfunction to lysosomal and neurite abnormalities.
Conclusions: These data underscore the evolutionarily conserved role of RAB7L1-LRRK2 pathway in the context of lysosome-related functions in diverse cellular contexts.

ADPD5-0978

MONITORING THE OUTCOME OF VIRAL ENCEPHALITIS IN LRRK2 MUTANT MICE: A ROLE FOR LRRK2 IN MICROBIAL SUSCEPTIBILITY OR HOST RESPONSE

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Objective

Leucine-rich repeat kinase-2 (LRRK2) has been implicated in modulating the risk of Parkinson's, Crohn's disease and leprosy. A proposed LRRK2 function lies within the innate immune system. To better study its role in complex diseases, we employed a recently described infection model using a neurotropic, respiratory-enteric-orphan (REO) virus, serotype-3-Dearing (Gauvin et al., 2013). We hypothesized that LRRK2 modulates disease severity.

Methods

After nasal administration of REO-virus to suckling lrrk2 knock-out mice and wild-type littermates (Tong et al., 2009), we used holocranomicroscopy (ie, whole skull mounts) to monitor infection from rhinitis to terminal encephalitis. To probe for genotypic differences, we quantified anti-REO antibody positivity by Aperio-ImageScope software examining serial, 4 micrometer-thin sections. We juxtaposed these data with available viral titres of select organs.

Results

The rate of neuronal infection at the time of euthanasia (day 11) was higher in knock-out mice for three regions examined. Mean percentage counts for anti-REO antibody-positive neurons in knock-out (versus wild-type) mice of thalamus, midbrain, and cerebellum measured 4.72(3.12), 8.20(2.00) and 0.37(0.20), respectively; corresponding p-values for the differences (0.17-6.20%) were calculated at 0.0871, 1.75x10(-8) and 0.1895, respectively. These neuropathological results were associated with preliminary findings of higher viral titres in the liver, lungs and brain of knock-out (versus wild-type) mice three days post inoculation.

Conclusions

REO-T3-Dearing virus-infected lrrk2 knock-out mice show significantly greater rates of neuronal infection in the midbrain by day 11 when compared to wild-type animals. We conclude that LRRK2 modulates host susceptibility and/or response after nasal exposure to a neurotropic pathogen.

ADPD5-1050

ROLE OF LRRK2 IN THE CONTROL OF DOPAMINE RECEPTOR TRAFFICKING

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Objectives: To date, the molecular mechanism of LRRK2 toxicity is largely unknown. Different lines of evidences suggest a potential role of LRRK2 in the control of neuronal vesicle trafficking. Our objective is to analyse the LRRK2 involvement in the control of dopamine receptor trafficking and to understand the molecular mechanisms involved in this process. The dopamine receptor levels into the membrane are the sum of new synthesized receptors reaching the membrane and those cycling between the membrane and the vesicle compartments. Moreover the vesicle contents can be either recycled to the membrane or degraded.
Methods: SHSY5Y cell lines stably expressing the Dopamine D1 (DRD1) or D2 receptors (DRD2) transduced by recombinant adenovirus expressing LRRK2WT or different pathological mutants or dead kinase. 48 hours after transduction the cells were treated by dopamine and the receptor trafficking was analysed by biotin protection assay (BPA) at different time points.
Results: The expression of LRRK2 pathological mutants affects the DRD1 trafficking in a dose dependent manner. In particular, the expression of mutant LRRK2s alter the DRD1 physiological equilibrium between the vesicle and membrane pool. The alteration in DRD1 cell trafficking parallels an alteration in ERK signalling. No significant effect was observed analysing both trafficking and signalling of DRD2 in the presence of LRRK2 pathological mutants.
Conclusions: Taking into account the relevance of dopamine receptor trafficking in the dopaminergic system physiology, our results highlight a important mechanism of toxicity due to the expression of LRRK2 pathological mutants.

ADPD5-1052

IN VIVO STUDY OF THE POTENTIAL NEUROTOXICITY OF FUNCTIONAL DOMAINS OF LRRK2 USING A VIRAL VECTOR APPROACH

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Mutations in the leucine-rich repeat kinase 2 gene (LRRK2) are the most common genetic causes of autosomal dominant PD, which is characterised by a progressive loss of dopaminergic neurons (DAn) in the substantia nigra pars compacta (SNc). The neurotoxicity of mutant forms of LRRK2, especially the G2019S mutation, is at least in part, related to an increase in its kinase catalytic activity. Transgenic mouse models of LRRK2 mutations despite their interest for studying PD pathogenesis, show limited neurodegeneration of DAn in the SNc with no motor symptoms, which precludes rapid testing of neuroprotective interventions (e.g. LRRK2 inhibitors). As an alternative to transgenesis, we used lentiviral (LV) and adeno-associated viral (AAV) vectors to overexpress the C-terminal part of wild type (WT) or G2019S mutant LRRK2. These vectors were stereotaxically injected in the rat SNc. Histological evaluation showed that 20-60% of the tyrosine-hydroxylase-(TH)-positive DAn were transduced, and expressed high levels of the kinase containing domains. Longitudinal evaluation (10 and 25 weeks post injection) of the rats injected with LVs and AAVs coding the wild type RCK-WD40 fragment or its mutant form (G2019S) showed: 1) no performance change in motor tests, and 2) no loss of TH-positive DAn in G2019S group as compared to wild type and sham-operated controls. We are currently testing the hypothesis that the addition of Ser^{1292} to our constructs could reinforce the G2019S-induced increase in kinase activity of LRRK2, potentially leading to neurotoxicity and motor dysfunctions.

ADPD5-1204

LRRK2 KINASE INHIBITION REDUCES ENDOGENOUS LRRK2 PROTEIN LEVELS IN VIVO

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LRRK2 (Leucine-rich repeat kinase 2) kinase inhibitors are proposed as potential PD drugs. Still many ambiguities exist concerning their therapeutic effects. For example, LRRK2 kinase inhibition reduces cellular LRRK2 phosphorylation to the same extent as several pathogenic LRRK2 mutations. Moreover, it has recently been shown that the beneficial effect of LRRK2 kinase inhibition on LRRK2-induced toxicity can be attributed to reduced LRRK2 protein levels rather than inhibition of kinase activity (Skibinski et al. 2014 J Neurosci 34(2):418-433). To further define the molecular consequences of LRRK2 kinase inhibitor treatment, we have explored the 'long-term' effects of pharmacological LRRK2 kinase inhibition.

Treatment of LRRK2 (WT or G2019S) overexpressing SH-SY5Y cells with different LRRK2 kinase inhibitors induced LRRK2 dephosphorylation via recruitment of PP1 (Lobbestael et al. 2013 BJ 456:119-128) prior to reduction of its protein levels. We confirmed these data for endogenous LRRK2 from rodent brain, where we observed LRRK2 dephosphorylation and reduced LRRK2 protein levels after systemic administration of LRRK2 kinase inhibitor. Treatment of an inhibitor-resistant LRRK2 variant did not affect LRRK2 phosphorylation and stability, indicating that our findings result from LRRK2-specific inhibitor effects.

Our findings shed light on the 'long-term' effects of LRRK2 kinase inhibition and have important implications regarding the use of these inhibitors as therapeutic agent. Currently, we are investigating the effects of kinase inhibition on other LRRK2 mutants as well as the mechanisms of the inhibitor-induced reduction of LRRK2 levels. Further insight in the relation between LRRK2 kinase activity and LRRK2 stability, may point to new therapeutic targets.

ADPD5-1857

HG-11-31-01 RESCUES IN VIVO DOPAMINE RELEASE DEFICITS IN LRRK2 G2019S KNOCK IN MICE

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The physiological and pathological roles of LRRK2 are yet to be fully determined, but evidence points towards the autosomal dominant Parkinsonism LRRK2 mutations causing a gain in kinase function, impacting neuronal maintenance, vesicular dynamics and neurotransmitter release. We created G2019S knock-in (KI) mice and have performed comprehensive in this model up to 24 months of age. We find elevated brain kinase activity in heterozygous and homozygous mice. Although normal at 6 months, by 12 months of age, *in vivo* basal and amphetamine-induced extracellular release of dopamine (DA) is reduced in both heterozygous and homozygous mice and we speculate that exocytotic release from the vesicular pool is impaired.

The aim of this study was to administer brain-permeable kinase inhibitor HG-11-31-01 to G2019S KI mice and measure amphetamine-stimulated extracellular DA levels using *in vivo* microdialysis. Our data indicates that LRRK2 inhibitor HG-11-31-01 significantly restores amphetamine-stimulated dopamine release in G2019S homozgous KI mice to levels that are similar to wild-type mice treated with vehicle. As expected, HG-11-31-01 had no effect in wild-type mice, but interestingly no effect was noted in heterozgous G2019S KI mice. This is most likely explained by the mutant G2019S-specific preference of this compound and/or alterations in accessability of compound binding due to mutation-induced protein conformational changes. We conclude that restoration of DA levels in homozgous LRRK2 G2019S KI mice by HG-11-31-01 suggests a direct *in vivo* link between LRRK2-mediated phosphorylation and dopamine release.

ADPD5-2253

NEURONAL AND NON-NEURONAL IMPLICATIONS OF LRRK2 DYSFUNCTION

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Objectives: Autosomal dominant mutations in the multi-domain kinase LRRK2 are the most common genetic cause of Parkinson's disease (PD). The missense mutations in the LRRK2 protein are believed to impart a gain-of-function at the level of LRRK2 kinase activity, but the functional role of LRRK2 in the cell remains unclear and the impact of disease linked mutations are not known. The goal of our work is to better define the biochemical and functional properties of LRRK2 and understand how pathogenic mutations alter these processes and ultimately how they relate to disease. **Methods**: Using a combination of genetic and pharmacologic tools, we have interrogated the role of LRRK2 in regulating autophagic flux. These include stable shRNA-mediated knockdown of LRRK2 in various cell lines, tissues from mutant LRRK2 knockin mice, and a panel of LRRK2 kinase inhibitors. **Results**: The stimulation of macroautophagy results in the recruitment of endogenous LRRK2 to autophagosome membranes. The resultant kinase activity that follows this LRRK2 activation is important to but not required for the autophagic degradation of insoluble proteins. **Conclusions**: Our data demonstrate that endogenous LRRK2 plays a vital role in the regulation of autophagic flux in a variety of cell types, and that reductions in LRRK2 expression or kinase activity interfere with protein clearance. In addition, when expressed at endogenous levels pathogenic PD-linked mutation in LRRK2 likewise results in dysregulation of autophagy. These data may provide mechanistic insight into the proteinopathy observed in certain LRRK2 animal models and patients carrying mutations in LRRK2.

ADPD5-1929

IDENTIFICATION OF NOVEL FBXO7 INTERACTING PROTEINS

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Objectives: Parkinson disease-15 (PARK15), or parkinsonian-pyramidal syndrome, is caused by mutations in the FBXO7 gene. Although FBXO7 function is implicated in various cellular processes (i.e. cell cycle regulation and mitophagy), mechanistic insight into FBXO7 signaling pathways and consequences of PARK15 mutations is limited. The goals of our study are identification of novel FBXO7 interacting proteins and investigation of the effect of PARK15 mutations on the detected interactions and related cellular processes.
Methods: TAP-tag and GST-tag wildtype and mutant (R378G) FBXO7 proteins were generated. DNA expression constructs of TAP-tagged FBXO7 proteins were transfected into human neuroblastoma cell lines (SH-SY5Y) and FBXO7 interacting protein were isolated by sequential pull down using FLAG and Streptactin peptide coated beads. GST-tagged proteins were used to pull down FBXO7 protein interactors from brain extracts. Various experimental paradigms were tested (i.e. investigation of cytoplasmic vs. nuclear interaction, phosphorylation dependent interactions, mitophagy dependent interactions). After pull down, eluates were fractionated by PAGE, Coomassie stained and processed for Mass Spec analysis. Candidate interactors were further validated in relevant follow up experiments.
Results: Several FBXO7 protein interaction networks have been identified. Our data confirm previously identified interactions of FBXO7 with proteins from the SCF E3 ubiquitin ligase complex (CUL1, SKP1) and proteasome (PSMF1). In addition, novel FBXO7 interacting proteins involved in various functional pathways (i.e. mitochondrial and nuclear function) have been identified and are currently validated.
Conclusions: Identification of novel FBXO7 protein interactors implicates SCF complex-dependent and -independent roles for FBXO7 function in various subcellular compartments (i.e. nucleus, mitochondria, cytoplasm).

ADPD5-1185

DOPAMINERGIC TOXIN MPTP ACTIVATES ASK1-P38 MAPK DEATH SIGNALING PATHWAY THROUGH TNF-DEPENDENT THIOREDOXIN OXIDATION IN A PARKINSONISM MOUSE MODEL

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Activation of apoptosis signal-regulating kinase 1 (ASK1) and downstream death signaling MAPK cascade are implicated in selective degeneration of dopaminergic neurons in substantia nigra in PD. ***Objectives:*** Since upstream activator(s) of ASK1 are not delineated, we investigated the same using the MPTP mouse model of Parkinsonism. ***Methods:*** Male C57/BL6 mice were treated with a single dose of MPTP and status of death signaling cascade was assessed over 12 hr in ventral midbrain (VMB) and striatum (ST). We identified the critical hub of this cascade using protein covariation network analysis (PCNA). ***Results:*** MPTP selectively activated ASK1 and its downstream p38 MAPK in a time-dependent manner in VMB alone. This potentially occurs through selective protein thiol oxidation of redox-sensitive thiol disulfide oxidoreductase, thioredoxin (Trx1) resulting in release of its inhibitory association with ASK1. Levels of tumor necrosis factor (TNF), a known activator of ASK1, increased 30 min after MPTP in VMB but not ST. PCNA analysis using protein states as nodes revealed TNF to be an important mediator of ASK1 cascade. Blockade of MPTP-mediated TNF signaling in VMB through intrathecal administration of TNF-neutralizing antibody prevented downstream Trx1 oxidation and ASK1-p38 MAPK activation. ***Conclusions:*** TNF activates ASK1-p38 cascade through protein thiol oxidation. Thus, preventing the increase of TNF levels and thence the downstream signaling through Trx1 oxidation leading to ASK1-p38 MAPK activation may be critical for neuroprotection in PD. Importantly, network analysis, such as PCNA may yield new insights into causes and effects of protein network perturbations in complex disease states.

02v. Cell, Molecular & Systems Biology: metabolomics

ADPD5-0855

L-DOPA INDUCES METABOLIC SWITCHES IN A TH POSITIVE NEURONAL CELL MODEL

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OBJECTIVES:

The symptoms of PD can be managed for several years by administering L-3,4-dihydroxyphenylalanine (L-DOPA). This drug has been shown to be toxic to dopaminergic neurons *in vitro* and *in vivo.* The focus of our study was to investigate the effects of L-DOPA treatment on the metabolism of a TH positive neuronal cell model.

METHODS:

The fate of various carbon sources was investigated using stable isotope-labeled metabolites such as glucose, glutamine or pyruvate. The LUHMES cells, a human TH positive neuronal cell model, were treated with concentrations of L-DOPA as measured in the plasma of PD patients under L-DOPA treatment.

RESULTS:

The results show clear switches in cellular metabolism of the neurons subjected to L-DOPA treatments. On the one hand, a switch from glucose to glutamine to fuel the tricarboxylic acid (TCA) cycle was observed. On the other hand, the production of lactate was increased and very interestingly, the L-DOPA treatment induced a switch from glucose to extracellular pyruvate-derived carbons for lactate production.

CONCLUSIONS:

Our data suggest that LUHMES cells undergo major perturbations of the central carbon metabolism, including decreased TCA cycle activity and a switch from glucose to glutamine as carbon source for the TCA cycle. This phenomenon has also been observed in cancer cells which rely on glycolysis solely for lactate production. In our case however, L-DOPA does not induce an increase of glycolytic activity for lactate production, but instead, the uptake of pyruvate from the medium was channeled to lactate production.

02w. Cell, Molecular & Systems Biology: transcriptomics

ADPD5-1435

META-ANALYSIS OF SUBSTANTIA NIGRA TRANSCRIPTOME DATA: SEARCHING FOR NEW BIOMARKERS OF PD

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The understanding of the genetic basis of the PD and the correlation between genotype and phenotype has revolutionized knowledge about the pathogenetic mechanisms of neurodegeneration, opening up exciting new therapeutic and neuroprotective perspectives. Genomic knowledge for PD is still very open and can provide a good start for studies of the molecular mechanisms that underlie the gene expression variations and the epigenetic mechanisms that may contribute to the complex and characteristic phenotype of PD. Here we use the software TRAM (Transcriptome Mapper), to analyse publicly available microarray data of PD patients and controls substantia nigra, to identify chromosomal segments (Map mode) and gene clusters (Cluster mode) which are biologically relevant in the two different conditions. TRAM integrates original methods for parsing, normalizing, mapping and statistically analyzing expression data; in addition, it is able to easily generate maps showing differential expression between two sample groups, relative to two different biological conditions. We performed a systematic meta-analysis of 143 samples from pool A (patients with PD) and 119 samples from pool B (healthy controls), for a total of respectively 4,128,764 data points (gene expression value) and 3,417,633 data points, relative to 37,580 distinct loci for wich A/B ratio value was determinable. Results obtained included 5 significantly over-expressed segments and 90 over/under-expressed clusters. A list of statistically significant over/under-expressed genes has been generated, including coding genes, ncRNAs and uncharacterized transcripts. This study offers a new approach for the regional analysis of gene expression in neurodegenerative diseases.

02w. Cell, Molecular & Systems Biology: transcriptomics

ADPD5-1715

PURINE METABOLISM GENE DEREGULATION IN PD IS STAGE- AND REGION-DEPENDENT

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Objective: Purines are the core of the DNA, RNA, nucleosides, and nucleotides which participate in a wide variety of crucial metabolic pathways including energy metabolism and cell signalling and form co-factors of several enzymatic reactions. The present study analyses mRNA expression of twenty-two genes involved in purine metabolism, in the *substantia nigra*, putamen and cerebral cortex area 8 in PD at different stages of disease progression. This information permits a better understanding of the primary regulation of purine-related genes and their possible implications in the pathogenesis of Parkinson's disease (PD).
Methods: RT-qPCR and immunohistochemistry were performed in the present study.
Results: Expression of adenylate kinase 2 (*AKA2*), *AK3*, *AK4*, adenine phosphoribosyltransferase (*APRT*), ectonucleoside triphosphate diphosphohydrolase 1 (*ENTPD1*), *ENTPD3*, non-metastatic cells 3, nucleoside-diphosphatese kinase 3 (*NM1*), *NME7*, and purine nucleoside phosphorylase 1 (*PNP1*) mRNA was reduced in the *substantia nigra* at stages 3-6. In contrast, *ADA* mRNA was up-regulated in the frontal cortex area 8 at stages 3-4, as were *AK1*, *AK5*, *NME4*, *NME5*, *NME6*, 5'-nucleotidase (*NT5E*), *PNP1* and prune homolog *Drosophila* (*PRUNE*) at stages 5-6. Gene down-regulation in the *substantia nigra* may be interpreted, in part, as a consequence of dopaminergic cell damage leading to neuronal cell death since *ENTPD3*, *NME1, NME7*, *AK1*, and *PNP1* are expressed in neurons as revealed by immunohistochemistry. Gene up-regulation in the frontal cortex suggests a primary manifestation or a compensation of altered purine metabolism in this region at advanced stages of PD.
Conclusions: Purine metabolism gene deregulation in PD is stage- and region-dependent.

02w. Cell, Molecular & Systems Biology: transcriptomics

ADPD5-1746

ARE MALE BIOLOGICALLY PREDISPOSED TO PARKINSON'S DISEASE: A PERIPHERAL TRANSCRIPTOMIC STUDY

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Objectives

Parkinson's disease (PD) is characterized by a combination of genetic and environmental factors. PD occurs more often in men than female with a sex ratio from 1.5 to 2. Epidemiologic studies have proposed the male gender as a risk factor of PD. The aim of this study is to determine if men could have a biological predisposition to PD.

Methods

We already proposed that the transcriptome profile in peripheral blood mononuclear cells (PBMC) could reflect the gene expression deregulation occurring in the brain. Transcriptome study was realized from PBMC of 9 male and 9 female sporadic PD patients and 7 male and 10 female controls (Whole Human Genome 44k Agilent microarray). Analyses of differential expression were performed with GeneSpring software. Genes with significant difference of expression (fold change >1.2 and Welch t-test $p< 0.05$) were analyzed using Ingenuity Pathway Analysis software which identified significantly deregulated cellular pathways.

Results

Genes implicated in the estrogen receptor and the androgen signaling pathways were deregulated in both male and female sporadic patients. There were less deregulated genes and pathways in PBMC from the male PD patients compared to male controls than in female PD patients. Deregulations of autophagic and cytoskeleton pathways were observed between male and female PD patients.

Conclusions

This preliminary transcriptomic study suggests that male could present a gene expression profile making them susceptible to PD development. This result needs confirmation in a second and larger set of population.

02x. Cell, Molecular & Systems Biology: synaptic plastcity

ADPD5-0977

RETROMER-DEPENDENT NEUROTRANSMITTER RECEPTOR TRAFFICKING TO SYNAPSES IS ALTERED BY THE PARKINSON'S DISEASE VPS35 MUTATION P.D620N

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OBJECTIVE: Vacuolar protein sorting 35 (VPS35) is a core component of the retromer complex, crucial to endosomal protein sorting and intracellular trafficking. Recently we linked a VPS35 mutation (p.D620N) to familial parkinsonism. Although retromer function is not very well defined in neurons, we aimed to test the hypothesis that VPS35 has neuron-specific functions, which are perturbed by this mutation.

METHODS: We examined retromer localization and interactions in primary neurons and mouse brain by Western blot and immunocytochemistry. Effects of the mutation were tested by transient over-expression of GFP-fused VPS35 (+/-p.D620N) in primary neurons and by endogenous levels of mutant VPS35 in a novel knock-in mouse model and human patient derived iPS cell dopamine-like neurons.

RESULTS: Endogenous VPS35 localizes to dendritic spines in cortical and striatal neurons and regulates the surface trafficking of synaptic excitatory AMPA-type neurotransmitter receptors (AMPARs). Some retromer functions are unaffected by the p.D620N mutation but the mutation alters VPS35 intracellular trafficking and subcellular localization as well as altering synaptic transmission and AMPAR recycling. We found altered VPS35 binding with known regulatory partners were induced by the p.D620N mutation which may explain partial loss-of function.

CONCLUSIONS: Perturbations to synaptic function induced by the VPS35 p.D620N mutation may produce chronic pathophysiological stress and eventual neurodegeneration in this form of familial parkinsonism

02y. Cell, Molecular & Systems Biology: modeling of disease progression

ADPD5-0864

OPTIMIZATION OF DIFFERENTIATION PROTOCOLS FOR HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED DOPAMINERGIC NEURONS

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Objective: PD is the second most common neurodegenerative disease. It is characterized by a slow and progressive degeneration of dopaminergic (DA) neurons in the substantia nigra (SN) pars compacta and of nerve terminals in the striatum, eventually leading to advanced motor symptoms. Generation of DA neurons *in vitro* could offer a great option for biomedical applications including disease modeling, or drug screening.
Methods: Various differentiation protocols were investigated for the differentiation of human induced pluripotent stem cells (hiPSC) into DA neurons. The most promising protocols used two small molecules, LDN and SB, inhibiting SMAD signaling by TGFß and BMP pathways thereby inducing neural fate. Furthermore, small molecules involved in patterning and correct localization of mesencephalic DA neurons were supplemented at different timepoints and in various concentrations. Additionally, we included a sorting step aiming at a pure population of DA neurons. Differentiated neurons were characterized and evaluated by immunocytochemistry and qRT-PCR.
Results: Using dual SMAD inhibition and small molecules for patterning including SHH, FGF8 or CHIR lead to high amounts of DA neurons after at least 30d of differentiation. Sorting steps were challenging to establish since high cell death rates occur.
Conclusion: Differentiation of hiPSC into a high yield of DA neurons is still a challenging process. However, based on our experiments, high amounts of TH-positive neurons could be generated.

02y. Cell, Molecular & Systems Biology: modeling of disease progression

ADPD5-1957

IMPULSE CONTROL DISORDERS IN PARKINSON'S DISEASE ARE ASSOCIATED WITH DYSFUNCTION IN STIMULUS VALUATION BUT NOT ACTION VALUATION

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Introduction: A substantial subset of Parkinson's disease (PD) patients suffers from impulse control disorders (ICDs), which are side effects of dopaminergic medication. Dopamine plays a key role in reinforcement learning processes. One class of reinforcement learning models, known as the actor-critic model, suggests that two components are involved in these reinforcement learning processes: a critic, which estimates values of stimuli and calculates prediction errors, and an actor, which estimates values of potential actions.
Objectives: To understand the information processing mechanism underlying impulsive behavior.
Methods: We investigated stimulus and action value learning from reward and punishment in four groups of participants: on-medication PD patients with ICD, on-medication PD patients without ICD, off-medication PD patients without ICD, and healthy controls.
Results: Analysis of responses suggested that participants used an actor-critic learning strategy and computed prediction errors based on stimulus values rather than action values. Quantitative model fits also revealed that an actor-critic model of the basal ganglia with different learning rates for positive and negative prediction errors best matched the choice data. Moreover, whereas ICDs were associated with model parameters related to stimulus valuation (critic), PD was associated with parameters related to action valuation (actor). Specifically, PD patients with ICD exhibited lower learning from negative prediction errors in the critic, resulting in an underestimation of adverse consequences associated with stimuli.
Conclusions: These findings offer a specific neurocomputational account of the nature of compulsive behaviors induced by dopaminergic drugs.

02y. Cell, Molecular & Systems Biology: modeling of disease progression

ADPD5-1958

COGNITIVE CORRELATES OF PSYCHOSIS IN PATIENTS WITH PARKINSON'S DISEASE

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Introduction and Objectives. Psychosis and hallucinations occur in 20–30% of patients with
Parkinson's disease (PD). In the current study, we investigate cognitive functions in relation to the occurrence of psychosis in PD patients.
Methods. We tested three groups of subjects – PD with psychosis, PD without psychosis and healthy controls – on working memory, learning and transitive inference tasks, which are known to assess prefrontal, basal ganglia and hippocampal functions.
Results. In the working memory task, results show that patients with and without psychosis were more impaired than the healthy control group. In the transitive inference task, we did not find any difference among the groups in the learning phase performance. Importantly, PD patients with psychosis were more impaired than both PD patients without psychosis and controls at transitive inference. We also found that the severity of psychotic symptoms in PD patients [as measured by the Unified Parkinson Disease Rating Scale Thought Disorder (UPDRS TD) item] is directly associated with the severity of cognitive impairment [as measured by the mini-mental status exam (MMSE)], sleep disturbance [as measured by the Scales for Outcome in Parkinson Disease (SCOPA) sleep scale] and transitive inference (although the latter did not reach significance).
Conclusions. Although hypothetical, our data may suggest that the hippocampus is a neural substrate underlying the occurrence of psychosis, sleep disturbance and cognitive impairment in PD patients.

02z. Cell, Molecular & Systems Biology: other

ADPD5-0286

MOLECULAR CHARACTERIZATION AND ANALYSIS OF THE PORCINE NURR1 GENE

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Introduction:
NURR1 is a member of the nuclear receptor superfamily and is a regulatory factor of differentiation, migration and maturation of mesencephalic dopaminergic neurons. NURR1 plays a crucial role in nigrostriatal dopmaine neuron development and is therefore believed to be associated with the pathogenesis of neurodegenerative diseases linked to the dopamine system of the midbrain.

Aims:
The aim of this study was to characterize the porcine NURR1 gene.

Results:
Here we report the isolation and characterization of porcine *NURR1* cDNA amplified by reverse transcriptase polymerase chain reaction (RT-PCR). The porcine *NURR1* cDNA encodes a protein of 598 amino acids which shows an extremely high similarity to bovine, human and mouse (99 %) NURR1. NURR1 transcript was detected in various organs and tissues with a differential expression. Also the developmental expression was examined in normally fertilized pigs and in cloned pigs. Two single nucleotide polymorphisms (SNPs) were identified the porcine *NURR1* gene, a missense C/T SNP in exon 3 resulting in an amino acid substitution of a leucine to a phenylalanine residue (L57F) in the porcine NURR1 sequence. Another non-synonymous SNP was identified in exon 3. This C/A SNP leads to an amino acid change from a proline to a histidine residue (P82H). None of the identified SNPs have been observed in human NURR1. The methylation level of the NURR1 gene was determined and displayed significant difference between brain and liver.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-0565

EXOSOMAL SORTING OF ALPHA-SYNUCLEIN FOR EXTRACELLULAR RELEASE IS REGULATED BY SUMOYLATION

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Extracellular α-Synuclein has been implicated in interneuronal propagation of disease pathology in Parkinson's Disease. How α-Synuclein is released into the extracellular space is still unclear. Here, we show that α-Synuclein is present in extracellular vesicles in the central nervous system (CNS). We find that sorting of α-Synuclein in extracellular vesicles is regulated by sumoylation and that sumoylation acts as a novel sorting factor for targeting of both, cytosolic and transmembrane proteins, to extracellular vesicles. We provide evidence that the SUMO-dependent sorting utilizes the endosomal sorting complex required for transport (ESCRT) by interaction with phosphoinositols. Ubiquitination of cargo proteins is so far the only known determinant for ESCRT-dependent sorting into the extracellular vesicle pathway. Our study reveals a novel function of SUMO protein modification as the first ubiquitin-independent ESCRT sorting signal, regulating the extracellular vesicle release of α-Synuclein but presumably constituting a mechanism with more general implications for cell biology.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-0642

PRION-LIKE SPREADING OF ALPHA-SYNUCLEIN AGGREGATES FROM THE OLFACTORY BULB TO CENTRAL BRAIN REGIONS IN WILD-TYPE MICE.

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In Parkinson's disease (PD), proteic aggregates develop in the brain. Those aggregates are composed of misfolded α-Synuclein (α-syn) arranged into fibrils rich in beta-sheet structures. Based on post-mortem analysis of PD brains, Braak and collaborators suggested that in PD, pathological aggregates develop in a stereotypic pattern, and propagate via neural pathways. Importantly, Braak observed that α-syn aggregates appear first in the olfactory bulb (OB) and the gut, and then spread to interconnected brain regions over several years. Interestingly, olfactory dysfunction accompanies the appearance of pathology in olfactory structures early in the course of PD. Thus, the olfactory system is a relevant model to study early stages of PD, and investigate spreading of α-syn aggregates throughout the brain.

Several recent articles demonstrate that injection of fibrillar α-syn into the striatum, cortex or substantia nigra of wild-type mice induce α-syn aggregation, but the induction of pathology in the OB, and the possible spread of pathology to other brain regions has not been explored yet.

Here, we test the long-term effect of fibrillar α-syn stereotactic injection into the OB of wild-type mice.

We demonstrate that certain species of α-syn recruit endogenous α-syn into pathological aggregates, that then spread sequentially, first to structures directly connected to the OB, and then to structures secondary connected to the OB. Moreover, spreading of pathology is accompanied by progressive and specific behavioral deficits.

Our results support the idea that α-syn spreading from OB to central structures can occur, and could be involved in the progression of PD pathology.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1031

CATHEPSIN D DEFICIENCY-INDUCED LYSOSOMAL DYSFUNCTION ACCELERATES TRANSCELLULAR TRANSMISSION OF ALPHA-SYNUCLEIN

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Deposition of α-synuclein aggregates is a pathological feature of PD. Both pathological and genetic studies suggested lysosomal dysfunction as one of the key features of PD and other neurodegenerative disorders. Recent studies suggested that cell-to-cell transmission of α-synuclein aggregates was the underlying mechanism of the progression of PD. In previous studies, we showed that α-synuclein aggregates can be transferred to neighboring cells through the endolysosomal pathway and broken down in lysosomes. Here, we tested the hypothesis that lysosomal dysfunction promotes cell-to-cell transmission of α-synuclein, thereby leading to the extensive accumulation and secretion of α-synuclein aggregates. Using zinc finger nucleases targeting either *ATP13A2*, a genetic cause of early onset PD, or *CTSD*, a gene encoding lysosomal hydrolase cathepsin D, we generated *ATP13A2-/-* and *CTSD+/-* cell lines. While the depletion of *ATP13A2* did not show alterations in lysosomal degradation, the heterozygous mutation of cathepsin D exhibited the accumulation of autophagic vacuoles and the lysosomal substrates such as p62 and polyubiquitinated proteins. Degradation rate of exogenous dextran was slower only in *CTSD+/-* cells. These results indicate that the lysosomal dysfunction occurs only in *CTSD+/-* cells. Consistent with this, the heterozygous depletion of cathepsin D potentiated the cell-to-cell transmission of α-synuclein, whereas *ATP13A2* deficiency did not increase the transmission of a-synuclein aggregates. These results suggest that the state of lysosomal function is the major determinant of the rate of transcellular transmission of α-synuclein aggregates.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1518

HOLOCRANOMICROSCOPY PROTOCOL VISUALIZES ALPHA-SYNUCLEIN AND TAU EXPRESSION IN OLFACTORY EPITHELIUM: IMPLICATIONS FOR TESTING THE BRAAK HYPOTHESIS

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Objectives: Hyposmia occurs early in several neurodegenerative disorders. The nasal cavity's involvement in the pathogenesis of Parkinson disease and Alzheimer's has not yet been studied in part due to difficulty visualizing olfactory and respiratory epithelia.
Methods: After optimizing a formic acid-based method that decalcifies bone and enhances select antigen accessibility, we processed intact mouse heads into 4 micrometer-thin sections for microscopy applications.
Results: Using a standardized protocol for 'holocranomicroscopy', we made 5 observations: 1) Expression of alpha-synuclein and tau in olfactory neurons of the nasal cavity; 2) Correlation between human SNCA allele numbers and alpha-synuclein levels throughout the cranium of PAC1-transgenic mice. There, 4 SNCA alleles induced abnormalities in movements, cognition and odor processing; 3) Formation of thioflavin T-positive amyloid-beta plaques in intracranial portions of the olfactory nerve in mutant human APP-cDNA-transgenic mice; 4) The tracking of infection following inoculation with a respiratory-enteric-orphan virus from the nasal cavity to cranial nerves-1 and -5 and encephalitis; and 5) Improved sensitivity in monitoring intracranial neurogenesis after BrdU administration.
Conclusion: Holocranomicroscopy (Fig.1) allows for the routine visualization of skull and soft tissue components in intact mouse heads including nasal epithelia, cranial nerves and brain. Our protocol permits the experimental study of interactions between human susceptibility alleles and naturally occurring pathogens to explore the etiologies of sporadic Parkinson's ('Braak hypothesis') and late-onset Alzheimer disease.

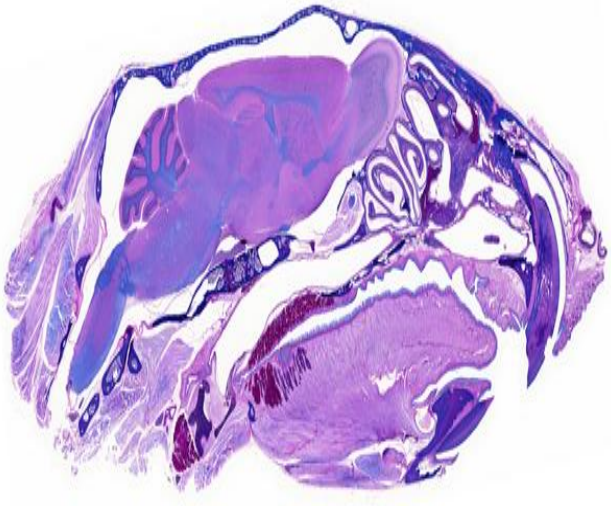

Figure 1: Luxol Fast Blue stained, sagittal section of intact mouse head.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1552

HEPARAN SULFATE IS INVOLVED IN CELLULAR UPTAKE OF ALPHA-SYNUCLEIN AGGREGATES

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Objectives: Spreading of the pathology to increasingly larger areas of the brain has been proposed to be caused by a prion-like seeding mechanism in neurodegenerative diseases like Alzheimer´s and Parkinson´s disease. It has been shown that protein aggregates involved in these diseases can transfer from one cell to another, but not much is known about how the secretion or internalization occurs. The objective of the present project was to investigate if heparan sulfate proteoglycans (HSPGs) is involved in cellular internalization of alpha-synuclein aggregates, and if this is true only for aggregates with an amyloid structure or also for oligomeric species.
Methods: Alpha-synuclein in oligomeric or fibrillar conformation was added to the cell media of rat neuroblastoma B103 cells or CHO-cells deficient in HS altogether or in N- or 2-O-sulfation of the HS chains. Cellular uptake was determined by ELISA on cell lysates. Colocalization of HSPGs and alpha-synuclein aggregates was studied through confocal microscopy.
Results: Cellular uptake of fibrillar alpha-synuclein was almost completely abolished by the addition of heparin to the cell media of B103 cells, while internalization of oligomers where not inhibited to the same extent. Both oligomeric and fibrillar alpha-synuclein were seen to colocalize with HS in what seems to be endocytic vesicles. All of the CHO mutants showed strongly inhibited uptake compared to wt cells, but there was no significant difference between the different mutants.
Conclusions: HSPGs are involved in the cellular uptake of alpha-synuclein aggregates and the amyloid fibril structure is probably important for this interaction.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1643

DIFFERENTIAL PATTERN OF NEURONAL LOSS IN THE CHOLINERGIC BASAL FOREBRAIN IN LEWY BODY DISORDERS

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Introduction: The basal forebrain cholinergic system consists of the medial septum/diagonal band nucleus (MS/DBN), nucleus basalis of Meynert (nbM) and nucleus subputaminalis (NSP). These nuclei project to the hippocampus and various cortical regions. A posterior-anterior gradient of cortical cholinergic deficit in Lewy body disorders (LBD) has been reported on imaging studies. However, it is not clear whether this pattern is reflected by the neuronal loss in various basal forebrain cholinergic nuclei.
Objectives: To characterise the pattern of neuronal loss in different subdivisions of the cholinergic basal forebrain in Lewy body disorders.
Methods: Tissue sections containing the basal forebrain from 93 PD, 100 PDD, 14 DLB and 15 age-matched controls were stained using immunohistochemistry with choline acetyltransferase (ChAT) antibodies. ChAT-positive neurons in the MS/DB, NSP and nbM were quantified, blind to the clinical diagnosis.
Results: A significant reduction of ChAT-positive neurons was found in LBD cases, with demented cases showing a greater loss. All subdivisions of the nbM were equally affected in PDD whereas the anterior and posterior nbM have a greater loss in PD. DLB cases have a significantly lower number of ChAT-positive neurons in the MS/DBN. A trend of decrease in ChAT-positive neurons was observed in the NSP among LBD cases.
Conclusions: We have identified a differential pattern of cell loss in various subdivisions of the cholinergic basal forebrain in LBD cases. This could possibly explain the specific cognitive profile presented by LBD patients and support findings from recent imaging studies.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1648

ROLE OF HSPGS IN EXOSOME-ASSOCIATED OLIGOMERIC ALPHA-SYNUCLEIN TRANSMISSION

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Objectives: Misfolding and aggregation of alpha-synuclein (αsyn) resulting in cytotoxicity is a hallmark of PD and related synucleinopathies. The recent body of evidence indicates that αsyn can be released from neuronal cells by nonconventional exocytosis involving extracellular vesicles such as exosomes. The transfer of αsyn between cells has been proposed to be an important mechanism of disease propagation in PD. The process by which αsyn is internalized into target cells remains largely unknown; although heparin sulfate proteoglycans (HSPGs) have been proposed to play a role. Here, we tested the hypothesis that HSPGs act as internalizing receptors for exosome-associated oligomeric αsyn.
Methods: Exosomes were purified from conditioned media of stable cells secreting αsyn oligomers. A novel bimolecular protein complementation assay was used to detect exosomes containing αsyn oligomers. Recipient cells were treated with exosomes containing αsyn oligomers and internalization was monitored. The role of HSPGs in exosome-associated αsyn internalization was investigated using both pharmacological and genetic approaches.
Results: We demonstrate that cell-derived exosome-associated αsyn oligomers can be efficiently internalized by recipient cells. Interestingly exosome-free αsyn oligomers isolated from conditioned medium were not internalized but remained bound to the extracellular surface. HSPG manipulation using genetically modified recipient cells (CHO-M1, CHO-745) did not attenuate internalization of exosome-associated αsyn oligomers.
Conclusions: Our data suggest that exosome-associated oligomeric forms of αsyn is preferentially taking up by recipient cells and that this process may be independent of HSPGs. Exosome-associated αsyn appears to be internalized via alternative endocytic pathways that are yet to be elucidated.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1847

IDENTIFICATION OF EXTRACELLULAR VESICLE BIOMARKERS AND CANDIDATE THERAPEUTIC TARGETS IN PD CELLULAR MODELS AND CLINICAL SAMPLES

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Objective: There is increasing evidence that PD is characterized by multi-focal, progressive neurodegeneration that often begins in olfactory and vagal neurons, clinically presenting as a movement disorder only when substantia nigra neurons are severely compromised. Efforts to restore or replace neuronal populations post-presentation are likely to suffer from the existing and progressive load of misfolded and aggregated alpha-synuclein.

Methods: We suggest that the development of disease altering medications must focus upon identification of early markers of disease spread between neurons and characterization of these molecular mechanisms as candidate therapeutic targets. We are investigating the potential of extracellular vesicles (exosomes and ectosomes) to mediate transfer of different alpha-synuclein variants between multiple cell-types and attempting to identify molecular markers of toxic vesicles as candidate therapeutic targets.

Results: We have isolated and characterized extracellular vesicles from neuronal and glial cell lines, patient-derived fibroblast cultures, CSF and plasma, and have analyzed the physical characteristics by atomic force microscopy and electron microscopy. Dye labeled exosomes can be visualized and transferred between distinct cell types. Proteomic analysis of exosomes from patient derived fibroblasts, neuronal and glial cells identifies hundreds of proteins that are being analyzed for alterations in content and quantity following various cellular stresses.

Conclusions: Isolation and characterization of extracellular vesicles from patient biofluids and fibroblast, neuronal and glial cell cultures combined with proteomics and metabolomics analysis of membrane components represents a strong potential to identify candidate therapeutic targets to reduce the progressive spread of protein aggregation diseases.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-2133

ASSESSMENT OF THE ROLE OF PARKINSON'S DISEASE ASSOCIATED ATP13A2/PARK9 IN THE IN VIVO INTER-NEURONAL SPREAD OF ALPHA-SYNUCLEIN PATHOLOGY

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Objectives: Parkinson's disease (PD) is characterised by Lewy bodies containing aggregated alpha-synuclein. Clinical progression in PD correlates with an increasing number of brain regions displaying alpha-synuclein pathology. Intrastriatial injection of alpha-synuclein proto-fibrils into wild-type (WT) mice results in alpha-synuclein pathology spreading to inter-connected brain regions. Elevated ATP13A2 levels reduce alpha-synuclein toxicity and facilitate the extracellular release of alpha-synuclein *in vitro* while ATP13A2$^{-/-}$ patients have early onset PD. This *in vivo* study assessed the impact of ATP13A2 loss on alpha-synuclein pathology resulting from injected alpha-synuclein proto-fibrils.

Methods: WT and ATP13A2$^{-/-}$ mice were stereotaxically injected into the striatum with recombinant alpha-synuclein proto-fibrils or PBS. At 30 or 90 days post-injection (dpi), brains were fixed, and sections stained and analysed for phosphorylated alpha-synuclein (pS129) pathology.

Results: At 30 dpi, both WT and ATP132$^{-/-}$ mice injected with alpha-synuclein proto-fibrils showed pathology at the injection site (striatum), as well as the amygdala, cortex and thalamus, and at 90 dpi, considerable pathology was also observed in the substantia nigra. Intriguingly, the severity of alpha-synuclein-positive aggregates, particularly in the substantia nigra differed between the genotypes. The PBS injected brains showed no pS129 pathology.

Conclusions: The alpha-synuclein pathology from injected alpha-synuclein fibrils displayed a genotype specific effect with the loss of ATP13A2 modulating the extent and degree of (alpha-synuclein) pathology. This strongly suggests that ATP13A2 influences the inter-neuronal spread of alpha-synuclein toxicity and highlights possible therapeutic approaches to slow or prevent PD progression.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-2223

LACK OF RELATIONSHIP BETWEEN NEURONAL INJURY AND NEURON-TO-NEURON ALPHA-SYNUCLEIN PROPAGATION

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Objectives:
Progressive spreading of alpha-synuclein (a-syn) from the lower brainstem to more rostral brain regions is a pathological feature of Parkinson's disease. The aim of this work was to determine if caudo-rostral a-syn propagation in a rat model was dependent upon or enhanced by neuronal injury/degeneration.
Methods:
Overexpression of human a-syn (ha-syn) was induced in the rat medulla oblongata (MO) by injections of AAV vectors carrying ha-syn DNA into the vagus nerve. This overexpression triggered a caudo-rostral protein spreading that was assessed by counting the number of axonal projections immunoreactive for ha-syn in the pons, midbrain and forebrain.
Results:
By varying AAV vector preparations and injection titers, two experimental conditions were obtained. Both conditions resulted in comparable levels of ha-syn overexpression. They differed, however, in regard to neuronal injury that occurred in the MO after treatment with one but not the other experimental paradigm. Spreading occurred regardless of cell injury. Interestingly, the count of ha-syn-containing axons in regions rostral to the MO was significantly higher in the absence than the presence of cell damage and neurodegeneration.
Conclusions:
Passive release from injured neurons is not essential for triggering a-syn transmission, nor does it exacerbate protein spreading. Data are consistent with the possibility that cell-to-cell transfer of a-syn involves active mechanisms within intact neurons and is therefore more pronounced when neuronal integrity is maintained.

03b.Pathophysiology & Disease Mechanisms: prion-like mechanisms

ADPD5-1384
A NOVEL APPROACH TO STUDY ALPHA-SYNUCLEIN UPTAKE AND ITS CONSEQUENCES

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Objectives: Despite a growing body of evidence indicating that uptake of extracellular alpha-Synuclein (aSyn) can initiate the neurodegenerative cascade and major cardinal features of PD, very little is known about the physio(patho)logical changes associated to this process. Here we present a novel approach to i) study the ability of aSyn to be internalized and ii) characterize the cellular responses triggered by aSyn uptake.
Methodology: neuronal cells were treated with different aSyn species, and i) internalized aSyn was quantified by Selected Reaction Monitoring-mass spectrometry, ii) high-throughput gene and protein expression analysis were conducted and possible associations among differentially expressed genes and proteins were studied using bioinformatics tools.
Observations: We found that aSyn fibrils are efficiently internalized, in a dose and time-dependent manner. On the contrary, we did not observe uptake of monomers or oligomers. Gene expression analysis revealed subtle changes in cells treated with extracellular monomers and oligomers, while a pronounced response was observed in cells treated with aSyn fibrils. Differentially expressed proteins of cells treated with fibrils are functionally related and can be grouped into well-defined clusters according to their involvement in general but also neuron-specific biological processes such as calcium signaling, mitochondrial homeostasis and synaptic transmission, among others.
Conclusions: aSyn fibrils are efficiently internalized by neuronal cells triggering a pronounced and functionally orchestrated cellular response. Future biological follow-ups targeting differentially expressed genes will shed light into the precise contribution of internalized aSyn on the biological processes affected by aSyn uptake.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0729

ZNT3 AND ALPHA SYNUCLEIN IN LEWY BODY DEMENTIA

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Introduction & methods : The loss of zinc transporter 3 (ZnT3) in mice has been implicated in age-related cognitive decline and pathological aggregation. We investigated the importance of ZnT3 and a postsynaptic marker, PSD95, in people with Parkinson's disease dementia (PDD n=31), dementia with Lewy bodies (DLB n=44), Alzheimer's disease (AD n=16) and controls (n=24), using standardized cognitive assessments during life, semi-quantitative scoring of Abeta, tau and alpha-synuclein at post-mortem semi-quantitative western blotting and immunohistochemistry in three cortical regions. Concentrations of monomeric alpha synuclein were determined by commercially available ELISA. Furthermore, given the emerging relationship between zinc and depression we examined the relationship of ZnT3 and scores for depression.

Results: DLB and PDD were characterized by significant reductions of PSD95 ($p<0.05$) and ZnT3 ($p<0.001$) in prefrontal cortex compared to controls and AD. Strong associations were observed between ZnT3 and PSD95 levels in prefrontal cortex and cognitive impairment ($p=0.001$ and $p=0.002$ respectively). Associations were also seen between ZnT3 levels in cingulate cortex and severity of amyloid-beta ($p=0.003$) and tau pathology ($p=0.011$). Concentrations of monomeric alpha synuclein correlated positively with those of ZnT3 in all three regions (Rs 0.34-0.45, $p<0.01$). Reductions in ZnT3 in BA9 were significantly associated with elevated depression scores in the study cohort (beta=-0.351(SE=0.393) $p=0.0004$).

Conclusions: This study has identified Zn^{2+} modulation as a novel target for the treatment of cognitive impairment and depression in DLB and PDD, and the potential for synaptic proteins to be utilised as biomarkers for the differentiation of DLB and PDD from AD.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0975

LRRK2 KNOCK-OUT MICE ARE PROTECTED FROM ALPHA-SYNUCLEIN FIBRIL-INDUCED COGNITIVE IMPAIRMENT

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OBJECTIVES: Investigate the effect of alpha-synuclein (aSyn) fibril inoculation on motor and cognitive behavior in mice, with subsequent evaluation of aSyn neuropathology. Assess the involvement of LRRK2 in synuclein pathology using LRRK2 knock-out (KO) mice.

METHODS: aSyn pre-formed fibrils (aSyn-PFFs or PBS control) were unilaterally delivered by stereotactic injection into the dorsolateral striatum of LRRK2 KO mice and their non-transgenic (NT) littermates. Motor ability was evaluated by open field, cylinder and drag tests. Cognition was assessed in a novel object recognition paradigm. Testing was at 30 and 90 days post-injection (dpi). Animals were then transcardially perfused with PBS, brains removed, fixed in NaCl/EtOH and paraffin embedded. Sections (6 um) were stained for pSer129-aSyn to assess inclusion pathology.

RESULTS: Striatal administration of aSyn-PFFs induced cognitive deficits in the absence of motor impairment in NT mice at 30dpi. At this time LRRK2 KO mice were protected from PFF-induced cognitive deficits. Genetic deletion of LRRK2 conferred long-lasting protection, as KO mice retained normal cognitive ability at 90dpi. Pathological aSyn inclusions were not detected at 30dpi, but were observed at 90dpi in aSyn-PFF treated mice.

CONCLUSIONS: We reveal a cognitive deficit that is produced soon after aSyn-PFF injection in NT mice. This manifests in the absence of motor dysfunction and prior to the detection of aSyn neuropathology. Interestingly, genetic deletion of *Lrrk2* protects mice from this cognitive impairment. The data indicate that LRRK2 is required for the immediate pathogenicity of aSyn fibrils and that LRRK2 silencing may confer protection against synucleinopathy

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0980

ENDOGENOUS LEVELS OF G2019S MUTANT LRRK2 ALTER SYNAPTIC TRANSMISSION AND BEHAVIOUR

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Objective: We recently reported that glutamate transmission is elevated in cortical neuron cultures from LRRK2 G2019S knock-in (KI) mice, a finding correlated with alterations to the phosphorylation state of the presynaptic release regulator synapsin1. We sought to test whether results generated *in vitro* are supported by neurophysiological, behavioural and biochemical assays *in vivo*.

Methods: Motor and cognitive function was assessed in KI mice by open field exploration, spontaneous rearing, novel object location / recognition memory paradigms. Electrophysiological recordings in acute brain slices assessed changes in synaptic transmission and plasticity in striatal projection neurons (SPNs). Nigrostriatal dopamine function was probed by voltammetry in brain slices and microdialysis in behaving mice. Neurophysiology and behaviour were probed pharmacologically.

Results: As *in vitro* we found increased glutamatergic transmission in KI SPNs. Mice also exhibited alterations to the dopamine system and behavioural hyperactivity. Relative to littermates, KI mice exhibited differential responses to dopamine receptor pharmacology at both the behavioural and synaptic level.

Conclusions: At physiological expression levels, the LRRK2 G2019S mutation alters neuronal function and produces a consistent glutamatergic synaptic phenotype *in vitro* and *in vivo*. In mice, alterations to the glutamate and dopamine systems are accompanied by behavioural phenotypes. Characterising the manifestation, and natural history, of mutant-induced changes is crucial to understanding the pathophysiology of LRRK2. Accurate animal modeling and insights such as those presented here are required for the design of neuroprotective strategies and may help to provide intervention against this and potentially other forms of PD

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-2220

LONG-TERM IN VIVO IMAGING OF DENDRITIC SPINE DYNAMICS IN A MOUSE MODEL OF PARKINSON'S DISEASE

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OBJECTIVES:
alpha-Synuclein is considered to be a crucial player in the disease progression of Synucleinopathies like Parkinson's disease (PD) or Lewy body dementia (LBD) and also has been reported in modulating brain plasticity. Alterations in distal neuronal compartments like dendritic spines during the course of neurodegenerative disease progression could hold important implications for the functioning of neural networks. In fact, cognitive decline in PD and LBD is a common symptom and could be attributed to impairments in cortical circuitries and synaptic plasticity.

METHODS:
We investigate how wild-type human alpha -Synuclein overexpression affects the dynamics of dendritic spines in the mouse somatosensory cortex. Imaging of layer V apical dendrites through a chronic cranial window is performed in PDGF-h-a-syn x GFP-M mice and in three different age groups. Using long-term *in vivo* two-photon microscopy, we are able to precisely follow the structural changes of dendritic spines over several weeks.

REULTS:
We find that overexpression of alpha-Synuclein profoundly affects spine dynamics as early as at 3 months of age. Compared to controls, the alpha-Synuclein overexpressing mice show decreased spine density in all analysed age groups. This might not only provide a link to dementia associated with PD and LBD, but also permits to investigate the effects of putative PD drugs *in vivo*.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-0551

VPS35 DYSFUNCTION IMPAIRS LYSOSOMAL DEGRADATION OF ALPHA-SYNUCLEIN AND EXACERBATES NEUROTOXICITY IN A DROSOPHILA MODEL OF PD

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Objectives: Recently, a missence mutation of *Vacuolar Protein Sorting 35* (*VPS35*) was identified as the cause of PARK17. VPS35, a core component of the retromer, mediates retrograde transport of cargoes from the endosome to the *trans*-Golgi network. One of the best-characterized cargoes for the retromer is the cation-independent mannose 6-phosphate receptor (CI-MPR), which participates in the delivery of lysosomal hydrolases such as cathepsin D (CTSD). Because CTSD is the lysosomal endopeptidase responsible for alpha-synuculein (aSYN) degradation, the insufficient activation of CTSD by VPS35 malfunction may affect the clearance of aSYN in lysosome.
Methods: The silencing of *VPS35* was performed in HEK293 cells by target-specific siRNA. The expression of retromer components, CTSD and aSYN were evaluated by Western blotting and immunostaining. The effect of VPS35 on aSYN-induced neurotoxicity, *Drosophila* homologue of *VPS35* (*dVPS35*) was silenced using RNAi. VPS35-deficient flies were mated to the transgenic flies expressing human aSYN under pan-neural promoter *elav* or eye-specific promoter *GMR*. Two assays were used to screen for modifiers: eye degeneration and climbing behavior.
Results: *VPS35* RNAi altered distribution of CI-MPR and perturbed the maturation process of CTSD in parallel with the accumulation of aSYN in the lysosomes. Furthermore, *dVPS35* silencing not only induced the accumulation of the detergent-insoluble aSYN species in the brain but also exacerbated both locomotor impairments and eye degeneration in the flies expressing human aSYN.
Conclusions: These findings indicate that retromer may play a role in aSYN degradation by modulating CTSD activity and might thereby contribute to the pathogenesis of PD.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-0596

MITOCHONDRIAL QUALITY CONTROL IN NEURONAL CELLS: THE FUNCTIONAL INTERPLAY BETWEEN PGC-1ALPHA AND PARKIN

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As mitochondrial dysfunction plays a key role in the pathology of Parkinson's disease (PD), it is crucial to understand the precise role of factors that control the number and quality of mitochondria in neuronal cells. Parkin is an E3 ligase which actively participates in the mitophagic degradation of dysfunctional mitochondria. PGC-1alpha is a transcription co-activator functioning as the main regulator of mitochondria biogenesis. Here, we are interested in the functional interaction between PGC-1alpha and Parkin in mitochondrial quality control in the context of neuronal cells.

In cortical neuronal cultures, we found that Parkin overexpression further increases the number of mitochondria in cells overexpressing PGC-1alpha. In addition, there is a significant upregulation in the mitochondrial reserve respiratory capacity when PGC-1alpha and Parkin were co-overexpressed. The combined overexpression of both proteins also leads to a rapid recovery of mitochondrial membrane potential following uncoupling stress.

In vivo, we unilaterally overexpressed PGC-1alpha and Parkin in the rat substantia nigra. In conditions of PGC-1alpha overexpression, we found a significant protective effect of Parkin on the dopaminergic function. Although the loss of dopaminergic marker and survival of nigral neurons expressing PGC-1alpha was not significantly rescued by Parkin compared to control, there was a significant difference in the neuron survival between wild-type Parkin and the PD-associated mutants K161N and R42P.

Overall, these results highlight the concerted role of Parkin and PGC-1alpha on mitochondrial function. We propose that this functional interaction may have an important role in the survival and activity of nigral dopaminergic neurons in PD.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-0655

SUMOYLATION AS A MODIFIER OF AUTOPHAGY IN ALPHA-SYNUCLEIN DISEASE

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Objectives. Small ubiquitin-like modifier-1, SUMO-1, conjugates appear in lysosomes in neural cells in response to protein aggregates. We previously identified lysosomal Hsp90 as a major target for SUMOylation in multiple system atrophy (MSA) tissue, mouse and cell culture models. In the unilateral rotenone mouse PD model, aged mice showed higher baseline SUMOylation and a proportionately lower increase in SUMOylated proteins in response to lesion than the younger adult cohort. In the current study, we investigated whether SUMOylation dependent modulation of lysosomes varies in Parkinson's disease human olfactory neurosphere-derived (hONS) cell lines compared to cell lines from unaffected individuals. We also examined the influence of inhibiting SUMOylation on protein aggregate response and autophagy in a neuroblastoma cell model. **Methods.** Immunofluorescence and Western analysis was used to investigate SUMO-1 and alpha-synuclein distribution and expression in PD-derived and control hONS cell lines subjected to proteasome inhibition. Potassium depolarization of SH-SY5Y neuroblastoma cells was used to induce calcium influx and alpha-synuclein aggregates and subjected to anachardic acid, ginkgolic acid and spectinomycin pre-treatment to specifically inhibit SUMOylation. **Results.** We found that neurosphere-derived cell lines from PD cases have a higher baseline level of lysosome SUMOylation than cells from unaffected individuals and show reduced induction in response to proteolytic stress. In the neuroblastoma cell model, we observed reduced frequency of alpha-synuclein aggregates (p, 0.01) and upregulation of LC3-positive macroautophagosomes with SUMOylation inhibitors. **Conclusions.** Lysosomal SUMOylation is raised in PD cell lines and SUMO pathway Inhibitors may promote clearance of alpha-synuclein aggregates by autophagy.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-1512

VACUOLAR PROTEIN SORTING 35 (VPS35) INTERACTS WITH LEUCINE-RICH REPEAT KINASE 2 (LRRK2) TO REGULATE AUTOPHAGY

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Objectives: VPS35 is one of the latest causative genes of Parkinson's disease (PD). The protein is a key component of the retromer complex that prevents lysosomal degradation of specific proteins by their sorting and trafficking back from endosomes either to the plasma membrane, or to the Golgi. This pathway is known to be involved in APP processing and Alzheimer's disease. However, reports on how VPS35 contributes to PD pathogenesis are very limited. Therefore, our goal is to address this fundamental question.

Methods: We take advantage of the power of Drosophila genetics to analyze genetic interactions in vivo. We then assess locomotor activity, lifespan and sensitivity to rotenone in the double or triple transgenic lines. To determine if Vps35 is required for proper function of dopaminergic neurons, we knocked down expression of Vps35, using a Vps35-RNAi line.

Results: Knocking-down Vps35 expression impaired locomotor activity, which suggests that the pathogenic VPS35 mutation may act in a dominant negative fashion. Importantly, overexpression of Vps35 rescued the phenotype of pathogenic mutant LRRK2, including the eye phenotype, locomotor activity and shortened lifespan, highly suggesting that these two genes are part of a common pathway. Finally, we genetically manipulated key components in the cascade that initiates autophagy and determined that LRRK2 and VPS35 are most likely part of the same pathway regulating autophagy.

Conclusions: Our data present in vivo evidence that Vps35 interacts with pathogenic mutant LRRK2 and that these two genes most likely share a common endolysosomal pathway that is involved in regulating autophagic response.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-1940

AUTOPHAGY IS INVOLVED IN AGE-RELATED SENSITIVITY TO PARKINSONIAN PATHOGENESIS

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Objective To explore the underlying mechanisms of autophagy in high sensitivity to MPTP-caused neurotoxicity in aged C57BL mice.
Methods 3 and 16 months old male C57BL mice were intraperitoneally injected with MPTP four times a day at 2-h intervals. Pole climbing tests was performed after MPTP exposure. TH-positive neurons and fibers in SNpc and striatum were detected with immunostaining. TEM was used to examine autophagosome. Western blotting was used to examine the protein levels of TH, α-synuclein, LC3-II, P62 and HDAC6 as well.
Results Compared with 3-month-old PD mice, 16-month-old PD mice showed significant shorter pole-climbing time after MPTP treatment. Age-associated changes in 16-month-old PD mice included the reduction in the densities of TH positive cells/fibers in SNc and striatum, lower TH protein expression, and α-synuclein accumulation as well. Under TEM, we observed increased lipofuscin and swollen mitochondria in 16-month-old parkinsonian mice. In 16-month-old mice treated with saline, the protein levels of LC3-II and P62 declined while P62 protein increased with age. Compared with 3-months-old PD mice, 16-months-old PD mice showed lower protein expressions of LC3-II and HDAC6.
Conclusion Ageing aggravated the DA neurons injury caused by MPTP. 16-month-old mice showed a high sensitivity to MPTP neurotoxicity. In 3-month-old mice, autophagy stress mediated by HDAC6 weakened the cytotoxicity of MPTP to a certain degree. While in 16-month-old mice, autophagy decompensation may be responsible for the higher sensitivity to MPTP neurotoxicity in aged mice.
Study Supported by: National Natural Science Foundation of China (No. 81171213 and 81301091).

03e. Pathophysiology & Disease Mechanisms: proteasome and ubiquitin

ADPD5-1779
IMPACT OF IMMUNOPROTEASOMES ON THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES

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The ubiquitin-proteasome-system (UPS) plays a central role in clearance of misfolded and oxidant-damaged proteins, which comprise a hallmark of many neurodegenerative diseases. Upon stimulation by interferons (IFN) catalytic subunits of the 20S proteasomes (s-proteasomes) can be replaced by alternative subunits, giving rise to the catalytically active immunoproteasomes (i-proteasomes). Since i-proteasomes play an important role in clearance of oxidant-damaged proteins that preferentially accumulate upon inflammatory stimulation, and neuroinflammation is a common finding in neurodegenerative diseases, we tested the expression of i-proteasomes during disease progression in mouse models of Alzheimer's disease (AD) and Parkinson´s disease (PD) and analyzed the impact of deficient i-proteasome function on disease pathogenesis by genetically ablating the beta5i(LMP7) i-proteasome subunit.
We demonstrate that expression of i-proteasome subunits in the brain increases upon aging, but is significantly accelerated by accumulation of misfolded proteins in AD and PD mice.
Genetic ablation of the LMP7 subunit and therefore i-proteasome activity attenuated the secretion of proinflammatory cytokines by microglia in AD mice but revealed no major impact on cerebral beta-amyloid burden compared to AD mice with functional i-proteasomes. In contrast, PD mice lacking functional i-proteasomes highlight an increase of aggregated alpha-Synuclein accompanied by worsening of associated pathology.
Our studies show that i-proteasomes are upregulated during the course of neurodegenerative diseases and that i-proteasome deficiency has a differential impact on course and associated pathology in models of intracellular and extracellular proteinopathies. These data underscore the importance of considering all aspects of disease associated pathology and highlight i-proteasomes as potential therapeutic targets in neurodegenerative diseases.

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-1652

HYPERIN ATTENUATES LIPOPOLYSACCHARIDE-INDUCED MICROGLIA ACTIVATION THROUGH INHIBITION OF P38 AND NFκB SIGNALING

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Objectives:Hyperin is an active compound isolated from *Rhododendron brachycarpum* G. Don (Ericaceae). In this study, we aimed to investigate its potentiality in inhibition of neuroinflammation.
Methods:BV2 microglial cells were pretreated with hyperin and stimulated with lipopolysaccharide (LPS).
Results:The results showed that hyperin significantly inhibited LPS-induced production of nitric oxide (NO) and pro-inflammatory cytokines such as TNF-α and IL-1β. Further analysis showed that inducible nitric oxide synthase (iNOS) and mRNA expression of TNF-α and IL-1β were attenuated by hyperin pretreatment. Analyses in signaling pathways demonstrated that hyperin led to suppression of LPS-induced p38 MAPK and p65/NFκB activation, but had little effect on the activation of JNK1/2 and Erk1/2. Interference with specific inhibitors revealed that hyperin attenuated the LPS-induced production of NO, TNF-α and IL-1β via both p38 and NFκB pathways.
Conclusions:Collectively, our data suggest that hyperin may serve as an anti-inflammatory agent and contribute to the suppression of neuroinflammation in disorders such as Parkinson's disease.

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-2268

ROTENONE ATTENUATES THE TOXICITY AND THE ELEVATION OF REACTIVE OXYGEN SPECIES INDUCED BY PARAQUAT IN SK-N-SH NEURONAL CELL LINE.

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Parkinson's disease (PD) is a chronic neurodegenerative disorder which affects 1–3% of the elderly population worldwide with the majority of PD cases (90–95%) being sporadic. Recent epidemiological data suggests a link between the exposure to environmental pesticides toxicants such as paraquat and rotenone and an increased risk in developing PD.

The mechanism of paraquat-induced dopaminergic cell damage involves increased production of oxygen-free radicals via a redox cycling mechanism leading to the formation of the superoxide anion in both the cytosol and the mitochondrial matrix. Rotenone is a lipophilic and natural pesticide, able to cross the blood-brain barrier. Rotenone inhibits complex I of the mitochondrial electron transport chain leading to the formation of ROS such as superoxide, subsequently causing reduced glutathione levels and oxidative stress.

Objectives: We have investigated the synergic toxicity of rotenone and paraquat.

Methods: The synergic effect of paraquat and rotenone was studied on SK-N-SH neuronal cell line. For this, SK-N-SH cells were co-treated with paraquat (70 mM) and rotenone (0.1 mM, 10 mM) for 24 hours.

Results: We found that paraquat induced cell death, the elevation of reactive oxygen species (ROS), of glutathione and of mitochondrial superoxide levels. The presence of rotenone at 0.1 mM attenuated the toxicity induced by paraquat, blocked the elevation of ROS and of glutathione induced by paraquat. At this concentration, rotenone did not induce the alteration in ROS steady-state levels.

Conclusion : These results demonstrate that a low dose of rotenone could counteract the toxicity of the pesticide paraquat.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0557

MODULATING AMPK ACTIVITY HAS NEUROPROTECTIVE EFFECTS IN THE IN VITRO AND IN VIVO MODELS OF PARKINSON'S DISEASE

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1. Objectives

Our objective was to establish how AMPK activity modulation affects the survival of primary cortical neurons and dopaminergic cells in the rat substantia nigra after exposure to rotenone or alpha synuclein over-expression.

2. Methods

We used AAV vectors to over-express three variants of the catalytic alpha2 subunit of the AMPK, wild-type (WT), dominant negative (K45R) and constitutively active (CA) in mouse primary cortical neurons and in the rat *substantia nigra*. Parkinsonian degeneration was induced by either AAV-mediated over-expression of human alpha-synuclein (*in vivo* and *in vitro*) or 2.5nM rotenone (*in vitro*).

3. Results

We discovered that over-expression of WT alpha2 subunit leads to a significant reduction in the AICAR-induced catalytic activity of the AMPK complex in neuronal cells, which predominantly express the alpha1 subunit. This was even more evident for the K45R subunit.

In primary cortical neurons exposed to sub-toxic concentrations of rotenone, basal oxygen consumption is increased in cells expressing the WT and K45R alpha2 subunits. Furthermore, WT alpha2 prevents reduction of mitochondrial load in cells exposed to the toxin.

In vivo, we found that over-expression of the aforementioned forms of AMPK's alpha2 subunit in the rat *substantia nigra* significantly protected dopaminergic neurons against alpha-synuclein-induced neurodegeneration.

4. Conclusions

This study indicates that modulation of AMPK activity may show significant neuroprotective benefits in various models relevant to PD. Our results also strongly support further exploration of the roles of both the alpha1 and alpha2 subunits of AMPK in the context of metabolic perturbations observed during PD progression.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0703

CURCUMIN SHOWS PROTECTIVE EFFECTS AT A MITOCHONDRIAL LEVEL IN A PINK1 SIRNA-MEDIATED CELLULAR MODEL OF PARKINSON'S DISEASE

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1. Objectives

Mutations in the *PINK1* gene are responsible for autosomal recessive Parkinson's disease (PD). The project aimed to determine the effect of a *PINK1* knock down (KD) model of PD and the effects of the antioxidant curcumin on mitochondrial function.

2. Methods

A siRNA-mediated KD in a SH-SY5Y neuroblastoma cell line was used for all functional studies. PINK1 KD cells and controls were either i. treated with 25µM paraquat for 24hours or ii. pre-treated with 2µM curcumin for 1hour before the paraquat treatment. Thereafter, cell viability was measured by an MTT assay, and mitochondrial respiration experiments were performed on the Seahorse XF Analyser.

3. Results

The PINK1 KD model showed a significant decrease in cell viability ($p<0.01$, 23.13%), maximal mitochondrial respiration ($p=0.0276$) and ATP production ($p=0.0156$). All cells treated with paraquat alone showed an expected decrease in viability, basal and maximal respiration, ATP production and spare capacity. Curcumin pre-treatment in control cells significantly increased the cell viability ($p<0.001$), the maximal respiration ($p=0.0248$) and the ATP production ($p=0.004$). In PINK1 KD cells, although curcumin did not significantly increase the cell viability or respiration parameters, it did significantly increase the ATP production ($p=0.0253$). These results suggest a protective effect of curcumin against paraquat.

4. Conclusions

PINK1 siRNA-mediated KD in SH-SY5Y cells can be used as a model of PD to study aspects of mitochondrial dysfunction. Furthermore, curcumin should be considered as a possible therapeutic target for PD, as it exhibits protective effects against paraquat at a mitochondrial level.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-1501

A STUDY OF MITOCHONDRIAL QUALITY CONTROL IN IPSC-DERIVED HUMAN DOPAMINERGIC NEURONS WITH A PATHOGENIC MUTATION IN OPA1

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Objectives:
The viability of dopaminergic neurons is critically dependent on the function of mitochondrial quality control mechanisms, which have been implicated in Parkinson's disease. While many studies have investigated parkin-mediated mitophagy, very little is known about the significance of mitochondrial fusion-fission in human dopaminergic neurons or the neuronal response to defects in this process. To address this question we generated iPSc-derived dopaminergic neurons from patients with haploinsufficiency in the GTPase Opa1, which regulates mitochondrial fusion and cristae morphology.
Methods:
iPSc-derived dopaminergic neurons were differentiated in the presence of neurotrophic factors following dual SMAD inhibition. Neurite length and mitochondrial fragmentation were assessed by fluorescent microscopy. Mitochondrial ultrastructure was imaged by TEM and mitochondrial respiration was assessed by Seahorse. Parkin-mediated mitophagy was analysed by fluorescent microscopy following nucleofection of YFP-tagged parkin.
Results:
We successfully generated iPSc-derived human neurons expressing neuronal (Tuj1, Tau, alpha synuclein) and dopaminergic (TH, GIRK2) markers. Although the percentage of dopaminergic neurons generated was not affected by Opa1 haploinsufficiency, patient neurons displayed reduced neurite length and branching. Initial functional assays indicated that neurons with Opa1 mutation exhibit a reduction in OXPHOS and disorganized cristae morphology. Surprisingly, these defects did not correlate with mitochondrial fragmentation or recruitment of parkin.
Conclusion:
We describe a unique model for the study of mitochondrial dysfunction in human dopaminergic neurons. Detailed understanding of the cellular changes in this model could provide novel fundamental insights into the pathophysiology of Opa1 mutations and the adaptive response of human dopaminergic neurons to mitochondrial damage.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0424

LEVELS OF CSF IL-6 ARE DECREASED AND CORRELATE WITH MMSE AND CSF LEVELS OF ALPHA-SYNUCLEIN IN DLB PATIENTS.

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Objectives: Inflammatory processes have previously shown to influence cognition and dementia progression. An involvement of interleukin (IL)-6 has in particular been suggested, as altered levels of IL-6 in cerebrospinal fluid (CSF) have been found in patients with Alzheimer's disease (AD). Also an association between cognitive decline and levels of IL-6 in CSF have been reported. The current study aimed to investigate whether clinically diagnosed patients with dementia with Lewy bodies (DLB) display altered CSF IL-6 levels in comparison with AD patients and non-demented controls and whether the IL-6 levels are correlated with cognitive status and biomarkers for AD and synucleinopathy.

Methods: Immunoassays measuring levels of IL-6 (Multiplex electrochemiluminiscence, Mesoscale), AD markers P-tau, T-tau and amyloid beta 1-42 (ELISA, Innogenetic) and alpha- synuclein (ELISA, Invitrogen) were used to analyze CSF from AD patients (n=53), DLB patients (n=32) and controls (n=46). Cognitive status was evaluated using the Mini Mental State Examination (MMSE).

Results: Our analysis showed significantly lower levels of IL-6 in CSF from DLB patient compared to CSF from both controls and AD patients. The IL-6 levels were additionally negatively correlated with MMSE in both AD and DLB patients, but not in controls. Also a positive correlation between alpha synuclein levels and IL-6 levels was detected in CSF from DLB patients.

Conclusion: Our findings support previous studies demonstrating a link between inflammatory processes and dementia progression and further strengthen the hypothesis that IL-6 is involved in dementia pathology and cognitive decline.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0450

MICROGLIAL ACTIVATION IN MULTIPLE SYSTEM ATROPHY

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Objectives:
Multiple system atrophy (MSA) is an adult-onset neurodegenerative disease characterised by aggregation of alpha-synuclein in oligodendrocytes to form glial cytoplasmic inclusions. MSA is classified according to the most severely affected brain regions: striatonigral degeneration (SND), olivopontocerebellar atrophy (OPCA) or as a mixed form. Microglial activation is reported to occur in MSA. Here we determine which microglial activation state, phagocytic M1 or anti-inflammatory M2, dominates in MSA brain and how this relates to severity of pathology.

Methods:
Patient brain donation enabled detailed post-mortem neuropathological examination including immunohistochemical and double label immunofluorescence microscopy in paraffin embedded brain tissue. Double immunofluorescence using a series of M1 (CD68, TSPO and TNF-alpha,) and M2 (Arginase-1, TSPO and YM1) microglial activation markers together with microglial marker Iba-1 was quantified and analysed in severely and mildly affected regions of six control, SND and OPCA MSA brains.

Results:
Microglial activation was observed in all regions examined in both MSA cohorts. Microglia were identified as M1 (positive for CD68 or TNF-alpha and Iba-1 or TSPO) or M2 (positive for Arginase-1 or YM1 and Iba-1 or TSPO). TSPO was determined to be a reliable marker of microglial activation. A trend towards increased M1 activation in MSA compared to M2 was observed by colocalisation of TSPO with CD68 immunoreactivity. This increase was significant in the substantia nigra in SND and OPCA MSA subtypes.

Conclusions:
Neuroinflammation is identified in MSA brain and may contribute to disease pathogenesis. Our results suggest that a specific microglial activation state may dominate in MSA brain.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0658
ALPHA-SYNUCLEIN AS AN EXTRACELLULAR TRIGGER OF NEUROINFLAMMATION IN MULTIPLE SYSTEM ATROPY

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Objectives. As degenerating neurons release alpha-synuclein it may mediate astrocyte activation or result in intracellular inclusion bodies following endocytosis. Multiple system atrophy (MSA) exhibits widespread astrogliosis together with glial alpha-synuclein cytoplasmic inclusions (GCIs) in mature oligodendrocytes. In the current study, astrocyte activation was analyzed by quantitative morphometry and by using molecular markers. **Methods.** We quantified astrocyte activation by morphometric analysis of MSA cases, and investigated the correlation to distance to nearest GCI as a proxy for extracellular alpha-synuclein. We obtained 'skinned' three-dimensional models of GFAP-positive astrocytes in MSA and control tissue (n = 75) from confocal z-stacks and measured the astrocyte process length and thickness and radial distance to GCI. **Results.** Astrocytes proximal to GCI-containing oligodendrocytes (r < 25 μm) had significantly (p, 0.05) longer and thicker processes characteristic of activation than distal astrocytes (r > 25 μm), with a reciprocal linear correlation (m, 90 μm^2) between mean process length and radial distance to the nearest GCI (R^2, 0.7). In primary cell culture studies, alpha-synuclein addition caused ERK-dependent activation of rat astrocytes and perinuclear alpha-synuclein inclusions in mature (MOSP-positive) rat oligodendrocytes. Activated astrocytes were also observed in close proximity to alpha-synuclein deposits in a unilateral rotenone-lesion mouse model. Moreover, unilateral injection of MSA tissue-derived alpha-synuclein into the mouse medial forebrain bundle resulted in widespread astrocyte and microglial activation in the alpha-synuclein-injected, but not sham-injected hemisphere. **Conclusions.** Taken together, our data suggests that the action of localized extracellular concentrations of alpha-synuclein may underlie both astrocyte and oligodendrocyte MSA pathological features.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0967

LAXATIVES ARE ASSOCIATED WITH LOWER RIGIDITY IN IDIOPATHIC PARKINSONISM

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Aim. To investigate whether improving intestinal-transit might reduce rigidity in idiopathic parkinsonism (IP). Two-thirds of IP-patients are hydrogen-breath-test-positive for small-intestinal-bacterial-overgrowth at presentation. Flexor-rigidity is greater the higher the circulating natural-killer cell count, an effect modulated by CD4+ count. Both counts are higher with breath-test-positivity.

Methods. Relationships of interventions to rigidity were explored using generalised linear mixed models. Surveillance yielded 1461 measures of torque required to extend/flex forearm in 78 IP-patients over 369 person-years. Maintenance osmotic laxative (macrogols) was exhibited in 55 (193 person-years); bulk-forming (ispaghula husk/methylcellulose/sterculia) in 58 (216); enterokinetic agent (prucalopride) in 26 (43); guanylate cylase-C receptor agonist (linaclotide) in 12 (6). Dopaminergic agonists were exhibited in 48 (250), MAO-B inhibitors in 45 (241), levodopa combinations in 31 (149), amantadine in 24 (81), anti-cholinergics (low dose) in 31 (177), propranolol in 11 (39), antidepressant in 13 (20).

Results. There was a significant temporal increase (6 (95% CI 2, 10) % per year, p=0.006) in flexor-rigidity, a consequent increase in the ratio, flexor/extensor, denoting flexed posture (4 (2, 8) % per year, p=0.04). Exhibition of laxatives stemmed the increase in flexor-rigidity. Anti-parkinsonian medication had no additional effect. There was a significant decrease in ratio with propranolol (-19 (-30, -6) %, p=0.006), reflecting an increase in extensor-rigidity (20 (2, 40) %, p=0.03). The ratio increased with antidepressants (28 (12, 46) %, p<0.001), reflecting a tendency to decreased extensor-rigidity (-13 (-24, 0) %, p=0.06).

Conclusions. Laxative may reduce rigidity by reducing inflammation directly or its inhibitory effect on responsiveness to anti-parkinsonian medication.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1110
NEUROINFLAMMATORY MARKERS IN LEWY BODY DEMENTIAS
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Introduction: Neuroinflammation is an important feature of Alzheimer's disease (AD). However, little is known about the inflammatory status of Lewy body dementias, including Parkinson disease dementia (PDD) and dementia with Lewy bodies (DLB).
Methods: Levels of beta-amyloid ($Abeta_{1-42}$), phosphorylated tau (pS396 tau) as well as inflammatory cytokines and chemokines in neocortical brain homogenates from aged controls, AD, PDD and DLB cases were measured using ELISA and Luminex assays.
Results: AD and DLB both showed significant $Abeta_{1-42}$ and p396 tau burden, at levels significantly higher than controls. In contrast, only AD showed significantly higher levels of inflammatory markers, while neuroinflammatory markers are unchanged in both PDD and DLB.
Discussion: Both PDD and DLB have a relatively mild neuroinflammatory response compared with AD. The efficacy of anti-inflammatory therapeutic approaches may therefore be more limited in PDD and DLB.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1617

THE NEUROPROTECTIVE EFFECT OF MYRACRODRUON URUNDEUVA STANTARDIZED EXTRACT IS IN PART RELATED TO ITS ANTI-INFLAMMATORY PROPERTIES

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Objetive: Relate the neuroprotective effect with anti-inflammatory activity of *Myracrodruon urundeuva* stantardized extract.**Methods:** Male Wistar rats were submitted to an unilateral striatal injection of 6-OHDA. The groups used: sham-operated (SO), 6-OHDA and 6-OHDA treated with SD (10 and 20 mg/kg, p.o., for 2 weeks). The animals were subjected to apomorphine-induced rotations and rota rod tests; and then euthanized for striatal dissections and dopamine and DOPAC determinations; and immunohistochemical (tyrosine hydroxylase – TH) assay. The anti-inflammatory *in vitro* effect was investigated on human neutrophil degranulation induced by phorbol mirystate acetate (PMA; 0.1 µM); the levels of myeloperoxidase (MPO) were determinated at 620 nm. The cytotoxicity assay of SD (0.1-100 µg/mL) was performed by the MTT test in human neutrophils. The data were analized by ANOVA and Newman-Keuls test or the Student's t test and considered significant at $p<0.05$. **Results:** SD reduced the number of apomorphine-induced circling behavior and number of falls. SD increased TH immunoreactivity and reduced the DA depletion observed in the striatal lesioned side of the 6-OHDA-group. SD also inhibited MPO release by neutrophils, at all concentrations tested, with 67% inhibition at the lowest concentration (0.1 µg/mL), when compared to the control group. Similar results were observed with INDO. The treatment of human neutrophils with SD did not significantly reduce the viability of cells, as determined by the MTT test. **Conclusion:** The present study showed that SD has a neuroprotective effect in the DP model and this may be related, at least in part, to its anti-inflammatory property.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1665

PROFILE OF TIME COURSE OF RIGIDITY FOLLOWING EXHIBITION OF DIFFERENT LAXATIVE CLASSES IN IDIOPATHIC PARKINSONISM

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Aim. To investigate whether stemming of the temporal increase in flexor rigidity [6 (95% CI 2, 10) % per year, p=0.006] by laxatives (AD/PD 2015), in idiopathic parkinsonism (IP), is attributable to particular pharmacological classes.
Methods. The surveillance data (1461 measures of torque required to extend/flex forearm in 78 IP-patients over 369 person-years) was further explored with respect to exhibition of maintenance osmotic and bulk-forming (ispaghula husk/methylcellulose/sterculia) laxatives; an enterokinetic agent (prucalopride); and guanylate cylase-C receptor agonist (linaclotide). Natural logarithms of rigidity values were used as the dependent variable in mixed effects, random coefficient models. Linear splines, pre- and post-intervention, were used to assess temporal trends. All analyses were adjusted for anti-parkinsonian and other relevant medicinal interventions.
Results. Bulk-forming laxatives [exhibited in 58 (216 person-years)] and prucalopride [26 (43)] simply stemmed the increase in flexor-rigidity. Exhibition of macrogols [55 (193)] tended to shift rigidity values downwards (-7 (95% CI (-13, 1) %, p=0.07) as well as stemming the increase. Exhibition of linaclotide [12 (6)] tended to reverse the temporal trend (-42 (-78, 4) % per year, p=0.07).
Conclusions. Laxatives are an integral part of the anti-parkinsonian armamentarium, not only because constipation affects quality of life but also because they appear to ameliorate a cardinal sign. Moreover, osmotic laxatives and linaclotide tended to reversal the upward trend in flexor rigidity, despite anti-parkinsonian medication.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1797

EFFECT OF STANDARDIZED EXTRACT OF MYRACRODRUON URUNDEUVA IN CORTICAL NEUROINFLAMMATION OBSERVED IN RATS SUBJECTED TO MODEL OF PD

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Objetive: evaluate the effect of standardized extract of M. urundeuva in hippocampal changes observed in rats subjected to an experimental model of Parkinson's disease. **Methods:** The standardized extract of *Myracrodruom urundeuva* (SEMU) (20 and 40 mg/kg,p.o), was tested in a experimental model of PD induced by 6-hidoxidopamine (6-OHDA, 12 µg/µl). Male Wistar rats (200 g) were divided in 3 groups (n=8): lesioned group and 6-OHDA treated with SEMU 20 mg/kg and 40 mg/kg (p.o,for 15 days). At the end of the treatments, the brain was dissected for performing cortical histological sections and subsequently, underwent immunohistochemistry for: iNOS and COX-2. For all histological methods, there was a count of marked cells for quantitative analysis. The results were expressed as means ± SEM. One-Way ANOVA and Student Newman Keuls as the *post hoc* test were used and results considered significant at $p<0,05$. **Results:** The number of cells stained for COX-2 and iNOS was significantly lower on SEMU (40mg/kg) treated animals (22,83 ± 1,70; 50,67 ± 3,04; respectively) compared to 6-OHDA group (66,83 ± 1,88; 78 ± 2,88, respectively); Values found in the group SEMU 20 were: 29,67 ± 1,56; 73,17 ± 2,12, respectively ,showing a tendece for a dose dependency.**Conclusion:** SEMU provide decrease in cortical inflammation, responsible in part for non-motor symptoms of PD.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-1833

IL-10 MODIFIES DISEASE PROGRESSION AND PATHOLOGY IN A MOUSE MODEL OF ALPHA SYNUCLEINOPATHY

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An invariant feature of the pathological cascade in alpha-synucleinopathies is reactive gliosis and broad activation of the innate immune response in the CNS. Though proinflammatory innate immune activation has been postulated to accelerate alpha-synuclein (aS) pathology in mice, there is a paucity of data exploring the therapeutic potential of dampening immune activation. In primary mixed neuronal cultures treated with exogenous alpha-synuclein fibrils, rAAV mediated expression of IL-10, a master anti-inflammatory cytokine, suppresses proinflammatory chemokine, cytokine and other innate immune factor gene induction (Figure 1). Based on this data that IL-10, an anti-inflammatory cytokine, markedly dampens innate immune activation in vitro, we postulated that IL-10 based therapies may have beneficial outcome in aS mouse models. We have tested the effect of spinal cord targeted delivery of rAAV-IL-10 in the homozygous M83 mice expressing the A53T mutan alpha synuclein. An additional 'seeded' model using the peripheral to central propagation of aS pathology in hemizygous M83 mice were also used (See, Sacino et al, Proc Natl Acad Sci, 2014). Our results, which have been reproduced in at least two cohorts, show that IL-10 expression leads to accelerated disease progression (i.e., paralysis and/or moribund state) in both the homozygous and seeded hemizygus M83 transgenic mice (Figure 2). Biochemical analysis of the spinal cords reveal that IL-10 modulates innate immune activation and also affects the ubiquitination of soluble aS. These intriguing results reveal the unexpected and complex interplay between innate immunity and proteostasis in mouse models of synucleinopathies.

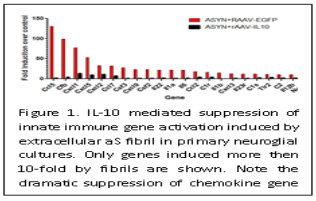

Figure 1. IL-10 mediated suppression of innate immune gene activation induced by extracellular aS fibril in primary neuroglial cultures. Only genes induced more then 10-fold by fibrils are shown. Note the dramatic suppression of chemokine gene

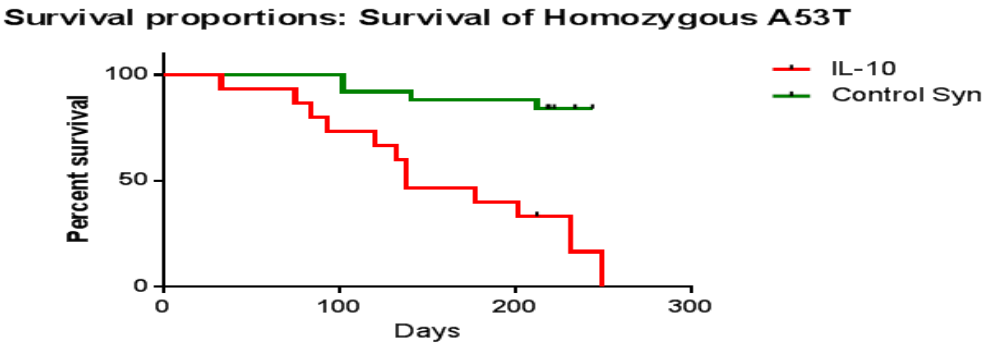

Figure 2: Spinal cord directed expression of rAAV-IL-10 accelerates disease process in homozygous aS M83 mice compared to rAAV-EGFP expressing mice. n=12/group.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-0700

LYSOSOMAL CHOLESTEROL ACCUMULATION MIGHT BE BENEFICIAL TO PATIENT SUFFERING FROM PARKINSON'S PATHOLOGY, YET CHOLESTEROL COULD BE A RISK FACTOR TO NORMAL INDIVIDUALS.

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Objective: Parkinson's disease (PD) is progressive degenerative disease of the central nervous system and till now there is no cure for the disease. According to recent studies, statins, the widely used cholesterol lowering drugs, might be beneficial in PD. However, due to contradictory finding the role of cholesterol in PD is still inconclusive. The true challenge is to understand the cholesterol homeostasis in PD.
Methods and results: We have found that MPP+-induced cell death is accompanied by cholesterol accumulation in pre-apoptotic cells in a lysosomal like pattern in BE(2)-M17 neuroblastoma cells. We hypothesize that increased cholesterol level is an important mechanism to protect membrane integrity in response to early apoptotic stress. To study this we generated a model system in which lysosomal cholesterol was increased using U18666A. We found lysosomal cholesterol to protect the cells from MPP^+ induced cell death by delaying leakage of lysosomal content to the cytosol. However high cholesterol also stimulated formation of α-Syn aggregates, which indicates that cholesterol could be a potential risk factor to induce PD pathology. Treatment with the cholesterol lowering drug lovastatin marginally reduced cholesterol and protected cell by inhibition of ROS production rather than by decreasing cholesterol level.
Conclusions: Our study clearly shows the dual role of cholesterol in PD. Increased understanding of cholesterol equilibrium is necessary to elucidated if statin drugs would be beneficial in PD patients.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-0838

ABNORMAL LIPID ALTERATIONS IN LIPID RAFTS RELATED TO PARKINSON'S DISEASE. CORRELATION WITH PROTEIN REARRANGEMENTS

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Our previous work has demonstrated that structural lipids of lipid raft microdomains are altered in cortical areas of PD, even at early preclinical stages. These alterations may be associated with cognitive deterioration during neuropathological progression, as an indicative of the potential correlation with changes in the proteins associated with these microstructures.

Here, using a murine model of PD treated with MPTP as neurotoxic, we have purified lipid rafts from different brain areas, and analyzed their lipid composition. Moreover, we have analyzed by immunoblotting and immunoprecipitation whether lipid changes may correlate with redistribution and interactions within these domains of alpha-synuclein, a main hallmark of this disease known to be integrated in these neuronal domains.

Different brain areas (septum, frontal and posterior cortices and cerebellum) of mice at different ages, 6 and 14 months, were investigated. The potential associations of alpha-synuclein with other raft protein markers related to toxic pathways have also been analyzed.

Our data suggest that, as previously shown for Alzheimer's disease, structural alterations of lipid rafts also affect the behaviour of proteins interacting within these structures, altering their normal behavior and dynamics, and ultimately contributing to neuronal dysfunctions.

Supported by SAF2010-22114-C02-01/02. ACA holds a fellowship from Fundación CajaCanarias.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1191

ALTERED LIPID LEVELS PROVIDE EVIDENCE FOR MYELIN LIPID DYSFUNCTION IN MULTIPLE SYSTEM ATROPHY

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Objectives: Multiple system atrophy (MSA) is a rapidly-progressive neurodegenerative disease characterized by parkinsonism, cerebellar ataxia and autonomic failure. A pathological hallmark of MSA is the presence of alpha-synuclein deposits in oligodendrocytes, the myelin-producing support cells of the brain. Although lipid constitutes 78% of myelin and myelin dysregulation is recognized as an important early pathological event in MSA, changes in lipid level/distribution in MSA brain are unknown. In this study we assessed the quantitative changes in myelin lipids in MSA brain.
Methods: We undertook a lipidomic analysis of MSA myelin by liquid chromatography-mass spectrometry. We quantitatively measured three groups of lipids, sphingomyelin, sulfatide and galactosylceramide, which are all important in myelin integrity and function, in affected (under the motor cortex) and unaffected (under the visual cortex) white matter regions in MSA (n=8) and control (n=10) brains.
Results: For all three groups of lipids, most of the species were severely decreased (40–69%) in affected but not unaffected MSA white matter. An analysis of the distribution of lipid species showed no significant shift in fatty acid chain length/content with MSA.
Conclusion: Myelin lipid dysfunction may contribute to MSA pathology.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1426

MOUSE MODEL BASED ON GM1 DEFICIENCY MANIFESTS MOVEMENT AND NON-MOVEMENT SYMPTOMS OF PARKINSON'S DISEASE; ALLEVIATED BY GM1 ANALOG FACILITATING GDNF

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Objectives: To demonstrate (a) 3 non-movement symptoms in a mouse model of Parkinson's disease (PD) based on ganglioside GM1 deficiency; (b) these symptoms relate to impaired GDNF signaling; (c) alleviation of symptoms with LIGA20, membrane permeable analog of GM1.

Methods: Use of genetically altered heterozygous (HT) mice with one disrupted allele for *B4galnt1*, GM2/GD2 synthase, which reduces GM1 (and other ganglio-series gangliosides) to ~50% of wild type. Immunohistochemistry (IHC) was employed for alpha-synuclein (aSyn), tyrosine hydroxylase (TH), and pRet. T maze determined cognition dysregulation. Constipation was determined by fecal matter content in colon and fecal water content.

Results: (I) Gastrointestinal; constipation was indicated in HT mice and more severely in knockout (KO) mice; also enteric neurons showed aSyn aggregates and depleted pRet. (II) Cardiac sympathetic denervation was indicated by (a) aSyn aggregates in superior cervical ganglion; (b) reduced TH^+ nerve fibers in cardiac tissue. (III) Cognitive impairment was revealed by T maze performance. As with movement impairment and nigral/striatal pathology, these 3 symptoms including restoration of pRet were alleviated by LIGA20.

Conclusions: LIGA20, serving as replacement therapy for deficient GM1, activated deficient GDNF signaling as seen in restored pRet. This study revealed GM1 as associated with the Ret/GFRa1 receptor complex and necessary for formation of this GDNF receptor. IHC of nigral sections from PD patients revealed significant deficiency of GM1 and pRET in TH^+ neurons. Additonal analyses suggested systemic GM1 deficiency as major risk factor in PD. The HT mouse manifests accurate recapitulation of PD.

Support: NIH 2RO1 NS033912

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1542

MECHANISMS OF DOPAMINERGIC NEURODEGENERATION INDUCED BY PD-LINKED MUTATIONS IN VPS35

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Mutations in the *vacuolar protein sorting 35 homolog* (*VPS35, PARK17*) gene were recently identified as a cause of late-onset, autosomal dominant Parkinson's disease (PD). Here, we explore the pathogenic consequences of PD-associated mutations in VPS35 using a number of model systems. The common D620N missense mutation in VPS35 does not compromise its protein stability or vesicular localization, or the vesicular sorting of the retromer cargo, sortilin, SorLA and CI-M6PR, in rodent primary neurons or patient-derived human fibroblasts. Studies in yeast suggest that analogous PD-linked mutations in VPS35 do not act through a loss-of-function mechanism. The overexpression of human VPS35 in primary cultures induces neuronal cell death and increases neuronal vulnerability to PD-relevant cellular stress. Furthermore, D620N VPS35 induces marked dopaminergic neurodegeneration and axonal pathology following viral-mediated gene transfer in a rat model of PD. Studies in mice reveals that genetic deletion of α-synuclein attenuates D620N VPS35-induced dopaminergic neurodegeneration and axonal damage. Additional studies also reveal a functional interaction of VPS35 with the dominant PD gene product, LRRK2. Collectively, these data suggest that dominant mutations in *VPS35* lead to neurodegeneration in PD consistent with a gain-of-function mechanism in a manner that may involve α-synuclein. Our studies support a key role for VPS35 in the development of PD and link together three dominant PD gene products (VPS35, α-synuclein and LRRK2) in a putative common pathogenic pathway.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1987

A COMPREHENSIVE MOLECULAR INTERACTION LANDSCAPE REVEALS A KEY ROLE FOR LIPID SIGNALING IN THE ETIOLOGY OF PARKINSON'S DISEASE

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Objectives
Parkinson's disease (PD) is characterized by degeneration of midbrain dopamine neurons and caused by a complex interplay of genetic and environmental factors. We aimed to elucidate this interplay and the underlying pathophysiological mechanisms by integrating genetic and functional data from both sporadic and familial PD patients.
Methods
We conducted bioinformatics and systematic literature analyses of the 429 top-ranked genes from 12 published genome-wide association studies (GWAS) of PD (involving 13,534 cases and 47,148 controls). Of these, 249 genes encode proteins that interact in a molecular landscape. Other studies - such as expression studies in postmortem brain tissue of PD patients and functional studies in (genetic) cell and animal models of PD - provided corroborating evidence for these PD GWAS gene-encoded proteins and for additional PD-related proteins (including those encoded by genes associated with familial forms of PD) to be included in the molecular landscape.
Results
The uptake, processing and signaling of lipoproteins, their metabolites and derivatives plays a key role in the generated molecular PD landscape. These lipids and lipid-signaling molecules regulate mitochondrial and lysosomal function, as well as the immune response, and are important modulators of cellular stress and apoptosis.
Conclusions
The biological signaling cascades and processes within the molecular PD landscape integrate classical (i.e., familial PD gene-derived) functional themes with PD GWAS and expression data, functional data from cell and animal models, and lifestyle-related environmental risk factors. As such, the PD landscape provides unique clues for the development of novel PD treatment strategies.

03l. Pathophysiology & Disease Mechanisms: translational regulation

ADPD5-0303
PARKINSON'S DISEASE: LOST IN TRANSLATION

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Proteins conduct the actions and functions of a cell. Protein synthesis is dynamic and tightly controlled in order to respond to the needs of the cell. Cells direct and regulate protein synthesis in response to hormones, nutrient availability and following episodes of intracellular stress by changing which proteins are translated. Alterations in the regulation of mRNA translation and protein synthesis can have serious effects on the function and health of a cell. Disease causing mutations in eukaryotic translation initiation factor 4 gamma-1 (EIF4G1) and LRRK2 when expressed in mice lead to age dependent dopaminergic neurodegeneration. Additionally, disease-causing mutations in EIF4G1 and LRRK2 result in altered mRNA translation and protein expression. Understanding the changes in mRNA translation and protein expression may provide clues to disease pathways that result in Parkinson's disease, a common age-related progressive neurodegenerative disorder with an unknown etiology.

03m. Pathophysiology & Disease Mechanisms: micro RNAs

ADPD5-0330

MICRO-RNAS - REGULATORS OF SIGNAL CASCADE OF ACTIN REMODELING AS BIOMARKERS OF NEURODEGENERATIVE DISORDERS AND TRIGGERS OF STRESS RESPONSE

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Objectives: Neurodegenerative disorders (NDs) affect both elderly (Alzheimer's, AD; Parkinson's, PD; Huntington's HD, diseases) and newborns (spinal muscular atrophy, SMA). Three main diagnostic symptoms of AD, PD, HD are memory defects, motor impairments, amyloid inclusions. A deregulation of specific microRNAs (miRs) has been shown to be concomitant to a certain disorder. Since one miR can change expression of hundreds of target genes, NDs can be considered as an RNA disorder. Surprisingly, studies in nematodes and Drosophila, rather than in mammals, allows the elucidation of specific miRs as biomarkers of NDs. Among these are let-7/miR-184 for PD, target genes are Parkin and PINK. miR-34 is believed to be a biomarker of AD, the main gene targets being TAU, CREB, HSP70. Poorly studied miR-9 has been indicated as a biomarker of HD and SMA. **Methods:** Isolated in our lab temperature-sensitive mutant a*gn*^{ts3} of the *agnostic* locus harboring *dlimk1* manifests the triad of NDs diagnostic symptoms mimicking PD with Dementia and Leavy Bodies at normal temperature. Therefore, microRNAs libraries from a*gn*^{ts3} and three wild type strains were obtained and compared. **Results:** a*gn*^{ts3} manifests a suppressed content of let-7/miR-184 and increased content of miR-34. A novel binding site for miR-34 is created due to an S-element (Tc1/mariner family) insertion near to *dlimk1* 3'-UTR. **Conclusions:** Heat shock acts as a trigger between pathologic and normal states and can completely abolish the triad of NDs diagnostic symptoms via miRs regulation of actin remodeling.

03n. Pathophysiology & Disease Mechanisms: kinases and phosphatases

ADPD5-0662

DOWN-REGULATION OF P21-ACTIVATED SERINE/THREONINE KINASE1 IS INVOLVED IN LOSS OF MESCENPHALIC DOPAMINE NEURONS.

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Objectives: Although the roles of p21-activated serine/threonine kinase 1 (PAK1) have been reported in Alzheimer's disease (AD), details in Parkinson's disease (PD) are restrictive. Thus, we tried to determine a role of PAK1 in PD.
Methods: We took advantage of SH-SY5Y cells, cultured dopamine (DA) neurons, 6-OHDA–induced hemiparkinsonian model rat, lentiviral expression system, along with a variety of molecular and cellular biological tools.
Results: Expression of a dominant-negative form of PAK1 (PAK1-DN) decreased the viability and increased cell death by oxidative stresses. PAK1-DN reduced the protein level of Bcl-2, an anti-apoptotic protein, through an ubiquitin/proteasome-dependent mechanism. Although the down-regulation of Bcl-2 induced by PAK1-DN was rescued by extracellular signal-regulated kinase 2 (ERK2), a phosphomimetic form (S70E) of Bcl-2 by PAK1 was also resistant with PAK1-DN expression, indicating that the Bcl-2 level might be regulated by both PAK1-ERK signaling and PAK1. Oxidative stress by 6-OHDA treatment decreased the phosphorylation of PAK1. Conversely, expression of an active form of PAK1 (PAK1-CA) could rescue behavioral defects and loss of DA neurons in hemiparkinsonian rat model.
Conclusions: Our data suggest that the down-regulation of PAK1 activity could involved in the loss of mesencephalic DA neurons, suggesting a new molecular mechanism in pathogenesis of PD.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-0423

H-3 RECEPTOR ANTAGONIST AND JNK-3 INHIBITOR: A NEW THERAPEUTIC APPROACH TO TREAT PARKINSON'S DISEASE.

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Background: Parkinson disease is a progressive loss of neuromelanin containing dopaminergic neurons from the substantia nigra *pars compacta*. Reports suggest that the highest density of H_3-receptors is found in basal ganglia and H_3-antagonists can increase the turnover of dopamine in basal ganglia. c-Jun N-terminal kinase (JNK) pathway plays an important role in stress mediated neurotoxicity, inflammation; while blockade of JNK by specific inhibitors may prevent or effectively slow-down the progression of PD. **Objective:** The study is aimed to evaluate the effectiveness of conessine as H3 antagonist and 1,9-pyrazolanthrone as JNK-3 inhibitor. **Methods:** By using Maestro 9.3 version of Schrodinger suite, binding affinity of Conessine is evaluated with histamine H-1 [PBD ID: 3RZE], H-2 [PBD ID: 1JQD] and H-3 receptors. The structure of H-3 receptor is not available in Protein Data Bank and was prepared by homology model using Maestro 9.3 version of Schrodinger suite. The receptors of human c-jun-n-terminal kinase JNK1 [PDB ID: 1UKH], JNK2 [PDB ID: 3E7O] and JNK3 [PDB ID: 2EXC] are taken from the Protein Data Bank and further modified for Glide docking calculations. **Results:** It reveals conessine shows better G score of -3.96 with H3 receptor and 1,9-pyrazolanthrone selective inhibiter of JNK-3. Conessine demonstrated functional antagonism of H3receptor in an in-vivo pharmacological model. The enzyme inhibitory assay in SH-SY5Y human neuroblastoma cell line shows that 1,9-pyrazolanthrone inhibits JNK-1, 2 with good affinity towards JNK-3 (IC_{50}- 0.05 µM). **Conclusion:** This study supports targeting H-3 receptor and JNK-3 is an important strategy in treatment of neurodegenerative disorders.

03r. Pathophysiology & Disease Mechanisms: vasculature & neoangiogenesis

ADPD5-1101

CEREBROSPINAL FLUID ANGIOGENIC BIOMARKERS IN PARKINSONS DISEASE

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Background: Existing evidence suggests that Parkinson´s disease (PD) is accompanied by angiogenesis in the affected brain areas. However, studies on well-characterized clinical cohorts are needed in order to establish the role of angiogenesis in PD pathology.

Methods and materials: We analyzed cerebrospinal fluid (CSF) levels of several angiogenic factors in 38 healthy controls and 100 patients with PD, including 18 PD dementia (PDD) patients, from the prospective Swedish BioFinder study. VEGF, PlGF, sVEGFR-1, sVEGFR-2, Ang2, and IL-8 were quantified using ultra-sensitive electrochemiluminescence immunoassays. In addition, we assessed white matter lesions and microbleeds with magnetic resonance imaging (MRI) and blood brain barrier (BBB) integrity using CSF/serum albumin ratio. Angiogenic factors were also measured in additional cohort of healthy controls (n=64) and PD patients with and without dementia (n=87).

Results: We found high CSF levels of VEGF, PLGF, sVEGFR-2, and lower levels of Ang2 in non-demented PD patient group and in PDD group compared to controls after controlling for the confounding effects of age and gender. Angiogenesis markers, including VEGF and PlGF, were associated with gait difficulties and poor memory in PD. We also found robust associations of angiogenic markers with BBB dysfunction, white matter lesions (WMLs) and microbleeds in PD patients. Moreover, VEGF and PlGF positively correlated with neurodegeneration and glial activation markers, NFL and MCP-1. The main findings were confirmed in the validation cohort.

Discussion/conclusion: The results presented here provide new evidence implicating angiogenesis in PD and uncover potential drug targets for future therapies.

03s. Pathophysiology & Disease Mechanisms: neurogenesis and stem cells

ADPD5-1295

DISSECTING THE ROLE OF DJ-1 IN ADULT NEUROGENESIS BY MODULATION OF ENDOGENOUS NEURAL STEM CELLS WITH LENTIVIRAL VECTORS

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Objectives: The endogenous neural stem cell (eNSC) pool in the adult brain provides an attractive cell source for neuroregeneration. We demonstrated labeling of the eNSC in the subventricular zone (SVZ) of adult mouse brain by stereotactic injection of lentiviral vectors (LV). In the present study we employ this technology to investigate the role of DJ-1. DJ-1 protects neurons against oxidative stress and cell death and loss-of-function mutations in DJ-1 lead to autosomal recessive early-onset PD.

Methods: We generated HIV-based LV expressing an eGFP reporter and a short-hairpin RNA against DJ1 (LV-shDJ1) or a mismatch control (LV-shDJ1MM). In a first step, we transduced primary neurosphere cultures derived from adult eNSC isolated from the SVZ. Next, LV-shDJ1 and LV-shDJ1MM were injected in the SVZ of 2 months old mice. eGFP allowed to follow the fate, morphology and number of newly generated neurons.

Results: Knockdown of DJ-1 resulted in reduced proliferation, size and renewal capacity of the neurospheres. Moreover, DJ-1 deficient neural progenitors showed elevated ROS levels and impaired mitochondrial homeostasis. In vivo, we observed reduced proliferation of the eNSC in the SVZ and a decrease in the number of newborn neurons in the OB.

Conclusion: Our data suggests that DJ-1 plays a relevant role in the adult neurogenesis pathway by protecting neural progenitors from high levels of reactive oxygen species (ROS). To dissect the role of DJ-1 in a cell-specific manner, we now employ Cre conditional LV in nestin-Cre transgenic mice, which switches on the knockdown solely in nestin-positive eNSC.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-0505

EXTENSIVE UPTAKE OF ALPHA-SYNUCLEIN OLIGOMERS IN PRIMARY ASTROCYTES – A CENTRAL PATHOLOGICAL MECHANISM IN PD?

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Objectives: In order to clarify the role of glial cells, other than microglia, in Parkinson's disease and dementia with Lewy bodies, we have studied uptake, degradation and toxic effects of oligomeric alpha-synuclein in a co-culture system of neurons, astrocytes and oligodendrocytes.

Methods: Primary neurons, astrocytes and oligodendrocytes, derived from E14 wild-type mouse cortices, were exposed to 500 nM (Cy3- or pHrodo-labeled) recombinant HNE-induced alpha-synuclein oligomers for 24 h. The cells were then thoroughly washed and the uptake and degradation of alpha-synuclein was studied at different time points, 0-12 days after exposure, by immunostainings, confocal microscopy, time-lapse microscopy, electron microscopy, Western blot and ELISA.

Results: Alpha-synuclein oligomers were taken up by all three cell types, but most readily by astrocytes, which accumulated large amounts of oligomers intracellularly. Cy3-labeled alpha-synuclein oligomers engulfed by astrocytes were shown to co-localize with the lysosomal marker Lamp-1, indicating that alpha-synuclein are degraded by lysosomes. Supporting this, alpha-synuclein oligomers labelled with pHrodo, an indicator dye which only fluoresces in the acidic environment of active lysosomes, were visualized 24 hours after exposure. Moreover, Western blot and ELISA of cell lysates showed that degradation of alpha-synuclein occurs 2-4 days after exposure. Interestingly, electron microscopy revealed that the oligomers caused mitochondrial damage, but without being lethal to the glial cells.

Conclusion: The intracellular processing of oligomeric alpha-synuclein by astrocytes emphasizes an important and possibly protective role of astrocytes in the presence of toxic alpha-synuclein oligomers.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-1419

INVOLVEMENT OF THE TRANSCRIPTION FACTOR NFAT IN PD

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Parkinson's Disease (PD) is the second leading cause of dementia worldwide and the mechanism that underlie the neurodegeneration are unknown. Alpha-synuclein (α-syn) is described as the culprit in PD. Apoptosis and inflammation are known to be key events in neurodegeneration of PD, which can be triggered upon an increase in intracellular Ca^{+2} caused by α-syn. Although, the Nuclear Factor of Activated T-Cells (NFAT) is activated by Ca^{+2} influx, its contribution to PD is poorly understood. NFAT proteins directly regulate the gene expression involved in the control of apoptosis and in the inflammatory process. Therefore, the main goal of the present project is to evaluate the involvement of NFAT in the neurodegenerative process induced by aggregates of α-synuclein. Our results have shown that only oligomers of α-synuclein were able to mediate increase in cell death in primary cultures of dopaminergic neurons from embryonic mice (E15) or N2A cells differentiated into dopaminergic cells and this effect was partially reversed when these cultures were pretreated with cyclosporine or VIVIT, two inhibitors of NFAT In addition we examined the involvement of monomers, oligomers and fibers in the synaptic events. We found that only oligomers and fibers showed to be capable of causing a decrease in Synapsin I, a protein involved in pre-synaptic events and this effect was likewise reversed when these cultures were pretreated with a NFAT inhibitor. In conclusion, these results suggest that the NFAT may have a role in the process of cell death and synaptic loss mediated by α-synuclein aggregates.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-1460

MULTIVARIATE ANALYSES OF AMYLOID-BETA OLIGOMER POPULATIONS INDICATE A CONNECTION BETWEEN PORE FORMATION AND CYTOTOXICITY

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This work established well-controlled aggregation conditions of amyloid-beta 1–40 or amyloid-beta 1–42 peptides over a 20-day period and characterized these preparations with regard to their beta-sheet content, degree of fibril formation, relative abundance of various oligomer sizes, and propensity to induce membrane pore formation and cytotoxicity. Using this multivariate data set, a systematic and inherently unbiased partial least squares (PLS) approach showed that for both peptides the abundance of oligomers in the tetramer to 13-mer range contributed positively to both pore formation and cytotoxicity, while monomers, dimers, trimers, and the largest oligomers (>210 kDa) were negatively correlated to both phenomena. Multivariate PLS analysis is ideally suited to handle complex data sets and interdependent variables such as relative oligomer concentrations, making it possible to elucidate structure function relationships in complex mixtures. This approach, therefore, introduces an enabling tool to the field of amyloid research, in which it is often difficult to interpret the activity of individual species within a complex mixture of bioactive species.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-1468

THE SLEEP MODULATING PEPTIDE OREXIN-B IS PROTECTIVE FOR VULNERABLE MIDBRAIN DOPAMINE NEURONS

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Objectives: Orexin-A and orexin-B (also known as hypocretin-1 and -2) are small hypothalamic neuropeptides involved in the control of sleep, wakefulness and energy homeostasis (Tsujino and Sakurai, Front Behav Neurosci, 2013). The loss of orexin containing neurons in Parkinson disease (PD) is therefore likely to explain some of the sleep disturbances associated to this disorder (Fronczek et al, Brain, 2007; Thannickal et al, Brain, 2007). Here, we tested the possibility that dysfunction of the orexin system in PD may also have an impact on neurodegenerative events affecting brainstem dopamine (DA) neurons.

Methods: To this aim, we used a model system of rat midbrain cultures in which DA neurons degenerate spontaneously and progressively as they mature (Toulorge et al, Faseb J, 2011).

Results: We found that orexin-B provides partial but robust protection to spontaneously dying DA neurons whereas orexin-A has only marginal effects. Rescued neurons accumulated DA efficiently by active transport suggesting that they were also functional. The effect of orexin-B was comparable in intensity to that provided by GDNF but independent of it. It was instead attributable to activation of G protein-coupled orexin receptors and to downstream signaling events requiring intracellular calcium mobilization. In addition to its own protective action for DA neurons, orexin-B had also the potential to reveal that of the alkaloid nicotine via a mechanism involving α7 nicotinic acetylcholine receptors.

Conclusions: Altogether, our data suggest that a relationship may exist in PD between degenerative events affecting hypothalamic orexin neurons and brainstem DA neurons.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-2000

S-NITROSYLATION OF PARKIN AS A NOVEL REGULATOR OF EXTRACELLULAR ALPHA-SYNUCLEIN-EVOKED DOPAMINERGIC CELL DEATH.

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Objectives:
Parkin encodes an E3 ubiquitin ligase, and disruption in parkin-mediated protein ubiquitination may contribute to accumulation of damaged protein and cell death. It was suggested, that parkin is inactivated in sporadic form of Parkinson's disease (PD) via S-nitrosylation or oxidative and dopaminergic stress, leading to the accumulation of defective proteins. However, the role of alpha-synuclein (ASN), the key protein component in Lewy bodies, in parkin modulation is unknown. Therefore, the aim of this study was to investigate the effect of extracellular ASN on parkin post-translational modifications and dopaminergic cell death.
Methods:
The experiments were performed using spectrophotometrical, spectrofluorometrical, immunochemical methods and real-time PCR analysis.
Results:
Our results indicated, that ASN is liberated from the cells into extracellular space and that this extracellularly acting protein leads to dopaminergic PC12 cells death. We first demonstrated that extracellular ASN leads to free radicals generation, increased cytoplasmic calcium concentration and decreased intracellular ATP level. Furthermore, stress response genes activation was observed. Finally, we showed substantial increase of parkin S-nitrosylation in ASN treated cells.
Conclusions:
Taken together, our data indicate that ASN affects parkin function through its S-nitrosylation. We suggest, that ASN-induced cellular stress could contribute to parkin deregulation and in consequence to accumulation of defective proteins and propagation of neurodegeneration. These findings may thus provide a molecular link between ASN liberation, oligomerization and parkin dysfunction in sporadic PD.
Supported by NCN grant: 2012/05/B/NZ3/02047 and European Union from the European Social Fund under the Operational Program Human Capital NCBIR grant POKL.04.03.00-00-060/12.

03v. Pathophysiology & Disease Mechanisms: metal ions

ADPD5-0906

MITOCHONDRIAL COMPLEX I INHIBITION INDUCES IRON ACCUMULATION AND CELL DEATH THROUGH THE ACTIVATION OF IRON REGULATORY PROTEIN 1

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Unpaired mitochondrial function, oxidative stress and iron accumulation are common features of several neurodegenerative disorders, between them AD and PD. It was recently reported that inhibition of mitochondrial complex I results in decreased iron-sulfur cluster (ISC) synthesis and activation of Iron Regulatory Protein 1 (IRP1), a key regulator of cellular iron homeostasis. These findings established a causal relationship between decreased mitochondrial activity and iron homeostasis, a finding particularly relevant for the understanding of neuropathological events where mitochondrial dysfunction and iron accumulation are pathognomonic signs. Objectives. To evaluate the functionality of IRP1 activation and its role in the death of SHSY5Y dopaminergic neuroblastoma cells subjected to complex I inhibition.
Methods. Complex I was inhibited with rotenone. IRP1 activity was evaluated by EMSA. The oxidative tone was evaluated fluorescent maleimides. Iron homeostasis proteins were evaluated by Western blot. shRNA was used to silence IRP1 expression.
Results. Activation of IRP1 by complex I inhibition associated with increased levels of Transferrin Receptor 1 (TfR1) and Divalent Metal Transporter 1 (DMT1), and decreased levels of Ferroportin 1 (FPN1), together with increased iron accumulation, oxidative stress and cell death. Silencing of IRP1 largely abolished the rotenone-induced changes in DMT1, TfR1, FPN1 and iron accumulation, and protected cells from cell death induced by complex I inhibition.
Conclusions. These results support the notion that IRP1 activation, secondary to inhibition of mitochondrial ISC synthesis, may underline the events leading to iron accumulation observed in PD. Financed by grants FONDECYT 1130068 and CONICYT-PIA ACT1114.
Conclusions. These results support the notion that IRP1 activation, secondary to inhibition of mitochondrial ISC synthesis, may underline the events leading to iron accumulation observed in PD. Financed by grants FONDECYT 1130068 and CONICYT-PIA ACT1114.
Results. Activation of IRP1 by complex I inhibition associated with increased levels of Transferrin Receptor 1 (TfR1) and Divalent Metal Transporter 1 (DMT1), and decreased levels of Ferroportin 1 (FPN1), together with increased iron accumulation, oxidative stress and cell death. Silencing of IRP1 largely abolished the rotenone-induced changes in DMT1, TfR1, FPN1 and iron accumulation, and protected cells from cell death induced by complex I inhibition. Conclusions. These results support the notion that IRP1 activation, secondary to inhibition of mitochondrial ISC synthesis, may underline the events leading to iron accumulation observed in PD. Financed by grants FONDECYT 1130068 and CONICYT-PIA ACT1114.
Methods. Complex I was inhibited with rotenone. IRP1 activity was evaluated by EMSA. The oxidative tone was evaluated fluorescent maleimides. Iron homeostasis proteins were evaluated by Western blot. shRNA was used to silence IRP1 expression.Results. Activation of IRP1 by complex I inhibition associated with increased levels of Transferrin Receptor 1 (TfR1) and Divalent Metal Transporter 1 (DMT1), and decreased levels of Ferroportin 1 (FPN1), together with increased iron accumulation, oxidative stress and cell death. Silencing of IRP1 largely abolished the rotenone-induced changes in DMT1, TfR1, FPN1 and iron accumulation, and protected cells from cell death induced by complex I inhibition. Conclusions. These results support the notion that IRP1 activation,

secondary to inhibition of mitochondrial ISC synthesis, may underline the events leading to iron accumulation observed in PD. Financed by grants FONDECYT 1130068 and CONICYT-PIA ACT1114.
Objectives. To evaluate the functionality of IRP1 activation and its role in the death of SHSY5Y dopaminergic neuroblastoma cells subjected to complex I inhibition.Methods. Complex I was inhibited with rotenone. IRP1 activity was evaluated by EMSA. The oxidative tone was evaluated fluorescent maleimides. Iron homeostasis proteins were evaluated by Western blot. shRNA was used to silence IRP1 expression.Results. Activation of IRP1 by complex I inhibition associated with increased levels of Transferrin Receptor 1 (TfR1) and Divalent Metal Transporter 1 (DMT1), and decreased levels of Ferroportin 1 (FPN1), together with increased iron accumulation, oxidative stress and cell death. Silencing of IRP1 largely abolished the rotenone-induced changes in DMT1, TfR1, FPN1 and iron accumulation, and protected cells from cell death induced by complex I inhibition. Conclusions. These results support the notion that IRP1 activation, secondary to inhibition of mitochondrial ISC synthesis, may underline the events leading to iron accumulation observed in PD. Financed by grants FONDECYT 1130068 and CONICYT-PIA ACT1114.

03v. Pathophysiology & Disease Mechanisms: metal ions

ADPD5-0941

LASER ABLATION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY TO MEASURE ZINC, COPPER, AND MANGANESE IN THE HUMAN PARKINSON'S DISEASE SUBVENTRICULAR ZONE

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1. Objectives. In Parkinson's disease (PD) there are changes in essential biometal concentrations in affected brain regions. Specifically, zinc and iron are increased in the PD substantia nigra, while copper is decreased compared to controls. In addition, occupational manganese exposure has been linked to PD, and manganese neurotoxicity shows similar symptoms to PD. However, detecting metals in the human brain has long been a challenge on the basis of spatial resolution and accuracy of measurement.
2. Methods. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to quantify copper, zinc, and manganese concentrations in the subventricular zone (SVZ), a region on the lateral wall of the lateral ventricle that is home to a population of neural progenitor cells, in age-matched PD (n = 5) and control (n = 5) brains. LA-ICP-MS allows quantitative measures of metal species to be acquired at a detailed anatomical level.
3. Results. There were no apparent changes in copper in the PD SVZ compared to the control SVZ; however, there was a significant increase in zinc in the PD vs. control SVZ ($p < 0.01$), and an even greater increase in manganese in the same region in PD compared to controls ($p < 0.001$). No significant changes were seen in the neighbouring caudate nucleus.
4. Conclusions. In this study, biometals that have been implicated in the pathology of PD are increased in the PD SVZ. The implications of increased concentrations of these metals in the SVZ will be discussed.

03v. Pathophysiology & Disease Mechanisms: metal ions

ADPD5-1044

IRON CHELATION PROTECTS SYMPATHETIC NERVE CELLS AGAINST OXIDATIVE STRESS INDUCED BY DOPAMINE AND 6-HYDROXYDOPAMINE

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The oxidative stress-induced loss of dopamine(DA)-secreting neurons in the substantia nigra is one of hypotheses of Parkinson's disease (PD) pathophysiology. These neurons are particularly prone to oxidative stress due to DA. It belongs to the catecholamines that are able to undergo spontaneous oxidation giving rise to reactive intermediates (aminochromes) and reactive oxygen species (ROS). No less important is the local impairment in iron ions balance, when free or loosely bound iron ions can aggravate the ROS-mediated toxic injury.

PC12 rat pheochromocytoma cell line differentiated with nerve growth factor into sympathetic neurons was used in our studies. We examined relationship of DA and 6-hydroxydopamine (6-OHDA) to free iron ions in pathobiochemistry of PD and the possibility of neuroprotection by iron chelation. Our cellular experiments assessed the ability of DA, 6-OHDA and their oxidised forms to induce injury to PC12 cells (LDH assay) and production of ROS inside PC12 cells (dichlorofluorescein assay), as well as, the ability of strong cell-permeable iron chelator salicylaldehyde isonicotioyl hydrazone (SIH) and boronic ester prochelator BSIH to suppress their harmful effects. Both SIH and BSIH were able to dose-dependently and significantly protect differentiated PC12 cells from the toxicities of DA, 6-OHDA and their oxidation products, suggesting that catecholamines can significantly contribute to oxidative injury of neurons in PD, which can be prevented by iron chelation.

This study was co-financed by the European Social Fund and the state budget of the Czech Republic. Project no. CZ.1.07/2.3.00/30.0061.

03v. Pathophysiology & Disease Mechanisms: metal ions

ADPD5-2174

TOXICOGENOMIC ANALYSIS OF ZEBRAFISH (DANIO RERIO) EMBRYOS REVEALS PATHWAYS INVOLVED IN MANGANESE-INDUCED DEMENTIA

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Introduction and objectives: Manganese (Mn) is essential for living organisms, playing an important role in nervous system function, bone mineralization, protein and energy metabolism, metabolic regulation and cellular protection. Nevertheless, chronic and/or acute exposure for this metal, mainly during early life stages can lead to neurotoxicity, but the mechanism for these events is still unclear. We hypothesize that exposure to Mn species induces differential neurotoxicogenomic alterations. Thus, the aim of the present study was to compare the toxicity of several and representing common aquatic chemical species of manganese, and to understand the mechanism of Mn-induced neurodisorders. **Methods**: The toxic effects of the manganese (Mn(II)-Chloride, Mn(II)-Citrate and Mn(III)-Citrate) in embryos of *Danio rerio* was verified by chemical speciation, chemical fractioning, neurobehavioral and toxicogenomic approaches (DNA microarray and RT-PCR). The embryos of zebrafish were exposed during different development stage from 2 hours post fertilizations –hpf to 122 hpf, for 48 h, 72 h and 120 h. **Results**: We found a stage-specific increase of lethality, being more significant for embryos exposed for Mn(II)-Citrate, specially > 48 hpf. Additionally, we verified that the zebrafish embryos exposed for manganese (48-120 hpf) showed significant Mn bioaccumulation, behavioral (locomotor and cognitive) impairment and finally disruption of genes associated with protein metabolism pathway. These genes (*bcat2, cenpj, dpp4, eif2s1, ell2, erbb2ip, mmp2, myl6, sgce, slc14a2 and tcea3*) are potentially associated with dementia (Parkinsonism and Alzheimer´s) too. **Conclusion:** These toxicogenomics findings suggest that early-life exposure for manganese may contribute to late-life neurodegeneration.

03w. Pathophysiology & Disease Mechanisms: calcium homeostasis

ADPD5-0660

EXCLUSION OF ALPHA-SYNUCLEIN AGGREGATES FROM CALBINDIN-D28K CELLS IN THE UNILATERAL ROTENONE-LESIONED MOUSE MODEL OF PD

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Objectives. Neurodegeneration in Parkinson's disease is associated with formation of neuronal inclusion bodies composed mainly of aggregated alpha-synuclein protein. Expression of the Ca(II)-buffering protein, Calbindin-D28K (CB), correlates with neuronal survival in PD and elevated intracellular free Ca(II) can trigger alpha-synuclein aggregation and inclusion body formation in model systems pointing to the influence of Ca(II) regulation in neurodegeneration. In the current study, the relationship between CB expression and alpha-synuclein aggregation was examined in a unilateral mouse PD model, whereby lesion of the medial forebrain bundle of C57 black mice (WT, neurturin-/-, Aged WT) with the mitochondrial inhibitor, rotenone, was performed to trigger neurodegeneration and alpha-synuclein inclusions. **Results.** Immunofluorescence of brain tissue sections revealed more frequent CB-positive cells within the lesioned hemisphere than within the control hemisphere. Large alpha-synuclein aggregates (>1µm) were 4-fold more frequent in the lesioned hemisphere compared to the non-lesioned hemisphere (p, 0.01). NTN-/- mice showed a greater frequency of CB-positive cells than WT mice, especially in the lesioned hemisphere. Alpha-synuclein inclusions were almost completely absent in CB-positive cells. Western analysis showed total CB expression was 20% higher (p, 0.05) in treated compared to control hemisphere in the aged animals and 25 % higher in aged compared to young mice in the lesioned hemisphere (p, 0.05), but not significantly different between young and aged in the unlesioned hemisphere. **Conclusions.** The findings show alpha-synuclein aggregation is excluded from CB-positive neurons and that the increased sensitivity of aged animals to lesion is not related to differential CB expression.

03w. Pathophysiology & Disease Mechanisms: calcium homeostasis

ADPD5-0726

CALCIUM TRIGGERS NUCLEATION OF ALPHA-SYNUCLEIN ON SYNAPTIC VESICLES AND THUS INCREASES THE VULNERABILITY OF DOPAMINERGIC NEURONES IN PARKINSON'S DISEASE

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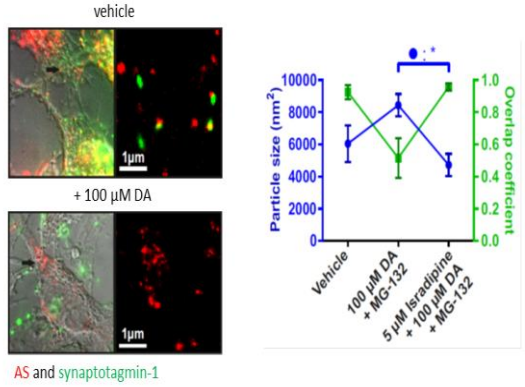
Objectives: It has been reported that alpha-synuclein (AS)-phospholipid interactions can enhance the formation of AS fibrils. However, AS-lipid interactions lead to the formation of an extended α-helix and thus the question remains as to how AS undergoes the structural conversion from an α-helical structure to a β-sheet-rich fibril.
Methods: We apply super-resolution microscopy on dopaminergic neurones to image AS aggregation on synaptic vesicles (see Fig.). Having performed NMR and other biophysical and biochemical measurements in combination with small unilamellar vesicles (SUV) that resemble synaptic vesicles we propose a model of AS function/dysfunction at the presynaptic terminal.
Results: We show that Ca^{2+} can enhance endogenous AS aggregation on synaptic vesicles in a PD related model system which can be prevented by treating the cells with the Ca^{2+} channel inhibitor isradipine (see Fig.). We give evidence that Ca^{2+} can influence AS-phospholipid interactions and thus provide a link through which a conversion from an α-helical to a β-sheet-rich state may become feasible. However, preventing aggregation does not prevent neuronal death and thus we show that other factors such as mitochondrial stress and free radical production are more causative of cell death.
Conclusions: Controlling Ca^{2+} homeostasis by isradipine treatment and providing patients suffering from PD with anti-oxidants may result in a better neuroprotective strategy than preventing AS fibril formation.

Fig. DA induced AS aggregation is Ca2+ dependent and initiated on synaptic vesicles.



03w. Pathophysiology & Disease Mechanisms: calcium homeostasis

ADPD5-0798

DO L-TYPE CALCIUM CHANNEL CAV1.3 AND ITS SPLICE VARIANT PLAY A ROLE IN SELECTIVE DEGENERATION OF DOPAMINERGIC NEURONS SEEN IN PD?

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Objectives - Dopaminergic neurons of substantia nigra pars compacta (SNpc) show selective vulnerability to neurodegeneration in PD. Influx of calcium during pacemaking in SNpc through L-type calcium channel Cav1.3 has been proposed to lead to neurodegeneration through increased intracellular calcium. Cav1.3-42A, an alternatively spliced short variant of Cav1.3-42 opens during pacemaking leading to increased calcium entry and has been implicated in PD pathogenesis. Our study examines expression of Cav1.3 in SNpc in mouse and human autopsy samples from controls and PD patients.

Methods – Brain regions from C57BL/6J mice and human samples from patients with PD confirmed at autopsy and matched controls (brain bank, ICM, Paris) were used in the study. qRT-PCR, in situ hybridization and immuno-histochemistry were used for quantitation and localization.

Results – In mouse SNpc and VTA Cav1.3-42A was expressed in greater amounts compared to cortex and co-localized with tyrosine hydroxylase (TH) positive neurons. However, in human autopsy brains, the expression levels of Cav1.3-42 and 42A were similar across brain regions. Further, no significant difference was observed in expression levels of Cav1.3-42 and 42A between controls and PD patients across midbrain regions even upon normalization with dopamine active transporter (DAT) to account for loss of TH positive neurons.

Conclusions – Our results indicate that there is no difference in the expression of Cav1.3 42 or 42A between midbrain and cortex and across midbrain regions from PD brains, making it unlikely for Cav1.3 to confer selective vulnerability to SNpc in PD.

03w. Pathophysiology & Disease Mechanisms: calcium homeostasis

ADPD5-2163

STRONG CAUSAL RELATIONSHIP BETWEEN THE STORE-OPERATED CALCIUM ENTRY (SOCE), INTRACELLULAR CALCIUM STORES AND ER STRESS (UPR) IN DOPAMINERGIC NEURONS.

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Store-operated Ca^{2+} entry (SOCE) is essential for the refilling of intracellular Ca^{2+} stores, but its relation to endoplasmic reticulum (ER) stress, and its role in degeneration of dopaminergic (DA) neurons is poorly understood. In this study, human DA neuronal SH-SY5Y cell line was used to test the role of SOCE in the depletion of Ca^{2+} stores, ER stress, unfolded protein response (UPR), and cell viability, which may determine whether DA neurons will live or die. We found that the main components of SOCE (Orai1, STIM1 and PLA2g6) are present and fully functional in DA neurons, and thapsigargin (TG)-induced depletion of ER stores results in Orai1/STIM1 co-localization and activation of SOCE. Inhibition of SOCE causes depletion of Ca^{2+} in ER and significant ER stress (UPR). Dose-dependence of TG effects revealed that ER Ca^{2+} depletion precedes ER stress and loss of cell viability. More than 85% of Ca^{2+} need to be lost from ER to initiate UPR (CHOP expression), and lead to cell death. TG-induced depletion of the stores (rather than activation of SOCE) is the cause of UPR, and it can be rescued by molecular up-regulation of SOCE (Orai1 overexpression). Thus, there is a strong causal relationship between Orai1-mediated SOCE, ER Ca^{2+} stores, ER stress/UPR and cell viability in the model DA neurons. Our results suggest that Orai1 and SOCE-dependent refilling of Ca^{2+} in the ER is essential for the prevention of ER stress, and impairment of SOCE may be a trigger for DA neuronal death.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-0552

GABA TRANSMISSION REGULATES ALPHA-SYNUCLEIN SECRETION FROM THE GLUTAMATERGIC TERMINALS IN MOUSE STRIATUM.

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Recent studies have suggested that pathologically relevant species of alpha-synuclein (AS) can be propagated between neurons in a prion-like manner. Due to this evidence, extracellular AS is considered the most promising target to hinder disease progression in PD. We have previously shown that AS is readily released in human and mouse brain parenchyma, even though the physiological role of the secreted AS has not been yet elucidated. Given the importance of extracellular AS levels, we aimed to investigate the mechanism of AS secretion *in vivo*. In mouse striatum, we have found that AS is exclusively localized to the glutamatergic terminals. However, absence of AS from the lumen of brain-isolated synaptic vesicles suggested that they are unlikely to mediate its release. To dissect the mechanism of AS release, we have used reverse microdialysis to locally administer reagents that target specific cellular pathways. Using this approach, we have shown that AS secretion is a calcium-regulated process that also dependents on the activation of SUR1-sensitive K_{ATP} channels. SUR1 is distributed in the cytoplasm of GABAergic neurons from where the K_{ATP} channel regulates GABA release. Using a combination of specific agonists and antagonists, we were able to show that, in the striatum, GABA release via the SUR1-KATP channels on the GABAergic neurons could regulate AS secretion from glutamatergic terminals. Importantly, this mechanism is mediated by the activation of the presynaptic $GABA_B$ receptors on the glutamatergic terminals. We believe that understanding the mechanism of AS secretion will help us identify new therapeutic targets for PD.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-0751

STRIATAL CHOLINERGIC INTERNEURONS CONTROL MOTOR FUNCTION AND BASAL GANGLIA OUTFLOW IN EXPERIMENTAL PARKINSONISM

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Striatal cholinergic interneurons (CINs) are considered as key players in basal ganglia-related functions and pathologies. Their contribution to motor dysfunction in Parkinson's disease (PD), although supported by the symptomatic relief provided by anticholinergic drugs, remains elusive. Here, we used an optogenetic approach to address the implication of CINs in the normal and pathological functioning of the basal ganglia network. Using a behavioral approach, we demonstrated that CINs inhibition alleviates haloperidol-induced catalepsy and motor asymmetry in the unilateral 6-hydroxydopamine-induced lesion model. As possible cellular substrates for this antiparkinsonian effect, in vivo electrophysiological recordings reveal that CINs inhibition normalizes the burst activity in the main basal ganglia output structure, the substantia nigra pars reticulata, and strengthens cortical information transfer through the direct striatonigral pathway. Slice electrophysiological recordings demonstrate that, in PD state, CINs become hyperexcitable and their photoinhibition potentiates cortical synaptic transmission onto striatal medium spiny neurons. CINs inhibition in non-lesioned mice had no impact on the behavioral and physiological parameters examined, suggesting that the role of these interneurons in motor function is highly dependent on dopamine tone. Our findings, without excluding indirect pathway contributions to PD, point to the CINs modulation of cortical information processing through the direct pathway as a critical component involved in the control of basal ganglia outflow and motor dysfunction in PD state. We are currently investigating whether thalamic inputs, which provide another major source of excitatory drive to the striatum, are also modulated by CINs modulation in control and PD states.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-1399

DOPAMINE SYNTHESIS BY NON-DOPAMINERGIC NEURONS IN THE STRIATUM OF PARKINSONIAN MICE

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Objectives were to determine whether: (i) striata of parkinsonian and control mice contain neurons expressing the tyrosine hydroxylase (TH) gene and possessing TH only or aromatic L-amino acid decarboxylase (AADC) only, (ii) presumptive monoenzymatic TH-neurons and AADC-neurons synthesize dopamine (DA) due to L-DOPA transfer from the former to the latter.
The study was performed in C57BL/6 mice at the presymptomatic and early symptomatic stages of parkinsonism induced by 2-fold and 4-fold s.c. injections of MPTP, respectively, at the individual dose of 12 mg/kg. Striatal neurons were immunostained for TH and AADC, TH mRNA was measured with PCR RT. Our hypothesis about DA synthesis in the monoenzymatic neurons was tested by incubating slices with L-leucine which inhibits L-DOPA membrane transporter.
Results:
1. The axons containing both enzymes, TH only or AADC only, were observed in striata of MPTP-treated and control mice in the proportion (i) 55:22:23 in the control, (ii) 34:44:22 at the presymptomatic stage, (iii) 29:47:24 at the symptomatic stage showing an increase of the fraction of TH axons in parkinsonian mice.
2. Total DA amount in slices and incubation medium dropped under L-leucine administration proving DA synthesis by monoenzymatic axons.
3. TH gene expression was observed in the striatum at the symptomatic stage though no TH-immunostainig was observed in cell bodies.
Thus, DA is synthesized in the striatum of intact and parkinsonian mice by bienzymatic and monoenzymatic axons.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-1876
EARLY DYSFUNCTIONS PRECEDING NEURODEGENERATION IN ALPHA-SYNUCLEINOPATHY

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Objective: Adenoviral vector (AAV) mediated overexpression of human wild-type alpha-synuclein (alpha-syn) in the substantia nigra (SN) of normal animals has been shown to lead to a time-dependent DA neuronal loss and motor symptoms in rats, mimicking the progression of Parkinson's disease (PD) neuropathological cascade. Mild cognitive impairment is knwon to precede motor symptoms and dementia in PD, and therefore is considered as an early clinical marker of the pathology. In this study we explored the mechanisms through which alpha-syn accumulation leads to the development of dementia in PD.

Methods: AAV-mediated overexpression of human wild-type alpha-syn was induced in different brain regions in adult naive mice, and compared with AVV-GFP injected control. Animals were subjected to behavioral tasks at different time points after the injection, and the brain processed to detect neurodegeneration.

Results: We identified selective memory impairments, which were specifically related to the neuronal dysfunctions of the targeted regions. The cognitive defects observed, however, were not associated to neurodegeneration.

Conclusions: These findings provide *in vivo* evidence showing that cognitive impairment precedes alpha-syn overexpression-mediated neurodegeneration. They suggest the interesting hypothesis that alpha-syn overexpression might be the cause not only of dementia and motor symptoms occurring in the end-stage stage of PD, but also of cognitive impairment occurring in the very early stage of the disease. Supported by a grant from the Fondazione con il Sud (2011-PDR-13).

03y. Pathophysiology & Disease Mechanisms: aging

ADPD5-1959

THE EFFECTS OF CLINICAL MOTOR VARIABLES AND MEDICATION DOSAGE ON WORKING MEMORY IN PARKINSON'S DISEASE

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Objectives: We investigate the relationship between disease duration, dopaminergic medication dosage, and motor disability (UPDRS score) with WM in PD.
Methods: We recruited three groups: unmedicated, medicated PD patients, and healthy controls. All subjects were tested on three WM tasks: short-delay WM, long-delay WM, and the n-back task. Further, PD encompasses a spectrum that can be classified either into akinesia/rigidity or resting tremor as the predominant motor presentation of the disease. In addition to studying medication effects, we tested WM performance in tremor-dominant and akinesia-dominant patients. We further correlated WM performance with disease duration and medication dosage.
Results: No difference between medicated and unmedicated patients in the short-delay WM task, but medicated patients outperformed in the long-delay WM and n-back tasks. Interestingly, we also found that akinesia-dominant patients were more impaired than tremor-dominant patients at various WM measures, which is in agreement with prior studies of the relationship between akinesia symptom and basal ganglia dysfunction. Moreover, the results show that disease duration inversely correlates with more demanding WM tasks (long-delay WM and n-back tasks), but medication dosage positively correlates with demanding WM performance.
Conclusions: Our results show that WM impairment in PD patients depend on cognitive domain (simple vs. demanding WM task), subtype of PD patients (tremor- vs. akinesia-dominant), as well as disease duration and medication dosage. Our results have implications for the interrelationship between motor and cognitive processes in PD, and for understanding the role of cognitive training in treating motor symptoms in PD.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-0547

THE P300 AND DUAL TASK STUDIES REFLECT DEFICITS IN THE ALLOCATION OF ATTENTIONAL RESOURCES IN PATIENTS WITH PARKINSON'S DISEASE

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Objectives: The aim of this study is to investigate the attentional deficits of PD patients by measuring the P300 using the dual task platform, and considered the effects of performing dual tasks on their daily activities.
Methods: Eighteen PD patients without dementia, and twenty age-matched healthy controls were included in this study. The cognitive functions of all subjects were assessed using the MMSE, HDS-R, TMT part-A, and FAB. Daily experiences reflecting attention were interviewed. Auditory and visual odd-ball tasks were used for the ERP recordings. All subjects executed three single tasks and a dual task. The behavioral indicators included the response time (RT) for auditory tasks and percentage of correct answers for each task.
Results: The duration of time required for TMT performance was significantly prolonged in the PD patients as compared with that of the control subjects. PD patients showed more prolonged latencies and larger amplitudes of the P300 in all tasks, a delayed RT for the auditory task, a high error rate in performing all tasks, and more difficulties in performing dual tasks in daily activities compared with the control subjects.
Conclusions: Prolonged latencies of the P300 reflect slow cognitive processing. PD patients needed larger attentional resources to execute the tasks, and their capacity for attentional resources was smaller than that of the control subjects. As a result, the PD patients showed the performance errors and difficulties in performing daily activities. These results indicate that deficits in the allocation of attentional resources are present in the PD patients.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-0638

TWO AUTOPSY CASES OF DLB PRESENTING PROLONGED REFRACTORY DELIRIUM IN CLINICAL COURSE

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(Background) In the diagnosis criteria of DLB, the fluctuating cognition with pronounced variations in attention and alertness has been listed as one of core features. Sever conscious level down would be shifted to delirium state. In this study, we discussed about the etiology of consciousness level clinico-neuropathologically from two autopsy cases of DLB presenting the prolonged delirium. **(Method)** Investigate for two autopsy cases of DLB. (Case 1) Ninety one years old female. At age of 89y.o., she admitted the internal medicine due to dysfunction of hart. Until then, she has been healthy mentally and physically. Since first admitted day, she showed delirium state with psychomotor excitement. After three months treatment, her physical condition had recovered, but the delirium was observed intermissive. The delirium symptoms was gradually worsen and died at 90 y.o. due to pneumonia. (Case 2) Seventy four years old male. At age of 71 y.o., he was pointed out the impairment of memory and sometimes presented the visual hallucination. Gradually, the sever fluctuation of consciousness level was constantly observed and died at 74 y.o. due to pneumonia. (**Result)** Neuropathological diagnosis as follows: (Case1) 1) Lewy body disease, 2) Argyrophilic grain disases, (Case2) 1) Lewy body disease, 2) tauopathy. In common, neuropathological findings were observed in brainstem including dorsal nucleus of vagus or locus coeruleus.**(Conclusion)** Patients presenting the prolonged delirium in the clinical settings may be included DLB, especially may be having the pathology in the brain stem that concerning the level of consciousness.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-0646

THE REGULATION OF ASIC1A ON DOPAMINE RELEASE AND ITS IMPLICATION IN THE PATHOGENESIS OF PARKINSON'S DISEASE

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Objective: To study the distribution of acid-sensing ion channel 1a (ASIC1a) in dopaminergic neurons and its role in regulating dopamine release, and to explore its pathogenic role in Parkinsons disease development.
Materials and Methods: Immunofluorescence and Confocal(CLSM) were used to observe the distribution of ASICs in MES 23.5 cells; whole cell patch clamp technique was used to test the electronic properties of ASIC1a current; High Performance Liquid Chromatography (HPLC) was used to determine DA level; and Western blot was performed to study the expression of ASIC1a, pCaMKII proteins.
Results: ASIC1a co-localized to TH-postive in MES23.5 cell. Inhibitors of ASICs Amiloride and PcTx1 blocked the acid-induced current in a dose-dependent manner; the current density of ASICs increased after MPP+ (100uM) incubation for 1h, this was attenuated by PcTx1 (10nM, $P<0.05$). Compared to control group (PH7.4), extracellular application with pH6.0 solution induced a significant increase of DA release ($P<0.05$), which was diminished by PcTx1 pretreatment; simultaneously, PcTx1 blocked the DA release caused by MPP+ (100uM). Moreover, the intracellular calcium level increased after MPP+ application, and this was blocked by PcTx1. Pretreatment of KN-93 could reverse the ASICs current increase induced by MPP+; In addition, we observed the level of ASIC1 and pCaMKII proteins increased after MPP+ treatment for 24h ($P<0.05$).
Conclusion: Functional ASIC1a expresses on dopaminergic cells, and intracellular DA may be exhausted by MPP+ due to over activation of ASIC1a through Ca2+-CaMKII pathway. ASIC1 channel may represent as a target for regulating DA release and thereby protection against DA neuron losses.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1960

FACTORS UNDERLYING PROBABILISTIC AND DETERMINISTIC STIMULUS-RESPONSE LEARNING PERFORMANCE IN MEDICATED AND UNMEDICATED PATIENTS WITH PARKINSON'S DISEASE

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Objective: Prior studies have not tested individual differences or effects of medication dosage on stimulus-response learning in patients with Parkinson's disease (PD). In the current study, we investigated the effects of motor variables (including tremor, akinesia, and disease duration) as well as dopaminergic medication dosage on learning in unmedicated PD patients, medicated PD patients, and healthy controls. Method: We tested all subjects on probabilistic and deterministic learning tasks, and also collected awareness measures data using postexperimental questionnaires. Importantly, we tested learning performance in tremor-dominant and akinesia-dominant PD patients, and further correlated learning performance with disease duration and medication dosage. Results: Results show that akinesia-dominant patients were more impaired than tremor-dominant patients at probabilistic rewardbut not punishment-based learning, which is in agreement with prior studies of the relationship between akinesia and basal ganglia dysfunction. We also found no difference between medicated and unmedicated PD patients in reward- or punishment-based deterministic learning, but medicated patients were better than unmedicated patients at reward-based probabilistic learning. Our results show that awareness measures explain differences among probabilistic and deterministic learning performance. Moreover, we found that disease duration and motor severity are inversely correlated, and medication dosage is positively correlated, with reward-based probabilistic learning. Conclusion: Our results suggest that stimulus-response learning performance in patients with PD depends on the type of learning (probabilistic vs. deterministic), medication status (on vs. off medication, dopaminergic medication dosage), disease duration as well as motor severity and subtype in PD patients (tremor- vs. akinesia-dominant).

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-2165

IMPAIRMENT OF PARK14-DEPENDENT CALCIUM SIGNALING IS A NOVEL TRIGGER FOR AUTOPHAGIC DYSFUNCTION AND PARKINSON'S DISEASE

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The origins of age-dependent Parkinson's disease (PD) remain enigmatic despite recent success in identification of some genetic triggers (PARKs) and several major hallmarks of this incurable neurodegenerative disease. In search for the new keys to this complex process, we focused on a poorly understood PARK14 (*Pla2g6* gene), and discovered a previously unknown sequence of pathological events that can lead to age-dependent PD. Here we present the results of integrated genetic, molecular, biochemical, imaging, live cells and live animals studies. We demonstrate that a human PD-associated mutations, or truncation of the N terminus of PARK14 (PLA2g6) results in impairment of the store-operated Ca^{2+} entry and sustained depletion of intracellular Ca^{2+} stores, which we found to be a novel trigger for autophagic dysfunction, progressive loss of dopaminergic (DA) neurons in substantia nigra pars compacta (SNc), and age-dependent L-DOPA-sensitive PD-like motor dysfunction in a new PLA2g6 $ex2^{KO}$ mouse model. Discovery of a novel Ca^{2+} signaling pathway leading to DA neuronal death, and creation of a mouse model that mimics human age-dependent PD, opens unique opportunities for unveiling new mechanistic details of this multifaceted process.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-1145

ALPHA-SYNUCLEIN PROTOFIBRIL-SELECTIVE ANTIBODY PREVENTS MOTOR RELATED DEATH IN ALPHA-SYNUCLEIN A30P TG MICE

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Background: Large soluble alpha-synuclein oligomers (protofibrils) have been shown to have pronounced neurotoxic properties. Such species are therefore attractive therapeutic targets for disease modification in Parkinson's disease and dementia with Lewy bodies, where alpha-synuclein deposition is believed to be a central pathogenic hallmark. Several mechanisms for disease modification using immunotherapy have been suggested.

Objectives: To investigate whether treatment with a protofibril-selective antibody can prolong survival in h[A30P]alphaSYN tg mice and to evaluate possible mechanisms of action of a protofibril-selective antibody using *in vitro* assays.

Methods: Starting at 12 months of age, h[A30P]alphaSYN tg mice were administered bi-weekly i.p. injections (10 mg/kg) of the protofibril-selective antibody mAb47. The occurrence of motor symptoms and survival was recorded during the study until termination of animals for ethical reasons.

The ability of mAb47 to mediate protofibrillar uptake in microglia was investigated using BV-2 cells. In addition, the ability to block cell-to-cell spreading of alpha-synuclein protofibrils was studied in H4 neuroglioma cells in the presence of mAb47.

Results: The survival of mAb47-treated h[A30P]alphaSYN tg mice was significantly prolonged compared to placebo treated animals. Uptake of alpha-synuclein protofibrils in BV-2 microglia cells was increased in the presence of mAb47 and uptake was abrogated when blocking Fc-gamma receptors. Also, the cell-to-cell spreading of alpha-synuclein protofibrils was reduced after mAb47 treatment.

Conclusions: Treatment with the alpha-synuclein protofibril-selective antibody mAb47 prevented motor related death and increased overall survival of h[A30P]alphaSYN tg mice. Also, mAb47 enhanced uptake in a microglial cell line and decreased cell-to-cell spreading of alpha-synuclein protofibrils.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-1245

CHARACTERISATION OF THE CELLULAR UPTAKE OF ALPHA-SYNUCLEIN OLIGOMER/PROTOFIBRIL SELECTIVE ANTIBODIES

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Objectives

Immunotherapy targeting aggregated alpha-synuclein has emerged as a potential therapeutic strategy against Parkinson's disease and other alpha-synucleinopathies. We have developed alpha-synuclein oligomer/protofibril selective antibodies and demonstrated that they can lower levels of toxic alpha-synuclein in human cell lines and, upon intraperitoneal administration, in spinal cord of transgenic mice. However, it is not known whether they exert their effects intra- or extracellularly. Here, we have further evaluated treatment related mechanisms with emphasis on whether the antibodies are internalised by cultured cells.

Methods

Wild type or alpha-synuclein-BiFC overexpressing H4 human neuroglioma cells were treated with a set of monoclonal oligomer/protofibril selective alpha-synuclein antibodies, linear epitope monoclonal alpha-synuclein antibodies or with an irrelevant isotype control. Cells were incubated with antibody for up to 24h in regular growth media at 37°C. Presence of treatment antibody was analysed with immunofluorescence. Antibody levels in conditioned media were measured by sandwich ELISA.

Results

The alpha-synuclein oligomer/protofibril selective antibody mAb47 showed the highest degree of intracellular presence, which reached its maximum after four hours of incubation. The other antibodies displayed different degrees of uptake. Induction of oligomers with the BiFC system led to an increased uptake of alpha-synuclein antibodies, partly overlapping with the localisation of the oligomers. Along with the cellular uptake the extracellular antibody levels decreased continuously, as measured by ELISA.

Conclusions

The oligomer/protofibril selective alpha-synuclein antibody mAb47 was readily internalised by cells in culture, especially if oligomer formation was induced. Ongoing studies will elucidate by which mechanisms the antibodies are internalised by the cells.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-2219

ACTIVE IMMUNIZATION AGAINST ALPHA-SYNUCLEIN AMELIORATES DEGENERATIVE PATHOLOGY AND PREVENTS DEMYELINATION IN A MSA MODEL

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Objective: MSA is a neurodegenerative disease characterized by parkinsonism, ataxia and dysautonomia. Histopathologically, hallmarks of MSA include abnormal α-syn accumulation within oligodendroglia, leading to neuroinflammation, demyelination and neuronal death. To date, no disease-modifying treatment modalities for α-synucleinopathies, including PD and MSA, are known. Recent studies suggest that immunotherapy increases α-syn clearance and might constitute a viable synucleinopathy therapy. The main objective of this study was to evaluate effects of active vaccination using novel AFFITOPE®-vaccines on reducing MSA-like pathology in the MBP-α-syn transgenic mouse model.

Methods: AFFiRiS has developed novel active immunogens (AFFITOPE®-vaccines) for treating synucleinopathies by targeting α-syn. AFFITOPEs® are short immunogenic peptides that don't carry the native α-syn-epitope but rather a sequence mimicking the original α-syn-epitope. AFFITOPE®-peptides, when conjugated to KLH and adjuvanted with aluminum, induce long-term, sustained, α-syn-specific antibody responses suitable for synucleinopathy treatment. We have previously shown that active AFFITOPE®-vaccination technology was effectively reducing behavioral deficits, α-syn accumulation and inflammation in two synucleinopathy-models.

Results: We used AFFITOPE®-vaccines for immunizing MBP-α-syn mice, a MSA model expressing α-syn in oligodendrocytes. AFFITOPE®-vaccination resulted in production of specific anti-α-syn antibodies that crossed into the central nervous system and recognized α-syn aggregates within glial cells. AFFITOPE®-vaccination resulted in decreased accumulation of α-syn, reduced demyelination in neocortex, striatum and corpus callosum, and reduced neurodegeneration. Clearance of α-syn involved activation of microglia and reduced spreading of α-syn to astroglia.

Conclusions: Results on AFFITOPE®-vaccination in different synucleinopathy-models indicate a potential disease-modifying effect, which is currently tested within a Phase I study in MSA-patients.

04d. Therapeutic Targets & Mechanisms for Treatment: alpha-synuclein

ADPD5-0440
PROLYL OLIGOPEPTIDASE MODULATES ALPHA-SYNUCLEIN OLIGOMERIZATION VIA DIRECT PROTEIN-PROTEIN INTERACTION

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Objectives
Prolyl oligopeptidase (PREP) modulates alpha-synuclein aggregation *in vitro* and *in vivo*. Small-molecule PREP inhibitor KYP-2047 reduces alpha-synuclein aggregation in two A30P alpha-synuclein transgenic mouse strains. The aim of this study was to explore the mechanistic basis of PREP-based modulation of alpha-synuclein oligomerization.

Methods
We combined a live-cell alpha-synuclein dimerization assay, based on protein-fragment complementation assay (PCA), with a novel cell-free method, microscale thermophoresis (MST), to explore the effects of PREP on alpha-synuclein. PCA detects protein-protein interaction based on the reconstitution of reporter protein (Gaussia luciferease) fragments fused to proteins of interest (alpha-synuclein or PREP). MST is a novel cell-free technology analyzes interactions of biomolecules based on their movement in a microscopic temperature gradient.

Results
Our results demonstrate that PREP binds directly to alpha-synuclein and enhances its dimerization. Pharmacological inhibition of PREP reduces its self-oligomerization resulting in enhanced interaction of PREP and alpha-synuclein, and reduced alpha-synuclein dimerization.

Conclusions
These results suggest that via its self-oligomerization and direct interaction with alpha-synuclein, PREP acts as a seeding point for alpha-synuclein aggregation. Our results also provide a mechanism of action for KYP-2047, a PREP inhibitor previously shown to reduce alpha-synuclein aggregation *in vivo*. Taken together, our results support PREP inhibition as a potential new therapy for synucleinopathies. The novel combination of a live-cell method and a cell-free method complement each other, and provides a powerful platform for studying pathophysiologically relevant protein-protein interactions.

04d. Therapeutic Targets & Mechanisms for Treatment: alpha-synuclein

ADPD5-0682

ADIPONECTIN, THE ANTI-DIABETIC ADIPOKINE, HAS A THERAPEUTIC EFFECT IN MODELS OF ALPHA-SYNUCLEINOPATHIES.

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Objectives
The main objective of this study was to determine whether adiponectin (APN), a adipokine with anti-diabetic and anti-atherosclerosis properties, may protect against a-synucleinopathies, such as PD and dementia with Lewy bodies (DLB).
Methods
Biochemical and histological studies were carried out for various experimental systems, including autopsy brains of Parkinson's disease (PD) and dementia with Lewy bodies (DLB), cellular and transgenic-mouse models of α-synucleinopathies.
Results
First, the immunoreactivities of APN were detected in Lewy bodies derived from PD and DLB, suggesting the involvement of APN in the pathogenesis of α-synucleinopathies. Second, APN was protective in vitro; aggregation of α-synuclein (αS) in B103 neuroblastoma cells expressing αS, was significantly ameliorated by treatment with recombinant APN. In addition, APN was protective against various neurotoxicities (i.e. ER stress, mitochondrial toxicity and proteasome inhibition). Furthermore, phosphorylation levels of Akt and other molecules situated in insulin receptor signaling pathway were stimulated by APN in these cells. Finally, various neuropathological features in αS transgenic mice, including αS aggregation and movement disorder, were significantly ameliorated by nasal treatment of the mice with APN.
Conclusions
Our results suggest that APN may act as anti-neuropathogenic in the pathogenesis of α-synucleinopathies and that APN signaling pathway might be a therapeutic target of α-synucleinopathies.

04d. Therapeutic Targets & Mechanisms for Treatment: alpha-synuclein

ADPD5-1116

VPS35 PROTEIN ACTS ON ALPHA-SYNUCLEIN ACCUMULATION AND CLEARANCE IN NEURONS

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Parkinson's disease (PD) is the second most common neurodegenerative disorder and its prevalence is expected increase as our population ages. Recently, two distinct VPS35 mutations have been described in familial PD, the P316S mutation (Prol316Ser) and the missense mutation D620N (Asp 620 Asn). The VPS35 gene encodes for a subunit of the retromer system. Furthermore, VPS35 plays an important role in sorting proteins of the retrograde trafficking cargo in early endosomes to the trans-Golgi network. Defects in retrograde trafficking and endosomal dysfunction have been described in PD and related synucleophaties. In order to understand the role of the VPS35 protein in PD we investigated the effects of VPS35 modulations on α-synuclein uptake, accumulation and clearance in neurons, neuronal cell line and in α-synuclein transgenic mice. Our results suggest that manipulating VPS35 expression levels may induce abnormalities in retrograde transport and endosomal function causing accumulation in the neurons. Moreover, our results suggest that VPS35 influences α-synuclein degradation pathway and may affect aggregation. Together our results suggest an important role for VPS35 in the uptake, transport and degradation of α-synuclein and thus may represent a target for therapeutic strategies in PD. Moreover our studies reveal unexpected genetic and functional interactions between two genes associated to PD, VPS35 and EIF4G1, and we described a functionally connection of the genes to α-synuclein pathobiology in yeast, warms and mouse.

04d. Therapeutic Targets & Mechanisms for Treatment: alpha-synuclein

ADPD5-1149

DEVELOPMENT OF NOVEL SMALL MOLECULES AS DRUG CANDIDATES FOR PD

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Objective: design of novel disease modifying therapeutics for PD
Background: For PD and several other synucleinopathies, a pathological hallmark is the presence of alpha-synuclein (aSyn) deposits. Several studies suggest that synucleinopathies as well as other protein aggregation diseases share key molecular features, including aggregate toxicity. Previous studies from our group demonstrated that oligomeric toxicity is strongly influenced by the capacity of oligomers to grow, indicating that the aggregation process *per se* is a major contributor to the toxicity.
Methods: We set out to discover small molecule entities (SME) targeting both pathological aSyn oligomer formation, growth and toxicity. The Morphomer platform developed by AC Immune enables the generation of compounds targeting and modifying the beta-sheet conformation of aggregation-prone proteins and rendering them harmless.
Results: We generated interesting drug candidates with appropriate biophysical CNS properties that reduced the cytotoxicity of aSyn aggregates by a decrease of their beta-sheet content. We then confirmed that our lead compound was able to bind human Lewy bodies (LB) and assessed its affinity for aSyn. Our biological rationale was validated *in vivo* in two transgenic mice models (PDGF-aSyn (D-Line) and Thy1-aSyn (TNWT61)) with our lead, which was able to significantly reduce *in vivo* the formation of aSyn pathological structures in a dose dependant manner accompanied by improvement of neuronal marker (tyrosine hydroxylase) in the substantia nigra and rescuing of body weight loss linked to the disease model.
Conclusion: These findings will guide the design of novel disease modifying therapeutics for PD and other synucleopathies.

04d. Therapeutic Targets & Mechanisms for Treatment: alpha-synuclein

ADPD5-1667

PASSIVE IMMUNOTHERAPY AGAINST ALPHA-SYNUCLEIN REDUCES TRANS-SYNAPTIC ALPHA-SYNUCLEIN PROPAGATION AND AXONAL DEGENERATION IN A COMBINED VIRAL VECTOR AND TRANSGENIC MODEL OF SYNUCLEINOPATHY

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Background
Parkinson's Disease (PD) and Dementia with Lewy bodies (DLB) are characterized by progressive accumulation of alpha-synuclein (alpha-syn) in neurons. Recent studies have proposed that neuron-to-neuron transmission of alpha-syn plays a role in the pathogenesis of PD/DLB. Therefore, we explored the effect of passive immunization against alpha-syn in a new mouse model of trans-synaptic propagation.
Methods
For these experiments, non-transgenic (non-tg), alpha-syn knock-out and human alpha-syn tg (line 61) mice received unilateral intra-cerebral injections with a lentiviral (LV)-alpha-syn vector construct followed by systemic administration of control IgG or the monoclonal antibody 1H7 for 3 months. Cerebral alpha-syn accumulation and axonopathy was assessed by immunohistochemistry and effects on behavior by Morris water maze.
Results
Unilateral LV-alpha-syn injection resulted in axonal propagation of alpha-syn in the contra-lateral site with subsequent behavioral deficits and axonal degeneration. Passive immunization with 1H7 antibody reduced the trans-synaptic propagation, impairment in water maze behavior and axonal degeneration.
Conclusion
Together this study supports the notion that an antibody against alpha-syn is capable of reducing propagation of alpha-syn, thus ameliorating PD-like pathology and improving the associated deficits in a new alpha-syn transmission model. This work was funded by NIH grant AG18840 and Prothena Biosciences.

04d. Therapeutic Targets & Mechanisms for Treatment: alpha-synuclein

ADPD5-1690

REPURPOSING DRUGS FOR ANTI-ALPHA SYNUCLEIN AGGREGATION

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Objectives:
PD is a brain disorder characterized by the presence of alpha-synuclein (a -syn) aggregates forming intraneuronal inclusions known as Lewy bodies and Lewy neurites. Symptomatic treatments are available, but there are no disease-modifying therapies. Therefore, we aim to find compounds that inhibit the aggregation of a-syn and might halt the progression of PD.
Methods:
Recently, we published the a-syn protein misfolding cyclic amplification (PMCA), an *in vitro* system to produce a-syn fibrils in a fast, efficient and reproducible manner. We demonstrated that known anti-amyloid compounds inhibited the formation of a-syn aggregation by PMCA. We are now using the a-syn PMCA to screen previously used drug libraries to identify inhibitors of a-syn aggregation. Moreover, we are using cell assays to test the effects of the candidate drugs in culture.
Results:
Using a-syn PMCA as a high-throughput assay, we have screened hundreds of compounds and have found some that inhibit the aggregation of a-syn.
Conclusion:
Our results indicate that by using a non-biased assay such as the a-syn PMCA to screen for compounds with anti-a-syn aggregation properties, we might be able to find disease-modifying agents.

04d. Therapeutic Targets & Mechanisms for Treatment: alpha-synuclein

ADPD5-2183

IDENTIFICATION OF NOVEL ALPHA-SYNUCLEIN BINDING SMALL MOLECULES BY STRUCTURE-BASED VIRTUAL SCREENING

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1. Objectives: The aggregation of alpha-synuclein (alphaSyn) is linked to the onset and pathology of Parkinson's disease (PD). In a recent 3D structure of an 18 residue-long fragment of alphaSyn, the alphaSyn fragment monomer is stabilized into a beta-hairpin structure by beta-wrap proteins (Mirecka et. al., 2014, Angew.Chem.Int.Ed.Engl., 53, 4227). This fragment of alphaSyn covers a critical, fibril-forming part of alphaSyn and also contains multiple disease-related mutations. The availability of this experimental structure prompted us to apply structure-based virtual screening for the identification of novel alphaSyn ligands.

2. Methods: OpenEye programs were used to define small molecule binding sites in the alphaSyn structure (PDB ID: 4BXL), and to perform high throughput structure-based docking calculations. A small molecule CNS-like fragment library was prepared in Mcule. Stereoisomers, major tautomers and protomers were generated by ChemAxon. Calculations were run at the Hungarian supercomputing centre (NIIF Institute).

3. Results: Two possible small molecule binding sites were identified in close proximity of residues Tyr39 and His50. The generated CNS-like fragment small molecule library contained 22,521 fragments. From the docking calculations highest-ranked compounds with a favorable interaction profile were analyzed and 68 compounds were selected for experimental testing.

4. Conclusions: We have identified potential binders of alphaSyn *in silico* using structure-based virtual screening. These *in silico* hits can be potentially PET imaging agent candidates as well as hit candidates as alphaSyn aggregation inhibitors for PD therapeutics.

This work was supported by the Hungarian Brain Research Program -KTIA_NAP_13-2014-0009.

04d. Therapeutic Targets & Mechanisms for Treatment: alpha-synuclein

ADPD5-2248

MODELING PARKINSON'S DISEASE WITH INDUCED PLURIPOTENT STEM CELLS

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As a powerful platform for understanding of disease mechanism and consequent evaluation of disease-modifying therapies aimed at both familial and sporadic PD patients, we have recently generated ventral mesencefalic DA neurons (vmDAn) from idiopathic (ID) PD-iPSC lines, familial LRRK2-PD iPSC lines and control hiPS cell lines. With the aim to characterize PD-dependent neuronal alterations, we first differentiated control and PD-specific iPSC toward midbrain dopaminergic neurons following the protocol recently implemented in our laboratory that relies on lentiviral-mediated forced expression of the ventral midbrain determinant LMX1A, together with DA neuron patterning factors. All iPSC lines tested generate vmDAn, with the characteristics of A9 ventral midbrain DA neurons as revealed by expression of G protein-activated inward rectifier potassium channel 2 (GIRK2) and FOXA2. We then co-cultured neural progenitor cells -derived iPS over a monolayer of mouse embryo cortical astrocytes, which supported viable cultures of vmDA neurons for up to 75 days. Under these conditions, Ctrl-iPSC gave rise to vmDAn that were morphologically homogeneous and showed the expected features of mature vmDAn, including complex dendritic arborizations. However, DAn differentiated from ID-PD or LRRK2-PD iPSC developed over long-time culture a range of altered morphologies that were not evident at shorter time-points. When we measured the number of vmDAn and the length of neuritis, we found that both the number and length of neurites of Ctrl-iPSC-derived DAn were significantly higher than those of DAn differentiated from ID-PD or LRRK2 iPSC.
We have also observed an abnormal α-synuclein accumulation, and alterations in the autophagy machinery. We are now analyzing whether DAn degeneration in PD is truly a cell-autonomous phenomenon, or whether it is influenced by an altered cross-talk between DAn and glial cells, by exploring combinations of DAn and astrocytes differentiated from PD patient-specific and control iPSC. The generation of genuinely human experimental models of PD provides a platform that will disclose new opportunities not only for basic research but also for drug discovery and cell therapy in PD.

04g. Therapeutic Targets & Mechanisms for Treatment: kinases

ADPD5-0267

AN EARLY ENDOSOME REGULATOR, RAB5B, IS AN LRRK2 KINASE SUBSTRATE

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1. Objectives: Leucine-rich repeat kinase 2 (LRRK2) has been identified as a causative gene for Parkinson's disease (PD). LRRK2 contains a kinase and a GTPase domain, both of which provide critical intracellular signal-transduction functions. We showed previously that Rab5b, a small GTPase protein that regulates the motility and fusion of early endosomes, interacts with LRRK2 and co-regulates synaptic vesicle endocytosis. We investigated meaning of this interaction.

2. Methods: Using Rab5b recombinant proteins, we applied *in vitro* kinase assays with mass spectrophotometry analysis. GTPase, neurite outgrowth and EGFR degradation assays were used to test physiological meaning of Rab5 phosphorylation by LRRK2, using nonphosphorylatable or phosphomimetic mutant Rab5 recombinant proteins.

3. Results: We show here that LRRK2 phosphorylates Rab5b at its Thr6 residue. Phosphorylation of Rab5b by LRRK2 on the threonine residue was confirmed by Western analysis using animals or cells stably expressing LRRK2 G2019S. The phosphomimetic mutant T6D recombinant protein exhibited reduced GTP binding and increased GTPase activity compared to that of the wild type Rab5b protein, suggesting that LRRK2-mediated phosphorylation of Rab5b makes the Rab5b signal weaker than that of the unphosphorylated form. Neurite outgrowth analysis and epidermal growth factor receptor (EGFR) degradation assays showed that Rab5b T6D exhibited phenotypes that were expected to be observed in the inactive Rab5b, including longer neurite length, and less degradation of EGFR.

4. Conclusion: These results suggest that LRRK2 kinase phosphorylates Rab5b and its kinase activity functions as a Rab5b GTPase activating protein and thus, negatively regulates Rab5b signaling.

04g. Therapeutic Targets & Mechanisms for Treatment: kinases

ADPD5-0668

INHIBITION OF C-ABELSON TYROSINE KINASE (C-ABL) AS A POSSIBLE STRATEGY FOR TREATMENT OF PD: STUDY IN MPTP-INDUCED MICE MODEL

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Objective: c-Abelson tyrosine kinase (c-Abl) is known to phosphorylate Cdk5 at Tyr15 (Tyr15-Cdk5) and thereby facilitates the Cdk5 activity. We found that Cdk5 with Tyr15 phosphorylation (Cdk5-pTyr15) is enriched in the mouse striatum, where dopaminergic stimulation inhibited phosphorylation of Tyr15-Cdk5 by acting through the D2 class dopamine receptors. To see if c-Abl is involved in the pathophysiology of Parkinson's disease (PD) and inhibition of this kinase would be a possible strategy for PD treatment, we examined the effect of c-Abl inhibitors in 1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine (MPTP) mouse model of PD in this study.
Methods: Male C57Bl/6 mice were subjected of MPTP treatment to establish PD model. Inhibitors of c-Abl, imatinib or nilotinib, were administered by intraperitoneal injection with 25 mg/kg. After behavioral examinations assessing motor dysfunction, biochemical and immunohistochemical examinations were performed.
Results: Administration of c-Abl inhibitors normalized motor dysfunction induced by MPTP in mice. Both of the inhibitors reduced the MPTP-induced phosphorylation of Cdk5 protein at Tyr15 (Cdk5-pTyr15), together with its substrate DARPP32 phosphorylated at Thr75. Most of these effects were as same as those of L-dopa (15 mg/kg), showing the c-Abl inhibitors possess equivalent potential compare to other PD therapeutics. Our results suggested use of c-Abl inhibitors is a possible strategy for future PD treatment.
References: Morigaki et al., *Neuroscience* 189:25-31 (2011); Yamamura et al., *Front Cell Neurosci* 7:12 (2013); Tanabe et al., *Front Cell Neurosci* 8:50 (2014)

04i. Therapeutic Targets & Mechanisms for Treatment: other enzymes

ADPD5-2053

NEUROPROTECTIVE EFFECTS OF DELAYED START ADMINISTRATION OF THE CLASS III HDAC INHIBITOR NICOTINAMIDE IN THE LACTACYSTIN MODEL OF PARKINSON'S.

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Objective(s): Determine whether the class III pan-HDAC inhibitor nicotinamide is neuroprotective in the lactacystin model of Parkinson's using a delayed start treatment model.

Background: Epigenetic changes, both DNA methylation and histone modification, occur in the Parkinson's brain. It has been hypothesised that histone hypoacetylation through overactivity of histone deacetylases (HDAC's) leads to chromatin condensation and repression of gene expression leading to neuronal death. Hence, HDAC inhibitors (HDACI's) have been proposed as neuroprotective agents. Nicotinamide, a naturally occurring substance well tolerated by humans, a pan HDAC class III (Sirtuins) inhibitor and has been demonstrated to protect against MPTP toxicity when given as a pre-treatment which does not mirror the potential clinical setting.

Methods: 250mg/kg or 500mg/kg nicotinamide was administered i.p. for 28 days starting 7 days after the induction of a lactacystin lesion to the substantia nigra (SNc). Forepaw-use asymmetry, amphetamine induced rotations, MRI brain scans was assessed at baseline and weekly during the study. At the end of the study SNc dopaminergic neuronal counts were performed by tyrosine hydroxylase immunohistochemistry/stereological counting. RT-PCR was utilised to assess gene expression.

Results: Despite nicotinamide reversing the lactacystin induced histone hypoacetylation and stimulating gene expression of glial derived neurotrophic factor (GDNF) and the anti-apoptotic factor Bcl2, nicotinamide failed to induce any neuroprotective effects when assessed by MRI, behaviour or via SNc dopaminergic neuronal counts.

Conclusions: Unlike the pan-class I/IIa HDACI valproate the pan-class III HDACI nicotinamide is not neuroprotective in the lactacystin model of Parkinson's, suggesting pan-inhibition of Sirtuins is not neuroprotective.

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-1282

THE ANTI-INFLAMMATORY ACTIONS OF NORADRENERGIC AGENTS AS A TARGET TO PREVENT NEURODEGENERATION IN PARKINSON'S DISEASE

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Neuroinflammation is recognised as a key player in the progression of Parkinson's disease (PD) and interest is increasing in inflammatory models of PD including lipopolysaccharide (LPS)-induced degeneration of dopaminergic neurons in the substantia nigra (SN). In recent years there has been an additional focus on the degeneration of noradrenergic neurons. Degeneration within the locus coeruleus, the main noradrenergic cell body region within the midbrain, is evident in PD, and a loss of noradrenaline (NA) appears to exacerbate both the demise of DA neurons and the motor symptoms of PD. NA is potentially involved in an array of compensatory, anti-inflammatory and possibly neuroprotective mechanisms in PD. Moreover as a number of drugs that can increase noradrenergic function are already in clinical use for various conditions, the potential for targeting NA for treating the symptoms of PD is high. In the current investigation we have observed that a combination of treatment with the noradrenaline reuptake inhibitor atomoxetine and the alpha$_2$-adrenoceptor (AR) antagonist idazoxan, a combination which serves to enhance the extrasynaptic availability of noradrenline, exerts anti-inflammatory effects in the LPS model of PD. Functional deficits in motor function were partially rescued following treatment. Furthermore there was a complete restoration of nigral DA levels and reduced striatal DA depletion. In addition, IBA-1 immunohistochemical staining of microglia in the SN displayed a greater percentage of ramified processes in rodents treated with atomoxetine/idazoxan, compared with saline treated animals which displayed an amoeboid morphology, indicating the anti-inflammatory actions of NA leading or contributing ultimately to neuroprotection.

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-2090

DIFFERENTIAL OCCUPANCY OF RAT BRAIN 5-HT2A AND 5-HT2C RECEPTORS BY PIMAVANSERIN: RELEVANCE TO EFFICACY IN RAT MODELS OF ANTIPSYCHOTIC-LIKE ACTIVITY

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Pimavanserin is an inverse agonist of 5-hydroxytryptamine (5-HT) 2A receptors (5-HT_{2A}Rs), currently being investigated for Parkinson's disease-related psychosis (Hacksell et al., *Neurochem.Res.* **39**:2008, 2014). Its efficacy in animal models of antipsychotic-like activity can only partially be explained in terms of 5-HT_{2A}Rs inhibition. Since pimavanserin is also a 5-HT_{2C}R inverse agonist (Vanover et al., *JPET* **317**:910, 2006), in the present study its occupancies of 5-HT_{2A} and 5-HT_{2C} receptors were compared in parallel, at doses commonly used to reveal its antipsychotic properties. Adult, male, Wistar Han rats were treated with increasing doses of pimavanserin (0.1, 0.3, 1, 3 and 10 mg/kg, ip) and euthanized after 30 min. 5-HT_{2A}Rs and 5-HT_{2C}Rs occupancy was determined by means of *ex vivo* [^{3}H]MDL100907 and 5-HT_{2C}-selective [^{3}H]mesulergine autoradiography, respectively.

Pimavanserin dose-dependently occupied 5-HT_{2A}Rs, with a dose resulting in 50% of maximal occupancy (OD_{50}) at 0.11±0.01 mg/kg, corresponding to a pimavanserin concentration of 0.53±0.28 ng/ml in plasma and 16.8±1.1 ng/g in the brain. For 5-HT_{2C}Rs, the occupancy was significantly lower, as revealed by an OD_{50} of 1.59±0.32 mg/kg, while its concentration in plasma and in the brain was 24.0±5.8 ng/ml and 276±55 ng/g, respectively.

In conclusion, we describe here the differential occupancy of pimavanserin at 5-HT_{2A}Rs and 5-HT_{2C}Rs for the first time. Our occupancy data correlate well with the respective affinities of the compound (5-HT_{2A}R>5-HT_{2C}R). The significant 5-HT_{2C}Rs occupancy by pimavanserin (at doses commonly used to reveal its antipsychotic properties) will be considered in the context of the pharmacokinetic/pharmacodynamic relationship in our own behavioral data and existing literature.

04k. Therapeutic Targets & Mechanisms for Treatment: nicotinic & other ionotropic receptors

ADPD5-1622

MODULATION OF SYNAPTIC GLUN2A-CONTAINING NMDA RECEPTORS IN L-DOPA INDUCED DYSKINESIA

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Objectives: In PD, an aberrant subunit composition of striatal NMDA receptors (NMDARs) play a key role in the regulation of the abnormal motor behavior observed in L-DOPA induced dyskinesia (LID). Research focused on identification of compounds able to reduce NMDAR activity. However, so far, clinical trials provided conflicting results. Accordingly, the identification of novel approaches to rescue the alterations of the glutamatergic synapses underlying LID has become an urgent need in the field.
Methods: We have evaluated a new approach to reduce synaptic GluN2A-containing NMDARs in L-DOPA treated dyskinetic animals. In particular, we have analysed the role of GluN2A binding with Rabphilin-3A (Rph3A), a novel GluN2A interacting protein, involved in NMDAR stabilization at synaptic membranes. Rph3A/GluN2A interaction and Rph3A synaptic levels have been analysed in L-DOPA-treated animals. A cell-permeable peptide corresponding to the GluN2A domain involved in the interaction with Rph3A (TAT2A40) has been administered in vivo to reduce the formation of Rph3A/GluN2A complex.
Results: L-DOPA treated dyskinetic animals are characterized by an increase level of Rph3A in the excitatory postsynaptic membranes and by an augmented formation of GluN2A/Rph3A complex, leading to an aberrant synaptic localization of GluN2A-containing NMDARs. The concomitant treatment with L-DOPA and TAT2A40 ameliorates significantly the dyskinetic behaviour in L-DOPA treated rats. In particular, a single injection with the TAT2A40 peptide induces a long-lasting (3 days) reduction of LIDs.
Conclusions: Modulation of GluN2A/Rph3A complex could represent an innovative strategy to reduce GluN2A-containing NMDARs in L-DOPA-treated dyskinetic animals and, consequently, the onset of LIDs.

04l. Therapeutic Targets & Mechanisms for Treatment: sigma-1, metabotropic, muscarinic and other GPCRs

ADPD5-2054

NEUROPROTECTIVE AND BEHAVIOURAL EFFECTS OF MGLUR8 AGONIST DCPG IN THE LACTACYSTIN AND LPS MODELS OF PARKINSON'S DISEASE:

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Objective: Ascertain the neuroprotective/behavioural effects of targeting metabotropic glutamate receptor subtype 8 (mGluR8) in the lactacystin and LPS rat model of Parkinson's utilising the agonist (*S*)-3,4-dicarboxyphenylglycine (DCPG).
Background: Excess glutamatergic signalling in the basal ganglia and neuroinflammation are features of neurodegenerative process in Parkinson's. Evidence suggests that pharmacological activation of the mGluR4 subtype can provide significant neuroprotection and reduce behavioural deficits in traditional toxin rodent models of Parkinson's by reducing neuronal excitability. However, it is not known whether targeting mGluR8 can trigger similar effects.
Methods: Rats were unilaterally lesioned with the proteasome inhibitor lactacystin or Lipopolysaccharides (LPS) in the substantia nigra (SNc).DCPG was administered systemically for 14 days starting 4 days after lactacystein lesioning and for 7 days starting the next day after LPS lesioning. Forelimb-use asymmetry tests and amphetamine-induced rotations were used to assess behavioural deficits, whilst immunohistochemistry and stereological quantification of SNc dopaminergic neurons and microglia was performed.
Results: DCPG treatment was associated with a small neuroprotective effect on the loss of dopaminergic neurons in both the lactactacystin (~10% neuroprotection) and LPS (~24% neuroprotection. This was associated with a small attenuation in forelimb-use asymmetry but not in amphetamine-induced rotations in both models. DCPG treatment was also associated with a modest but not significant reduction in SNc microglial activation in both models.
Conclusion: Although the mGluR8 agonist DCPG did induce neuroprotection in both animal models the degree of neuroprotection was too small to support the concept that targeting mGluR8 receptors are a worthwhile neuroprotective target for treating Parkinson's.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-0395

PARKINSON'S DISEASE: THERAPEUTIC TARGETING OF TOLL-LIKE RECEPTORS

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Microglia play opposing roles in Parkinson's disease (PD) pathogenesis: in early stage they exert neuroprotection mediated through alpha-synuclein (Sa) phagocytosis, while as the disease progresses, they fail in Sa clearance and induce neuroinflammation and neurodegeneration. Innate immunity plays important role both in Sa-clearance and inflammation Sa-mediated. Toll-like receptors (TLRs) promote Sa clearance in the early stage, while during the disease they result chronically activated by accumulated Sa and induce a pro-inflammatory cascade leading to neurodegenerative changes. This current knowledge could allow to select specific TLR immunomodulators, to treat PD, mainly blocking neuroinflammatory cascade.
Specific small TLR antagonists, perhaps able to cross the blood brain barrier could also inhibit TLRs expressed by microglia, then exerting their anti-inflammatory effects directly on CNS. In addition, some of these TLR modulators exhibit high affinity, have the potential to be given to patients as an inhaled drug, a skin patch or a pill. Recently, small interfering RNA-selective compounds have become more straightforward because of the significant progress made in predictive modeling for new therapeutic approaches.
Modulation of TLR expression with small molecules acting as TLR antagonists might represent an alternative approach in PD therapy, especially in the late state, in order to decrease the inflammatory response, slowing the progression of the desease. Moreover, these TLR-targeting drugs have fewer side effects and lower or no toxicity compared to drugs with non specific anti-inflammatory effect commonly used in PD treatment.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-1313

INHIBITION OF THE BRAIN INFLAMMATORY RESPONSE BY THE ANTIBACTERIAL DRUG DOXYCYCLINE

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Objectives: Our previous work showed that the antibacterial drug doxycycline (DOX) confers neuroprotection in a mouse model of Parkinson disease by inhibiting glial cell activation (Lazzarini et al, Glia, 2013). In the present work, we aimed to get further insights into the molecular mechanisms behind the anti-inflammatory effect of DOX.
Methods: To do that we used a model system of microglial cells in which the inflammatory process is triggered by low concentrations of the bacterial lipopolysaccharide (LPS).
Results: Our data show that DOX significantly inhibits the response of microglial cells to LPS treatment. In particular, we demonstrate that DOX diminishes the synthesis of two key pro-inflammatory cytokines, TNF-alpha and IL-1 beta. DOX treatment also leads to a reduction in the levels of reactive oxygen species and nitric oxide. The effects of DOX are associated to decreased phosphorylation levels of p38 and NF-kB p65, two master regulators of inflammation.
Conclusions: These results suggest that DOX besides its well-known antibacterial activity can also operate as an anti-inflammatory drug that may be useful for treating neurodegenerative diseases such as Parkinson disease.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-1485

PRETREATMENT WITH PHOSPHODIESTERASE INHIBITOR (IBUDILAST) PREVENTS THE NEUROINFLAMMATION AND AMELIORATES MOTOR DISTURBANCE IN THE MPTP MODEL OF PD

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Objective: To estimate a possible preventive effect of Ibudilast (IBD), a non-selective (3, 4, 10, 11) phosphodiesterase inhibitor in the MPTP model of Parkinson's disease.

Methods: 3 months-old male C57Bl/10Tar mice were treated IBD b.i.d. for 9 days with subcutaneous injections [0, 20, 30, 40 or 50 mg/kg], beginning 2 days prior to MPTP (60 mg/kg) intoxication. Locomotor activity was examined by the RotaRod performance test. Expression of mRNA cytokines (TNFα, IL-6, IL-1β) and neurotrophic factor (GDNF) in the striatum were examined by the Real Time RT-PCR method. Micro- and astroglia activation markers (Iba-1 and GFAP, respectively) were estimated by Western blot analysis. Tyrosine hydroxylase (TH) expression and dopamine (DA) metabolism were evaluated by Western blot analysis and high-performance liquid chromatography, respectively.

Results: Compared to control, IBD in the dose of 40 mg/kg significantly improved motor abilities in MPTP mice. In addition, IBD attenuated astroglial reactivity and decreased release of all investigated pro-inflammatory cytokines in the striatum in a dose-dependent manner. IBD (40 mg/kg) also promoted production of GDNF. However, IBD administration did not change DA metabolism and striatal TH expression and had no impact on microglial activity in MPTP-treated mice.

Conclusions: IBD may have preventive effects mostly as a glial cell attenuator and promoter of neurotrophic factor. However, long-term observation will be needed to confirm its potential role in the restoration of neuronal function and promotion of dopaminergic cell survival.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0273

THE EFFICACY AND SAFETY OF COENZYME Q10 IN PREVENTING THE PROGRESSION OF EARLY PD: A META-ANALYSIS

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Objectives: The objective of this study is to assess and summarize the available evidence on the efficacy and safety of Coenzyme Q10 administration in the prevention of the progression of early Parkinson's Disease.

Methodology: A Literature search in several databases was conducted for relevant randomized controlled trials on the use of Coenzyme Q10 in Parkinson's Disease. Three randomized controlled trials met the inclusion criteria. The efficacy of Coenzyme Q10 were measured using the total and the component scores of the Unified Parkinson Disease Rating Scale on follow-up. On the other hand, safety were measured using the withdrawal rate and the associated adverse reactions during the therapy of CoQ10. The Review Manager Software was utilized for the meta-analysis.

Results: Compared to Placebo, treatment of CoQ10 did not show any significant difference in the mean scores of the UPDRS mental and ADL scores. Interestingly, the UPDRS motor score showed a significant difference between Coenzyme Q10 and placebo, but no significant difference when a subgroup analysis between high-dose (-4.03 [-15.07-7.01], p-value= 0.47, I^2=67%, P for heterogeneity=0.08) and low-dose Coenzyme Q10 (0.53 [-0.89-1.94], p-value= 0.47, I^2=34%, P for heterogeneity=0.22) was done. Overall, there was no significant difference in the total UPDRS score (0.68 [-0.61-1.97], p-value= 0.30, I^2=0%, P for heterogeneity=0.70). The most common side effects of the use of Coenzyme Q10 are anxiety, back pain, headache, sore throat, nausea, dizziness and constipation.

Conclusions: Coenzyme Q10 did not show enough evidence to prove its efficacy in early PD, however, its safety can be secured.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-1673

NEUROPROTECTIVE ACTIVITIES OF NEPALESE AND NATIVE AMERICAN TRADITIONAL MEDICINE

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Parkinson's disease (PD) affects more than five million people worldwide, but there are no treatments to stop or slow the neurodegenerative process. With 80% of the world population using medicinal plants as a primary source of healthcare, the development of plant-based therapies would critically impact the lives of these patients. The objective of our research is to identify plant-based therapies that would be readily available to these populations but could also be developed into more modern drugs. Nepalese healers and local people as well as members of Native American tribes (Blackfeet and Lumbee) were interviewed to document their use of medicinal plants to treat symptoms related to PD. The neuroprotective activity of a selection of botanical extracts was assessed in cell culture models relevant to PD. Extracts with the greatest neuroprotective activity were further characterized in terms of mechanisms of action including activation of the Nrf2 antioxidant pathway in primary cortical astrocytes and primary midbrain cultures and rescue of mitochondrial function. Among the 50+ plants documented in our ethnopharmacological studies, we identified six promising candidates characterized by distinct chemical compositions. These botanical extracts alleviate dopaminergic neuron death and neurite loss elicited by rotenone and/or paraquat, two PD-related toxins. Furthermore, the extracts activate critical neuroprotective pathways such as the Nrf2 antioxidant response. Further mechanistic studies will provide a deeper understanding of the neuroprotective activities of these extracts and suggest strategies for designing herbal remedies to slow or stop PD progression.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-2050

POTENTIAL MOLECULAR MECHANISM OF BERBERISE VULGARIS EXTRACT, BERBERINE AND BERBERINE CHITOSAN NANOPARTICLES IN ALZHEIMER TREATMENT.

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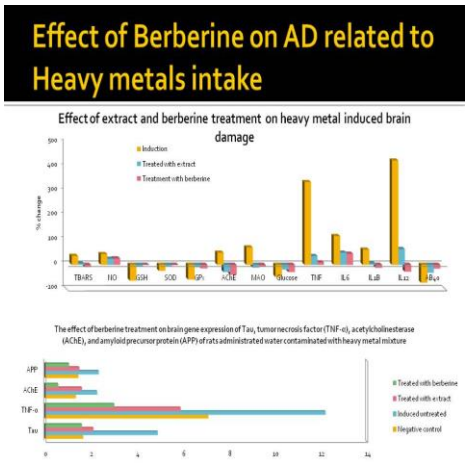
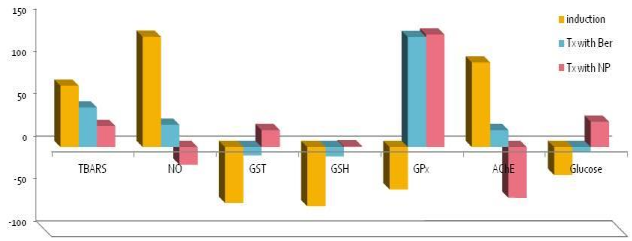
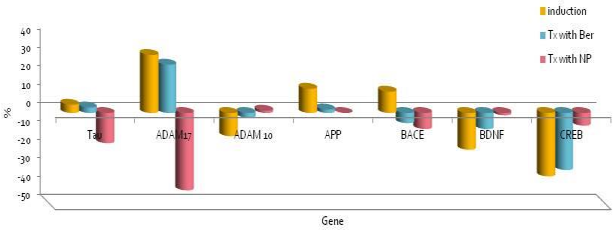
Major characteristics of Alzheimer's disease (AD) aresynaptic loss, cholinergic dysfunction, and abnormal protein depositions in thebrain in form of toxic non-soluble amyloids and hyperphosphorylated tau. Ourmain focus in the present study is to assess the therapeutic effect ofberberise vulgaris extract, berberine and berberine nanoparticle againstAD-like disease by tracking its effect on the oxidative stress-inflammatorypathway and MAPK pathway as well as AD hall markers. AD induced in rats by oraladministration of 5 ml of contaminant water for 3 months or insulin resistancefor one month. Then the treatment with tested compounds was carried out foranother one month. Firstly, we examined the berberine effect *in silico* and we found that berberine was potent anti-acetylcholine and inhibited bothTNF-alpha-converting enzyme and COX-2. Our biochemical and molecular parametersshowed that tested compounds inhibited the AChE and down-regulated itsexpression that could be returned its ability to act as potent antioxidants inbrain tissue. Matching with *-in silico* study, berberine normalized theproduction of TNF- alpha. IL12, IL 6 and IL 1β and ADAM 10 and ADAM 17. Finally,Berberine activated the production of APP-40 that acts as antioxidants forbrain tissue and inhibited the production of APP-42 fragment that responsiblefor Beta-amyloid plaques formation. Altogether, our data confirmed the use ofberberine as well as berberine nanoparticles, as drug candidate for AD likedisease treatment as berberine can lower Amyloid beta via multiple mechanisms



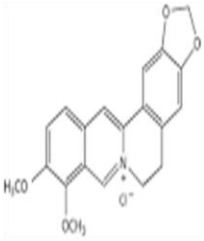
Effect of Berberine nanoparticles on AD treatment

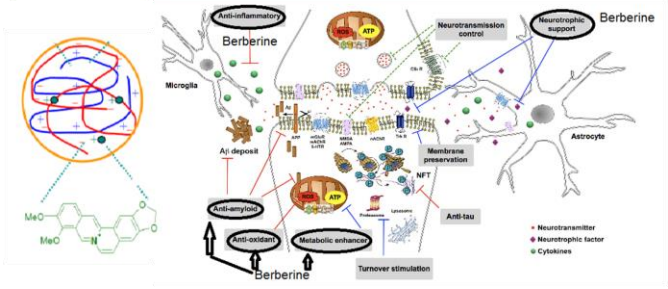


Effect of berberine or Ber-NP on relative gene expression in AD-like disease induced in animal



Neurotransmitter control	• Inhibits AChE
Neurotrophic factor	• Increases BDNF
Antioxidants	• Reduces ROS • Increases antioxidants
Anti-inflammatory	• Inhibits the production of TNF, ADAM, IL 1B, iNOS and COX
Metabolic enhancer	•Increases ATP, NADP
Neuron Plasticity	• increases CREB





04q. Therapeutic Targets & Mechanisms for Treatment: cell transplantation

ADPD5-0413

NEURO-PEPTIDE TREATMENT WITH CEREBROLYSIN ENHANCES THE SURVIVAL OF GRAFTED NEURAL STEM CELL IN THE THY1-ALPHA SYNUCLEIN TRANSGENIC MODEL OF PD

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Stem cell grafting has been investigated as a neuro-restorative therapy in Parkinson's Disease (PD) and other a-synucleinopathies but graft cell survival is hampered in a toxic neural environment where a-synuclein can propagate and trigger toxicity. Supplying neurotrophic factors to grafts has been proposed to augment survival. We investigated Cerebrolysin (CBL), a peptidergic mixture with neurotrophic-like properties, as an adjunct to stem cell therapy in the mThy1-a-synuclein tg model of PD.
Fetal mouse cortex-derived neural stem cells (NSC) were tagged with GFP and BrdU and injected into the striatum of non-tg and a-synuclein tg mice. Simultaneously, mice received saline or CBL (i.p.) until sacrifice.
Groups were assessed at 1 and 3 months post-grafting, Vehicle-treated a-synuclein tg mice displayed decreased NSC survival compared to vehicle-treated non-tg mice. Less than 5% of grafted NSCs in vehicle-treated a-synuclein tg mice were BrdU or GFP-positive. CBL enhanced NSC survival in both groups above baseline. Most surviving NSCs remained as neuroblasts and were DCX and PCNA-positive. Grafted NSCs in CBL-treated animals displayed lower caspase-3 activation and TUNEL-positive cells as compared to vehicle-treated a-synuclein tg mice. No tumor growth was detected. Levels of a-synuclein were similar in vehicle- and CBL-treated tg mice. Transfer of a-synuclein from host to graft cells was similar between vehicle- and CBL-treated groups.
CBL increases NSC survival in the a-synuclein environment. The mechanism may involve neurotrophic effects, as CBL did not affect total a-synuclein levels. This indicates that CBL can support graft cell survival and may support their therapeutic efficacy.

04q. Therapeutic Targets & Mechanisms for Treatment: cell transplantation

ADPD5-0690

INTRACAROTID INFUSION OF MESENCHYMAL STEM CELLS IN AN ANIMAL MODEL OF PARKINSON'S DISEASE: BEHAVIOURAL AND NEUROPROTECTIVE ASSESSMENT

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Mesenchymal stem cells (MSCs) have been proposed as a potential therapeutic tool for Parkinson's disease (PD). Systemic administration of MSCs has been tested in patients with degenerative parkinsonism. These studies reported slightly positive results, but left open questions regarding the actual capacity of MSCs to reach the brain and exert neuroprotective effects.

Objectives We infused autologous MSCs into the internal carotid of rats bearing a 6-hydroxydopamine-induced lesion of the nigrostriatal tract. The aim was to evaluate: 1) rat (r)MSC homing in the brain with or without transient blood-brain barrier (BBB) permeabilization, 2) symptomatic and neuroprotective efficacy of rMSC infusion.

Methods rMSCs were infused 14 days after 6-hydroxydopamine injection. Hyperosmolar solution of mannitol was used to transiently permeabilize the BBB. Behavioral impairment was assessed by stepping test and response to apomorphine. Seven and twenty-eight days after cell infusion, animals were sacrificed and the lesion in the striatum and substantia nigra was evaluated.

Results A noteworthy percentage of cells was found only within the brains of animals pre-treated with mannitol. Infused rMSCs showed a scattered localization. Cell infusion did not reduce the 6-hydroxydopamine-induced neurodegeneration or the motor impairment at the stepping test, but induced progressive normalization of the pathological (rotational) response to apomorphine.

Conclusions BBB permeabilization is required to grant passage of rMSCs into the brain. MSCs infusion does not modify the progression of PD-like neuronal damage, while it may affect behavioral responses associated with dopaminergic stimulation. These aspects should be further investigated before considering any translation into the clinical setting.

04q. Therapeutic Targets & Mechanisms for Treatment: cell transplantation

ADPD5-0832

COMBINING NEURONAL-PRIMED MESENCHYMAL STEM CELL AND GENE THERAPY IN HEMI-PARKINSONIAN RHESUS MONKEYS

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Objectives The treatment situation of PD is grim. Therefore, a treatment method which is safe but also fundamentally to restore the function of the nigrostriatal system and can greatly ease the treatment of their symptoms is in urgent needed.**Methods** We investigated a novel in vitro neuronal differentiation strategy with the use of LIM homeobox transcription factor 1, alpha (LMX1A) and Neurturin (NTN). Neuronal-primed ASCs and MSCs derived from rhesus monkey (rASCs), USCs derived from human were implanted into the striatum and substantia nigra of methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-lesioned hemi-parkinsonian rhesus monkeys. Monkeys were monitored with the use of behavioral tests and health measures until the fourth month after implantation.**Results** The differentiated cells derived from ASCs, MSCs and USCs transcribed and expressed a variety of dopaminergic neuron-specific genes. Compared with MSCs and ASCs, USCs are more easily differentiated into neuron like cells. The Neuronal-primed adult stem cells were transplanted into the brains of PD monkeys may play a therapeutic role to ameliorate the symptoms of PD. Single-photon emission computed tomography analysis and postmortem analysis revealed that the grafting of adult stem cells combined with rAd-NTN-TH had neuroprotective effects compared with rAd-NTN-TH or stem cells alone. **Conclusions** These findings may lead to cellular sources for autologous transplantation of Parkinson disease. Combined transplantation of gene vectors such as adenovirus with therapeutic genes and induced mesenchymal stem cells expressing inducible gene may be a better therapy candidate for the treatment of Parkinson’s disease.

04q. Therapeutic Targets & Mechanisms for Treatment: cell transplantation

ADPD5-1007

NEURAL STEM CELL TRANSPLANTATION RESCUES COGNITIVE AND MOTOR DYSFUNCTION IN ALPHA-SYNUCLEIN MICE BY ENHANCING BDNF, DOPAMINERGIC, GLUTAMATERGIC AND NEUROGENIC SYSTEMS

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Objectives: Stem cell transplantation for Parkinson's disease (PD) has primarily focused on dopamine replacement strategies. While this approach can improve PD-related motor deficits in both models and patients, it has achieved little to no impact on the cognitive aspects of the disease. Here, we explore the potential of neural stem cell (NSC) transplantation in an α-synuclein transgenic model of PD dementia (PDD) and dementia with Lewy bodies (DLB).

Methods: To examine the potential of NSC transplantation on α-synuclein induced cognitive and motor dysfunction, NSCs were transplanted into the striatum of 12-month old α-synuclein transgenic mice. To further investigate the role of neurotrophic support in NSC-mediated benefits, a line of $BDNF^{shNRA}$-NSCs was also developed and examined. One month following transplantation, performance in multiple motor and cognitive domains was assessed and pathology examined.

Results: Our findings reveal that transplantation of predominantly gliogenic NSCs into aged α-synuclein transgenic mice dramatically improves both cognitive and motor function. In addition, NSC-derived BDNF is critical for this process and changes in dopaminergic, glutamatergic, and neurogenic function differentially contribute to motor and cognitive recovery.

Conclusions: Considered together, our data show that striatal NSC transplants can dramatically improve both motor and cognitive function in a transgenic model of PDD/DLB by elevating levels of BDNF and enhancing dopaminergic, glutamatergic, and neurogenic systems. These studies demonstrate that transplantation of gliogenic NSCs could offer a promising new approach to treat both the motor and understudied yet devastating cognitive symptoms of synucleinopathies.

04q. Therapeutic Targets & Mechanisms for Treatment: cell transplantation

ADPD5-1026

HUMAN ADIPOSE-DERIVED STEM CELLS: POTENTIAL IN TREATMENT OF PD

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Treatment of PD using stem cells has focused by many researchers for a long time, but the ideal therapeutic strategy is not developed yet. And the consistency and high reliability of the experimental results verified by the animal models have been considered greatly important factors for the stability of human adipose-derived stem cells (hASCs), a kind of mesenchymal stem cells (MSCs) isolated from adipose tissue.
The aim of this study is to investigate preventive and therapeutic potential of hASCs for PD and is to identify the factors related to this therapeutic effect. Using the PD toxin models induced by 6-hydroxydopamine (6-OHDA), we demonstrate that the behavioral performances were significantly improved at 3 weeks after the intravenous injection of hASCs. Also, dopaminergic neurons were rescued and the increased structure-modified mitochondria in PD mice model were decreased by injection of hASCs. Furthermore, inhibited mitochondrial complex1 activity by 6-OHDA was restored in the brain of hASCs injected PD mice model. Taken together, our findings suggest that intravenous injection of hASCs may have a therapeutic potential for PD by recovering of mitochondrial dysfunctions.

04q. Therapeutic Targets & Mechanisms for Treatment: cell transplantation

ADPD5-1507

TRANSPLANTATION OF HUMAN NEURAL STEM CELLS IN IMMUNODEFICIENT ALPHA-SYNUCLEIN TRANSGENIC MICE: EXAMINING THE ROLE OF NEUROTROPHIC SIGNALING IN COGNITVE RECOVERY

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Objectives: We recently found that transplantation of syngeneic murine neural stem cells can dramatically improve cognitive and motor deficits in an α-synuclein transgenic (ASO) model of PD dementia (PDD) and dementia with Lewy bodies (DLB) via a neurotrophic mechanism. To examine xenotransplantation of clinically relevant human neural stem cells (hNSCs), we have now generated and examined a immunodeficient ASO mouse line.
Methods: ASO mice were backcrossed onto a Rag2/IL2rg double knockout background to generate a xenotransplantation-compatible PDD/DLB model that lacks B-cells, T-cells and NK-cells. The resulting mice develop similar α-synuclein pathology and behavioral deficits as the parental ASO strain, yet allow robust engraftment of human NSCs (hNSCs). Either WT-hNSCs or glial derived neurotrophic factor (GDNF)-secreting-hNSCs were then transplanted into the striatum of 6-month old Rag-ASO mice, and performance on multiple motor/cognitive tasks was assessed one month later.
Results: We find that transplanted hNSCs engraft well and begin to differentiate in Rag-WT and Rag-ASO mice. Both WT-hNSCs and GDNF-NSCs rescue motor function with similar magnitude in Rag-ASO mice. Fascinatingly, while both cells types also improve cognitive performance, transplantation with GDNF-NSCs provides substantially greater improvements than WT-hNSCs.
Conclusions: Our findings indicate that hNSC-derived neurotrophic support promotes behavioral recovery in a novel xenotransplantation-compatible transgenic model of PDD/DLB. Our data also suggest that enhancement of hNSC neurotrophin secretion may be critical for improving α-synuclein-related cognitive deficits. Together, these studies aim to enhance our understanding of the potential impact of hNSCs on PDD/DLB related cognitive dysfunction and further elucidate the therapeutic potential of NSC transplantation.

04r. Therapeutic Targets & Mechanisms for Treatment: protein aggregation

ADPD5-1776
IN VITRO KINETIC STUDIES FOR NEW DRUG TESTING IN AMYLOIDOGENIC DISORDERS

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With increasing evidence of protein aggregation as an early sign in neurodegenerative and prionopathies pathogenesis, the expectation of a common therapy has become a primary goal for drugs development. The aim of this work is to establish a consistent *in vitro* protocol for testing the effect of new potential drugs through kinetic studies.
Using alpha-synuclein as model protein, the aggregation process is followed by classic biophysical methods such as Thioflavin-T binding fluorescence and turbidity measurements. The kinetics obtained are described according to the recently proposed crystallization-like model [1] , quantitatively characterizing the weight of nucleation and growth steps.
True inhibition happens when the kinetics of amyloid fibrillization are retarded by decreasing rates of fibril formation and elongation. Apparent inhibition may however result from, e.g., the alteration of thermodynamic properties such as the solubility of the amyloidogenic protein. Apparent inhibitors markedly influence protein aggregation kinetics measured in vitro, yet they are likely to lead to disappointing results when tested in vivo. This is because cells and tissues media are in general much more buffered against small variations in composition than the solutions prepared in lab [2].

[1] R. Crespo, F. a Rocha, A. M. Damas, and P. M. Martins, “A generic crystallization-like model that describes the kinetics of amyloid fibril formation.,” *J. Biol. Chem.*, vol. 287, no. 36, pp. 30585–94, Aug. 2012.
[2] P. M. Martins, “True and apparent inhibition of amyloid fibril formation.,” *Prion*, vol. 7, no. 2, pp. 136–9, 2013.

04s. Therapeutic Targets & Mechanisms for Treatment: misfolding and chaperones

ADPD5-0327

EFFECTS OF MOLECULAR CHAPERONES IN FIBROBLASTS FROM PATIENTS WITH SPORADIC AND GLUCOCEREBROSIDASE-RELATED PD

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Objectives: Parkinson's disease (PD) pathogenesis is associated with a combination of genes and environmental stressors. In particular, heterozygous mutations in GBA gene, encoding for lysosomal enzyme glucocerebrosidase (GCase), are responsible for misfolding and loss of function of the protein and have been associated with higher risk of developing PD, indicating that impaired lysosomal clearance is critical in disease pathogenesis. The aim of this study is highlighting changes affecting proteins linked to proteostasis and GCase folding, especially saposin c, in fibroblasts derived from patients with sporadic and GBA-related parkinsonism, at baseline and after administration of molecular chaperone Ambroxol.
Methods: fibroblast primary cultures were established from skin biopsies obtained from sporadic (n=5) or GBA-related (n=5) PD patients and age/gender-matched healthy controls (n=7). Fibroblasts were treated with Ambroxol according to McNeill et al (Brain, 2014). Protein levels and distribution of GCase, saposin c and saposin c precursor (PSAP) were assessed by immunochemical analysis. Activity of GCase and cathepsin D, responsible for PSAP cleavage, were measured using the fluorogenic substrate 4-Methylumbelliferyl-β-D-glucuronide-hydrate and a commercial kit, respectively.
Results: protein levels for GCase, PSAP and saposin c are unchanged among groups at baseline and increase with Ambroxol. Basal GCase activity is reduced in fibroblasts from GBA-related patients. Ambroxol administration enhances both GCase and cathepsin D activity.
Conclusions: studying the effects of Ambroxol in patient-derived fibroblasts, which share the same genetic complexity of the neurons, might improve our understanding of molecular chaperone-induced responses in sporadic and GBA-related PD and identify novel therapeutic targets for disease-modifying treatments.

04t. Therapeutic Targets & Mechanisms for Treatment: gene therapy

ADPD5-1188

NOVEL GENE THERAPY TO TARGET THE PROTEOSTASIS NETWORK IN PARKINSON DISEASE

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Parkinson´s disease (PD) is the second most common neurological disorder. PD is characterized by the lost of the dopaminergic neurons of the *substantia nigra*, leading to a gradual decrease in the levels of the neurotransmitter dopamine and progressive alterations in the control of voluntary movements. Recent evidence implicates adaptive responses to endoplasmic reticulum (ER) stress in this disease, mediated by the unfolded protein response (UPR) pathway. Here we investigated a novel combination of UPR transcription factors to selectively modulate the homeostasis state of dopaminergic neuron without altering apoptosis programs.

We generated 6 different fusion proteins between of UPR components XBP1s and ATF6f. We evaluated the levels of expression and subcellular localization of these proteins. The transcriptional activity and specificity were evaluated by luciferase assay and real time PCR.

We observed a nuclear localization of all fusion proteins and different of expression levels. The transcriptional activity was different between these variants and we identified a version of ATF6f-XBP1s that induces a strong UPR transcriptional activity. This novel transcription factor is capable of increasing the levels expression of particular cluster genes that were describe as target of heterodimer between ATF6f and XBP1s.

Our results describe a novel molecule that effective tunes selective UPR of a particular cluster gene associate to ATF6f/XBP1s heterodimer activity.

Supported by FONDEF-D11I1007, PAI-7912010006, FONDECYT-1140549, Millennium Institute P09-015-F, The Michael J. Fox Foundation for Parkinson Research, and Ring Initiative ACT1109.

04u. Therapeutic Targets & Mechanisms for Treatment: RNAi-based therapy

ADPD5-0904

NOVEL NON-VIRAL VECTORS TARGETING THE CENTRAL NERVOUS SYSTEM MEDIATE RNA INTERFERENCE-KNOCKDOWN OF ALPHA-SYNUCLEIN IN A PD MODEL

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Objective: To develop novel non-viral vectors for the systemic delivery of siRNA into the central nervous system (CNS)

Methods: The efficacy of our novel vectors in siRNA delivery into neurons was first tested *in vitro* using flow cytometry, immunofluorescence analysis and western blotting on cultured neuroblastoma cells. Brain specific delivery of *SNCA* siRNA and gene knockdown *in vivo* was confirmed by ELISA and Western blot analyses of mice brain lysates as well as by behavioral tests and immunohistochemistry analysis in a Parkinson's disease mouse model.

Results: We developed two novel non-viral vectors named C2-9r and RI-C2-9r that can efficiently deliver siRNA into neurons. Delivery of siRNA against alpha-synuclein gene *(SNCA)* using these vectors enabled 60% knockdown of alpha-synuclein expression in neuronal M17 cells, which resulted in a significant resistance to MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mediated cytotoxicity ($P<0.001$). Our vectors confer superior stability to systemically delivered siRNA *in vivo*, and thereby mediate enhanced alpha-synuclein protein knockdown by 60%-90% within different brain regions. Further, we provide compelling evidence for the *in vivo* therapeutic application of our vectors to silence *SNCA* in the CNS of a Parkinson's disease mouse model and confer protection against dopaminergic neurodegeneration.

Conclusion: We have successfully designed novel vectors that provide an effective and clinically relevant tool for non-viral gene therapy of brain diseases.

04u. Therapeutic Targets & Mechanisms for Treatment: RNAi-based therapy

ADPD5-0929

STRIATAL NURR1 SILENCING AS A NOVEL ANTIDYSKINETIC TARGET FOR PARKINSON'S DISEASE

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Objective. We have demonstrated that levodopa-induced dyskinesia (LID) in parkinsonian rats is associated with aberrant 're-wiring' of corticostriatal input to the striatum. Further, using microarray gene analysis we have identified that one particular synaptic plasticity gene, Nr4a2 (aka: Nurr1), normally not expressed in striatum shows a >30-fold induction in the striatum of levodopa treated parkinsonian rats that developed LID but not in those that remained dyskinesia-free.
Methods. Toward understanding potential clinical utility of this novel striatal target, we are examining the ability of rAAV-Nurr1-shRNA in our rat model of LID to provide antidyskinetic efficacy against moderate and high dose levodopa. The impact of striatal Nurr1 silencing on motor function and corticostriatal remodeling associated with LID are also being monitored.
Results. To induce LID, we first employed a sub-chronic escalating levodopa dosing paradigm and either viral vector mediated overexpression or silencing (shRNA) of Nurr1 in the DA-depleted, dorsolateral striatum. Nurr1 overexpression reduced the threshold for, and increased the LID severity score for equivalent levodopa administrations. Conversely, silencing of striatal Nurr1 by shRNA attenuated peak dose LID. Additional studies examining the impact of striatal Nurr1 silencing on chronic, long-term levodopa administration are ongoing.
Conclusions. Silencing striatal Nurr1 may present an experimental therapeutic that is not palliative but addresses an underlying cause of LID. We propose that Nurr1, acting as a transcription factor is a nexus of divergent signaling that underlie changes in ultrastructure and subsequent physiologic output. (Supported by Udall Center grant P50NS058830 and the St. Mary's Foundation)

04v. Therapeutic Targets & Mechanisms for Treatment: synaptic plasticity and repair

ADPD5-1816

REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION FOR ON-FREEZERS WITH ADVANCED PD. A LONG-TERM FOLLOW UP

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BACKGROUND AND OBJECTIVES: Freezing of gait is experienced in about 30% of PD patients within 5 years, and nearly in 60% after 10 years. Treatment of “off” time FOG is relatively straight forward, but “on-freezing” is a difficult to treat scenario. Objectives. Evaluation of the efficacy and safety of rTMS in management of “on-freezing” in patients with advanced PD

METHODS. This study was performed in two phases, first phase was a randomized, double-blind, placebo-control, which included 22 Egyptian patients received 12 rTMS sessions over 4 weeks (either real or sham) over the leg area of motor cortex contralaterally to the more affected side in addition to rehabilitation program involving specific gait training techniques. In the second open label long term phase, we enrolled 20 patients (all are included in phase I) who received over a 6 months period 3 cycles of rTMS, (12 sessions each) which were performed every other month. Primary efficacy variables are FOG Q (SF), motor section and total score of UPDRS, 2ry outcomes are gait variables (Cadence, number of falls, stride length, stride time and turn time), and on-time.

RESULTS: improving of FOG Q, with significant decrease in number of falls and widened stride length ($P<0.001$) were detected with rTMS. The total score of UPDRS and other gait variables were not changed. No serious adverse events were recorded.

CONCLUSIONS: These results indicate that rTMS has a positive long term effects in on-freezers with advanced PD with subsequent decrease of number of falls.

04v. Therapeutic Targets & Mechanisms for Treatment: synaptic plasticity and repair

ADPD5-1990

EXERCISE-INDUCED NEUROPLASTICITY IN THE MPTP MOUSE MODEL OF PARKINSON'S DISEASE

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Objectives

Parkinson's disease (PD) is characterized by degeneration of dopamine (DA) neurons in the substantia nigra, resulting in hypokinetic rigidity. Physical exercise has been shown to improve motor function in PD patients and DA-related neuronal signaling in rats. In this study, we aimed to determine which underlying molecular processes are involved in exercise-induced adaptive neuroplasticity and motor function improvement.

Methods

Mice were treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrimidine (MPTP) that destroys DA nigrostriatal neurons, providing a model of reduced striatal DA innervation similar to human PD. To capture the behavioral effects and the underlying specific molecular pathways involved in exercise-induced neuroplasticity, the behavioral outcome (i.e. motor performance) in MPTP-treated and control mice following forced treadmill running was correlated with genome-wide mRNA expression analyses of multiple brain areas.

Results

Forced treadmill running improved motor performance in MPTP-treated but not in MPTP-untreated control mice. Preliminary analysis of the mRNA expression data showed that both running and MPTP particularly affected 'mitochondrial (dys)function' and 'oxidative phosphorylation' related processes.

Conclusions

Based on these preliminary data, forced running appears to improve motor function in MPTP-treated mice. Both MPTP treatment and forced exercise affect mRNA expression patterns related to 'mitochondrial (dys)function' and 'oxidative phosphorylation', processes that have also been implicated in human PD pathogenesis. Hence, these data may lead to the development of new treatments for PD, involving physical exercise and/or neuroplasticity.

04w. Therapeutic Targets & Mechanisms for Treatment: adult neurogenesis

ADPD5-1413

INHIBITION OF LEUKOTRIENE RECEPTORS RESTORES COGNITION IN AN ANIMAL MODEL OF PDD

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Leukotrienes, inflammatory mediators of inflammation, are well-studied in the field of asthma. In the brain, elevated leukotriene levels are reported in acute as well as chronic lesions (eg Alzheimer´s disease), and increasing concentrations of the leukotriene-producing enzyme 5-LOX have been demonstrated in the aged brain and in Parkinson´s disease (PD). Here, elevated leukotriene signaling might be involved in neuroinflammatory processes and in the development of dementia in PD (PDD).

With respect to aging, we recently demonstrated that a 6-week oral treatment of young and aged rats with the leukotriene receptor inhibitor montelukast resulted in reduced neuroinflammation, enhanced neurogenesis, and remarkably, in a restoration of cognitive functions in aged rats. Now, we examined if montelukast has beneficial effects on cognition in a PDD animal model. We used 6 month-old PDGF-ß α-Syn transgenic mice, which show microgliosis, deficitis in neurogenesis, and cognitive impairments. Mice were treated orally with montelukast or vehicle daily for 6 weeks, and several behavioural tests were performed. First, we analyzed CNS pharmacology and showed that, after oral administration, montelukast entered the brain of the treated animals. In respect to cognition, the 6-week treatment with montelukast lead to significant improvement in learning and memory in the Morris Water maze in PDD mice. Ongoing histological analyses of the hippocampus will validate if the beneficial effects of montelukast on cognition in PDD mice are accompanied by increased hippocampal neurogenesis and/or decreased neuroinflammation. Based on the current behavioural data, we suggest montelukast as a promising drug to ameliorate dementia in PDD.

04w. Therapeutic Targets & Mechanisms for Treatment: adult neurogenesis

ADPD5-1864
MODULATION OF CYCLIC AMP AS A NEW THERAPEUTIC STRATEGY FOR PARKINSON DISEASE TREATMENT

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1. Objectives . Parkinson disease (PD) is the second most prevalent neurodegenerative disease and no effective treatment is available to date. In our search for new innovative drugs, we have found that PDE7 inhibitors are good drug candidates for this disease [1]. We want now to check if the enhancement of intracellular levels of cAMP could stimulate neurogenesis events on PD animal models.
2. Methods. Hemiparkinsonian rats orally treatment with S14, a small heterocyclic molecule that inhibits PDE7. Phenototypic and biochemical analysis determining neurotrophic factors modulation and neurogenesis induction.
3. Results. After a S14 dose-response curve looking for phenotypic scores, we have observed a great neuroprotection of dopaminergic neurons in the *substantia nigra pars compacta*, accompanied by higher levels of neurotrophic factors and a consistent increase of the neurogenesis with in this brain area.
4. Conclusions. Modulation of cAMP is a good therapeutic strategy to the future treatment of Parkinson disease. PDE7 inhibitors, and specifically our drug candidate S14, merit to be developed till clinical trials to assess its therapeutic potential in Parkinson disease patients.

[1] Morales-Garcia JA, Redondo M, Alonso-Gil S, Gil C, Perez C, Martinez A, Santos A, Perez-Castillo A. Phosphodiesterase 7 inhibition preserves dopaminergic neurons in cellular and rodent models of Parkinson disease. PLoS One. 2011 Feb 24;6(2):e17240.

04x. Therapeutic Targets & Mechanisms for Treatment: deep brain stimulation

ADPD5-0607

SUBTHALAMIC NUCLEUS STIMULATION VERSUS PREOPERATIVE MEDICAL THERAPY FOR PATIENTS WITH PD: A SYSTEMATIC REVIEW

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Objectives: Subthalamic nucleus stimulation is one of the most promising therapies for PD. This study aimed to compare the efficacy of bilateral subthalamic nucleus stimulation versus preoperative medication in the treatment of Parkinson's disease by making a comprehensive review and meta-analysis of published literature.
Methods: A systematic literature search up to December of 2013 was performed in PubMed, Ovid and Chinese Biomedical Retrieval System. The clinical outcomes were assessed using unified Parkinson's disease rating scale.
Results: A total of 27 studies comprised of 861 patients with one year follow-up were identified for systematic review. After one year of stimulation, activities of daily living and motor scores were improved by 46 and 54% in the off-medication state, respectively, compared with preoperative off-medication scores. Average reduction in levodopa equivalent dose intake following surgery was 48%, with dyskinesia and fluctuation reduced by 64 and 50%, respectively. Meta-analysis showed that bilateral subthalamic nucleus stimulation was as well as preoperative medication in improving tremor, rigidity, postural stability and total motor scores, but was less effective than preoperative medication on bradykinesia, gait and activities of daily living scores. Combined therapy (stimulation plus medication) achieved better improvement than preoperative medication. Severe surgical side effect was intracranial hemorrhage in 4.0% of patients. Common stimulation-induced adverse effects were weight gain (21.1%), hypophonia (16.8%), depression (9.3%) and eyelid apraxia (6.2%).
Conclusions: Bilateral subthalamic nucleus stimulation is an efficacious therapy as an adjunct to medication. Carefully selection of Parkinson's disease patients may reduce the incidence of adverse events.

04x. Therapeutic Targets & Mechanisms for Treatment: deep brain stimulation

ADPD5-1936
STRIATAL GLUTAMATE AND GABA AFTER HIGH FREQUENCY SUBTHALAMIC STIMULATION IN PARKINSONIAN RAT

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Background: High frequency stimulation of the subthalamic nucleus is recognized as an effective treatment of advanced Parkinson's disease. However, the neurochemical basis of its effects remains unknown. The aim of this study is to investigate the effects of high frequency stimulation of the subthalamic nucleus in intact and 6-hydroxydopamine-lesioned hemiparkinsonian rat model on changes of principal neurotransmitters, glutamate, and gamma-aminobutyric acid in the striatum.
Methods: The authors examined extracellular glutamate and gamma-aminobutyric acid change in the striatum on sham group, 6-hydroxydopamine-lesioned group, and 6-hydroxydopamine-lesioned plus deep brain stimulation group using microdialysis methods. High-pressure liquid chromatography was used to quantify glutamate and gamma-aminobutyric acid.
Results: The results show that high frequency stimulation induces a significant increase of extracellular glutamate and gamma-aminobutyric acid in the striatum of 6-hydroxydopamine-lesioned plus deep brain stimulation group compared with sham and 6-hydroxydopamine-lesioned group.
Conclusions: The clinical results of high frequency stimulation of the subthalamic nucleus are not restricted to the direct subthalamic nucleus targets but involve widespread adaptive changes within the basal ganglia.

04x. Therapeutic Targets & Mechanisms for Treatment: deep brain stimulation

ADPD5-2017

CAN DEEP BARIN SITMULATION PRESERVE WORKING ABILITES IN PARKINSON'S DISEASE?

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Objective: There is a debate on the potential advantageous effects of bilateral subthalamic deep brain stimulation (DBS) in the treatment of Parkinson's disease with early fluctuations. Our investigation aimed to evaluate if DBS therapy was able to preserve the working capabilities.
Methods: We reviewed the data of 40 young (<60 yearsold) PD patients who underwent DBS implantation at University of Pécs and had an at least 2 years followup. Patients were categorized into two groups based on their working capabilities at time of surgery: 'Active job' group (n=20) and 'No job' group (n=20). Baseline characteristics were comparable. Severity of motor symptoms (UPDRS3), quality of life (EQ5D) and presence of active job were evaluated preoperatively and 2 years postoperatively.
Results: Although similar (approximately 50%) improvement was achieved in the severity of motor and major nonmotor symptoms in both groups, the postoperative quality of life was significantly better in the 'Active job' group (0.687 vs. 0.587, medians, $p<0.05$). Majority (80%) of 'Active job' group members were able to preserve their job 2 years after the operation. However, only a minimal portion (5%) of the 'No job' group members was
able to return to the world of active employees ($p<0,01$).
Conclusion: Although our study has several limitations, our results fits well with the conclusions of EarlyStim study. In patients with active job the appropriately 'early' usage of DBS might help preserve working abilities in a twoyear timeframe and gain higher improvement in quality of life.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1049

INTRATHECAL BACLOFEN INJECTION RELIEVES DYSTONIC CAMPTOCORMIA IN PARKINSON'S DISEASE.

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Objectives: Axial symptoms in Parkinson's disease (PD) are more difficult to treat. Especially, camptocormia is one of the most resistant symptoms to various therapies, for example botulinum toxin therapy or deep brain stimulation (DBS). Xylocain injection to lateral oblique abdominal muscle is reported to be effective to camptocormia in case the bent point is upper thoracics. In this study we tried intrathecal baclofen injection to find the efficacy on camptocormia in PD.

Methods: 7 patients (3 male and 4 female) with camptocormia, who had no cause other than PD, were included. Mean disease period was 11.7year and its SD was 4.69. Intrathecal baclofen injection was performed by by lumbar puncture. Injections were consisted three steps, first 50, second 75, and three 100microgram. Each patients was infected two steps or three steps. Postural change was evaluated photographically before and 3-4 hours after injection.

Results: In 6 of 7 patients, camptocormia was improved after baclofen injection. 2 patients started to show improvement at first step (50microgram), 3 other patients started to improve at second step, and 1 patient showed improvement only at 3rd step (100microgram). One patients denied third step after two steps whose were not effective to camptcormia.

Conclusion: From the results of this study, intrathecal baclofen injection has potentiality to treat camptocormia in patients with PD.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-2254

THERAPEUTIC EFFECT OF PEPTIDE AMPHIPHILE NANOFIBERS IN A RAT MODEL OF PARKINSON'S DISEASE

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Objectives: Parkinson's Disease (PD) is characterized by progressive degeneration of dopaminergic nigrostriatal neurons and reduction in striatal dopamine levels. Peptide nanofibers are new generation biomaterials that have been shown to induce neurite outgrowth in vitro and to stimulate axonal growth and reduce apoptosis in spinal cord injury in vivo. In this study, we tested the effect of a synthetic peptide amphiphile (PA) nanofiber system that can mimic the activities of laminin (LN) and heparan sulfate proteoglycans (GAG) in a rat model of PD.

Methods: Adult male Sprague-Dawley rats (250-300 g) received two intrastriatal injections of 6-hydroxydopamine (6-OHDA; 8 ug) or L-ascorbic acid (LAA; 0.3%) and one week later randomized into three groups with equal number of rotations for 30 min after intraperitoneal d-amphetamine (5 mg/kg) injection as follows: 6-OHDA+PA; 6-OHDA+Sucrose; and LAA+PA. Nanofibers were administered in two consecutive fragments as PA-LN and PA-GAG by microinjections to the 6-OHDA lesion sites. Rotational behavior test was repeated 6 weeks later followed by sacrificing rats and obtaining striata for further neurochemical and immunohistochemical studies.

Results: Our results revealed for the first time that the PA-LN and PA-GAG combine and form a peptide gel in vivo. In addition, Parkinsonian rats receiving site-specific injections of PA nanofibers showed significantly (by 18%; $p<0,05$) reduced number of rotations 6 weeks after treatment.

Conclusion: These data suggest that PA nanofibers may have therapeutic potential in PD.

05a. Drug Development & Clinical Trials: active vaccination

ADPD5-1820

RESULTS FROM A PHASE I STUDY TO ASSESS THE TOLERABILITY, SAFETY AND IMMUNOLOGICAL AND CLINICAL ACTIVITY OF AFFITOPE® PD01A IN PATIENTS WITH EARLY PARKINSON'S DISEASE

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Objectives: This 12- month study was designed to assess safety and tolerability of AFFITOPE® PD01A, the first alpha-synuclein (aSyn)-targeting vaccine candidate tested clinically, in early Parkinson's disease. This single-center, parallel group, patient blinded Phase I study also assessed immune and clinical response.
Methods: 32 patients were enrolled: 24 randomly assigned to 15 µg or 75 µg PD01A, and 8 untreated patients served as a control group. Active patients received 4 subcutaneous injections at weeks 0, 4, 8 and 12 with final assessments at week 52. Primary objective was to test the safety and tolerability of two doses of PD01A. Secondary endpoints encompassed the vaccine-specific immune response as well as clinical parameters (UPDRS Ia, II, III, PDQ-39, PD-NMS, IGE). Each patient was evaluated regularly over 12-months.
Results: All 32 patients completed the study. All 24 patients in the active groups received four vaccinations as planned. Both doses tested were found to be safe and well tolerated. The most frequent adverse events were local reactions to vaccine administration, which all were self-limited. The active groups performed similarly to each other and showed significant effects against the control group on multiple immunological and efficacy outcomes. 62.5% of treated patients demonstrated increased levels of aSyn-specific antibodies, and these immune responders showed statistically significant treatment effects over non-responders for several efficacy outcomes.
Conclusions: This is the first clinical trial assessing an aSyn-targeting agent. PD01A was found to be safe and well-tolerated at both doses tested. Results are consistent with a disease-modifying effect of PD01A.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-0356

CORRECTION OF NON-MOTOR FLUCTUATIONS IN PATIENTS WITH PD

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1. Objectives: To evaluate the effect of dopamine receptor agonists and amantadine on vegetative, sensory, mental non-motor fluctuations at Parkinson's disease.

2. **Methods :** 20 patients, different sex, middle ages - 58,4 ± 6,01 years, akinetic - rigid form of the disease. Duration of PD - 6,8 ± 1,5 years, all patients received standard therapy with L-Dopha 4 times a day (daily dose of 250 - 375mg).

Middle estimation degree of weight at PD on the Hoehn and Yahr scale was 3,5±0,2. Used UPDRS (I, II, III A and B parts), Mini-Mental State Examination (MMSE), BDI. Patients were divided into groups: 1). was treated with amantadine sulfate 500 ml intravenous infusion for 5 days, 2). got treatment of the pramipaxole| prolonged action (Mirapex® ER) 1,5 mg/per day, 3). received amantadine sulfate orally 200 mg per day. Clinical and neuropsychological testing was performed at baseline, after 5 days and one month after treatment.

3. **Results:** the study of non-motor symptoms (UPDRS I, II parts) showed a significant decrease in scores at patients of all three groups, but the effect was more rapid at using intravenous form (from 25,2 ± 2,1 to 20,3 ± 2,4).

The therapy showed a significant improvement in cognitive function, reduction of depressive disorders.

Conclusions: amantadine sulphate and non-motor manifestations of pramipexole reduce fluctuations at Parkinson's disease. More rapid effect in sensory and autonomic fluctuations was at amantadine sulfate, in mental at pramipexole.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-0746

DIETARY INTERVENTIONS WITH STRONG PREVENTIVE AND THERAPEUTIC EFFECTS ON BOTH MOTOR AND NON-MOTOR SYMPTOMS IN A MOUSE MODEL OF PARKINSON'S DISEASE

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Introduction. Administration of dietary precursors and cofactors for membrane synthesis may enhance synapse formation relevant for neurodegenerative disorders. Indeed, preventive treatment with the precursors UMP/DHA reduced rotational behavior in the 6-OHDA Parkinson's Disease (PD) model. Here we examined the efficacy of dietary interventions in the rotenone model of PD, inducing both motor and non-motor symptoms, including gastrointestinal dysfunction. In addition, we tested the effects of an extended nutritional formula based on the same precursors.

Method. Male C57BL/6J received a unilateral rotenone injection in the striatum (5.4 ug). Dietary interventions started either 1 week before (preventive) or 3 weeks after surgery (therapeutic), when PD-like symptoms had developed. Readout parameters included behavioral tasks as the rotarod, novel object discrimination, grip strength, and inverted screen test, as well as histological examination of brain and gut.

Results. Our results show that the preventive UMP/DHA treatment improved rotenone-induced disturbances in rotarod performance and intestinal transit. Similar effects were found in the therapeutic setting, indicating that the diets did not interfere with rotenone toxicity. The therapeutic treatment also alleviated rotenone-induced deficits in novel object discrimination, grip strength, and inverted screen test. Our extended nutritional intervention was more effective than the diet providing UMP/DHA only.

Conclusion. This is the first study demonstrating a therapeutic effect of specific dietary interventions in a mouse model of PD. The improved efficacy of the extended nutritional intervention suggests synergies within our specific multi-nutrient approach.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-2119

CAPSAICIN PRE-TREATMENT CONFERS NEUROPROTECTION IN A MODEL OF PARKINSON'S DISEASE

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Introduction: Parkinson's disease (PD) is characterised by progressive loss of motor function and dopaminergic neuron (DN) density. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is neurotoxic to DN in the mouse *substantia nigra* yielding PD-like symptoms. Red peppers contain capsaicin that can protect neurons in an *in vitro* model of acute oxidative insult (Fong and Witting *unpublished*). We ***aim*** to test whether capsaicin prevents neuronal loss and brain function in a mouse model of PD.
Methods: Male C57BL/6 mice were given vehicle (DMSO) or capsaicin (1 mg/kg) 6 h prior to saline or MPTP (25 mg/kg/day) treatment over 2 consecutive days. Gait was assessed on a subset of mice six days after the onset of treatment. Serum and various organs were harvested for biochemical and histological (DN counting using stereological post tyrosine-hydroxylase staining) analyses.
Results: Serum GGT and creatinine were below detection while serum urea decreased slightly in MPTP-treated animals although this did not reach significance. Cerebral levels of IL-6, TNF-α and p-mTOR (anti-apoptotic mediator) were also unchanged. Total DN counts decreased slightly in the MPTP-treated animals while in capsaicin-treated mice DN were not different to controls. Notably, capsaicin restored rear paw brake timing indicating an improved gait.
Discussion: Neither MPTP nor capsaicin caused detectable liver or kidney damage. The dose of MPTP employed only induced a marginal decline in DN density, yet this was sufficient to affect gait as measured by front/rear paw brake time. Pre-treatment with capsaicin significantly improved gait but had no meaningful impact on DN density.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-2328

Combination of acamprosate and baclofen as a promising therapeutic approach for Parkinson's disease

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Objectives: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterised by the loss of dopaminergic nigrostriatal neurons but which involves the loss of additional neurotransmitter pathways in brain and the autonomic nervous system. Mono- or polytherapeutic symptomatic interventions in PD patients have declining efficacy long-term and no influence on disease progression. The systematic analysis of available genetic and functional data as well as the substantial overlap between Alzheimer's disease (AD) and PD features led us to repurpose and explore the effectiveness of a combination therapy (ABC) with two drugs – acamprosate and baclofen – that was already effective in AD animal models, for the treatment of PD. Results: We showed in vitro that ABC strongly and synergistically protected neuronal cells from oxidative stress in the oxygen and glucose deprivation model, as well as dopaminergic neurons from cell death in the 6-hydroxydopamine (6-OHDA) rat model. Furthermore, we showed that ABC normalised altered motor symptoms in vivo in 6-OHDA-treated rats, acting by protecting dopaminergic cell bodies and their striatal terminals. Interestingly, ABC also restored a normal behaviour pattern in lesioned rats in a symptomatic fashion. Conclusion: Our results demonstrate the potential value of combining repurposed drugs as a promising new strategy to treat this debilitating disease.

05j. Drug Development & Clinical Trials: neurotransmitter modulators

ADPD5-0459

EFFECTS OF DOPAMINERGIC MEDICATION ON COGNITIVE FUNCTION IN DRUG-NAIVE DE NOVO PATIENTS WITH PD

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Objectives: There is a correlation between cognitive and motor functions in Parkinson's disease (PD). However, the effects of dopaminergic medication on cognitive function in PD patients are uncertain, especially in drug-naive *de novo* PD patients. We examined the cognitive effect of dopaminergic medication in drug-naive PD patients. **Methods**: Dopaminergic drugs (levodopa, dopamine agonist, selegiline) were introduced to 16 drug-naive PD patients and increased to an optimal dose for motor symptoms. Patients were tested prior to and after 4-7 months of drug initiation. Motor function was assessed using the Unified Parkinson's Disease Rating Scale (UPDRS; total score and subscores of tremor, rigidity, bradykinesia, gait and postural instability). Cognitive function was assessed using the Japanese version of Montreal Cognitive Assessment (MoCA-J: total score and subscores of delayed recall, attention, visuospatial function, executive function, language and orientation). Score changes from baseline for both motor and cognitive assessment were compared. **Results**: Dopaminergic drugs ameliorated the UPDRS total score in all participants. MoCA total score improved in 12 patients, but 4 patients had deteriorated by one point. Amelioration in gait score showed a significant negative correlation with that of attention. Amelioration in bradykinesia score also showed a negative correlation with visuospatial function. However, there was a significant positive correlation in amelioration score between gait and language. **Conclusions**: Dopaminergic treatment can relieve cognitive function in drug-naive PD patients. However, the dopaminergic effects on cognitive and motor functions are domain specific.

05j. Drug Development & Clinical Trials: neurotransmitter modulators

ADPD5-1985

EFFICACY OF COMBINATION THERAPY WITH LEVODOPA AND SERTRALINE ON PARKINSONIAN RATS

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Objective: Parkinson's disease is a neurodegenerative condition. In this disease nigro striatal dopaminergic pathway and some other neurotransmitter systems (i.e. serotonergic system) are involved. Long-term administration of Levodopa in parkinsonian patients leads to development of some motor disorders such as L-dopa induced dyskinesia and off-phenomena. Hence employment of strategy for effective treatment of this disease is of interest.
In this investigation we attempted to evaluate the effect of sertraline, as a selective serotonin reuptake inhibitor, on anti-cataleptic effect of L-dopa in 6-hydroxydopamine (6-OHDA) lesioned rats.
Methods: Catalepsy was induced by unilateral infusion of 6-OHDA (8µg/2µl/rat) into the substantia nigra, compact part (SNc). Male wistar rats weighting 180-200g were used in the present study. These rats received intraperitoneally (i.p.) L-Dopa (15mg/kg) twice daily for 20 consecutive days, and anti-cataleptic effect of L-dopa was evaluated by bar-test on days 5, 10, 15 and 20. On the day 21, these rats co-injected with three different doses of sertraline (0.5, 1and 2mg/kg, i.p.) and L-dopa (15mg/kg, ip).
Results: L-dopa exerts anti-cataleptic effect only until day 15, and its effect was vanished on the day 20. Sertraline (1 and 2 mg/kg i.p.) markedly attenuated the cataleptic behavior ($P<0.001$). Furthermore, the effect of sertraline (2 mg/kg, i.p.) on anti-cataleptic effect of L-dopa (15mg/kg, ip) was antagonised by NAN-190 (0.5 mg/kg, i.p.) as a 5-HT_{1A} receptor antagonist.
Conclusion: Sertraline improves the anti-cataleptic effect of L-dopa in the 6-OHDA-lesioned rats and probably this effect mediated through activation of $5HT_{1A}$ receptors.

05j. Drug Development & Clinical Trials: neurotransmitter modulators

ADPD5-1998

EFFECTS OF ISTRADEFYLLINE ON NON-MOTOR SYMPTOMS AND QUALITY OF LIFE IN PARKINSON'S DISEASE PATIENTS

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Objective: To clarify the effect of istradefylline for non-motor symptoms (NMS) and quality of life (QoL) in Parkinson's disease (PD) patients. **Background**: Istradefylline decreases daily OFF time and improves the UPDRS Part III in PD patients, but the efficacy for NMS and QoL in patients with PD has not been investigated enough. **Methods**: We investigated out-patients with PD at Hoehn-Yahr (HY) stage 2-3, who had over 1 hour of OFF time / day and were taking above 300 mg of levodopa/decarboxylase inhibitor / day. The subjects received istradefylline 20 or 40 mg / day for 12 weeks. We estimated the Self-Rating Depression Scale (SDS), the Apathy Scale (AS), the Sagamihara-Keio Apathy Scale (SKAS), the Parkinson's Disease Sleep Scale-2 (PDSS-2), the Non-Motor Symptom Scale for Parkinson's Disease Study 2 (NMSS-2), the Parkinson's Disease Questionnaire (PDQ-39) and UPDRS, before, 8 and 12 weeks after the intervention. **Results**: 24 subjects (10 males, mean age 71.5 years, disease duration 9.5 years, HY 2.2, MMSE 28.3, FAB 14.5) were included. The summary index of PDQ-39 improved from the baseline (26.3) by 4.3 (p = 0.006) and the mobility, stigma and social support domains became significantly better. The UPDRS total and the UPDRS Part III improved from the baseline (28.0 and 13.0) by 5.7 (p = 0.002) and 3.7 (p = 0.000) significantly. The SDS, AS, SKAS, PDSS-2 and NMSS-2 scores did not change remarkably. **Conclusion**: Istradefylline improved QoL and motor symptoms in PD patients, but its efficacy for NMS was not proven.

05m. Drug Development & Clinical Trials: antiepileptics

ADPD5-1595

A CASE OF PARKINSON'S DISEASE THAT GABAPENTIN IMPROVED BOTH VISUAL HALLUCINATIONS AND PAIN

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OBJECTIVES: Parkinson's disease (PD) is the most common neurodegenerative disease. Visual hallucinations (VH) and pain are common non-motor symptoms. Although dopaminergic dysfunction has traditionally been considered as one of the causes of these symptoms, the detail mechanisms have not been understood well. General treatments for VH, decrease of dopamine agonists and use of antipsychotic medication, often lead to exacerbation of motor symptoms and excessive sedation. Gabapentin, an anti-epilepsy drug, effects glutamic acid neuron system and gamma-amino butyric acid neuron system. Gabapentin also has an analgesic effect. We report our experience that gabapentin improved both VH and pain in a patient with PD.
METHODS: A case report.
RESULTS: The case is an 81 year-old Japanese male, who was diagnosed with PD at the age of 67. His Hoehn and Yahr staging scale was IV. He suffered from pain caused by hallucinations of insects invading his body. In spite of the general treatments, VH and pain persisted. Moreover, exacerbation of motor symptoms and excessive sedation hindered a further attempt. Gabapentin was administered to ease his pain. After that, not only pain but also VH disappeared without any adverse effects. He has been managed without recurrence of VH and pain for 10 months until now.
CONCLUSIONS: To our knowledge, this is the first report suggesting positive effects of gabapentin for VH and pain. VH and pain in PD might be associated with non-dopaminergic pathways. Further experience and study are needed.

05q. Drug Development & Clinical Trials: deep brain stimulation

ADPD5-2229

LEVODOPA PHARMACOKINETICS DURING ORAL INTAKE IN ONE PATIENT WITH PARKINSON'S DISEASE

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Objective: One patient was accidentally given oral levodopa during a study aiming for better understanding of the basal ganglia and of the mechanisms of deep brain stimulation of the subthalamic nucleus (STN DBS) with and without levodopa treatment in patients with Parkinson's disease (PD). The results from the accidental oral levodopa intake are presented. *Methods*: Five patients with advanced PD were included in the study. During planned STN DBS surgery three microdialysis catheters were implanted in the right putamen and in the right and left globus pallidus interna. During three study days microdialysis was performed continuously and STN DBS was performed according to a specific protocol. On the third day levodopa was given intravenously. After DBS surgery but before STN DBS was started one of the patients was accidentally given levodopa/benserazide and entacapone tablets at several occasions. *Results*: The levodopa levels increased promptly in CNS after each levodopa intake. Immediately the levodopa seemed to be metabolized to dopamine since these levels correlated well with the levodopa concentrations. *Conclusion:* The results indicate that PD patients still have capacity to metabolize levodopa to dopamine despite advanced disease with on-off symptoms and probably also pronounced nigral degeneration.

05w. Drug Development & Clinical Trials: drug-delivery systems

ADPD5-0548

ROTIGOTINE TRANSDERMAL PATCH IS EFFECTIVE AND SAFE IN PD WITH DEMENTIA.

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Objective
Safety of dopamine agonist (DA) in demented patients has not been elucidated yet, because the previous RCTs excluded such patients. We aimed to assess the influences on daytime sleepiness and other adverse effects (AEs) of transdermal patch of rotigotine in patients with PD with dementia (PDD).
Methods
This was an open-label study. We enrolled PDD patients (MMSE score <26 points) with unsatisfactory control of motor symptoms. Treatment with transdermal patch of rotigotine was titrated to optimal dose (4-8 mg/24 hour) over 2-4 weeks. The primary outcome was the Epworth Sleepiness Scale (ESS) for evaluation of daytime sleepiness. The secondary outcomes included Hoehn &Yahr (H&Y) stage, time spent with dyskinesia by MDS-UPDRS Part IV, Clinical Global Impression of Change (CGIC) of motor symptoms, AEs of nausea/vomiting, syncope, hallucination, and skin reactions requiring treatment, and compliance.
Results
The subjects were 6 PDD patients (age 77±6 years, H&Y stage 3±1, mean±SD). ESS did not change significantly after the treatment (12.2±5.4 before treatment (n=6), 10.7±2.3 with rotigotine 4 mg/24 hour (n=6), and 16.0±8.7 with rotigotine 8 mg/24 hour (n=3)). CGIC improved after the treatment. Other secondary outcomes did not worsen after the treatment.
Conclusions
Twenty four-hour transdermal delivery of rotigotine at doses up to 8 mg/24 hours does not worsen the daytime sleepiness and other AEs in PDD patients. This is achieved together with satisfactory improvement in motor symptoms, demonstrating that this new modality of non-ergot DA is well tolerated and beneficial in PDD patients.

05w. Drug Development & Clinical Trials: drug-delivery systems

ADPD5-1921

CONTROLLABLE SYNTHESIS AND CHARACTERIZATION OF LEVODOPA-LOADED POLY(LACTIC-CO-GLYCOLIC ACID) NANOPARTICLES FOR PARKINSON'S DISEASE TREATMENT

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Abstract:

1. Objectives

Although several progressive treatments have been developed, many effective drugs have failed in clinical examinations due to the penetration ability across the blood-brain barrier. As an attractive strategy, local and controlled delivery of levodopa using biodegradable polymeric nanoparticles has attracted more attention in Parkinson's disease treatment. In this study, in order to obtain desired surface morphology and particle size of poly(lactide-co-glycolide) (PLGA) nanoparticles, and high emulsifying effects, vitamin E TPGS and different amounts of chitosan have been used.

2. Methods

The particle size, surface morphology and phase composition correlated with different percentages of chitosan and different pH were characterized. In addition, the *in vitro* cytotoxicity of the samples using PC12 cell line was investigated. In the next step, six groups of mice received intravenous tail-vein administration of levodopa-loaded poly(lactic-co-glycolic acid) nanoparticles to monitor the *in vivo* blood-brain barrier transport behavior.

3. Results

According to the results, with the increase of chitosan ratio, more effective *in vitro* therapeutic effects could be observed, which achieved high cell viability. In addition, the levodopa-loaded poly(lactic-co-glycolic acid) nanoparticles showed high efficacy while the same doses of levodopa provided no significant effect in the *in vivo* models.

4. Conclusions

The proposed drug delivery system represents a promising agent for potential chronic administration in the Parkinson's disease therapy. This demonstrates the great prospect of chitosan as ligand in the brain targeting that could be effectively optimized for a better functionality.

05w. Drug Development & Clinical Trials: drug-delivery systems

ADPD5-2301

WILLINGNESS TO PAY FOR A NEW DRUG DELIVERY IN PARKINSON PATIENTS

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Objective:The Swedish reimbursemant system operates a system where prices are set based on the expected value to the consumer.This value can be measured using willingness to pay(WTP).
Aim:To assess PD patients´ WTP for newly developed microtablets of levodopa in combination with a drug-delivering device(M/E) compared to standard treatment with levodopa combined with the COMT-inhibitor entacapone (L/e).
Method: 2000 randomly included PD patients had a postal questionnaire covering demographics, disease-specific issues, views on medication and WTP in diffrerent hypothetical scenarios. The first scenario was M/E with no change in effects or side effect; the second scenario was M/E with same effect and less side effects;and the third scenario was M/E with improved effects with less side effects. These scenarios were coupled to different costs to choose from.
Results: 999(50%) patients responded, mean age 71 years, mean PD duration of 9 years. 50% of all patients preferred M/E before L/e in scenario one with increasing preference to scenario three. The average monthly WTP among all respondents in scenario one was SEK 230, and SEK 226 in L/e, both with an almost longitudinal doubling up to scenario three. Duration of PD-related sypmtoms, high education, and high medication intake implied a higher WTP in all scenarios in contrast to age, sex, and extra doses of levodopa
Conclusion: WTP for M/E increased gradually with high medication intake and education as well as with expected increased reduction of PD symptoms.

05y. Drug Development & Clinical Trials: other

ADPD5-0957

NPT088, A GAIM-IMMUNOGLOBULIN FUSION, REDUCES ALPHA-SYNUCLEIN DEPOSITS AND INCREASES TYROSINE HYDROXYLASE IN ALPHA-SYNUCLEIN OVEREXPRESSING TRANSGENIC MICE

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Objectives. NeuroPhage Pharmaceuticals is developing novel therapeutics for the treatment of neurodegenerative diseases, including Parkinson's and Alzheimer's disease. NPT088 is a clinical candidate with potential disease-modifying activity that disrupts and clears a variety of amyloid aggregates in the brain. In addition to targeting Abeta and tau aggregates, NPT088 disrupts alpha-synuclein fibrils in biochemical, cell-based and animal studies. The current study investigated the effects of chronic systemic administration of NPT088 on neuropathological endpoints in transgenic mice overexpressing human alpha-synuclein.

Methods. mThy-1-h-alpha-synuclein transgenic mice (~3 months old at start of dosing) received weekly intraperitoneal injections of NPT088 (2 or 20 mg/kg) or PBS for 13 weeks. Age-matched wildtype mice received PBS injections. Effects on proteinase K (PK) resistant alpha-synuclein and tyrosine hydroxylase immunoreactivity were assessed in various brain regions, including frontal cortex, striatum and hippocampus. NTP088 effects on alpha synuclein were also assessed using western blot analyses.

Results. NPT008, at both doses (2 & 20 mg/kg), significantly improved neuropathology in alpha-synuclein transgenic mice following chronic systemic treatment. NPT088 significantly reduced PK resistant alpha-synuclein in striatum, frontal cortex and hippocampus. A significant reduction in PK resistant alpha-synuclein (LB509) was also shown by western blot. In addition, NPT088-treated mice had significantly greater levels of tyrosine hydroxylase immunoreactivity than PBS-treated mice, similar to levels of wildtype mice.

Conclusions. Chronic NPT088 treatment of alpha-synuclein mice produced significant reductions of alpha-synuclein with greater levels of tyrosine hydroxylase compared to PBS treatment. NPT088 was well-tolerated and produced no adverse effects. IND filing is planned for 3Q2015. MJFF-funded research.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0650

TOPOGRAPHY OF CORTICAL THINNING ASSOCIATED WITH WHITE MATTER HYPERINTENSITIES IN PARKINSON'S DISEASE

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Background: Although white matter hyperintensities (WMHs) are associated with cognitive impairments in Parkinson's disease (PD), the relationships between WMHs and cortical atrophy in regard to cognitive impairments are unknown. Here, we investigated the topography of cortical thinning related to deep (DWMHs) and periventricular WMHs (PWMHs) and their differential impacts on cognitive performance in PD.
Methods: We enrolled 242 patients with non-demented PD and evaluated WMH scores using a semi-quantitative visual rating system. The patients were divided into low-, moderate-, and high-grade groups based on WMH severity for total WMHs (TWMHs), DWMHs, and PWMHs, and cortical thickness was measured using a surface-based method according to the WMH severity. Additionally, the correlations between WMH-associated cortical thinning and neuropsychological performance were analyzed.
Results: The detailed neuropsychological test demonstrated that PD patients with high-grade WMHs showed poorer performance on frontal lobe-based cognitive tasks compared with those with low-grade DWMHs. The areas of cortical thinning were more extensive in patients with DWMHs, involving the entire frontal areas and restricted temporoparietal areas, whereas in patients with PWMHs, cortical thinning was localized in the small frontal areas. A multiple regression analysis of the relationships between WMH-associated cortical thickness and cognition revealed that DWMH-associated frontal thickness had an independent effect on frontal lobe-based cognition, while frontal thickness related to PWMHs did not have a significant correlation with cognitive tasks.
Conclusions: These data suggest that in patients with PD, DWMHs are closely coupled with decreased cortical thickness in the frontal areas and may lead to declines in executive function.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0652

EFFECT OF OLFACTORY IMPAIRMENT AND WHITE MATTER HYPERINTENSITIES ON COGNITION IN NON-DEMENTED PARKINSON'S DISEASE

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Background: Although white matter hyperintensities (WMH) and olfactory dysfunction are independently associated with the cognitive impairments in Parkinson's disease (PD), the effects of simultaneous presence of these abnormalities remain unknown. Thus, we investigated the different effects of deep WMH and periventricular WMH on olfactory and cognitive performance and evaluated the additive effects of the concurrent presence of WMH and olfactory dysfunction on cognitive performance in PD.
Methods: We enrolled 171 patients with non-demented PD whose WMH scores were assessed using a semi-quantitative visual rating system. The olfactory and cognitive performance was assessed using the Cross-Cultural Smell Identification (CCSI) test and the Seoul Neuropsychological Screening Battery. Additionally, the additive effects of concurrent WMH and olfactory dysfunction on cognitive performance were investigated using binary logistic regression.
Results: The deep WMH exhibited a significant negative correlation with the CCSI score (p=0.026) but the total WMH and periventricular WMH did not. A multiple regression analysis revealed that the total WMH (β=-0.109, p=0.011) and deep WMH (β=-0.153, p=0.020) severities had significant negative correlations with semantic fluency. A logistic regression analysis revealed that the simultaneous presence of severe olfactory dysfunction and deep WMH was associated with a greater risk for the semantic fluency impairments (odds ratio=15.909, p= 0.0005) compared to patients with mild deep WMH or high CCSI scores.
Conclusions: These data indicate that deep WMH was closely coupled with olfactory impairments and cognitive decline in PD. Moreover, the concurrent presence of high-grade deep WMH and severe olfactory impairments has a greater influence on semantic fluency.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0797
ABERRANT CEREBRAL NETWORK TOPOLOGY AND MILD COGNITIVE IMPAIRMENT IN EARLY PD
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Objectives: To assess whether mild cognitive impairment (MCI) is associated with disruption in large-scale structural networks in newly diagnosed, drug-naïve patients with Parkinson's disease (PD).
Methods: Graph theoretical analyses were applied to 3T MRI data from 123 PD patients and 56 controls from the Parkinson's Progression Markers Initiative (PPMI). Thirty-three patients were classified as having MCI using the Movement Disorders Society Task Force criteria, while the remaining ninety patients were classified as cognitively normal. Global measures (clustering coefficient, characteristic path length, global efficiency, small-worldness) and regional measures (local clustering coefficient, local efficiency, hubs) were assessed in the structural networks that were constructed based on cortical thickness and subcortical volume data in each group.
Results: Patients with MCI showed a marked reduction in the average correlation strength between cortical and subcortical regions compared to controls. These patients had larger characteristic path length and reduced global efficiency in addition to a lower local efficiency in frontal and parietal regions compared to cognitively normal patients and controls. A reorganization of the highly connected regions in the network was observed in patients with and without cognitive deficits.
Conclusions: This study shows the earliest stages of cognitive decline in PD are associated with a disruption of the large-scale coordination of the brain network and with a decrease of the efficiency of parallel information processing. These changes are likely to herald further cognitive decline and provide support to the view of PD as a disconnection syndrome capable of spreading along the connections of the cerebral network.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1292

DIFFUSION MRI ALTERATIONS IN PRODROMAL DEMENTIA WITH LEWY BODIES

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Introduction: Diffusion Tensor Imaging (DTI) is an imaging modality which allows to visualize *in vivo* the bundles structures. We investigate the early diffusion MRI alterations in prodromal stage of dementia with Lewy bodies (DLB) using a newly proposed tensor-based method, in comparison to the standard SPM scalar-based approach.

Methods: Twelve controls were compared to 22 patients affected by DLB in prodromal stage. Voxelwise analyses were carried out on 1) scalar measures (FA and MD) using SPM and 2) a novel tensor-based method [1], that we recently proposed and which extends the general linear model to tensor images, taking into account the whole tensor information. We also compared hallucinated DLB *vs* no hallucinated DLB.

Results: The results show diffusion alterations in DLB as compared to healthy controls in insular cortex, anterior cingulate cortex and occipital lobe. Hallucinations seem to be more specifically related to modifications in anterior cingulate cortex, occipital lobe and temporal lobe. The tensor-based method appears to be more sensitive to detect the involved regions.

Conclusion: This study highlights the early diffusion alterations in the prodromal stage of DLB, that are mainly located in the grey matter and in the insular cortex, which seems to be an important region for this pathology, as proven in others studies. We also demonstrate the interest of specifically designed tensor-based methods to detect subtle diffusion alterations.

[1] A. Bouchon *et al*, General linear models for group studies in diffusion tensor imaging, ISBI Beijing, China, April 2014.

06a. Imaging & Biomarkers: structural MRI

ADPD5-2277

HOT CROSS BUN SIGN IN A PATIENT WITH PARKINSONISM AND CEREBELLAR ATAXIA

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Objectives:

The "Hot cross bun" sign is a cruciform hyperintensity in the pons, especially on transverse T2-MR images of the brain. We discussed the differential diagnosis of the "hot cross bun" sign in a patient presenting with cerebellar ataxia, parkinsonian symptoms and cranial MR showing hot cross bun sign and cerebellar atrophy.

Case:

A 49-year-old male presented with 8 year history of gait disturbance, drop attacks and urinary incontinence. Three years ago he was diagnosed as Parkinson's disease and his symptoms gradually worsen, responding poorly to dopaminergic treatment. Medical history included diabetes mellitus and polyneuropathy. The patient was being treated with levodopa/carbidopa/entacapone 150 mg (5x1) on admission. Neurological examination revealed hypophonic and heavily slurred speech, slow saccades, hyperreflexia and right extensor plantar response. Bradymimia, severe bradykinesia, rigidity, postural instability and Romberg sign were also noted. The patient could not stand up without assistance. MRI revealed prominent serebellar atrophy and 'hot cross bun" sign in the pons on T2/FLAIR sequences (Figure 1, 2).

Discussion:

The hot cross bun sign reflects degeneration of transverse pontocerebellar fibers, regardless of the underlying pathogenic process. Although it has been reported to be specific for multiple system atrophy, it is also present in other neurodegenerative diseases including spinocerebellar ataxia, cerebrotendinous xanthomatosis, HIV-associated progressive multifocal leucoencephalopathy, leptomeningeal carcinomatosis, variant Creutzfeldt-Jakob or vascular diseases. Findings of cranial images in correlation with careful clinical examination narrows this differential diagnoses. Our patient has been diagnosed probable multiple system atrophy mostly depending on exclusion of other diseases.

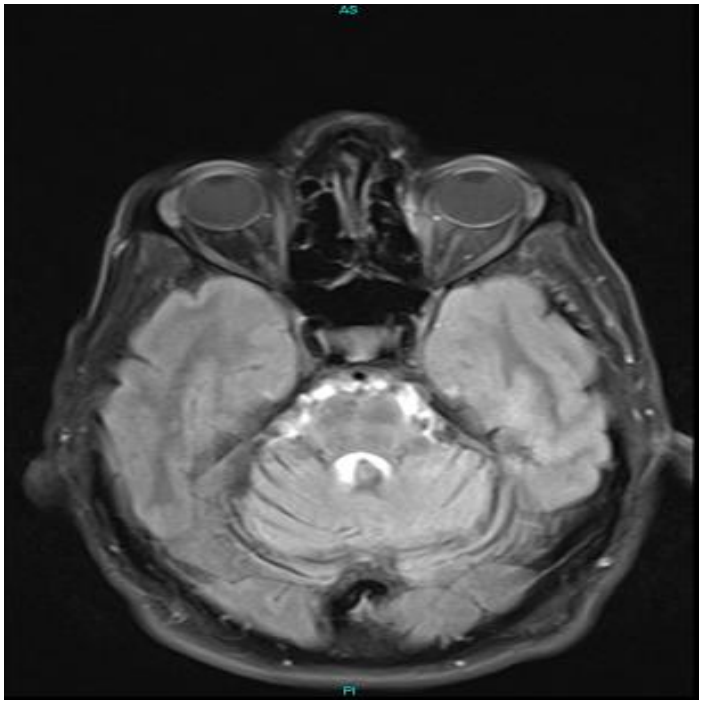

Figure 1

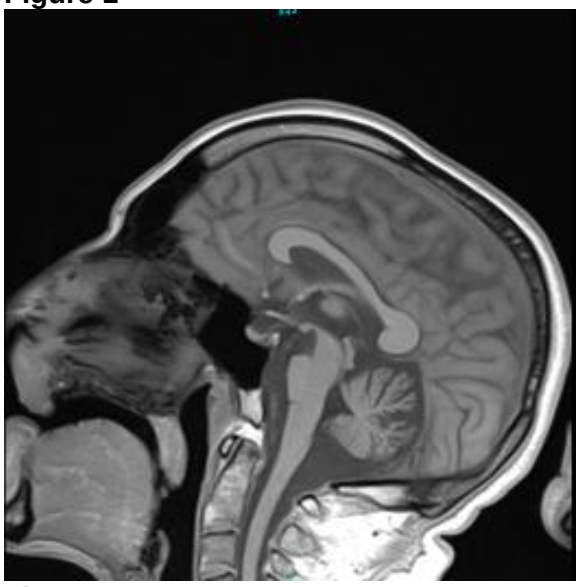

Figure 2

06a. Imaging & Biomarkers: structural MRI

ADPD5-2294
REFINING STRUCTURAL BRAIN CONNECTIVITY FOR ALZHEIMER'S DISEASE AND PARKINSON'S DISEASE CLASSIFICATION
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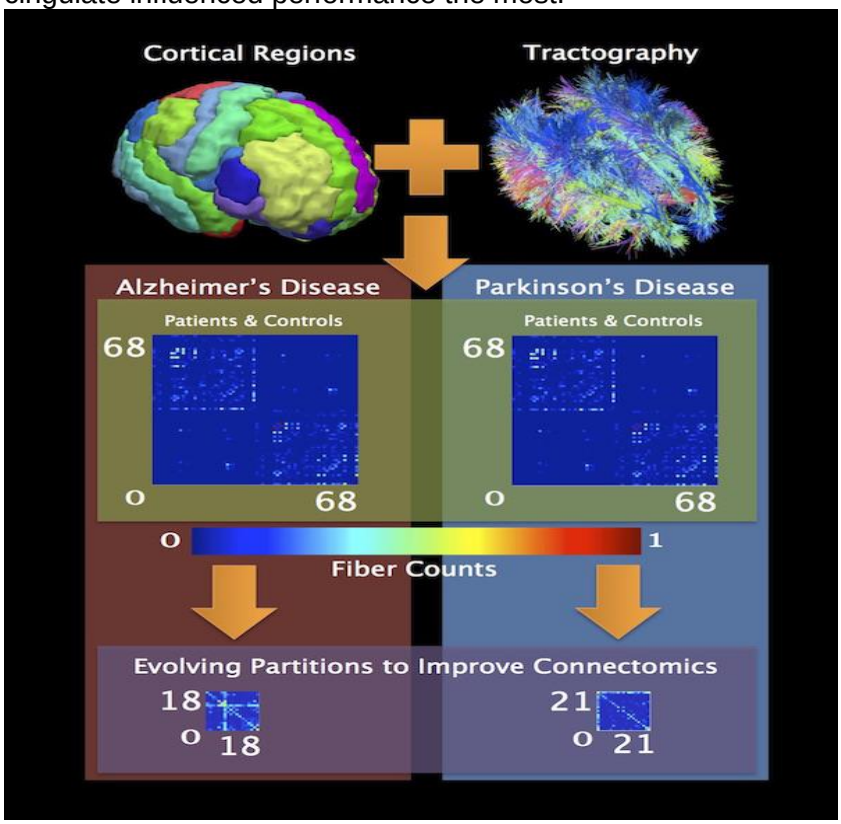
Objectives: We adaptively refine the partition of the brain for connectivity-based classification of Alzheimer's disease (AD) and Parkinson's disease (PD).
Methods: Structural and diffusion MRI at 3T was analyzed for (1) 47 AD patients and 51 age- and sex-matched controls from ADNI (73.8±7.5Y; 48F), and (2) 62 PD patients and 62 age- and sex-matched controls from PPMI (60.8±9.8Y; 45F). We computed anatomic connectivity among 68 cortical regions from the Desikan-Killiany atlas using tractography to compute the number of fibers between regions. Each individual's 68x68 connectivity matrix was normalized by fiber count and optimized by merging regions using a novel algorithm. "Evolving Partitions to Improve Connectomics"(EPIC).
Results: The baseline classification accuracy for AD vs. controls based on 68x68 connectivity matrices was 62.75%; EPIC-based matrices of size 18x18 yielded no significant change in accuracy. For PD vs. control classification, the initial accuracy was 73.38% and the 20x20 EPIC-derived architecture did not boost classification performance (see Figure). Significance was assessed by a corrected repeated k-fold cross-validation t-test.
Conclusions: In both AD and PD, the EPIC algorithm combines most of the cortical regions into a single connectivity component to remove redundant predictors. In PD, this composite region influenced classification accuracy the most; in AD the anterior cingulate influenced performance the most.



06a. Imaging & Biomarkers: structural MRI

ADPD5-2304

3T MRI VOLUMETRY OF WHOLE SUBSTANTIA NIGRA BY PROTON DENSITY, AND ITS NEUROMELANIN-RICH PARS BY T1-MAGNETIZATION TRANSFER, IN PARKINSON'S DISEASE

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Background

The neuromelanin-rich substantia nigra (SN) can be identified by T1 hyperintensity after magnetization transfer (MT) pulse (Ogisu 2013). In this pilot study, we implemented two volumetric sequences, PD and T1-MT, to measure respectively the whole SN and the neuromelanin-rich SN in Parkinson's disease.

Methods

17 patients with Parkinson's disease (8M,9F, 54.7±8.5yrs) and 14 normal controls (6M,8F, 50.8±14.1yrs) were studied by 3T MRI (Siemens Skyra). Whole brain T1 MPRAGE and two axial volumetric slabs, PD and T1-MT, perpendicular to the fourth ventricle floor, were acquired.
After multiplying the mean midbrain signal for a standard normalization factor, an individual signal threshold was obtained, and applied to segment the SN in every subject in both sequences. SN volumes were compared between patients and controls by two tailed t-test. In patients, SN volumes were correlated with the total UPDRS score (average 38.0±12.2) in off phases.

Results

While proton-density volumetry did not differentiate SN of patients (L 397.1mm^3±109,6, R 339.2+87,4) from controls (L 365.4±76.7, R 341.4±69.0), T1-MT volumetry significantly (p±45, R 138.3±52.9) from controls (L 239.2±42.8, R 221.5±55.8). Moreover, in this limited sample of patients there was a trend for correlation between decreased volume of neuromelanin-rich left SN and UPDRS-III score (Pearson -0.5, p=0.053).

Discussion

3T MRI with PD and T1-MT can identify substantia nigra and its neuromelanin-rich pars. Using a volumetric acquisition and a semiautomatic segmentation, the neuromelanin-rich pars differentiates patients from controls with statistical significance, higher than previously reported (Ogisu 2013).

06b. Imaging & Biomarkers: functional MRI

ADPD5-1015

REGIONS OF CEREBRAL HYPOPERFUSION RELATED TO ORTHOSTATIC HYPOTENSION ARE STRONGLY ASSOCIATED WITH DOMAIN-SPECIFIC COGNITIVE IMPAIRMENT IN LEWY BODY DISORDERS

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Orthostatic hypotension (OH) is the most common dysautonomia associated with Lewy Body Disorders (LBD). The contribution of OH to cognitive impairment in LBD, however, remains uncertain. **Objective:** To examine the interrelationships of cerebral blood flow (CBF), cognitive function, and OH. **Methods:** Fifteen LBD patients (76.3±6.9 years, 4 women) underwent 3T MRI to quantify CBF using arterial spin labeling. CBF estimates were also performed on matched adults with small vessel disease (SVD) to establish group differences. OH was quantified as the change in systolic blood pressure (delta-SBP) between supine and standing postures. Cognitive function was assessed by the Montreal Cognitive Assessment (MoCA), Rey-Osterrieth Complex Figure Test, Digit Symbol Substitution Test and Wisconsin Card Sorting Task. **Results:** Compared to SVD, LBD exhibited lower grey matter CBF (46±12 vs. 59±21 mL/100g/min, p=0.047), but similar global cognitive status (MoCA: 19.1±4.8 vs. 22.8±7.0, p=0.104). Within LBD, a voxel-wise relationship between delta-SBP and CBF identified four regions of interest. In the right lingual gyrus and bilateral precuneus, as well as the left frontal and precentral gyri, a greater SBP drop was associated with lower CBF ($p<0.001$ [uncorrected], $k_E \geq 8$ voxels). Lower CBF within the identified regions was associated with poorer cognitive performance (false discovery rate, $q \leq 0.05$), as follows: lingual (visuospatial, attention); precuneus (visuospatial, executive function); frontal (visuospatial, executive function); and precentral (visuospatial, attention, executive function). **Conclusions:** Domain-specific cognitive impairment was strongly related to regionally-specific cerebral hypoperfusion. These findings provide new insight into the relevance of OH on cognitive function in LBD and the salient brain regions involved.

06b. Imaging & Biomarkers: functional MRI

ADPD5-1232
DOES DORSAL STRIATUM MEDIATE STIMULUS-RESPONSE LEARNING OR DECISION-MAKING?

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We tested the prevalent contention that dorsal striatum (DS) mediates later-stage stimulus-response learning when responses become automatic or habitual. Using functional magnetic resonance imaging, we examined striatal brain activity during later-stage stimulus-response learning, once performance accuracy was greater than 90%. Seven males and seven females (mean age 22) learned to associate abstract images with left or right button-presses during an initial study phase. These pairings were reinforced by feedback in Session 1 and practiced without feedback in Session 2. Session 3 measured whether stimulus-response associations had achieved habit status. DS activity correlated with stimulus-response events only during Blocks 1-3 of Session 1 when response times and accuracy suggested a level of deliberation in responding. No significant DS activity occurred for Blocks 4-12 or in Session 2 when responses times and accuracy had reached plateau, though stimulus-response associations had not reached automaticity in Session 3.

We conclude that DS underlies decision-making that continues to require deliberation and not stimulus-response learning or habit formation. DS activation ceased to occur in late stages of Session 1 and in Session 2, when responding was fast and highly accurate but *before* relations became automatic based on performance in Session 3. In Parkinson's disease, DS is significantly dopamine depleted. Increasingly, DS is shown to mediate cognitive functions. Elucidating DS-mediated cognition improves our understanding of cognitive dysfunction in Parkinson's disease. These results clarify the cognitive profile in Parkinson's disease and guide dopaminergic therapy. Indeed, learning seems spared but decision-making is impaired, reviewing cognitive studies in PD.

06b. Imaging & Biomarkers: functional MRI

ADPD5-1256

DORSAL STRIATUM MEDIATES COGNITIVE FLEXIBILITY, NOT COGNITIVE EFFORT: AN EVENT-RELATED FMRI STUDY IN HEALTHY YOUNG ADULTS

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Whether dorsal striatum (DS) mediates cognitive flexibility or cognitive effort is unclear due to a pervasive confound. Neuropsychological tasks with greater cognitive flexibility demands require increased cognitive effort. We manipulated cognitive flexibility and effort separately to specify the role of DS in decision making.

Sixteen healthy young adults completed a number Stroop task, measuring functional magnetic resonance imaging data. Participants selected the physically larger number of a pair. Smaller physical size differences between a number pair increase cognitive effort for the discrimination but not demand for flexibly shifting attention or responding. We also investigated the effect of conflict between the physical and numerical dimensions of targets *(e.g.,* **2** in larger print, compared to 6 in smaller print). Selections in this incongruent case are more cognitively effortful but they also require flexibly shifting attention to physical size, suppressing conflicting responses related to numerical magnitude. Both increased cognitive flexibility and effort yield slowed and more error-prone responses. The aim was to determine whether DS differentially mediates cognitive flexibility and cognitive effort, however.

As expected, behavioural interference effects occurred for both increased cognitive flexibility and cognitive effort. Despite similar magnitude interference, DS only mediated the interference arising due to increased cognitive flexibility requirements in the incongruent case. DS was not preferentially activated for smaller relative to larger physical size differences between number pairs.

DS underlies cognitive flexibility specifically, not merely cognitive effort.

Specifying DS-mediated functions increases understanding of cognitive dysfunction in PD and clarifies the impact of dopaminergic therapy on cognition in Parkinson's disease.

06b. Imaging & Biomarkers: functional MRI

ADPD5-1336

BRAIN STRUCTURAL AND FUNCTIONAL CHANGES AFTER ACTION OBSERVATION THERAPY IN PD PATIENTS WITH FREEZING OF GAIT

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Objective. To assess brain functional and structural changes following action observation therapy (AOT) in PD patients with freezing of gait (PD-FoG).

Methods. 23 PD-FoG patients underwent a 4-week (W4) rehabilitation training. Subjects were randomized into 2 groups: in AOT-group, therapy consisted of AO combined with practicing the observed actions; control-group performed the same training combined with landscape-videos observation. At baseline (T0) and W4, patients underwent: clinical/motor functional evaluations, 3D-T1-weighted and functional MRI. At T0, 15 controls performed the same MRI protocol. FMRI tasks consisted of: foot simple-movement; observation of videos showing a man with FoG; motor-imagery in the same circumstances as observation task. Clinical/motor assessments were repeated at week8 (W8).

Results. At W4, both groups showed reduced FoG severity and walking-speed improvement, with AOT-group showing additional UPDRSIII, balance, and quality of life improvements. At W4, AOT and the control-group showed increased grey matter (GM) volume of bilateral parietal regions and primary motor cortex, respectively. FMRI showed that AOT was associated with increased recruitment of primary sensorimotor/premotor cortices, mirror neuron system (MNS) and caudate nucleus bilaterally during simple-motor and motor-imagery tasks. At W4, control-group showed reduced recruitment of primary sensorimotor areas and parietal regions during all tasks. Only in AOT, functional brain changes were associated with clinical improvements at W4 and predicted clinical evolution at W8.

Conclusions. AOT has a positive additional effect on walking ability recovery of PD-FoG patients. In PD, AOT promotes brain structural/functional plasticity of both the primary sensorimotor and MNS.

Funding. Jacques and Gloria Gossweiler Foundation.

06b. Imaging & Biomarkers: functional MRI

ADPD5-1492

CEREBRAL PERFUSION IN PRODROMAL AND NON-PRODROMAL DEMENTIA WITH LEWY BODIES: AN ARTERIAL SPIN LABELING STUDY.

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Objectives

In the scope of dementia with Lewy bodies (DLB), brain functional analyzes aim to explain how the brain activities adapt to the neural degeneration. Still, functional disturbances at the early stage of the pathology are poorly understood. Since they may impact the diagnosis and medical care, we investigate how much cerebral perfusion, as a possible biomarker, is early affected and reflects cognitive decline.

Methods

Twenty-eight prodromal (mild cognitive impairment) patients with DLB, 10 DLB patients with dementia, and 12 healthy controls received a multimodal magnetic resonance imaging including a 6 minutes resting-state pulsed arterial-spin labeling sequence. Spatially normalized cerebral blood flow maps were corrected for age and global mean value then analyzed using voxel-wise ANOVA.

Results

We showed that prodromal patients did not significantly differ from healthy controls, although we observed a tendency to a diffuse increased perfusion ($p<0.001$, uncorrected). Non-prodromal patients differed from healthy controls by a hypo-perfusion in the left insula ($p<0.05$, FWE-corrected). Non-prodromal patients also showed less perfusion than prodromal patients in the middle frontal gyrus, post-central gyrus and superior temporal sulcus ($p<0.05$, FWE-corrected).

Conclusions

Cerebral perfusion distinguishes prodromal from patients with dementia. The tendency of a higher perfusion in prodromal patients might reveal a compensatory phenomenon in these slightly affected patients. Patients with dementia showed a decreased perfusion in other brain parts than the areas usually reported as altered in Alzheimer disease. Therefore, cerebral perfusion imaging might be suitable for clinical and scientific investigations.

06j. Imaging & Biomarkers: PET - other

ADPD5-0293

DIFFERENTIAL DIAGNOSIS OF PD AND PARKINSON PLUS SYNDROME BY 18F-FP-(+)-DTBZ PET IMAGING

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Objectives: The diagnosis of Parkinson's disease (PD) and Parkinson plus syndrome (PP), including multiple system atrophy (MSA), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS), is based on clinical symptoms. To develop an objective tool to distinguish PD from PP is critical. We have proved that positron emission tomography (PET) imaging with ^{18}F-FP-(+)-DTBZ, a novel radiotracer targeting vesicular monoamine transporter 2 (VMAT2), may sever as a potential marker in investigating the monoaminergic integrity in PD. Our previous studies also suggested that the regional K1 obtained by early-phase ^{18}F-FP-(+)-DTBZ PET imaging is highly correlated with regional cerebral blood flow.
The objective of this study is to evaluate the capability of ^{18}F-FP-(+)-DTBZ PET imaging in the differential diagnosis of PD and PP by the early-phase (brain perfusion) and delayed-phase (monoaminergic integrity) imaging.
Methods: We enrolled 22 patients with MSA-parkinsonism type (MSA-P), 39 PSP patients, 16 CBS patients, 75 PD patients and 20 age-matched healthy subjects (HC). The regional standardized uptake volume ratio (SUVR) was calculated with occipital as reference from MRI-based spatially normalized ^{18}F-FP-(+)-DTBZ.
Results: In all subjects, PSP patients had the lowest VMAT2 availability in bilateral caudate, anterior putamen and substantia nigra (SN). The delayed-phase SUVRs of the striatum and SN had no difference between PD and MSA-P group. Nevertheless, the early-phase SUVRs of ^{18}F-FP-(+)-DTBZ in contralateral caudate and putamen in MSA-P patients were significantly lower than those of the PD group.
Conclusion: The dual imaging of ^{18}F-FP-(+)-DTBZ PET could provide more information and power to distinguish PD from PP.

06j. Imaging & Biomarkers: PET - other

ADPD5-0605

QUANTITATIVE EVALUATION OF THE SEVERITY OF PD BY [11C]CFT PET IMAGING AND SPM ANALYSIS

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Objective To investigate the clinical value of [^{11}C]CFT PET in the diagnosis and assessing the severity of PD.
Methods Thirty-eight patients with PD at various Hoehn & Yahr (H&Y) stages were included and underwent a [^{11}C]CFT PET scan. The correlation between [^{11}C]CFT uptake and Unified Parkinson's Disease Rating Scale part III (UPDRS III) of PD patients was evaluated by calculating Pearson's regression coefficient. Statistical Parametric Mapping (SPM) analysis was performed to compare the difference of dopamine transporer (DAT) distribution between early and advanced PD patients.
Results There was a significant reduction of [^{11}C]CFT uptake in the bilateral striatum of PD patients. There was a significant negative correlation between clinical scores of UPDRS III, rigidity, bradykinesia, posture, gait and [^{11}C]CFT uptake in the striatum. The SPM analysis of early PD (H&Y 1-2) patients, compared with the normal controls, revealed a significant and asymmetric decrease of [^{11}C]CFT uptake in the striatum, predominantly in the putamen and caudate nucleus contralateral to the onset limb, and the posterior area of ipsilateral putamen. There was a significant symmetric decrease of [^{11}C]CFT uptake in both putamen and caudate nucleus in advanced PD (H&Y 3-5) patients, compared with normal controls. Compared with early PD patients, the reduction of DAT was more severe in bilateral caudate nucleus and the ipsilateral putamen in the advanced PD patients.
Conclusions [^{11}C]CFT PET can serve as a suitable biomarker to represent the severity of PD in early and advanced stages.

06j. Imaging & Biomarkers: PET - other

ADPD5-0854

CONSTRUCTION AND EVALUATION OF A NEW TEMPLATE-BASED SPATIAL NORMALIZATION FOR 18F-FP-(+)-DTBZ (AV-133) IMAGING

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Objectives
18F-FP-(+)-DTBZ (AV-133) is a PET VAMT2 radiotracer for studying dopaminergic nerve-terminal integrity. The summed AV133 images acquired from the early time frames can provide estimate for relative perfusion information. The aim is to construct and evalute a new tempate from the early time frame images for 18F-DTBZ quantitation.
Methods
Seventy-three subjects were divided into two cohorts: 18F-FP-(+)-DTBZ images from the first cohort (6 HC and 25 PD) for constructing templates, and from the second cohort (11 HC and 31 PD) for validation. Four templates were constructed including normal (HC_{tmp}), PD patients (PD_{tmp}), all subjects (MIX_{tmp}), and perfusion ($pAV\text{-}133_{tmp}$). Each subject's MRI images were first coregistered to PET images. Then, the MRI images were normalized to MNI template (MNI_{tmp}), and resulted parameters were applied to coregistered PET images to obtain the spatially normalized PET images. The four templates were constructed from the spatially normalized PET images. The performance of the four PET templates were evaluated in terms of template-based quantitation. Occipital was selected as a reference to compute standardized uptake value ratios (SUVR) within target VOIs, including caudate, anterior and posterior putamen. The percentage difference of SUVR within each target between the four PET templates and MNI_{tmp} .
Results
The pAV133 template generated the minimal and stable SUVR %difference among all PET templates as compared to MNI_{tmp}.
Conclusions
The pAV-133 template of AV-133 imaging shows better performance in stable and accurate quantitation, therefore is potential for clinical applications.

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-0507

THE DEGREE OF CARDIAC MIBG UPTAKE IS CORRELATED WITH THAT OF CARDIAC SYMPATHETIC DENERVATION IN PATHOLOGICALLY-VERIFIED LEWY BODY DISEASE

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Objective: The aim of this study is to quantify the degree of cardiac sympathetic denervation in pathologically-veirfied Lewy body disease (LBD) and to examine the relationship between the degree of cardiac sympathetic denervation and cardiac MIBG uptake.

Methods: Twenty-three subjects with pathologically-verified LBD (17 men and six women, mean age at death: 77.5±6.9 years) who were performed MIBG cardiac scintigraphy in life, were enrolled in this study. One subject with multiple system atrophy and one Alzheimer's disease were served as controls. The sections of the left ventricular anterior wall from the specimens were immunostained with anti-thyrosine hydroxylase (TH) and anti-neurofilament (NF) antibodies. We quantified the immunoreactive areas of the residual cardiac nerve axons and examined the relationship between the degree of cardiac nerve axons and H/M ratios on MIBG cardiac scintigraphy.

Results: 1) Cardiac MIBG uptake in the early and delayed phases were reduced in 90.9% and 95.7% of the subjects with LBD, respectively. 2) The area of TH-immunoreactive axons was correlated with the degree of cardiac MIBG uptake both in early (correlation coefficient (r) =0.57, $p<0.01$) and delayed ($r=0.54$, $p<0.01$) phases. The area of NF-immunoreactive axons was correlated with the degree of cardiac MIBG uptake both in early ($r=0.65$, $p<0.01$) and delayed ($r=0.58$, $p<0.01$) phases.

Conclusions: This study confirms that MIBG cardiac scintigraphy is a useful imaging tool to make a clinical diagnosis of LBD and can assess the degree of cardiac sympathetic denervation.

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-0550

PARKINSON'S DISEASE IS DISTINGUISHABLE FROM OTHER RELATED NEURODEGENERATIVE DISORDERS ON 123-I-MIBG MYOCARDIAL SCINTIGRAPHY

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Objectives: The aim of this study was to investigate the diagnostic significance of 123-I-metaiodobenzylguanidine(MIBG) in differentiating PD from other related neurodegenerative diseases.
Methods: In total 718 patients were recruited based on their medical records, including 530 patients in the PD group(88 patients with DLB, 58 patients with Hoehn & Yahr(H&Y) stage 1(PD1), 106 patients with stage 2(PD2), 230 patients with stage 3(PD3), and 48 patients with stage 4(PD4)), 42 patients with Alzheimer's disease(AD), 19 patients with corticobasal syndrome(CBS), 33 patients with multiple system atrophy(MSA), 41 patients with progressive supranuclear palsy(PSP), 24 patients with normal pressure hydrocephalus(NPH), 18 patients with essential tremor(ET), and 13 patients with vascular parkinsonism and dementia(VP). SPECT images were obtained at 30 minutes(early image) and four hours (delayed image) after injection, and the H/M ratio, and washout rate were evaluated.
Results: The H/M ratios for the early and delayed images were compared for each disease using a one-way ANOVA(Tukey's test) . The H/M ratios of PD group was significantly lower than those in other diseases. The upper confidence limit of 99% was 2.17 on the early images, and 2.03 on the delayed images. A two-way ANOVA(Bonferroni test) showed a significantly lower delayed H/M ratio than early H/M ratio in the PD group, but not in the other groups. In the PD group, the H/M ratio decreased in the order of PD1, PD2, PD3, DLB and PD4.
Conclusions: The present findings indicate that MIBG scintigraphy can be used to clearly differentiate PD patients from those with other related disorders.

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-0553

TIMING OF ABNORMALITIES ON CARDIAC 123I-MIBG SCINTIGRAMS IN PATIENTS WITH DEMENTIA WITH LEWY BODIES

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Objectives: Dementia with Lewy bodies(DLB) is characterized clinically by dementia, fluctuating consciousness, parkinsonism and hallucinations. This study was undertaken to investigate the relationship between abnormal MIBG scintigram findings and parkinsonism.Methods: Eighty-eight DLB patients, 56-90 years of age, with a MMSE score of 21.3±5.5, who underwent MIBG scintigraphy were enrolled. The subjects were divided into two groups, 65 patients with parkinsonism(DLB-P), and 23 patients without parkinsonism(DLB-N). SPECT images were obtained at 30 minutes(early image) and four hours (delayed image) after injection, and the H/M ratio, and washout rate were evaluated. Results: The duration of disease until the MIBG examination was not significantly different between the DLB-P and DLB-N groups. The H/M ratios on the early and delayed images, and washout rate in the DLB-P and DLB-N groups were as follows: 1.72±0.30, 1.62±0.62 (NS), 1.45±0.33, 1.30±0.21($P<0.05$), and 47.7±7.0, 50.3±4.6($P<0.05$), respectively. The frequency of visual hallucinations, non-visual hallucinations, delusions, and REM sleep behavior disorder (RBD) was not significantly different between the groups.Discussions and Conclusions: The decreased H/M ratios on delayed images and increased washout rates in the DLD-P group suggest that the presence of parkinsonism may be related to a decreased H/M ratio. However, the fact that 23 patients with DLB(26%) showed a decreased H/M ratio prior to the appearance of parkinsonism indicates that the other factors must be considered. Further investigation is therefore necessary to clarify this issue.

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-1017

IOFLUEPANE (123I) UPTAKE AND COGNITIVE DECLINE IN PATIENTS WITH PD.

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Background: PD are known to affect not only motor function but also cognitive ability. On the other hand, there is no useful measurement of cognitive decline other than neuropsychological examinations. Recent studies showed that iofluepane (^{123}I) injection is diagnostically effective in patients with movement disorders and dementia, and that striatal dopamine transporter binding of iofluepane (^{123}I) provides an accurate and highly sensitive measure of dopamine degeneration. However, it is uncertain whether information on dopamine transporter has role in describing cognitive decline in Parkinson's disease.
Objective: To elucidate the relationship of cognitive function and dopamine degeneration.
Method: A series of 9 patient with Parkinson's disease were included. Disease duration, age, and the maximum value of modified Hoehn and Yahr staging score (H-Y score) were obtained to know disease progression and disease burden. To measure cognitive performance, Mini-Mental State examination (MMSE) and Addenbrook's cognitive examination-revised (ACE-R) were performed. Brain MRI were performed to exclude cerebral vascular diseases or other causes of Parkinsonism. In addition, we analysed striate binding ratio (SBR), which is obtained from iofluepane (^{123}I)-SPECT, and correlation coefficient were calculated.
Result: SBR showed strong positive correlation to ACE-R ($r=0.77$, $p<0.05$) and MMSE ($r=0.73$, $P<0.05$). We also found strong negative correlation to disease duration ($r=-0.80$ $p<0.05$) but there were no correlation to H-Y score.
Conclusion: Iofluepane (^{123}I)-SPECT is possibly useful to know the disease progression and SBR will help to confirm the possible decline of cognitive performance in patients with Parkinson's disease.

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-1020

[(123)I]FP-CIT SPECT AND [(123)I]MIBG SCINTIGRAPHY BETWEEN LEWY BODY DISEASE AND OTHER NEURODEGENERATIVE DISORDERS

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Objectives: To compare myocardial scintigraphy imaging using ^{123}I-metaiodobenzylguanidine (MBG) scintugraphy and striatal dopaminergic imaging using N-(3-fluoropropyl)-2β-carbomethoxy-3β-(4-^{123}I-iodophenyl)nortropane (FP-CIT) single photon emission computed tomography (SPECT) in patients with Lewy body disease (LBD).
Methods: Twenty patients (Parkinson disease (PD);n=5, Dementia with Lewy bodies (DLB); n=5, idiopathic REM sleep behaviour disorder (RBD);n=4, Alzheimer disease (AD);n=3, Progressive supranuclear palsy (PSP);n=3) were prospectively investigated. All patients performed both methods for differential diagnosis between LBD and other neurodegenerative disorders.
Results: There was no significant difference between these two methods in patients with LBD. The overall sensitivity, specificity, accuracy, positive and negative predictive values in LBD were 90%, 92%, 90%, 90%, 90%, respectively for ^{123}I-MIBG scintigraphy. For ^{123}I-FP-CIT SPECT, the overall sensitivity, specificity, accuracy, positive and negative predictive values in LBD were 90%, 58%, 72%, 70%, 78%, respectively.
Conclusions: LBD usually present both myocardial and striatal dopaminergic impairments. ^{123}I-MIBG scintigraphy method provides a very high diagnostic accuracy for differentiating between LBD and other neurodegenerative disorders, which is clearly superior to the accuracy of ^{123}I-FP-CIT SPECT. The combination of ^{123}I-MIBG scintigraphy and ^{123}I-FP-CIT SPECT imaging may lead to a improvement in distinguishing LBD from other neurodegenerative disease.

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-1139

INITIAL NEGATIVE DAT SPECT IN DEMENTIA WITH LEWY BODIES: A DLB SUBTYPE?

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Objective

Low striatal dopamine transporter (DAT) binding uptake on SPECT imaging is a suggestive feature in diagnostic criteria for dementia with Lewy bodies (DLB). Little is known about patients meeting criteria for probable DLB with normal DAT-scans (clin+DAT-).There has been no record of follow-up scans in these patients. This study aims to describe these patients and compare them to DLB patients with positive DAT-scans (clin+DAT+).

Methods

Sixty-seven DLB-patients with DAT-scans ([^{123}I]FP-CIT SPECT) available were selected from the Amsterdam Dementia Cohort. Stable diagnoses were confirmed based on clinical follow-up. Clin+DAT- patients were checked for follow-up imaging. DAT-scans were evaluated independently by two nuclear medicine physicians. Clin+DAT- patients were matched for age and disease duration 1:2 with clin+DAT+ patients. Cross-sectional data on cognition, extrapyramidal signs, hallucinations, fluctuations, orthostatic hypotension and REM-sleep behavior disorder (RBD) were compared.

Results

Seven DAT-scans were reported negative (10.4%). 43% Of clin+DAT- patients presented with extrapyramidal signs (vs 64% of clin+DAT+ patients), 57% with orthostasis (vs 36%), 86% with hallucinations (vs 42%), 57% with fluctuations (vs 50%) and 100% with (suspected) RBD (vs 42%). MMSE scores were comparable. In five clin+DAT- patients, a second DAT-scan was performed. All follow-up scans were abnormal, consistent with DLB diagnosis.

Discussion

This study is first to describe a subset of DLB patients with initial negative DAT-scans, which turned positive during disease progression. We hypothesize that clin+DAT- cases could represent a subtype of DLB with possibly a different severity or spread of alpha-synuclein pathology. Recognizing this phenotype may be important for clinical management.

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-1289

METABOLIC ALTERATIONS IN THE RIGHT HIPPOCAMPUS DETECTED IN VIVO BY 1H MR SPECTROSCOPY IN THE MPTP MODEL OF PD

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Objective: To determine the effects of MPTP-induced nigrostriatal neurodegeneration on the biochemical profile in the hippocampi (Hpc) in mice.
Methods: One year old male C57Bl/10Tar mice were injected *i.p.* with MPTP (40 mg/kg) or equivalent vehicle volume. One week later brains of the control and MPTP-injected mice (n=10/group) were scanned with Bruker BioSpec 70/30 Avance III system equipped with 7T magnet. Structural MR images were acquired with T2-weighted TurboRARE sequence. Spectroscopic recordings were then carried out in two volumes of interest (1.6 x 1.8 x 2.7 mm^3 each) comprising left and right Hpc. Metabolite concentrations were estimated with LC model and expressed as ratios to total creatine concentration.
Results: Compared to controls, MPTP mice displayed significantly lower glutamate (Glu) and combined glutamate+glutamine (Glx) resonance signals and slightly lower glycerophosphocholine + phosphocholine (GPC+PCh) signals in the right Hpc. However, no significant differences were observed in the left Hpc.
Conclusions: Reduced Glu and GPC+PCh in the right Hpc may signify deficits in neuronal metabolism and/or firing rate and possible neuronal shrinkage toward the right hippocampus.

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-0418

COMPLEMENTARY ROLE OF 99MTC-TRODAT-1 SPECT AND FDG-PET CT: REDEFINING THE MANAGEMENT OF PATIENTS WITH PARKINSON'S DISEASE AND OTHER MOVEMENT DISORDERS.

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Objectives: To investigate the potential usefulness of 99mTc-TRODAT-1 SPECT and FDG PET CT imaging in the evaluation of subjects with suspected or diagnosed parkinson's disease and other movement disorders with the following objectives:

· Documenting the complementary role of 99mTc-TRODAT-1 and FDG PET CT imaging in various neurodegenerative movement disorders
· Early diagnosis of parkinson's disease
· Pre clinical diagnosis of parkinson's disease in high risk groups
· Differentiation of mono symptomatic tremor of parkinson's disease from other causes
· Differential diagnosis of different parkinson plus syndromes

Methods: 200 patients with movement disorders and 50 age matched healthy volunteers were evaluated. 99mTc-TRODAT-1 was prepared from a lyophilized kit and SPECT imaging performed using a double-head camera. Uptake in the striatum and its sub regions was calculated and compared with that of healthy volunteers. 150 patients were evaluated by FDG PET CT on a separate day by GE Discovery STE PET CT system.

Results: Reduced striatal uptake of 99mTc-TRODAT-1 was found in idiopathic parkinson's disease subjects with a significant rostro-caudal gradient. Significant reduction of 99mTc-TRODAT-1 was found in atypical parkinson's disease and other parkinson plus syndromes. FDG PET CT images showed variable striatal FDG uptake in subjects suspected or diagnosed as idiopathic Parkinson's disease. However, different patterns of cortical/sub cortical hypo metabolism was noted in parkinson plus syndromes.

Conclusion: 99mTc-TRODAT-1, in conjunction with FDG PET CT, may serve as a useful imaging agent for the early detection of parkinson's disease and differentiation of different parkinson plus syndromes.

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1348

CORPUS CALLOSUM DAMAGE AND MOTOR FUNCTION IN PD

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Objective: To investigate corpus callosum (CC) damage and its relationship with motor impairment in PD.

Methods: We enrolled 173 PD (98 with Hoehn and Yahr score [HY]=1-1.5, 37 with HY=2-2.5, 29 with HY=3-3.5, 9 with HY=4-5) and 39 healthy controls (HC). Diffusion tensor (DT) MRI tractography was performed to obtain the CC and its main three partitions: CC-genu, CC-body, and CC-splenium. Fractional anisotropy (FA) and mean diffusivity (MD) were measured. Age-adjusted group comparisons were assessed. Pearson's correlations were used to explore the relationship between CC DT MRI metrics and UPDRS III score.

Results: All PD showed decreased FA and increased MD of the whole CC and its partitions. Such a damage was more marked with increasing PD severity, being only mild in PD with HY=1-1.5 (prevalent in CC-body) and severe (similar in all CC partitions) at the later stages of the disease. UPDRS III score correlated significantly ($p<0.001$) with FA of CC, CC-genu, CC-body, and CC-splenium and MD of CC, CC-body, and CC-splenium.

Conclusions: PD is associated with damage to the CC that increases with disease worsening. In PD patients, the best predictor of CC deterioration of motor functions is the involvement of the CC-body, which includes the transcallosal motor tracts. Assessing CC alterations may improve the understanding of the pathogenetic mechanisms associated with motor impairment in PD.

Funding. Italian Ministry of Health (#GR-2009-1577482); Ministry of Science and Technology of the Republic of Serbia (#175095).

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1349

WHITE MATTER MICROSTRUCTURAL DAMAGE AND COGNITIVE IMPAIRMENT IN PARKINSON'S DISEASE

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Objectives: To investigate white matter (WM) damage and its relationship with cognitive impairment in Parkinson's disease (PD).

Methods: We enrolled 168 PD patients (105 without cognitive impairment [PD-ncog]; 48 with MCI [PD-MCI] and 15 with dementia [PD-DEM]), and 41 healthy controls (HC). PD and controls underwent a clinical and neuropsychological evaluation. Tract-based spatial statistics were used to perform a voxel-wise analysis of fractional anisotropy (FA) and mean diffusivity (MD), adjusted for subject's age. PD were compared with controls and between each other.

Results: All PD showed decreased FA and increased MD in the splenium of corpus callosum (CC), right frontal WM and internal capsule. In PD-MCI, WM damage spreads to anterior regions of CC, bilateral frontal, anterior temporal and parietal WM. WM damage increased in severity in PD-DEM, involving frontal and parietal WM . PD-DEM *vs* PD-ncog showed alterations in the body of CC There were no differences in DT MRI metrics in PD-DEM *vs* PD-MCI.

Conclusions: WM damage appears to be limited to the splenium of CC and to part of the right frontal WM in PD-ncog. WM damage in PD becomes more severe with worsening of cognitive performance. WM damage spreads in the anterior regions in PD-MCI, showing the maximum severity in PD-DEM patients. Assessing WM alterations may improve the understanding of the mechanisms associated cognitive impairment in PD

Funding. Italian Ministry of Health (#GR-2009-1577482); Ministry of Science and Technology of the Republic of Serbia (#175095).

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1364

BRAIN STRUCTURAL AND FUNCTIONAL ABNORMALITIES IN PARKINSON'S DISEASE PATIENTS WITH FREEZING OF GAIT

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Objective. To assess brain functional and structural alterations in Parkinson's disease patients with freezing of gait (PD-FoG).

Methods. T1-weighted, diffusion tensor (DT) MRI and resting state (RS) fMRI were obtained from 22 PD-FoG patients and 36 controls. Grey (GM) and white matter (WM) damage were assessed with SPM8 and FSL. RS fMRI data were analyzed using a model free approach investigating sensorimotor and cognitive networks. MRI differences between patients and controls, and the relationships between MRI and clinical/cognitive variables were assessed.

Results. Compared with controls, patients showed WM damage in the corpus callosum and cingulum, WM underneath frontal, parietal and occipital cortices and corticospinal tracts, bilaterally. GM volume loss was not detected in patients relative to controls. RS fMRI analysis showed that PD-FoG is associated with a decreased functional connectivity relative to controls: in the bilateral superior frontal gyrus and precuneus within the default mode network; in the bilateral insula, right cingulum, left thalamus and putamen within the ventral-attentional network; and in the left inferior occipital gyrus within the visual-associative network. More severe motor disability and lower cognitive scores were associated with greater WM damage and reduced functional connectivity.

Conclusions. PD-FoG patients showed a widespread pattern of WM damage and altered functional connectivity involving mainly the frontal and parietal regions. MRI alterations were related with patient clinical and cognitive profiles, supporting the theory of FoG as the result of poor integration between motor programming, visuo-spatial and attentional abilities.

Funding. Jacques and Gloria Gossweiler Foundation.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-0635
DELTA BAND EEG ACTIVITY IS INCREASED IN INCIDENT PD DEMENTIA
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Objectives: We previously showed that in PD, low EEG background rhythm predicts future decline in cognition. Our objectives: 1) determine if spectral EEG measures changed with incident PD dementia, and 2) compare the degree of change among the spectral EEG measures.
Methods: 71 PD subjects participated as part of Arizona Study of Aging and Neurodegenerative Disorders which utilizes annual neuropsychological and biennial EEG evaluation. We excluded subjects with dementia at baseline and EEG examinations with deep brain stimulation and barbiturate or benzodiazepine use. Initial and follow-up EEG (mean 3.9 years apart) were analyzed for EEG measures of background rhythm frequency (BRF) and relative power (percentage) in delta (2.5-4 hz), theta (4-8 hz), alpha (8-13 hz), and beta (13-30 hz) bands. Neuropsychological evaluation was used to determine PD dementia by movement disorder society criteria. Change from baseline among subjects with incident dementia was compared to that of subjects without incident dementia by using the two-sample *t* test.
Results: Delta increased by a mean of 6.9 % (SD 8.3) among the 13 subjects who developed dementia, versus only 1.4 % (SD 4.6) among the 58 subjects who did not develop dementia (P=.002). The change in BRF, theta, alpha, and beta did not differ between the same groups.
Conclusions: Delta bandpower showed a significant specific increase among spectral EEG measures in those subjects that developed dementia among spectral EEG measures. In addition to being used as a potential biomarker, the pathophysiological significance of EEG delta activity in PD should be studied.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-1360

ELECTRO-PHYSIOLOGICAL DIFFERENTIAL DIAGNOSTIC MARKER FOR AD AND DLB

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Objective
Demonstrate the applicability and properties of an electro-physiological based biomarker for differential diagnosis of AD and DLB.
Methods
In a database, EEGs from 209 healthy (NRM), 22 individuals with DLB, 26 individuals with PDD, and 560 clinical subjects of other types than PDD and DLB, have been entered. Applying statistical pattern recognition (SPR) to a large set of EEG features classifiers contrasting clinical cohorts in a pairwise manner are developed. For each classifier, the SPR finds an optimal combination of the EEG features which separate the two groups under consideration. The accuracy, sensitivity, and specificity of each classifier are estimated using 10–fold cross validation.
Results
The receiver operating characteristic (ROC) curves for the “NRM vs clinical“ classifier system have the following qualities: area under curve (AUC) is 0.91(0.04), specificity (SPE) is 0.85(0.06), sensitivity (SEN) is 0.85(0.04), and the accuracy (ACC) is 0.85(0.05). The standard deviation is given in parentheses. For the “PDD/DLB vs other clinical types“ classifier system the results are: AUC=0.92(0.01), SPE=0.84(0.05), SEN=0.88(0.03), and ACC=0.86(0.02). Applying the same methodology to the DLB and PDD groups showed no separation.
Conclusion
We have introduced an EEG based biomarker for PDD and DLB combined into one group that shows good sensitivity and specificity and it is argued that this method can be a good addition to available methods. EEG is a standard method that is inexpensive and readily available and is therefore attractive for use in various settings.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-1532
INVESTIGATIONS INTO SYNTACTIC PROCESSING AND ITS RELATIONSHIP TO GENERAL COGNITION IN PD
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1. Objective:
Recent work has demonstrated language deficits in PD. Friederici et al. (2003), for example, reported reduced P600 amplitudes -- an event related brain potential (ERP) component related to syntax -- to word category violations in speech in PD, and concluded that basal ganglia circuitry is crucial for late/integrative syntactic processes. We assess the generality of this conclusion by examining the processing of morphosyntactic violations during reading in combination with performance on an extensive neuropsychological battery.
2. Methods:
We recorded the EEG while individuals with PD (Mean MoCA=26.2) and age-matched controls read sentences containing morphosyntactic violations (and grammatical controls). We correlated P600 effect (violations minus controls) amplitudes and latencies with various neuropsychological measures.
3. Results:
Statistically indistinguishable P600 effects were observed in both groups. P600 parameters for both groups were significantly correlated with Executive Function/Working Memory (EF/WM) scores: higher scores were associated with earlier and larger P600 effects (PD, r=.8~.9; controls, r=.6~.7).
4. Conclusions:
We found no evidence for P600 effect amplitude reductions in individuals with PD with severity equal or greater to those in previous reports. Preliminary analyses suggest that in PD syntactic P600 amplitude and latency are better predicted by scores on tests of EF/WM than grammaticality judgment accuracy. This suggests that the link between the basal ganglia and syntactic processing is more complicated than presumed to date.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-1848

EEG FINDINGS IN DEMENTIA WITH LEWY BODIES, ALZHEIMER'S DISEASE AND DEMENTIA ASSOCIATED WITH PD.

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Objectives

We tried to elucidate the diagnostic value of conventional electroencephalogram (EEG) in the clinical overlap between dementia with Lewy bodies (DLB), dementia associated with PD and Alzheimer's disease (AD).

We used Grand Total EEG score (GTEs), a simple EEG scoring method.

Methods

20 patients with mild AD, 26 with DLB and 26 with PDD were studied with EEG. GTEs was calculated for every patient. The three groups were compared in terms of GTEs, age, neuropsychological assessmens, disease onset and duration, drug exposure, etc..

Results

We found a clear-cut difference in the GTEs between DLB and PDD. There was a difference in median GTE score of DLB, PDD and AD subjects.

Frontal intermittent rhythmic delta activity (FIRDA), was similar in DLB and PDD, but was absent in AD patients.

Conclusions

DLB and PDD showed marked electroencephalographic differences in term of GTEs. GTEs and FIRDA were significatively different between patients with DLB and PDD compared to AD, according to previous reports.

From a strictly clinical point of view, EEG with the GTEs may help to orientate clinicians towards the correct diagnosis and management of these dementia disorders.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-1981

SLEEP ALTERATIONS AS A BIOMARKER OF PARKINSON DISEASE.

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Background: Disturbed sleep is a frequent complain of patients with Parkinson Disease (PD; 1) but the relationship and underlying mechanisms remain unclear. The aim of our study is to understand how PD can impact sleep and to select the best sleep parameter as a biomarker for PD.
Methodology: Rats were injected bilaterally with AAV- α-Synuclein in Substantia Nigra and compared to AAV-GFP control animals (2). Both groups were implanted with electroencephalographic (EEG) and electromyographic (EMG) electrodes. Sleep architecture and spectral profile of the vigilance states were analyzed over 24h.
Results: Five weeks after injection, the AAV- α-Synuclein rats presented a fragmented sleep pattern compared to the AAV-GFP littermates with disruption of the spectral profiles. Moreover, these animals have an important increase of muscle tone during their sleep.
Conclusion: Together, our results demonstrate that AAV- α-Synuclein rats have similar alteration of their sleep pattern as PD patients and confirm the model as translationally relevant for PD. This model will then be used to characterize the development of sleep disturbances as an early marker of disease in correlation with α-Synuclein accumulation. In addition, our data highlight the importance of sleep alteration in PD and validate the potential of EEG/sleep characteristics to provide a valuable animal-clinical interface in PD.

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06q. Imaging & Biomarkers: MR spectroscopy

ADPD5-1503

REDUCTIONS IN BRAIN PHOSPHOCREATINE LEVELS ARE SIMILAR IN PD AND METHAMPHETAMINE DEPENDENCE

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Objectives: Accumulation of α-synuclein plays a critical role in early-onset PD. Overexpression of α-synuclein is toxic to neurons and depletes striatal dopamine, causing oxidative stress as well as mitochondrial damage (Kirik, 2002). Interestingly, methamphetamine, a potent psychostimulant, also produces significant overexpression and conformational changes of α-synuclein (Tavassoly, 2012). Further, converging evidence implicates a similar pattern of mitochondrial dysfunction associated with PD and methamphetamine toxicity. Thus, this study measured *in vivo* high energy phosphorus metabolites in methamphetamine users to compare profiles of oxidative phosphorylation between methamphetamine toxicity and PD.

Methods: Phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) data were acquired in 56 methamphetamine-dependent subjects (age=33±7) and 23 healthy comparison subjects (age=31±7). Chemical shift imaging was completed on a 3-Tesla scanner. Bilateral basal ganglia spectroscopic data were quantified using AMARES.

Results: In the basal ganglia, methamphetamine users had significantly decreased (-6.2%, p=0.001) phosphocreatine (PCr, an indicator of high energy phosphate buffer stores) levels compared to healthy controls. This finding was similar to prior reports of PD studies, in which reduced PCr levels in early and advanced PD were consistently noted.

Conclusions: These preliminary data are consistent with oxidative damage that has been reported in both PD and methamphetamine addiction. Interestingly, a recent large-scale epidemiologic study reported that the risk of Parkinson's disease is significantly higher in methamphetamine users (Callaghan, 2012), further supporting our findings. Due to the oxidative stress observed in PD and MA toxicity, neuroprotective agents may have benefits through a common pathophysiology. Further evaluation of novel interventions for maintaining high energy phosphate is warranted.

06q. Imaging & Biomarkers: MR spectroscopy

ADPD5-1758
INCREASED THALAMIC GABA LEVELS CORRELATE WITH PD SEVERITY
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Objectives: The loss of dopamine producing cells in PD leads to alterations in basal ganglia pathway firing patterns. We used Magnetic Resonance Spectroscopy (MRS) to determine if basal ganglia gamma-aminobutyric acid (GABA) levels are altered and related to disease severity in unmedicated patients.
Methods: Subjects were divided into two groups: PD patients (mild-to-moderate PD, n=18, age: 62.67±8.27 y) and controls (n=15, age: 59.67±8.62 y). Three PD subjects were medication-naïve while the rest withheld medication for at least 12 hours before testing. Disease severity was measured using the Unified Parkinson's Disease Rating Scale (MDS UPDRS-III). All subjects underwent MRS on a 3-T Siemens Trio MR scanner. J-edited GABA MRS data was acquired from 25 mm x30 mm x25 mm volumes of interest placed in the right thalamus and right striatum. GABA levels were quantified and expressed as ratios to creatine (Cr).
Results: GABA/Cr was significantly higher (p=0.018) in the thalamus of the PD group (0.31±06) compared to the control group (0.26±05). No significant group differences were seen in striatal GABA levels. A significant positive correlation was found between thalamus GABA/Cr and UPDRS-III scores (p=0.029, Spearman's rho =0.375).
Conclusion: The increased inhibitory output from the internal pallidal segment in the indirect pathway is responsible for hypokinetic symptoms in PD and this is reflected as higher GABA values detected in the thalamus by MRS. The correlation of thalamic GABA measured by MRS with UPDRS scores indicates that GABA MRS holds potential to be a non-invasive biomarker for assessing PD severity.

06r. Imaging & Biomarkers: other

ADPD5-0235
CLINICAL CORRELATES OF RAPID EYE MOVEMENT SLEEP WITHOUT ATONIA IN PARKINSON'S DISEASE
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Objective: Rapid eye movement (REM) sleep behavior disorder (RBD) is characterized by REM sleep without atonia (RWA) and is associated with Parkinson's disease (PD) progression.
Methods: We quantified tonic and phasic RWA by performing polysomnography (PSG) in 198 PD patients and correlated the findings with their clinical characteristics.
Results: All PD patients were categorized into quartiles of tonic and phasic RWA. Although none of the differences were significant, we found that patients with higher RWA tended to be older, had longer PD disease durations, were more likely to have RBD, had higher Hoehn & Yahr (H&Y) stages, took higher doses of parkinsonian medications, had poorer cognition, experienced worse quality of life, and had more severe sleep disturbances. After adjusting for age, sex, and PD duration, patients in the highest quartile of RWA were more likely to be severe PD patients (H&Y scale ≥3.0) compared to those in the lowest quartile.
Conclusions: These findings provide evidence that RWA was associated with PD severity, especially with regard to tonic muscle activity.

06r. Imaging & Biomarkers: other

ADPD5-0953

CEREBROSPINAL FLUID LEVELS OF YKL-40 PROTEIN IN DEMENTIA WITH LEWY BODIES.

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Objectives: to investigate the cerebrospinal fluid levels of YKL-40, an inflammatory biomarker, in Dementia with Lewy bodies (DLB) and prodromal DLB (prod-DLB), and its relationship with Alzheimer´s disease (AD) biomarkers in this group of patients.
Methods: We defined prod-DLB as mild cognitive impairment (MCI) with at least one suggestive feature of underlying synucleinopathy (Parkinsonism/REM-sleep behaviour disorder/visual hallucinations). We measured Ab42, total-tau, p-tau, and YKL-40 in CSF from 145 participants with amnestic MCI (aMCI, n=43), AD (n=57), DLB (n=15), prod-DLB (n=6) and cognitively normal controls (n=24). Biomarkers were compared between groups defined by clinical diagnosis or CSF-profile (tau/Ab42 ratio above or below 0.52)
Results: CSF YKL-40 was higher in prod-DLB patients compared to DLB (299.06 vs. 230.79, p=0.008). The prod-DLB group showed higher YKL-40 than controls (299.07 vs. 208.25, p=0.001) and a trend towards higher levels compared to aMCI (299.07 vs. 256.13, p=0.079). No difference was detected in YKL-40 between DLB patients and controls (p=0.20), although there was a trend towards lower levels in DLB compared to AD (230.79 vs. 258.36, p=0.079). Within the composite group DLB/prod-DLB, there were no differences in YKL-40 levels according to CSF-profile (tau/Ab42 ratio above or below 0.52, p=0.247). YKL-40 levels correlated with total-tau and p-tau in all groups.
Conclusion: YKL-40 is increased in CSF in prodromal DLB, suggesting that inflammation is an early process in the disease. This feature is also observed in AD and aMCI. The YKL-40 levels do not seem to be affected by the AD underlying co-pathology in the DLB/prod-DLB group.

06r. Imaging & Biomarkers: other

ADPD5-1042

PHOSPHOLIPID BINDING FOR ANALYSIS OF ERYTHROCYTE ALPHA-SYNUCLEIN AND DIAGNOSIS OF PARKINSON'S DISEASE

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A quantitative phospholipid-ELISA assay was developed to investigate the pathophysiology of alpha-Synuclein (a-Syn) expressed in red blood cells (RBC). The assay is based on the biochemical property of a-Syn to specifically bind acidic phospholipids for its capture from samples. We though to study whether RBC-expressed a-Syn, detected by its ability to bind phospholipids, may differ between patients with PD and healthy controls. Twenty one individuals with PD and fifteen healthy controls, with similar demographic features, were recruited to this study. Total and proteinase K-resistant a-Syn levels were determined in samples of packed red blood cells (PRBCs) by the phospholipid-ELISA assay.

The results indicated a significantly lower ratio of total-to-proteinase K-resistant a-Syn levels in PD patients than in the healthy control group. However, there was considerable overlap between the two groups. Of note, the higher levels of proteinase-K-resistant a-Syn detected in RBC from PD patients may support an involvement of systemic mechanisms in the pathophysiology of PD.

We concluded that the biochemical property of a-Syn that enables it to bind phospholipids and the occurrence of proteinase K-resistant a-Syn in PRBCs, provide promising tools for the diagnosis of PD.

06r. Imaging & Biomarkers: other

ADPD5-1074

CHARACTERISATION OF COGNITIVE DECLINE OVER 5 YEARS IN AN INCIDENT PARKINSON'S DISEASE COHORT: RESULTS FROM THE CAMPAIGN STUDY

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Objectives

Parkinson's disease (PD) is associated with cognitive deficits in memory, attention and executive function. However, little is known about the pattern of decline over time, which is important for selecting appropriate test outcomes for disease and symptom modifying treatment trials.

Methods

In this population-based cohort of incident PD patients, detailed neuropsychological assessments were performed at baseline, 3.5 years and 5 years from diagnosis. Participants were assessed with pentagon copying, phonemic and semantic verbal fluency and three CANTAB® tasks measuring memory (Spatial Recognition Memory (SRM), Pattern Recognition Memory (PRM)), and executive function (One Touch Stockings of Cambridge (OTS)). Participants also completed the MMSE®.

Results

MMSE® scores declined over five years (baseline, N=142; effect size =-0.93) as did pentagon and semantic fluency scores (effect sizes= -0.13 and -0.19 respectively). In the more cognitively-able participants who completed all tasks over all visits ('completers', n=71) there was minimal decline in MMSE® scores (effect size= -0.1) but significant decline in CANTAB® SRM (effect size= -0.38) and OTS (effect size= -0.33). Semantic fluency scores declined for completers (effect size= -0.16), although there was significant improvement in PRM.

Conclusions

A decline in SRM and OTS was detected in the more cognitively-able subgroup, suggesting that this group show impairments that are frontal-specific. These data suggest that although MMSE® is sensitive to decline in cognition in PD, it does not detect decline in more-able, non-demented patients. Findings are discussed in relation to depression, treatment and QoL outcomes.

06r. Imaging & Biomarkers: other

ADPD5-1104

PRELIMINARY DEMONSTRATION OF THE BIOMARKER POTENTIAL OF METAL ACCUMULATION MEASURED WITH QUANTITATIVE SUSCEPTIBILITY MAPPING (QSM) IN PARKINSON'S DISEASE

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OBJECTIVE

Determine the PD landscape of metal overload with QSM—a new MRI contrast—in relation to white matter (WM) alterations probed with DTI and grey matter loss with structural MRI, with investigation of its biomarker value.

METHODS

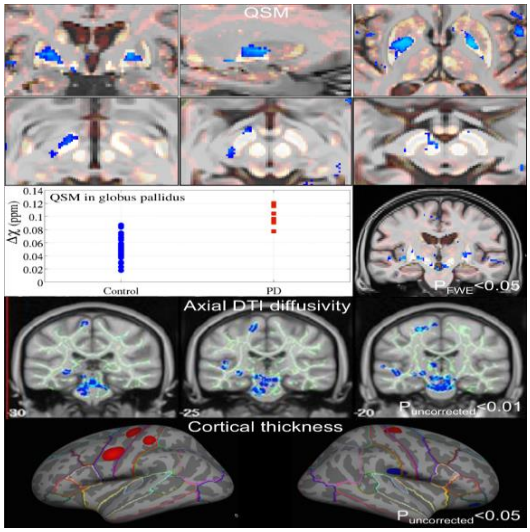
Eight PD patients—age = 63(9)—and thirty-four matched controls were recruited. All MRI measurements were performed in a Siemens Verio 3T scanner. T2 images were inspected to exclude vascular pathology. Structural MPRAGE scans were used for FreeSurfer-v5.3 cortical thickness analysis, SPM12b-based voxel-based morphometry and FSLv5.0-FIRST deep grey matter nuclei volumetry. Parametric DTI maps—obtained from an optimised diffusion-imaging scheme—were introduced in TBSS for a group-level evaluation of WM integrity. QSM—sensitive to metal overload (predominantly that from tissue iron), deoxygenated-blood products and calcifications—was calculated and analysed with *state-of-the-art* methods (Acosta-Cabronero et al. 2013).

RESULTS

Magnetic susceptibility was profoundly increased in PD in the globus pallidus (almost complete group separation, see plot). Strong abnormalities were also observed in the substantia nigra and red nucleus while the insular and motor cortices were also affected at $P_{FWE}<0.05$. Only statistically weak DTI (meso-striatal) and structural abnormalities (motor cortex) were found.

CONCLUSIONS

Metal deposition measured with QSM shows strong potential as an imaging biomarker for the diagnosis of PD unlike more established MRI measures which only show weak group-level changes.



06r. Imaging & Biomarkers: other

ADPD5-1369

CSF CATECHOLAMINES AS BIOMARKERS IN EARLY UNTREATED PD

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Objectives: Development of biomarkers for early PD continues to be an important area of research, with the eventual goal of identifying patients for early therapeutic intervention. Previous studies have suggested that certain catecholamines in the cerebrospinal fluid (CSF) might serve as biomarkers for PD. To further examine the potential use of CSF catecholamine levels as a biomarker for PD we studied both early untreated and treated PD patients.
Methods: CSF was obtained from normal controls and PD patients participating in several studies of aging and PD. Catecholamines and metabolites were purified from CSF using the alumina extraction method, separated by HPLC, and measured using electrochemical detection.
Results: Three groups of patients were studied: controls (N=40, mean age 63 years); never treated PD (N=20, mean age 63 years); treated PD (N=49, mean age 68 years). Treated PD patients had significantly higher DOPA, dopamine, norepinephrine, and DOPAC levels and untreated PD patients had significantly lower DOPAC levels (table).

	Controls	**PD no Treatment**	**PD Treated**
DA (pg/ml)	11.2 (17.3)	8.6 (5.5)	58.9 (76.3)*#
DOPA	689.8 (164.4)	600.1 (185.5)	1,173,311 ($3.1x10^6$)*#
DOPAC	465.6 (162.5)	346.8 (163.7)*	2290.8 (3300.8)*#
NE	121.3 (55.2)	123.2 (59.2)	179.9 (107.0)*#
DHPG	2165.9 (635.4)	2140 (815.9)	2525.1 (1134.3)

Mean(SD), $p<0.05$ (versus control*, between PD groups#)
Conclusions: It would appear that CSF catecholamines are not a useful biomarker in actively treated PD patients, however it is possible that CSF DOPAC may have a role as a biomarker for early PD detection.

06r. Imaging & Biomarkers: other

ADPD5-1379
COLONIC ALPHA-SYNUCLEIN: A POTENTIAL DIAGNOSTIC BIOMARKER IN PARKINSON'S DISEASE
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Objectives: Alpha-synuclein deposition has been identified in colonic biopsies taken from Parkinson's disease (PD) patients, occasionally years prior to the development of motor symptoms. However, these findings are from very few cases and we want to validate detection of alpha-synuclein pathology in the gastrointestinal (GI) tract as a pre-symptomatic diagnostic marker of PD.
Methods: Oxford Parkinson's Disease Centre (OPDC) cohort is a prospective, longitudinal study comprising patients with early idiopathic PD (n=1200), healthy controls (n=300) and participants at risk of Parkinson's (n=300). To date, 135 participants have given written consent to use their previous GI tract biopsies, making this potentially the largest study of its kind. We have retrieved and stained 51 colonic biopsies (36 PD, 15 controls) so far with an optimized neuronal marker, calretinin and alpha-synuclein antibodies for both its normal (LB509) and phosphorylated (WAKO) forms.
Results: Calretinin showed optimal decoration of mucosal nerve fibers and submucosal ganglionic cells in all samples superior to any other neuronal markers tested. All of our 36 PD patients showed staining for both forms of alpha-synuclein. However, the samples were fairly superficial, sparsely revealing the submucosal plexus and the detected alpha-synuclein staining was variable and frequently diffuse with no clear co-localization with calretinin. We also detected alpha-synuclein accumulation in plasma and vascular endothelial cells. Moreover, some controls also showed alpha-synuclein staining.
Conclusions: Issues of specificity and sensitivity remain to be solved. We plan to address these issues by applying alternative techniques to immunohistochemistry that could better detect the disease-specific neuronal alpha-synuclein accumulation.

06r. Imaging & Biomarkers: other

ADPD5-1790
THE NINDS PD BIOMARKERS PROGRAM
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Objectives: PD affects at least one-half million patients across the United States. Currently available pharmacological and surgical treatments provide some symptomatic relief, but do not impact progression. The National Institute of Neurological Disorders and Stroke (NINDS) Parkinson's Disease Biomarkers Program (PDBP) was established to advance discovery of biomarkers that will improve the efficiency and outcome of Phase II clinical trials for PD and advance therapeutic development.
Methods: The PDBP is a longitudinal program gathering clinical data and biospecimens in subjects who span the disease spectrum, as well as from control subjects. Ten projects are supported under the PDBP; six are clinical. PDBP has enrolled >1000 well-characterized participants. Each clinical site follows a standardized protocol for subject visits and biospecimen collection.
Results: There are >250 cerebrospinal fluid samples and collectively more than 3800 RNA, DNA, plasma and serum samples available for analysis. These biospecimens and associated quality control data are available through the NINDS Repository catalog in the PDBP data management resource (DMR) at no cost, after a straightforward application and review process with a fast turn-around.
Conclusion: The PDBP is a resource with a collection of detailed clinical data and extensive associated biospecimens on subjects with PD and controls. Researchers gain access to the PDBP DMR data by requesting an account. They can subsequently request biospecimens through a straightforward process. More information about requesting an account, ordering biospecimens and more generally, the NINDS PDBP, can be found on the PDBP website (https://pdbp.ninds.nih.gov).

06r. Imaging & Biomarkers: other

ADPD5-1826

UNILATERAL AND BILATERAL SUBTHALAMIC NUCLEUS STIMULATION (STN DBS) EFFECTS ON SACCADIC EYE MOVEMENTS

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Objective: The goal of this study was to assess changes of saccadic eye movements in unilateral and bilateral subthalamic nucleus stimulation (DBS STN) in PD (ICD-10; G20).
Material and Methods: Patient was qualified for surgery (ICD-10; 02.931, 00.36, 86.95): implantation of stimulating electrodes (model 3387 Medtronic) to the STN on the left side and after three months on the right side. Before and after treatment, the patient was examined using a Saccadometer (Ober Consulting Poland, Advanced Clinical Instrumentation Cambridge UK), allowing the measurement of eye position with the time resolution of 1 msec .
Results: Before surgery the patient showed an asymmetry of the saccades, depending on the direction of movement of the eyes. After the surgery saccadic parameters have changed. The latency of left saccades was reduced. The duration of left saccades was increased and right has not changed. The amplitude of left saccades was reduced. The peak velocity of left saccades was reduced.
Conclusions: The results show that left DBS-STN significantly impacts on the saccadic refixations, in particular its asymmetry depending on the direction of eye movement. This study shows that the STN plays an important role in the control of eyeball movement. Its function is probably related to the determination of contralateral range of motion (amplitude) and velocity, as well as the ipsolateral latency.

06r. Imaging & Biomarkers: other

ADPD5-2177

IDENTIFICATION OF POST-TRANSLATIONALLY MODIFIED ALPHA-SYNUCLEIN PROTEIN IN BIOFLUIDS OF PARKINSON'S DISEASE PATIENTS USING A TARGETED AND QUANTITATIVE MASS SPECTROMETRY APPROACH.

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Parkinson's disease (PD) is a movement disorder characterized by the progressive loss of dopaminergic neurons and the presence of intracellular protein inclusions (Lewy Bodies) found in the brain of affected patients. Protein aggregation and post-translational modifications (PTMs), such as the site specific phosphorylation of alpha-Synuclein protein have been reported to be strongly linked to PD pathogenesis. Therefore, pathologically modified alpha-Synuclein species represent a primary target for the diagnosis and treatment of PD. We aimed at conducting a comprehensive study, using targeted and quantitative mass spectrometry approaches, to identify and map the pattern of alpha-Synuclein PTMs in plasma and red blood cells from PD patients compared to healthy control subjects. More specifically, we focused on the pattern of PTMs in the blood in order to identify if these modifications correlate with alpha-Synuclein PTM's observed in the brain and cerebrospinal fluid (CSF) during disease progression. The use of full-length, heavy isotope-labelled (^{15}N) alpha-Synuclein protein and peptide standards with site-specific modifications, combined with selected reaction monitoring (SRM) have enabled us to specifically identify single or multiple site-specific modifications of alpha-Synuclein. We have developed a multiplexed SRM assay which allows us to monitor several PTMs during a single analytical run. The identification of a specific isoform or PTMs pattern that correlate with PD or DLB could provide novel insights into the mechanism of the disease development, contribute to the identification of novel therapeutic targets and most importantly, could provide a diagnostic marker to detect and monitor the progression of PD and related synucleinopathies.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-0554

DOES COGNITIVE DECLINE IN PD STARTS BEFORE DIAGNOSIS? A POPULATION-BASED STUDY.

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Objective: New concepts propose that PD starts decades before motor signs (pre-motor PD) with non-motor symptoms such as REM sleep behaviour disorder, hyposmia, constipation etc. It is accepted that 20-30% of newly diagnosed PD subjects have some cognitive decline but it isnot known if it starts before diagnosis. Thanks to the very long-term follow-up study of the population based PAQUID study (Perez et al. Alzheimers Dement. 2012 ;8:463-9), we challenged the occurrence of cognitive symptoms over a 14-year period before the diagnosis of PD.

Methods: This is a case-control study nested in the PAQUID cohort. Of the 3,777 initial subjects of the cohort, 43 have developed a PD during the 14 years of follow-up. These cases were matched to 86 elderly control subjects. The evolution of scores on cognitive, functional, and depression scales was described throughout the 14-year follow-up using a semiparametric extension of the mixed-effects linear model.

Results: We have not found significant cognitive decline or emergence of depressive symptoms in future PD subjects before clinical diagnosis compared with controls. Only psychomotor speed was found to significantly 4 years before PD diagnosis. Also, there was no difference in the impact on daily activities, except in using public transportation two years before diagnosis of PD.

Interpretation: This study shows that slower psychomotor speed occurs 4 years before motor diagnosis while other cognitive functions seemed preserved until diagnosis. We could not confirm pre-motor depression in PD. Limits of our study are that of the test used and subjects numbers.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-2126

NON-MOTOR SYMPTOM BURDEN IN PARKINSON'S DISEASE: A LONGITUDINAL STUDY

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Background: Non-motor symptoms (NMS) are common among patients with Parkinson's disease however little is known about their progression in terms of severity or burden.
Objective: This study was aimed to establish the progression of NMS burden and factors affecting it.
Methods: Parkinson's disease patients were prospectively enrolled and followed-up for up to 18 months. Non-motor symptoms scale (NMSS) was used to evaluate the burden of non-motor symptoms.
Results: There was a significant reduction of total NMS burden (from 25.0 to 17.0, p-value <0.001)) over the follow-up period. Similarly all NMS domains except domains 2 (sleep/fatigue) and 3 (mood/cognition) showed significant reduction of scores. In the univariate analysis, Hoehn & Yahr staging, disease duration, Schwab & England Activities of Daily Living score and UPDRS motor scores were individually predictive of change in total NMS burden. However, in multivariable analysis only the latter two were significantly predictive of change in the total NMS burden.
Conclusion: There was a significant reduction of total NMS burden over the study period. The severity of motor and activity of daily living impairment were the best predictors of NMS change.

07b. Epidemiology, Risk Factors, Genetics & Epigenetics: environmental

ADPD5-1464

GENOME WIDE GENE-VITAMIN D INTERACTION ANALYSIS SUGGESTS POTENTIAL ROLE OF MELANOMA RELATED GENES IN PARKINSON DISEASE

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Objectives: Vitamin D (vit D) deficiency has been associated with increased Parkinson disease (PD) risk. We sought to identify novel genes contributing to PD via a genome-wide interaction analysis with vit D deficiency.
Methods: We imputed up to 7.2 million single nucleotide polymorphisms (SNPs) in 477 PD cases and 430 controls from a previous genome-wide association study. Vit D metabolites were measured using mass spectrometry. Joint tests of gene-environment interaction were conducted by comparing a full model containing SNP dosage, vit D deficiency (plasma 25(OH)D <20 ng/ml), an interaction term, and covariates (age, sex and sampling season) to a restricted model with only vit D deficiency and covariates.
Results: Vit D deficiency was associated with PD (Odds Ratio (OR)=2.7, P<0.0001). The strongest evidence for interaction was found at rs7312710 in *FBRSL1* (P=2x10^{-6}). The effect of rs7312710 on PD depended on vit D status: the minor allele is associated with increased risk in vit D deficient individuals (OR=2.3, P=0.0004) and with decreased risk in vit D non-deficient individuals (OR=0.7, P=0.0036). The second strongest interaction was found in *C10orf11* with a similar pattern. Both genes are implicated in melanin production and risk of melanoma.
Conclusion: Our study demonstrates vit D-gene interactions and suggests that PD and melanoma share biological pathways (i.e. melanin production) that when perturbed modify risk to both diseases. This is supported by 1) our previous pathway analysis implicating melanogenesis pathway in PD pathogenesis; 2) increased incidence of melanoma in PD patients; 3) substantia nigra is enriched with melanin-positive neurons.

07b. Epidemiology, Risk Factors, Genetics & Epigenetics: environmental

ADPD5-1680

ASSESSING PLASMA LEVELS OF HEAVY METALS IN PATIENTS OF PD

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Background : PD is a progressive neurodegenerative disorder. Oxidative stress contributes to the cascade, leading to dopamine cell degeneration in PD. Metals play a key role in the intracellular oxidative balance. However their implication in the degeneration process remains unknown.
Objective : To assess Aluminum, Cadmium, Copper, Lead, Manganese, Mercury and Zinc concentrations in serum of a group of PD patients in order to determinate, in comparison with age-matched controls, whether alteration in their levels could be involved in PD.
Method : A serum level of 7 trace elements (Al, Cd, Cu, Lead, Mn, Mercury and Zinc) was investigated in 192 patients with PD and 90 matched controls (non-neurodegenerative disease). We compared these parameters in PD patients with controls, and we also compared the variations within the PD group according to age, illness duration, stage of the disease and levodopa intake.
Results : Patients with PD had significantly lower Zn levels compared to controls. Other heavy metal levels in PD patients did not differ significantly from those of controls. Levodopa therapy, age, stage, and illness duration did not significantly influence the measured parameters.
Conclusions : These results suggest that low plasma level of Zn could be a marker of PD or at least, a risk factor for the development of this disease.

07f. Epidemiology, Risk Factors, Genetics & Epigenetics: inflammation

ADPD5-0352

NIGRAL NEURODEGENERATION RESULTED FROM AXONAL DAMAGE AND INFLAMMATION AFTER DIFFUSE BRAIN INJURY

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Objectives: Many epidemiological studies have demonstrated traumatic brain injury (TBI) as a risk factor for developing sporadic Parkinson's disease (PD). In this study, we elucidated that TBI-induced axonal injury and inflammation in the medial forebrain bundle (MFB) and inflammation in the nigrostriatal system may contribute to dopaminergic neurodegeneration in the substantia nigra.
Methods: midline fluid percussion injury (mFPI) rodent model, ELISA, immunohistochemistry, HPLC, locomotor behavioral test
Results: In response to mFPI, a significant increase of cytokines and chemokine (IL-1β, IL-6, TNFα and CXCL1) was detected in the striatum and substantia nigra at 1-6 hours post-injury. Meanwhile, impaired axonal transport via antibodies targeting amyloid precursor protein (APP) was found in the MFB, which connects the substantia nigra and striatum, at 24 hours post-injury. Additionally, we found long-lasting increases of both Iba1 and OX-6 stained activated microglia in the MFP, striatum and substantia nigra at 1, 7, and 28 days post-injury. Importantly, dopaminergic neurons identified with both TH immunostaining and retrograde tracer Fluoro-Gold labeling were significantly decreased in the substantia nigra at 28 days post-injury.
Conclusions: Current studies suggested that a single moderate diffuse brain injury resulted in dopaminergic axonal damage in the MFB and inflammatory responses in the nigrostriatal system, potentially leading to dopaminergic neurodegeneration in the nigra, which may associate with the risk of developing late-onset PD.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1261

CHARACTERIZATION OF GENETIC VARIABILITY IN THE PARK10 LOCUS

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Objective: A recent genome wide association study (GWAS) using autopsy-confirmed cases and controls (Beecham et al, Neurology 2014, in press) replicated the previously identified PARK10 locus and reduced its size from 10.6 Megabases to ~100 kilobases (kb), a risk association which has not been seen in GWAS studies using only clinically-diagnosed patients. As part of an ongoing next generation sequencing project in Parkinson Disease (PD), we examined the PARK10 region in a dataset of cases and controls with only a clinical diagnosis, to characterize the region.
Methods: Genomic sequencing was done on a 160 kb region spanning PARK10 in 333 PD cases and 167 controls. Variants were characterized using SeattleSeq, GWAVA, CADD and RegulomeDB for functionality, and analyzed for association.
Results: 77 variants with potential effect on functionality were identified. One (rs11206279; eQTL) showed a trend for association with PD, but was not replicated in the original autopsy-confirmed dataset. However, a small region of Hardy-Weinberg disequilibrium (HWD) was seen near the most associated marker in the PARK10 locus. This region contains multiple known transcription factor binding sites and previously reported copy number variants. Importantly, this HWD was observed in cases, but not in controls.
Conclusion: No obvious functional sequence variant changes potentially contributing to PD were seen in this clinically-diagnosed-only dataset, though the additional heterogeneity compared to the autopsy-confirmed data could be problematic. However, the experiments suggest that initial follow-up experiments using the autopsy-confirmed PD and control dataset should be focused on the identified area of HW disequilibrium.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1800

IDENTIFICATION OF PROGNOSTIC BIOMARKERS FOR PERSONALISED MEDICINE IN PD

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Long-term studies demonstrate that the progression of PD varies greatly from one patient to another. This makes it impossible for clinicians to accurately predict the course of the disease in each individual, and to properly evaluate treatment efficacy or clinical trials. Thus, there is a great need for prognostic biomarkers to establish personalized medicine in PD.

In this project we have worked with The Norwegian ParkWest study, one of the world's longest running longitudinal studies of PD, to search for prognostic biomarkers of cognitive decline in PD. Our aim was to look for differences in DNA that distinguish between individuals who developed dementia relatively quickly after PD diagnosis and individuals who remained cognitively normal. We have taken both a targeted approach, analyzing known candidate biomarkers, and an unbiased approach, using partial exome sequencing of 1045 genes and a two-step extreme trait design.

We identified genetic variants in phenotypic extremes and prioritized them using Ingenuity Variant Analysis software. Variants with predicted effects on protein structure and function and association with cognitive decline were subsequently genotyped in the entire Norwegian ParkWest cohort. These and the known candidate variants were analyzed for association with both disease risk and the time of onset of dementia in PD. This work is an important step in the development of early prognostic biomarkers of disease heterogeneity. We will discuss how genetic variability impacts heterogeneity of disease progression in our cohort and the potential to identify new therapeutic targets to prevent or delay disease progression.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1995

BOOSTING POWER TO DETECT PARKINSON'S DISEASE GENETIC RISK VARIANTS BY CONDITIONING ON GENETIC DETERMINANTS OF BRAIN STRUCTURE

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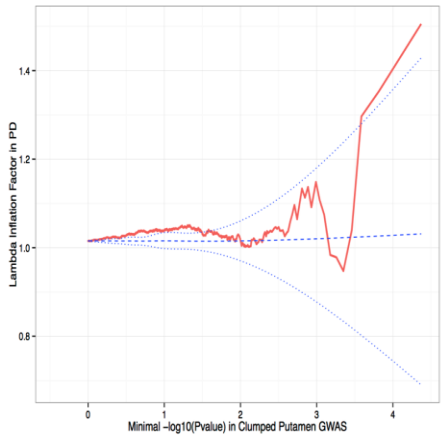
Objectives. As the basal ganglia are implicated in Parkinson's disease (PD), we tested for genetic overlap between PD risk variants and common variants associated with putamen volume. Given evidence of pleiotropy, we show how to boost power to detect PD risk variants by conditioning on ENIGMA's brain volume GWAS findings.
Methods. We used continuous inflation analysis (CIA) to examine genetic overlap (pleiotropy) between a published genome-wide association study (GWAS) of Parkinson's disease (Nalls et al., 2014) and our recent GWAS of putamen volume (Hibar et al., 2015). Next, we performed a pleiotropy-informed conditional false discovery rate (FDR) analysis conditioning on the putamen GWAS results to search for novel Parkinson's disease risk variants (FDR q-value < 0.05).
Results. We found significant evidence of overlap in the genetic determinants of Parkinson's disease and putamen volume using CIA (Figure 1). With pleiotropy-informed conditional FDR, we found 16 significant, previously undetected, Parkinson's disease risk variants (implicating genes like ITGA2B, DLG2, and KTN1).
Conclusions. Shared genetic factors influence brain structure and Parkinson's disease risk. We can leverage this overlap by incorporating recently-discovered information about gene variants that influence brain structure to boost power to detect gene variants that affect risk of developing Parkinson's disease. Several genetic variants discovered in this analysis represent novel biological targets for further investigation.
Figure 1. Continuous inflation analysis (CIA) plot of PD enrichment conditioned on putamen volume GWAS. Points outside of 5th and 95th percentile (blue dotted lines) are considered to be significantly enriched.



07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-2037

AN ASIAN-SPECIFIC ALDH2 SINGLE NUCLEOTIDE POLYMORPHISM AND PARKINSON'S DISEASE

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Objective: To determine whether an Asian-specific ALDH2 single nucleotide polymorphism rs671(A) can increase the risk of development of Parkinson's disease (PD).
Methods: An association analysis of rs671(A) in a large cohort of Taiwanese patients with PD (n = 627) and age matched controls (n = 480) with TaqMan genotyping was performed.
Results: The genotype distribution of rs671(A) in PD patients and control subjects was consistent with Hardy-Weinberg equilibrium. Compared with the GG genotype, the frequency of the genotypes that render ALDH2 inactive (AA and AG genotypes) was not significantly different between the patient and control groups. The adjusted odd ratios (ORs) for the genotypes with lower ALDH2 activity (AA and AG genotypes) were not statistically significant (OR = 1.0450, 95% CI = 0.8231-1.3268, p = 0.7176).
Conclusion: Our results show that the single nucleotide polymorphism rs671(A) alone is not associated with the development of PD.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-2265

LEFT-SIDE DEBUT OF PARKINSONISM SYMPTOMS AS A SPECIFIC CLINICAL FEATURES OF MUTATION IN GLUCOCEREBROSIDASE GENE.

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Introduction. Glucocerebrosidase (GBA) gene mutation represent a genetic risk factors for the development of Parkinson's disease (PD), but feature of parkinsonism symptoms according with mutation remain poorly understood.
Aim. The aim of the study was to detect specific clinical features in patients with PD carrying GBA gene mutation.
Materials and methods: 12 patients with PD carrying GBA gene mutation and 20 PD patients without this mutation were observed. In all patients a complex assessment of the cognitive status (MoCA), movement disturbances (UPDRS III scale) were applied.
Results. There were no differences in cognitive status according MoCA scale in groups carrying and without mutation (24.72±3.2 and 24,85 ±3.2 accordingly). In group with GBA mutation was detected only moderate cognitive impairment and no cases of dementia, in spite of the fact that duration of illness in some cases was high (9,6±4,6 years). No differences were found in movement disturbances in both group (46,1±19,6 vs 44,4±18,3 accordingly), but PD patients carrying GBA gene mutation more often had left-side debut of parkinsonism symptoms (83,3% vs 55% in group without GBA mutation) ($p<0,05$).
Conclusion. We decided only left-side debut of parkinsonism symptoms as specific clinical features in patients with PD carrying GBA gene mutation, but not cognitive impairments and movement disturbances at all.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-2315

GENETIC VARIANTS ASSOCIATED WITH PARKINSON'S DISEASE IN SOUTHERN SPAIN

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1.- Objectives

Increasing evidence supports an extensive and complex genetic contribution to Parkinson's disease (PD) etiology. Our aim was to evaluate the association between certain genetic variants and the risk to develop Parkinson's disease (PD) in a cohort of Andalusian population.

2.- Methods

We performed a case-control association study by genotyping 64 single nucleotide polymorphisms (SNPs) in 117 patients diagnosed with PD and 408 controls from southern Spain. The SNPs included were selected from PD traditionally related genes (SNCA, LRRK2, PARK2, DJ-1, VPS37) besides those proceeding from genome wide association studies (GWAS) as MAPT, GBA, HLA-DOA, STK39, ACMSD and GAK. Genotyping was carried out using Taqman assays in an OpenArray Real-Time PCR platform. Data analysis includes logistic regression adjusted by multiple testing.

3.- Results

Significant differences were observed in the allele frequencies between PD patients and controls after multiple testing adjustment for the following SNPs. HLA rs206769 ($p= 1.187x\ 10^{-10}$; OR= 0.2135), SNCA rs2736990 (p= 0.009268; OR= 1.75), SNCA rs356204 (p= 0.022; OR= 1.673), SNCA rs356219 (p= 0.03002; OR= 1.752), LRRK2 rs34637584 ($p= 0.001774; 5.87x\ 10^{9}$), LRRK2 rs28903073 (p= 0.0181; OR= 11.09). No significant differences were found in allele distribution between cases and controls for the rest of the SNPs analyzed.

4.-Conclusions

Our findings suggest that SNPs SNCA rs2736990, SNCA rs356204, SNCA rs356219 and LRRK2 rs34637584, LRRK2 rs28903073 likely contribute to PD susceptibility in Andalusian population whereas HLA rs206769 might be protective against neurodegeneration.

07j. Epidemiology, Risk Factors, Genetics & Epigenetics: GWAS

ADPD5-0932

GENOME-WIDE INTERACTION STUDY IDENTIFIES MAPK10 AS A GENETIC MODIFIER FOR THE PROTECTIVE EFFECT OF COFFEE AGAINST PD

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OBJECTIVE: Clinical trials for neuroprotective treatment of PD have all failed, many due to low efficacy. Drug-efficacy varies among individuals due to genetic difference in drug response. When efficacy is averaged over all genotypes, the effect becomes diluted. Our goal is to find genetic markers to enable pre-selection of subjects for clinical trials. Here, the aim was to identify genes that influence efficacy of caffeine. Caffeine is inversely associated with risk of developing PD, and clinical trials have suggested caffeine and adenosine A_{2A} agonists may benefit some patients. **METHODS:** We used the NeuroGenetics Research Consortium dataset (1450 cases and 930 controls with genetic and exposure data) in a genome-wide gene-environment study. We tested interaction between seven-million genetic-variants and caffeine-intake on the risk of PD. **RESULTS:** Significant interaction was detected with variants in the *MAPK10* gene on chromosome 4 (P=4E-8) indicating association of coffee with PD varied significantly by *MAPK10* genotype. Considering *MAPK10* and previously-identified *GRIN2A* together separated the individuals into three distinct genotypic groups: high-responders showed 87% risk reduction with coffee (OR=0.13, P=2E-5), moderate-responders had 52% risk reduction (OR-0.48, P=4E-6); and one-half of the study population were non-responders (OR=1.0, P=0.76). **CONCLUSIONS:** Results suggest genotyping two variants, at a cost of $1 per subject, may distinguish responders from non-responders, which if confirmed, will enable pharmacogenomic trials for prevention and treatment. This study provides the first genetic link between PD and *MAPK10 (JNK3),* a brain-specific stress-induced gene whose activation is required for neuronal apoptosis.

ADPD5-1411

IDENTIFICATION OF LOW-FREQUENCY GENETIC VARIANTS WITH LARGE EFFECTS ON AGE-AT-ONSET OF PARKINSON'S DISEASE

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OBJECTIVES: Parkinson's disease (PD) is a common neurodegenerative disorder with variable age-at-onset. If age-at-onset can be delayed by a few years, a significant number of cases may never occur, and among those who develop PD, fewer will experience physical disability, dementia and psychosis which affect 75%-90% of individuals as disease progresses. **METHODS:** To identify genetic modifiers of age-at-onset, we performed a GWAS using 1,985 PD cases from NGRC followed by replication in 3,986 PD cases from eight datasets. Unlike GWAS for risk, there is no consensus on most suitable method for analysis of age-at-onset. After assessing six different methods, we chose Cox proportional-hazards-model for GWAS. We used 1,986 NGRC controls to rule out association with age, and the moving average frequency plots to confirm association is with age-at-onset and not risk. **RESULTS:** We identified and replicated two regions that showed association with age-at-onset in familial PD (P_{NGRC}<3E-8, 3E-4≤$P_{Replication}$<0.05), but not in non-familial PD (P_{NGRC}≥0.79, $P_{Replication}$≥0.15). The signals mapped to *LHFPL2* on chromosome 5q14.1 and *TPM1* on chromosome 15q22.2. The alleles had low frequencies (MAF_{LHFPL2}=0.016; MAF_{TPM1}=0.012) and were associated with 6-15 years earlier onset (*LHFPL2*: NGRC=12.3 years, Replication=6.37 years; *TPM1:* NGRC=15.3 years, Replication=6.0 years). **CONCLUSIONS:** In summary, we uncovered evidence for the existence of uncommon variants with large effects on age-at-onset of PD. The candidate loci identified, *TPM1* (which regulates muscle-contraction) and *LHFPL2* (highly-expressed in brain tumors), are both biologically plausible considering that PD is a movement disorder and it shares genetically-controlled pathways with cancer.

07l. Epidemiology, Risk Factors, Genetics & Epigenetics: whole genome sequencing

ADPD5-0925

PARKINSON DISEASE VARIANT DATABASE (PDVD) WITH MULTIPLE EVIDENCE LEVELS TO RANK THE SIGNIFICANCE OF SEQUENCE VARIANTS

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Objective: Next generation sequencing (NGS) has significantly increased the rate of rare sequence variants (SV) reported in PD patients. But interpretation of the functional significance of SV can be difficult. Current databases do not address this question and/or do not accommodate NGS data. The PDVD is designed to address these needs.
Methods: SV included will come from the literature and existing NGS databases. SV are ranked in each of three evidence levels: 1) "Genetic evidence" (e.g. population frequency, family segregation) and 2) "functional evidence" extracted from literature and data repositories and 3) in-silico analyses for all variants to determine potential functional effect of the variants ("in-silico evidence") (e.g. PolyPhen2 for nonsynonymous, RegulomeDB and GWAVA for non-coding variants).
Results: Each variant is placed in one of six categories, based on the strength of evidence from the three evidence-levels, and this data will be available through a website. Summary data for each dataset and/or for each SV will be linked to the NGS laboratory or publication, to facilitate collaborative efforts.
Conclusions: The PDVD will allow users to quickly evaluate variants and initiate collaborations. The summary data format avoids individual identifiers to allow rapid availability of NGS data to the PD research community. The database meets recommendations by NINDS PD2014. Funded by the US Department of Defense.

Table 1. Rankings for SV

	Category 1	Category 2	Category 3
Genetic support	Highest	High	High
In-silico support	High	High or Intermediate	High or Intermediate
Functional support	Any	High	Intermediate or Low
	Category 4	**Category 5**	**Category 6**
Genetic support	Intermediate	Low	Low
In-silico support	Any	High	Intermediate or Low
Functional support	Any	Intermediate or Low	Low

07l. Epidemiology, Risk Factors, Genetics & Epigenetics: whole genome sequencing

ADPD5-1161

EXOME SEQUENCING AND 2ND SNP-GWAS OF PD

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PD is a complex disorder caused by multiple genetic variants. We reported a genomewide association study (GWAS) in a total of 2,011 cases and 18,381 controls from the Japanese, which detected 4 PD-risk loci; PARK16, BST1, α-synuclein, and LRRK2 (Satake et al, Nat Genet 2009).

To search for further PD-risks in exonic areas, we performed exome sequencing of 755 PD patients using Sureselect and HiSeq2500. Moreover, in parallel, to identify further common variant PD-risks, we performed Japanese 2nd SNP-GWAS, which expanded our previous one.

Average depth of our data is x 126, and 94.4 % of whole exon sequence was covered by 10 x or more reads. At first, using exome sequencing data of 625 PD cases and 259 controls, we tested association between PD and exonic SNVs within the 4 PD-loci reported by SNP-GWAS. Genetic variants with strong PD-risk did not exist within these 4 PD-loci, indicating that these 4 PD-loci will contribute to this disease as common SNP variants. Further, in 2nd GWAS using 1,948 cases and 28,990 controls, we identified a novel susceptibility locus with P <5 x 10-8. Expression level of a gene within the locus was reduced when the risk SNP exists. In a fly model, knockout of the gene worsened motor function.

We will test association between whole exonic SNVs and PD to identify novel PD-genes harboring rare-variant risks. Our GWAS and in-vivo model data showed that this gene is a novel PD-risk.

07o. Epidemiology, Risk Factors, Genetics & Epigenetics: histone modification, DNA methylation

ADPD5-1203

ALPHA-SYNUCLEIN METHYLATION IN PARKINSON'S DISESASE: EFFECT OF SEX AND L-DOPA

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1. Objectives

Mounting evidence suggests a prominent role for alpha Synuclein (*SNCA)* expression in the aetiology of Parkinson's disease (PD). In addition to the dominant point mutations in the *SNCA* gene, increasing gene dosages of *SNCA* induce familiar PD, and polymorphisms in regulatory elements of *SNCA* (REP1) increase the risk of sporadic PD. *SNCA* gene expression is controlled at multiple levels including DNA methylation. We performed a thorough analysis of *SNCA* intron 1 methylation in DNA from peripheral blood of 975 individuals.

2. Methods

DNA methylation of *SNCA* intron 1 was analysed using bisulfite specific PCR and pyrosequencing. Lymphocytes were treated with l-DOPA *in vitro* and analyzed for DNA methylation and *SNCA* expression.

3. Results

We detected significant hypomethylation in PD and the difference was more obvious in female individuals. Methylation of *SNCA* in blood decreases with age in healthy individuals. A genetic polymorphism in the investigated sequence stretch (rs3756063) was correlated with methylation and increased the specificity of the observed differences. Male PD patients, who had been exposed to higher doses of l-DOPA, displayed higher levels of *SNCA* methylation, while female PD patients on l-DOPA showed a much weaker effect. These trends prompted us to mimick the presumed effect of l-DOPA *in vitro*. Overall methylation of *SNCA* increased with l-DOPA treatment and decreased *SNCA* mRNA levels.

4. Conclusions

Our data put epigenetic mechanisms on the map of mechanisms determining the individuals' suscebtibility toward PD and point to several hitherto unappreciated phenomena.

07p. Epidemiology, Risk Factors, Genetics & Epigenetics: other epigenetic factors

ADPD5-0628

DNA METHYLATION AS AN EPIGENETIC BIOMARKER FOR ALZHEIMER'S AND PD

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Objective: Biomarkers that aid in earlier diagnoses of Alzheimer's (AD) or Parkinson's disease (PD) could facilitate earlier diagnosis and treatment of these diseases. Here we characterize the global methylation profiles from patients with post-mortem neuropathologic confirmation of either AD or PD and confirm specific methylation changes in a prospective cohorts of patients.
Methods: Genome-wide methylation profiles were obtained on blood samples from 19 neurologically normal controls, 20 AD and 19 non-demented PD patients using the Illumina Infinium 450K Methylation BeadChip. Consented subjects gave blood samples and subsequently followed through end of life. Neuropathology analyses confirmed the respective clinical diagnoses. A second validation cohort of 15 controls, 10 AD, and 15 PD patients was prospectively recruited based purely on clinical criteria.
Results: We obtained robust data on over 480,000 CpG methylation sites in the form of beta values, which represent the ratio of methylated CpG to the sum of methylated plus nonmethylated CpG at a given site. Thus, these values range from 0 (unmethylated) to 1 (fully methylated). Significant methylation differences in the discovery cohort of neuropathological-confirmed cases and controls were then confirmed in the second prospectively recruited cohort.
Conclusions: Methylation profiles in the blood of individuals with AD or PD and healthy controls show distinct differences. Further validation efforts on larger sample sets, and characterization of methylation status in patients at varying stages of disease, will help to establish whether methylation status at specific loci could be leveraged as a biomarker to track disease progression or aid in disease diagnosis.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0218
CLINICAL COURSE OF MIGRAINE IN PARKINSON PATIENTS
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Migraine is a public health problem of great impact on both the patient and society. The overall migraine prevalence in western countries is 6–8% in men and 15–25% in women. The dopamine theory of migraine pathogenesis, first proposed by F. Sicuteri in 1977. It has been shown that the most migraine symptoms can be induced by dopaminergic stimulation. Moreover, there is dopamine receptor hypersensitivity in migraineurs, as demonstrated by the induction of yawning, nausea, vomiting, hypotension, and other symptoms of a migraine attack by dopaminergic agonists at doses that do not affect nonmigraineurs. We decided to investigate the course of migraine in Parkinson's disease. To study the role of the dopaminergic system in the pathogenesis of migraine, the course of migraine is evaluated after the onset of PD in 25 migraineurs. Our result shows that the migraine attacks became shorter and milder after the onset of PD. Approximately 60% of PD patients reported an improvement in or remission of migraine after PD onset. These findings suggest that PD might somehow shorten the clinical course of migraine, and call for a larger survey.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0240

WHITE MATTER LESIONS ARE CORRELATED TO OLFACTORY DYSFUNCTION IN DE NOVO PD

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Objectives
It is well known that white matter lesions (WMLs) are often found in patients with Parkinson's disease (PD). We assessed the relationships between WMLs and several clinical features including olfactory dysfunction in patients with de novo PD.
Methods
We enrolled 36 patients with de novo PD (14 men and 22 women, 74.4 ± 7.7 years, disease duration 1.8 ± 1.6 years); and examined them using the Unified Parkinson's Disease Rating Scale (UPDRS), mini-mental state examination (MMSE), frontal assessment battery (FAB), and Odor Stick Identification Test for the Japanese (OSIT-J). Based on the fluid attenuated inversion recovery (FLAIR) MR image of the brain, we graded deep and subcortical white matter hyperintensity (DSWMH) from 0 to 4. Furthermore, we compared the clinical symptoms between the following 3 groups: grade 0+1: 10 patients, grade 2: 10 patients, grade 3+4: 16 patients. Using DSWMH (grade 0+1 vs. grade 2+3+4) as the objective variable, we conducted logistic regression analysis for the clinical parameters.
Results
Grade 0+1 patients had significantly lower age and higher MMSE, FAB, and OSIT-J than the other patients ($p < 0.01$). Disease duration and UPDRS part III score did not differ between the 3 groups. Logistic regression analysis showed that the OSIT-J had the strongest correlation with DSWMH ($p < 0.05$).
Conclusions
Age, cognitive function, and olfactory function were correlated with WMLs in de novo PD. Specifically, olfactory dysfunction was highly correlated to WMLs. It may suggest that pathogenesis of olfactory dysfunction was associated with that of WMLs in PD.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0471

RISK OF PD FOLLOWING SEVERE CONSTIPATION: A NATIONWIDE POPULATION-BASED COHORT STUDY

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Introduction: Constipation is a non-motor symptom of PD. We investigated the association between the severity of constipation and subsequent risk of PD in a population-based sample.
Methods: 551,324 participants free of PD, dementia, and stroke were retrospectively ascertained between January 1, 2005 and December 31, 2005 using the Taiwan National Health Insurance Research Database. The association between constipation at the beginning of the study and the incidence of PD was examined using a Cox regression model. Information regarding comorbidities and concomitant medications use was adjusted in the proportional hazards models.
Results: After an average follow-up of 5.5 years, 2,336 incident PD cases were diagnosed. The crude incidence rate of PD per 1,000,000 person-days was 1.57 for subjects without constipation and 4.04, 5.28, and 12.67 for mild, moderate, and severe constipation, respectively. After adjusting for age, sex, comorbidities, and concomitant medication use, patients with constipation were more likely to develop PD than subjects without constipation; the adjusted hazard ratio (aHR) was 3.28 (95% CI: 2.14-5.03), 3.83 (2.51-5.84), and 4.22 (2.95-6.05) for individual constipation severity categories. Constipation severity was also associated with an increased likelihood of PD in the time-varying analysis; the aHR was 2.84 (2.43-3.33), 5.22 (4.61-5.92), and 10.47 (9.46-11.58) for mild, moderate, and severe constipation, respectively ($p < 0.0001$). After excluding PD patients diagnosed within 3 years of constipation, the association remained significant.
Conclusions: Our study suggests that the severity of constipation is associated with a future diagnosis of PD in a dose-dependent manner.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0503

CARDIAC CONDUCTION SYSTEM CHOLINE ESTERASE INHIBITOR HYPERSENSITIVITY IN PATIENTS WITH DEMENTIA WITH LEWY BODIES (DLB)

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Objective

Patients with DLB have shown hypersensitivity to antipsychotic drugs and a wide variety of autonomic dysfunction. To assess the potential sensitivity to choline esterase inhibitors (ChE-Is) resulting in bradycardia, we performed electrocardiography in patients with Alzheimer's disease (AD) or DLB before and after administration of a ChE-I; we then compared the changes in their heart rate (HR) changes.

Patients and Methods

Eleven patients with DLB and 24 with AD were examined. These patients had no history of drug treatment affecting the cardiac conduction system, arrhythmias, or cardiac disease. All patients were treated with a ChE-I. Electrocardiography was performed before and after administration of the ChE-I. The HR, PR interval, QRS time, and QT time were measured and the differences were computed between the pre- and post-administration time points.

Results

The mean decrease in HR after the administration of the ChE-I was 12 ± 3/min and 3 ± 5.7/min in the DLB and AD groups, respectively. This indicated a statistically significant decrease in HR in the DLB group than that in the AD group ($p < 0.05$).

Discussion

The ChE-I-induced significant decrease in the HR in patients with DLB in this study may be due to both autonomic dysfunction and hypersensitivity of the cardiac conduction system to ChE-Is in patients with DLB. When administering ChE-Is to patients with DLB, to avoid the risk of bradycardia, electrocardiography should be performed not only before, but also periodically after, ChE-I administration to monitor changes in HR.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0973

MOLECULAR MECHANISMS UNDERLYING HAPLOTYPE-SPECIFIC REGULATION OF GENE EXPRESSION AT THE MICROTUBULE ASSOCIATED PROTEIN TAU LOCUS

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Objectives
The microtubule associated protein tau (*MAPT*) locus is defined by two major genetic haplotypes called H1 and H2. Genetic associations have been identified between H1 haplotype polymorphisms and several neurodegenerative diseases. The expression of exon 3-containing *MAPT* transcripts is two-fold higher from the H2 allele compared to H1 in brain areas highly affected by neurodegeneration. This project aims to use whole genomic locus expression vectors to identify the sequence variants responsible for the difference in splice phenotypes between the haplotypes and the splice factors that interact with the functional variants.
Methods
MAPT vectors were expressed in SK-N-F1 neuroblastoma cell line and the relative expression levels of exon 3-containing transcripts were determined by qRT-PCR. Biotin-labelled RNA oligonucleotides will be used in RNA electrophoretic mobility shift assays and affinity purification of RNA-binding proteins followed by mass spectrometry to identify trans-acting nuclear factors interacting with the haplotype-specific sequence variants.
Results
Intronic single nucleotide polymorphisms (SNPs) rs1800547 and rs17651213 were exchanged in three possible combinations between H1 and H2 wild-type *MAPT* vectors containing the whole *MAPT* locus (~143 kb). Wild-type vectors expressed in SK-N-F1 cells showed greater expression of exon 3-containing transcripts from H2 compared to H1. This expression pattern was reversed when only rs17651213 was exchanged between the H1 and H2 vectors.
Conclusions
A sequence variant responsible for the difference in exon 3 expression between the H1 and H2 MAPT haplotypes was identified through expression studies. This finding provides mechanistic insight into how sequence variations associated with neurodegeneration affect pre-mRNA transcript production.

08a. Animal Models: transgenic mice

ADPD5-0523
ESCRT REGULATES AUTOPHAGIC CLEARANCE OF PROTEIN AGGREGATES IN NEURODEGENERATIVE DISEASES

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Objectives: ESCRT (endosomal sorting complex required for transport) play a key role in the later step of the autophagosome, amphisome, and endosome maturation. The aim of this study is to investigate whether ESCRT dysfunction is associated with the abnormal accumulation of protein aggregates and subsequent neurodegeneration in animal model.
Methods: We specifically deleted the ESCRT-0 component, hepatocyte growth factor-regulated tyrosine kinase substrate (hrs), in neurons of the adult forebrain by using conditional knockout mice on calcium/calmodulin-dependent protein kinase II alpha (CaMKII)-Cre-expressing background. The locomotor activity was evaluated by footprint analysis, hind-limb extension and hanging wire tests. The neuronal cell loss was determined by hematoxyline-eosin staining. The intraneuronal accumulation of ubiquitinated proteins, autophagic substrate p62, and neurodegenerative disease-related proteins such as α-synuclein, TDP-43, tau, and huntingtin was examined by immunostaining and Western blot analyses. 8-Hydroxydeoxyguanosine (8-OHdG) and phosphorylated p38/SAPK were used as the indicators for oxidative stress and the stress-kinase activation, respectively.
Results: The locomotor performance in the hrsflox/flox; CaMKII-Cre mice was significantly impaired compared to that in hrs+/+; CaMKII-Cre mice. Histological analysis showed the prominent neuronal loss in CA1/CA3 regions of the hippocampus. Furthermore, we observed a striking accumulation of detergent-insoluble α-synuclein, TDP-43, tau, and huntingtin as well as ubiquitinated proteins and p62 in the brain of hrsflox/flox; CaMKII-Cre mice. These histopathological changes were accompanied by the increased expression of 8-OHdG and phospho-p38/SAPK.
Conclusions: These findings suggest that the functional disruption of ESCRT machinery compromises autophagic/lysosomal degradation of aggregate-prone proteins and acquires cytotoxic activity leading to neuronal cell death.

08a. Animal Models: transgenic mice

ADPD5-1151

THY-1 ALPHA-SYNUCLEIN MICE – DISEASE PROGRESSION AFTER WEANING

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Objectives

Thy-1 Alpha-Syn mice are characterized by strong alpha-synuclein pathology combined with severe and stable motor deficits. Although early affliction was described (Chesselet et al. 2012), only little is known about early post weaning disease progression and nothing is published about brain pathological correlates of the progressive PD motor symptomatic.

Methods

Male Thy-1 Alpha-Syn mice completed a comprehensive behavioral test battery at 1.5*, 2, 3 and 6 months of age. Brains of 2 and 6 months old mice were sampled and investigated for soluble and insoluble alpha-synuclein load, neuro-inflammation, tyrosine hydroxylase (TH) levels, lysosomes (Lamp-1) and agyrophilic neurons by Campbell Switzer (CS) staining. Pathology in relevant brain regions was correlated to behavioral deficits.

Results

Motor deficits appear age-dependently and deficits are measurable as early as at 6 weeks of age in wire suspension. Those are followed by impaired nest-building, beam walk and marble burying performance (2 months), RotaRod and pasta gnawing deficits (3 months) and hyperactivity (6 months). Deficits in predominantly CNS related tasks correlated with progressive intrasomal alpha-synuclein accumulation and aggregation in the substantia nigra, while deficits with peripheral involvement such as wire suspension were unrelated to brain pathological changes.

Conclusions

Peripherally triggered deficits e.g. diminished wire suspension, precede deficits with CNS involvement e.g. reduced RotaRod stamina or hyperactivity in the Open Field paradigm. The latter directly correlate with certain brain pathological changes, whereas perinuclear accumulation of alpha-synuclein as well as agyrophilic aggregates turned out to be the crucial drivers during early disease progression.

*only in wire suspension test

08a. Animal Models: transgenic mice

ADPD5-1212

ALPHA SYNUCLEIN OVEREXPRESSION LEADS TO DOPAMINERGIC AND CHOLINERGIC DEFICITS AND REDUCED LOCOMOTOR ACTIVITY IN A MOUSE MODEL OF PD

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Objectives: Parkinson´s disease (PD) is the second most common neurodegenerative disease and the most common movement disorder in humans, with yet no therapy available beside symptomatic treatment. PD is characterized by accumulation of alpha synuclein aggregates at presynaptic sites and loss of midbrain dopamine neurons. However, several other neurons degenerate in the course of the disease and different transmitter systems are affected.

Methods: To investigate PD related neuronal dysfunction a novel transgenic mouse model overexpressing human alpha synuclein under the control of the mouse Thy 1 promotor was generated.

Results: Transgenic animals exhibited widespread alpha synuclein accumulation in brain and spinal cord. We observed neuronal cell loss in the motor cortex and a decrease in the number of cholinergic interneurons in the striatum as well as lower extracellular striatal dopamine levels, with no changes in vesicular dopamine content. Further, transgenic mice showed deficits in motor functions that progress during ageing. Accordingly, the inhibitory and stimulatory effects of dopaminergic and cholinergic agonists/antagonists on locomotor activity were more pronounced in sensitized transgenic mice compared to control animals.

Conclusions: Our results indicate that alpha synuclein overexpression can alter cholinergic and dopaminergic functions and provides a useful model to test new drugs and therapeutic strategies for the treatment of synucleinopathies.

08a. Animal Models: transgenic mice

ADPD5-1723

BEHAVIORAL, GASTRO-INTESTINAL AND HISTOPATHOLOGICAL FINDINGS IN A53T a-SYNUCLEIN MOUSE MODEL OF PARKINSON'S DISEASE AFTER LPS EXPOSURE

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Multiple transgenic animal lines exist for Parkinson's Disease (PD). However, many of the models show only a mild phenotype or do not express non-motor changes associated with PD. However, there is evidence that PD affects various non-motor related changes in clinical disease, including disturbances in colon motility and fecal output.

We attempted to enhance disease progression of A53T mouse model by using additional LPS challenge followed by behavioral monitoring and nuclear imaging. In addition, we monitored colon motility as a non-motor endpoint.

Twelve month-old A53T transgenic mice and corresponding wild type mice were tested for baseline behavior followed by LPS challenge over 3 consecutive weeks. Behavioral monitoring by beam balance test and beam traversing were performed and non-motor effects of the A53T transgene as well as additional challenge by LPS were monitored by a fecal output assay. Peripheral TSPO ligand (CLINDE) binding activity was analyzed by SPECT/CT imaging over time. Brains were collected and processed for dopamine and its metabolites from the striatum and histological evaluation of tyrosine hydroxylase and GAP 43 positive cells from substantia nigra (SN). Expression of α-synuclein was monitored and evaluated between non-challenged and LPS-challenged A53T mice.

This study focused on the validation of a previously published mouse model of PD in mice with novel endpoints and additional challenges. We present data on the progression of the PD phenotype up to 16 months of age, the effect of LPS on the phenotype, and whether biochemical, histopathological, and nuclear imaging markers correlate with behavioral changes.

08a. Animal Models: transgenic mice

ADPD5-2164
A NOVEL PARK14 TRANSGENIC MOUSE MODEL OF AGE-DEPENDENT PARKINSON'S DISEASE.

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One of the major limitations in the field of Parkinson's disease (PD) is the absence of a mammalian model that closely mimics progression of age-dependent human PD. Our recent studies focused on a poorly understood PARK14 (PLA2g6), and store-operated Ca^{2+} signaling. Here we present *in vivo* and *in vitro* evidence for progressive loss of dopaminergic (DA) neurons in substantia nigra pars compacta (SNpc), and age-dependent PD-like motor dysfunction in a new PLA2g6 (PARK14) $Ex2^{KO}$ mouse model, in which specific Ca^{2+} signaling function of PLA2g6 was genetically impaired. Comparative analysis of ageing $Ex2^{KO}$ and WT littermates (using balance beam, pole, rotarod, open field and other behavioral tests) showed progressive PD-like motor dysfunction at the age that closely mimics the onset of idiopathic PD in humans. Importantly, L-DOPA reversed the motor deficits in KO^{Ex2} mice in dose and age-dependent manner. Motor dysfunction in $Ex2^{KO}$ animals was not a result of muscle, or neuroaxonal dystrophy. Stereological analysis of TH-positive neurons in SNpc revealed progressive loss of dopaminergic neurons in ageing $Ex2^{KO}$, but not WT animals. Thus, PLA2g6 $Ex2^{KO}$ mice develop age-dependent PD-like neurodegeneration with progressive loss of DA neurons in SNpc, as well as specific motor dysfunction that closely mimic parkinsonism in humans. This mouse offers a novel mammalian model of age-dependent PD.

08a. Animal Models: transgenic mice

ADPD5-2250

BEHAVIOURAL DEFICITS IN THE SYNERGY MOUSE: A BI-GENIC MODEL OF PARKINSONISM AND DEMENTIA WITH LEWY BODIES

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Objective: Our goal is to recapitulate several pathogenetic features of human Lewy body disorders, including behavioural and biochemical abnormalities, in a pre-clinical mouse model. SYNERGY mice combine three disease-causing traits (4 insertions of PAC1-*SNCA*^{A53T} alleles*; homozygous Gba1*^{D409V} alleles; *Snca*-null). We hypothesized that SYNERGY mice will show age-dependent behavioral deficits that correlate with elevated expression of human alpha-synuclein (SNCA) in the brain downstream of mutant *Gba1* function.

Methods: Comprehensive motor, cognitive and olfactory testing is being carried out at 1½, 3, 6 and 9-12 months of age (moa). SYNERGY mouse performance is compared to both littermate controls (PAC1-*SNCA*^{A53T}; *Gba1*$^{wt/wt}$; *Snca*$^{-/-}$) ('SYNERGY controls') and wild-type mice bred in parallel on the same mixed background (*Snca*$^{+/+}$*; Gba1*$^{wt/wt}$) ('WT mice'). Behavioural performance will be correlated with SNCA load in the brain.

Results: At 1½, 3 and 6 moa SYNERGY mice and SYNERGY controls showed significant deficits in task-dependent motor performance, as seen in the rotarod, vertical pole, and nest building tests. At 3 moa, SYNERGY mice displayed normal cognition; based on our characterization of both parental strains, we expect the onset of cognitive deficits by 6 moa.

Conclusions: Our behavioural analyses to date suggest that SYNERGY mice have motor deficits as early as 1½ moa that correlates with the increased expression of human SNCA. A significant mutant *Gba1* effect on motor outcomes is not yet apparent by 6 moa. Further behavioural, biochemical and histopathological characterizations are ongoing. We propose that SYNERGY mice have the potential to be a relevant model of Lewy body disorders.

08b. Animal Models: transgenic rats

ADPD5-1724

TARGETED OVER-EXPRESSION WITH AAV-MEDIATED HUMAN a-SYNUCLEIN IN A RAT MODEL OF PARKINSON'S DISEASE – VALIDATION OF NOVEL FINE MOTOR FUNCTIONAL EFFECTS

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Targeted over-expression of human α-synuclein using viral-vector mediated gene delivery into the substantia nigra of rats and non-human primates has been reported to lead to dopaminergic cell loss and the formation of α-synuclein aggregates reminiscent of Lewy bodies. In the context of these findings, we further validate long term functional and motor deficits in AAV-mediated α-synuclein transfection model in rats during chronic follow-up period.

Recombinant AAV expressing human α-synuclein (A53T) was stereotaxically injected unilaterally into substantia nigra of Wistar rats. Rats were allowed to recover for 3 weeks prior to initial baseline behavioral testing with rotational asymmetry test, stepping test and cylinder test. Similar behavioral test battery was applied again at weeks 5, 8 and 12. In addition to traditionally used rat PD model tests, MotoRater test system, a high speed kinematic motor performance monitoring was applied at multiple time-points during the follow-up period. Evaluation focused on animal gait, swimming, wading and ladder climbing between groups. Furthermore, integrity of the dopamine active transport (DAT) system was evaluated by using ^{123}I- β-CIT and SPECT/CT imaging on weeks 3, 8 and 12 after AAV- α-synuclein transfection.

This study focused on the validation of previously published AAV- α-synuclein transfection model in rats but with the addition of novel end-points. We describe the long term phenotype of AAV- α-synuclein transfected rats with traditionally used behavioral tests but also by using novel fine motor analysis techniques and DAT imaging which provide new insight to uni- and bilateral effects of AAV α-synuclein transfection.

08c. Animal Models: primate models

ADPD5-0837

IDENTIFICATION OF COGNITIVE IMPAIRMENTS IN MPTP-TREATED MARMOSETS USING THE WISCONSIN GENERAL TESTING APPARATUS

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Objectives: Most patients with PD develop dementia, including impairments in attention, executive and visuospatial functions. Although the motor symptoms are well modelled in animals, the cognitive dysfunction is poorly defined, and there are limited treatment strategies. Therefore, the purpose of this study was to characterise cognitive performance in the MPTP-treated marmoset model of PD using the Wisconsin General Testing Apparatus.

Methods: Naïve (n=6) and MPTP-treated (n=6) common marmosets were tested on a series of cognitive tasks using the Wisconsin General Testing Apparatus (WGTA). Marmosets were evaluated on a simple visual discrimination (VD) task, followed by 2 reversals of this newly learnt VD task and a visuospatial (VS) conditional discrimination task. The mean number of trials and response latencies to criteria (90% correct) were recorded for each marmoset.

Results: Both groups of marmosets performed the VD task similarly to criteria ($P>0.1$, NS; Figure 1). However, when the VD task was reversed, MPTP-treated marmosets were significantly impaired on both the first ($P<0.05$) and second reversal ($P<0.05$). During the VS test, MPTP-treated marmosets demonstrated robust impairments in learning this task ($P<0.01$). Response times per trial for each test were not different between the groups ($P>0.1$, NS), suggesting that MPTP-treated marmosets were motivated and not impeded by their motor deficit to perform the cognitive tasks.

Conclusions: MPTP-treated marmosets demonstrated cognitive impairments on tasks sensitive to executive function and visuospatial conditional learning using the WGTA. This cognitive battery in the MPTP-treated primate may offer the opportunity of validating novel therapeutic strategies for PD-dementia.

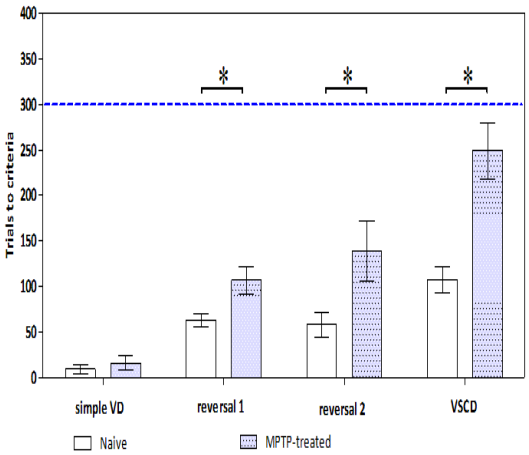

Figure 1. Total number of trials to criteria for naive and MPTP-treated common marmosets following simple VD task, two reversals and a VS conditional discrimination task. Data are mean ± SEM (n=6). * P<0.05 vs naive

08c. Animal Models: primate models

ADPD5-0998

NON-HUMAN PRIMATE MODEL OF PARKINSON'S DISEASE BASED ON VIRAL VECTOR MEDIATED OVEREXPRESSION OF ALPHA-SYNUCLEIN: UPDATE ON BEHAVIORAL AND PET MEASURES

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Objectives. To develop a new non-human primate model of PD based on viral vector mediated overexpression of A53T alpha-synuclein (aSyn) to serve as a preclinical testing platform for evaluating potential therapeutics.

Methods. 12 cynomolgus macaques were enrolled in the study (female, 9yrs old). Each received baseline behavioural assessments on motor activity, non-human primate parkinsonian disability rating scale (MPPrs) and were trained to conduct a fine motor task (mMAP test). Behaviour was assessed once monthly. Baseline PET scans were obtained using 18F-labelled AV133 (VMAT-2) and FDG (fluorodeoxyglucose) and were both conducted every 2 months. Animals were bilaterally injected with either AAV1/2 A53T aSyn or empty vector (EV) (1.7 x 10exp12 gp/ml) into each hemisphere of the substantia nigra (SN). Animals were sacrificed 8 months post surgery.

Results. By 5 months post surgery, A53T aSyn macaques showed 45% less motor activity in the 2-4 hr period of a 4 hr observation, compared to EV controls ($P<0.05$). This deficit persisted through to the final month (53%, 56% and 60%, respectively, to month 8, [all, $P<0.05$]). No significant effect was observed on mMAP performance at any time point. PET imaging showed a reduction in striatal VMAT-2 signal in A53T aSyn macaques compared to controls that emerged by month 2 and persisted to month 6. Further PET imaging and postmortem analyses are ongoing.

Conclusions. These data begin to form the basis of a macaque model of PD alpha-synucleinopathy that can be employed to evaluate disease modifying potential of novel disease modifying therapeutics.

08c. Animal Models: primate models

ADPD5-1753

HAEMATOLOGICAL EVALUATION OF A NEW MODEL OF PARKINSON DISEASE (SAPAJUS APELLA)

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Introduction: Parkinson´s disease (PD) is a neurodegenerative disorder and it is characterized by a progressive degeneration of nigral dopaminergic neurons followed by motor deficits. The use of MPTP treatment for PD models causes the similar effect in the brain and the central dopaminergic system has a crucial role in regulation of the immune processes as well as hematopoiesis. The possible role of this regulation in the hematopoieses must be investigated in animal models for PD treated with MPTP to evaluate the health status of the animals used in PD researches.

Objectives: Evaluate the hematological parameters in cappuchin monkey treated with MPTP.

Methods: 10 male capuchin monkey (mean age=12 years) received MTPT injection (0.4mg/kg, twice a week) during 1 month. Blood samples were collected before and after each treatment, the following parameters were were: red blood cells numbers, hemoglobin, platelets, MCV, MCH, MCHC, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils. Intra-individual comparisons were made with T test and p value < 0.05 was considered as significant.

Results: Intra-individual comparisons showed that there were no significant differences between in the most all hematologic parameters evaluated before and after the treatment in each monkey, with an exception to the values of lymphocytes were we observed a significant decrease after MPTP treatment ($p<0,05$) (Figure 1).

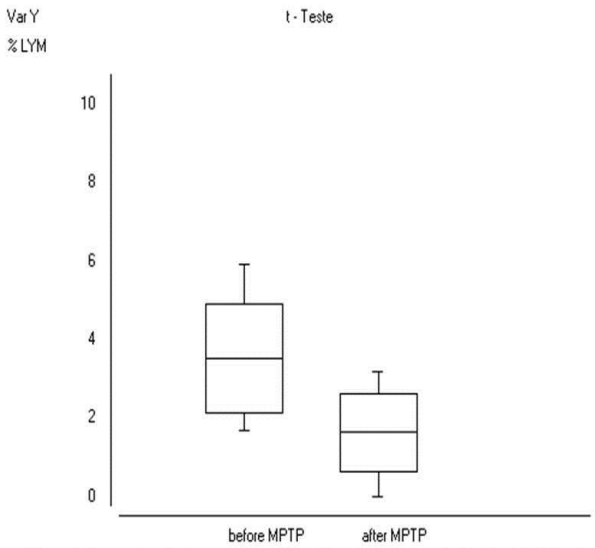

Figure 1: Comparison between values of lymphocytes before and after the MPTP treatment in capuchins monkeys (T Test /p<0,05)

Co**nclusions**: We observed that MPTP treatment in capuchins monkeys causes a significant decrease in lymphocites values.

08c. Animal Models: primate models

ADPD5-2212

COGNITIVE EFFECTS OF ATOMOXETINE IN THE CHRONIC LOW-DOSE (CLD) 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP)-TREATED MACAQUE MODEL OF PARKINSON'S DISEASE

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Objective. Atomoxetine (ATX), a selective norepinephrine reuptake inhibitor, is approved for the treatment of attention deficit hyperactivity disorder and has shown beneficial effects on working memory in healthy monkeys. Parkinson's disease (PD), in which there is widespread degeneration of the catecholamine system, is characterized by both motor symptoms and impairment of cognitive performance. The purpose of the study was to examine the effect of ATX in the gold standard primate model of cognitive defects in PD, the so-called chronic low-dose (CLD) MPTP macaque model.
Methods. The cognitive effects of ATX were assessed in 5 CLD macaques, displaying cognitive deficits and mild parkinsonian motor deficits. Three doses of ATX (0.3, 1.0 or 3.0 mg/kg) were tested in the Variable Delayed Response (VDR) and the Continuous Performance Task (CPT) on a touch-screen computerized system.
Results. No significant overall effect of ATX was reported on the VDR or CPT tasks. However, when using a best dose analysis, ATX significantly increased the percent of correct responses at a medium delay in the VDR task and decreased the number of commission errors on both the easy and difficult versions of CPT task.
Conclusion. Although no clear cognitive improvement was found after ATX administration in the CLD macaque, the best dose analyses reflected a small positive effect on working memory and on the control of impulsive responding. These modest effects of ATX are consistent with the available clinical literature seem to suggest of lower likelihood of response to ATX for patients with PD-MCI.

08d. Animal Models: drosophila

ADPD5-0742
SUPPRESSION OF SNCA EXPRESSION IN THE BRAIN ARRESTED PATHOLOGY PROGRESSION IN DROSOPHILA MODELS OF FAMILIAL AND SPORADIC PD
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Objectives. Parkinson disease (PD) is a neurodegenerative disorder characterized by the dysfunction and loss of dopaminergic neurons in the substantia nigra and accompanied by the presence of cellular inclusions known as Lewy bodies. An important component of the Lewy bodies is the protein α-synuclein (*SNCA*). SNCA is normally present in the human brain, but is physically altered in PD. However, the biological functions are worthy of investigation.
Methods. In our study, transgenic *Drosophila melanogaster* was established as a model to analyze PD-like pathology caused by *SNCA* overexpression. The following fly strains were used in this study: *UAS-SNCA.WT* carries human SNCA gene; *UAS-SNCA.A30P* carries human SNCA with A30P mutation; *UAS-SNCA.A53T* carries human SNCA with A53T mutation. We used the TARGET system, a method for temporal and regional gene expression targeting in *Drosophila*. Flies were raised at the restrictive temperature 18^{o}C throughout their development, two weeks of adult life at 29^{o}C to induce the expression of SNCA and next two weeks at restrictive temperature 18^{o}C to stop the expression SNCA.
Results. We showed a strong deficit of synaptotagmin 1 and n-synaptobrevin mRNA in brain of flies that expressed *SNCA*. Transgenic lines of *Drosophila* that expressed wild type *SNCA* and mutant *SNCA* exhibited a progressive neurodegeneration of dopaminergic neurons. After the stop expression of *SNCA* the level mRNA presynaptic proteins is not restore but the neurodegeneration is slightly slowed.
Conclusions. We found that suppression of transgene expression in the brain arrested in part alpha-synuclein pathology progression.
This work is supported by RFBR grant 12-04-00898.

08f. Animal Models: pharmacological & lesion models

ADPD5-0367

SYMPTOMATIC AND NEUROPROTECTIVE EVALUATION OF A CANDIDATE COMPOUND IN THE UNILATERAL 6-OHDA LESION RAT MODEL OF PD

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Parkinson's disease (PD) is the second most common neurodegerative disease, affecting 1% of the population over 55 years of age. PD is characterized by the loss of dopaminergic neurons and a decline in striatum dopamine content and is associated to motor symptoms: akinesia, bradikinesia, rigidity and tremor and postural abnormality. Key neurotoxic models of PD were produced by the unilateral intranigral administration of the toxin 6-OHDA representing the most commonly used. The hallmark of this rat preclinical model is the high reproducibility of the dopaminergic lésion >90% dopamine content (attested by a immunoTH study in the striatum) allowing to test the symptomatic efficacy of a candidate compound as well as neuroprotective effect in preventive condition. In our experimental conditions, the symptomatic efficacy of a test compound is tested with the three different akinetic tests 2 weeks after 6-OHDA administration: initiation time, stepping and cylinder tests, in comparison with the reference compound L-DOPA. The neuroprotective effect of the test compound is evaluated by counting the number of TH positive cells in the SNc. Finally, the mechanism of neuroprotection of the test compound is investigated by two neuroinflammation biomarkers (GFAP and IBA1) by immunohistochemical studies.

08f. Animal Models: pharmacological & lesion models

ADPD5-1155

PROLYL OLIGOPEPTIDASE INHIBITION ATTENUATES THE TOXICITY OF PROTEASOMAL INHIBITION IN ALPHA-SYNUCLEIN OVEREXPRESSING CELLS AND IN PARKINSON'S DISEASE MOUSE MODEL

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Objectives: A model for PD based on proteasome inhibition was studied in cell culture and in mice. The properties of a prolyl oligopeptidase (PREP) inhibitor, KYP-2047, that has autophagy enhancing and alpha-synuclein (aSyn) aggregation inhibiting features, was tested in these models.
Methods: SH-SY5Y cell lines overexpressing A53T or A30P aSyn were exposed to a proteasome inhibitor, lactacystin (LC, 10 µM) for 24 hours ± 1 µM KYP-2047. LDH test was done to measure cell viability. Aggregation and ubiquitinylation markers were quantified by western blot.
LC (2µg) was injected stereotaxically above the mouse substantia nigra. KYP-2047 was administered for 5 days and week after the surgery motor tests (cylinder and 2-hour locomotor activity) were performed. Immunohistochemistry was used to examine changes in aSyn, tyrosine hydroxylase (TH) and glutamate decarboxylase (GAD).
Results: In aSyn overexpressing cells, KYP-2047 reduced the LC-induced increase in aSyn oligomers and ubiquitin levels leading to improved cell viability
In mice, LC reduced the distance travelled and rearing in locomotor test but not in KYP-2047 treated mice. Immunohistochemical analysis revealed significant accumulation of aSyn and GAD increase in substantia nigra, and significant striatal TH loss.
Conclusions: Our results show that nigral stereotaxic injection of LC in mice produces rapid loss of dopaminergic cells with aSyn accumulation and motor deficits making it a suitable PD model. Moreover, PREP inhibition prevented the LC induced behavioral changes in mice and improved cell viability in aSyn cellular model, proposing a role for PREP inhibition as a potential drug therapy in PD.

08f. Animal Models: pharmacological & lesion models

ADPD5-1432

DEVELOPMENT OF A TEST-SYSTEM BASED ON PARKINSONIAN MICE FOR A SEARCH OF NEUROPROTECTORS

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Objectives were to specify (i) timing characteristics of the degeneration of nigrostriatal dopaminergic neurons, axons and cell bodies, separately in MPTP-treated mice, (ii) functional characteristics of surviving neurons, (iii) validity of a test system for screening of neuroprotectors.

Methods. The study was performed in C57BL/6 mice at the symptomatic stage of parkinsonism induced by 4-fold s.c. injections of MPTP at the individual dose of 12 mg/kg. Dopaminergic neurons were studied by using quantitative and semi-quantitative immunocytochemistry for tyrosine hydroxylase (TH) and HPLC for DA assay.

Results. According to our data, the number of TH-immunoreactive striatal axons begins to decrease shortly after the first injection of MPTP going down up to 43% by the 6th h following the last injection. This was accompanied with a decrease of TH content in individual axons under maintaining of DA at the control level. The number of the nigral TH-cell bodies decreased for 43% from the 3rd to the 6th h following the last MPTP injection in parallel with a slight decrease of DA content in the substantia nigra and the maintaining of intraneuronal TH at the control level. Neurodegeneration was almost prevented by the nomifensine injection 30 min prior to MPTP administration confirming the validity of this parkinsonian model as a test system for a search of neuroprotectors.

Conclusions. Thus, a test-system for a search of neuroprotectors was developed in this study.

08f. Animal Models: pharmacological & lesion models

ADPD5-1528

EVALUATION OF OLFACTORY DISCRIMINATION IN A HEMI-PARKINSONIAN 6-HYDROXIDOPAMINE MODEL OF PARKINSON'S DISEASE

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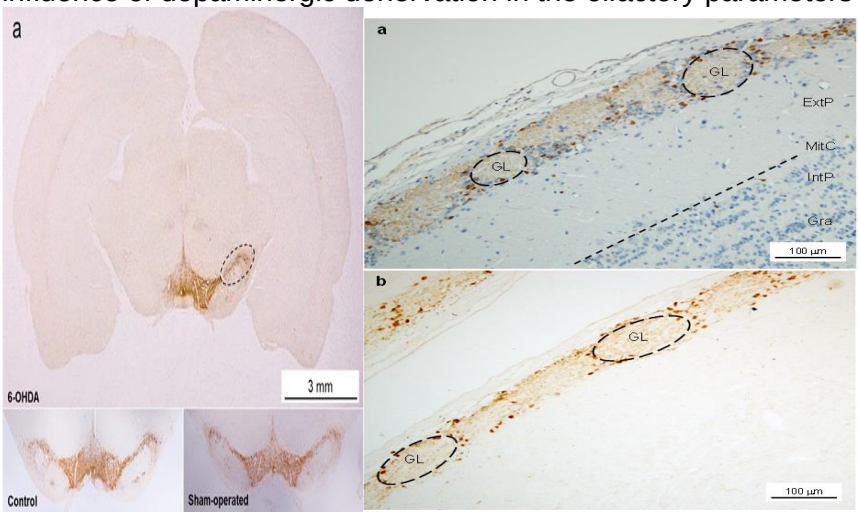
OBJECTIVE: Evaluate the histopathology and behavior of the olfactory system in rats submitted to dopaminergic lesion induced by 6-hydroxydopamine (6-OHDA),

METHODS: Forty eight adult male Wistar rats were divided into groups: (1) 6-OHDA lesioned, (2) sham-operated and (3) control group (without any procedure). Groups 1 and 2 were submitted to stereotaxic surgery for unilateral microinjection of 6-OHDA and ascorbic acid solutions in the medial forebrain bundle. The extension of the dopaminergic lesion was evaluated at 21, 28 and 57 days after surgery. During each time period, motor function (rotational behavior and open field) and olfactory discrimination function (Coconut milk test) were evaluated. We performed both cell counting in the substantia nigra pars compacta (SNpc) and measured optical density (OD) in the OB, for tyrosine hydroxylase (TH) immunoreactivity.

RESULTS: All 6-OHDA groups of each time period exhibited a reduction of dopaminergic neurons in the SNpc ($p<0.001$, compared to control and sham-operated groups) with a mean reduction of 73%. Pearson's correlation demonstrate a modest, non-significant, negative correlation ($p= 0.33$) between the number of TH positive cells and rotations. We did not detect olfactory dysfunction ($p=0.31$, for treatment and time factors) or any significant alterations in the amount of TH positive cells in the glomerular layer of the ipsilateral OB ($p=0.80$, compared to contralateral OB).

CONCLUSION: The 6-OHDA model still represents a very useful tool in PD pathophysiology research, regarding DA denervation. However, we did not observed influence of dopaminergic denervation in the olfactory parameters investigated.



ADPD5-1692

DESYMPATHYZATION OF THE HEART IN MPTP-TREATED MICE AT THE PRESYMPTOMATIC AND SYMPTOMATIC STAGES OF PARKINSONISM

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Objectives were to determine the noradrenaline (NA) concentration in the heart and the heart force in response to NA in C57BL/6 mice at the presymptomatic and early symptomatic stages of parkinsonism two weeks following 2-fold and 4-fold s.c. injections of MPTP, respectively, at the individual dose of 12 mg/kg. NA was assessed with HPLC ex vivo, while the heart force was measured with tensometry in vitro. According to our data, NA concentration decreased by 23% in the atria and by 21% in the ventricles at presymptomatic stage and by 34% and 31%, respectively at the symptomatic stage suggesting a heart desympathyzation. NA strengthens the atrium force by 86% and the ventricle force by 61% at the presymptomatic stage, whereas this reaction slightly decreased at the symptomatic stage up to 15% and 35%, respectively. It means that the heart sensitivity to NA increased significantly under desympathyzation that is more prominent at the presymptomatic stage. Thus, the heart desympathyzation is developed in MPTP-treated mice at the presymptomatic stage of parkinsonism being even more prominent at the early symptomatic stage.

08f. Animal Models: pharmacological & lesion models

ADPD5-2046
REGULATION OF TYROSINE HYDROXYLASE IN A LOW DOSE ROTENONE MODEL OF PARKINSON'S DISEASE

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Objectives:
Tyrosine hydroxylase (TH, the rate limiting-enzyme in catecholamine synthesis) is regulated acutely via phosphorylation of 3 serine residues – Ser19, 31 and 40, and chronically via changes in TH protein levels. In this study we aimed to investigate how TH is regulated in the brain and adrenal gland in a low dose rotenone model of Parkinson's disease (PD).
Methods:
Male Sprague-Dawley rats received intraperitoneal injections (2 mg/kg) of rotenone (*n*=5) or vehicle (*n*=4) 5 days/week for 4 weeks. The rearing test was performed weekly. Adrenal glands and selected brain regions were collected postmortem for western blot analysis of TH protein and TH phosphorylation.
Results:
Rearing behaviour decreased in the rotenone group by week 3 (*p=0.003*), with further decreases in rearing by week 4 (*p<0.0001*). TH was unchanged in the substantia nigra (SN) and striatum but pSer40 was decreased in the striatum (*p=0.04)* of rotenone rats. In the olfactory bulb, TH protein levels decreased by 60% (*p=0.01*) while pSer31 levels increased by 40% (*p=0.02)* in the rotenone group. In the adrenal gland, TH and phenylethanolamine-N-methyltransferase (another catecholamine synthetic enzyme) levels increased by 80% (*p=0.02) and 100% (p=0.02)* respectively in the rotenone rats.
Conclusions:
This study provides evidence that TH regulation in the olfactory bulb and adrenal gland (the hypothesised early regions affected in PD) are altered before any changes in TH regulation are detected in the SN. The low dose rotenone via intraperitoneal injections therefore may have potential to model the early changes of PD.

08f. Animal Models: pharmacological & lesion models

ADPD5-2074

FUNCTIONAL STATE OF THE NIGROSTRIATAL SYSTEM IN MICE AT THE PRESYMPTOMATIC AND EARLY SYMPTOMATIC STAGES OF PARKINSONISM

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The objective was to evaluate functional state of the nigrostriatal system in parkinsonian mice at presymptomatic and early symptomatic stages, induced by 2-fold and 4-fold MPTP injections (12 mg/kg per injection). We evaluated in substantia nigra (SN) and striatum uptake, spontaneous and stimulated release of 3H-dopamine (DA) on slices; D2 and MAO A&B gene expressions; enzymatic activity of MAO A&B. Moreover, intercellular DA was measured in striatum with microdialysis.
At presymptomatic stage, despite a loss of 29% neurons in SN, most parameters were unchanged though MAO A&B gene expressions decreased. In striatum, intercellular DA decreased by 61% as a result of: (i) a decrease of DA spontaneous release, gene expression and activity of MAO B; and (ii) an increase of MAO A gene expression, DA stimulated release and uptake. When converting the data on the number of cell bodies and axons, it was found that DA spontaneous release compensatory increased by 43% and 216%. In SN at symptomatic stage, no differences in most parameters were observed compared to presymptomatic one, though D2 and MAO B gene expressions decreased. We observed in striatum a decrease of DA intercellular level, DA stimulated release and uptake, MAO A&B gene expressions, and an increase of MAO A&B activity. Thus, an absence of motor disorders at presymptomatic stage is partly explained by an increase of DA spontaneous release and decrease of MAO B activity in striatum whereas motor dysfunctions appeared to be triggered by a decrease of DA release and increase of MAO A activity.

08f. Animal Models: pharmacological & lesion models

ADPD5-2103

BIOCHEMICAL AND BEHAVIORAL EFFECTS OF COMBINED TREATMENT WITH PAROXETINE AND L-DOPA IN UNILATERALLY 6-OHDA-LESIONED RATS

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The aim of the present study was to examine rotational behavior and monoamine metabolism in motor (striatum, substantia nigra) and limbic (prefrontal cortex, hippocampus) brain structures of 6-OHDA-lesioned rats, treated chronically with paroxetine jointly with L-dopa. The experiment was performed on male Wistar rats injected unilaterally with 6-OHDA (16ug/4ul) into the medial forebrain bundle. Two weeks later, the animals were tested for the rotational behavior induced by apomorphine. Rats exhibiting more than 100 contralateral turns/1h were administered paroxetine (5mg/kg) or L-dopa (12mg/kg), alone or in combination, once daily for 21 consecutive days. Rotational behavior was recorded after the first and the penultimate doses of the examined drugs. Dopamine (DA), serotonin (5-HT) and their metabolites were determined using HPLC in the motor and limbic brain structures 1h after the last drug injections.
Combined administration of paroxetine and L-dopa resulted in decrease in the number of contralateral rotations compared to the L-dopa-treated group. Joint treatment with paroxetine and L-dopa increased DA levels on the ipsi- and contralateral side of the substantia nigra and on the contralateral side of the remaining structures more visibly than did L-dopa alone. Paroxetine alone enhanced 5-HT level in the contralateral striatum and substantia nigra while combined treatment decreased it. In limbic structures paroxetine+L-DOPA lowered 5-HT content compared to the effect of L-DOPA alone. The obtained data are discussed in the context of PD therapy.
Study supported by the Project “Depression-Mechanisms-Therapy” – POIG.01.01.02-12-004/09-00.

08f. Animal Models: pharmacological & lesion models

ADPD5-2225

A NEW CHEMICAL MODEL OF PARKINSON'S DISEASE

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Objective: Parkinson's disease (PD) is a complex genetic disorder, associated with environmental risk factors and aging. Current animal genetic or toxin-based models have so far failed to recapitulate all of the features of PD, thus impeding attempts to develop neuroprotective or disease-modifying treatments. Here we describe a new chemical for induction of PD in mouse, with a slower kinetics than other toxins, thus more relevant regarding physiopathology.

Methods: The effect of 96h exposure to the Pyridinium on a neuroblastoma cell line (SH-SY5Y) was investigated on cell death (caspases activation, oxidative stress), and on α-synuclein aggregation. The consequences of a 7-day *per os* exposure to Pyridinium (20mg/kg/day) in mouse were evaluated by biochemical (mitochondrial dysfunction) and histological studies (phosphorylated α-synuclein, Tyrosine hydroxylase, Tau (using an antibody that recognizes an early pathological conformation)).

Results: Pyridinium exposure induced SH-SY5Y cell death, increased alpha-synuclein aggregation, and inhibited mitochondrial complex I. The EC_{50} of Pyridinium on cell survival was in the millimolar range but these effects appeared after 96 hours versus 24 hours for MPP+.

Five weeks after mice exposure to Pyridium, mitochondrial respiratory control ratio, and complex I activity were decreased (24% and 48% respectively) simultaneously to an increase of Ser-129-phosphorylated α-synuclein and loss of striatal dopaminergic neurons. Misfolded Tau in hippocampus indicated an associated tauopathy.

Conclusion: With its slower kinetics, the use of Pyridium can significantly address critical gaps in our understanding of the etiology of PD, in the identification of new early biological markers and development of novel effective treatments currently lacking.

08g. Animal Models: natural & seminatural models

ADPD5-0217
DECREASED SPREADING DEPRESSION SUSCEPTIBILITY IN PARKINSON RAT MODEL

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Parkinson disease (PD) is known by a major loss of dopaminergic nigrostriatal neurons and by an increased turnover of neurotransmitter by surviving neurons of the nigrostriatal tract. The clinical diagnosis of PD is based on the identification of some combination of the cardinal motor signs of bradykinesia, rigidity, tremor, and postural instability. Spreading depression (SD) known as an evoked neuronal activity and changes in ionic, metabolic and hemodynamic characteristics of the brain. Pronounced release of dopamine during SD and the probable role of dopamine in SD process suggests that disruption of dopaminergic pathway in PD may cause SD to behave differently. To test this possibility, we induced dopaminergic lesion by bilateral intracerebral stereotactic injection of 6 µL of 6-hydroxydopamine in the medial forebrain bundle (MFB). After 4 days, SD was induced by the injection of 3M KCl and SD propagation was followed using two ion-sensitive microelectrodes placed in the parietal and occipital cortex. Eliciting SD in rat model was associated with a significant increase in the threshold of SD and a decrease in the propagation velocity and duration of accompanying extracellular DC changes. The present data show that rat model of Parkinson's disease are less prone to SD.

08h. Animal Models: other

ADPD5-1397

TREADMILL TRAINING ATTENUATES DOPAMINERGIC DEFICITS AND ELEVATES BDNF AND GDNF BIOSYNTHESIS IN CHRONIC MPTP MOUSE MODEL OF PD.

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PD is neurodegenerative motor disorder, the symptoms of which are partly due to the progressive degeneration of the nigrostriatal dopaminergic pathway. In the initial stages, motor symptoms of PD can be controlled by pharmacological intervention, however as the disease progresses, this is associated with loss of efficacy and emergence of side effects. Therefore, the first step of an ideal therapy must be to prevent this cell death. Accumulating clinical evidence suggests that physical exercise can provide this much needed treatment, and studies of PD animal models of dopamine deficiency associated with the motor symptoms support this hypothesis. It is suggested that these neuroprotective effects are likely to involve the activation of signaling cascades by neurotrophic factors. The aim of this study was to investigate the effects of treadmill exercise-induced behavioral and neurochemical changes in C57/BL6 mice treated for five weeks with 12,5mg/kg 1-methyl-4-phenyl1,2,3,6-tetrahydropyridine (MPTP) in combination with 250mg/kg probenecid. Mice were subdivided into four groups: (i) control, (ii) sedentary (non-exercised with induction of PD), (iii) exercised before during and after the induction of PD, and (iv) exercised only after the induction of PD. Brains were collected for immunohistochemistry for tyrosine hydroxylase, VMAT-2 transporter, BDNF and GDNF neurotrophins in substantia nigra and striatum. Exercised groups exhibited reduced damage to the dopaminergic system and excessive increase of BDNF and GDNF expression. These results suggest that the chronic MPTP protocol is a model of progressive PD, which may be suitable to investigate chronic pathological processes and neuroprotective strategies in PD.

08h. Animal Models: other

ADPD5-1514

CLINICAL EVALUATION OF A NEW MODEL OF PD (SAPAJUS APELLA) TREATED WITH MESENCHYMAL STEM CELLS

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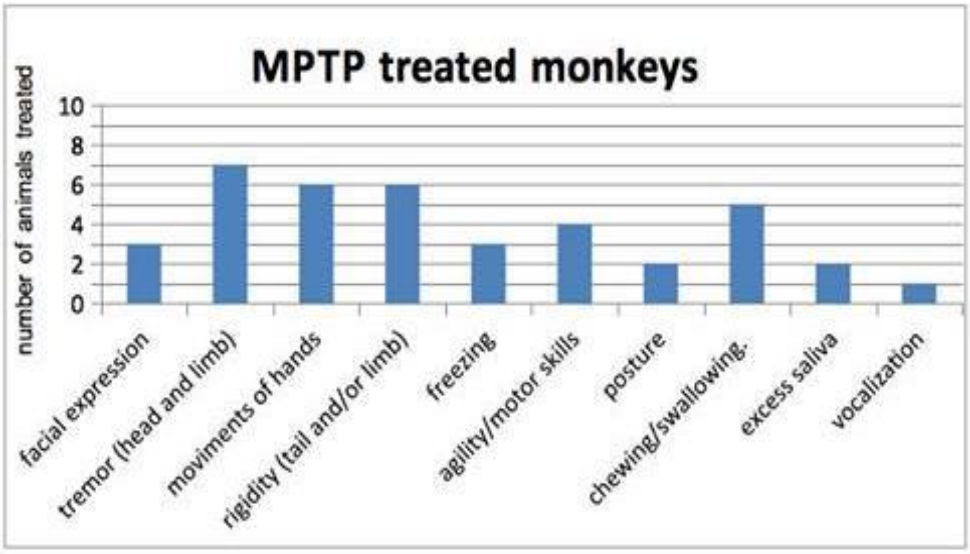
Introduction: Stem cell therapy has the potential to significantly impact the development of disease-modifying treatments for PD. However, it´s very important the development of new animal models of PD, before the scientific comunity start to speculate the use of stem cell therapy.

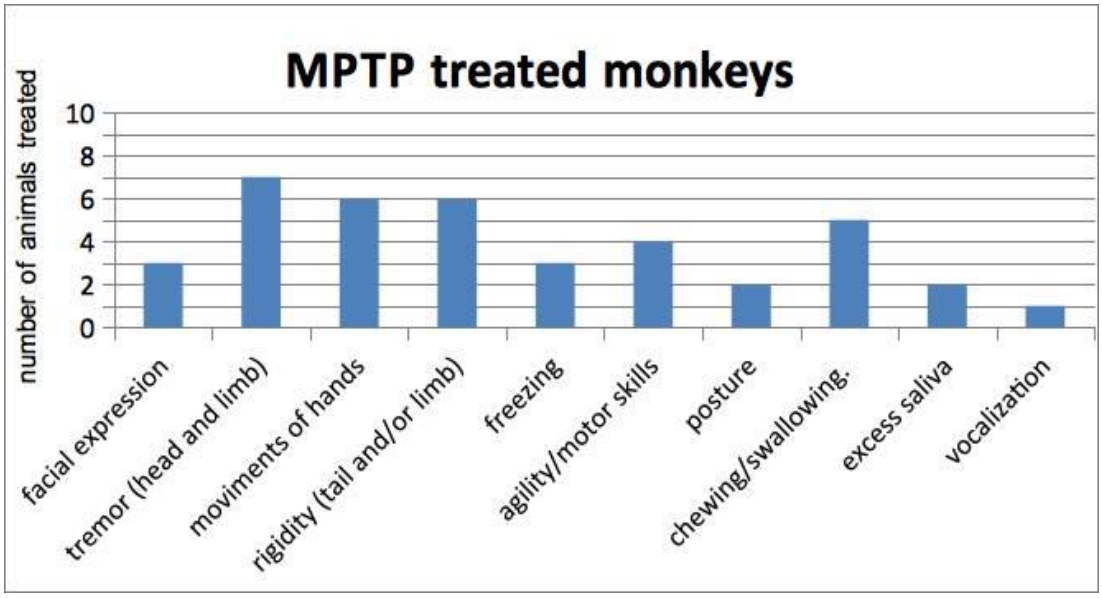
Objectives: This study was design to propose capuchin monkey (*Sapajus apella*) as model of PD and also to evaluate the potential use of autologus mesenchymal stem cells (MSC) therapy in PD treatment.

Methods: 10 male capuchin monkey (mean age=12 years) received MTPT injection (0.4mg/kg, twice a week) during 1 month. After this period, the animals were divided in 2 groups One group (n=5) received a single dose of MSC via intracarotid and the other (n=5) received the vehicle solution instead of MSC. During all period MPTP treatment (30 days) and for 30 days after MSC therapy the clinical aspects were evaluated in accordance with the UPDRS scale adapted.

Results: Clinical aspects related with PD were present in almost all of the MPTP-animals (Figure 1). Futhermore, after MSC therapy, most of the animals present significant reduction (p>0,005 – t test) of the evaluated clinical asptects of PD (Figure 2).

Co**nclusions**: Capuchin monkeys seems to be a promise new animal model of PD. The use of autologus MSC therapy shown to have a great potential on the treatment of PD.


MPTP treated monkeys
number of animals treated
10
8
6
4
2
0
facial expression
tremor (head and limb)
moviments of hands
rigidity (tail and/or limb)
freezing
agility/motor skills
posture
chewing/swallowing.
excess saliva
vocalization


MPTP treated monkeys
number of animals treated
10
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rigidity (tail and/or limb)
freezing
agility/motor skills
posture
chewing/swallowing.
excess saliva
vocalization

08h. Animal Models: other

ADPD5-2205

PROGRESSIVE NIGROSTRIATAL NEURODEGENERATION ASSOCIATED WITH ALPHA-SYNUCLEIN PATHOLOGY INDUCED BY AAV-MEDIATED OVEREXPRESSION OF MUTANT ALPHA-SYNUCLEIN IN MICE, RATS AND MARMOSETS

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Objective: Animal models are an essential asset for pathophysiological research as well as validation of therapeutic strategies of human diseases. The absence of adequate in vivo experimental models has severe repercussions for therapeutic intervention success. Despite progress in animal modelling, no mammalian model recapitulates the required age-dependent phenotypes associated with Parkinson's disease (PD).

Methods: We selected an elegant series of models of ascending complexity with three different strains of mice (C57Bl/6J, SAMP8 and SAMR1 for senescence-accelerated mouse prone or resistant, respectively), adult rats and marmoset monkeys at various ages. Each model received a stereotatic injection in the substantia nigra pars compacta of adeno-associated virus (AAV) serotype 9 carrying mutant human alpha-synuclein (A53T; 10^{10} GC/ml) under the neuron specific synapsin promoter including a WPRE enhancer element. Sixteen weeks after injection, we systemically investigated species and age-dependent differential susceptibility of dopamine neurons regarding the extent of neuronal loss. We also assessed alpha-synuclein spreading.

Results: We successfully induced dopaminergic degeneration in all species. Mice were less susceptible to alpha-syn-mediated toxicity compared to rats and monkeys. Both SAMP8 and SAMR1 present a strong alpha-synuclein staining which was not correlated with the occurrence of degeneration. High-resolution kinematic analyses revealed that rats exhibited the hallmarks of Parkinsonian syndromes during gait, including periods of freezing, postural instability, and reduced speed of motion. In marmosets, old animals were more susceptible to degeneration at both fibers and cell body levels.

Conclusion: According to the species used, we observed differences in the PD-related neurodegeneration progression over time associated with alpha-synucleinopathy.

09a. Patient Care & Support: caregiver support

ADPD5-0370
QUALITY OF LIFE EVALUATIONS IN PD: SELF/CAREGIVER AGREEMENT
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Background: Self-reports about quality of life (QoL) of patients with Parkinson's disease (PD) themselves and of their spouses as caregivers may be subjective, and not always reliable. Objective: To examine patient-spouse agreements on the items of the PD Quality of life Questionnaire (PDQ-39), the Scale of Quality of Life of Care Givers (SQLC), and the Multidimensional Caregiver Strain Index (MCSI). Methods: Patients and their spouses were separately interviewed and completed the same questionnaires for mutual characterization of each other. Their scores were examined with approximate agreement coefficients and interclass correlations (ICC).Results: 12 patient-spouse pairs were assessed. Spouses were younger (age: 70.6 ± 5.3 vs. 75.8 ± 6.7 years, P=0.06), both groups had a similar education level (14.7 and 14.3 years). Approximate agreements among QoL items were strong for PDQ-39, SQLC and MCSI (75.4 ±14 %; 78.1 ±14.1 % and 78.2 ±14.3 %, respectively), as well as within each of the couples: 75.8-78.6%. Patient-spouse opinions had better concurrence for physical condition (PDQ items: 3, 8, 12-15, ICC 95% CI – 0.637-0.713) and depression in sick persons (PDQ items: 17, 23, 24, ICC 95% CI – 0.6-0.874). Divergences in assessments in the items of SQLC and MCSI, designed for spouses were considerable (averaged ICC – 0.1)). Individual agreements within each of the couples were modest (averaged ICC– 0.41 for PDQ, and 0.429 for SQLS). Conclusions: PD patients and their spouses-caregivers had accetable approximate agreements for most of QoL items oriented to patients. However, the inner patient-spouse interrelationships have appeared complex.

09a. Patient Care & Support: caregiver support

ADPD5-0629

A COMPARISON OF CAREGIVING OUTCOMES IN PARKINSON'S DISEASE AND PARKINSON'S DISEASE DEMENTIA

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The growing incidence of cognitive impairments in persons with Parkinson's disease (PD) creates unique challenges for caregivers. Therefore, the relationship between symptom presentation and caregiver outcome deserves to be investigated.

OBJECTIVE: determine the different contribution of stressors to role strain, mediators and outcomes in PD compared to PD dementia (PDD) caregivers.

METHODS

Community-living PD and PDD spouse caregivers providing 3^{+}hour of care/week for >1-year were recruited (N=55). Caregivers' completed questionnaires that assessed: characteristics (age, sex, duration), stressors (ADL, cognitive impairment, motor dysfunction, memory-behaviour problems), role-strain (care-hours), mediators (assistance, self-efficacy, coping) and outcomes (depression, burden, life-satisfaction). ANOVAs determined PD-PDD group differences, while individual stepwise regression models determined stressors that best-explained caregiver outcomes.

RESULTS

Persons with PDD had greater cognition impairment, ADL and IADL disability, motor dysfunction, and more bothersome memory and behaviour problems ($p<0.01$). PDD caregivers provided more care-hours ($p=0.04$); experienced greater burden, depression and decreased life-satisfaction than PD ($p<0.006$). There were no differences in characteristics, assistance, formal/informal supports, coping strategies and self-efficacy between groups. IADL ability and cognitive impairment best-explained PDD caregiver outcomes ($F(1,51)=29.9;R^2=0.55;p=0.02$). Specific IADL (shopping, food-preparation, household-chores, transportation, medication) that best-explained cognitive decline were food-preparation and shopping ($R^2=0.53;p=0.003$). For caregiver outcomes, food-preparation explained 32% of burden, household-chores explained 16% of depression, and food-preparation and transportation explained 35% of life-satisfaction ($p<0.02$).

CONCLUSIONS

PDD dementia caregivers experience declining IADL ability due to cognitive impairments, leading to negative outcomes. This research supports the importance of equipping caregivers with coping skills relevant to symptom presentation to decrease negative outcomes and improve life-satisfaction.

09a. Patient Care & Support: caregiver support

ADPD5-1593
UNDERSTANDING OF THE DISEASE AND THE CARE GIVER BURDEN IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is progressive degenerative disorder and the management of PD is aiming for the improvement of daily activities of patients and their family. The purpose of this study is to evaluate the patients and their caregivers' understanding of the disease and its impact on the caregiver burden.

Caregivers of PD patients were consecutively recruited from outpatient clinic. Caregivers were confined to one of patients' family member, who were main member in patients' care. Questionnaire to evaluate the understanding PD was developed by movement disorder specialists based on current reviews and guidelines, covering pathology and nature of the disease, symptomatology, and treatment. Structured interview about demographic and socioeconomic characteristics was also done with questionnaire covering caregiver burden, understanding the disease, general quality of life (QoL), and depression.

Total 83 caregivers were recruited (age 62.2 ± 12.2 years, 55 men) and most of them were spouses of patients (79/83). Caregivers' understanding showed significant difference according to sex (men better than women). Caregiver burden was significantly correlated with their understanding, especially economic aspect of burden and understanding about pathology and nature of the disease. Depression and QoL of caregiver showed significant correlation with caregiver burden. Patients' understanding and QoL was also correlated with caregiver burden.

Understanding of patients and caregivers about their disease showed negative correlation with caregiver burden. Education about the disease to patients and their caregivers could improve daily burden of the disease and should be an essential part of the proper management of PD.

09a. Patient Care & Support: caregiver support

ADPD5-2196
FACTORS INFLUENCING MUTUALITY IN PARKINSON´S DISEASE DYADS
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Objectives
The aim of this ongoing project is to explore the quality of the relationship (mutuality) between patients with Parkinson´s disease (PD) and their partners and how it is affected by the motor and non-motor symptoms, other perceived difficulties, caregiver burden, and health-related quality of life (HRQoL).
Methods
The dyads (n=34) completed validated scales measuring mutuality (MS), caregiver burden (CBS), caring difficulties (CADI), depression (GDS), activities in daily life (ADL) and Parkinson´s disease HRQoL (PDQ8). Associations were evaluated using Pearson´s correlations to identify variables significantly related to mutuality. Multiple regression analysis was used to estimate the associations between independent factors and mutuality.
Results
Mean age of the PD patients was 69.6(SD=8.3) and for the partners 69.2(SD=8.3) years. Most of the partners 21/34 (62%) were women and had been cohabiting with the PD patient for 38.9(SD=14) years. The mean Hoehn & Yahr score was 2.5(SD=.7) with an average PD duration of 9.4(SD=6.5) years. Mutuality score was significantly negatively associated with CBS (r=-.71), CADI (r=-.73), GDS (r=-.64), ADL (r=-.41), PDQ8 (r=-.41). Mutuality did not strongly correlate with motor and non-motor symptoms, or disease duration. CBS, GDS, PDQ8 and ADL explained 55 % of the variance in mutuality and of the included variables CBS had the largest contribution (beta =-.56, p =.008).
Conclusion
Neither severity of symptoms nor disease duration were associated with impaired mutuality as were higher depression and caregiver burden scores in dyads with mild to moderate PD. Further attempts are needed to improve mutuality in dyads with depression.

09b. Patient Care & Support: mobile applications

ADPD5-1621

NEW MOBILE HEALTH SYSTEM FOR AT-HOME REHABILITATION OF WALKING IN PERSONS WITH PD

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Background: Mobile health technologies have the potential to empower patients with PD to exercise on their own while receiving online supervision on the correctness of their motor output, thereby promoting long-term adherence and enhancing training effects. Aim of this study was to test the effectiveness of a newly developed auditory bio-feedback system (ABF) for gait training.

Methods: ABF was developed within the FP7/CuPiD project (grant agreement n°288516) and consists of three wireless inertial sensors (easily wearable on shoes and trunk) able to accurately measure in real-time gait spatio-temporal parameters and trunk inclination. The sensors connect to a smartphone and ABF as a whole acts as a 'virtual clinician' continuously assessing and vocally correcting patients' ineffective or unsafe gait patterns. 20 PD subjects were enrolled to participate in a 6-week at-home program in which they train with ABF for 30 minutes at least 3 times/week. Gait analysis pre-post training was performed to assess system efficacy.

Results: The first 14 patients (mean age: 64.5±8.3 yrs; 3 women, disease duration 10.84±5.84 yrs) who completed training using the system were able to successfully use ABF and reported high satisfaction. Comfortable walking speed improved by 9.34%±2.79% (p=0.01), step length improved by 7.05%±1.74% (p=0.03) and the 2-minute walk test improved by 11.65%±3.93% (p=0.03).

Conclusion: Initial findings suggest that patients with PD are able to autonomously use ABF and that it is a promising tool for teaching effective gait patterns. This now needs confirmation in a controlled study.

Fig.1: ABF System

Fig.2: Android app

09f. Patient Care & Support: cognitive training

ADPD5-0985

THE QIGONG APPROACH FOR INPATIENTS WITH PD: TWO CASE REPORTS

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Introduction

Qigong is an exercise based on traditional Chinese medicine. Qigong combines fast or slow motions and motions at rest such as those during meditation. Qigong is beneficial for the mind and body. Qigong exercise relieves stress and ensures relaxation. Further, Qigong reduces muscular strain and suppresses ataxic movement.

Objective

We aimed to investigate the effect of Qigong exercises in two inpatients with PD.

Description

1. Procedure

Two inpatients with Parkinson's disease underwent a Qigong session for 30 minutes every week. In addition, we taught them Qigong self-exercises to be performed for 5 minutes.

2. Case reports of inpatients with Parkinson's disease

(1) Case A: Female, 75 years

This patient had a 20-year clinical history of Parkinson's disease. She performed all Qigong exercises eagerly. After 6 months, she achieved body balance and stability, and her ataxia improved. Therefore, she was moved from the hospital to a welfare facility.

(2) Case B: Male, 65 years

This patient had a 10-year clinical history of Parkinson's disease. Despite his initial hesitation to undergo Qigong exercises, the patient practiced these exercises every week. His activities of daily living (especially visiting the toilet and bathing) improved after performing Qigong. Subsequently, he started performing the Qigong exercises eagerly. After four months, he was discharged from the hospital, and currently, because of Qigong, he is able to perform his hobbies (painting and karaoke) at home.

Conclusion

These cases suggest that Qigong may reduce muscular strain stress and help cope with Parkinson's disease.

09h. Patient Care & Support: functional foods

ADPD5-0358
QUALITY OF LIFE BEFORE AND AFTER PHONOAUDIOLOGIC THERAPY IN PATIENTS WITH PARKINSON DISEASE

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Objective: To evaluate quality of life (QOL) in individuals with Parkinson's disease (PD) before and after therapy for swallowing disorders. **Methods:** Patients with PD who accepted to participate in all intervention sessions and research steps were selected from the Movement Disorders Outpatient Clinic at Hospital de Clínicas de Porto Alegre. Patients with language and/or hearing conditions that prevented therapy comprehension were excluded. Four individual sessions of 30 minutes each with orientations concerning feeding and a postural maneuver (head down) were performed. Quality of Life in Swallowing Disorders (SWAL-QOL) were also applied before and after therapy. **Results:** Ten individuals were evaluated, 80% male, mean age of 62,2 years (±11,3), mean of 7,5 (±4,3) years of education and mean disease duration of 10,7 (±4,7) years. 30% were classified as H&Y 2, 50% as H&Y 3 and 20% as H&Y 4. It was identified an increase in scores of all domains, including a significant difference in domain 4 (symptom frequency) p=0,025. In domain 5 (food selection), there was a trendp=0,95. Correlations between the differences before and after therapy of each domain and variables as gender, age, H&Y and education were tested. There were significant correlations between domain 10 (sleep) and gender (p=0,047), and H&Y (p=0,024). Besides, a tendency to correlation was seen between domain food selection and H&Y (0,051). **Conclusion:** From these results, an improvement in QOL concerning swallowing of PD patients after phonoaudiologic therapy can be observed. Furthermore, the lower the disease stage, the greater are the improvements after therapy.

09h. Patient Care & Support: functional foods

ADPD5-2157

CORRELATION BETWEEN QUALITY OF LIFE AND ALTERATIONS IN SPEECH, SWALLOWING AND COGNITION IN INDIVIDUALS WITH PARKINSON'S DISEASE

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Objective: to evaluate the impact of clinical alterations in speech, swallowing and cognition in the quality of life (QL) of patients with Parkinson's disease.
Methods: This is a quantitative, transversal, descritive study. Patients with Parkinson's disease participated in this study and all individuals under went speech, swallowing and cognitive evaluation, they answered the PDQ-39 questionaire and had their disease severity definied according to the Hoehn&Yahr scale.
Results: 51 PD individuals were evaluated, 28 (54,9%) were male. Average age was 61,7 years (±10,4) and mean disease duration since diagnosis was 10,2 years (±5,5). In the phonoaudiologic evalatuation, 28 (54,9%) had speech disturbancesand 38 (74,5%) hadswallowingimpairment. QL scoreswerelower as disease severity stage sincreased. Among all evaluated aspects, only cognition had na impact in the perception of QL of these individuals, even though speech alterations were the main reason to seek phonoaudiologic treatment.
Conclusion: Cognitive alterations directly reflect the perception of QL of patients with PD. Speech disturbances, however, are the main complaint, but with no impact on QL. Swallowing impairment does not have an impact on QL.

09i. Patient Care & Support: other

ADPD5-1493
COMPARISONS OF COGNITIVE DEFICITS IN PD AND METHAMPHETAMINE TOXICITY
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Objectives: A growing body of literature suggests that mild cognitive impairment is present at the time of diagnosis of PD. In animals, MPTP administration produces significant cognitive deficits even in the absence of Parkinsonian motor impairment. Studies of methamphetamine users also have reported impaired cognitive function without motor symptoms. This may be due to potential similarities in pathophysiology between PD and methamphetamine toxicity. This study examined the profile of cognitive deficits in methamphetamine users compared to those observed in PD patients.
Methods: Fifty-two abstinent methamphetamine and thirty-two healthy subjects completed cognitive assessments including Wisconsin Card Sorting Test (WCST), Stroop Test, and Wechsler Memory Scale (WMS). Performance on several functional domains including executive function, cognitive flexibility, attention, and memory was compared to cognitive impairment found in PD patients from prior studies.
Results: Relative to controls, methamphetamine users made more errors ($p=0.04$) on the WCST and required more time on Stroop Interference ($p=0.03$). WMS revealed that both immediate and delayed memory as well as auditory and visual working memory were significantly decreased in methamphetamine users (all $p<0.01$). Cognitive profiles in early PD are notable for attentional, executive, and visuospatial deficits (Svenningsson, 2012).
Conclusions: These results suggest similar cognitive deficits in both PD patients and methamphetamine abusers, and lend support to the evidence that PD and methamphetamine toxicity share common features of alterations in the frontal dopaminergic systems. Further research is needed to identify the neurophysiological basis of these deficits in order to develop novel pharmacotherapeutics to address these shared symptoms.

09i. Patient Care & Support: other

ADPD5-2102

E-MOTION: A RELIABILITY ASSESSMENT FOR A PROTOTYPE SOFTWARE TO COMPLEMENT THE CLINICAL EVALUATION OF PATIENTS WITH PARKINSON'S DISEASE

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Objective: To compare a software prototype, e-Motion with a benchmark reference, multiple-camera 3D motion capture system to track a gait pattern.
Background: Parkinson's disease (PD) is characterized by alterations in gait pattern that may increase the risk of falls. The gait pattern's variations cannot be objectively measured in the clinical examination, therefore, is necessary to adapt devices to measure objectively, valid and replicable changes in gait patterns.
Methods: we developed the "e-Motion Capture System", a prototype software able to calculate motor (stride and step length) and spatiotemporal (velocity and acceleration), parameters that are affecting quality of life in patients with PD. This analysis was performed to compare the spatial locations of the ankles from a volunteer under indoor controlled conditions.
Results: The index for the reliability to both systems and both laterality (N=599) averaged together 0.96 (IC95% 0.94 - 0.97). To left ankle, (N=103), the index was 0.96 (IC95% 0.88 - 0.98), and to right ankle, (N=112) the index was 0.97 (IC95% 0.85 - 0.99). According to guideline for the evaluation of intra-class correlation coefficients, the inter-rater agreement between e-Motion and 3D motion capture system shows an excellent agreement.
Conclusions: The e-Motion Capture System could develop and quantify measurements of motor and spatial-temporal variables sensitive to change in the timeline of the disease. In addition, this tool is useful to complement the clinical assessment and measure efficacy of pharmacological and non-pharmacological interventions of patients with PD.

09i. Patient Care & Support: other

ADPD5-2156
ASSESSMENT OF NONVERBAL AND VERBAL APRAXIA IN PATIENTS WITH PARKINSON'S DISEASE
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Objective: To assess the prevalence of nonverbal and verbal apraxia in patients with Parkinson's disease (PD), and analyze the correlation between these conditions and patient age, education, duration of disease and PD stage, as well as to evaluate the correlation between the two types of apraxia and the frequency and types of verbal praxic errors made by patients in the sample. **Method:** This was an observational prevalence study. The sample comprised 45 patients with PD seen at the Movement Disorders Clinic of the Clinical Hospital of Porto Alegre, Brazil. Patients were evaluated using the Speech Apraxia Assessment Protocol, and PD stages were classified according to the Hoehn and Yahr (H&Y) scale. **Results:** The prevalence of nonverbal apraxia and verbal apraxia in the present sample was 24.4%. There was no significance between the nonverbal apraxia with age, education, duration of disease, PD stage (H&Y), and genre. The correlation between verbal apraxia and education was significant ($p \leq 0.05$), but there was no significance with age, duration of disease, PD stage (H&Y) and genre. The most frequent types of verbal praxic errors were omissions (70.8%). The analysis of mode and place of articulation showed that most errors occurred during the production of vibrant (57.7%) and dentoalveolar (92%) phonemes, consecutively. **Conclusion**: patients with PD presented nonverbal and verbal apraxia, and made several verbal praxic errors. Verbal apraxia, but not nonverbal apraxia, was correlated with education levels.

10a. Other: cell, molecular & systems biology

ADPD5-1158

6-HYDROXYDOPAMINE CAUSES CELL DEATH IN RETINOIC-ACID INDUCED DIFFERENTIATED SH-SY5Y CELLS VIA DOPAMINE TRANSPORTER

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Objectives: 6-hydroxydopamine (6-OHDA) is the most used neurotoxin to study Parkinson's Disease (PD). Even though this neurotoxin disrupts the nigrostriatal pathway in vivo by uptake via dopamine transporter (DAT), the toxicity mechanism of 6-OHDA in vitro remains controversial. This may be related to lower levels of DAT in cell lines, such as the human neuroblastoma SH-SY5Y. Our group established a dopaminergic differentiation induced by retinoic acid (RA) treatment in SH-SY5Y cells. Herein, we aim to validate RA-differentiated SH-SY5Y cells as model for PD studies and evaluate the role of DAT in 6-OHDA-induced neurotoxicity.

Methods: SH-SY5Y cells differentiation was induced by the combination of ten micromolar of RA in cell culture medium supplemented with one percent of fetal bovine serum during seven days. We evaluated cellular proliferation, neurite outgrowth, gene expression, dopamine transporter (DAT) immunocontent and 6-hydroxydopamine cytotoxicity.

Results: We found that SH-SY5Y differentiation decreases cellular proliferation and increases neurite outgrowth. Moreover, RA-differentiated cells express higher levels of dopaminergic synapse markers, DAT immunocontent and is more sensitive to 6-OHDA compared to undifferentiated SH-SY5Y cells. Later, we observed that the pharmacological inhibition of DAT significantly reduces hydrogen peroxide production and cell death in response to 6-OHDA. Thus, demonstrating a pivotal role for DAT in 6-OHDA neurotoxicity in RA-differentiated SH-SY5Y cells.

Conclusions: Our data support that RA-differentiated SH-SY5Y cells is a suitable in vitro model to study PD pathophysiology since not only it mimics the dopaminergic cell features but also the 6-OHDA-induced cytotoxicity in vivo.

Acknowledgements: MCT/CNPq Universal (470306/2011-4), PRONEN/FAPERGS (1120325), CAPES (14581/13-2).

10a. Other: cell, molecular & systems biology

ADPD5-1429

THE COMMUNITY DRIVEN PD MAP PROJECT: FROM A KNOWLEDGE REPOSITORY TO A RESEARCH TOOL

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Objectives: As the knowledge on molecular mechanisms involved in Parkinson's disease (PD) is complex and heterogeneous it needs to be efficiently organized. Therefore the "PD map" compiles literature-based information on PD into an easy to explore and freely accessible molecular interaction map (http://pdmap.uni.lu). The involvement of the scientific community is crucial for such a resource. To further promote the use of the PD map, we developed new, research-facilitating functionalities.

Methods: PD map content curation and development of associated tools are community-driven. Therefore the PD map offers straightforward, interactive online features to provide feedback on content and functionalities. Furthermore, direct interaction with the scientific community in hands-on curation workshops facilitate the development of user-oriented functionalities.

Results: Here we present applications of new PD map research tools. (1) With the drug interaction tool user can now search for drug names or brands. Potential targets are fetched from drug databases and displayed in the PD map. (2) To visualize user data on the PD map, experimental data-sets can now be uploaded and displayed, thus putting large scale data into the context of PD-related molecular pathways. (3) The export function enables to export selected areas of the PD map as xml.file, preserving annotations and information on the underlying literature.

Conclusion: By forming a resource for computational analysis and a platform for community interactions, the PD map will become a hub for the PD-community to deal with the exponentially increasing information on PD and allowing the research community to open new avenues in PD research

10b. Other: disease mechanisms

ADPD5-0337
REWARD SENSITIVITY DEFICITS UNDERLIE APATHY IN PD
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Objectives
Apathy is present in up to 70% of Parkinson's disease (PD). Its mechanisms are poorly understood and objective detection methods that do not rely on questionnaire reports are lacking, often leading to a misdiagnosis of depression, inadequate disease monitoring and poor outcomes. Here we used eye movement and pupil modulation in response to reward as objective metrics of motivation. Differences in reward sensitivity in PD patients ON dopaminergic medication were compared to OFF, and the relation to apathy assessed.
Methods
20 patients with idiopathic PD, tested ON and OFF medication, and healthy age-matched participants made saccadic eye movements for different monetary rewards. Eye position, saccadic peak velocity and pupil diameter were measured using an infrared eye tracker, while apathy was indexed by Lille Apathy Rating Scale (LARS) scores.
Results
Controls and PD patients ON demonstrated increased saccadic velocity and pupil diameter as reward magnitude increased. This reward sensitivity was blunted in PD patients OFF dopaminergic medication. Crucially, PD cases who were more apathetic on the basis of LARS scores, exhibited significantly less reward sensitivity than more motivated PD patients.
Conclusions
Saccadic velocity and pupil diameter are influenced by reward magnitude, with the degree of modulation varying with motivation levels across individuals. These indices provide novel, objective behavioural measures for assessing apathy in PD. The use of dopaminergic medication may be an effective treatment for apathy by increasing reward sensitivity, independent of effects on motor control.

10b. Other: disease mechanisms

ADPD5-0463
DYSFUNCTIONAL PROCESSING OF TIME IN PARKINSON DISEASE
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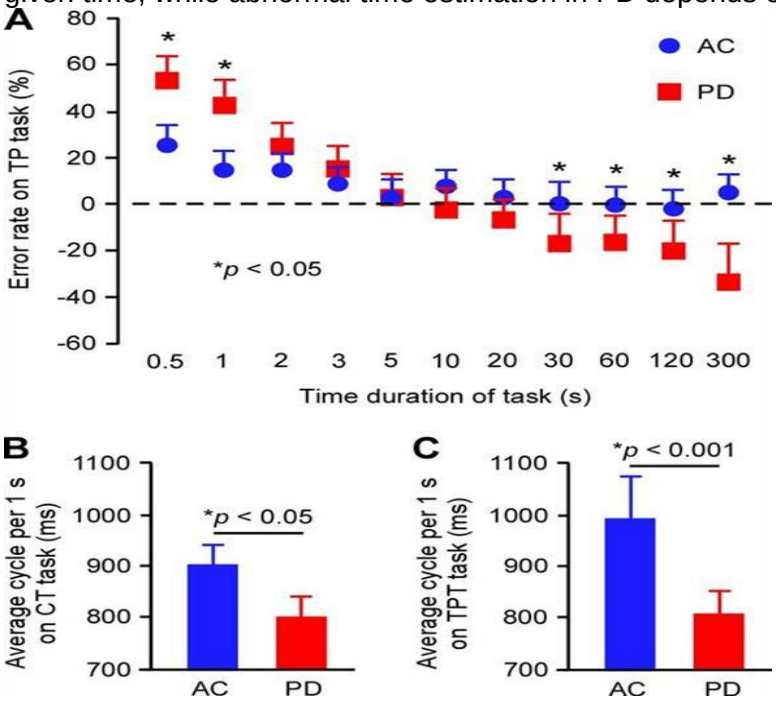
Objectives: Temporal information processing is inefficient in patients with Parkinson' disease (PD). This results in perception of shortened time (Riesen & Schnider, 2001). The pathophysiology of PD has been described as linked with the basal ganglia (Lalonde & Hannequin, 1999), and patients have tremor (Yanagisawa & Nezu, 1987) and muscular rigidity (Lee, 1989). We hypothesized that disordered basal ganglia are linked to time counting dysfunction and this in turn leads to abnormal estimation of mental time. Methods: Nineteen PD patients and 19 age-matched controls (AC) took part. Temporal function was measured by subjective time production (TP) in 0.5–300 seconds tasks, subjective time by tapping (TPT) for 60 seconds, and one second cycle tapping (CT) for 60 seconds. Results: TP in PD was shorter than in AC. An average cycle per second of TPT in PD was shorter than AC (Fig. 1A). While the CT cycle in AC was longer than PD (Fig. 1C), the AC cycle was closer to one second compared with TPT (Fig. 1B). The CT cycle in PD was similar to TPT. Conclusions: The results suggest that shorter time estimation in PD is caused by a shortened time counting cycle. Furthermore, normal time estimation in AC depends on flexible time counting within a given time, while abnormal time estimation in PD depends on stabilized time counting.



10b. Other: disease mechanisms

ADPD5-1199
NIGRAL CELL LOSS FOLLOWING NORADRENERGIC DENERVATION
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Objectives: In Parkinson's disease, besides dopaminergic cell loss, there is an extensive noradrenergic cell loss in the locus coeruleus. Although the noradrenaline system have been recognized as part of the pathology in Parkinson's disease, the consequence of noradrenergic degeneration as well as the temporal relation between noradrenergic and dopaminergic cell loss remains unclear. There are also implications of noradrenaline acting as a neuroprotector. We aim to investigate the effect of reduced noradrenergic input on nigral dopamine neurons.
Methods: Sprague-Dawley rats were injected with DSP4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine], causing selective degeneration of noradrenergic neurons. The effects were analyzed with *in vivo* voltammetry and post-mortem tissue analysis.
Results: Assessment of the noradrenergic nerve fiber density demonstrated, on average, 90 % denervation 3 months post DSP4-treatment. Evaluation of the dopaminergic system using *in vivo* chronoamperometry showed significantly increased striatal dopamine release in DSP4-treated rats compared to controls. *In vivo* amperometry with enzyme-based multisite microelectrodes was performed to test whether the increased dopamine release was due to an altered striatal glutamatergic input. The recordings revealed significantly decreased glutamate release in denervated rats. After sacrifice, the brains have been collected and processed for immunohistochemistry. Stereological cell counts of tyrosine hydroxylase-positive neurons in DSP4-treated rats demonstrated a significantly reduced number of noradrenergic locus coeruleus neurons and most interestingly also a significantly reduced number of nigral dopamine neurons compared to controls.
Conclusions: These findings support the hypothesis of noradrenaline acting as a neuroprotector for nigral dopamine neurons, as well as its plausible role in the etiology of Parkinson's disease.

10b. Other: disease mechanisms

ADPD5-1986

A NOVEL DINUCLEOTIDE COMPLEX REPEAT IN THE SNCA GENE CONFERS RISK FOR LEWY BODY PATHOLOGY IN ALZHEIMER'S DISEASE AND AFFECTS SNCA EXPRESSION PROFILES

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Genetic association of the *SNCA* gene with several Lewy-body (LB) related disorders were reported. However, the actual genetic variant/s that underlie the observed association remains largely elusive. Structural variants (SVs), particularly microsatellites are among the most polymorphic loci in the human genome and have been implicated in many Mendelian diseases, including neurodegenerative conditions. However, studies about their possible role in complex human disorders are underrepresented. We recently showed that tagging-SNPs across *SNCA* locus were significantly associated with increased risk for LB-pathology in AD. Building on these results, herein, we embarked on an effort to identify SVs within *SNCA* that contribute to LBV/AD-risk. Using an algorithm to catalogue and score SVs in *SNCA*-intron4, followed by deep sequencing, we identified a novel, highly polymorphic, complex CT-microsatellite. We showed in autopsy cases with LBV/AD compared with AD-only controls that certain alleles at this complex CT-microsatellite conferred risk to develop LBV/AD ($p<0.0001$). We further correlated the genetic association with biological functions, and expression analysis demonstrated that the CT-microsatellite site acts as a splicing regulator of exon5, whereas the risk allele was significantly associated with elevated levels of exon5 skipping ($p=0.01$). We also detected significantly higher levels of the alternative-splicing event in LBV/AD brains compared to AD controls ($p=0.0002$). In conclusion, we discovered a novel complex CT-microsatellite in *SNCA* that contributes to LB-pathology, possibly via splicing regulation of *SNCA*. Studies of the relevant of this CT-microsatellite to other LB-spectrum disorders and experiments using biological system and genome-editing approach to validate its regulatory effect are underway.

10b. Other: disease mechanisms

ADPD5-2015

QUANTITATIVE AND FIBER-SELECTIVE EVALUATION OF PAIN AND SENSORY DYSFUNCTION IN PATIENTS WITH PARKINSON'S DISEASE

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Introduction: Pain and sensory disturbances affect many patients with Parkinson's disease (PD). The present study aimed to evaluate the pain and sensory sensitivity of each class of afferent fibers in PD patients and determine the effects of dopaminergic therapy on pain and sensory sensitivity.

Methods: Current perception threshold (CPT) and pain tolerance thresholds (PTT) at three frequencies, 2000 Hz, 250 Hz, and 5 Hz, to stimulate Aβ fibers, Aδ fibers, and small C-polymodal fibers, respectively, were measured in 72 PD patients and 35 healthy controls.

Results: CPT was higher at all three frequencies and PTT was lower at 2000 Hz and 250 Hz in PD patients with pain versus healthy controls ($P < 0.05$). CPT was higher at 2000 Hz and 250 Hz and PTT was lower at 2000 Hz and 250 Hz in PD patients without pain versus healthy controls ($P < 0.05$). PD patients with pain exhibited higher CPT at 5 Hz and 250 Hz than PD patients without pain ($P < 0.05$). Dopaminergic therapy did not affect CPT or PPT in PD patients ($P > 0.05$). Conclusions: Abnormal Aδ fiber- and Aβ fiber-dependent sensory inputs may exist in PD. Abnormal sensory inputs via C fibers and Aδ fibers might be associated with the presence of pain in PD. Because dopaminergic therapy failed to mitigate these sensory and pain dysfunctions, mechanisms not involving the dopaminergic pathway are likely to be implicated

10b. Other: disease mechanisms

ADPD5-2249

EVIDENCE FOR SUBTYPE-SPECIFIC IMPAIRMENT IN EMOTION PROCESSING IN PARKINSON'S DISEASE

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Objectives
Impaired facial emotion recognition in Parkinson's disease (PD) has been reported for negative basic emotions. However, results are heterogeneous and to our knowledge none of the studies investigated impairment in different PD subtypes, although previous reports suggest a more benign course of disease in tremor-dominant compared to akinetic-rigid PD. The aim of the present study was to evaluate emotion recognition abilities of PD patients considering the variable of motor subtype.
Methods
Detailed clinical and neuropsychological assessments (e.g. Unified Parkinson's Disease Rating Scale, UPDRS; Hoehn-&-Yahr-Scale; cognitive tests; questionnaires on quality of life, anxiety and depression) were acquired in tremor-dominant and akinetic-rigid PD patients and healthy controls matched for age, gender and education. Emotion recognition abilities were assessed with the Ekman-60-faces-test.
Results
Our preliminary behavioral data showed worse performance in 17 akinetic-rigid (mean age: 67.6±6.7 years; 9 male; mean UPDRS-motor: 26.8±9.7) compared to 12 tremor-dominant patients (62.8±14.2 years; 6 male; UPDRS-motor: 20.4±6.2) in the recognition of surprise (p=.008) and anger (p=.008). There were no significant differences regarding age, education, disease duration, UPDRS scores and IQ. L-dopa dose was significantly higher (p=.009) in akinetic-rigid PD but did not correlate with performance in emotion recognition. Compared to the 17 healthy controls (62.1±7.5 years; 8 male), recognition of surprise (p=.005) and anger (p=.035) was enhanced in tremor-dominant patients.
Conclusion
Our results indicate that disease-related motor subtypes may play a role in emotion processing in PD. Enlargement of the study sample and the identification of respective neural correlates using functional MRI will provide further insight.

10c. Other: preclinical research

ADPD5-0687
MODIFICATIONS OF THE INTESTINAL DOPAMINERGIC TRANSMISSION IN A RODENT MODEL OF PD.
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Objectives: gastrointestinal (GI) dysfunction, constipation in particular, is the most common nonmotor symptom of PD. In this study we explored the relationship between central dopaminergic denervation, GI dopaminergic function and GI motility in a PD-like model of nigrostriatal lesion, focusing on the potential role of the dorsal motor nucleus of the vagus (DMV).
Methods: Sprague-Dawley rats received a stereotaxic injection of saline or neurotoxin 6-hydroxydopamine (6-OHDA) into the medial-forebrain-bundle. Peristalsis was evaluated in isolated colonic segments, in baseline conditions and following exposure to combinations of D2 receptor (DRD2) agonist sumanirole and antagonist L-741626. Dopamine levels, DRD2 and choline acetyltransferase (ChAT) expression were assessed in the ileum and colon of all animals. Investigation of neuronal activity in the dorsal motor nucleus of the DMV was studied by analysis of FosB/Delta FosB expression in tyrosine hydroxylase and ChAT positive neurons.
Results: peristalsis showed no differences at basal recording between 6-OHDA and control groups. However, 6-OHDA lesioned animals completely lost the inhibitory response to sumanirole. A trend toward a reduced expression of DRD2 was observed in the colon of 6-OHDA lesioned rats, which was associated with increased tissue levels of dopamine and enhanced ChAT expression. A slight, but consistent, increase in FosB/Delta FosB expression was observed in the DMV of 6-OHDA lesioned rats.
Conclusions: our results demonstrate that selective lesion of the nigrostriatal dopaminergic pathway triggers alterations of GI dopaminergic system. Neuronal activations within the DMV may be the link between nigrostriatal lesion and neurochemical/functional changes in the GI tract.

10c. Other: preclinical research

ADPD5-0698

NANOSCALE CAPILLARY ISOELECTRIC FOCUSING FOR THE DETECTION OF DIFFERENTIALLY SIALYLATED SERPINA1 IN CEREBROSPINAL FLUID AS AN EARLY DIAGNOSTIC MARKER FOR PD DEMENTIA

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PD with dementia (PDD) encompasses together with dementia with Lewy bodies (DLB) 10-15% of all degenerative dementia. About 30% of patients with Parkinson's disease (PD) develop a dementia in the course of the disease which in turn drastically reduces quality of life and does often lead to an earlier hospitalization of the patients. However, up to date, diagnosis of PDD is merely done by clinical parameters. Recently, we described by using two dimensional gel electrophoresis (2D and 2D immunoblot) that isoforms of the serine protease inhibitor Serpin A1 are differently sialylated in the cerebrospinal fluid (CSF) of healthy individuals and PD compared to PDD. However, 2D electrophoresis is time-consuming and therefore not suited as a high-throughput approach. To overcome this problem we used a new nanoscale capillary isoelectric focusing (CIEF) approach for the detection of differentially sialylated Serpin A1 isoforms. So far we analyzed 102 patients with PDD, PD and non-demented controls. The CIEF approach gave mainly similar results like the 2D immunoblots. In addition using the CIEF approach we were able to specifically quantify each SerpinA1 isoform's expression level. We found a significant difference in the expression levels of certain SerpinA1 isoforms between PDD, PD and controls. By CIEF we are now able to analyze up to 96 samples per day compared to 4-8 samples by our 2D immunoblot. The detection of different Serpin A1 isoforms by CIEF is rapid, sensitive and allows a large scale analysis of biosamples.

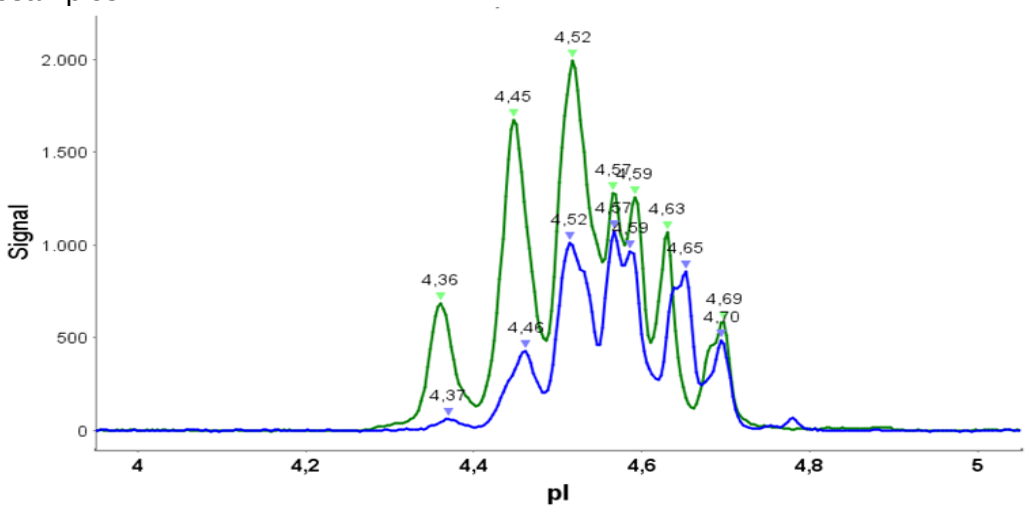

Figure: Analysis of CSF SerpinA1 using CIEF. Overlay of a control (blue) and PDD (green) electropherogram showing the difference in the SerpinA1 expression level of certain isoforms between the two groups. pI, isoelectric point.

10c. Other: preclinical research

ADPD5-0812

GASTRIC DYSFUNCTION AND PARKINSON'S DISEASE: EVIDENCE FROM AN IN VITRO STUDY IN 6-OHDA RATS.

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OBJECTIVES: Gastroparesis is frequently observed in patients with Parkinson's Disease (PD). To investigate *in vitro* the mechanisms underlying PD-associated gastric dysmotility, pharmacological/electrical field stimulation (EFS) responses were studied in preparations from 6-hydroxydopamine (6-OHDA) rats.
METHODS: Thiopental-anaesthetized male Sprague Dawley rats were injected intracranially with 6-OHDA or saline and sacrificed after 8 weeks. Stomach circular/longitudinal strips and pylorus were pharmacologically-stimulated with increasing concentrations of bethanechol/prostaglandin F2 alpha (PGF2). The relaxing effect of dopamine (DA) was studied on hypertone induced by PGF2α/bethanechol. EFS was performed on pre-contracted tissues in the absence/presence of atropine plus guanethidine (NANC-conditions), L-NAME (NO-synthase inhibitor) and protease alpha-chymotrypsin. The experiments were carried out in the respect of Italian Law on the use of animals for experimental ends (D.Lgs26/2014).
RESULTS: Pharmacological stimuli**:** Maximal responses to bethanechol/PGF2-alpha were markedly reduced in gastric circular muscles of 6-OHDA rats. In gastric longitudinal strips/pylorus from 6-OHDA rats, DA induced a smaller relaxation compared to controls. EFS: EFS-induced relaxation of pylorus was lower and insensitive to L-NAME in 6-OHDA rats compared to control. EFS relaxation of gastric longitudinal muscle was almost abolished by L-NAME and alpha -chymotrypsin in 6-OHDA rats.
CONCLUSIONS: The data obtained in rat tissues excised after 8 weeks from 6-OHDA administration indicate that the loss of responsiveness to contractile agents in gastric circular muscles, the impairment of pyloric nitrergic control and the increase of inhibitory peptidergic neurotransmission in longitudinal strips can altogether contribute to the gastric dysmotility we observed *in vivo* in the same experimental model and detected in PD patients.

10c. Other: preclinical research

ADPD5-1570
PREVALENCE OF SMELL IMPAIRMENTS AND SUBSTANTIA NIGRA HYPERECHOGENEICITY IN LITHUANIANS, BORN IN 1964

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Objectives: to determine the prevalence of smell impairments and changes in substantia nigra (SN) echogeneicity on TCS in healthy adult population and to evaluate the link between the two.
Methods: 295 subjects – 59% women and 41% men, born in 1964 (aged 48,42 ± 0,04 at the moment of the study) , participating in a population study "Connection between genetic and lifestyle risk factors for chronic disease risks since childhood" were screened for smell impairments, using Sniffin' Sticks 12 test, as well as for SN echogeneicity changes using TCS. They all underwent neurological examination with focus on extrapyramidal signs and none was found to have PD at the moment of the study.
Results: sufficient acoustic temporal bone window allowed complete TCS results to be obtained in 90.5% of cases. In 6.1% of examined cases unilateral or bilateral enlargement of SN area (hiperechogeneicity) was detected (using the cutoff value of 0.26 cm^2). In additional 18.8% of cases SN area was found to be marginal unilateraly or bilaterally (0.20-0.26 cm^2). 24.1% of participants had at least mild hiposmia. Changes in SN echogeneicity correlated positively with smell impairments ($p<0.05$). 9.8% of participants were found to have both - smell and SN echogeneicity impairments.
Conclusions: almost ¼ of the studied population was found to have at least mild smell impairments. In similar proportion of cases hyperechogeneicity of SN was detected. A positive correlation between those symptoms was observed.

10c. Other: preclinical research

ADPD5-1971

NEUROPROTECTIVE TRIALS IN PRODROMAL PARKINSON'S DISEASE AND DEMENTIA WITH LEWY BODIES WITH REM SLEEP BEHAVIOR DISORDER

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Objective
To delineate risk and risk factors for conversion from idiopathic REM sleep behavior disorder (RBD) to Parkinson's disease, Dementia with Lewy bodies, and Multiple System Atrophy, in order to enable practical planning of neuroprotective trials against neurodegenerative synucleinopathy.
Methods
In a 10-year prospective cohort, we tested prodromal Parkinson's markers in 89 patients with idiopathic RBD. With Kaplan-Meier analysis we calculated risk of neurodegenerative synucleinopathy, and compared our values to recent estimates from a 300-patient RBD study group multicenter study. Using Cox proportional hazards, we tested the ability of prodromal markers to identify patients at higher disease risk, and then designed stratification strategies to optimally select patients for definitive neuroprotective trials.
Results
The risk of defined neurodegenerative synucleinopathy was high; 30% developed disease at 3 years, rising to 66% at 7.5 years. This was similar to the RBD study group's multicenter estimate (25% at 3 years, 41% at 5 years). Age (HR=1.07), olfactory loss (HR=2.8), color vision (HR=3.1), and subtle motor dysfunction (HR=3.9) identified higher risk of disease conversion. Stratification with prodromal markers increased risk of neurodegenerative disease conversion by 200%, and combining markers allowed sample size reduction in neuroprotective trials by >40%. With a moderately-effective agent (HR=0.5), a 2-year trial can demonstrate definitive reductions in neurodegenerative disease with only 80 patients per group.
Conclusions
Using stratification with simply-assessed markers, RBD patients can identify extremely high likelihood of prodromal synucleinopathy, enabling design pf neuroprotective trials against Parkinson's disease and Dementia with Lewy bodies.

10c. Other: preclinical research

ADPD5-2052
PARKINSON'S UK TISSUE BANK: A UNIQUE TISSUE RESOURCE FOR PARKINSON'S DISEASE RESEARCH
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Background: Parkinson's UK Tissue Bank was established in 2002 to collect and supply high quality human brain, spinal cord, DNA and CSF samples to researchers. Tissue is collected through a donor programme with almost 10,000 potential donors. To date we have collected almost 800 Parkinson's related brains and we are currently collecting ~110 cases per year with short post-mortem delays. Neuropathological examination utilise the latest international criteria, are graded according to Braak along with the degree Alzheimer's type co-pathology. Extensive medical histories exist on all cases.
Objectives: To act as an open access tissue resource for researchers so as to foster research towards a better understanding/cure for Parkinson's. We have supplied tissue to hundreds of key research projects e.g. GWAS sequencing project. A number of different tissue formats are available i.e. snap frozen, formalin fixed - paraffin embedded, and formal fixed – cryopreserved tissue.
Access: The Tissue Bank welcomes applications from both for profit and not for profit organisations. Tissue request forms can be requested via the tissue bank manager, all applications are reviewed by an independent scientific review board. The Tissue Bank via its review board can issue its own ethical approval for the use of tissue, under the MREC scheme, thus saving the need for researchers to get separate ethical permission in many cases. We encourage potential users to discuss their requirements with the Tissue Bank manager Dr D. Gveric or its Scientific Director Professor D Dexter. e-mail us at pdbank@imperial.ac.uk or Tel: +44 207 594 9732

10d. Other: diagnostics

ADPD5-0307

FOLLOW-UP STUDY OF MCI PATIENTS DEVELOPING AD OR DLB (2): CHARACTERISTICS OF NEUROPSYCHOLOGIAL EXAMINATIONS AT BASELINE

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Objective: We conducted a long-term follow-up study to investigate the characteristics of neuropsychological examinations on MCI patients who developed Alzheimer's disease (AD) or dementia with Lewy bodies (DLB) during the follow-up period.

Methods: The subjects were 96 elders who visited our memory clinic between November 2006 and December 2012 and were diagnosed as MCI (FAST lower than 3, CDR lower than 0.5, MMSE higher than 24). All the subjects underwent neuropsychological examinations (MMSE, WAIS-III, WMS-R) within a month from their first visit to memory clinic and were followed the mean of 44.8 months. The subjects were divided into three groups; AD-converters (AD group: n=23), DLB-converters (DLB group: n=13) and non-converters (NC group: n=60). We compared the results of neuropsychological examinations at baseline among three groups.

Results: Compared to NC group, AD group exhibited significantly lower scores in MMSE, Verbal comprehension in WAIS-III, Verbal memory, Visual memory and Delayed recall in WMS-R, and DLB group showed significantly lower scores in Processing speed in WAIS-III and Visual memory in WMS-R. There was no significant difference between AD group and DLB group.

Conclusion: Specific patterns of cognitive decline can be observed in MCI patients before they fulfill clinical diagnostic criteria of AD or DLB. Detailed neuropsychological examinations can be helpful for early detection of AD or DLB in MCI patients.

10d. Other: diagnostics

ADPD5-0308
FOLLOW-UP STUDY OF MCI PATIENTS DEVELOPING AD OR DLB (1): COMPARISON OF [18]F-FDG PET IMAGES AT BASELINE

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Objective: We conducted a long-term follow-up study to investigate the patterns of hypometabolism on MCI patients who developed Alzheimer's disease (AD) or dementia with Lewy bodies (DLB) during the follow-up period.
Methods: The subjects were 96 elders who visited our memory clinic between November 2006 and December 2012 and were diagnosed as MCI (FAST lower than 3, CDR lower than 0.5, MMSE higher than 24). All the subjects underwent [18]F-FDG PET within a month from their first visit to memory clinic and were followed the mean of 44.8 months. The subjects were divided into three groups; AD-converters (AD group: n=23), DLB-converters (DLB group: n=13) and non-converters (NC group: n=60). We compared the patterns of hypometabolism at baseline among three groups.
Results: Compared to NC group, AD group exhibited significant hypometabolism in the posterior cingulate cortex, and DLB group showed significant hypometabolism in the precuneus. DLB group exhibited significant hypometabolism in the occipital lobe compared to AD group.
Conclusion: The present study indicated that specific patterns of hypometabolism can be observed in MCI patients before the patients fulfill clinical diagnostic criteria of AD or DLB; MCI patients who progress to AD tend to show hypometabolism predominantly in the posterior cingulate cortex, and those who progress to DLB are likely to exhibit hypometabolism predominantly in the precuneus and occipital lobe. [18]F-FDG PET can increase the opportunities for early detection of AD or DLB in MCI patients.

10d. Other: diagnostics

ADPD5-0421

THE RELATIONSHIP BETWEEN MONTREAL COGNITIVE ASSESSMENT AND BIOCHEMICAL BIOMARKERS IN PATIENTS WITH PARKINSON'S DISEASE

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Objectives: To clarify the relationship between Montreal cognitive assessment (MoCA) and blood biochemical biomarkers in patients with Parkinson's disease (PD).
Methods: MoCA and Mini-Mental State Examination (MMSE) were examined in PD patients and apolipoprotein E (APOE) phenotypes, serum β-amyloid 1-40 (Aβ40) and 1-42 (Aβ42) and plasma homocysteine (Hcy) were measured. These cognitive scores and serum or plasma levels were statistically analyzed among 3 groups divided with APOE4 or APOE2 phenotypes at first and then 3 groups divided with Aβ ratio (Aβ42/Aβ40) tertiles.
Results: Total of 150 PD patients were enrolled (65 males and 85 females). Their mean age were 67.9 y. Mean total scores of MoCA (23.2) was significantly lower than those of MMSE (28.2) ($p<0.01$). In total scores of MMSE, APOE4 homozygous group scored significantly lower than heterozygous group ($p<0.05$) although there was no significant difference among APOE2 groups. In total scores of MoCA, there was no significant difference in both APOE4 and APOE2 groups. In total scores of MoCA or MMSE, there were no correlations with serum Aβ40, Aβ42, and plasma Hcy. Among groups with lower, middle and higher tertiles of Aβ ratio, there was no significant difference in total scores of MoCA, MMSE and plasma Hcy.
Conclusions: MoCA is a sensitive battery to detect mild cognitive impairment in PD but there was no significant relationship with well-known biochemical biomarkers in patients with dementia in our preliminary study. It was suggested that cognitive dysfunctions in PD detected with MoCA could be different from those in other dementias.

10d. Other: diagnostics

ADPD5-0506

ASSESSMENT OF NEUROPSYCHOLOGICAL FEATURES IN MILD DEMENTIA WITH LEWY BODIDES

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Objectives)
Dementia with Lewy bodies (DLB) is frequently accompanied by visual hallucination, parkinsonism, or cognitive fluctuation. However, discerning DLB from Alzheimer's disease (AD) without these prominent clinical features is difficult and challenging for clinicians, particularly at the beginning of disease. This study aimed to investigate the validity of neuropsychological testing in differentiating DLB from AD.
Methods)
The participants included 30 mild DLB and 211 mild AD outpatients of the memory clinic at Keio University hospital (Clinical Dementia Rating = 0.5 or 1, age from 60 to 89). All the participants received brain MRI and neuropsychological testing including mini mental state examination (MMSE), Raven's colored progressive matrices (RCPM), Rey auditory verbal learning test, logical memory (LM) subtest of Wechsler memory scale-revised, Rey-Osterrieth complex figure test (ROCFT), modified Stroop test, trail-making test, and verbal fluency test (VFT). The geriatric depression scale (GDS) was used to evaluate the subjective mood.
Results)
No significant differences were obtained between DLB and AD in age (77.8±5.4 years in DLB and 76.1±8.0 years in AD), education (13.0±3.3 years and 12.9±2.8 years, respectively), GDS scores (5.1±3.3 and 4.9±3.9, respectively), and scores of MMSE (21.3±4.5 and 21.1±4.8, respectively). Analysis of neuropsychological testing scores demonstrated that DLB showed significantly lower scores in RCPM, copy of ROCFT and VFT, while they showed significantly higher scores in LM than AD.
Conclusions)
DLB patients performed poorer visuospatial functions and executive functions than AD. In contrast, they performed better cognitive functions in logical memory. These findings are compatible with an analysis of previous studies.

10d. Other: diagnostics

ADPD5-0584
COGNITIVE AND AFFECTIVE THEORY OF MIND IN DEMENTIA WITH LEWY BODIES

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Introduction: "Theory of mind" (ToM) refers to the ability to attribute thoughts (cognitive component) or feelings (affective component) to others. This function has been studied in many neurodegenerative diseases but not in dementia with Lewy bodies (DLB). The aim of our study was to assess ToM in patients with DLB and to search neural correlates of deficits.
Methods: Twenty-nine patients with DLB (DLB group), on early stage of the disease, and 15 healthy elderly adults (HC group) were included in the study. We used the Faux Pas recognition (FPR) test, the Reading the Mind in the Eyes (RME) test and the facial emotion recognition of Ekman's test to assess the two components of ToM. We studied correlations between ToM scores and gray matter volume and we compared DLB impaired and not impaired patients using voxel-based morphometry.
Results: DLB group performed significantly worse than HC group for the FPR test ($p=0.02$) and the RME test ($p=0.02$), but there was no significant differences for the Ekman's test ($p=0.60$). The main region associated with ToM impairments was the prefrontal cortex but we also found correlations with orbitofrontal cortex, precuneus, superior temporal sulcus, occipital cortex, fusiform gyrus, insula, amygdala, cerebellum and basal ganglia ($p<0.001$, uncorrected, minimum cluster size of 25 voxels).
Conclusion: This study is the first to show early impairments of affective and cognitive ToM in DLB. Among patients with ToM difficulties, we found atrophy in brain regions classically involved in ToM but also in less reported regions, as basal ganglia and cerebellum.

10d. Other: diagnostics

ADPD5-0701

UTILITY OF SELF-ADMINISTERED QUESTIONNAIRE TO ASSESS OLFACTORY DYSFUNCTION IN JAPANESE PATIENTS WITH PD

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Objective
Olfactory dysfunction is a common non-motor feature in PD. Recent study reported the usefulness of a questionnaire-type olfactory test in the evaluation of olfactory dysfunction in PD, however its utility for Japanese PD patients is not validated. The aim of this study was to analyze the usefulness of a recently developed self-administered odor questionnaire (SAOQ) in assessment of olfactory function in Japanese PD patients.
Methods
Twenty-nine PD patients (14 females and 15 males) were recruited in this study. General cognitive function was examined using the Addenbrook's Cognitive Examination-Revised. Motor impairment was assessed using the Hoehn and Yahr stage and Unified Parkinson's Disease Rating Scale. Olfactory function was measured using the SAOQ, which consisted of 20-smell items familiar to Japanese, and the Open Essence (OE, Wako Pure Chemical Industries, Tokyo, Japan). Internal consistency of SAOQ in PD patients was assessed using Cronbach's alpha. Internal validity was assessed using Pearson's correlation coefficient by correlating SAOQ scores with OE values. The level of statistical significance was set at $p < 0.05$.
Results
Cronbach's alpha was 0.90. SAOQ scores correlated significantly with OE values ($r = 0.45$, $p < 0.05$), but not with age ($r = -0.02$, $p = 0.93$). Furthermore, there was no significant difference in SAOQ scores between female and male in the Chi-square test ($p = 0.46$).
Conclusion
This SAOQ is useful in evaluating olfactory dysfunction in Japanese PD patients.

10d. Other: diagnostics

ADPD5-0779

CONVENTIONAL ALZHEIMER'S BIOMARKERS IN CSF (ABETA42, TAU AND PHOSPHORYLATED-TAU) ARE NOT PATHOLOGICAL IN MILD COGNITVE IMPAIRMENT PATIENTS WITH PRODROMAL DLB

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1) Objectives

Dementia with Lewy bodies (DLB) is the second most frequent dementia after Alzheimer's disease (AD). The differential diagnosis between these two diseases is particularly difficult especially early in the disease. AD biomarkers (Aβ42, Tau and Phospho-Tau181) in cerebrospinal fluid (CSF) appear to be also disrupted in other neurodegenerative diseases such as DLB. In the literature, DLB patients present an isolated Aβ1-42 decreased. However, all these results were obtained in demented patients. So, we propose to explore conventional Alzheimer's biomarkers in DLB patients with a prodromal stage.

2) Methods

Patients were classified according to the criteria of McKeith for DLB patients and the criteria of Dubois for Alzheimer's patients. The results of lumbar puncture were not considered for the classification of patients. The patients were categorized according to their pathology: AD, DLB, or mixed AD and DLB pathology and depending on their state of dementia: prodromal or dementia stage. Once every patient classified in a group, we analyzed the results of their biomarkers.

3) Results

For demented patients, we find a decrease of Abeta42 in CSF patients with DLB. However for the prodromal stage of DLB, the levels of Abeta42 are identical to those of controls.

4) Conclusion

For the first time, we show an analysis of the levels of AD biomarkers in DLB patients with prodromal stage. Thus, we have shown that at this stage the DLB patients had no pathological profile. The profile changes at the demented stage, with an isolated decrease of Abeta42.

10d. Other: diagnostics

ADPD5-0899
IMPROVING THE CLINICAL DETECTION AND DIAGNOSIS OF LEWY BODY DEMENTIA USING A COMPOSITE RISK SCORE

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Lewy Body Dementia (LBD) is a challenge to diagnose, particularly outside of expert centers with delays in diagnosis approaching 18 months leading to significant burden to patients and caregivers. While consensus criteria have excellent specificity, there is no standardized way to assess symptoms. We developed the Lewy Body Composite Risk Score (LBCRS) to improve the ability to detect LBD in clinic and research populations. The LBCRS was derived from clinical features of LBD in autopsy-verified cases. We tested the LBCRS in a consecutive series of 204 patients presenting for initial evaluation of memory, mood, motor and behavioral complaints. Patients (mean age 77.5±8.0y, mean education 15.5±3.6y, 52% female) received comprehensive evaluations including Clinical Dementia Rating, Unified Parkinson's Disease Rating Scale, Neuropsychiatric Inventory, Functional Assessment Questionnaire, Mayo Fluctuations and Sleep Questionnaires, Epworth Sleepiness Scale and Neuropsychological testing. Diagnoses include subjective cognitive impairment (6), MCI (58), AD (79), LBD (39), vascular dementia (5), FTD (9), and other dementias (8). The LBCRS was completed independent of the clinical evaluation. Mean LBCRS scores were significantly different between LBD and AD (6.2±2.2 vs. 2.5±1.3, p<.001) and between MCI-LBD vs MCI-AD (3.2±1.0 vs. 1.1±0.7, p<.001). The LBCRS was able to discriminate between different causes of dementia. Using a cut-off score of 3, Area under ROC for LBD vs. AD 0.93 (0.88-0.98), Area under ROC for MCI-LBD vs. MCI-AD 0.96 (0.91-1.0). The Lewy Body Composite Risk Score increases diagnostic probability that Lewy bodies are a contributing dementia pathology and should improve clinical detection and enrollment for clinical trials.

10d. Other: diagnostics

ADPD5-1269

DIFFERENTIAL DIAGNOSIS BETWEEN ALZHEIMER'S DISEASE AND DEMENTIA WITH LEWY BODIES: PLANNING SUPPORT DURING THE COPY OF THE REY COMPLEX FIGURE.

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Objectives: Patients with Lewy Bodies Dementia (DLB) present more constructive deficits and also earlier executive impairment than Alzheimer's disease (AD) patients (Metzger-Baddeley, 2007). We aim to demonstrate that mechanisms affected in the constructive apraxia are different in AD and DLB.
Methods: 12 patients (mean age: 82.5 years +/-5.65) and 10 patients DCL (mean age: 83.08 +/-3.12) matched for education level and dementia severity (MMSE score) made the copy of the Rey Complex Figure under two conditions: with or without planning support. The Boston Qualitative Scoring System (BQSS) was used.
Results: Constructive apraxia (AC) frequency is about 80 % for DLB group against 33 % for AD patient. Qualitative approach shows higher planification impairments for DLB patients (BQSS planning score = 0.90/4*). Planning support permitted to normalize the performances of all AD patients. On the contrary, disorders persist for half of DLB patients.
Discussion: Constructive disorders involve planning impairment in AD. Even if planning disorders are commune in DLB, we suppose that other visuo-perceptive, visuo-spatial or purely constructive processes may be damaged in DLB.
Conclusion: Planning support is useful to differentiate DLB from AD patients. Constructive disorders which remain in copy condition with planning support are a strong argument for DLB.

10d. Other: diagnostics

ADPD5-1305

NEUROPSYCHOLOGICAL CORRELATES OF PAREIDOLIAS IN DEMENTIA WITH LEWY BODIES

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Background

Pareidolias are complex visual illusions frequently experienced by patients with Dementia with Lewy bodies (DLB) that are considered an analogous phenomena to visual hallucinations. Few studies have explored the neuropsychological correlates of pareidolias.

Objective

To study the neuropsychological correlates of pareidolias in DLB patients in a Memory Disorders Unit.

Methods

We included 43 subjects (55.8% women) with a clinical diagnosis of mild to moderate DLB evaluated in our Memory Unit between October 2013 and July 2014. All subjects underwent formal cognitive evaluation with an extensive neuropsychological battery including behavioral (NPI) and functional (IDDD) scales. The Scenery and Noise Pareidolias test was performed to evaluate the presence of pareidolias (Uchiyama et al., 2012). Correlations and group comparisons (Mann-Whitney U-test) between patients with or without pareidolia, were performed.

Results

Mean age was 79.2 years and mean education was 8.6 years. Mean MMSE score was 24.6 with a moderate functional interference (IDDD: 54.07). Scenery pareidolias were present in 79.1% and Noise pareidolias in 81.4% of the patient sample. DLB patients with pareidolias (scenery) performed worse in visuoperceptive, visuoespacial, frontal task and language ($p<0.05$). Visual illusions (scenery and noise) were correlated with visual hallucinations (NPI) ($p=0.002$ and $p=0.008$ respectively).

Conclusions

The presence of pareidolias in DLB is associated with lower cognitive performance. Pareidolias parallel hallucinations in DLB. Further studies are required to determine the underlying mechanisms.

10d. Other: diagnostics

ADPD5-1330

MEMORY AND NAMING PERFORMANCE DISTINGUISHES SUBTYPES OF DEMENTIA WITH LEWY BODIES FROM ALZHEIMER'S DISEASE

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OBJECTIVE: To determine whether learning, memory, and naming performance distinguishes subgroups of dementia with Lewy bodies (DLB) from those with Alzheimer's disease (AD) dementia.
METHODS: Patients with probable DLB (n=206) and AD dementia (n=343) were recruited through the Mayo Clinic Alzheimer's Disease Research Center. All patients had MMSE ≥ 20. DLB patients were subdivided into impaired versus normal naming performance. Age-adjusted scores from the Auditory Verbal Learning Test (learning over trials, delayed recall, recognition memory) and Boston Naming Test were examined.
RESULTS: The DLB group was older than the AD dementia group (mean years = 73.3(9.4) vs. 71.8(6.9), $p<0.05$), with no difference in education. Learning efficiency, delayed recall, and recognition memory was significantly better in DLB than in AD dementia ($p<0.01$). DLB patients with impaired naming were indistinguishable from patients with AD dementia on delayed recall, while DLB patients with normal naming had significantly better delayed recall, learning, and recognition memory ($p<0.01$). Unlike delayed recall performance, learning efficiency and recognition memory was not dependent on naming performance in DLB.
CONCLUSIONS: As a group, DLB outperforms AD dementia in learning efficiency, delayed recall and recognition memory. Nonetheless, there is a subgroup of patients who meet criteria for probable DLB but who demonstrate a pattern of impaired naming and impaired delayed recall that is indistinguishable from AD dementia. Further work is needed to determine if DLB patients with this memory and naming profile are more likely to have co-morbid AD pathology.

10d. Other: diagnostics

ADPD5-1681
COGNITIVE DYSFUNCTION IN PARKINSON'S DISEASE WITH SWEDDS
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Background : Patient diagnosed with Parkinson's disease (PD) on clinical grounds who subsequently turn out to have normal dopamine transporter imaging have been referred to as SWEDDs (scans without evidence of dopaminergic deficits). Cognitive dysfunction is frequent manifestation in Parkinson's disease. In this study we determined the similarities and differences in the cognitive dysfunction between PD and SWEDDs.
Objective : To assess cognitive dysfunction in patients of Parkinson's disease with SWEDDs, in comparison with Parkinson's disease and age-matched controls.
Method : This study enrolled 34 patients with SWEDDs, 104 patients with early PD and 29 healthy controls. Neuropsychological tests covering different cognitive domains were performed on all subjects for evaluation of cognitive function.
Results : PD with/without SWEDDs patients reported lower cognitive performances than healthy controls in several cognitive domains (attention, executive function, verbal and visual memory). But there are only one test of attention (digit span_forward) shows difference between PD and PD with SWEDDs group. Other cognitive domains were nealy correspond with two groups.
Conclusions : Cognitive dysfunction in PD with SWEDDs were not previously reported. This study shows that parkinson's disease have multi-domain cognitive dysfunction, additionally presynaptic dopaminergic deficits were not contributed cognitive dysfunction in early parkinson's disease.

10d. Other: diagnostics

ADPD5-1717
RISK SCORE APPROACH FOR THE PREDICTION OF PROGRESSION TO DEMENTIA IN NON-DEMENTED PATIENTS WITH PD
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Backgrounds: Parkinson's disease (PD) is often associated with mild cognitive impairment and dementia. We investigated the individual risk of PD patients to develop dementia using baseline neuropsychological tests.
Methods: A total of 274 non-demented patients with PD underwent baseline neuropsychological evaluation and followed for mean duration of 2.2 (1.1) years. Each neuropsychological score was categorized with cut-offs at 1.0 standard deviation (SD) as well 1.5 SD below age- and education-matched norms. Cox regression analysis was used to make a risk score system combining baseline neuropsychological tests predicting future progression to dementia.
Results: Twenty-two patients (8.0%) progressed to dementia during follow-up period. Univariate Cox regression analyses adjusted for age, sex, and education showed that digit span forward (-1.0 SD), digit span backward (-1.0 SD), Rey-Osterrieth Complex Figure (RCFT) copy test (-1.0 SD), Controlled Oral Word Association Test (COWAT) animal (-1.0 SD), COWAT phonemic (-1.0 SD), contrasting program (-1.0 SD), go-no-go test (-1.0 SD), and RCFT delayed recall (-1.5 SD) significantly predicted progression to dementia. Calculated risk score (range 0-13) based on multivariate Cox regression analysis predicted progression to dementia well (area under curve = 0.74, 95% CI = 0.63-0.86).
Conclusion: Risk score system is a useful approach for the prediction of dementia risk among PD patients, but should be validated and further improved to increase its predictive value. This approach showed that baseline neuropsychological score in PD patients significantly predict future progression to dementia and could help to identify candidates for future interventional studies for PD dementia.

10d. Other: diagnostics

ADPD5-2047
A CHALLENGING CASE : IS IT PARKINSON PLUS SYNDROME, UNIPOLAR MAJOR DEPRESSION WITH PSYCHOTIC FEATURES OR AN OVERLAP SYNDROME?

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A 63 year old lady presented to the hospital with 6 months worsening paranoid delusions, visual hallucinations and bilateral leg pain. She was treated with dopaminergic agents for presumed Parkinson's Disease for 8 years (initially diagnosed based on unilateral hand motor symptoms limited to pincer grip and unscrewing a bottle cap). She was subsequently diagnosed with restless leg syndrome and in the past 3 years her dopaminergic agents had been up-titrated to treat restless leg syndrome; in spite of which she continues to report disabling leg pain. After detailed examination and investigations, it was deemed that the pain was inconsistent with restless leg syndrome and was thought to be a functional problem. It became increasingly evident that her symptoms were related to Unipolar Major Depression with psychotic features. She had insight but exhibited a myriad of neuropsychiatric symptoms predominantly paranoia, manipulative and attention seeking behaviors and selective augmentation of behavioral symptoms in the presence of her husband. A Single-photon emission computed tomography (SPECT) of the brain revealed generalized symmetrical posterior parietal and occipital lobes hypoperfusion, which in the current clinical setting favored a possible diagnosis of Lewy Body Dementia. Dopaminergic agents were weaned off successfully and, despite some signs of Parkinsonism (mild bradykinesia and rigidity), her functionality was not affected. Citalopram and Quetiapine were carefully up-titrated concomitantly. She was transferred to the Elderly Mental Health Service for ongoing in-patient care, where she continues to exhibit neuropsychiatric symptoms with an emerging functional mobility difficulty.

10d. Other: diagnostics

ADPD5-2144
NONMOTOR SYMPTOMS IN DE NOVO PARKINSON DISEASE COMPARING TO NORMAL AGING

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Introduction: Nonmotor symptoms (NMSs) are common in Parkinson disease (PD), affecting patient's quality of life. The prevalence and domains of NMSs in untreated de novo PD remains unclear, especially comparing to normal aging. The objective was to determine NMSs in untreated de novo PD patients.
Methods: We performed a case-control study to evaluate the frequency and severity of NMSs in untreated de novo PD patients (n=71) and age-matched normal controls (n=60) using the Non-Motor Symptoms Scale (NMSS). The motor section of the Unified Parkisnon Disease Rating Scale (mUPDRS) and the Hoehn and Yahr (HY) stage were also obtained in PD patients
Results: The number of NMSs and the NMSS scores were significantly higher in the PD patients than in controls ($p<0.001$). There was no correlation of the NMSS scores with age and sex in both group and additionally with mUPDRS score and HY stage in PD patients group. Mood/cognition, attention/memory and gastrointestinal domains are the most frequent in PD patients and rarely seen in controls.
Conclusion: NMSs in untreated de novo PD patients are more prevalent and severe with different domain involvement comparing to normal aging.

10d. Other: diagnostics

ADPD5-2298

A UNI-RHINAL ODOR DETECTION TEST HELPS DETECT AND TRACK ALZHEIMER'S DISEASE AND ALSO HAS A DISTINCT PATTERN IN PARKINSON'S DISEASE

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This study was a continuation of a pilot study of a quick, non-invasive, and reliable diagnostic test for early Alzheimer's disease (AD) (Stamps, Bartoshuk, Heilman, 2013) that included more advanced stages of AD as well as Parkinson's disease (PD) patients. The participants included controls (N=31), patients with probable AD (N=36), mild cognitive impairment (N=33), other causes of dementia (N=30), or PD (N=22). Patients with a comorbid dementia or history of a brain tumor, stroke, seizures, nasal polyps, or severe head injury were excluded. Odor detection was tested one nostril at a time. A container of 14g of peanut butter was raised up from the bottom of a 30 cm ruler 1cm at a time. Upon detection, the distance from their nostril to the stimulus was measured. Patients with AD were significantly more impaired at detecting an odor with their left nostril (mean detection distance = 6.2 cm) than with their right (mean detection distance = 14.3 cm) ($p<0.001$). The mean difference of the left nostril's detection distance minus the right nostril's detection distance was -8.3 cm which was significantly different from patients with other types of dementia, controls, and Parkinson's patients ($p<0.0001$). As AD progressed, the asymmetry decreased as the right nostril became more impaired until both nostrils were symmetrically impaired at the more severe stages. The odor detection distance of the left nostril did not change with disease progression. Parkinson's patients were bilaterally and significantly impaired across both nostrils compared to the other groups ($p<0.001$) independent of disease stage.

10f. Other: clinical trials

ADPD5-1043
THE AUTONOMIC SCALE IN PD AND MULTIPLE SYSTEM ATROPHY PATIENTS : CORRELATIONS BETWEEN ONE SCALE TO THE OTHER.
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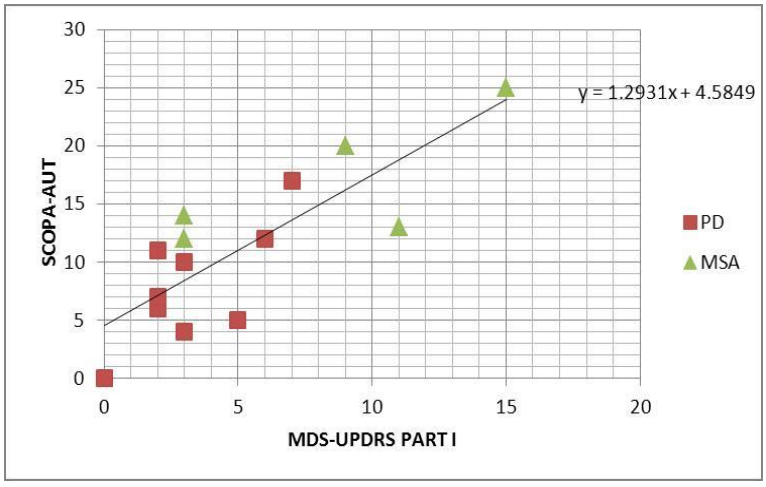
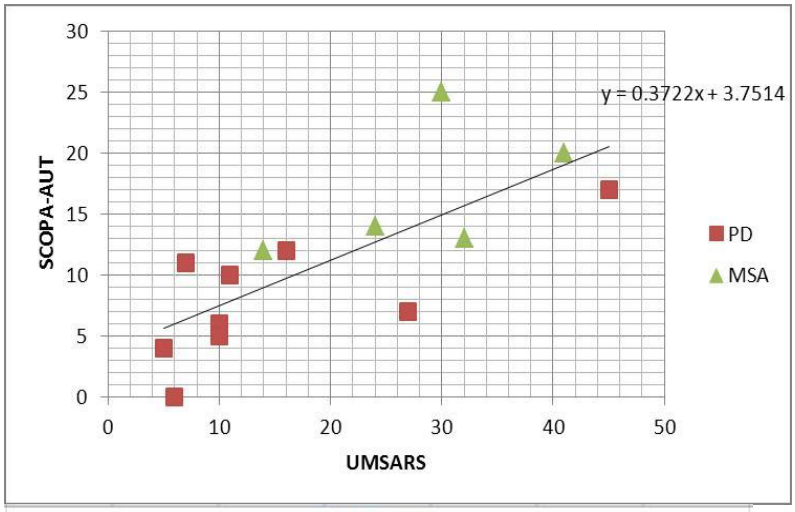
Background : PD is classically diagnosed with motor deficits, but wide range of non-motor symptoms, especially autonomic symptoms, commonly affect patients with PD and multiple system atrophy (MSA). There are several scales to assess autonomic symptoms in PD and MSA . The aim of this study is to determine equation models for the conversion of scores from one scale to the other.
Methods : 14 patients who first visited to our movement clinic with parkinsonism (bradykinesia, resting tremor, rigidity, postural instability) were evaluated with the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) Part I and Scales for Outcomes in Parkinson's Disease-autonomic symptoms (SCOPA-AUT), and Unified MSA rating scale(UMSARS) I-IV. Linear regression was used to develop equation models. Spearman's rho was used to assess the relation between autonomic scales. 9 patients finally diagnosed to PD and 5 patients to MSA.
Results : MDS-UPDRS PART I and SCOPA-AUT sacle have strong correlation (r=0.743) and UMSARS and SCOPA-AUT have very strong correration (r=0.848) coefficients.
Conclusion : SCOPA-AUT scale is briefer and shorter than UMSARS and more practical to administer than UPDRS. And it can be performed by patient or observer without involvement of researcher or doctor. With the equation models identified in this study, scores from UPDRS non-motor examination or UMSARS can be converted to SCOPA-AUT scale score.





Correlations

			SCOPA	UPDRS	UMSARSD	KOGS
Spearman's rho	SCOPA	Correlation Coefficient	1.000	.743	.848	.385
		Sig. (2-tailed)	.	.002	.000	.174
		N	14	14	14	14
	UPDRS	Correlation Coefficient	.743	1.000	.702	.415
		Sig. (2-tailed)	.002	.	.005	.140
		N	14	14	14	14
	UMSARSD	Correlation Coefficient	.848	.702	1.000	.556
		Sig. (2-tailed)	.000	.005	.	.039
		N	14	14	14	14
	KOGS	Correlation Coefficient	.385	.415	.556	1.000
		Sig. (2-tailed)	.174	.140	.039	.
		N	14	14	14	14

10f. Other: clinical trials

ADPD5-1811
ANOSOGNOSIA IN AMNESTIC-MILD COGNITIVE IMPAIRMENT AND MILD COGNITIVE IMPAIRMENT IN PD

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A diagnosis of Mild Cognitive Impairment (MCI) often starts with cognitive concerns raised by the patient or a caregiver although patients often lack awareness of the cognitive changes they are experiencing. It is unclear what neuropsychological characteristics among MCI subjects relate to reduced insight into their cognitive and behavioral changes. We hypothesized that subjects with amnestic MCI (a-MCI) would be more likely to lack awareness of their cognitive deficits (anosognosia) than individuals with MCI related to Parkinson's disease (MCI-PD).
Methods: Subjects included 18 a-MCI and 10 MCI-PD subjects in an ongoing study. Anosognosia was assessed by comparing responses of patients and caregivers on a questionnaire in 3 domains (cognitive, physical, behavioral), with higher scores indicating a higher level of anosognosia. Depression assessed by Beck Depression Inventory II.
Results: Anosognosia scores did not differ significantly between the groups (all p's > .11). No relationship between depression and anosognosia was noted in the a-MCI group, while a greater severity of depression was associated with lower anosognosia scores (better insight) in the MCI-PD group. In the a-MCI group, poorer delayed recall and recognition of a word list were associated with a higher score for cognitive abilities and with the total anosognosia scores. Among MCI-PD subjects, poorer word list learning was associated with a higher rate of anosognosia for cognitive abilities.
Conclusions: Findings from this study are preliminary, pending further data collection. However they do suggest an association between depression and greater insight, and between certain types of memory impairment and greater anosognosia.

10f. Other: clinical trials

ADPD5-2033
OPTIMIZING THE PLACEBO RESPONSE IN PARKINSON'S DISEASE CLINICAL TRIALS
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Background: Substantial improvement of Parkinsonian symptoms is observed in placebo groups of many clinical trials leading to a number of negative and failed studies.
Objectives: To analyze the factors contributing to increased placebo responses in PD clinical trials conducted at Quintiles between 2000- 2014.
Methods: Quintiles has been involved in the development of multiple PD medications available on the market. Of the 70 trials conducted only the large phase III pivotal studies were selected for the purposes of this analysis.
Results: Twenty Pivotal Phase III studies were performed of which 15 in Advanced and 5 in Early PD patients. All studies compared investigational product to placebo in a parallel design. Of the total number of pivotal studies, 12 were positive, while 8 were either negative or failed. Of these, 5 presented with high placebo responses. The most frequent factors potentially contributing to improvements on placebo were: high patient/rater expectations, study methodology, investigational product mechanism of action and rater dependent factors. Regional differences in the placebo response, observed in some of the studies, were not necessarily related to ethnicity but rather to the lack of awareness of the placebo effect in clinical trials.
Conclusion: The assessment of PD pivotal studies conducted at Quintiles has shown that 60% of these were positive while 25% had high placebo responses. This is in concordance with the literature were placebo improvements were observed in up to 50% of patients. Controlling some of the factors contributing to this effect, would improve the chance of successful trials.

10h. Other: other

ADPD5-0281
PATTERNS OF NEUROPSYCHOLOGICAL PROFILES ACCORDING TO SEVERITY OF DEPRESSIVE SYMPTOMS IN NEWLY DIAGNOSED PD

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Objectives: Depression is common non-motor symptoms in patients with Parkinson's disease (PD). Depression is not only associated with only poor quality of life, but also associated with decreased cognitive functions and motor decline. In our study, we analyzed clinical neuropsychological profiles according to severity of depressive symptoms in newly diagnosed Parkinson's disease patients.
Methods: 90 newly diagnosed PD patients were enrolled. We divided 38 patients of depressive group and 52 patients of non-depressive group according to their geriatric depression scale by score of 18. Age, sex, education, age at onset, disease duration, Korean mini-mental status examination and Hohen and Yahr stage were analyzed by t-test. Each neuropsychological tests were analyzed with analysis of covariance (ANCOVA) controlled by educational years. Pearson's correlation coefficient was calculated to assess the correlation between depression scores and Z-scores of neuropsychological tests.
Result: There was no difference between depressive group and non-depressive group in age, education years, disease severity, age at onset and disease duration. The score in semantic fluency was lower in depressive group compared with non-depressive group. The depression scores were correlated with semantic fluency scores and there was a trend that depressive scores were correlated with semantic memory scores.
Discussion: Although depressive symptoms are generally associated with executive dysfunctions, it is also recognized that depression relates to poorer semantic fluency ability. Our result suggests that depressive symptoms in newly diagnosed PD patients are associated with frontotemporal dysfunction, revealed by impairment in semantic fluency and semantic memory tests.

10h. Other: other

ADPD5-0545

CLINICAL EVALUATION OF PATIENTS WHO HAD DEMENTIA WITH LEWY BODIES IN THE PSYCHIATRIC WARD OF A GENERAL HOSPITAL

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Objectives: As the number of elderly patients with dementia in Japan increases, the number of inpatients with dementia with Lewy bodies (DLB) is also expected to increase. We performed a retrospective investigation of patients with DLB who were admitted to our hospital and examined issues encountered during treatment.
Methods: Subjects included 25 patients who were admitted to our center and who received a diagnosis of probable DLB at the time of discharge. The medical records of these patients were used to obtain information regarding their diagnoses, clinical manifestations, treatment and outcome.
Results: On admission, 52% of patient was diagnosed having DLB and 36% having mood disorders. Most common BPSD was depression. A decrease in body weight due to diminished food intake was noted in 13 patients. The symptoms of dysautonomia included constipation, orthostatic hypotension or changes in blood pressure, and abnormal sweating. Deep vein thrombosis (DVT) was diagnosed in four. Acetylcholinesterase inhibitors (ChEIs) ameliorated BPSD in 72% of patients. Antidepressants were effective in seven out of 13. 16 patients had positive outcomes, three worsened, and nine were transferred to a different hospital.
Conclusions: It is important to distinguish cases of DLB among cases of mood disorders diagnosed in elderly patients. ChEI was useful in many patients with BPSD, but a wide variability in the treatment response was observed in patients who received psychotrohics. More than half of patients also manifested physical complications. Therefore, it is important to take both the physical and psychological needs of the patient into account.

10h. Other: other

ADPD5-0991
EXECUTIVE FUNCTIONING AND CAREGIVER AND SELF-REPORT OF NEUROPSYCHIATRIC SYMPTOMS IN PD
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[2]Neurology, Korea University College of Medicine, Ansan, Korea
[3]Psychology, University of Virginia, Charlottesville, USA
Objectives: To examine patient and caregiver reports of current neuropsychiatric behaviors on the Frontal Systems Behavior Scale (FrSBe) and their relationship to objective measures of executive functioning.
Methods: Data from 58 patients with Parkinson's disease (PD) were examined. An Executive Composite Score was created using T-scores from four cognitive measures of executive functioning (Wisconsin Card Sorting Test Perseverative Errors, Letter Fluency, Trails B, and Stroop Color-Word Test Interference). Correlations between patient and caregiver-derived FrSBe subscale scores (Apathy, Disinhibition, and Executive Dysfunction) and the FrSBe Total Score were examined. All FrSBe scores were ratings of current functioning. Multiple linear regression analyses were used to predict executive functioning based on FrSBe ratings while controlling for depression.
Results: Patient and caregiver Total Score (.470), Apathy (.306), Disinhibition (.430), and Executive Dysfunction (.469) were significantly correlated ($p<.05$). Patient Total Score (beta = -.535, $p<.005$), Apathy (beta = -.493, $p<.001$), Disinhibition (beta = -.532, $p<.005$), and Executive Dysfunction (beta = -.565, $p<.001$) were significant predictors of the Executive Composite Score. Caregiver ratings of the same behaviors did not predict the Executive Composite Score. Given these findings, patient FrSBe subscale scores were entered into a stepwise multiple regression equation while controlling for depression. Patient self- report of executive dysfunction alone was found to be a significant predictor of the Executive Composite Score (Beta = -.461, $p<.001$).
Conclusions: PD patients had better insight into their cognitive and behavioral limitations than caregivers. Patient self-assessment of behavioral symptoms of executive dysfunction predicted objective neuropsychological performance.

10h. Other: other

ADPD5-1098

MILD COGNITIVE IMPAIRMENT IN PARKINSON DISEASE AND PROGRESSION TO DEMENTIA

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[2]Clinical Psychology Neurobiology and Methodology, University of La Laguna, La Laguna, Spain
[3]Unidad de Trastornos Estrapiramidales. Servicio de Neurologia, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain

Objective: Cognitive impairment and dementia are frequent in PD. New diagnostic criteria for PD-MCI have recently been proposed by the Movement Disorder Society (MDS) Task Force (Litvan et al., 2012). Our aim was to study the rate of PD-MCI according to new criteria and the progression to dementia.
Methods: 43 patients with idiopathic PD and 20 normal controls. All patients met the clinical criteria for the diagnosis of PD. Those who met the criteria of MDS for the diagnosis of dementia associated with PD were excluded. PD-MCI diagnosis was based on level 2 of the criteria proposed by MDS Task Force. Neuropsychological testing includes two tests for each of the following five cognitive domains: attention and working memory, executive, language, memory, and visuospatial functions. Cognitive impairment was operationalized as 1,5 standard deviations or more below the mean of the control group. Follow-up assessments were conducted 6-8 years after base-line in 39 patients.
Results: Fifteen patients (34.9%) were diagnosed as PD-MCI at base-line. Follow-up assessment showed that eight of PD-MCI patients (20,5%) developed dementia. In addition, 17.9% of patients without MCI at base-line also developed dementia at follow-up.
Conclusions: Our preliminary results show that the percentage of PD-MCI patients in this study and rate of progression to dementia are comparable with previous studies. In our study an important number of patients with PD will develop dementia in 6-8 years (38,4%). The percentage of patients developing dementia was slightly higher in the PD-MCI group compared to patients without MCI at baseline.

10h. Other: other

ADPD5-1144
DEPRESSION SEVERITY IN PD PATIENTS MODULATES PATIENT AND INFORMANT PERCEPTIONS OF NEUROPSYCHIATRIC BEHAVIORS AND EXECUTIVE DYSFUNCTION

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[2]Psychology, University of Virginia, Charlottesville, USA
[3]Neurology, Korea University College of Medicine, Ansan, Korea

Objectives: PD patients and informants differ in their perceptions of non-motor symptoms. The effects of time and depression on their perceptions remains unclear. We investigated 1) the relationship between patients' and informants' ratings on the Frontal Systems Behavior Scale (FrSBe) and 2) the effects of time and depression on symptoms. ***Methods:*** Using linear mixed effects models, controlling for differences in perceived pre-PD symptomatic functioning, we examined the relationship between 83 PD patients' and informants' ratings on the FrSBe Total and subscale scores (Apathy, Disinhibition, and Executive Dysfunction) as well as effects of patient depression on ratings of current (CURRENT scores) and Pre-PD symptomatic behaviors (BEFORE scores). Depression was categorized as absent, mild, or moderate/severe using established cutoff scores on the Geriatric Depression Scale or Beck Depression Inventory-II. ***Results:*** Patient and informant FrSBe Total and subscale scores were significantly correlated (p<.0001). Patients with moderate/severe depression at the time of evaluation had higher BEFORE Total FrSBe scores than those without depression (p<.001), but they did not differ on subscale scores. More severely depressed patients had significantly higher CURRENT Total FrSBe and subscales scores than those without depression (p<.01 - p<.0001). Patients' level of depression did not affect informant BEFORE FrSBe scores, but informant CURRENT Total FrSBe and subscale scores were significantly higher when patients were moderately/severely depressed (p<.001 - p<.0001). ***Conclusions:*** Patient-perceived neuropsychiatric symptoms and executive impairment were associated with informant observations. Patient depression levels at evaluation significantly modulated patients' and informants' perceptions of behavior, particularly in more severely depressed patients.

10h. Other: other

ADPD5-1611
RESTLESS LEGS SYNDROME IN CELIAC DISEASE PATIENTS.
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Objectives: Celiac disease is an autosomal recessive autoimmune enteropathy triggered by ingestion of gluten. Celiac disease has a prevalence of 1-2% and is associated with various neurological syndromes, including neuropathy, cerebellar ataxia, migraine, restless leg syndrome (RLS). RLS is a disease of unknown etiology. RLS has a prevalence of 5 to 15% in a population. Purpose of the study is the prevalence of RLS among patients with celiac disease.
Methods: We examined 200 patients with celiac disease and 100 patients of control group with reflux esophagitis. The diagnosis of RLS was made according to the diagnostic criteria of the International RLS Study Group. Patients underwent neurological examination and instrumental examination, and they are filled with RLS rating scale.
Results: We found that RLS occurs more than 5 times more often in patients with celiac disease compared with the control group (21 ± 2,9 and 4 ± 2, respectively, p <0,001). The proportion of men with RLS and age older than 40 years was significantly predominated in the group of celiac disease patients. In patients with celiac disease and RLS statistically significant prevailed male gender, by age - persons older than 40 years.
Conclusions: RLS occurs frequently in patients with celiac disease. Probably, celiac disease is a risk factor for the development of RLS syndrome. The role of iron deficiency, inflammation and immune dysfunction in the pathogenesis of RLS in celiac disease should be revealed.

10h. Other: other

ADPD5-1612
SEVERE RESTLESS LEGS SYNDROME AS AN EARLY NON-MOTOR SIGN OF THE PD.
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Objectives: Restless legs syndrome (RLS) is a common sensory-motor disorder. Undiagnosed and untreated RLS leads to serious disruption of sleep. The pathophysiology of RLS is multifactorial. One of the causes of secondary RLS is Parkinson's disease. Purpose of the study is the prevalence of severe RLS in patients with early Parkinson's disease (disease duration of less than three years).
Methods: We examined 57 patients with early PD (study group) and 100 patients of the control group without Parkinson's disease. The diagnosis of RLS was made according to the diagnostic criteria of the International RLS Study Group. Patients underwent neurological examination and instrumental examination, and they are filled with RLS rating scale and the Johns Hopkins' assessment scale of the RLS severity.
Results: We found that RLS occurs in more than 4 times more likely in patients with early Parkinson's disease compared with the control group (26% and 6%, respectively). The proportion of patients with the third degree of RLS severity was significantly prevailed in the study group.
Conclusions: Severe RLS may be an early sign of Parkinson's disease.

10h. Other: other

ADPD5-1926
CHANGES IN RETINAL MORPHOLOGY AND VISUAL FIELD IN EARLY PARKINSON'S DISEASE
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Visual symptoms are common non-motor symptoms of idiopathic Parkinson's disease (PD). Here, we investigated changes in retinal morphology and visual field in early PD patients and control individuals. Peripapillary retinal nerve fiber layer (RNFL) thickness, macular thickness and volume, and foveal thickness were measured using optical coherence tomography (OCT), and mean deviation of visual field (MD) was measured using an automatic visual field analyzer. PD patients were in an 'ON' period during ophthalmic evaluation, and their clinical characteristics were evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn & Yahr (H&Y) scale during an 'OFF' period. Compared with control individuals, PD patients showed a decrease in RNFL thickness on average and in specific regions (i.e., inferior and superior quadrants; 5-, 7-, 10- and 11-o'clock positions). Mean total macular thickness and volume was also significantly decreased in PD patients compared with control individuals, but there was no difference between groups in foveal thickness. MD scores were significantly lower in PD patients than in control individuals. Average RNFL thickness was negatively correlated with PD duration and H&Y stage, macular thickness and volume were negatively correlated with UPDRS motor score and H&Y stage, and MD score was positively correlated with PD duration and H&Y stage. Our results show that patients with early PD exhibit retinal dysmorphology and visual field defect.

10h. Other: other

ADPD5-2230

ASSESSMENT OF LOCOMOTOR DEFICITS AND THERAPEUTIC IMPROVEMENT IN PARKINSON'S DISEASE PATIENTS AND PRE-CLINICAL MODELS

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Objective: Parkinson's disease (PD) patients experience progressive motor impairments, including severe gait deficits. MPTP-treated monkey model of PD played a pivotal role for the safe and efficacious translation of therapeutic interventions developed in rodents to viable clinical applications for humans. However, the lack of consensus on a methodology and the almost exclusive reliance on clinical scores of PD has often hindered successful translation of interventions to clinical settings. There is a critical need to develop translational methodologies to enable robust and reliable evaluation of therapies across species.

Methods: The connection-free platform allowed us to monitor muscle synergies and kinematics while the subjects walked on a treadmill, along a corridor, and across horizontal ladders of varying complexities. Using principal component analysis (PCA) applied on a large number of parameters; we could objectively quantify and rank task-specific deficits of gait patterns across a wide range of PD severities. To assess the translational value of the developed analyses, we recorded Parkinsonian patients and rats with alpha-synuclein-mediated nigrostriatal degeneration under the same conditions as MPTP monkeys.

Results: This analysis also uncovered kinematic parameters that were specifically improved after the administration of L-DOPA and others that remain resistant to dopamine replacement therapy. We found striking similarities in the pattern of gait deficits across species, and on the therapeutic effects mediated by L-DOPA.

Conclusion: The developed methodologies establish a highly-sensitive analytic framework to evaluate safety and efficacy of therapeutic interventions to alleviate locomotor deficits in animal models of PD and in Parkinsonian patients.

01d. Protein Misfolding & Aggregation: TDP-43

ADPD5-1107

CLUSTERIN IS A TOPOLOGICALLY DYNAMIC CHAPERONE THAT PARTICIPATES IN BOTH INTRACELLULAR AND EXTRACELLULAR PROTEOSTASIS

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Objectives

Under certain stress conditions clusterin, a normally secreted chaperone, can be redirected from the secretory pathway to intracellular compartments. Here we investigate whether clusterin plays an active role in intracellular proteostasis under these conditions.

Methods

We generated a model of extracellular clusterin expression in *Drosophila* in order to probe the effects of clusterin on the neurodegeneration associated with the cytosolic aggregation of the amyotrophic lateral sclerosis (ALS)-associated 43 kDa TAR DNA-binding protein (TDP-43). We then extended these studies to evaluate the ability of clusterin to suppress cytosolic aggregation of other intracellular proteins, and to investigate the ability of clusterin to localise to the cytosol in response to TDP-43 aggregation in both a human glial cell line and in the neurons and glia of the human spinal cord.

Results

We demonstrate that co-expression of clusterin significantly reduces the aggregation and neurotoxicity of TDP-43 in *Drosophila* motor neurons. These effects are accompanied by a dramatic reduction in clusterin secretion and its redistribution to the cytosol, processes which depend on the ability of TDP-43 expression to induce ER stress. Furthermore, the aggregation and neurotoxicity of mutant tau R406W and extended polyglutamine tracts are also rescued by clusterin expression. We also observe intracellular clusterin in motor neurons of ALS-patient postmortem tissue and in human cultured astrocytes, where clusterin and TDP-43 physically interact in the cytosol.

Conclusions

Clusterin is first identified chaperone to exhibit intra- and extracellular activity, rescuing the neurotoxicity associated with the intracellular aggregation of TDP-43, mutant tau and extended polyglutamine tracts.

01d. Protein Misfolding & Aggregation: TDP-43

ADPD5-1538

DISTINCT PHOSPHO-TDP-43 BRAIN DISTRIBUTION IN TWO CASES OF FTLD, ONE ASSOCIATED WITH ALS.

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Introduction: In the last few years, the TDP-43 protein (TAR DNA-binding protein 43) has been implicated in some neurodegenerative diseases pathogenesis. It's an intranuclear protein involved in many distinct cellular processes, like: gene transcription, alternative splicing, mRNA stabilization, microRNA biogenesis, apoptosis and cell division. Its molecule, when altered, shows modified pattern of distribution, as well as functioning, along the Central Nervous System (CNS) structures. The Fronto temporal Lobar Degeneration (FTLD) and the Amyotrophic Lateral Sclerosis (ALS) are two examples of TDP-43 proteinopathy.

Material and methods: We studied two donated brains from patients with clinical diagnosis of FTLD , one associated with ALS. Both patients had been followed by the *Neurology Clinic of ISCMPA.* After fixation, macroscopic examination with sampling of different encephalic regions were performed. Specific regions were chosen to immunohistochemistry (IHC) technics with anti-Aβ, AT8, anti-α-syn and anti-phospho-TDP-43 on the *Laboratory of Pathology of UFCSPA*.

Results: Both brains showed anti-phospho-TDP-43 positivity but not equally distributed over the encephalic zones. FTLD case presented TDP-43-phosphorylated on the frontal cortex, hippocampus and entorhinal cortex; in ALS-FTDL case the abnormal protein was also seen on brainstem. ALS-FTDL also presented Aβ and AT8 positivity in hippocampus and entorhinal cortex (Braak I and II).

Discussion: The hypothesis supported by scientific literature that these neurodegenerative diseases can have the same etiology with distinct encephalic region involvement is also corroborated by the present study.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-0869

FRONTOTEMPORAL DEMENTIA: TAU PROTEINS ARE MISSING

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1.Objectives
Frontotemporal dementia (FTLD) is a clinical syndrome characterized by progressive deterioration in behaviour, personality and/or language. Molecular basis of FTLD is very heterogeneous and leads to a classification based on the composition of protein aggregates made of TAU proteins, TDP-43 or FUS/TLS. Using biochemical approaches, we showed that some cases presenting with TDP-43 pathology (FTLD-TDP) display a partial or total loss of TAU proteins expression that can be referred as “TAU less”. Our goal is to determine if this “TAU less” group can be a part of the current FTLD classification.
2.Methods
FTLD brains (n=34) were obtained from the Lille Neurobank and the GIE NeuroCEB of Paris. Western Blot was performed on frontal area with antibodies directed against TAU and TDP-43. In order to assess neuronal death, protein expression of the NSE, Aconitase, Histone H3 and Neurofilaments was studied as well. Human Tau mRNA was analysed by QPCR. Finally, 2D-DIGE was performed on FTLD brains with or without loss of TAU proteins.
3.Results
We demonstrate that loss of TAU proteins mainly affects FTLD-TDP brains with specific genetic background. However, neither a decrease in TAU mRNA level nor a massive neuronal death were able to explain this phenotype suggesting a dysfunction in post-transcriptional and/or -translational regulation of TAU. Interestingly, proteomic analysis highlighted 15 proteins deregulated in “TAU less” brains including synaptic and astrocytic proteins.
4.Conclusion
In conclusion, our results suggest that “TAU less” group may define a new variant in the FTLD classification.

02d. Cell, Molecular & Systems Biology: TDP-43

ADPD5-1176

FLUORESCENCE-BASED ASSAY DEVELOPMENT TO SCREEN DRUGS AGAINST AMYOTROPHIC LATERAL SCLEROSIS DISEASE

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Amyotrophic Lateral Sclerosis disease (ALS) is characterized by the death of both upper and lower motor neurons in the motor cortex of the brain, the brain stem, and the spinal cord. Prior to their destruction, motor neurons develop intracellular protein inclusions in their cell bodies and axons. These inclusions often contain ubiquitin, and generally incorporate one of the ALS-associated proteins like SOD1, TAR DNA binding protein (TDP-43, or TARDBP) or FUS. Innoprot has developed a novel fluorescence cell-based assay for High Content Screening to screen compounds that can prevent or decrease the protein TDP43 or FUS aggregation into the stress granules after induction a cytotoxic stress. In this work we used both models to screen a library of 1200 compounds. Arimoclomol and Riluzole compounds were used as positive controls for the fluorescent TDP43 and FUS aggregation model. After the screening campaign, positive compounds were chosen for further testing, based on the strength of the initial response and the lack of cytotoxicity. Our results indicated that the pharmacological inhibition or modulation TDP43 or FUS aggregation implicated in ALS is a valid strategy for drug screening.

02y. Cell, Molecular & Systems Biology: modeling of disease progression

ADPD5-1177

MODELLING MOTOR NEURON EXCITOTOXICITY IN THE AXON

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Objectives

Amyotrophic lateral sclerosis (ALS) is characterised by degeneration of upper and lower motor neurons. Evidence suggests that excitotoxicity may play a major role in this process. It remains unclear how motor neurons become exposed to excitotoxicity and whether this may be mediated by more than one cellular compartment (dendrites or axons).

Methods

Spinal motor neurons were obtained from the spinal cords of E13.5 mice and plated into microfluidic chambers with distal skeletal myocytes. Kainic acid (KA, 100µM) was delivered to either the somatodendritic or axonal compartment for 24 hours. Targeted excitotoxicity *in vivo* was delivered to the spinal cord (10mM kainic acid) or skeletal muscle (10mM glutamate) in Thy1-YFP mice. Pathology was assessed at 28 days post-surgery.

Results

KA delivered to the somatodendritic compartment *in vitro* resulted in a significant ($P<0.05$) increase in distal axon degeneration (55.8%±2.58% KA; 43.4%±2.08% control). This was accompanied by widespread cellular degeneration within the treated somatodendritic compartment. Distal axon excitotoxicity resulted in no significant ($P>0.05$) degeneration (41.0%±3.22%). KA delivered to the spinal cord *in vivo* resulted in a significant ($P<0.05$) increase in neuromuscular junction degeneration (30.85%±0.07% KA; 2.20±0.59% control). Somatodendritic KA resulted in a significant ($P<0.05$) loss of motor neuron cell bodies and motor deficits. Excitotoxicity delivered to the skeletal muscle did not induce degenerative or functional changes.

Conclusions

This work identifies specific vulnerability of the motor neuron cell bodies to excitotoxicity. These results suggest that therapeutics aimed at reducing excitotoxicity could be more effective if targeted to the motor neuron cell bodies.

03b.Pathophysiology & Disease Mechanisms: prion-like mechanisms

ADPD5-2016

THE MECHANISM OF TDP-43 SPREADING IN ALS

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1. Objectives: One hallmark of ALS is cytotoxic aggregates of the TDP-43 protein in neurons, comparable to aggregates of bona-fide amyloidogenic proteins in other neurodegenerative diseases, such as Aβ, tau and α-synuclein. Biophysical studies have shown that TDP-43 is indeed amyloidogenic. Recently, the aggregates of these amyloids, as well as of TDP-43, were shown to spread in the brain during disease progression. Experimental evidence indicates that this occurs via a cell-to-cell transfer, apparently comparable to prion transmission. However the mechanism of this intercellular transmission is unknown and may not necessarily be shared by all amyloids. Our goal is to characterize the mechanism of intercellular transmission of TDP-43 in ALS and to identify proteins mediating it.
2. Methods: We have generated neuronal SH-SY5Y cells expressing TDP-43 (full length and A315T mutant) tagged with either GFP, HA or APEX, the latter being based on enzymatic activity hence confers enhanced sensitivity.
3. Results: We found that tagged TDP-43 localizes to the nucleus like endogenous TDP-43, and that tagged TDP-43 is transferred to naïve cells. We shall focus on the use of APEX as a tag and employ GFP and HA for corroborating APEX-tagged TDP-43 results. Notably, the APEX tag allows labeling (by biotin) of proteins located in close proximity (but not distant) to the TDP-43-APEX fusion protein and we have shown its feasibility.
4. Conclusion: This novel cell culture model should shed light on the details of the mechanism of cell-to-cell transmission of TDP-43 and may suggest candidate targets for ALS therapeutics.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-1140

LINKING OF AUTOPHAGY AND UBIQUITIN-PROTEASOME SYSTEM (UPS) IN NEUROTOXIC YEAST CELL DEATH MODELS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with progressive degeneration of motor neurons, resulting in atrophy of musculature and death. Mutations in the gene TDP-43 have been associated with ALS. The RNA-binding protein TDP-43 accumulates in the cytoplasm of neurons of ALS patients as abnormal ubiquitinated, phosphorylated and insoluble inclusions.

We investigate the link between the cytotoxicity of TDP-43, UPS and autophagic activities in an easy to handle *S. cerevisae* cell death model.

Methods and Results: To determine the cytotoxicity of TDP-43 we measured growth deficiency in drop dilution assays. Yeast wild type (wt), *atg* (autophagy related) and UPS knock-out strains were transformed with human TDP-43 or vector control plasmids. In order to increase autophagy, growth media contained rapamycin blocking the TORC1 signalling pathway. We observed enhanced TDP-43 cytotoxicity with increased autophagy and with decreased UPS. TDP-43 cytotoxicity was relieved by deletion of *ATG* genes. We used the GFP-Atg8 processing assay to study autophagic turnover upon TDP-43 expression. wt and Δ*atg1* were transformed with TDP-43 and GFP-Atg8. We found an increase of processed GFP upon expression of TDP-43.

Conclusions: Our results suggest that autophagy and UPS influence TDP-43-triggered cytotoxicity. UPS plays a cytoprotective role whereas autophagy plays a detrimental role. We believe that insights gained by applying yeast as cell death model promote a better understanding of the relation between autophagy and UPS in ALS.

03h. Pathophysiology & Disease Mechanisms: metabolism and insulin

ADPD5-0520
THIAMINE DEFICIENCY – UNEXPECTED CAUSE OF DEATH IN TWO PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Objectives: Hypovitaminoses by virtue of undernourishment still represent clinical relevant diseases with a broad spectrum of symptoms. We discuss two patients with amyotrophic lateral sclerosis (ALS) who unexpectedly died not because of respiratory failure, but central dysregulation traced back to acute Wernicke Encephalpathy (WE), surprisingly found by autopsy.
Methods: The two ALS patients underwent neuropathological examination after death that was performed with permission and according to German law.
Results: The sole macroscopic abnormality was a reddish colouration of the corpora mamillaria and the periventricular thalamus. The spinal cord was macroscopically unremarkable. Microscopically, the hypothalamus featured multiple fresh hemorrhages in the mammillary body, exhibiting the pattern of an acute WE. The Prussian blue staining did not detect siderophages as sign of resorption of a former bleeding. As neuropathological correlate for ALS, we found a reduced number of spinal cord motor neurons, phospho-TDP43-positive preinclusions in pyramidal neurons of the frontocentral and parietal cortex and pTDP43-positive skein-like inclusions in few ventral horn neurons of the thoracic spinal cord, confirming the clinical diagnosis of ALS.
Conclusions: Here, we report thiamine deficiency causing acute WE with fatal outcome in two patients with ALS. Both objected parenteral gastrostomy that predestines them to the high risk group due to their dysphagia. As thiamine deficiency represents a potential life threatening complication in the course of ALS, it is important to raise awareness for possible prevention by supplementation.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-0782

OVERACTIVATION OF WNT5A SIGNALING IN FTLD-TDP: CROSS-TALK OF PROGRANULIN AND TNF-ALPHA SIGNALING PATHWAYS.

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Objectives: Frontotemporal lobar degeneration with TDP-43 pathology (FTLD) is the most common cause of dementia in people under 65 years of age. Loss-of-function *progranulin* gene (*GRN*) mutations have been identified as the major cause of FTLD-TDP, however little is known about the relationship between progranulin (PGRN) deficiency and neuronal loss in FTLD-TDP. Previously we reported enhanced proliferative activity associated with the activation of Wnt5a signaling in PGRN deficient cells. The objective of this work has been elucidating the relationship between PGRN deficiency, Wnt5a signaling and cell proliferation in immortalized lymphoblasts from carriers of the c.709-1G>A *GRN* mutation (Asymptomatic and FTLD-TDP).
Methods: Cell proliferation was assessed by cell counting. mRNA levels were evaluated using real-time PCR and protein levels by immunoblotting. Co-Immunoprecipitation Assay was used for analyze the interaction between PGRN and its receptors.
Results: PGRN deficient cells showed increased expression of Wnt5a that seems to be related with overactivation of the TNF/NF-κB signaling. We observed a competition between PGRN and TNFα for binding both TNFα receptors. Blocking TNFa signaling with antibodies against TNFRI or TNFRII, as well as inhibiting NF-κB with wedelelactone, resulted in a decrease of both Wnt5a expression and proliferation in PGRN deficient lymphoblasts. In other hand, the addition of exogenous TNFα increased the Wnt5a expression and proliferation in control cells.
Conclusion: Our results revealed an important role of NF-κB signaling in PGRN-associated FTLD-TDP and confirm that PGRN can binds to TNFα receptors regulating the expression of genes, such as Wnt5a, implicated in the onset and progression of FTLD-TDP.

03v. Pathophysiology & Disease Mechanisms: metal ions

ADPD5-1013

CUMULATIVE FUNCTIONAL COPPER DEFICIENCY IN SPINAL CORDS OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) MODEL MICE AND POST-MORTEM HUMAN ALS TISSUE

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Objectives: We recently reported that increasing copper bioavailability both therapeutically and genetically acted to protect spinal cord motor neurons, whilst concomitantly improving locomotor function and survival of ALS model mice. These outcomes indicate that copper deficiency contributes to the pathogenesis of ALS. For the present study we directly assessed the extent of copper deficiency in ALS. **Methods:** We assessed levels and copper-dependent activity of the anti-oxidant SOD1, the ferroxidase ceruloplasmin and mitochondrial cytochrome c oxidase in the spinal cords of SOD1G37R ALS model mice from pre-symptom through to late symptom stages of disease progression. We also assessed post-mortem spinal cord samples from human cases of sporadic ALS. For mouse tissue analyses, non-transgenic littermate and wild-type SOD1 overexpressing mouse spinal cords, and non-disease affected livers were used as controls. Spinal cords from healthy controls were included in the human tissue analyses. **Results:** All analyses revealed a strong disparity between protein levels measured and their copper-dependent activity, consistent with a broad functional copper deficiency in ALS. This disparity was evident at an early age in the ALS model mice and was cumulative as disease progressed. **Conclusions:** These results indicate the cause of ALS phenotype in mutant SOD1 mice is not restricted to mutant SOD1 toxicity and may be driven, at least in part, by broader consequences of functional copper deficiency. The fact that the human ALS tissue we examined included only sporadic cases of the disease indicates the role of functional copper deficiency in ALS extends beyond mutant ALS cases.

04e. Therapeutic Targets & Mechanisms for Treatment: TDP-43

ADPD5-1077

AN ANTI-MISFOLDING TDP-43 PROTEINS DRUG WITH NEW MECHANISM

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An Anti-misfolding TDP-43 Proteins Drug with New Mechanism

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Our strategy is to maintain the functional conformation of the prone-to-aggregate domain of disease proteins throughout lifespan, could result in improved therapeutic outcomes. The functional substitution of the non-amyloid prion-like domain TDP-43 with the amyloid prion domain sup35 revealed a potent common structure for treating TDP-43 proteinopathies by using off-amyloid stabilizers[1,2,3]. These stabilizers may simultaneously prevent prion-like spread in neurodegenerative diseases. We selected few off-amyloid compounds from compound library and examined the effects of redirecting non-amyloid, misfolded TDP-43 protein into functional proteins. We successfully obtained two compounds via cell-based disease models. In this presentation, we will discuss mechanism-based target identification, drug discovery and pharmacological interventions for TDP-43 proteinopathies.

Reference:

1. I-F Wang*, H-Y Chang, S-C Hou, G-G Liou, T-D Way & C-K James Shen. (2012) The self-interaction of Native TDP-43 C-terminus inhibits its degradation and contributes to early pathogenesis. *Nature Communications.* 3, 766.

2. H-Y Chang, S-C Hou, T-D Way, C-H Wong & I-F Wang*. (2013) Deregulation of heat shock proteins associates with functional and pathological aggregation of TDP-43. *Nature Communications.* 4, 2757.

3. A NOVEL METHOD FOR STABILIZING TDP-43 PROTEIN (US PATENT FILING DATE, 2013).

04e. Therapeutic Targets & Mechanisms for Treatment: TDP-43

ADPD5-1589
REGULATION OF NUCLEAR TDP-43 BY NMDA RECEPTOR
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The dysfunction of TAR DNA-binding protein-43 (TDP-43) is implicated in neurodegenerative diseases. However, the function of TDP-43 is not fully elucidated. Here we show that the protein level of endogenous TDP-43 in the nucleus is increased in mouse cortical neurons at the early stage but return to basal level at the late stage after glutamate accumulation-induced injury. The elevation of TDP-43 level is resulted from a downregulation of phosphatase PTEN. We further demonstrate that activation of NR2A-containing NMDA receptors (NR2ARs) leads to PTEN downregulation and subsequent reduction of PTEN import from cytoplasm into the nucleus after glutamate accumulation. The decrease of PTEN protein level in the nucleus contributes to a reduced association of PTEN with TDP-43 and thereby mediates the elevation of nuclear TDP-43. We provide evidence that the elevation of nuclear TDP-43, mediated by NR2AR activation/PTEN downregulation, confers protection against cortical neuronal death at the late stage after glutamate accumulation. Thus, this study reveals a NR2AR/PTEN/TDP-43 signaling pathway by which the nuclear TDP-43 promotes neuronal survival. These results suggest that upregulation of nuclear TDP-43 represents a self-protection mechanism to delay neurodegeneration at the early stage after glutamate accumulation and that prolonging the upregulation process of nuclear TDP-43 may have therapeutic significance.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1277

DIFFUSION TENSOR IMAGING AND CORTICAL THICKNESS MEASUREMENTS AS A FUNCTION OF DISEASE SEVERITY IN AMYOTROPHIC LATERAL SCLEROSIS

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OBJECTIVE

Assess the sensitivity of cortical thickness measurements versus diffusion tensor imaging to detect pathologic changes in ALS.

METHODS

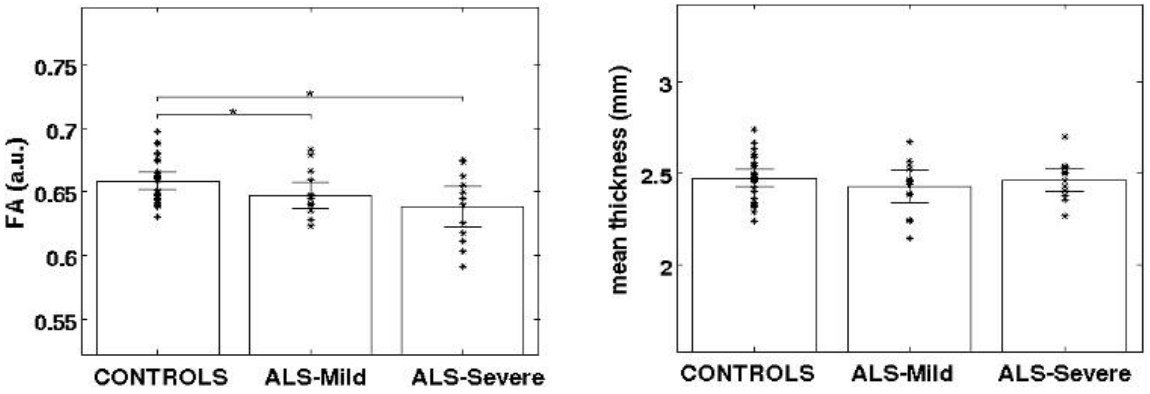
Twenty-six ALS-limb onset patients (mean age=57.4) and twenty-nine matched controls were included. Patients were divided into two groups of 13 according to severity (ALS-Mild ALSFRS-R > 38, ALS-Severe ALSFRS-R<=38). MRI measurements were performed in a Siemens Verio 3T scanner. DTI data used TBSS to evaluate white matter integrity while cortical thickness in structural images was measured using Freesurfer. Statistical comparisons were done in regions of interest comprising the corticospinal tracts for DTI and the precentral gyrus for cortical thickness.

RESULTS

Corticospinal DTI analysis showed significant fractional anisotropy (FA) differences between the ALS groups' means compared to controls (fig. 1-left) with a non-significant trend that the more advanced had lower values than the mild group. Significant differences in cortical thickness were not identified and there was no suggestion of greater reduction in the more advanced group.

CONCLUSIONS

The results indicate that corticospinal FA reductions are more sensitive, and more closely aligned to disease severity than cortical thickness of the motor strip. This should not, however, be interpreted as meaning degeneration of white matter is more biologically pronounced than grey matter—it is quite possible that results are due to differential sensitivities of the two scan modalities.



06a. Imaging & Biomarkers: structural MRI

ADPD5-2228

CORTICOSPINAL TRACT INTEGRITY IN RELATION TO EXTRA-MOTOR NEUROCOGNITIVE IMPAIRMENT IN AMYOTROPHIC LATERAL SCLEROSIS: A DIFFUSION TENSOR IMAGING AND NEUROPSYCHOLOGICAL STUDY

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Objectives: Diffusion tensor imaging (DTI) is a most promising structural MRI method to detect amyotrophic lateral sclerosis (ALS) changes both in pyramidal tracts and in extra-motor regions. Thus, we evaluated motor and extra-motor involvement in non-demented patients with ALS using DTI and their relation to neuropsychological measures.
Methods: We included 21 patients with definite or probable ALS (revised El Escorial Criteria) and 11 healthy controls. Using 30-directional DTI at 3T MRI scanner, we analyzed fractional anisotropy-(FA), apparent diffusion coefficient-(ADC), axial diffusivity-(D_A) and radial diffusivity-(D_R) for corticospinal tract-(CST), corpus callosum-(CC) and uncinate fasciculus-(UF). Neuropsychological evaluation included tests of executive (Trail Making Test part B-TMTB; Stroop Test-SNST; Digit Span Forward and Backwards; Wisconsin Card Sorting Test-WCST) and memory functions (Babcock Story Recall Test-BSRT; Rey Auditory Verbal Learning Test-RAVLT).
Results: We found significant ($p<0.05$) between-group differences in FA (CC), ADC (bilateral CST,CC), D_A (right CST,CC,bilateral UF), and D_R (right CST,CC). Within the ALS group, CST DTI indices (FA,ADC,D_A,D_R) did not correlate with CC or UF indices. CST indices were related to memory but not executive performance. CC FA was negatively associated with BSRT, whereas CC diffusivity indices were negatively associated with Digit Span Forward and RAVLT. UF integrity was positively associated with TMT-B, SNST and several memory scores.
Discussion: DTI identifies degeneration in both motor and extra-motor white matter tracts in ALS, with CST integrity being unrelated to extra-motor integrity (CC, UF). Motor integrity is associated to memory performance while extra-motor integrity is related to patients' both executive and memory cognitive profile.

06a. Imaging & Biomarkers: structural MRI

ADPD5-2289

SUBCORTICAL SHAPE SEPARATES ALZHEIMER'S DISEASE, FRONTO-TEMPORAL DEMENTIA AND HEALTHY ELDERLY SUBJECTS

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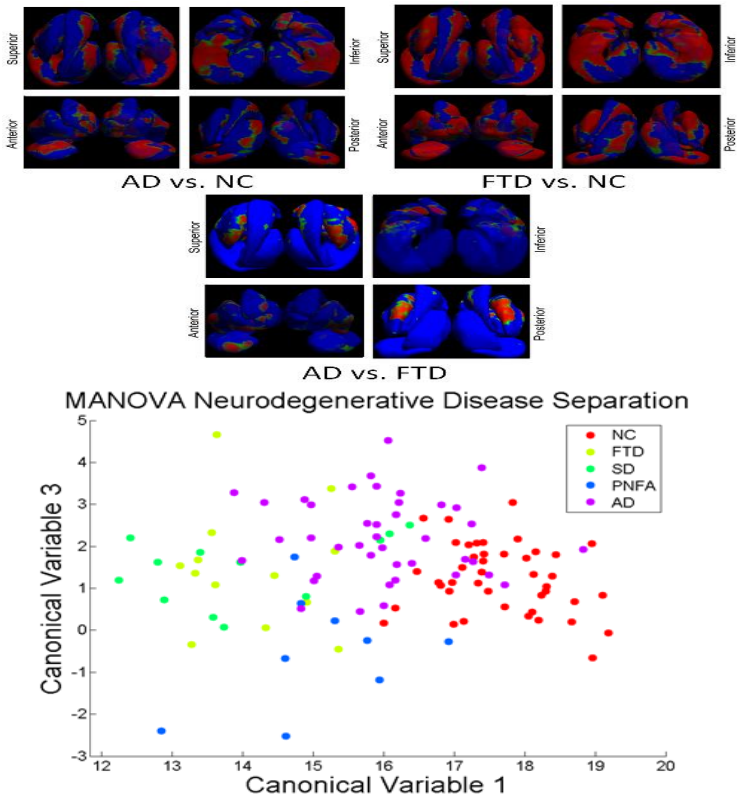
Data: 118 T1-weighted MR images of Alzheimer's (AD), Fronto-temporal dementia (FTD) patients and controls were parcellated with FreeSurfer. These structures were meshed, enforcing consistent topology: amygdala, hippocampus, pallidum, nucleus accumbens, thalamus, putamen, caudate.

Methods: Shapes were registered to a previously generated shape atlas, using the spherical fluid registration. Radial thickness (RDM) and surface Tensor-based Morphometry (TBM) were computed at homologous points. Mass-univariate group comparisons were performed: AD vs. NC, FTD vs. NC, AD vs. FTD, all corrected using False Discovery Rate (FDR). PCA & MANOVA were performed to distinguish among the three disease categories as well as the three FTD subtypes: behavioral variant (FTD); SD: semantic dementia; PNFA: progressive nonfluent aphasia (**Figure 2**).

Results: We created P-maps over the seven structures, thresholded at the FDR critical q: AD vs. NC q = 0.017, FTD vs. NC q = 0.03, AD vs. FTD q = 0.0072 (p-maps in **Figure 1**). Widespread atrophy is apparent throughout the basal ganglia, with stronger overall effect in the FTD cohort. The dorsal striatum differentiated the AD and FTD cohorts, in particular the behavioral variant and semantic dementia, subtypes characterized by social-emotional deficits.



06f. Imaging & Biomarkers: PET - glucose

ADPD5-0518

GLUCOSE METABOLISM IN EARLY ONSET VERSUS LATE ONSET BEHAVIORAL VARIANT FRONTOTEMPORAL DEMENTIA

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Backgrounds
: The aim of this study was to compare the overall glucose metabolism between early onset and late onset behavioral variant frontotemporal dementia (bvFTD) to investigate whether their metabolic deterioration was different even at the same dementia severity.
Methods
: Thirty one patients with early onset bvFTD (mean age: 61.6±4.9), 12 with late onset bvFTD (mean age: 75.3±5.3) and 65 healthy volunteers (mean age: 64.9±7.3) were recruited from the dementia clinics of 3 tertiary referral hospitals. Glucose hypometabolic patterns were evaluated by comparing patients with early onset bvFTD and late onset bvFTD with 65 healthy controls using voxel-based statistical parametric mapping.
Results
: There were no significant differences in Korean version of Mini-Mental State Examination, clinical dementia rating (CDR), and sum of boxes scores of FTD-CDR between early onset and late onset bvFTD patients. However, overall glucose hypometabolism of early onset bvFTD patients was much greater in magnitude and extent involving bifrontal and anterior temporal areas than that of late onset bvFTD patients.
Conclusions
: The results of greater hypometabolism in early onset than late onset patients with bvFTD even at the same severity of dementia were consistent with those of our previous research comparing glucose metabolism between patients with early onset and late onset Alzheimer's disease, reflecting greater functional reserve in younger than in older subjects.

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1335

CORTICAL THINNING AND WHITE MATTER TRACT DAMAGE IN RELATION TO COGNITION IN MOTOR NEURON DISEASES

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Objective. To assess the patterns of cortical thinning and white matter (WM) tract abnormalities in relation to cognition and behavioural symptoms in patients with motor neuron disease (MND).

Methods. 101 patients with motor neuron disease (MND) and 56 healthy subjects were studied. Patients were classified into MND with a pure motor syndrome (MND-motor) and those with cognitive/behavioural symptoms (MND-plus). A surface-based morphometry analysis was used to assess cortical thickness. Corticospinal tract (CST), corpus callosum (CC), and major association tracts diffusion tensor (DT) metrics were obtained. A Random Forest (RF) approach was used to identify the set of image features correlated with cognitive/behavioural deficits.

Results. There were 48 MND-motor and 53 MND-plus patients. Relative to controls, both patient groups showed cortical thinning of the bilateral precentral and postcentral gyri, cingulate cortex, inferior temporal and parietal areas. In all regions, there was a trend towards a more extensive involvement in MND-plus *vs* MND-motor. Both patient groups showed a damage of the motor CC fibers, but such a damage was greater in MND-plus cases. MND-plus patients also showed a severe involvement of the extra-motor WM tracts bilaterally. RF analysis showed that the best predictors of cognitive deficits and behavioural symptoms in MND patients were the DT MRI metrics of the frontotemporal tracts.

Conclusions. Cortical thinning and WM degeneration are highly dependent upon neuropsychological and behavioural symptoms in patients with MND. WM tract damage contributes to the severity of selective cognitive and behavioural manifestations more than cortical thinning.

Funding: Italian Ministry of Health (#RF-2010-2313220).

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1343

CONNECTED SPEECH PRODUCTION IN THE NONFLUENT VARIANT OF PRIMARY PROGRESSIVE APHASIA AND ITS RELATIONSHIP WITH WHITE MATTER DAMAGE

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Objective. To investigate the association between the different components of the connected speech and white matter (WM) damage in nonfluent variant of primary progressive aphasia (nfvPPA).
Methods. To assess the connected speech, we recorded speech samples from 11 nfvPPA patients while they described the image of the picnic picture subtest of the Western Aphasia Battery and analyzed them considering: lexical production rate and phonological/articulatory errors; pauses and repetitions; lexical typology; and syntactic structure. Diffusion tensor (DT) MRI metrics were obtained from the interhemispheric and major long association WM tracts.
Results. Speech samples in nfvPPA patients were characterized by slow rate, distortions, syntactic errors and reduced complexity of sentence production. The lexical production rate was positively related with the integrity of the left superior longitudinal (SLF) and inferior longitudinal (ILF) fasciculi and cingulum bilaterally; the Italian phoneme distortions were related with damage of the corticospinal tracts; the false starts were related with damage of the corpus callosum (CC); the lexical selection (such as the use of nouns, verbs, or prepositions) was related with DT MRI metrics of left SLF and CC genu; the syntactic complexity (i.e., mean length of utterance, ratio between number of words per sentence and number of sentences) and presence of morpho-syntactic errors were related with damage of the left SLF and ILF, and CC body.
Conclusions. We reported associations between particular aspects of connected speech and specific WM tract integrity in nfvPPA. This study underlines the relevant role of WM in nfvPPA.
Funding: GR#2010-2303035.

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1350

GREY AND WHITE MATTER MRI SIGNATURES OF THE FRONTOTEMPORAL LOBAR DEGENERATION CONTINUUM

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Objective. To identify overlapping and unique grey (GM) and white matter (WM) signatures within the frontotemporal lobar degeneration (FTLD) continuum, and discriminate likely FTLD-TAU and FTLD-TDP patients using structural and diffusion tensor (DT) MRI.

Methods. T1-weighted and DT MRI were collected from 121 subjects: 35 motor neuron disease (MND), 14 behavioral variant of frontotemporal dementia, 12 semantic and 11 nonfluent primary progressive aphasia, 21 progressive supranuclear palsy syndrome and 28 controls. GM atrophy was established using voxel-based morphometry. Tract-based spatial statistics was used to perform a WM voxel-wise analysis.

Results. All patient groups, with the exception of MND cases with a pure motor syndrome, shared a focal GM atrophy centered around the dorsolateral and medial frontal cortex and a largely overlapping pattern of WM damage involving the genu and body of the corpus callosum and ventral frontotemporal and dorsal frontoparietal WM pathways. Surrounding this common area, phenotype (symptom)-specific GM and WM regions of damage were found in each group. Patients with a high likelihood of an underlying FTLD-TAU had more severe WM damage relative to patients who are likely to harbor FTLD-TDP pathology, despite a similar pattern of GM atrophy.

Conclusions. In the FTLD spectrum, WM damage is more severe than GM atrophy. Frontal cortex and WM pathways represent the common target of neurodegeneration in these conditions. The topographic pattern of damage supports a 'prion-like' protein propagation through WM connections as underlying mechanism of the stereotyped progression of FTLD.

Funding: Italian Ministry of Health (#RF-2010-2313220, #GR-2010-2303035), CurePSP MD505-12_001.

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1359

DIFFERENTIATING THE SUBTYPES OF PRIMARY PROGRESSIVE APHASIA USING CORTICAL THICKNESS AND DT MRI MEASURES

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Objective. To test a multimodal MRI-based approach, comprising cortical thickness and white matter (WM) damage metrics, to discriminate the nonfluent (nfvPPA) and semantic (svPPA) variants of primary progressive aphasia (PPA).

Methods. T1-weighted and diffusion tensor (DT) MRI were obtained from 13 nfvPPA and 13 svPPA patients, and 23 controls. Cortical thickness and DT MRI indices from the long-associative and interhemispheric WM tracts were obtained. A random forest analysis was used to identify the image features associated with each clinical syndrome. Individual patient classification was performed using ROC analysis with cortical thickness, DT MRI, and a combination of the two modalities.

Results. Random forest analysis showed that the best markers to differentiate the two PPA variants at an individual patient level were diffusivity abnormalities of the left inferior longitudinal and uncinate fasciculi and cortical thickness measures of left temporal pole and inferior frontal gyrus. A combination of cortical thickness and DT MRI measures ('grey matter [GM]&WM' model) provided the best classification pattern (area under the curve 0.91, accuracy 0.89, sensitivity 0.92, specificity 0.85). Leave-one-out analysis validated these findings demonstrating that the 'GM&WM' model had an higher accuracy (0.86) compared with 'GM-only' (0.73) and 'WM-only' (0.69) models.

Conclusion. A combination of structural and DT MRI metrics provides a quantitative procedure to distinguish nfvPPA and svPPA at an individual patient level. The discrimination accuracies obtained are high enough to suggest that the 'GM&WM' model is potentially relevant for the differential diagnosis of PPA in the clinical practice.

Funding. Italian Ministry of Health (GR-2010-2303035).

06r. Imaging & Biomarkers: other

ADPD5-1125

QUANTITATIVE SUSCEPTIBILITY MAPPING (QSM) IN AMYOTROPHIC LATERAL SCLEROSIS

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OBJECTIVE

Determine whether there are alterations in metal content in ALS as measured with QSM.

METHODS

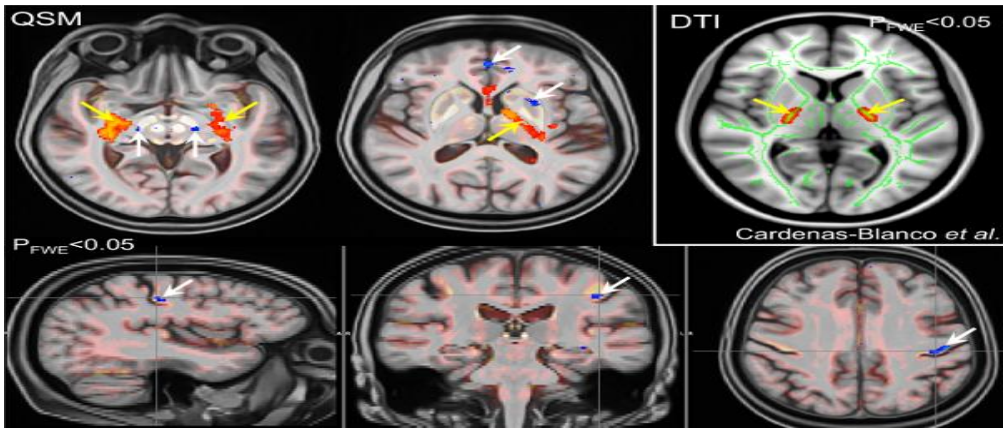
Twelve ALS patients (age=62±9 years) and thirty-four matched controls were analysed. All MRI measurements were performed in a Siemens Verio 3T scanner. T2* images were inspected to exclude cases with vascular pathology. Susceptibility weighted images—chiefly sensitive to the magnetic properties of metals—were reconstructed, post-processed and analysed using *state-of-the-art* quantitative methods.

RESULTS

Increased magnetic susceptibility (blue), suggesting metal accumulation, was found in the primary motor cortex as well as, putamen, substantial nigra and prefrontal areas. In addition, reduced magnetic susceptibility (orange) was identified in white matter including the cortico-spinal tract that co-localised with previously reported diffusion tensor abnormalities (Cardenas-Blanco et al. J Neurol in press).

CONCLUSIONS

QSM identified *in vivo* evidence for metal (most probably iron) misregulation in ALS. Notably the results suggest pathological accumulation in the primary motor cortex which could be contributing to a toxic insult; reduced susceptibility in white matter tracts suggests reduction of ferritin binding sites due to myelin/axonal loss. Increased susceptibility effects in the putamen, in contrast, appear to be common to a range of degenerative states, as we have previously observed them in Alzheimer's and in normal aging.



07c. Epidemiology, Risk Factors, Genetics & Epigenetics: metabolic

ADPD5-0522
THIAMINE DEFICIENCY – RELEVANT IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Objectives: By chance, we detected two patients with amyotrophic lateral sclerosis (ALS) who unexpectedly died because of central dysregulation traced back to acute Wernicke Encephalpathy (WE). Due to the two cases and their contingent cause of death, we were interested in prevalence of thiamine deficiency among ALS patients.
Methods: During two years, we examined laboratory markers of thiamine deficiency in 122 ALS inpatients without clinical symptoms of B1 deficiency to estimate prevalence. Here, transketolase and thiamine-pyrophosphat-effect (TPP-effect) were investigated in an external laboratory using EDTA-blood samples. Reference value was appointed to an increase of the TPP-effect >15% for slight and >20% for severe thiamine deficiency.
Results: We found a prevalence of B1 deficincy in 28% of ALS. Interestingly, none of the patients showed symptoms of WE. Contrary to our expectations, there was no different prevalence in the ALS-subgroups of bulbar versus spinal forms and no correlation between thiamine deficiency and severity of ALS symptoms, graduated by the ALS-functional rating score ALS-FRS-R.
Conclusions: ALS patients belong to the risk group for developing WE due to their dysphagia. In spite of absent typical WE-symptoms, prevalence of thiamine deficiency is 28% in our collective. Regarding the two patients who died due to WE and the prevalence we found for B1 deficiency, it is recommendable to pay attention to this possible fatal metabolic disease and to analyze thiamine levels in ALS patients at a low threshold.

07k. Epidemiology, Risk Factors, Genetics & Epigenetics: disease-causing mutations

ADPD5-0893

NGS RESEQUENCING USING A PANEL OF GENES ASSOCIATED WITH NEURODEGENERATIVE BRAIN DISEASE EXPLAINS 43% OF FAMILIAL FTLD PATIENTS

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Objectives: We aimed at determining the contribution of mutations in genes casually associated with neurodegenerative brain diseases (NBD) in a Belgian cohort of patients with frontotemporal lobar degeneration (FTLD).

Methods: To expedite the screening we designed a NBD gene panel for amplicon-based resequencing of all exons of 30 known genes. Targeted exons are amplified in multiplex PCR panels followed by massive parallel sequencing.

Results: In 423 FTLD patients we identified 146 rare protein-modifying variants. These included 50 variants that were definitely or probably pathogenic: 35GRN, 4MAPT, 5VCP, 1FUS, 2TARDBP, 1CHMP2B, 2PSEN1, and 4 possibly pathogenic: 2VCP, 2FUS. Together with C9orf72 repeat expansions, this explained 43% of familial FTLD patients and up to 62% of pathology confirmed cases. Interestingly, we detected 2 carriers with two mutations: one with a GRN together with a C9orf72 expansion mutation and another with a VCP and a C9orf72 expansion mutation. In our Belgian FTLD cohort the 2 most frequently mutated genes are GRN (8.3%) and C9orf72 (7.3%). Further, we identified novel missense mutations in APP (n=6), MAPT (n=13) and in LRRK2 (n=17) of which the impact on the FTLD phenotype is yet unclear.

Conclusion: The NBD gene panel considerably increased the speed and efficacy of mutation screening of causal genes, and will substantially improve molecular diagnostic testing in a medical setting. The assay is a highly accurate and cost-effective screening method offering a more complete genetic output. At this moment, exome sequencing cannot offer the same amount of coverage and fidelity for targeted gene screening.

08a. Animal Models: transgenic mice

ADPD5-2161

A NOVEL TDP-43 TRANSGENIC MOUSE MODEL OF FRONTOTEMPORAL LOBAR DEGENERATION AND AMYOTROPHIC LATERAL SCLEROSIS

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Objectives: To generate and characterise a novel neuronal-specific inducible TDP-43 transgenic mouse model with both neurodegeneration and behavioural deficits for the study of Frontotemporal Lobar Degeneration (FTLD) and Amyotrophic Lateral Sclerosis (ALS).
Methods: A novel mouse model was generated using the Tet-OFF Advanced system (ClonTech) driven by the neuronal-specific Thy1.2 promoter, to induce non-leaky expression of human TDP-43 with the A315T mutation. Neuronal expression of the mutant TDP-43 was confirmed with Western blot, degeneration was assessed by immunofluorescence, and mice were subjected to a battery of behavioural studies at 3 months of age.
Results: Western blot and immunofluroesence analysis confirmed expression of mutant TDP-43 in the cortex, hippocampus and, to a lesser degree, spinal cord and cerebellum of transgenic mice, which was absent in wild-type littermates. Transgenic mice showed considerable early onset degeneration of the cortex and hippocampus, and significant deficits in a number of behavioural tests compared to wild-type littermates: Morris Water Maze; Elevated Plus Maze; Rotarod; Pole Test. Interestingly, treatment of three-month-old transgenic mice with doxycycline (to suppress TDP-43 expression) reduced the behavioural deficits of the transgenic mice.
Conclusions: Our novel TDP-43 mouse model shows early onset of degeneration, behavioural deficits both in memory-based and motor-based tests, and decreased anxiety, similar symptoms to those experienced by FTLD and ALS patients. The alleviation of behavioural deficits in the absence of TDP-43 expression suggests that the presence of toxic TDP-43, as well as existing neurodegeneration, contribute to the neurodegenerative process underlying FTLD.

10d. Other: diagnostics

ADPD5-1670

INTEREST OF CSF BIOMARKER RATIOS IN THE DIAGNOSIS OF LATE ONSET BV-FRONTOTEMPORAL LOBAR DEGENERATION (BV-FTLD) VS ALZHEIMER'S DISEASE (AD)

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Background: Late-onset bv-FTLD (>65 ys) accounts for 3-18% of all bv-FTLD. New bv-FTLD clinico-radiological criteria show low sensibility for late-onset cases; AD is the main misdiagnosis. We investigated whether CSF biomarkers could help in diagnosing late-onset bv-FTLD.

Methods: a retrospective study (2007-2013) on clinically suspected amnestic AD or bv-FTLD with onset after 65 ys and no severe psychiatric or vascular burden. Patients were re-classified as AD or bv-FTLD according to the revised criteria (McKhann, 2011; Rascovsky, 2011); the CSF T-Tau/Abeta42 (>1.06) and P-Tau/Abeta42 (>0.2) cut-offs for diagnosis of MA *vs* FTLD (Bian, 2008; De Souza, 2011) were then applied.

Results: Fifty-seven patients were included. According to the international criteria diagnoses were AD (N=37; 65%), bv-FTLD (N=12; 21%), executive AD (N=4; 7%) or "neither AD nor bv-FTLD" (N=4; 7%). After applying MA *vs* FTLD CSF cut-offs the diagnoses were AD (N=22, 39%), FTLD (N=29, 51%) or undetermined (N=6, 10%). Most of the probable/possible AD with high/intermediate biomarker probability were confirmed as AD; all bv-FTLD were confirmed as bv-FTLD; some of the probable/possible AD with undetermined biomarkers had CSF cut off in the FTLD range or had conflicting CSF results; the undetermined cases had CSF cut off in the range of "non-AD".

Conclusions: Late onset bv-FTLD is possibly under diagnosed: CSF analysis, notably combined biomarkers and ratios, could suggest this diagnosis and differentiate it from AD. The actually proposed cut-off could possibly be further improved in relation to the population on study (late-onset disease) and the specificity of the differential diagnosis (bv-FTLD *vs* AD).

10f. Other: clinical trials

ADPD5-0219

DISEASE PROGRESSION MODEL TO CHARACTERIZE THE AMYOTROPHIC LATERAL SCLEROSIS TIME-COURSE USING THE ALSFRS RATING SCALE

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Objectives

To develop a longitudinal model describing Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) scores in ALS patients, to evaluate factors affecting disease progression, to detect presence of clusters in disease progression, to evaluate if data collected in an observational period (3 months) can predict disease deterioration at 12 months.

Methods

Patients (n=3936 with 29688 ALSFRS measurements) from PRO-ACT database (https://nctu.partners.org/ProACT/) were included in the analyses. A non-linear Weibull model described the ALS disease progression using 3 parameters: baseline ALSFRS, disease progression rate, rate of change in response. The presence of clusters in disease progression trajectories was investigated using a mixture modeling approach.

Results

Two clusters of disease deterioration trajectories were identified: slow progressors (56% of patients with 15% change from baseline); fast progressors (44% of patients with 46% change from baseline). Gender statistically affected disease progression rate: the woman progress ~10% faster than man. Logistic regression identified model predicted ALSFRS (change from baseline and value at week 3) as statistical significant predictors of slow/fast disease deterioration at 12 months ($p<0.0001$ and $p<0.005$, respectively).

Conclusions

The results confirmed previous analysis on ALSFRS-R scale [1] regarding: heterogeneity of measurements, presence of clusters in disease progression, and predictive power of measurements collected in an observational period on disease deterioration at 12 months. These findings indicate the interest of disease progression model for implementing population enrichment strategy to control the level of heterogeneity in patients included in a new trial.

[1] R. Gomeni R et al. *Amyotroph Lateral Scler Frontotemporal Degener. 2014;15(1-2):119-29*

ADPD5-0339

ASSOCIATION OF TAU WITH TIA-1 REGULATES STRESS GRANULE BIOLOGY AND TAU MISFOLDING

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Increasing evidence links neurological disease processes to dysfunctional n of neuronal RNA binding proteins (RBPs), RNA granules and stress granules (SGs). RBPs, such as T-cell intracellular antigen 1 (TIA1), contain prion-like, poly-glycine rich domains, which promote a process of protein aggregation that is normally physiological and reversible. Disease-linked mutations in RBPs increase the tendency of these proteins to aggregate, leading to formation of pathological SGs. Importantly, TIA1 and other SG proteins, co-localize with neuropathology in brain tissue of subjects with AD and ALS.

We now report a novel role for tau in regulating SG dynamics, and an equally novel ability of TIA-1 to induce misfolding of tau. Tau associates with TIA-1, a core nucleating RBP, promotes SG formation and reduces the movement of RNA granules containing TIA1, while deleting tau inhibits stress granule formation. TIA1 also regulates tau biology. Overexpressing TIA1 stimulates misfolding and phosphorylation of tau, formation of SGs that co-localize with insoluble tau. Live cell imaging indicates that TIA1 stabilizes granular tau and prolongs its half-life. Importantly, this system is modulated by translational signaling, being potentiated by puromycin or salubrinal, but inhibited by cycloheximide, which points to translational control as a novel tau regulatory pathway. The interaction of TIA1 with tau also stimulates neuronal degeneration, which is potentiated by oligomeric Ab. These results indicate tau contributes to the translational stress response, and raise the possibility that the association of tau with SGs contributes to the pathophysiology of tauopathies.

ADPD5-0705

SWEET NEUROBIOLOGY: THE ROLE OF ALTERATIONS IN PROTEIN GLYCOSYLATION IN ALZHEIMER'S DISEASE PATHOLOGY

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Human neurodegenerative diseases are associated with pathogenic oligomers and aggregates of misfolded proteins, eventually causing the progressive loss of neurons in the brain. One process which might be affected in neurodegenerative disorders is post-translational protein modification, known to be derailed during aging. Protein modifications such as phosphorylation, acetylation and glycosylation are critical to normal function of many proteins.

We focus on protein glycosylation. The majority of proteins synthesized in the rough ER undergo glycosylation, which is an enzyme-directed site-specific process. Protein glycosylation also occurs in the cytoplasm and nucleus as the O-GlcNAc modification, which, interestingly, competes with phosphorylation for the same amino acids.

We explore the role of glycosylaition in Alzheimer’s disease (AD), where several reports indicated vast alterations in protein glycosylation in AD.

Using an *in silico* approach we found that many genes encoding glycosylation-related enzymes exhibited different expression profile in brains of human AD patients as compared with healthy subjects. We next studied the effect of enhancing or reducing expression of the glycosylation-related genes in transgenic *Drosophila* over-expressing human tau which serve as an established AD model. We thus identifyed leading glycosylation enzymes, both augementing and ameliorating neurodegeneration in the *Drosophila* eyes which serve as a model. For example, overexpression of the *Drosophila* homolog of human RPN2 (involved in N-glycosylation) partially corrected eye neurodegeneration caused by over-expression of tau. We will next verify these effects of alterations in global protein glycosylation on AD pathology using human cultured cells over-expressing Aβ or tau as well as AD model mice.

ADPD5-0737

SWEET INHIBITION: TAU-DERIVED GLYCOPEPTIDES AS NOVEL INHIBITORS OF AMYLOID AGGREGATION

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Harmful amyloid oligomers and fibrils of certain disease-specific proteins are the hallmarks of various neurodegenerative diseases, e.g. Aβ and tau in Alzheimer's disease (AD). Inhibiting the misfolding and aggregation of these proteins is an attractive strategy for developing disease modifying therapeutics for these maladies.

In this study we rationally designed glycopeptides as novel inhibitors of tau aggregation. In this strategy the 'two-component' aggregation inhibitory molecules contain (i) the peptide backbone as an aromatic core that would target and bind the aromatic residues in the amyloidogenic monomer, thus confering target specificity, and (ii) sugar moieties that would provide steric hindrance component thus preventing aggregation of the target protein . Sugars were chosen as steric hindrance motif since sugar moieties on proteins have been shown to promote correct folding, and prevent aggregation, of the glycosylated proteins. Capitalizing on the specificity of peptides to bind target proteins we have synthesized various glycopeptides that target specifically the hexa-peptide core domain of tau (PFH6) and have shown their ability to inhibit its aggregation in vitro.

In the future we shall continue to study the tau-specific inhibitory effect of the novel glycopeptides and will assess their ability to disassemble per-formed PHF6 amyloid fibrils. In addition, we plan to feed the various inhibitory glycopeptides to available transgenic flies over expressing human tau, which serve as an established model of AD, and examine their ability to reduce tau aggregation in the treated flies and to ameliorate their AD-related symptoms.

ADPD5-0748

REGULATION OF TAU LOCALIZATION TO STRESS GRANULES

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Objectives: Stress granules (SG) are cytoplasmic messenger ribonucleoprotein particles (mRNP) that form when translation initiation needs to be inhibited, which typically occurs during a stress response. SGs contain RNA-binding proteins with self-aggregating properties, such as TIA-1, TTP and TDP-43. Recent evidence indicates that SGs colocalize with neuropathological Tau inclusions in Alzheimer's disease, and that TIA-1 and TTP bind to phosphorylated Tau. The objective of this study was to characterize the basic mechanisms underlying recruitment of Tau to SGs.
Methods: We have used protein-fragment complementation assay (PCA)-based Tau(0N4R) reporter dimers to follow subcellular localization of Tau derived from intracellular or extracellular sources in HEK293T cells. Arsenite was used as a positive control for induction of SG formation. SGs were revealed by TIA-1 immunostaining.
Results: In Tau overexpressing cells, Tau remains mostly cytosolic but it is strongly recruited to TIA-1-positive SGs upon arsenite treatment. The pseudohyperphosphorylated form of Tau (TauE14) displays partial TIA-1 colocalization also without arsenite treatment. Cells that do not express Tau but are exposed to Tau reporter dimer-containing conditioned media show intracellular Tau inclusions that colocalize with TIA-1, with and without arsenite treatment. Expression of TIA-1 shRNA in acceptor cells decreases the amount of Tau/TIA-1-positive SGs.
Conclusions: Cytosolic Tau can be recruited to stress granules upon stress or hyperphosphorylation. Internalized extracellular Tau dimers appear to escape from endocytic vesicles, followed by recruitment to the SGs. SGs may provide a novel therapeutic target in tauopathies.

ADPD5-0814

ASSESSING THE EFFECT OF A BRAIN-DERIVED TAU FRAGMENT ON MICROTUBULE BINDING AND TAU LOCALISATION IN CELLS

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Objectives

We have previously described a C-terminal tau fragment present in human tauopathy brain. Here we investigated the effects of this tau fragment (termed tau35) on microtubule binding and tau distribution in cells.

Method

Chinese hamster ovary (CHO) cells were transiently and stably transfected with plasmids expressing full-length (2N4R) human tau, tau35, or co-expressing full-length tau and tau35. Microtubule binding assays were used in these cells to compare the microtubule-binding ability of tau35 with that of full-length tau. The degree of microtubule bundling was also assessed using immunocytochemistry. Subcellular fractionation was used to isolate fractions containing either membrane-bound or cytosolic tau from the transfected cells.

Result

In stably transfected CHO cells, ~25% of 2N4R tau binds to microtubules, which reduces to ~15% when Tau40 and tau35 are expressed together. These results indicate a degree of competition by tau35, despite its reduced ability to bind to microtubules. Expression of full-length tau induces moderate bundling of microtubules in CHO cells. This bundling is strongly enhanced when tau35 is co-expressed with 2N4R tau, resulting in the formation of thick microtubule bundles under the plasma membrane and a smaller, more rounded, CHO cell morphology. Subcellular fractionation revealed that both 2N4R tau and tau35 associate with membranes in a similar proportion and this is unaffected by co-expression.

Conclusion

Our results suggest that the N-terminal half of tau is involved in tau-microtubule interactions. Tau35 associates with membranes similarly to 2N4R tau, although the distribution pattern of these two forms of tau may be altered.

ADPD5-0989

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF MISSING MOLECULES LINKING ABETA TO TAU

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It has not yet been clarified what are the molecular mechanisms and the causal relationship between Aβ aggregation, i.e. senile plaques, and the neurofibrillary tangles containing tau protein, both of which are pathological characteristics found in Alzheimer's disease (AD) brains. To identify the molecule(s) and the molecular mechanism(s) linking Aβ to tau, focusing on how Aβ induces tau aggregation, we have performed OMICS analysis of a newly generated AD model mouse, APP knock-in mouse (Saito *et al.*, *Nat. Neurosci.*, 2014). This is a mouse model free of APP overexpression and the induced pathologies are therefore associated with increased Aβ levels. In parallel, we performed a tau interactome analysis. This analysis was based on transgenic expression of tagged-tau in mice, which enabled the pull down of proteins interacting with tau *in vivo*. Several upregulated and downregulated proteins were identified by the two approaches. After *in vitro* validation of the targets, we have carried out an *in vivo* analysis of these proteins using adeno-associated virus system to overexpress the proteins in the mouse brains. In an alternative approach, transgenic mice of selected proteins were generated. Using these two systems, we have found one protein that is phosphorylated in an Aβ dependent manner, and which affects the phosphorylation status of tau. In addition, this protein is co-localized with phosphorylated tau in AD brains. We are currently analyzing the molecular mechanisms of this protein and how it induces tau phosphorylation and aggregation.

ADPD5-1054

DEVELOPMENT AND CHARACTERIZATION OF A GENE TRANSFER-BASED RAT MODEL OF TAUOPATHY

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With a prevalence of 40 million cases worldwide, Alzheimer's disease (AD) has become a major public health issue. Although the disease has been well characterized from an anatomo-pathological point of view, its causes remain elusive and an effective disease-modifying therapy is still awaited. The recent failure of several clinical trials suggests that orienting patients towards treatments at a prodromal stage would probably be more efficient. In this context, *in vivo* positon emission tomography (PET) imaging of neurofibrillary tangles (NFTs) would be of great use. Our aim is to generate a fast-developing model of tauopathy in the rat brain, independently of the beta-amyloid component of AD. This will help establish the biological substrate of current Tau-specific positron emission tomography (PET) probes and validate new ones. To that purpose, adeno-associated viral vectors (AAV) encoding the human Tau protein were injected in the hippocampus of adult rats. Overexpression of either the wild-type or the mutant protein induced Tau hyperphosphorylation and its abnormal misfolding, characteristics of early stages of tauopathy. In addition, NFT-like lesions were observed, as early as 2 months post-injection, both in animals expressing P301L mutant species and rats co-expressing a pro-aggregating peptide along with WT Tau. Further histochemical characterization of the model is ongoing and will be presented. This model, the first in rats to show fast aggregation of the wild-type form of Tau, will be useful to decipher the affinity of Tau tracers for different species of Tau fibrils.

ADPD5-1157

UBIQUITINATION-DEPENDENT AND -INDEPENDENT TAU CLEARANCE VIA P62/SQSTM1

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Objectives: For the clearance of tau proteins both the ubiquitin-proteasome system and autophagy are involved. Polyubiquitination of tau proteins would be an early and essential step for these processes. There is ubiquitin binding domains (UBA) within p62/SQSTM1 through which p62/SQSTM1 binds to tau proteins thereby transferring these into clearance machinery. To understand the exact underlying molecular mechanisms we performed this study.
Methods: Various mutant constructs of p62/SQSTM1 including p62ΔUBA, were established. Under the condition of ubiquitin overexpression the efficacy of wild type p62 and p62ΔUBA on tau clearance were evaluated.
Results: P62/SQSTM1 was demonstrated to enhance the clearance of tau proteins in neuronal cells, both total and phosphorylated form. The deletion of UBA within p62/SQSTM1 markedly diminished its efficacy on tau clearance, but not completely. Under the condition of ubiquitin overexpression, to some extent, the overall increased ubiquitination was linked to enhanced clearance of tau proteins. The remaining clearance of tau proteins even after deletion of ΔUBA constructs were thought to be mediated through ubiquitination-independent pathway. The direct physical interaction between tau proteins and p62ΔUBA were identified to be underlying mechanism.
Conclusions: We identified P62/SQSTM1 mediates the clearance of tau proteins. Both ubiquitination-dependent and –independent system are considered cooperatively mediating tau clearance.

ADPD5-1644

NEW METHOD BASED ON CAPILLARY ELECTROPHORESIS FOR IN VITRO EVALUATION OF PROTEIN TAU PHOSPHORYLATION BY GLYCOGEN SYNTHASE KINASE 3-B

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The hyperphosphorylation of tau protein is associated with the development of the neuronal pathology of Alzheimer's disease. Capillary electrophoresis (CE) has never been used to study tau phosphorylation.
This work aims to develop and optimize simple and reliable CE-based assays to evaluate tau phosphorylation by the human kinase, glycogen synthase kinase 3-β (GSK3β).
For this purpose, a novel pre-capillary CE assay was first developed. In-capillary CE-based enzymatic assay was also used since this approach is known to be time- and cost- effective. The enzymatic reaction was monitored by detecting and quantifying the product adenosine 5'- diphosphate (ADP). The influence of two classes of glycosaminoglycan (GAG), namely heparin and heparan sulfate, on reaction kinetics was also evaluated. Enzyme (GSK3β), substrates (tau and ATP) and GAG concentrations were optimized.
Results obtained by both CE approaches were comparable and were in excellent agreement with those reported in the literature using conventional radiometric and immunoblotting methods. In fact, CE results confirmed the inductory effect of the sulfated sugars heparin and heparan sulfate on tau hyperphosphorylation probably due to exposition of new sites phosphorylatable by GSK3β.
This study shows simple (no-labeling), rapid (less than 30 minutes *per* assay), economical (few tens of nanoliters) and eco-friendly (no-radioactivity) CE-based kinase assays allowing the comprehension of the abnormal phosphorylation of tau. It can be extended to the screening of different modulators of tau phosphorylation to highlight their function and to develop effective drugs for neurodegenerative disease treatments.

ADPD5-1711

IDENTIFICATION OF GENES AND SMALL MOLECULES THAT INTERFERE WITH TAU AGGREGATE FORMATION IN CELLULAR MODELS

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There is increasing evidence for prion-like transmissibility of disease-causing amyloidogenic proteins like Tau. With age formation of Tau inclusions has been shown to develop in a stereotypical manner in particular brain regions from where they appear to spread. Injection of brain extracts from transgenic mice with Tau pathology into the brain of human Tau transgenic mice induced the formation of Tau filaments and showed spreading of pathology from the sites of injection to neighboring brain regions. The transfer of aggregated intracellular Tau was also observed between co-cultured cells.

Objectives: The aim of this study was to build a platform of complementary cellular assays to study Tau aggregation and transmission screen for genes, pathways, or small molecules that interfere with transfer, build-up, or break-down of Tau aggregates. The assays are also used to study the role of Tau phosphorylation and the different Tau isoforms on the aggregation dynamics.

Methods: Cellular Tau aggregation assays were built based on imaging, AlpaLISA, BRET, and enzyme complementation both in HEK cells and in primary neurons. The cells were used to do genome-wide siRNA and shRNA screens and a compounds screen with around 500,000 compounds. The assays were also made with different Tau isoforms and mutations.

Results and conclusions: Several chemical series were identified that interfered with the Tau aggregation phenotype in the different cellular assays. Different genes were identified that play a role in Tau aggregation in the cellular context. Some of these genes have been implicated with AD before and will be explored further.

ADPD5-1858

ACTIVITY STIMULATES TAU SPREAD IN VIVO.

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Tau protein can transfer inter-neuronally and trans-synaptically between neurons in cell cultures and in mouse models, which is thought to explain the anatomical distribution and progressive spread of pathology observed in human Alzheimer's Disease. Moreover, hyperactivity has been suggested to drive disease progression in Alzheimer's disease. Here, we examined the hypothesis that increase neuronal activity drives taupoathie. Specifically, we used optogenetics to stimulate specific neural circuitry in vivo, recorded neuronal activity longitudinally, and demonstrated that increase neuronal activity can lead to exacerbated distribution of tau that is consistent with enhanced release and uptake of tau. Taken together, these data may explain the observation of focal tauopathy associated with epileptic seizures and there may be negative implications for stimulation therapies such as deep brain stimulation (DBS), or transcranial magnetic stimulation (TMS) that are currently in clinical trials in AD patients.

ADPD5-1859

MICRORNA-132/212 DELETION RECAPITULATES THE PATHOLOGICAL FEATURES OF TAUOPATHIES IN MICE

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Tauopathies are a class of neurodegenerative disorders characterized by the abnormal inclusions of Tau protein. Despite intensive research, the underlying mechanisms involved in tau metabolism dysregulation in vivo remain unclear. Recently, we and others have shown that the microRNA-132/212 cluster is strongly downregulated in tauopathy brains (e.g., Alzheimer's disease, progressive supranuclear palsy and frontotemporal dementia). In this study, we evaluated the effects of miR-132/212 deletion on tau expression, splicing, phosphorylation, and aggregation in mice. By Western blot analysis, we observed a significant increase in endogenous tau expression starting from P16 and until adulthood in miR-132/212 knockout (KO) mice. Using reporter assays and cell-based studies, we identified a functional miR-132 binding site within the tau 3' untranslated region, providing a mechanism for the abnormal regulation of tau expression. During development, 3R vs. 4R tau ratios are misregulated in the absence of miR-132/212, likely due to various mechanisms. Starting at 6 months of age, tau becomes hyperphosphorylated at several pathological epitopes (e.g., S422). At the age of 12 months, hyperphosphorylated tau was found in (sarkosyl) insoluble aggregates. Notably, similar results were obtained in miR-132/212 KO mice expressing human Tau P301L. Both biochemical and electronic microscopy analyses showed that miR-132/212 deficiency induced age-dependent changes in autophagy, as seen in tauopathy patients, which could explain in part the observed effects on tau aggregation. Collectively, these results provide strong evidence that loss of miR-132/212 function in the brain could contribute significantly to tau neuropathology in a large subset of neurodegenerative disorders.

ADPD5-1977

SELF-ASSEMBLED CYCLIC D,L-ALPHA-PEPTIDES AS CONFORMATIONAL INHIBITORS OF TAU AGGREGATION

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Alzheimer's disease is characterized by accumulation of misfolded amyloid-β (Aβ) peptides and hyperphosphorylated tau (ptau) proteins in the brain that are observed as amyloid plaques and neurofibrillary tangles, respectively**(1)**. Recent studies have shown that pathogenic amyloids share common structural features despite being composed of different proteins and amino acids, which may be responsible for their cross-interaction**(2)**. We have recently reported on the discovery of a novel self-assembled cyclic D,L-α-peptides that bind Ab, inhibit its aggregation and reduce its toxicity to rat pheochromocytoma PC12 cells by stabilizing the less-toxic form of the Ab**(3)**. We also demonstrated that the active cyclic peptides are recognized by the polyclonal antibodies that bind prefibrillar assemblies of amyloids, suggesting the immense structural similarity between the self-assembled cyclic D,L-α-peptide and the amyloids.

In this study, we further demonstrate that the active cyclic D,L-α-peptide (**CP-2)** can also bind and inhibit the aggregation of a tau-derived peptide, Ac-VQIVYK-NH_2 (AcPHF6)**(4)** that aggregates through β-sheet interactions to form toxic species similar to those formed by tau protein. Moreover, circular dichroism spectroscopy studies and cell toxicity assays suggested that upon co-incubation, **CP-2** changes the aggregation pathway of AcPHF6 to a "off-pathway" mechanism and protects PC12 cells from AcPHF6-induced toxicity, without exhibiting any toxicity by itself.

We also found that CP-2 is effective in reducing the Ab seeds – induced aggregation and toxicity of AcPHF6. Finally, co-localization studies clearly indicate that AcPHF6 and CP-2 can penetrate cells via similar mechanism through the endosome/lysosome compartment.

ADPD5-2081

NOVEL SANDWICH-ELISA FOR DETECTION OF AGGREGATED TAU PROTEIN

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Objectives: (a) development of an ELISA measuring concentrations of aggregated tau protein in tissue and cultured cells of different species, (b) characterization of tau oligomer and tau filament stability using the ELISA, (c) application of the ELISA to quantify tau aggregation during aging of tau transgenic P301L mice.

Methods: Monoclonal tau antibodies were generated by immunization with pseudo-phosphorylated tau fragments. Tau antibodies were screened for tau specificity using brain tissue of tau knockout mice. Usefulness of this ELISA is demonstrated by the characterization of the aggregate stability of tau oligomers and tau filaments after treatment with deaggregating reagents. The emergence of aggregated tau species in P301L tau-transgenic mice is quantified during development.

Results: The epitope of detection/capture antibody resides in the C-terminal portion of tau, which is conserved across species. The detection limit of the Sandwich-ELISA using tau oligomers and filaments is 100pg/ml. The detection of tau filaments is strongly compromised by pretreatment with 6M guanidinium and incubation at 134°C. Tau oligomers are more susceptible and can be destroyed by 1% mercaptoethanol. Tau aggregates in P301L mice are present at day of birth and increased 30-fold to postnatal day 240.

Conclusions: (a) the Sandwich-ELISA revealed a good specificity for aggregated tau protein with high sensitivity; (b) its application to tau aggregate stability revealed the different properties of tau oligomers versus tau filaments; (c) the detection of aggregated tau species in the presence of monomeric tau was exemplified in the developing brain of P301L tau transgenic mice.

01k. Protein Misfolding & Aggregation: clearance of misfolded proteins

ADPD5-0948

TARGETING THE LYSOSOME TO REDUCE TAU PATHOLOGY

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Objectives
In Alzheimer's disease, tau hyperphosphorylation leads to accumulation of soluble tau and formation of toxic oligomers, which ultimately results in aggregation. Tau oligomers can be degraded by the autophagy/ lysosomal pathway (ALP), which comprises a multi-step process starting with the formation of autophagosomes, followed by fusion with lysosomes to become autolysosomes and ultimately degradation of autophagic substrates. In the present study we are investigating how modulation of the ALP function can be employed to reduce tau phosphorylation and aggregation.
Methods
Hela cells and cortical neurons were transfected or transduced with tau constructs to study tau pathology. The ALP was modulated by using specific chemical and generic tools targeting the respective step of the pathway. Tau pathology was determined by immunocytochemistry and Western blotting to assess total tau and phospho-tau levels. A bimolecular complementation system was employed to visualize tau oligomerization in living cells and fluorescence was analyzed by confocal microscopy.
Results
In our cell models, we found that phosphorylated and aggregated tau accumulates upon lysosomal inhibition using Bafilomycin indicating that these tau species are degraded by the lysosome. Next, we are investigating the effect of ALP inhibition at different steps on tau phosphorylation and aggregation to determine potential targets for intervention. Currently, we are establishing different ALP activators targeting the autophagy initiation complex (Tat-Beclin) and lysosomal proteases (Cathepsin D and B).
Conclusions
Specific activation of the ALP may be a promising strategy to reduce tau in the pathology.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-0484

SIGMA-1 RECEPTOR REGULATES TAU PHOSPHORYLATION AND AXON EXTENSION BY SHAPING P35 TURNOVER VIA MYRISTIC ACID

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Dysregulation of cyclin-dependent kinase5 (cdk5) through its activator p35 has long been implicated in the pathophysiology of neurodegenerative disease because it causes abnormal hyperphosphorylation of cytoskeletal proteins such as tau and neurofilament, which ultimately lead to the emergence of neurofibrillary tangles. P35 has a short half-life and undergoes rapid proteasomal degradation; however, neurotoxic insults promote p35 conversion to a longer half-life p25 that is no longer anchored to the PM, allowing atypical phosphorylation by cdk5. Studies have revealed that brains of Alzheimer's and Parkinson's patients are deficient in sigma-1 receptor (Sig-1R) expression or exhibit *SIGMAR1* mutations in patients with motor neuron diseases; however, the specific role of the Sig-1R in neurodegeneration is still unclear. Here we examined the role of endoplasmic reticulum (ER) chaperone Sig-1Rs in the regulation of p35. We found that Sig-1R KO neurons exhibit greater basal levels of p35 expression and that this increase is likely due to a decreased degradation rate of p35 but not dysregulated calpain activity. Sig-1R KO neurons also display shorter axonal length and decreased cortical axon density. Furthermore, we show that myristic acid competes with the Sig-1R agonist (+)-pentazocine for Sig-1R binding. Treatment with myristate mitigated the p35 protein level changes, restored axonal elongation and diminished PHF-1 in the Sig-1R KO neurons. Taken together, these findings indicate that Sig-1Rs mediate the myristoylation of p35, thereby regulating the degradation of p35, which, in turn modulates cdk5 activity. This property of the Sig-1R appears important to the structural and functional maintenance of the axon.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-0494

BIPHASIC CHANGES IN CEREBROSPINAL FLUID LEVELS OF TAU IN TG4510 MICE

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Objectives: Increased Tau pathology is a major hallmark in Alzheimer's disease and tauopathies. However, it is unclear how the increase in Tau pathology might relate to the accumulation of CSF Tau, and what other factors may influence CSF Tau. To investigate this, we utilized the forebrain-inducible Tg4510 mice and conducted a longitudinal study to fine map the changes in Tau pathology, brain atrophy and CSF Tau.

Methods: Tg4510 mice at various ages were analyzed for Tau pathology and brain atrophy by ELISA and immunohistochemistry. CSF was collected from the same animals and subjected to ELISA to measure Tau and pT181 Tau.

Results: In the younger mice of 14 to 18 weeks of age, we observed a strong correlation between CSF levels of Tau and brain levels of insoluble Tau, but not total Tau. We also observed a strong correlation between levels of CSF Tau and CSF pT181 Tau. Surprisingly, in mice of older age (19 and 20 weeks), even though their insoluble Tau levels nearly doubled in a weekly basis, their CSF Tau levels reached plateau and did not increase further. Notably, this coincided with brain atrophy, which became significant from 20 weeks of age.

Conclusions: Our data demonstrated biphasic changes in CSF levels of Tau in the Tg4510 mice, where the initial increase in CSF Tau may attribute to increased Tau pathology while the subsequent plateau of CSF Tau in older mice may reflect a reduced Tau release from atrophied neurons.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-0632

TYPE 1 DIABETES-LIKE INDUCES TAU DEPENDENT COGNITIVE DEFICITS

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Introduction: The etiology of Alzheimer disease (AD) is complex and multi-factorial. Further compounding this problem is the fact that AD patients often suffer from a variety of additional co-morbidities. One of the more prominent co-morbid conditions is diabetes, and epidemiological studies reveal that diabetes patients have a significant risk of developing AD compared to healthy individuals, suggesting a strong association between this medical co-morbidity and AD. However, the underlying molecular mechanisms connecting these two disorders are still not well understood. Here, we seek to determine whether tau is a key molecular factor for insulin-dependent type 1 diabetes (T1D) to impair cognitive and synaptic function.
Methods: To properly address this question, we use a combination of newly developed genetically-modified models with biochemical, histological and behavioral approaches to elucidate the underlying molecular mechanism by which T1D-like disease promote tau pathology and impair cognitive and synaptic function.
Results: Our study shows that T1D-like disease induces cognitive impairment via tau dependent mechanisms, and its dysregulation causes reduction in synaptic proteins levels and cognitive decline. Concomitantly, we demonstrate the novel finding using tau null mice, that reduction of endogenous tau mitigates behavioral and synaptic impairments induced in T1D-like mice.
Conclusions: Therefore, our data reveal that tau is a key molecular factor necessary for T1D-like disease to induce cognitive decline, and represents a potential therapeutic target for diabetes and AD patients.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-0961

ABNORMAL PLASMA MEMBRANE BLEBBING AND ACTIN CYTOSKELETON REMODELING ARE PRODUCED BY THE EXPRESSION OF TAU PROTEIN IN GLIAL CELLS

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Abnormal aggregation of tau protein in glial cells has been reported in some tauopathies; however, the pathological meaning of these nonfibrillary aggregates still remains unsolved. In this study we evaluated whether full-length tau protein (Tau441) and its acid aspartic^{421}-truncated variant (Tau421) produce alterations in the normal organization of the cytoskeleton and plasma membrane (PM) when expressed in cultured C6 glioma cells. Glial cells were transiently transfected with plasmids (pcDNA3.1Zeo(-)) containing the sequence for either Tau441 or Tau421. After transfection, morphological changes and alteration in the normal organization of the cytoskeleton and PM were evaluated by multilabeling-immunofluorescence and confocal microscopy. By 48 hours after transfection, abnormal microtubule bundling was observed in most of the cells that either expressed Tau441 or Tau421. Moreover, both variants of tau produced extensive PM blebbing associated with cortical redistribution of the actin cytoskeleton (actin-F). These effects were reverted when tau-expressing cells were incubated with drugs that depolymerize the actin cytoskeleton. In addition, when glial cells showing tau-induced PM blebbing were incubated with inhibitors of the Rho-GTPase-Rho-associated kinase (ROCK) signaling pathway, both the formation of abnormal membrane blebs and the actin-F remodeling were avoided. These results may represent a new mechanism of tau toxicity, in which tau-induced microtubule bundling produces activation of the Rho-GTPase-ROCK pathway mediating the remodeling of cortical actin and MP blebbing. In the disease, this tau-induced blebbing of the PM may occur and contribute to the impairment of glial activity.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-0995

CASPASE-2-GENERATED TAU FRAGMENT IMPAIRS MEMORY FUNCTION IN TAUOPATHIES

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Objectives
Neurofibrillary tangles are composed of aggregated forms of tau, a microtubule-associated protein. They correlate well with neuron loss and brain dysfunction in Alzheimer's disease and other tauopathies. However, our previous study found that neurofibrillary tangles contribute very little to cognitive dysfunction in rTg4510 mice expressing tau P301L mutant. It was the aim of this study to find tau*, the culprit responsible for memory decline in the absence of neurodegeneration or neurofibrillary tangles.
Methods
Total tau proteins purified from the brains of rTg4510 mice are subjected to mass spectrometry for tau* identification. In vitro tau* is generated by caspase-2 cleavage assay. Miniature excitatory postsynaptic current is recorded to assess synaptic function. Antisense oligonucleotides is administered to suppress caspase-2 expression in the brains of rTg4510 mice. The memory function is measured by Morris water maze. We measured brain tau* levels by western blot assay.
Results
Here, we identified a 35kDa tau cleavage product as tau*. We found that tau* is abundantly present in the brains of rTg4510 mice and its levels correlate well with memory dysfunction. Tau* is generated by caspase-2-mediated cleavage of tau protein at Asp314. It mislocalizes to dendritic spines, where it disrupts synaptic function by impairing glutamate receptor trafficking. After the suppression of endogenous caspase-2 expression in the brains of young rTg4510 mice, tau* levels decreased and memory function recovered.
Conclusions
Thus, these data demonstrate that caspase-2-generated tau* impairs memory function independently of neurodegeneration or neurofibrillary tangles, which suggests that caspase-2 is a target for developing new therapeutics.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-1072

DISTRIBUTION OF ENDOGENOUS AND EXOGENOUS TAU IN THE MOUSE BRAIN

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Tau, a microtubule-associated protein, makes up neurofibrillary tangles (NFTs) in Alzheimer's disease (AD) and related tauopathies. In normal neurons, tau binds and stabilizes axonal microtubules, and confined to axons. In AD, tau is hyperphosphorylated, accumulate in neuronal somata and dendrites, and form NFTs. tau has been extensively studied in the cell biology, but it remains to be clarified why endogenous tau localizes to axonal microtubules. In order to investigate the distribution of tau, we explored the immunohistochemical technique, which can make possible to distinguish between the endogenous and exogenous (human) tau in the brain tissues. First, we examined the distribution of endogenous tau in the hippocampus of wild-type mouse. Tau is exclusively localized to the axonal compartment but barely in dendrites or neuronal cell bodies. Endogenous tau is clustered in presumably presynaptic portions but never found in dendritic spines. Next, we compared the distributions of endogenous and exogenous (human) tau in the brains of tau-transgenic (tau-Tg) mice that developed tau deposition resembling human tauopathy and tau-knock-in (tau-KI) mice that never led to tauopathy. In tau-Tg mice, exogenous tau localizes in dendrites and cell somata in a sharp contrast to endogenous tau. Unexpectedly, exogenous tau in tau-KI mice had the normal distribution. This may suggest that the missorting of exogenous tau is involved in the NFT formation in tau-Tg mice.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-1169

HYPOTHERMIA-INDUCED HYPERPHOSPHORYLATION – A CELLULAR MODEL TO STUDY TAU KINASE INHIBITORS

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Objectives

Tau hyperphosphorylation is a known hallmark of Alzheimer's disease (AD). Hence, developing and screening novel kinase inhibitors are in a focus of current AD research. To find promising drug candidates, inducible cellular models of Tau hyperphosphorylation are useful tools for studying central nervous system drug effects.

Methods

SH-SY5Y cells (SH) or SH cells overexpressing the longest isoform of human Tau 441 carrying two well-characterized mutations (V337M/R406W) were treated with several kinase inhibitors and were either kept under normo- or hypothermic conditions to induce Tau hyperphosphorylation. Afterwards, total Tau and its phosphorylated species pSer262, pSer202, pSer396 and pThr231 were analyzed in cellular lysates.

Results

We are able to show a decrease of temperature triggered hyperphosphorylation of different Tau phosphorylation sites in SH- and SH-Tau cells. Interestingly, these effects were more prominent in SH-Tau than in SH cells, indicating that the presence of mutated human Tau increases the therapeutic window. In contrast to unaltered total Tau and pThr231´Tau, pSer396 and pSer262 Tau levels were significantly enhanced in SH and more pronounced in SH-Tau cells under hypothermic conditions. Kinase inhibitors such as LiCl were able to attenuate the hypothermia-induced hyperphosphorylation, making the model suitable for screenings of novel kinase inhibitors.

Conclusions

Hypothermia induces Tau hyperphosphorylation in SH and SH-Tau cells, an effect that can be inhibited by kinase inhibitors. Since the phosphorylation was more prominent in SH-Tau cells, this *in vitro* model serves as valuable, fast, cost-effective and high-throughput screening tool for studying novel kinase inhibitors.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-1229

FUNCTIONAL VALIDATION OF BIN1 AS AN ALZHEIMER`S DISEASE RISK FACTOR

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Recent genome-wide association studies (GWAS) have revealed numerous Alzheimer`s Disease (AD) susceptibility loci. Many of these candidate genes such as PICALM, Bin1 and CD2AP are implicated in endocytosis and cell membrane-associated processes. However, the functional connections of the risk factors with AD mechanisms are still poorly understood. To validate potential risk factors, we have established a variety of *Drosophila* models, which mimic different aspects of AD pathogenesis, namely the accumulation and aggregation of Amyloid-beta (A-beta) peptides, the endocytosis and cleavage of the Amyloid Precursor Protein (APP) and the hyperphosphorylation and aggregation of tau. These models can be utilized to screen for pathway modulators employing RNAi and phenotypic readouts.

Using this approach we identified Amphiphysin, the *Drosophila* homologue of Bin1, as a specific modulator of tau-induced neurotoxicity. Amphiphysin binds lipid membranes and induces membrane curvature. However, its actual role in the adult brain remains elusive and little is known how Bin1 could contribute to the pathogenesis of AD. Knockdown of Amphiphysin aggravates phenotypes produced by the overexpression of tau in the fly and simultaneously increases tau level. These changes could not be detected in A-beta expressing flies. Interestingly, expression of human tau in both, the fly brain and neuronal cell culture, upregulates endogenous Amphiphysin, indicating that Amphiphysin has a novel role in alleviating aggregate stress or facilitating tau clearance. Currently we investigate the mechanism of how Amphiphysin might influence tau aggregation and degradation. This identification could shed light on novel pathological mechanisms and be a potential therapeutic target.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-1290

DOWN-REGULATION OF MAPT MEDIATED VIA RAAV SIRNA IN EXPERIMENTAL MODEL OF TAUOPATHY

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MAPT (Microtubule-Associated Protein Tau) is one of the principal components of neuron cytoskeletal system. It has been shown previously that differential tau isoform expression and phosphorylation regulates the assembly and stabilization of microtubules in fetal and adult neurons and participates on transport of proteinous or vesicular cargos along the microtubule. MAPT deficient animals do not show overt phenotype. However deficiency in tau protein induced in adult neurons could have serious consequences. We have therefore set to prepare siRNA for down-regulation of tau protein in terminally differentiated neurons transduced with rAAV viral vectors. We have designed and experimentally tested several small interfering (si) RNAs targeting different regions MAPT transcripts. The siRNAs were expressed from pSilencer2.1 vector under the control U6 promoter. The silencing efficiency of several *MAPT*-silencing constructs (siRNA1-siRNA7) was validated by transfection into HEK293 cell line, transiently expressing a His-tagged cDNA of *MAPT* gene. The level of tau protein down-regulation has been verified by Western blotting. The siRNA most efficiently inhibiting expression of MAPT was subsequently inserted into rAAV vector and used to produce viral particles to transduce rat primary neurons in culture. We have prepared the experimental tool for investigation of the role of endogenous tau in specific brain regions of adult rat and tool for testing the hypothesis whether or not the reduction of endogenous tau protein level could lead to alleviation of neurofibrillary degeneration seen in animal model of tauopathy.

This work was supported by grants: APVV-0677-12, VEGA 2/0194/14, VEGA 2/0161/13 and grant from Axon Neuroscience.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-1467

STRUCTURAL STUDY OF TAU PROTEIN FOCUSED ON ITS C-TERMINAL DOMAIN

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Objectives: Microtubule-associated protein tau plays a key role in the pathogenesis of AD and other tauopathies. As it was shown that C-terminal domain of tau protein inhibits its assembly into insoluble filaments, it is important to gain insights into the structural features of tau C-terminus.

Methods: 30 amino acid long tau peptide derived from tau C-terminus was co-crystallized with the Fab fragment of monoclonal antibody. The diffraction data were collected and the structure was solved by molecular replacement.

Results: The Fab fragment of monoclonal antibody DC39C with epitope in the C-terminal domain of tau protein was crystallized alone and in complex with tau peptide. Both crystallization and co-crystallization trials produced crystals amenable for diffraction data collection and structure solution.

Conclusions: Insights into the atomic structure of physiological and pathological tau conformations, as the structure of C-terminal tau peptide, may help to unravel the unanswered questions of tauopathy pathogenesis.

Supported by: APVV-0677-12

02b. Cell, Molecular & Systems Biology: tau

ADPD5-1506
LOSS OF TAU PROGRESSIVELY PRECIPITATES SCIATIC NERVE MORPHOFUNCTIONAL DEFICITS AND MOTOR IMPAIRMENT

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Objectives: Dementia is the cardinal feature of Alzheimer's disease (AD) but the clinical symptoms of this disorder also include a marked loss of motor function. While Tau abnormal hyperphosphorylation and malfunction are well-established key events in AD neuropathology, there is still an on-going debate on the impact of loss of normal Tau function in neuronal degeneration and subsequent behavioral deficits. Previous studies showed that animals lacking Tau (Tau-KO) exhibit motor deficits with controversial findings about motor-related CNS dopaminergic defect raising uncertainty on the underlying mechanisms. Interestingly, little attention has been devoted to a putative involvement of PNS primary afferents as the primary compartment of motor circuitry.
Methods: This study uses a battery of behavioral tests analyzing motor function in both young (4-6 months) and old (17-22 months) Tau-KO mice combined with a systematic morphofunctional analysis of their sciatic nerve.
Results: Herein, we provide evidence that the age-dependent motor impairment in Tau-KO animals is paralleled by ultrastructural and functional impairments of efferent fibers conveying motor-related information. Specifically, the sciatic nerves of old (17-22-months) Tau-KO mice showed increased degenerating myelinated fibers and diminished conduction properties in comparison to age-matched wild-type littermates and adult (4-6 months) Tau-KO and WTs. In addition, the sciatic nerves of Tau-KO mice exhibit a progressive hypomyelination (assessed by g-ratio) specifically affecting large-diameter, motor-related axons in old animals.
Conclusions: These findings suggest that loss of Tau protein may progressively impact on peripheral motor system adding to our understanding of peripheral neurological deficits in AD and other Tauopathies.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-1568

THE PRESENCE OF TAU PATHOLOGY AFFECTS THE ADAPTIVE IMMUNE RESPONSE

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Objectives:

The adaptive immune system and particularly lymphocytes, in addition to preventing and combating infections, are important in regulating brain functions like learning and memory. Importantly, Alzheimer's disease (AD) is associated with a decline in the effectiveness of lymphocytes. This work investigates the relationship between tau pathology, a pathological hallmark of AD and adaptive immunity.

Methods:

We have used a transgenic mouse that overexpresses human mutated (P301S) tau protein under the control of a neuronal-restricted Thy1.2 promoter. In this transgenic mouse, progressive tau pathology develops in several areas of the nervous system. The function of lymphocytes isolated from these mice and the *in vivo* response of lymphocytes to experimental autoimmune encephalomyelitis (EAE) were studied.

Results:

Naïve P301S mice showed a significant reduction of serum level of cytokines such as IFNgamma, TNFalpha, IL-2, and IL-10, normally associated with lymphocytic functions, compared to naïve wild-type mice. Lymphocytes isolated from naïve P301S mice released significantly less IFNgamma after stimulation with dynabeads and *Concanavalin* A. P301S mice showed a strong resistance to EAE induction with a delayed onset and a reduced magnitude of clinical symptoms. Consistently we found a significant reduction of demyelination, axonal degeneration, and microglia/macrophage activation in the spinal cord of treated P301S mice compared to treated wild-type mice.

Conclusions:

This work shows that the presence of pathological tau in the CNS affects adaptive immunity, which may explain the reduced lymphocytic functions in AD patients that could contribute to the cognitive decline.

02b. Cell, Molecular & Systems Biology: tau

ADPD5-1613
ERK/MAPK IS NOT A BONA FIDE TAU KINASE
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Alzheimer disease is characterized by the deposition of intracellular aggregates of hyperphosphorylated Tau protein. Tau hyperphosphorylation has been attributed in part to the deregulation of kinases and phosphatases activities. ERK1/2 was reported to be activated in the first stages of AD and was proposed as potential therapeutic target. However, while the phosphorylation of Tau by ERK1/2 has been demonstrated in cell-free system, it remains controversial *in vivo*. Here, we showed that pharmacological inhibition of ERK1/2 in mice and SH-SY5Y cells did not reduce basal levels of phospho-Tau or hypothermia-induced Tau hyperphosphorylation. We also found that activating ERK1/2 by hyperthermia did not correlate with increased Tau phosphorylation. Finally, ERK1/2 was inhibited but Tau phosphorylation was not altered by *Mek1* deletion *in vivo*. In conclusion, these results do not support the involvement of ERK1/2 in Tau phosphorylation under physiological conditions.

ADPD5-2006

POST-TRANSLATIONAL MODIFICATION(S) OF TAU PROTEIN IN APP KNOCK-IN MICE

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Objectives: Single locus amyloid precursor protein knock-in (*App*-KI) mouse models developed in our laboratory show Aβ amyloidosis without overproduction of APP and non-Aβ fragments in an age-dependent manner (Saito *et al.*, Nat Neurosci, 2014). We expect the models to be applicable to identifying neuropathological mechanism(s) for treatment of Alzheimer's disease (AD) because of essentially no artificial phenotypes compared to conventional *APP*-transgenic models. In this study, we aim to elucidate the mechanistic processes that link Aβ amyloidosis and tauopathy using *App*-KI mice.

Methods: We crossbred *App*-KI mice with Wtau-Tg mice (Kimura *et al.*, EMBO J, 2007), which express wild-type human 2N4R tau under the *Camk2a* promoter (termed App×Tau mice). Because the Wtau-Tg mice do not carry FTDP-17-causing mutations, we consider the double mutant mice as more relevant models of AD.

Results: The Wtau-Tg mice have been shown to exhibit hyperphopshorylated tau but not to form neurofibrillary tangles (NFTs). The 24-month-old App×Tau mice did not exhibit NFTs despite robust Aβ pathology. Yet, the aged App×Tau mice showed greater tau hyperphosphorylation than single Wtau-Tg mice.

Conclusions: Possibly, we need more time than 24 months to reconstitute tauopathy in the model mice because it takes approximately a decade for Aβ amyloidosis to induce tauopathy in humans. We are currently investigating other post-translational modifications of tau based on MS analysis and will present the latest results obtained.

02q. Cell, Molecular & Systems Biology: protein degradation, proteasome & autophagy

ADPD5-1066

HISTONE DEACETYLASE INHIBITORS DECREASE P35 EXPRESSION THROUGH PROTEASOME PATHWAY.

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Alzheimer's disease (AD) is a neurodegenerative disorder arising from amyloid beta depositions and neurofibrillary tangles formation within the neurons with ultimate impairment on memory function. Histone deacetylase (HDAC) inhibitors have been reported to restore learning and memory in AD model mouse. Here, we investigated the molecular mechanism through which HDAC inhibitors made the therapeutic effects on taupathy using primary neuronal cells and SH-SY5Y neruoblastoma cells. We observed that the treatment of HDAC inhibitors, such as FK-228, valproic acid, and trichostatin A, decreased p35 expression and subsequent tau phosphorylation in SH-SY5Y cells and mice primary cortical neuronal cells with a dose-dependent manner. However, PCR analysis revealed there was no alteration in p35 mRNA expression under HDAC inhibitors treatment. MG-132 and lactacystin, the proteasome inhibitors, restored the HDAC inhibitor-induced downregulation of p35 expression. Furthermore, HDAC inhibitors increased mRNA and protein expression of SKP1A E3 ubiquitin ligase. Moreover, SKP1A siRNA recovered the HDAC inhibitor-induced downregulation of p35 expression. This study demonstrates that the overexpression of SKP1A by HDAC inhibitors might induce p35 degradation through proteasome activation.

ADPD5-1195

MOLECULAR CHARACTERISATION OF BRIDGING INTEGRATOR 1 (BIN1) INTERACTION WITH TAU

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BIN1 locus is identified as the second genetic determinant for Alzheimer's disease (AD). Bin1 involvement in the mechanism of the pathogenesis is unknown. We previously showed that BIN1 might modulate AD pathology through the Tau pathway.

To understand the functional consequences of the Bin1/Tau interaction, the present study investigates at the molecular level the details of this interaction by using GST-pull down and High resolution Nuclear Magnetic Resonance (NMR) spectroscopy approaches.

GST pull down assay were performed with GST fusion proteins of Tau or Tau domains (2N, PRD and 4R) and Bin1 and Bin1 SH3 domain. Immobilized GST fusion proteins were incubated with cell lysates overexpressing Bin1 iso1 or Tau 2N4R. Pull-downed proteins were analysed by immunodetection.

^{1}H, ^{15}N 2D NMR spectra were acquired at 900MHz on ^{15}N Tau 2N4R or ^{15}N Tau[165-245] fragment mixed with Bin1 SH3 and reversely ^{15}N Bin1 SH3 mixed with Tau and Tau[165-245]. Interaction mapping was performed based on the chemical shift perturbations observed in these 2D spectra due to the interaction.

GST pull down assays show that Bin 1 interacts with the proline rich domain of Tau (PRD, 165- 245), and reversely Tau with the Bin1 SH3 domain. Molecular details of the Bin1 SH3/Tau interaction were obtained by NMR spectroscopy that allow to define the peptide recognize by the Bin1 SH3 within the PRD.

Here, we report that BIN1 SH3 domain interacts with the PRD of Tau and mediates Bin1 interaction with Tau. The study of the regulation of this interaction is underway.

02u. Cell, Molecular & Systems Biology: network biology

ADPD5-2064

AGE-ASSOCIATED ALTERATIONS IN GLUTAMATERGIC SIGNALING IN TAUP301L MICE

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Objectives. We previously demonstrated that rTg(TauP301L)4510 mice exhibit an increase in glutamate release and a decrease in glutamate uptake in the hippocampus prior, and that these alterations in glutamatergic signaling correlate with cognitive deficits. Because hippocampal hyperexcitability is also observed in cognitively impaired aged humans, we hypothesized that similar durations of P301L tau expression would produce exacerbated glutamate dysregulation and cognitive deficits in “aged” compared to “young” TauP301L mice.

Methods. We compared the effects of 5 months of P301L tau expression when mice were “young” (2-7M: tau expression ON) versus “aged” (15-20M: tau expression ON). To examine glutamate regulation *in vivo*, we used amperometry coupled to ceramic-based microelectrode arrays (MEAs). Hippocampal glutamate regulation was correlated with performance in the hippocampus-dependent Morris water maze and radial arm water maze.

Results. In young TauP301L mice, we observed glutamate dysregulation, which correlated with cognitive deficits, as previously reported by our lab. Despite similar durations of tau expression, in aged TauP301L mice we observed exacerbated cognitive deficits and glutamate dysregulation. We also observed age-related cognitive decline and glutamate dysregulation in aged transgene negative littermate controls.

Conclusions. When put into context of recent findings that presynaptic glutamate release is sufficient to drive tau release into the extracellular space (PMCID: PMC3949564), our findings of increased glutamate release in TauP301L mice suggests glutamate-mediated exocytosis of tau may be one mechanism for the trans-synaptic spread of tau pathology associated with synaptic activity and that hippocampal hyperactivity might be permissive for the development of AD.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-0298

CHARACTERIZATION OF TRANS-CELLULAR TAU PROPAGATION USING A DROSOPHILA NEURONAL CELL CULTURE SYSTEM

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One major hallmark of Alzheimer's disease (AD) is the hyperphosporylation and subsequent aggregation of the microtubule-associated tau protein. Interestingly, different tau species have recently been detected in the cerebrospinal fluid (CSF) of brains from AD patients and it has been proposed to trans-cellularly propagate, seeding further aggregation of naive tau molecules. Therefore, a major challenge in our understanding of tau abnormalities is to clarify the localization of aggregated tau, the interaction of aggregated with soluble tau within the cell as well as with neighboring cells and the toxic effects associated with this process.

We report the establishment of a *Drosophila* neuronal cell culture system to analyze the aggregation properties of different human tau variants. By expressing these phospho-tau variants, we can correlate the phosphorylation status with aggregation and toxicity, by employing cell culture and *in vivo Drosophila* models. Moreover, we demonstrate the appearance of human tau protein in the culture medium when it is expressed in *Drosophila* neuronal cells, similarly as observed in mammalian cell culture. The extracellular tau is mainly found as free soluble protein and is not vesicle associated. Additionally, tau molecules are detected in neighboring non-transfected cells, implicating a trans-cellular trafficking process. Our current work targets the question of whether tau secretion is dependent on the aggregation state as well as the identification and characterization of factors playing key roles in tau secretion and propagation. These answers will provide new insights in understanding the pathogenesis and the progression of the disease pathology.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-0320

INVESTIGATIONS ON THE ROLE OF DIFFERENTIAL AT8-PHOSPHORYLATION IN TAU-CONTAINING EXTRACELLULAR VESICLES ISOLATED FROM BRAINS OF RTG4510 TRANSGENIC MICE

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BACKGROUND
TAU aggregates induce intracellular misfolding and aggregation of endogenous TAU in recipient cells (Clavaguera, 2009; Frost, 2009, Guo, 2011). In Alzheimer's Disease (AD), TAU aggregates may potentially spread from neuron to neuron via extracellular vesicles (EVs), such as exosomes (30-130nm) or microvesicles (200-1000nm). TAU has been found associated with exosomes isolated from cerebrospinal fluid of AD patients (Saman, 2012). Here we present the properties of TAU-containing EVs isolated from mouse brain tissue.
METHODS
EVs were isolated from the brain extracellular space (Perez-Gonzalez, 2012) of rTg4510 mice expressing P301L TAU (Santacruz, 2005). EVs were analysed using Western blotting, electron microscopy, and the qNano (IZON). Standard methods were used for cell-culture experiments.
RESULTS
To determine a putative role in AD pathogenesis, we isolated exosomes (precipitated at 100,000g) and larger EVs (10,000g). Both types of EVs contain TAU. Surface protein shaving assays revealed that TAU is intravesicular and protected by membranes. Interestingly, TAU was found differentially phosphorylated in exosomes. Exosomes were positive for AT180, AT270, pS262 and pS422, but negative for AT8-phosphorylated TAU. Conversely, larger EVs showed strong positivity for AT8. We will present the downstream effects of the uptake of EVs by hTAU-expressing cells.
CONCLUSIONS
We detected more phosphorylated TAU in larger EVs which might indicate that the size of TAU aggregates is more compatible with larger EVs. We speculate on the possible relevance of AT8 in seeding TAU aggregation and consider it important to investigate whether the pathogenicity of TAU in larger EVs (AT8+) differs from that in exosomes (AT8-).

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-0583

TIME-DEPENDENT CHANGES IN THE SEEDING PROPERTIES OF TAU FROM TRANSGENIC P301S MICE

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Objectives
Neurofibrilliary tangles display a progressive and characteristic distribution pattern in the AD brain consistent with a 'prion-like' spread of pathology. Misfolded tau protein has the ability to act as a nucleating template causing soluble tau to form insoluble aggregates that transmit between neurones. The tau species that initiates propagation is unknown, as is the time of pathological onset when this species is produced and becomes disease-inducing. These studies aimed to identify the time-dependent changes in tau species and characterise the tau that is responsible for the seeding phenomenon in the TgP301S mouse model.

Methods
Tau species were separated from a time-course of young to old TgP301S mouse brain homogenates using sucrose gradients. Different species were identified by non-denaturing gel electrophoresis and Western blot using antibodies for total and post-translationally modified tau. Fractions were evaluated for seeding ability in a cell-based model of tau propagation.

Results
Intracellular tau aggregation was only observed when seeded with fractions containing aggregate-derived, hyperphosphorylated tau. The amount of this pathological tau species increased over time in TgP301S mouse brain, as did its seeding efficiency. Fractions from young TgP301S mice, devoid of aggregated, AT8-positive tau did not seed in the cell-based model.

Conclusions
The present study shows that there is a time-dependent change in the seeding properties of tau in the TgP301S mouse model. The increase in aggregated, hyperphosphorylated tau species results in greater seeding propensity and tau propagation. Interestingly, the small oligomeric tau species in these mice were not seed-competent.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-0663

PATTERNS OF INVOLVEMENT OF THE VISUAL SYSTEM OF TAU AND ALPHA-SYNUCLEIN PATHOLOGY: RELEVANCE FOR SPREADING AND IN VIVO DIAGNOSTICS

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Objectives: Spreading of misfolded proteins has been suggested for neurodegenerative diseases. The hierarchical distribution of protein deposits in Alzheimer`s (AD) and Parkinson`s disease (PD) supports this concept. However, the anatomical regions involved in these disorders have diverse anatomical connections. **Methods:** We evaluated the optic pathway as an excellent anatomical model, which follows a strict trajectory. We immunostained the optic nerve, lateral geniculate nucleus (LGN), and occipital cortex for AT8 (phosphorylated tau) and alpha-synuclein in AD, tauopathy, and PD cases. **Results:** Immunoreactivity for both proteins along the optic pathway was detected: the optic nerve showed immunopositivity in the majority of cases with tau (57%) or alpha-synuclein (50%) pathology. In addition, a significant number showed aggregates in the LGN (tau: 46%, alpha-synuclein: 93%). We observed a significant correlation between tau pathology in the LGN and occipital cortex and Braak stages in cases with AD-related changes. In tauopathies, which do not involve the occipital cortex, like argyrophilic grain disease or progressive supranuclear palsy, tau pathology was mainly represented by astrocytes restricted to the LGN. In PD cases, LGN alpha-synuclein pathology correlated with involvement of the optic nerve while positivity in the occipital cortex was present in 5 out of 14 cases. **Conclusion:** Our results support the concept of disease-spreading along neural pathways and have implications for the diagnostic evaluation of the visual system in neurodegenerative proteinopathies.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-0714

MODULATION OF CELL-TO-CELL PROPAGATION OF TAU DIMERS BY LATE-ONSET ALZHEIMER'S DISEASE RISK GENES

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Objectives: Prion-like cell-to-cell propagation of misfolded proteins in common neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease, is becoming increasingly evident. Although advances in late-onset AD genetics have identified a large number of new disease-associated genes and pathways, functional characterization of these genetic susceptibility factors is clearly lagging behind. Particularly the connection of GWAS-identified risk genes to AD pathogenesis remains poorly understood. As many of the LOAD risk genes are mechanistically associated with endocytosis and vesicular trafficking, we hypothesized that some LOAD risk genes may have a role in transcellular propagation of Tau oligomers.
Methods: We have developed a novel live-cell reporter system based on protein-fragment complementation assay (PCA), using split humanized *Gaussia princeps* luciferase, to study cellular release and uptake of Tau dimers. The Tau release/uptake PCA assays were combined with an RNAi screen in HEK293T cells to study functional connections between the top 12 LOAD risk genes and cellular release and uptake of Tau dimers.
Results: Surprisingly, *APOE* RNAi showed the strongest effect on cellular uptake of Tau dimers. Knockdown of several genes, including *TREM2*, had a significant effect on cellular release of Tau dimers. Mechanistic studies further suggested that endocytic recycling pathways may play an important role in transcellulur propagation of Tau.
Conclusions: Late-onset AD risk genes may be functionally connected to cellular pathways involved in cell-to-cell propagation of Tau dimers/oligomers, and thus modulate the progression of the pathology.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-0930

NEURON-TO-NEURON PROPAGATION OF TAU AGGREGATES

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Tauopathies are a diverse group of neurodegenerative diseases defined by accumulation of misfolded cytosolic aggregates of microtubule associated protein Tau. AD is the most common tauopathy. Recent studies have strongly suggested that neuron-to-neuron propagation of aggregated Tau is involved in disease progression. To understand intraneuronal propagation, the mechanisms behind aggregates movement towards potential sites for transfer, exit and entry at the recipient neuron must be studied. We want to study the potential role of the synaptic connection in neuron-to-neuron propagation of Tau aggregates. Recently it was shown that uptake of recombinant Tau fibrils triggered aggregation of overexpressed Tau in mouse primary neurons. We expanded this model into a microfluidic culture device, which is comprised of three somal compartments connected by a series of microgrooves separating three distinct neuronal populations. Tau aggregates propagation was analyzed between the three distinct compartments. We observed that after triggering Tau aggregation in neurons in a first channel, Tau pathology was propagated into synaptically connected neurons in adjacent channels. This suggests that transsynaptic intraneuronal Tau transmission occurred. Our experimental set-up models the typical Tau pathology spreading patterns observed in AD human brains. Using this tool, we are aiming to further study the role of different synaptic proteins in Tau transsynaptic transmission.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1786

MIMICKING ALZHEIMER'S DISEASE SPREADING VIA AN INTEGRATED MICROFLUIDICS-MEA DEVICE IN VITRO

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1. Objectives

The objectives are to model AD propagation *in vitro* and to provide a both morphological and electrophysiological observation platform to study AD spreading.

2. Methods

Using microtechnology, a Microfluidics-MEA device was designed and fabricated. It is a device combining a compartmentalized PDMS microfluidic system and a micro-electrode array (MEA) recording system. (Fig.1)

After fabrication, the device was sterilized and primary cortical neurons were plated. After 21 DIVs, Tau-hyperphosphorylation was induced via Okadaic acid (OA) to build a healthy-diseased culture model. Immunocytochemistry staining and MEA recording were applied to observe AD spreading

3. Results

The integrated Microfluidics-MEA device was successful in both morphological observation and electrophysiological recording.

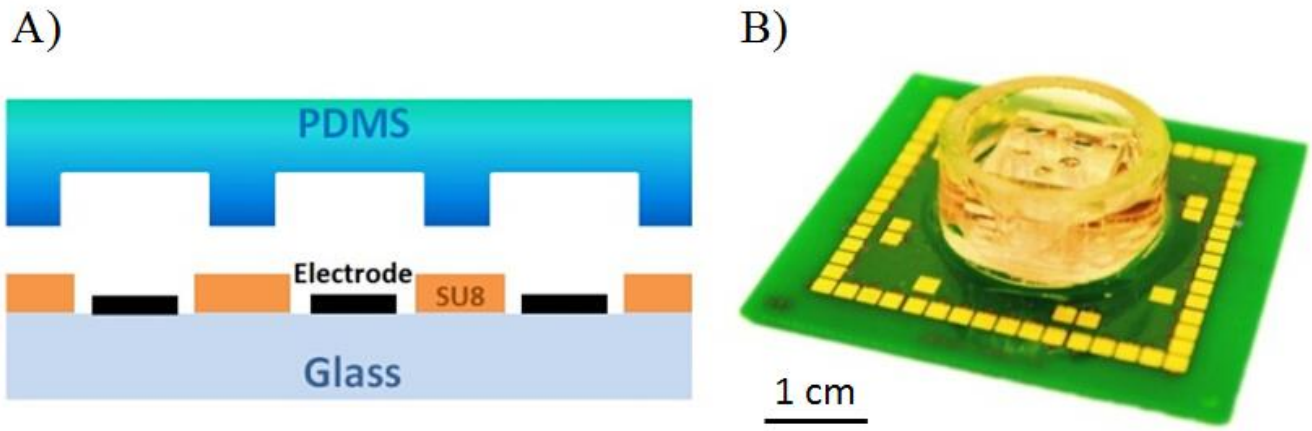

Figure 1: A) Schematic representation of the device's cross-section. B) Microfluidics-MEA device.

Inside the device, the conditions allow the soma and axon to physically connect while growing in spatially separated regions. Healthy (Fig.2, Region 3) and diseased (Fig.2, Region 1) populations were obtained, connected with axons only (Fig.2, Region 2).

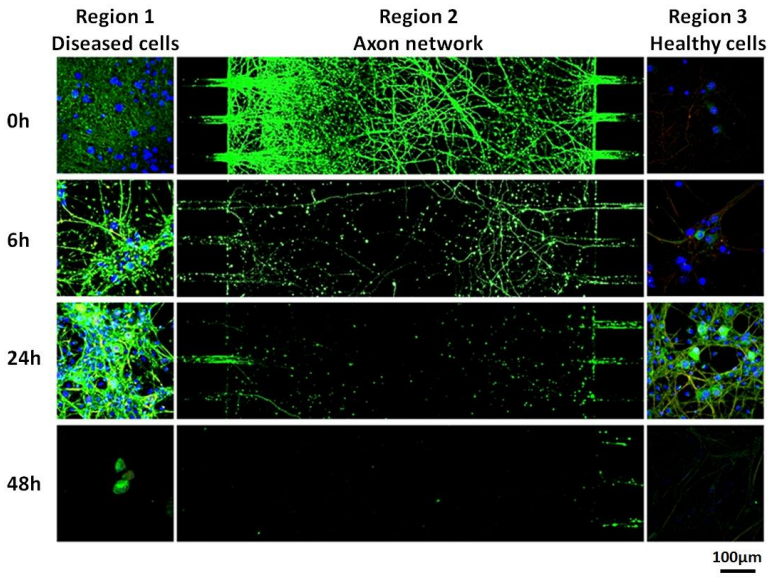

Figure 2. Disease spreading. (after 600 nM OA treatment for 75 min). Staining: hp-tau (pS262, green), nucleus (DAPI , blue)

The electrophysiological signals from the soma and axon were recorded separately and analyzed.

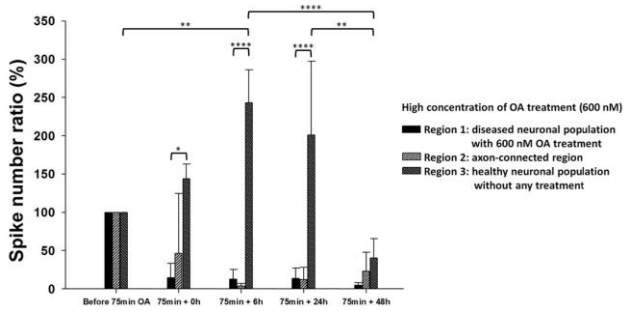

Figure 3. Spike number ratio acquired through recording. (N = 5, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.005$).

4. Conclusions

We reconstituted the structural and functional patterns of AD propagation by mimicking hp-Tau spreading in our Microfluidics-MEA device. The diseased population was directly affected by OA, causing morphological and electrophysiological effects on the healthy population through axon-only connections.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1999

FEASIBILITY OF PERIPHERAL TAU DETECTION TO DETERMINE BRAAK NEUROFIBRILLARY TANGLE STAGE

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Objectives: To determine the presence of tau in human peripheral tissues and its associated with Braak neurofibrillary tangle (NFT) stage.
Methods: Cases were chosen from a previous publication that contained phosphorylated tau deposits through the extent of their spinal cords (N=18; 16 Alzheimer's disease and 2 non-demented cases). From these cases, five peripheral postmortem human tissues samples (sigmoid colon, scalp, abdominal skin, liver, and submandibular gland) were subject to Western blot and ELISA assays for total tau species. Frontal gray matter was used as a frame of reference. In the peripheral area containing the highest total tau levels, a second confirmatory set of tissues having a variety of Braak NFT stages was examined (N=36; 12 Braak 0-II, 14 Braak III-IV, 10 Braak V-VI).
Results: The brain had the highest total tau levels, with 7889±2742ng/mg (average± standard deviation) of total protein, followed by the submandibular gland (120±25.9ng/mg), sigmoid colon (22±9.0ng/mg), abdominal skin (21±16.1ng/mg), scalp (14±5.7ng/mg), and liver (13±4.6ng/mg). Additional submandibular glands, having a variety of Braak NFT stages, showed a significant inverse association between Braak NFT stage and total tau levels (rho= - 0.55, P=0.002).
Conclusions: These results demonstrate that tau is present in measurable quantities within peripheral tissues but not to the levels that are detected within the brain. Of potential importance, there is the strong and significant inverse correlation between submandibular gland tau levels and Braak NFT stage. This study gives a glimpse into the etiopathogenesis of Alzheimer's disease from a peripheral perspective.

03b.Pathophysiology & Disease Mechanisms: prion-like mechanisms

ADPD5-1006

A FRET FLOW CYTOMETRY ASSAY DETECTS PROTEOPATHIC SEEDING

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Proteopathic seeding may underlie the pathogenesis of tauopathies such as Alzheimer's disease. This seeding activity is characterized by the ability of protein aggregates, or seeds, to template their conformations onto monomeric substrates, thereby amplifying the misfolded state. Via transcellular propagation, proteopathic seeds spread through the nervous system and may drive disease pathogenesis. To date, few methods can detect seeding activity in biospecimens with great specificity, sensitivity, and rapidity and thus the role of tau seeding in disease progression remains untested. We engineered an ultrasensitive, specific, and facile FRET-based flow cytometry biosensor assay based on expression of tau or synuclein fusions to CFP and YFP. These monoclonal biosensor cells specifically detect tau or synuclein seeding activity at femtomolar and picomolar levels, respectively. Due to seeding barriers, these biosensor cells are insensitive to cross seeding by heterologous amyloids and may thus serve as an ideal assay to measure specific proteinopathies from biospecimens. Our seeding assay readily detects tau seeding activity from brain lysates of the P301S tau transgenic mouse, an animal model of human tauopathy. Importantly, this brain-derived seeding activity is specific to tau, since tau immunodepletion abolishes activity. This seeding assay will permit investigations detailing the co-evolution of proteopathic seeding activity and disease progression.

03b.Pathophysiology & Disease Mechanisms: prion-like mechanisms

ADPD5-1263

TEMPLATED MISFOLDING OF TAU BY PRION-LIKE SEEDING ALONG NEURONAL CIRCUITS IMPAIRS SYNAPTIC AND COGNITIVE FUNCTION IN TAU TRANSGENIC MICE

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Prion-like seeding and propagation of Tau-pathology have been demonstrated in in vitro and in vivo models and may underlie the stereotyped progression of neurodegenerative Tauopathies. However, the involvement of templated misfolding of Tau in neuronal network dysfunction remains to be explored in detail. We have analyzed the repercussions of prion-like spreading of Tau-pathology via neuronal connections on synaptic and cognitive function in TauP301S transgenic mice.

We have recapitulated prion-like induction of Tau-aggregation by synthetic pre-aggregated Tau fibrils (PFFs) in primary neuronal cultures and organotypic cultures derived from TauP301S mice. We recapitulated prion-like seeding by PFFs in vivo in mice along functional neuronal connections. Injection of PFFs in entorhinal cortex resulted in spreading to different brain regions including hippocampal and isocortical brain regions, reminiscent for spreading of Tau-pathology in Alzheimer's Disease. In contrast, injection of PFFs in substantia nigra induced spreading of Tau-pathology to striatum, thalamus, brain stem, motor cortex and frontal cortex affecting distinct intrinsic functional connectivity networks. We performed calcium imaging analysis and electrophysiological analysis in Tau-seeded primary neurons and organotypic cultures and in acute hippocampal slices from PFF injected mice. This demonstrated aberrant synaptic and neuronal network function induced by templated misfolding of Tau, by synthetic Tau-seeds. Furthermore, templated Tau aggregation impaired cognitive function, reflected in impaired performance in the object recognition test 6 months post-injection.

Hence, our data demonstrate that templated misfolding of Tau by Tau seeds is sufficient to affect unique neuronal networks and contributes to synaptic and cognitive dysfunction in a model recapitulating spreading of Tau pathology.

03b.Pathophysiology & Disease Mechanisms: prion-like mechanisms

ADPD5-1387

GENERATION OF A TAU PRION STRAIN LIBRARY REVEALS VAST STRUCTURAL PLASTICITY IN THE TAU AMYLOID FOLD

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Neurodegenerative tauopathies are defined by the deposition of tau amyloids (neurofibrillary tangles). Prion-like propagation of aggregation likely contributes to their stereotyped progression along discrete neural networks, which may have important implications for the phenotypic diversity seen in this group of diseases. Previously, we showed that two unique tau conformations (strains 9 and 10) are stably propagated through living systems, maintaining their properties through months of cell division and upon passage through multiple generations of transgenic mice. However, the degree to which tau is capable of forming distinct conformations has been largely unexplored. In the present work, we have created a tau prion strain library using tau aggregates derived from diverse recombinant, transgenic mouse, and patient sources as inoculates. We utilized our previously established monoclonal HEK293 cell line stably expressing a tau repeat domain-YFP fusion protein to produce more than 15 distinct tau prion strains as assessed by inclusion morphology, limited proteolysis, and seeding propensity. In preliminary studies, we find that several of these strains induce distinct pathologies when inoculated into transgenic tau P301S mice. Based on these findings, we conclude that like prion protein, tau is capable of forming structurally diverse amyloid conformations that stably propagate themselves with high fidelity in living systems. Furthermore, the current resource may prove useful for examining many fundamental questions underlying strain biology in both cellular and animal models of tauopathy.

03b.Pathophysiology & Disease Mechanisms: prion-like mechanisms

ADPD5-1420

PROTEOPATHIC SEEDING ACTIVITY IS A PROXIMAL MARKER OF TAUOPATHY

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Transcellular propagation of toxic tau aggregates, or seeds, may underlie the stereotypic spread of pathology in Alzheimer's disease and related tauopathies. However, the critical role of tau seeding activity in disease progression remains untested. If proteopathic seeds are a causal agent in AD, we hypothesize that seeding activity is detectable in biological samples, precedes other pathological markers, and correlates with disease progression. To address these questions, we developed an ultra-sensitive and highly-quantitative FRET flow cytometry cell-based assay to measure seeding activity from brain homogenates. The assay detects robust seeding activity from AD brains, but not Huntington's or age-matched-control brains, demonstrating its utility in discriminating tauopathy vs. non-tauopathy human specimens. To address the potential causal role of seeding activity, we used P301S tauopathy mice to carry out a detailed time-course study. Using the same cohort of mice, we compared seeding activity from one hemisphere to several common histological markers of tau from the other hemisphere. All stains reliably identified tau pathology, although the age of onest was variable: Conformationally-abbarrent tau (MC1) was detected at 3 months; hyperphosphorylated tau (AT8 and PG5) was detected at 6 months; beta-sheet-rich amyloids (ThioflavinS) were detected at 9 months. Seeding activity, however, was consistently measured in four different brain regions at just 1.5 months, far preceding onset of other pathological markers. Seeding activity steadily increased with age and correlated well with all histological stains. Thus, the proximal appearance and progressive accumulation of seeding activity is consistent with a causal role of tau seeds in disease progression.

03b.Pathophysiology & Disease Mechanisms: prion-like mechanisms

ADPD5-1498

A NOVEL FIXED TISSUE SEEDING ASSAY DEMONSTRATES AGGREGATE CONFORMATION CAN AFFECT THE RATE OF SPREAD OF TAU PATHOLOGY

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Tauopathies are a group of diseases characterized by progressive neurodegeneration and the deposition of misfolded tau. We previously showed tau can form unique prion strains that induce distinct histopathology in a mouse tauopathy model. However, it is not known whether misfolded tau shares other important characteristics with prion protein. In this work, we have found misfolded tau derived from fixed tissue is stable and capable of seeding aggregation of naïve tau protein. To test the effect of fixation on the conformation of misfolded tau, we introduced material from fixed brain slices of P301S mice inoculated with our distinct tau strains (C9 or C10) into our original tau cell line (C1). This produced secondary strains with aggregates that have the same conformation as the original inoculum, verifying the stability of tau aggregate conformation after fixation. We next developed a novel technique to quantify the degree of tau pathology in fixed tissue using our FRET-based flow cytometry biosensor assay. This allowed us to quantify the rate of spread of tau pathology after inoculation with C9 or C10 into the hippocampus of P301S mice using isolated brain regions from fixed tissue slices. We found that C9 spread to the contralateral hippocampus more rapidly than C10, suggesting conformation may affect the rate of spread of tau pathology. Furthermore, our fixed tissue biosensor assay showed positivity in the contralateral hippocampus prior to the appearance of overt tau histopathology in that region, suggesting this technique may be more sensitive than traditional histopathology techniques.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0500

SYNAPTIC COMPENSATION FOLLOWING REFERENCE MEMORY IMPAIRMENT IN YOUNG ADULT TRIPLE TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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In Alzheimer's disease (AD) cognitive decline takes place over a period of several years with often a long period of mild cognitive impairment (MCI) before development of dementia probably due to synaptic loss which is interrupted with a transient compensatory increase during MCI. The (3xTg-AD) transgenic mice (hAPPSw, P301L tau, PS1 (M146V) knockin) show amyloid beta plaques and neurofibrillary pathology starting at around the age of 9-12 months. We studied the spatial reference memory of 12 week old 3xTg-AD and control mice using Morris Water Maze task, and neuronal and synaptic markers immunohistochemically in 10, 12, 13, 14, 15 and 16 weeks old animals. The 3xtg-AD mice showed impairment in reference memory accompanied by synaptic loss and reduced levels of b-III tubulin and MAP2 at 12 weeks of age. The cross-sectional analysis of the 6 different age groups showed a compensatory increase in synaptic markers relative to that in WT animals in a topographic and time manner in different regions of the brain. The WT animals were also subject to changes in the expression of the above mentioned markers. However, when studied longitudinally we found that in 3xtg-AD mice the compensatory phenomenon occurred in all the regions of the brain parallelly. These findings for the first time raise the intriguing possibility that AD causing mutated transgenes may initially cause an increase in synaptic markers as a compensatory mechanism for synaptic loss and this increase which though is transient could be the biological basis of long period of MCI.

03c. Pathophysiology & Disease Mechanisms: synapse pathology

ADPD5-0577

CSF LEVELS OF C-TERMINAL FRAGMENTS OF NEUROGRANIN ARE INCREASED AND CORRELATED WITH THE CSF ABETA1-42/ABETA1-40 RATIO IN ALZHEIMER'S DISEASE

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Objectives Abeta1-42 and protein tau are well-accepted CSF biomarkers to support early diagnosis of AD. However a robust surrogate for synaptic loss, an established correlate of cognitive decline, is not readily available. Previously a small soluble post-synaptic protein, namely neurogranin, has been shown to be such a promising surrogate CSF biomarker. Unexpectedly, deep-proteomic mass-spectrometry approaches demonstrated that neurogranin is also present in plasma/serum.
Methods We report the establishment of an ELISA for the C-terminal part of neurogranin to assess its diagnostic potential in an explorative study in paired CSF/plasma samples of cognitively healthy persons versus patients suffering from MCI due to AD (MCI), dementia due to AD (AD) or MCI/AD with high tau levels.
Results Using the new neurogranin ELISA we confirmed increased CSF levels in MCI and AD, and also observed a correlation with CSF tau. Additionally, we noted a negative relationship between CSF neurogranin (or tau) levels and the CSF Abeta1-42/Abeta1-40 ratio, as if gamma-secretase deficiency seems to correlate with axonal/synaptic loss. On the other hand, no significant differences were detected in plasma neurogranin levels between controls and the AD groups. Also, there was no correlation between CSF and plasma levels of neurogranin.
Conclusions This explorative study clearly warrants further clinical studies to address the additional diagnostic potential for AD of neurogranin in CSF. Furthermore, now a straightforward ELISA could be combined with mass-spectrometry to further advance neurogranin into a harmonized biomarker for synaptic pathology in CSF of AD patients.

03e. Pathophysiology & Disease Mechanisms: proteasome and ubiquitin

ADPD5-1183
ACTIVITY OF UBIQUITIN PROTEASOME SYSTEM IN ETIOPATHOGENESIS OF TAUOPATHY

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Common feature of neurodegenerative diseases such as Alzheimer`s disease is disturbance in protein turnover followed by intracellular accumulation of aberrant proteins. It can be caused either by impairment in the processes of protein synthesis or by aberrant posttranslational modifications accompanied with deficiency in the protein repair machinery.

In our study we characterized degradation pathways for physiological and pathologically truncated tau proteins and determined the activity of proteasome in context with the intracellular tau protein accumulation and clearance in cellular and animal models of human tauopathy.

In the cellular model we showed that pathologically truncated tau is degraded preferentially by ubiquitin-proteasome complex. Our study revealed that degradation of truncated tau is significantly inhibited (1.5x; $p<0.01$). Interestingly the over-expression of truncated tau leads to significant inhibition of ubiquitin proteasome system activity (~30%; $p < 0.001$). Our results on transgenic rats showed significant inhibition of proteasome activity in brain areas with advanced stage of neurofibrillary pathology (up to 2.15 times; $p<0.01$). Moreover the proteasome impairment negatively correlates with the load of sarkosyl insoluble tau in the afflicted brain areas of the transgenic animals.

In conclusion, our results clearly showed that truncated tau protein expressed in cellular and animal models of tauopathy caused significant impairment of proteasome. Regulation of processes involved in the intracellular protein degradation pathways may therefore represent a promising way for development of disease modifying compounds for treatment of human tauopathies.

This work was supported by Research grants: APVV-0677-12, VEGA 2/0194/14, VEGA 2/0161/13, Axon Neurosciences SE research grant

03f. Pathophysiology & Disease Mechanisms: oxidative damage

ADPD5-0823

TAU OLIGOMERS ALTER THE NUCLEIC ACID PROTECTIVE FUNCTIONS OF TAU IN VIVO.

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Objectives : An increase in oxidative DNA and RNA damage occurs in vulnerable neurons during the early stages of Alzheimer disease (AD). In addition to its well-known role in microtubule stabilization, Tau is an essential nuclear key player in the protection of neuronal genomic DNA and RNA integrity under ROS-producing stress (hyperthermic stress (HS). Our objective is to test the impact of Tau pathological forms on neuronal nucleic acid integrity in vivo.

Methods : THY-Tau22 mice expressing mild, moderate or severe Tau pathology were subjected to HS. Nucleic acid integrity was analyzed in mouse CA1 hippocampal and AD cortical neurons, using a TUNEL assay and immunolabeling for 8-OHdG. Immunolabeling for TOC1 and T22 antibodies was used to identified prefibrillar Tau oligomers. Methylene blue was i.p. injected in THY-Tau22 mice to prevent Tau polymerization.

Results : HS selectively induced DNA and RNA damage in neurons expressing prefibrillar Tau pathology. HS-induced nuclear nucleic acid damage was strictly correlated to the accumulation of prefibrillar Tau oligomers. Methylene Blue prevented HS-induced Tau oligomerization and nucleic acid damage.

Conclusion : We highlighted the existence of an early and limited time window of nucleic acid vulnerability to HS during the evolution of Tau pathology. Importantly, this study emphasizes a toxic role of prefibrillar Tau oligomers in impaired nucleic acid integrity in vivo. Our findings suggest that a loss of the Tau-mediated nucleic acid protective functions participate in the DNA and RNA damage observed in AD.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0608

NH2-TRUNCATED HUMAN TAU INDUCES DEREGULATED MITOPHAGY IN NEURONS BY ABERRANT RECRUITMENT OF PARKIN AND UCHL-1:IMPLICATIONS IN ALZHEIMER'S DISEASE

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Objects

Disarrangement in functions and quality control of mitochondria at synapses are early events in Alzheimer's Disease (AD) pathobiology. We reported that an AD-linked toxic 20-22 kDa NH_2-tau fragment mapping between 26 and 230 aminoacids of the longest human tau isoform (aka NH_2htau) early affects the Parkin-driven quality control of mitochondria (mitophagy) in post-mitotic neurons in correlation with bioenergetic deficits and *in vitro* synaptic pathology. However, whether the NH_2htau-enhanced autophagic turnover of mitochondria exacerbates neuronal death because of depletion of functional mitochondria or, alternatively, is a compensatory attempt to mitigate neuronal damage, still remains to be clarified.

Methods

Primary neurons; adenovirus-mediated infection; shRNA-mediated gene expression knockdown; Western blotting; immunofluorescence; immunoprecipitation; immunohystochemistry.

Results

We show that the extensive Parkin-dependent turnover of mitochondria occurring in NH_2htau-expressing post-mitotic neurons plays a pro-death role and that UCHL-1, the cytosolic ubiquitin-C-terminal hydrolase L1 which directs physiological remodeling of synapses by controlling ubiquitin homeostasis, critically contributes to mitochondrial and synaptic failure in this *in vitro* AD-model. Pharmacological or genetic suppression of improper mitophagy, either by inhibition of mitochondrial targeting to autophagosomes or by shRNA-mediated silencing of Parkin or UCHL-1 gene expression, restores synaptic and mitochondrial content providing partial but significant protection against the NH_2htau-induced neuronal death. In mitochondria from human AD synapses, the endogenous NH_2htau is stably associated with Parkin and with UCHL-1.

Conclusions

Our studies suggest that the NH_2htau fragment contributes to synaptic failure in AD neurons by aberrant recruitment of Parkin and UCHL-1 to mitochondria making them more prone to detrimental autophagic clearance.

03j. Pathophysiology & Disease Mechanisms: autoimmunity

ADPD5-1513
SPECIFIC ANTIBODIES AGAINST TAU PROTEIN

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Objectives
In past years, large attention was devoted to diagnostic markers as well as therapeutic approaches to neurodegenerative diseases, especially to Alzheimer disease (AD). The n ewest clinical studies are focused on tau protein. They used monoclonal antibodies (Abs) against a specific epitope of tau protein or polyclonal Abs like intravenous immunoglob ulins (IVIg). Some studies found that IVIg products contain Abs against tau protein. Acco rding to this findings, we were interested in the isolation these Abs and their characteriza tion.
Methods
We used a commercial product Flebogamma from Grifols. Specific Abs were purified ag ainst human recombinant tau protein (the longest isoform). Tau protein was covalently b ound to epoxy-
activated agarose resin at 5 mg of protein to 1 ml of resin. Specific Abs were characteriz ed by ELISA, Dot blot and Western blot analysis.
Results
We were able to immunopurify 1,6 mg/ml (0,88%) of specific anti-
tau Abs from 8 ml of 50 mg/ml of Flebogamma IVIg.
Conclusions
We found that in the commercial product Flebogamma
were specific Abs against tau protein. It is interesting that from the plasma of healthy don ors it is possible to isolate specific Abs against one protein that is associated with AD. A ccording to this, we decided to measure levels of Abs against tau protein in different gro ups: patients with Mild Cognitive Impairment, AD and Other Dementias as well as in heal thy elderly controls. This study was supported by GACR P304/12/G069, GACR 13-
26601S, IGA NT 13183 and MH CZ - DRO (PCP, 00023752), UK 266705/SVV/2014.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1333

THE ROLE OF TAU PROTEIN FRAGMENTS IN EARLY PHASE OF NEURODEGENERATION

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Epidemiological studies clearly indicate a strong relationship between Alzheimer's disease (AD) dementia and obesity. However, the mechanisms behind the association of fat metabolism and etiopathogenesis of AD are still not understood. Leptin, a major adipokine of white adipose tissue is supposed to play an important role in this mechanism. We have therefore investigated the link between selected metabolic parameters in peripheral blood and pathogenic changes in brain of transgenic rat model of human tauopathy. Brain tissue of transgenic rats expressing human truncated tau protein, whose primary sequence was designed according AD-core tau protein, was analysed in different stages of pathogenesis, preferentially in early pre-symptomatic phase. The data from brain stem and hippocampus of experimental animals were correlated with metabolic parameters in peripheral plasma. We found that pathological forms of human tau protein, if expressed in rat brain, leads to significant reduction of body weight accompanied with significant decrease in level of plasma leptin in asymptomatic animals. The level of plasma leptin inversely and significantly correlated with amount of hippocampal tau proteins hyperphosphorylated on several AD-associated phosphoepitopes. Our data show, that plasma leptin could serve as a convenient peripheral marker of early stage of neurofibrillary degeneration.

This work was supported by research grant from Axon Neurosciences SE and grant number APVV-0677-12

03m. Pathophysiology & Disease Mechanisms: micro RNAs

ADPD5-1193

PROFILING OF MICRORNA LEVELS IN RAT MODEL OF NEUROFIBRILLARY DEGENERATION

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Neurofibrillary tangles composed of post-translationally modified protein tau are major hallmarks of neurodegenerative diseases collectively known as tauopathies. Pathogenesis of these disorders is characterized by gene expression changes resulting to altered signaling pathways. Moreover, deregulation of microRNA levels has been also associated with many neurodegenerative diseases, suggesting possible employment of miRNAs as diagnostic biomarkers. In order to identify differentially expressed microRNA molecules during neurofibrillary degeneration we have performed complex miRNA profiling in rat model of tauopathy that recapitulates major features of neurodegeneration of AD type.

Transcriptomic analysis of brain tissue samples showed significantly increased expression of several particular miRNAs involved in the regulation of neuroinflammation (4,1-fold), innate immune response (2,6-fold), intracellular trafficking (2,4-fold) and signal transduction (2,3-fold). Interestingly, correlation analysis revealed strong positive relationship between dysregulated microRNA molecules.

The data suggest that expression of truncated tau protein leads to deregulation of signaling pathways during the neurodegeneration process manifested by altered miRNA expression. Moreover, identified miRNA markers can be potentially applied for the monitoring of the neurodegenerative cascades in Alzheimer's disease and related tauopathies.

This work was supported by Research grants: APVV-0677-12 and VEGA 2/0161/13, 2/0194/14 and research grant from Axon Neuroscience SE.

03n. Pathophysiology & Disease Mechanisms: kinases and phosphatases

ADPD5-1620

AMP-ACTIVATED PROTEIN KINASE ALPHA 2 SUBUNIT DEFICIENCY MODULATE TAU PATHOLOGY

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Objective: Neurofibrillary tangles (NFTs) are the pathological hallmark of neurodegenerative diseases commonly known as Tauopathies. NFTs result from the intracellular aggregation of abnormally and hyperphosphorylated tau proteins. Recently, we demonstrated that activated AMP-activated protein kinase (AMPK) co-localized with phosphorylated Tau in pre-tangle- and tangle-bearing neurons in Tauopathies. In addition, we showed that AMPK was a tau kinase *in vitro*. The goal of this study was to determine whether *in vivo* in the mouse brain AMPK was also involved in Tau phosphorylation.

Methods & Results: In order to determine if AMPK could affect tau phosphorylation and tangle formation *in vivo*, we crossed AMPKalpha2KO mice with the PS19 tau transgenic mice. Tau and phosphorylated Tau levels in Heat stable and insoluble fractions obtained from 8 months old animals were analyzed by ELISA, Western-blot and immunohistochemistry. We did not observed any significant effect of AMPKalpha2 deficiency on total or phosphorylated Tau levels in the heat-stable preparations. However, we observed a significant reduction in insoluble total and phosphorylated Tau in the AMPKalpha2KO mice as compared to controls.

Conclusion: We found that AMPK could regulate tau phosphorylation and tau aggregation *in vivo* in the mouse brain. Our results suggest that strategies aiming at inhibiting AMPK could be of therapeutic values for Tauopathies.

This work was supported by the French Fondation pour la cooperation Scientifique – Plan Alzheimer 2008-2012 (Senior Innovative Grant 2013) to VV, and by the National Institutes of Health Grant R01AG042508 to PM.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-1109

STUDY OF THE EFFECT OF GLUCOSE AND OXYGEN DEPRIVATION ON THE PHOSPHORYLATION OF TAU AND DEPOLARIZATION-INDUCED SIGNALING

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Background: Alzheimer's Disease (AD) is the most common form of dementia, with two major neuropathological features, including intracellular neurofibrillary tangles (NFTs) consisting of hyper-phosphorylated tau, and extracellular amyloid plaques. Hyper-phosphorylation of tau decreases its affinity for the microtubules, resulting in accumulation of the protein in the axons and disruption of the normal neuronal function. Cerebral blood hypoperfusion and subsequent elimination of oxygen and glucose supply to the brain are believed to be involved in the development of sporadic AD. Based on these data, we investigated the effects of glucose and oxygen deprivation on tau phosphorylation and depolarization-induced signaling.
Methods: Perfusion of acute brain slices from C57BL/6 mice with artificial cerebrospinal fluid supplemented with various oxygen and glucose concentrations. Total protein extracts were analyzed by SDS-PAGE.
Results: Hypoxia reduced the phosphorylation of tau in all the examined residues. This effect was more potent when oxygen deprivation was combined with low levels of glucose in the perfusion medium. However, glucose deprivation, alone, did not affect phospho-tau levels. Finally, hypoxia, but not hypoglycemia, resulted in complete attenuation of depolarization-induced neuroprotective signaling.
Conclusions: The hypophosphorylation of tau under hypoxic conditions could be attributed to an extensive down-regulation of energy metabolism, as a temporary neuroprotective mechanism against limited oxygen supply. Although these particular stress conditions failed to induce pathological phosphorylation of tau protein, they may exert their neurotoxic effects through impairment of depolarization-induced signaling, which is vital for normal neuronal function and survival.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-1180

GENE EXPRESSION ANALYSIS OF SIGNALING PATHWAYS IN A RAT MODEL OF TAUOPATHY

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The process of neurofibrillary degeneration in tauopathies is inevitably accompanied by dysregulation of signaling pathways leading to cellular stress and inflammation. In order to identify molecular hallmarks involved in these cascades we have employed the transgenic rat model of tauopathy expressing human truncated tau protein (AlzTau 151-391,4R).

In this study we have analyzed gene expression levels of inflammatory markers together with heat shock proteins in the brains of transgenic animals. We observed significantly increased gene expression of small heat shock protein (2.1-fold), cell adhesion molecule (2.8-fold) and activation of complement system (3.7-fold). Interestingly, the mRNA levels of these markers strongly correlated with the amount of sarkosyl insoluble tau. Immunohistochemical staining revealed localization of protein in individual cell populations what was quantitatively determined by stereological quantification.

The results suggest that misfolded tau protein induces variety of signaling pathways including cellular stress and neuroinflammation that are associated with neurodegeneration process of tauopathies.

This work was supported by Research grants: APVV-0677-12 and VEGA 2/0161/13, 2/0194/14 and research grant from Axon Neuroscience SE.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-2138

EFFECT OF REG-1ALPHA ON NEURONAL CELL DEATH IN CELLULAR AND ZEBRAFISH MODELS OF TAU HYPERPHOSPHORYLATION

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Objectives: We focus our interest on Reg-1alpha, a secreted glycoprotein, that is (1) differentially regulated during development, ageing and pathological processes, (2) more or less expressed in various organs depending on environmental factors, (3) specifically involved as a paracrine/autocrine factor in proliferation and differentiation of cells. The large majority of studies has been carried out in the digestive system. However, we have recently demonstrated that Reg-1alpha is involved in neurite outgrowth through its receptor EXTL3 and was able to regulate GSK-3beta pathway. Moreover, Reg-1alpha is overexpressed in human Alzheimer-disease brains and is associated with Tau intracellular inclusions. In this context, we investigated Reg-1alpha function and signaling pathway during the pathological processes in original cell culture and zebrafish models of tauopathy.

Methods: To understand the relation between Tau and Reg, we performed (1) overexpression of the human Tau cDNA corresponding to the splice variant 4R0N carrying P301L mutation and (2) pharmacological stimulation with okadaic acid to modulate the phosphorylation state of the system. We analyzed the inductive role of Reg-1alpha (recombinant protein) and the mechanisms involved in the degenerative processes. In addition, using the transgenic zebrafish hTauP301L line, injection of human Reg-1alpha mRNA effect was analyzed in embryos.

Results: Reg-1alpha overexpression in zebrafish hTauP301L resulted in severe defects during development and induced neuronal death. Moreover, we observed, in cellular models, abnormal neuritic morphologies and Reg-1alpha/hyperphosphorylated Tau colocalization. In these conditions, Reg-1alpha modifies the cellular phenotype and accelerates neuronal death in cells expressing tau-hyperphosphorylated by Akt/GSK-3beta pathway regulation and caspase3 activation.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-1504

PROPERTIES OF HUMAN OLIGODENDROCYTES GENERATED FROM IPSC LINES OF PATIENTS WITH MAPT MUTATIONS.

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Objectives Frontotemporal Dementia with Parkinsonism linked to Chromosome 17 (FTDP-17T) comprises 50% of all dementia cases under the age of 60 and involves the aggregation of the microtubule-associated protein tau. Research on tauopathies has mainly focused on the neuronal component overlooking the contribution of glial cells. Glial tangles in oligodendrocytes have been observed in various cases of FTDP-17T, and *in vivo* expression of tau in oligodendrocytes is sufficient to induce neurodegeneration and recapitulate tauopathy. Our aim is to generate oligodendrocytes from human induced pluripotent stem cells (iPSCs) of patients with FTDP-17T MAPT mutations (N279K and P301L) and to evaluate their electrophysiological and biochemical properties along side oligodendrocyte tau expression.
Methods We have optimised currently used protocols to generate human oligodendrocyte-lineage cells from iPSCs of control and FTDP-17T patients. Whole-cell patch clamp recordings were used to examine the effects of the N279K and P301L tau mutations on the membrane properties and ion-channel composition of these cells.
Results We obtained large amounts of human IPSC-derived oligodendrocyte using hypoxic culturing conditions. We managed to equally generate oligodendrocyte precursor cells (OPCs) from FTDP-17T and control iPSCs, both of which matured into myelin-interacting oligodendrocytes with neuronal axons. We are now investigating, tau expression, oligodendrocyte membrane properties, neurotransmitter signalling and the myelinating capacity of these human oligodendrocytes.
Conclusions Glial contribution to FTDP-17T has been underestimated. Relevant information can be obtained from the IPSC-oligodendrocytes that we have generated, thus helping to elucidate the role of glia in diseases with tau pathology.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-1354

THE PSP-RELATED TAU MUTATION A152T CAUSES HIPPOCAMPAL EXCITOTOXICITY

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Objectives:

We investigated the consequences of human full-length Tau with the rare point mutation A152T (hTau40/A152T, related to progressive supranuclear palsy) on hippocampal function.

Methods:

For pathophysiological characterization a combination of aged hTau40/A152T transgenic mice and cultured organotypic hippocampal slices was used.

Results:

Transgenic mice show Tau pathology including hyperphosphorylation, aggregation, neuronal loss and behavior deficits. In cultured slices Tau was found in both presynaptic and somato-dendritic compartments including dendritic spines. Furthermore we observed mossy fiber sprouting, an indicator for increased epileptiform activity. Pronounced epileptiform activity was indeed observed in such slices. In cultures extracellular glutamate started to rise early followed by neurotoxicity. In parallel we detected elevated levels of intracellular calcium in CA3 neurons, characterized by an enhanced calcium influx after membrane depolarization, sensitive to NR2B blockade. Glutamate increase was reduced to control levels by either inhibition of neurotransmitter release or blockade of voltage-gated sodium channels. In line with these changes, we observed enhanced basal synaptic transmission in the mossy-fiber pathway of acute hippocampal slices from aged hTau40/A152T mice with no change in short and long-term plasticity. Excitotoxic cell death in slices was ameliorated by long-term application of low-dose memantine and by administration of ceftriaxone, which stimulates glutamate uptake via glial Excitatory Amino-Acid-Transporter-2.

Conclusions:

hTau40/A152T causes pronounced excitotoxicity mediated by extrasynaptic NMDAR containing NR2B subunits due to an increase of extracellular glutamate, which is caused by enhanced presynaptic transmitter release.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-1989

IN VIVO IMAGING OF NEURONAL CIRCUIT'S DYNAMICS IN THE MOUSE TAUOPATHY MODEL

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OBJECTIVES:

In Alzheimer's brains, neurofibrilary tangles (NFTs) and tau pathology spread in stereotypical manner. Recent studies demonstrated that similar spreading pattern could be reproduced in animal models by injecting tau fibrils in specific brain areas where they serve as seeds for aggregation of endogenous tau. It is not fully clear what are the effects of tau pathology spreading on neuronal activity and functioning. In present study we address this question using two-photon in vivo imaging in tau seeding mouse model.

METHODS

We are able to induce NFTs formation by injecting recombinant tau fibrils in cortex of P301S transgenic mice. To study neuronal activity and neuronal circuit's dynamics we have established an approach which combines two-photon calcium imaging through cranial window with imaging in awake, head-fixed mice. In that way, we follow same neuronal populations over prolonged periods of time. Furthermore, by applying FSB, a dye which labels NFTs, we can directly visualize NFTs formation and spreading *in vivo*.

RESULTS

Our results suggest that formation of NFTs takes place in first two weeks after tau fibrils injection in cortex of P301S mice. NFTs could be detected throughout all cortical layers. Using *in vivo* two-photon calcium imaging we can reliably record activity from layer 2/3 neurons for more than 2 months after seeding.

CONCLUSIONS

In conclusion, our method enables *in vivo* investigation of tau pathology effects on neuronal functioning. Identifying and preventing early tau pathology induced changes in neuronal functioning could be potential therapy strategy for Alzheimer's disease and other tauopathies.

03y. Pathophysiology & Disease Mechanisms: aging

ADPD5-1739

HOW DOES AGEING CONTRIBUTE TO ALZHEIMER'S DISEASE?

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Ageing is the biggest risk factor for dementia of which Alzheimer's Disease (AD) is the most common form. There are currently no effective treatments that can halt the progression of AD, and despite decades of research the pathogenesis of the disease is poorly understood. AD is characterized by two pathological hallmarks: intracellular tau tangles and extracellular Abeta plaques. It is unclear how cellular changes that occur during ageing predispose us to the formation of these pathologies. Increasingly, the same cellular pathways that are implicated in ageing are also found to be altered in AD. These pathways include, among others, mTOR signaling, insulin signaling and autophagy.

In this study, the impact of these age associated pathways on tau mediated phenotypes and pathologies will be investigated. Components of the different cellular pathways will be assessed for age related changes in *Drosophila* expressing human tau. The tau mediated phenotypes that will be assessed include; longevity, climbing ability, circadian rhythms and memory function. Levels of tau, its phosphorylation state and solubility will be determined biochemically to assess tau pathology.

Preliminary results show that the expression of human tau induces the activation of dTOR (the TOR pathway in *Drosophila*), this effect becomes greater with age. Inhibiting dTOR using rapamycin significantly increases the lifespan of tau expressing flies. Conversely, activation of dTOR by dietary means, exacerbates tau-mediated locomotor deficits and shortens lifespan.

These findings demonstrate that a pathway implicated in ageing can influence tau-mediated phenotypes and pathology.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-0251

HYPERPHOSPHORYLATED TAU MAY PLAY A CRUCIAL ROLE IN THE PATHOGENESIS OF WHITE MATTER LESIONS IN ALZHEIMER'S DISEASE

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Background: Cerebral small vessel disease (SVD) is associated with the pathogenesis of white matter lesions (WML), however, WML have been observed in the absence of SVD in white matter adjacent to cortical areas exhibiting Alzheimer's disease (AD) pathology. We investigated the influence of AD pathology (hyperphosphorylated tau (HPT) and amyloid-beta (Abeta) plaques) and SVD on white matter integrity.

Method: We evaluated 40 *post mortem* human brains (15 normal aged controls; mean age 84.4±9.1 years; 9F:6M: 25 AD; 84.4±5.4 years; 15F:10M). All cases underwent *post mortem* T2* MRI assessment of white matter hyperintensity (WMH) according to the ARWMC scale and quantitative assessment (% binary area fraction of immunopositivity) of HPT (AT8) and Abeta (4G8) pathology and vessel wall fibrosis using Sclerotic Index (SI).

Results: ARWMC scores were significantly higher in AD compared to control cases ($P<0.05$), however, no significance difference was found in SI. For the overall cohort significant correlation was seen between ARWMC scores with AT8 and 4G8 immunopositivity in all major cortical regions ($P<0.05$). Regression analysis revealed AT8 immunopositivity was a significant predictor of ARWMC score in all major cortical regions ($P<0.01$) and overall hemisphere ($P<0.05$). SI was also shown to be a significant predictor of ARWMC for the overall hemisphere ($P<0.05$).

Conclusion: HPT pathology was found to be associated with WMH independent of concomitant SVD indicating a potential independent and/or combined pathogenic role of cortical neurodegenerative pathologies and SVD in the development of WML.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-1767

TAU ANTIBODY PROFILING IN A CELLULAR SEEDING ASSAY

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Objectives: A cellular seeding model was used to investigate the relation between activity, epitope, binding properties and phospho-dependency of anti-tau antibodies in preventing aggregation.
Methods: Cell homogenates of a HEK cell line stably expressing 2N4R-TauP30L-GFP aggregates were used to induce aggregation in a cellular seeding model expressing K18/P301L. Induced aggregation was detected by Bioluminescent Resonance Energy Transfer (BRET) ratio. For testing activity of anti-tau antibodies, cell homogenate and antibody were co-incubated with the cells.
Results: Co-incubation of anti-tau antibodies and cell homogenates resulted in a decreased BRET ratio for several antibodies. The antibodies with the highest activity were mostly total tau antibodies and only a small percentage were phospho-specific antibodies. The total tau antibodies with the highest activity mainly have an epitope located in the N-terminal part of tau indicating this could be a more exposed part of tau when part of an aggregate. Binding properties of the antibodies indicate as well that affinity/avidity is not the only driving force for activity.
Conclusions: A high affinity antibody does not guarantee that the antibody prevents aggregation based on the results obtained with this cellular seeding model. Other players are epitope and phospho-dependency of the antigen.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-0315

PREPARATION AND TESTING OF INHIBITORS OF THE FORMATION OF A NOVEL, NEUROTOXIC TAU FRAGMENT

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Objectives: Tauopathies are a group of incurable neurodegenerative conditions, characterized by the presence in the brain of neurofibrillary tangles composed of aggregated tau protein. Currently, *there are no disease-modifying treatments for tauopathies.* The development of such therapies requires an understanding of the molecular pathways that lead to synaptic dysfunction and neurodegeneration in tauopathies.

Methods: Our collaborators (Zhao and Ashe) recently obtained evidence that a cysteine protease cleaves tau to form a fragment believed to be tau* (35-kDa **t**au **c**leavage **p**roduct, henceforth TCP35). This fragment has been shown to mislocalize to dendritic spines in neurons and potentially disrupt synaptic function. Based on their reported cleavage site of tau, we have prepared a series of peptide aldehyde inhibitors with the goal of developing a structure-activity relationship (SAR) that will guide the further design of potent and selective inhibitors of this cysteine protease.

Results: Our initial results show that a reversible covalent pentapeptide based on the site of cleavage is a <500 nM and highly selective inhibitor of the protease target. Further SAR findings will be presented.

Conclusions: The identification of the cysteine protease responsible for the formation of a newly characterized neurotoxic tau fragment has paved the way for the development of new therapeutics for the treatment of tauopathies. We expect that preparation of potent inhibitors of the formation of TCP35 may be possible based on the cleavage sequence that has been identified.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-0379

THE AMYLOID-BINDING AGENT BTA-EG4 REDUCES PHOSPHORYLATED TAU LOAD AND LEVELS OF SYNAPTIC AMYLOID PRECURSOR PROTEIN IN MODELS OF ALZHEIMER'S DISEASE

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Objectives: The tetra (ethylene glycol) derivative of benzothiazole aniline, BTA-EG_4 acts as an amyloid-binding agent to reduce levels of beta-amyloid (A-beta), increase dendritic spine density and ameliorate cognitive decline in mouse models of Alzheimer's disease (AD). The effect of BTA-EG_4 on abnormal processing of tau has not yet been explored. Here, we sought to determine the impact of BTA-EG_4 treatment on disease-associated modifications in tau including its abnormal phosphorylation and localisation. Changes in amounts of pre- and post-synaptic proteins were also assessed, since tau is closely linked with synaptic degeneration in AD.
Methods: Primary cortical neurons and organotypic brain slice cultures from 3xTg-AD mice were treated with BTA-EG_4. Levels of tau and phosphorylated tau at several AD-relevant epitopes were quantified by western blotting. Crude synaptosomes were prepared from treated samples and levels of synaptic markers, tau and amyloid precursor protein (APP) were assessed by western blotting.
Results: Treatment with BTA-EG_4 was found to significantly reduce levels of total tau and tau phosphorylated at epitopes of relevance to AD. In addition, BTA-EG_4 treatment reduces levels of APP, but not tau, in synaptosomal preparations. The mechanism linking these changes in APP and tau is currently under investigation.
Conclusions: BTA-EG_4 freely permeates the blood brain barrier to bind A-beta and reduce A-beta-associated toxicity. We show here that BTA-EG_4 also reduces levels of total and phosphorylated tau, together with reducing APP load at synapses. These findings suggest that further preclinical exploration of BTA-EG_4 in models of AD is warranted.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-0764

DOES HYPERTENSION CONTRIBUTE TO TAU LOAD IN ALZHEIMER'S DISEASE? CROSS-SECTIONAL POSITRON EMISSION TOMOGRAPHY STUDY USING [11C]PBB3

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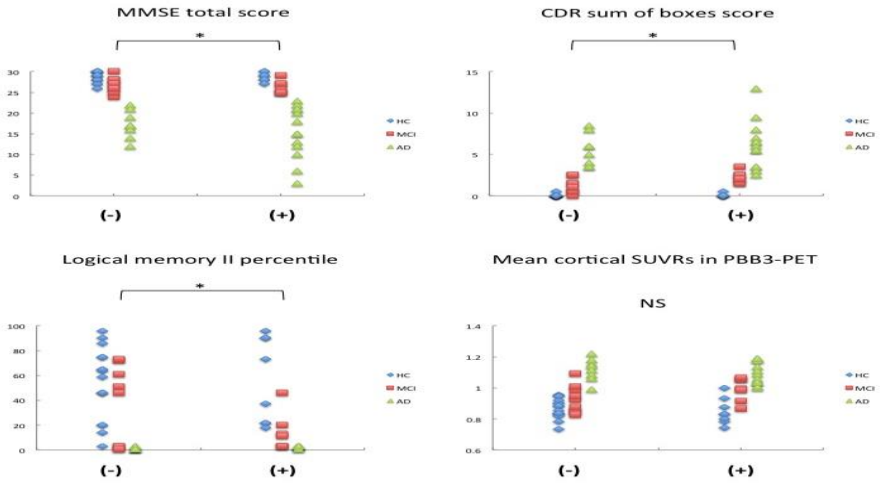
Objectives: Hypertension is known to be a risk factor for dementia, including Alzheimer's disease (AD). However, its pathological role in AD is unclear. This study aimed to investigate the influence of hypertension on the brain using tau deposition detectable by a novel positron emission tomography (PET) ligand, [11C]PBB3.
Methods: Brain magnetic resonance imaging scans, PET scans with the [11C]PBB3, and neuropsychological tests, such as the Mini Mental State Examination (MMSE), were performed on 61 subjects, including those with AD and mild cognitive impairments (MCI) and healthy controls (HCs). Subjects diagnosed with other types of dementia, such as vascular dementia, and those aged <50 years were excluded. In [11C]PBB3-PET imaging, standardized uptake value ratios (SUVRs) were determined as measures of radioligand retention using the cerebellar cortex as reference. Subjects were divided into hypertension-positive (HP) and negative (HN) groups based on clinical history, and comparisons were performed.
Results: The HP group included 13 subjects with AD, 7 with MCI, and 8 HCs, while the HN group included 8 subjects with AD, 9 with MCI, and 16 HCs. The MMSE scores in the HP group were significantly lower than those in the HN group. No significant difference was observed in the mean cortical SUVRs between the groups, not only in subjects with AD, but also in those with MCI and in healthy controls.
Conclusion: Although hypertension may be associated with cognitive decline, hypertension did not contribute to tau load in [11C]PBB3-PET. Further hypotheses involving other pathological mechanisms would be required.

Figure.



Comparisons between the hypertension-positive (+) and negative (–) groups among healthy controls (HC), subjects with mild cognitive impairment (MCI), and subjects with Alzheimer's disease (AD). MMSE, Mini Mental State Examination; CDR, Clinical Dementia Rating; SUVRs, Standardized uptake value ratios; *, $p < 0.05$ (Total group comparison using t-test); NS, Not statistically significant in the comparison.

ADPD5-0830

MOLECULAR AND FUNCTIONAL ANALYSIS OF AD-DERIVED PAIRED-HELICAL FILAMENTS

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Objectives: Although several studies demonstrate that Tau aggregates spread via transsynaptic transport, little is known about the molecular signature of the seed. The aim of this study is to characterize the biophysical and molecular profile of PHFs derived from *postmortem* AD brain and evaluate their seeding properties *in vivo*.
Methods: PHFs are enriched from p*ost mortem* AD brain tissue and analysed by Western blotting and ELISA. Immunopurified extracts are analysed by SDS PAGE and mass spectrometry to determine protein components and post-translational modifications. On the other hand, enriched PHFs are used for stereotactic injections of P301L mice.
Results: IP/MS analysis of Tau from *postmortem* AD brain confirmed phosphorylation of a number of epitopes including Ser202/Ser205 and Thr231. Besides Tau, a number of interacting proteins including heat-shock proteins, kinases, phosphatases and cytoskeleton proteins were identified. When PHFs are injected in the cortex, Tau pathology (confirmed by native PAGE, Western Blot and IHC) is observed from one to three months after injection in injected and non-injected hemispheres.
Conclusions: This study further characterized the molecular properties of PHFs from AD brain. Further analysis is required to identify other post-translational modifications including ubiquitination and acetylation. Injection experiments in Tau transgenic mice show that purified PHFs can trigger Tau aggregation *in vivo*. Further injection studies with fractionated AD brain samples are needed to get more insight in the molecular properties of this type of seed.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1030

CAPPING CYSTEINE INHIBITS TAU AGGREGATION AND NEURONAL LOSS.

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[Objective] Neurofibrillary tangles (NFT) are a pathological hallmark of Alzheimer's disease (AD); neuronal and synapse loss are key events during the process of NFT formation. With a view of developing a new therapy for AD, we screened an array of small molecules for their potential to inhibit the aggregation of toxic tau in an animal model of tauopathy, focusing on the mechanism of inhibitory action

[Methods] Tau-binding molecules were identified in a first screening of a natural compound derivatives library. Screened tau-binding compound was then tested by several tau aggregation assays, and confirmed a reducing granular tau oligomer level.

[Results] Epinephrine and pyrocatecholviolet that share 1,2-dihydroxybenzene in their structures, bound to tau and inhibited the toxic tau aggregation; compounds lacking 1,2-dihydroxybenzene did not show the latter capacity, indicating the requirement of 1,2-dihydroxybenzene for the inhibition of toxic tau aggregation. Binding assays revealed that 1,2-dihydroxybenzene bound to Cys residues in tau. Furthermore, tau modified by 1,2-dihydroxybenzene was not polymerized by heparin. They suggest that Cys residues contribute to tau aggregation. To confirm that 1,2-dihydroxybenzene-containing compounds penetrate the brain, we administered isoprenaline to P301L-tau transgenic mice. The treatment significantly decreased levels of Sarkosyl-insoluble tau, and neuronal loss.

[Conclusions]

1,2-dihydroxybenzene binds and caps the Cys residues in tau. Capped tau cannot interact with other tau molecules, thus inhibiting toxic tau aggregation. Administration of brain permeable 1,2-dihydroxybenzene inhibits Sarkosyl-insoluble tau and neuronal loss, and thus has the potential to alleviating brain dysfunction. These results suggest Cys as an important therapeutic target for AD.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1147

EFFECTS OF METHYLENE BLUE AND ANOTHER OXIDANT ON HUMAN TAU TRANSGENIC FLY MODELS

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Objective: Neurofibrillary tangles formed by aggregated tau are well correlated with the extent of cognitive decline in Alzheimer patients. Methylene blue (MB) is known to inhibit tau aggregation *in vitro*. However, the mode of action *in vivo* is still unknown. Using the fly models that express human wild type tau (hTau), we investigated the effects of MB and another oxidant that has similar oxidizing property on the tau accumulation and behavioral deficits caused by hTau expression. Methods: hTau flies were treated with 1 mM compound for 1 month. In prior to biochemical analysis of tau accumulation, their behavioral activity was analyzed. Results: We found that MB effectively reduced the tau accumulation and ameliorated the climbing deficits of hTau flies. However, it didn`t rescue the climbing ability completely and induced climbing deficits in wild type flies. Oxidizing property of MB is implicated in the dose-response effects of MB. To find a safer compound, we selected a compound that works similarly to MB but at the same time that is less toxic. Treatment with this compound reduced the tau accumulation and ameliorated the climbing deficits to the smaller extent than MB. Unlike MB, this compound didn`t affect the climbing ability of the wild type flies. Conclusion: These results suggest that the common oxidizing property play roles in the mode of action for suppressing tau accumulation *in vivo.* Compounds that have the similar oxidizing property, as MB and that are also nontoxic may become good therapeutic candidates for tauopathies including AD.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1386

PRO-AGGREGANT FULL-LENGTH TAU-DELTA-K280 CAUSES A TORPOR-LIKE PHENOTYPE IN HIPPOCAMPAL SLICE CULTURES: TREATMENT WITH TAU AGGREGATION INHIBITORS AND ADENOSINE A1-RECEPTOR ANTAGONISTS

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Objectives:
To investigate the mechanism of toxicity of pro-aggregant Tau and possible modes of treatment.

Methods:
Organotypic hippocampal slice cultures (OHSCs) from full-length pro- and anti-aggregant Tau (Delta-K280 or Delta-K280-PP) transgenic mice and littermate controls were prepared from P8 pups and cultured for 30 days *in vitro*. Treatment was applied for the entire *ex vivo* period. The OHSCs were analyzed using immunostaining, live cell imaging, qPCR and electrophysiology.

Results:
Both pro-and anti-aggregant Tau transgenic OHSCs show Tau missorting and phosphorylation (12E8, PHF-1, AT8, etc.). Local accumulations ('grains') of Tau in a pathological conformation (MC-1) are found in the neuropil of pro-aggregant Tau transgenic OHSCs only. The ATP level and (axonal) mitochondrial density was reduced in these OHSCs. Pro-aggregant Tau did not colocalize with 'classical' protein aggregation markers like P62, HSP70, vimentin or Tia-1. Instead, a qPCR based miniscreen pointed towards an impaired activity of neurons and astrocytes. Using a paired-pulse protocol (electrophysiology) we found impairment of the presynapse which could be counterbalanced by Tau aggregation inhibitors (BSC3094) or by preventing the downregulation of cellular activity using an adenosine A1-receptor antagonist (Rolofylline).

Conclusions:
In OHSCs, pro- and anti-aggregant Tau become missorted into somata and dendrites whereas pathological (MC-1 positive) Tau accumulates locally in pro-aggregant slices as grains. These grains do not colocalize with known aggregation markers. Instead, full length pro-aggregant tau but not its anti-aggregant tau counterpart causes a downmodulation of cellular activity and metabolism. This can be impaired by preventing aggregation or adenosine signaling via the adenosine A1-receptor.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1437
IN VIVO CHARACTERISATION OF A TOOL COMPOUND TARGETING TAU OLIGOMERS

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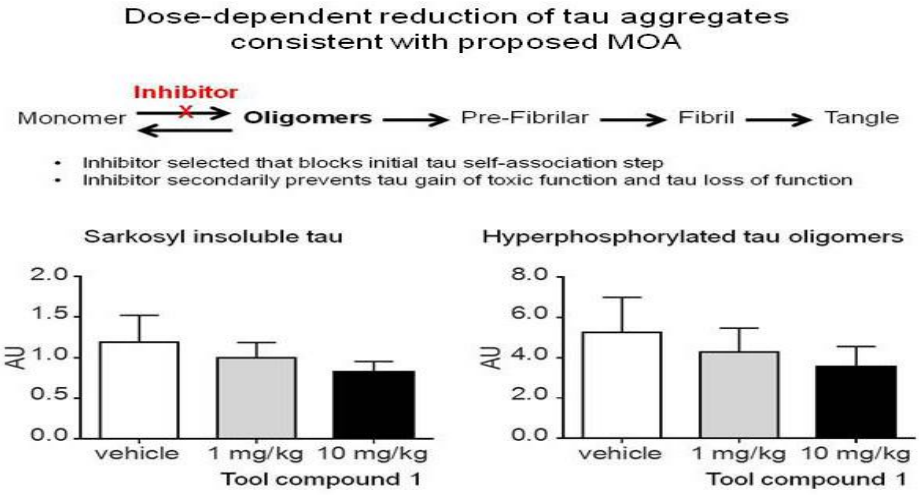
Objective: Tau oligomers are toxic to neurons, inhibit long term potentiation in hippocampal slices, impair the formation of associative fear memory in mice, and transmit tau pathology. Recent advances in research at Oligomerix and in the AD field strongly support targeting tau oligomers for drug discovery for the development of both symptom-modifying and disease-modifying therapeutics. Proof-of-concept studies in a mouse model of tauopathy were initiated with a tool compound inhibitor of tau oligomer formation to demonstrate target engagement in vivo and to validate the screening approach.
Methods: A highly diverse library of 100,000 drug-like small molecules was screened using AlphaLISA for detection of tau self-association, hits were validated and dose response and neurocytotoxicity assays were performed. Medicinal chemistry and secondary assays were used to select tool compounds for in vivo validation studies. Acute toxicity of selected tool compounds was assessed in wild type mice, and the JNPL3 mouse model (Taconic) was used to test compound efficacy for reduction of tau aggregates.
Results: Preliminary testing with Tool Compound 1 in wild type mice showed that it was non-lethal at the high, medium and low doses tested. Biochemical analysis of the brains of 5 month old JNPL3 mice treated with Tool Compound 1 for 8 weeks showed a dose dependent reduction in sarkosyl insoluble tau levels and also phospho tau levels.
Conclusions: These results are consistent with demonstration of target engagement in vivo. Based on the results of this initial study, a larger study has been initiated with 16 weeks of treatment.



04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1510

EPITOPE CHARACTERIZATION OF THE ANTI-TAU ANTIBODY AT8 THROUGH A PHOSPHOPEPTIDE CO-STRUCTURE AND PEPTIDE BINDING STUDIES

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Alzheimer's disease (AD) pathology is characterized by the development of hyperphosphorylated neurofibrillary tangles, which are comprised of extensively phosphorylated filamentous tau protein, or paired-helical filament tau (PHF-tau). AT8 is a PHF-tau specific monoclonal antibody that is a commonly used marker of neuropathology because of its recognition of abnormally phosphorylated tau. Previous reports described the AT8 epitope to include pS202/pT205. Our studies support and extend previous epitope findings by also identifying pS208 as part of the binding epitope. We characterized the phosphoepitope of AT8 through both peptide binding studies and with a co-structure with a phosphopeptide. A co-structure of AT8 Fab with a pS202/pT205 peptide shows that the interaction interface involves all six CDRs and tau residues 201-209. Both phosphorylation sites are recognized by AT8, with pT205 acting as the anchor. The structure further shows that phosphorylation at S208 can be accommodated by AT8. Crystallization of the Fab/peptide complex under acidic conditions shows that CDR-L2 is prone to unfolding and precludes peptide binding, and may suggest a general instability in the antibody. Phosphopeptide binding studies were performed using ELISA and surface plasmon resonance and it was found that AT8 bound to the triply phosphorylated tau peptide (pS202/pT205/pS208) 30-fold stronger than to the pS202/pT205 peptide, supporting the role of pS208 in AT8 recognition. The epitope characterization and binding analysis of AT8 further enhance the knowledge of this important diagnostic antibody and provide insight into the sequence of tau phosphorylation in the progression of AD and other tauopathies.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1517

26S PROTEASOME DYSFUNCTION AND COGNITIVE IMPAIRMENT DUE TO ABNORMAL TAU ACCUMULATION IS ATTENUATED BY RAISING CAMP

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Tau inclusions are a feature of tauopathies, implying a deficit in the cell's ability to clear tau either as a cause or consequence of the disease. Therefore, therapeutic activation of proteolytic pathways can be an effective treatment for tauopathies

Objective

The goal is to first investigate the status of proteasome clearance in tauopathy and to identify what type of tau species cause proteasome dysfunction. Secondly, to apply a therapeutic strategy by activating cAMP/PKA pathway to rescue proteasome activity *in vivo*.

Methods

The effects of tau accumulation on proteasome were studied in a model of tauopathy (rTg4510) and in a cross to a UPS reporter mouse (rTg4510/UbG76V-GFP). The function of affinity purified proteasomes was assessed by different assays. Immunohistochemistry analyses were performed to examine the proteolysis of UbG76V-GFP in tauopathy. To test whether activation of PKA/cAMP pathway by rolipram could prevent overt tauopathy development, we administered 0.03mg/kg rolipram for 21 days to mice at an early stage tauopathy.

Results

Accumulation of insoluble tau correlated with progressive decrease in the peptidase activity of 26S proteasomes, while levels of ubiquitinated proteins and undegraded UbG76V-GFP increased. Affinity purified proteasomes were less active in hydrolyzing ubiquitinated proteins. Loss of proteasome activity was prevented by administering rolipram, which activates cAMP/PKA and proteasome subunit phosphorylation. Enhancing proteasome function led to reduced levels of aggregated tau and improved cognitive performance in early stage tauopathy.

Conclusion

Proteasome function and proteolysis by the UPS decrease with worsening tauopathy, and stimulating proteasome activity through cAMP/PKA is a promising therapy for tauopathy.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1677

PROTOPINE, A PROMISING NOVEL HISTONE DEACETYLASE 6 INHIBITOR REDUCES TAUOPATHY IN VITRO AND IN VIVO

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Introduction: Alzheimer's disease (AD) is a neurodegenerative disease characterized by the appearance of tau-associated neurofibrillary tangles. We found a novel function of protopine (PRO), a brain permeable isoquinoline alkaloid, isolated from the Chinese medicinal herb Rhiozma Corydalis (Yanhusuo in Chinese), reduces tau aggregates by inhibiting HDAC6 and by inducing proteasomal mediated degradation in certain neuronal cells.

Methods: Primary cortical neurons, N2a and SHSY5Y–Tau-P301L cells were treated with PRO in the presence or absence of autophagy and proteasome inhibitors. Cell lysates were investigated for Tau-5, PHF-1, acetylated (Ac)-α-tubulin, Ac-histone 3 and Hsp70. Three-month old male JNPL3 mice received PRO (10mg/kg/day) or saline by intraperitoneal injection until 6 months. Rotarod was performed to assess the motor function of mice. The docking studies of PRO with HDAC6 was performed using Autodock program.

Results: We found that PRO induces acetylation of α-tubilin without influencing the levels of Ac-histone 3 in cells. From docking study, the binding affinity of PRO with HDAC6 was calculated to be -7.65kcal/mol with a Ki value of 2.5μM. PRO reduces the accumulation of tau and ubiquintinylated conjugates, and simultaneously increases the level of Hsp70 in SHSY5Y-P301L. PRO enhances tau degradation via proteasomal degradation, but not via autophagy. In JNPL3 mice, PRO treatment did not significantly influence animal body weight. Three months of PRO treatment significantly attenuated motor dysfunction of JNPL3 mice. Immunohistochemical and immunblot analyses of various tau proteins in mice brains are underway.

Conclusion: Based on our results, PRO deserves further exploration as a promising therapeutic agent for AD.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1752

RESCUE OF IMPAIRED LONG TERM DEPRESSION IN THY-TAU22 TRANSGENIC MICE

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Objectives: Cognitive decline, a hallmark of Alzheimer's disease (AD), and accompanying neuropsychiatric symptoms share dysfunctions of synaptic processes as a common cellular pathomechanism. Long-term potentiation (LTP) has been found to be a sensitive indicator for such synaptic dysfunctions. However, much less is known about how other forms of synaptic plasticity are affected by AD progression.
Methods: We used long-term field recordings of long-term depression (LTD) in the CA1-region *in vitro* and tested the effects of inhibitors of glycogen synthase kinase-3 beta (GSK3β) and of sodium selenate.
GSK3ß phosphorylation sites were analyzed by Western blotting.
Results: We found that an impairment of long-term depression (LTD), an alternative cellular mechanism for memory storage, can be reversed in THY-Tau22 mice by either inhibition of glycogen synthase kinase-3 (GSK3β) activity or application of the protein-phosphatase 2A (PP2A) agonist selenate. In agreement with these results we observed increased phosphorylation of GSK3β at Y216 and reduced total phosphatase activity in biochemical assays of hippocampal tissue of THY-Tau22 mice. Notably, L-LTD induction and pharmacological inhibition of GSK3ß appeared to downregulate GSK3ß-activity via a marked upregulation of phosphorylation at the inhibitory Ser9 residue.
Conclusions: Our results point to alterations in phosphorylation/ dephosphorylation homeostasis as key mechanisms underlying the deficits in LTD and hippocampus-dependent learning found in THY-Tau22 mice.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1777

PATHOLOGY AND HIGH-THROUGHTPUT PHENOTYPING OF THE INDUCIBLE TG4510 TRANSGENIC MODEL

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Objective: The rTg4510 mice, overexpressing human tau with the P301L mutation under control of a tetracycline responsive transacting element, exhibit many salient characteristics of fronto-temporal dementia and Alzheimer's disease in an age-dependent manner including cognitive impairment, motor deficits, tau hyperphosphorylation, neurofibrillary tangles, and neuronal loss in the forebrain.
Methods: PhenoCube® NeuroCube® and SmartCube® are high-throughput platforms that assess circadian, cognitive, motor behavior, anxiety, gait, and other domains using PGI's proprietary Computer Vision automated scoring system and machine learning algorithms to define phenotypic signatures. Other standard test followed published protocols. Immunohistochemistry with fluorescence or with DAB labeling was used to detect epitopes of interest in hippocampus and cortex.
Results: Using our proprietary behavioral testing platforms we have confirmed the phenotype described by other labs including hyperactivity and cognitive deficits. We also found deficits in a procedural learning and reversal T-maze, in addition to a pronounced nocturnal hyperactivity. The deficits in the Tg4510 are-age dependent unlike those of the tTa control, which are significant at the earlier ages. We present an assessment of pro-inflammatory transcripts expression, synaptophysin and phosphorylated Tau levels in the transgenic mice.
Conclusion: The tg4510 mouse represents a highly reproducible model of tauopathies that allows sensitive testing of drug effects on brain pathology and behavior. The option to switch off tau expression by doxycycline is a most suitable calibration for assessment of effects of new treatments.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-1910
MIRROR IMAGE PHAGE DISPLAY FOR SELECTION OF TAU-BINDING D-ENANTIOMERIC PEPTIDES FOR THERAPEUTIC APPLICATIONS IN NEURODEGENERATIVE DISEASES

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Objectives: A variety of neurodegenerative disorders, including Alzheimer's disease, are associated with neurofibrillary tangles composed of the tau protein. Inhibitors of pathological tau fibril formation could be useful for the development of therapeutics, provided that the inhibitors were specific enough to avoid interference with physiological processes.
Methods: We have developed biotechnological strategies to identify specific peptides out of large phage displayed peptide libraries by specialized selection procedures. In order to avoid disadvantages of natural peptides (e.g. protease sensitivity, immunogenicity), we have applied mirror image phage display to yield D-enantiomeric peptides that are extremely protease stable and not or less immunogenic than L-peptides.
Results: Employing mirror-image phage display with a large peptide library (> 1 billion different peptides), we have identified tau-fibril binding peptides consisting of D-enantiomeric amino acids. Here, we report on various D-enantiomeric peptides which bind to aggregating tau variants as well as full length tau fibrils and modulate the aggregation thereof.
Conclusions: These peptides might be an interesting starting point for therapy development.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-2092

TAU PROTEOLYTIC ACTIVITY: A MECHANISTIC TARGET FOR DRUG DISCOVERY

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Objectives: The particular structure and mechanisms by which tau oligomers exert their deleterious effects is an important focus of current research. The oligomeric forms of tau appear to be most closely associated with neuronal loss and memory impairment in mouse models of tauopathy and accumulate in AD brain. Importantly, extracellular tau oligomers have been shown to impair memory formation and to inhibit long-term potentiation in hippocampal slices. The general aim of this work is to elucidate and target toxic mechanisms of tau oligomers for drug discovery.
Methods: Recombinant human tau oligomers were purified. Autoproteolytic fragments were sequenced to determine cut sites. Peptides spanning a cut site were used as a substrate for assaying tau protease activity and for screening inhibitors. Protease inhibitors were used to characterize the class of protease. Truncated constructs and mutagenesis were used to localize the active-site region of tau. A cell assay was developed to screen inhibitors of tau protease activity.
Results: Tau acquires proteolytic activity enabling it to cut itself and other proteins upon formation of certain tau oligomer species. Tau oligomers belong to the class of serine proteases and are labeled with a functional probe for serine hydrolases. Antibodies specific for autoproteolytic tau fragments were developed. A point mutation in tau blocks tau cleavage in a cell assay for tau protease.
Conclusions: Tau protease activity may provide a mechanism for some of the toxic characteristics associated with tau oligomers. Peptide and cell based assays for targeting tau protease have been developed for drug discovery.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-2237

OVEREXPRESSION OF HUMAN TAU IN MOTONEURONS OF DROSOPHILA IMPAIRS LOCOMOTION WHICH CAN BE RESCUED BY NOVEL TAU AGGREGATION INHIBITORS

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1. Objectives: The aggregation of Tau is linked to the onset and pathology of Alzheimer's Disease (AD) and other neurodegenerative diseases. In this study, we over-express full-length human Tau protein in motoneurons of Drosophila melanogaster and apply it as an *in vivo* model to test novel drug-like compounds that we have shown to reduce Tau aggregation *in vitro*.
2. Methods: We expressed full-length human Tau protein in motoneurons of Drosophila using the UAS-Gal4 system. Climbing tests were carried out on cohorts of six larvae or adult flies. Kruskal-Wallis test was used for statistical analysis.
3. Results: Compared to the wild type animals, overexpression of Tau in motoneurons caused a strong impairment in the locomotion capabilities of both larvae and adult animals. Oral administration of two novel compounds to flies overexpressing Tau resulted in a statistically significant increase in the climbing ability of treated versus untreated animals.
4. Conclusions: The presented Tau Drosophila model is a practical *in vivo* model for the functional screening of drug candidates for their ability to restore locomotion activity. On the basis of our results, the two compounds that restore locomotion in Drosophila are promising lead candidates for efficacy studies in relevant mouse models of AD.
This work was supported by the Hungarian Brain Research Program: KTIA_NAP_13-2014-0009.

04c. Therapeutic Targets & Mechanisms for Treatment: tau

ADPD5-2285

IDENTIFICATION OF SMALL MOLECULE INHIBITORS OF TAU AGGREGATION BY TARGETING MONOMERIC TAU AS A POTENTIAL THERAPEUTIC APPROACH FOR TAUOPATHIES

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[7]MTA-TTK-NAP B - Drug Discovery Research Group – Neurodegenerative Diseases Institute of Organic Chemistry, Research Center for Natural Sciences, Budapest, Hungary

1. *Objectives.* A potential strategy to alleviate the aggregation of intrinsically disordered proteins (IDPs) is to maintain the native functional state of the protein by small molecule binding. However, the targeting of the native state of IDPs by small molecules has been challenging due to their heterogeneous conformational ensembles.
2. *Methods.* To tackle this challenge, we applied a high-throughput binding screen using chemical microarrays, to screen monomeric full length Tau ($hTau^{2N4R}wt$) for small molecule binders. Next, small molecule binders were tested for their ability to inhibit the aggregation of different tau constructs *in vitro* and in N2a cells.
3. *Results.* The screen identified a diverse set of novel fragment and lead-like small molecules capable of binding $hTau^{2N4R}wt$, some of which had the ability to inhibit the aggregation of $hTau^{2N4R}wt$, three repeat Tau construct, and pro-aggregant mutant four repeat Tau construct $Tau^{4RD}DK280$ *in vitro* and in N2a cells. a novel set of drug-like fragment and lead-like compounds that bound to Tau.
4. *Conclusion.* These results demonstrate that Tau is a viable receptor of small drug-like molecules. Moreover, these results support the potential and practical feasibility of the therapeutic strategy to target the early phases of the aggregation pathway of IDPs by a small molecule to reduce initial nucleation events, thereby eliminating the formation of potential toxic oligomers.

We thank the Wellcome Trust (UK), Medical Research Council (UK), Elan Pharmaceuticals (USA), the Canadian Institutes of Health Research (Canada), Hungarian National Brain Research Program (KTIA_NAP_13-2014-0009, Hungary) for funding this project.

04g. Therapeutic Targets & Mechanisms for Treatment: kinases

ADPD5-1287
CASEIN KINASE 1 INHIBITORS BENEFICIALLY MODULATE TAU PHOSPHORYLATION AND CELL SIGNALLING PATHWAYS IN A TRANSGENIC MODEL OF HUMAN TAUOPATHY

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Non-proline-dependent casein kinase 1 delta (CK1d) is highly elevated in Alzheimer's disease (AD) brain tissue where it plays an important, early role in phosphorylation of tau, and regulates aspects of the cell cycle which are re-activated in AD neurons leading to cell death. CK1d represents a potentially important target for drug development in AD and further analysis of the activity of different CK1d-mediated pathways is required.
We have developed two selective CK1d inhibitors, PS110 and PS278-05, that are orally available and enter the brain at therapeutic levels. We have undertaken in vivo studies in the TMHT mouse model of tauopathy and monitored the effects on tau and downstream CK1d targets using targeted mass spectrometry, western blotting and immunohistochemistry, and applied SysQuant®, a global phosphorylation workflow, to determine the cross-pathway effects of CK1d inhibition in mouse brain tissue.
Both CK1d inhibitors improved cognitive performance in the TMHT mouse.
The compounds have reduced overall phosphorylation of tau, reduced tangles and increased cell count in hippocampus, and reduced active CDK5 levels. CK1d compounds have modified key pathways including those linked to amyloid processing, oxidative phosphorylation and energy production. In vitro toxicity studies confirm that neither compound showed adverse effects, and in vivo trials show the compounds have good stability and absorption, and are long-lasting.
Inhibition of CK1d affects multiple cell signaling pathways and we will present data showing which pathways are most active in a mouse model of tauopathy and the relevance of these findings to CK1d as a target in human disease.

04i. Therapeutic Targets & Mechanisms for Treatment: other enzymes

ADPD5-1563

CHRONIC O-BETA-N-ACETYLGLUCOSAMINYLASE INHIBITION WITH THIAMET-G PREVENTS TAU PATHOLOGY AND HYPERACTIVITY IN RTG4510 MICE

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The abnormal hyperphosphorylation of the microtubule-associated protein tau plays a crucial role in neurodegeneration in Alzheimer's disease (AD) and aggregation of hyperphosphorylated tau into neurofibrillary tangles is a hallmark in AD. Besides kinases and phosphatases tau phosphorylation is regulated by O-GlcNAcylation, a posttranslational modification of proteins on the serine or threonine residues with β-N-acetylglucosamine (GlcNAc). O-GlcNAcylation is dynamically regulated by O-GlcNAc transferase, the enzyme catalyzing the transfer of GlcNAc to proteins, and N-acetylglucosaminidase (OGA), the enzyme catalyzing the removal of GlcNAc from proteins. Thiamet-G is a specific and brain permeable OGA inhibitor.
rTg4510 mice overexpress inducible human 4R0N mutant P301L tau and displayed age-dependent progression of tau pathology with tau hyperphosphorylation at AD-relevant epitopes, accumulation of the 64 kDa tau species, and neurofibrillary tangles in cortex and hippocampus. A subset of rTg4510 mice displayed a hyperactive phenotype both in a novel environment and during the diurnal cycle in the home cage, reminiscent of agitated behaviour. The percentage of hyperactive rTg4510 mice increased with age and was prevented by suppression of tau transgene indicating that this phenotype correlates with the progressive tau pathology. Treatment of rTg4510 mice with Thiamet-G increased overall O-GlcNAcylation levels in the brain indicative for sufficient target engagement. Further, Thiamet-G increased O-GlcNAcylation levels on mutant tau isolated from rTg4510 brains. Chronic Thiamet-G treatment significantly reduced soluble and insoluble 64 kDa hyperphosphorylated tau levels and reduced the number of hyperactive rTg4510 animals.
These results support OGA inhibition by Thiamet-G as possible treatment concept to reduce tau pathology and dysfunction.

04l. Therapeutic Targets & Mechanisms for Treatment: sigma-1, metabotropic, muscarinic and other GPCRs

ADPD5-0411

BENEFICIAL EFFECTS OF A2A ADENOSINE RECEPTOR TARGETING IN A MOUSE MODEL OF TAUOPATHY

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Objectives. Consumption of caffeine, a non-selective adenosine A_{2A} receptor ($A_{2A}R$) antagonist, reduces the risk of developing Alzheimer's Disease (AD) in Human and mitigates both amyloid and Tau burden in transgenic mouse models. However, the impact of selective $A_{2A}R$ blockade on the progressive development of AD-related lesions and associated memory impairment has not been investigated. In the present study, we explored the outcome of $A_{2A}R$ gene deletion in the THY-Tau22 transgenic mice, which progressively develop hippocampal Tau pathology and spatial memory defects.
Methods. $A_{2A}R$ KO animals and THY-Tau22 Tau transgenic mice were mated to obtain $A_{2A}R$ gene deletion in the Tau mouse model. We then analyzed the subsequent effects on both pathological (Tau phosphorylation and aggregation, neuro-inflammation) and functional impairments (spatial learning and memory, hippocampal plasticity, neurotransmitters profile).
Results. We found that deleting $A_{2A}Rs$ protects from Tau pathology-induced deficits in terms of spatial memory and hippocampal long-term depression. These effects were concomitant with a normalization of the hippocampal glutamate/GABA ratio, together with a global reduction in neuro-inflammatory markers and a decrease in Tau hyperphosphorylation. Interestingly, treatment of Tau mice with the specific $A_{2A}R$ antagonist MSX-3 also led to significant memory improvements as well as decreased Tau hyperphosphorylation.
Conclusions. By showing that $A_{2A}R$ genetic or pharmacological blockade improves the pathological phenotype in a Tau transgenic mouse model, the present data highlight A_{2A} receptors as important molecular targets to consider in Alzheimer's Disease and Tauopathies.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-0619

NEUROINFLAMMATION ENHANCES AUTOPHAGY AND ATTENUATES TAU PATHOLOGY IN A TAUOPATHY MOUSE MODEL

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OBJECTIVES:
Alzheimer's disease (AD) is pathologically characterized by extracellular amyloid-b deposits, intracellular hyperphosphoylated tau-formed neurofibrillary tangles, and microglia-dominated neuroinflammation. As the same as Ab, tau contributes to neurodegeneration. There is evidence showing that tau is degraded through autophagy, a cellular protective mechanism reported to be damaged in AD. In this project, we aimed to investigate the relationship between neuroinflammation and autophagy and the pathogenic role of neuroinflammation in tau pathology.
METHODS:
We established neuroinflammatory mouse models with both 3- and 17-month-old C57BL/6 mice by intraperitoneal injection of lipopolysacchride (LPS, 1mg/kg every two days for one week). We ablated IKKb protein in microglia in 17-month-old C57BL/6 mice using Cre-Lox technique to reduce aging-induced neuroinflammatory activation. Furthermore, we intraperineally injected human tau mutant (P301S)-transgenic mice with 0.15mg/kg LPS weekly by injection for 12 consecutive weeks to investigate the autophagy markers and the amount of phosphorylated tau with biochemical and histological methods. Additionally, we evaluated neuronal autophagy in SH-SY5Y cells expressing fusion proteins of mRFP-GFP-LC3b co-cultured with LPS-challenged macrophages.
RESULTS: We observed that, in both LPS-induced and IKKb ablation-reduced neuroinflammatory activation models, the autophagy activity as showed with protein levels of LC3-II, beclin1 and p62 in Western blot was always correlated with neuroinflammatory activity. Interestingly, the long-term administration of LPS at a low dose enhanced autophagy in P301S tau-transgenic mice, which was followed by a significant reduction of phosphorylated tau in the mouse brain. Similarly, LPS-activated macrophages enhanced LC3b-expressing cells.
Conclusion: Neuroinflammation enhances autophagy, and potentially degrading phosphorylated tau proteins in AD pathogenesis.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-1163

A MOUSE DOSING STUDY WITH PHARMACODYNAMIC AND EFFICACY OUTCOMES DEMONSTRATES THE POTENTIAL OF A NOVEL DRUG CANDIDATE TARGETING NEUROINFLAMMATION

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Objectives: Clinical studies and preclinical animal model investigations support the prevailing hypothesis that proinflammatory cytokine overproduction from activated glia can contribute to neuron degeneration and impaired cognitive function. Initial validation of proinflammatory cytokine overproduction as a targeted pathway raised the potential for new therapeutic approaches to acute and chronic brain disorders, such as traumatic brain injury (TBI) and Alzheimer's disease (AD). We previously reported that MW01-2-151WH (MW151), a novel small molecule candidate that can attenuate proinflammatory cytokine overproduction, is efficacious in mouse models of AD and TBI (BMCL17:414; J Neurosci 32:10201; J Neuroinfl 5:28; J Neurotrauma 27:1283). Dosing, including intervention time window, is a critically important next step.
Methods: We examined pharmacodynamic and efficacy outcomes after MW151 administration at different concentrations and post-injury time windows in a closed head injury (CHI) mouse model of diffuse TBI. Endpoints included glial activation, cytokine levels, and cognitive function.
Results: Post-injury administrations of MW151 at low doses suppressed the acute cytokine surge. MW151 ameliorated the cognitive deficits associated with the CHI, when given during the first 7 days post-injury.
Conclusions: Low dose MW151 intervention across an extended post-injury window can modify disease progression. Proinflammatory cytokine levels trending toward homeostasis is a viable pharmacodynamic endpoint.

05a. Drug Development & Clinical Trials: active vaccination

ADPD5-1115

PATIENT SATISFACTION IN A FIRST IN MAN STUDY OF A TAU IMMUNOTHERAPY AGENT

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Objectives: To examine the acceptability of a hospital setting for patients with Alzheimer's disease (AD), participating in an entry into man study of a novel immunotherapeutic agent, developed by AC Immune, targeting tau pathology.
Methods: It was considered necessary to conduct initial clinical testing of the vaccine in a hospital setting to maximise patient safety. Entry into man in AD patients was considered ethically acceptable and preferable compared to a study in healthy volunteers, as both the safety and the immunogenicity of the product are likely to depend on the study population.
Eight patients with mild AD were hospitalised for 24 hours in a special facility adjacent to the emergency care facility and intensive care unit of Turku University Hospital. The room was separate from other patient facilities. A member of the clinical study team accompanied the subject for the entire duration of the stay. Caregivers were allowed to stay in the same room overnight. Patient and caregiver experiences were assessed with an open interview.
Results: All subjects coped well in the facility and rated the hospital stay as pleasant and comfortable in all respects.
Conclusions: The use of an in-patient facility adjacent to the emergency and intensive care units provided an acceptable setting for AD patients participating in an entry into man study of a novel vaccine. Increased security of the setting in case of a medical emergency compared to a conventional phase I unit was achieved without compromising patient comfort and acceptability.

05e. Drug Development & Clinical Trials: tau modifyers

ADPD5-0434

NOVEL INHIBITORS OF AD RELEVANT KINASES IN RELATION TO TAU PATHOLOGY: IN VITRO ACTIVITY DATA AND FIRST IN VIVO RESULTS

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Objectives Close consideration has been taken into the tau pathology as perspective field for AD drug developments. Several protein kinases have been identified to cause pathologic tau phosphorylations. Resulting tau hyperphosphorylations finally lead to neurofibrillary tangles and the loss of tau function contributes to neuronal death. Novel protein kinase inhibitors related to tau pathology have been identified and characterized in *in vitro* studies of tau phosphorylation and tau content. First *in vivo* results of effectively influencing neuronal degeneration are presented.
Methods The target kinase activity of prepared lead structures was determined in *in vitro* assays. The kinase binding mode has been demonstrated by molecular modelling in enzyme docking studies. Cellular studies of toxicity, of tau protein phosphorylation and tau amounts will be presented. First *in vivo* results in mice with tauopathy will be reported.
Results Affinities of the more lipophilic compounds towards cdk1 and cdk5 were demonstrated by amino acid hydrogen bonding and additionally reasoned with hydrophobic interactions. A selective gsk3 beta affinity was shown for a methoxylated derivative by an exclusive binding to Arg141 of gsk3 beta. Demonstrated nontoxic inhibitor concentrations reduce tau amino acid phosphorylation and tau content. The most active compound proved to prevent further neuronal decay in a tauopathy mice model.
Conclusions Lipophilic 1-aza-9-oxafluorene lead structures effectively inhibited AD relevant kinases by demonstrated kinase interactions. The neuronal degeneration in demonstrated studies of motoneuronal abilities can be attenuated thus proving effective *in vivo* activities and a positive outcome of the kinase inhibition.

05e. Drug Development & Clinical Trials: tau modifyers

ADPD5-1267

DRUG CANDIDATES PROMOTING O-LINKED GLYCOSYLATION OF TAU FOR THE TREATMENT OF TAUOPATHIES

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Objective

The presence of neurofibrillary tangles (NFTs) is a characteristic hallmark of tauopathies which include amongst others Alzheimer's disease (AD) and progressive supranuclear palsy (PSP). NFTs are composed of aggregates of the microtubule-associated tau protein, and NFT pathology can be modified by inhibition the glycoside hydrolase, O-linked beta-N acetylglucosaminidase (OGA). Mechanistically, OGA inhibition blocks the removal of O-linked N-acetylglucosamine (O-GlcNAc) moieties from serine and threonine residues. This leads to an accumulation of O-GlcNAcylated tau protein that is less prone to aggregation. Following this rationale we sought to develop novel OGA inhibitors with the final aim to test the tau hypothesis in the clinic using PSP as initial indication.

Methods

A focused medicinal chemistry lead optimization campaign was performed starting from a series of novel OGA inhibitors. Candidate molecules with drug-like profiles were identified using a stringent assay cascade.

Results

The OGA inhibitor ASN-561 has been identified as a preclinical development candidate. ASN-561 has good potencies for inhibition of recombinant and cellular O-GlcNAcase enzymes combined with excellent brain penetration. ASN-561 demonstrated pharmacodynamic efficacy in wild-type mice upon single, oral dose utilizing O-GlcNAcylation of brain proteins as a readout for CNS target engagement. In JNPL3 tau transgenic mice ASN-561 increased the levels of tau which is O-GlcNAcylated at serine 400 up to 12-fold compared to vehicle at the highest dose. Preclinical studies supporting the investigation of the molecule in human clinical trials are currently being performed.

Conclusion

ASN-561 is an advanced preclinical OGA inhibitor with the potential to enter human Phase I safety and tolerability studies in 2015.

05e. Drug Development & Clinical Trials: tau modifyers

ADPD5-2288

DEVELOPMENT OF THE NOVEL SMALL MOLECULE TAU AGGREGATION INHIBITORS, PTI-51-CH3 (TAUPRO™) AND PTI-80, FOR THE TREATMENT OF TAUOPATHIES

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Accumulation of intracellular neurofibrillary tangles (NFTs) composed of aggregated tau protein is a key pathological hallmark of all tauopathies. PTI-51-CH3 (Taupro™) and PTI-80 have been identified and developed two potent small molecule tau aggregation inhibitors. Both compounds target the tau repeat domains that constitute the core of tau fibrils in NFTs, and have the ability to both prevent tau fibril formation and disaggregate pre-formed tau fibrils. The robust inhibitory activities usually occur at a PTI-compound:tau protein molar ratio of 0.3-0.4 in Thioflavin S fluorometry studies. In addition, PTI-51-CH3 and PTI-80 dose-dependently inhibit tau from forming beta-sheet-containing fibrils as determined by circular dichroism spectroscopy and electron microscopy. The inhibitory potency appears to be superior than those previously reported in the literature. As drug candidates, PTI-51-CH3 and PTI-80 also possess reasonable PK parameters in plasma and brain, and have reasonable brain exposure at the Cmax exceeding the estimated free-tau concentration range in neurons. Both compounds have safe drugability profiles with no cytotoxicity observed in cell culture studies, and no significant inhibition of CYP450 enzymes. PTI-51-CH3 and PTI-80 are currently being tested for in vivo efficacy in a transgenic mouse model. These mice express a human tau isoform with the FTDP-17 P301S mutation, that has commonly been used as a tauopathy animal model. Our results suggest that PTI-51-CH3 and PTI-80 are top pre-clinical candidates for future development as tau aggregation inhibitors for the treatment of Alzheimer's disease, progressive supranuclear palsy and other tauopathies.

Funded by ProteoTech Inc. and NIH Grant #AG039129

05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-0935

APPLICATION OF MICROSCALE THERMOPHORESIS (MST) TO DETECT PROTEIN FIBRIL - SMALL MOLECULE INTERACTIONS

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OBJECTIVES

Identifying novel small molecules bind to protein fibrils for the development of PET imaging agents for the diagnostics of Tauopathies such as Alzheimer's disease has proven challenging. For this purpose, we developed a sensitive biophysics based free solution methodology to quantify such interactions.

METHODS

MST binding experiments were conducted on a Monolith NT. 115 instrument. NT647-NHS dye was used to label both tau fibrils, generated in vitro, and intrinsically disordered tau monomers. Measurements were conducted using 25 nM protein with 0.05% Tween HEPES buffer titrating the unlabelled binding partner from < 1 nM to 100 times the K_D for the interaction. Enhanced gradient standard capillaries were used in each experiment using settings of 90% LED power and 40% MST power.

RESULTS

Smooth binding curves and binding affinities (K_D) were obtained for the interaction between tau fibrils and thiazine red (22±21 nM), lansoprazole (29±24 nM), a novel drug-like compound with K_D about 48 nM and between tau monomers and heparin (131±8 nM), consistent with data reported in the literature.[1,2,3]

CONCLUSIONS

MST shows promise as practical methodology for quantification of ligands binding to tau fibrils. K_D can be obtained with very small amounts of protein with repeatability of measurement results. The outlined MST methodology may be applied to other protein fibril systems and used for screening for small molecule binders of protein fibrils.

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05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-2060

TARGETING A2A RECEPTOR TO TREAT ALZHEIMER'S DISEASE: DESIGN, SYNTHESIS AND EVALUATION OF ANTAGONISTS

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Objectives.
Human A_{2A} adenosine receptor belongs to the superfamily of G protein coupled receptors and its blockade is highly relevant for therapeutics of Parkinson and Alzheimer diseases. Currently, only three compounds are still being tested in clinical phase for PD treatment. Even if they show good affinities for the receptor, there is still a need for improving their ADME properties by keeping their selectivity towards other adenosine receptors.
Methods.
Based on the recently published crystalline structure of the A_{2A} receptor complexed with ZM241385, a selective and high-affinity antagonist[1] and on a pharmacophoric model,[2] we designed new ligands using *in silico* docking studies starting from antagonists that we recently identified in our group.
Results.
Cyclic guanidine core was identified as a promising structure. We therefore developed an original 5-step synthesis enabling us to incorporate pharmacomodulations and to evaluate structure activity relationships. Affinity tests were then conducted on HEK293 cells membranes expressing A_{2A}R and cytotoxicity was evaluated on SY5Y cells.
Conclusions.
We designed a new chemical route to access our cyclic guanidine structures. For now, these molecules did not show better affinity than our firstly identified antagonist. SAR helped us to confirm the necessity of having a rigid cyclic structure and to identify substituent that seem to improve the affinity.

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05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-2063
IN SILICO DESIGN OF ADENOSINE A2A ANTAGONISTS
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Objectives.
Human A_{2A} adenosine receptor belongs to the superfamily of G protein coupled receptors and its blockade is highly relevant for therapeutics of Parkinson and Alzheimer diseases. Co-crystallization of A_{2A} receptor with selective antagonists was further analyzed from *in silico* studies to highlight polar and aromatic inter-molecular hotspots for design of novel antagonists.
Methods.
Docking-scoring processes were performed to select a suitable crystal structure and refine a virtual screening protocol trained from a library training set including most of known A_{2A} antagonists and thousands decoy ligands. This protocol was expected to screen new test sets like academic chemical libraries and *de novo* combinatorial libraries.
Results.
A preferential crystal A_{2A} structure was identified since molecular docking predictions of reference A_{2A} antagonists fit with experimental co-crystallized poses. After the training stage, the best screening performance was reached in the top-1% scored subset in term of enrichment and yield rate of true active by filtering docking poses satisfying aromatic stacking with Trp246 and hydrogen bond with Asn253. From a 2,200 molecules in-house library, these rules were applied to screen 7 virtual hits whose one exhibited a consistent A_{2A} binding affinity in the range of µM.
Conclusions.
Rich structural material about A2A receptor structure allows the design of suitable structure-based virtual screening protocols. Preliminary results of virtual screening onto a small in-house library let us expect very successful campaigns onto larger databases.

05v. Drug Development & Clinical Trials: structure-activity relationships

ADPD5-0403

LIGAND-INDUCED STRUCTURAL CHANGES OF THE HUMAN FARNESYL PYROPHOSPHATE SYNTHASE: IMPLICATIONS TO DRUG DESIGN FOR TAUOPATHY-ASSOCIATED NEURODEGENERATION

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Human farnesyl pyrophosphate synthase (hFPPS) is the gate-keeper of mammalian isoprenoids and the key target of bisphosphonate drugs. However, bisphosphonates suffer from poor “drug-like” properties and are mainly effective in treating skeletal diseases. Recent investigations have implicated hFPPS in various non-skeletal diseases, including tauopathy-associated neurodegeneration and progression of Alzheimer’s disease (AD). Consequently, the development of non-bisphosphonate inhibitors of hFPPS can provide molecular tools for validating this enzyme as a therapeutic target for AD. A structure-based, multistage screening protocol, based on differential scanning fluorimetry (DSF), NMR and crystallography, was developed in order to identify non-bisphosphonate inhibitors of hFPPS. These studies led to the discovery of a novel class of hFPPS inhibitors, which bind to a catalytically relevant allosteric pocket of the enzyme near the active site cavity. The synthesis and structure-activity relationship optimization studies of these compounds will be described. In addition, preliminary biological evaluation of these compounds for their ability to block tau metabolism in human immortalized neurons will be presented. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canadian Institute of Health Research (CIHR) and the Fonds de Recherché du Québec-Nature et Technologies (FRQ-NT).

06e. Imaging & Biomarkers: PET - tau

ADPD5-0880

DETECTION OF PHF-TAU PATHOLOGY WITH [18F]T807 IN BRAIN SECTIONS FROM FRONTO TEMPORAL DEMENTIA PATIENTS

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Objective: To establish the ability of the radiotracer [18F]T807, also known as [18F]AV-1451, to detect Tau aggregates in postmortem human FTD and FTLD brain tissue.
Methods: Frozen sections from FTD, FTLD, Alzheimer's disease (positive control) and age matched controls subjects were exposed to [18F]T807 and examined by autoradiography. Adjacent slides from the same tissue samples were stained with PHF Tau antibodies (AT8, AT100, 3R and 4R) in order to establish the presence of pathological Tau aggregation in the [18F]T807 positive regions. In addition, several fluorescent reporter compounds that compete with [18F]T807 binding were used to double stain these tissues to identify any colocalization between the tracer and PHF Tau aggregates revealed by the antibodies.
Results: The positive signals obtained by autoradiography from [18F]T807 in FTD and FTLD brain tissue were located in areas that contained PHF tau as determined by Tau antibodies. All [18F]T807 surrogate fluorescent compounds colocalized with immunofluorescence signal obtained with antibodies that stain pathological Tau aggregations but also with those that stain the 3R and 4R variants of the protein.
Conclusions: Our autoradiography results suggest that the radiotracer [18F]T807 is capable of binding Tau in FTD and FTLD tissue. Furthermore, fluorescent signals from T807 structurally related reporter compounds colocalized with tau antibodies AT8, AT100, 3R and 4R.

06e. Imaging & Biomarkers: PET - tau

ADPD5-1338

RELATIONSHIP BETWEEN MULTIPLE PET TRACERS' REGIONAL UPTAKE, INCLUDING THE NOVEL TAU BIOMARKER 18F-THK5117, AND NEUROPSYCHOLOGICAL PROFILE IN ALZHEIMER'S DISEASE TIME-COURSE

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Autopsy studies have shown that increasing cerebral tau pathology in Alzheimer's disease (AD) is associated with decline in cognitive functions. In contrast, the amount of amyloid plaques shows weak association with cognition in early AD, as suggested by amyloid PET imaging. So far, cerebral in vivo metabolism assessed using ^{18}F-FDG has been showing best correlations with cognitive performance, particularly memory. The recent development of novel radiotracers for in vivo assessment of tau pathology now offers a unique possibility to study the regional deposition of tau in the brain along with cognitive changes. The aim was to compare, at different stages of AD, the similarity/differences in regional hypometabolism with tau deposition measured by PET in relation to cognition.

Patients with AD as well as patients with Mild Cognitive Impairment (MCI) underwent three dynamic PET-scans using the novel tau tracer ^{18}F-THK5117, the amyloid ligand ^{11}C-PIB, and ^{18}F-FDG, as well as 3D structural MR. All participants also underwent a comprehensive neuropsychological examination covering five cognitive domains. Regional Standard Uptake Values normalized to cortical cerebellar uptake were calculated for the three PET tracers in each patient.

In this ongoing study, the relationship between PET tracers' regional uptake and cognitive scores in each clinical group is investigated.

In vivo measurement of PET tau pathology will result in new knowledge on the relationship between tau and cognitive functions across clinical stages of AD. This multi-tracer approach will give valuable information of regional brain changes and help reveal the yet unclear mechanisms linking pathological features and phenotype

06e. Imaging & Biomarkers: PET - tau

ADPD5-1406

REGIONAL DISTRIBUTION AND BINDING PROPERTIES OF 3H-THK5117 IN AD AUTOPSY BRAIN

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Objectives: To facilitate the early diagnosis of AD, it is important to understand the time course of each characteristic hallmarks of AD. In this study, we aim to comprehend the time course of Tau pathology with in vitro characterization of ^{3}H-THK5117, a PET tracer currently under development for Tau in vivo imaging. To interpret the relation with the other pathological hallmarks we also compared the binding of ^{3}H-THK5117 with ^{3}H-PIB (amyloid plaques) and ^{3}H-Deprenyl (astrocytosis).

Methods: The regional distribution of ^{3}H-THK5117 was determined with single concentration binding assay in differents brain regions on AD and control cases. Autoradiographies with ^{3}H-THK5117, ^{3}H-PIB and ^{3}H-Deprenyl were performed on whole frozen hemisphere sections from AD cases that, before death, had undergone in vivo ^{18}F-FDG and ^{11}C-PIB PET imaging.

Results: ^{3}H-THK5117 binding shows a difference in regional distribution with higher binding in AD compare to control. A negative correlation was observed between in vitro manual segmentation of ^{3}H-THK5117 autoradiography and in vivo FDG PET scan. The preliminary results for the laminar in vitro distribution showed a similar pattern for ^{3}H-THK5117 and ^{3}H-Deprenyl in hippocampus while we observed a different binding pattern for ^{3}H-PIB

Conclusions: THK5117 shows a high specific binding with difference between AD and control cases. The in vitro binding pattern is in accordance with Tau distribution in the brain observed with AT8 antiTau immunostaining. A similar regional pattern are observed with Deprenyl but not PIB. Finally, the negative correlation between ^{3}H-THK5117 and ^{18}F-FDG suggests a correlation between Tau deposit and neurodegeneration.

06e. Imaging & Biomarkers: PET - tau

ADPD5-1712

PRECLINICAL EVALUATION OF A NOVEL TAU PET TRACER [18F]THK-5351

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Objectives: *In vivo* imaging of neurofibrillar pathology will provide new insights into Alzheimer's disease (AD) and allow monitoring of disease progression and treatment effects. We developed [^{18}F]THK-5105 and [^{18}F]THK-5117 as tau PET tracers and successfully visualized tau deposition in the brain of AD patients. A drawback of these tracers is the non-specific white matter retention that may interfere with visual interpretation of PET images. We continued compound optimization and developed a novel tau PET tracer [^{18}F]THK-5351. **Methods:** In vitro binding of [^{18}F]THK-5351 to PHF-tau in the postmortem AD brain tissues was evaluated by in vitro binding assay and autoradiography. Binding kinetics were further evaluated and compared with [^{18}F]THK-5117. In vivo biodistribution of [^{18}F]THK-5351 in normal mice were performed. **Results:** [^{18}F]THK-5351 displayed high binding affinity with AD brain homogenates containing high amount of PHF-tau. In vitro autoradiography of [^{18}F]THK-5351 in AD brain sections showed the laminar distribution, which was completely different from that of [^{11}C]PiB. Microautoradiograpy also supported selective labeling of [^{18}F]THK-5351 to tau pathology in AD brain section. [^{18}F]THK-5351 showed faster dissociation from white matter and higher signal-to-background ratio than [^{18}F]THK-5117. Initial sufficient brain uptake of [^{18}F]THK-5351 and faster washout from normal mouse brain than [^{18}F]THK-5117 were observed. **Conclusions:** These results support [^{18}F]THK-5351 is a promising PET tracer for imaging tau pathology in humans and is expected lower background and higher contrast PET images of than [^{18}F]THK-5117.

06e. Imaging & Biomarkers: PET - tau

ADPD5-2173

TAU DEPOSITION AS MEASURED BY 3H-THK5117 IN CORTICAL LAYERS OF POST-MORTEM AD BRAIN IN COMPARISON WITH AMYLOID PLAQUES AND ASTROCYTOSIS

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Objectives
Tau pathology has been suggested to spread in Alzheimer's disease (AD) between interconnected neurons trans-synaptically. Thus, studying the distribution of tau in the different cortical layers of the brain can improve our understanding of tau's spreading patterns. Furthermore, by comparing the laminar distribution of tau, amyloid-beta and astrocytosis, the possible relationship between these pathological features can be understood.

Methods
The laminar distribution of hyperphosphorylated tau, fibrillar amyloid plaques and reactive astrocytes were assessed using the radiotracers ^{3}H-THK5117, ^{3}H-PiB and ^{3}H-L-deprenyl, respectively. The binding of each radiotracer through the cortical layers was quantified in autoradiograms of post-mortem whole hemisphere brain sections from three AD patients and one control.

Results
In a 60-year-old AD patient the ^{3}H-THK5117 binding showed a laminar distribution with highest binding in the superficial layers in most brain regions. In the temporal cortex, high ^{3}H-THK5117 binding was also observed in deeper cortical layers. The binding pattern was quite similar with that of ^{3}H-L-deprenyl (astrocytosis) while ^{3}H-PIB (fibrillar amyloid plaques) showed a more even distribution between the different cortical layers. In two other AD cases of 79 and 81 years of age, somewhat different binding patterns of ^{3}H-THK5117 were observed.

Conclusions
The present study reveals interesting patterns of cortical laminar distribution of tau deposition as measured by the tentative PET tracer THK5117 in autopsy AD brain, which coincides with astrocyte distribution but differs from amyloid deposition. The similarities and dissimilarities between these pathological markers in different cortical layers illustrate the complexity of AD pathology.

06j. Imaging & Biomarkers: PET - other

ADPD5-0225
RETINA EXAMINATION FOR TAU TANGLES AND BETA AMYLOID PLAQUES IN ALZHEIMER'S DISEASE

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Objective: To detect tau tangles and beta amyloid plaques in retina for the early diagnosis of Alzheimer's Disease (AD).
Methods: We examined 30 patients with mild cognitive insufficiency (MCI) and 15 age matched healthy controls. Age range was 64 - 85. Retina was examined by using fundus autofluorescence (FAF) and optical scanning tomography (OCT). FAF showed us lipofuscin which contained beta amyloid in AD and the layer of the accumulations was detected by OCT. Patients who had retinal lesions were given curcumin with Meriva for three days and FAF-OCT tests were repeated. Suspicious cases for AD were sent for brain PET- CT imaging.
Results: In 22 patients, tau tangles and plaques were observed on OCT. Curcumin stained the retinal accumulations in all 22 patients. Since curcumin binded to beta amyloid, it was proven that these plaques were related to AD. In all 22 patients, PET-CT results were consistent with bilateral temporo-parietal hypometabolism. No tangles or stained plaques were observed in the control group.
Conclusion: Our study suggests that tau tangles and beta amyloid plaques can be found in retinas of AD patients. This is the first study that reveals imagings of tau tangles and beta amyloid plaques in alive AD patients with FDA approved devices.

06j. Imaging & Biomarkers: PET - other

ADPD5-0226
RETINA EXAMINATION FOR TAU TANGLES AND BETA AMYLOID IN ALZHEIMER'S DISEASE

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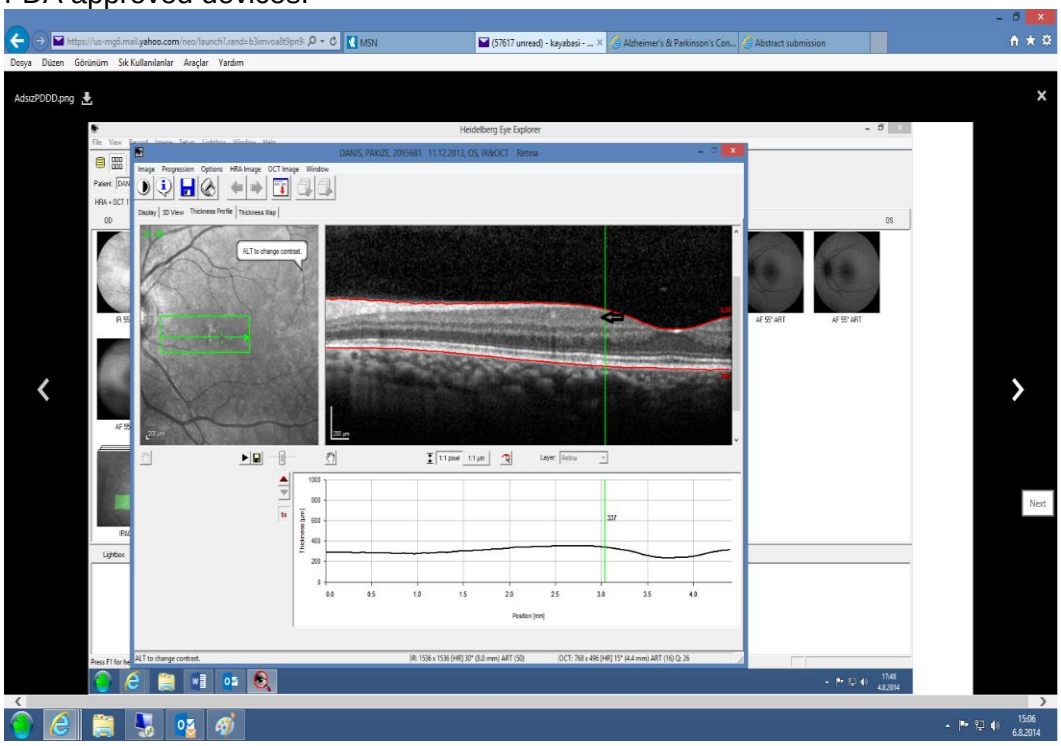
Objective: To detect tau tangles and beta amyloid plaques in retina for the early diagnosis of Alzheimer's Disease (AD).
Methods: We examined 30 patients with mild cognitive insufficiency (MCI) and 15 age matched healthy controls. Retina was examined by fundus autofluorescein (FAF) and optical scanning tomography (OCT) tests. FAF detected lipofuscin which contained beta amyloid in AD and the layer of the accumulations was detected by OCT. Patients who had retinal lesions were given curcumin with Meriva for three days and FAF-OCT tests were repeated. All the suspicious cases for AD were sent for brain PET- CT imaging.
Results: In 22 patients, tau tangles and plaques were observed on OCT. Curcumin stained the retinal lesions in all 22 patients. Since curcumin binded to beta amyloid, it was proven that the plaques were related to AD. All 22 patients had PET- CT results consistent with bilateral temporo-parietal hypometabolism. Tau tangles and curcumin staining was not seen in the control group.
Conclusion: Our study suggests that tau tangles and beta amyloid plaques can be seen in retina in an easier way and probably earlier than brain changes in AD. This is the first study that reveals tau plaque and beta amyloid imagings in alive AD patients with FDA approved devices.



06n. Imaging & Biomarkers : multimodal imaging

ADPD5-0422

FDG-PET CT AND TRODAT-1 SPECT IN DEMENTIA: REDEFINING THE TAUOPATHIES AND ALPHA SYNUCLEIONOPATHIES

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Objectives: To investigate the potential usefulness of FDG PET CT and 99mTc-TRODAT-1 SPECT imaging in the evaluation of subjects with suspected or diagnosed dementia with the following objectives:

- Documenting the complementary role of 99mTc-TRODAT-1 and FDG PET CT imaging in various neurodegenerative disorders
- Early diagnosis of dementia and minimal cognitive impairment
- Pre clinical diagnosis of dementia in high risk groups
- Differential diagnosis of different tauopathies and alpha-synucleinopathy

Methods: 50 patients with suspected or diagnosed dementia and 10 age matched healthy volunteers were evaluated using FDG PET CT and 99mTc-TRODAT-1. 99mTc-TRODAT-1 was prepared from a lyophilized kit and SPECT imaging performed using a double-head camera. FDG PET CT was done on a separate day by GE Discovery STE PET CT system.

Results: Different patterns of cortical and sub cortical hypo metabolism was noted in the different dementia sub types on FDG PET CT. Reduced striatal uptake of 99mTc-TRODAT-1 was found in all the alpha-synucleinopathies but in only few of the tauopathy.

Conclusion: FDG PET CT in conjunction with 99mTc-TRODAT-1, may serve as useful imaging agents for the early detection of dementia and differentiation of different tauopathies and alpha-synucleinopathy

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1301

MAPPING REGIONAL GREY AND WHITE MATTER DAMAGE IN PATIENTS WITH PROGRESSIVE SUPRANUCLEAR PALSY SYNDROME

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Objective. To investigate the pattern of grey matter (GM) atrophy and white matter (WM) damage in patients with probable progressive supranuclear palsy syndrome (PSPs) using MRI.

Methods. We enrolled 31 patients with probable PSPs and 22 matched healthy controls. Patients underwent clinical and neuropsychological evaluation, and brain structural and diffusion tensor (DT) MRI. The regional patterns of brain GM atrophy and WM microstructural damage were assessed using voxel-based morphometry and tract-based spatial statistics, respectively ($p<0.05$ FWE).

Results. PSPs patients were in a moderate stage of disease (Hoehn and Yahr score: 3.3) and showed mild to moderate cognitive impairment involving especially attentive-executive functions. PSPs patients did not show GM atrophy relative to controls. On the contrary, they showed a reduction of fractional anisotropy (FA) and an increase of mean, axial and radial diffusivities in the main WM tracts bilaterally, including body and splenium of corpus callosum, cingulum, inferior fronto-occipital, superior longitudinal and uncinate fasciculi, anterior and superior corona radiata, corticospinal tracts, and thalamic radiations. Superior cerebellar peduncles and internal capsules showed a significant increase of diffusivity values, but no FA changes.

Conclusions. In PSPs patients, WM microstructural damage is prominent compared to GM atrophy even in the moderate stage of the disease, suggesting that diffuse WM damage in tauopathies is not merely a function of disease severity. Regional differences in DT MRI metrics might reflect a different vulnerability of WM tracts. Our finding might provide new insight in understanding the pathophysiology of the disease and the clinical progression.

Funding: CurePSP MD505-12_001.

06r. Imaging & Biomarkers: other

ADPD5-0510

NON-PHOSPHORYLATED TAU IN THE CSF AS A NOVEL POTENTIAL BIOMARKER OF ALZHEIMER'S DISEASE

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Objectives: (a) technical validation of a first, to our best knowledge, ELISA method measuring concentrations of non-phosphorylated (non-P-Tau) molecules in the human cerebrospinal fluid (CSF); (b) testing if the CSF concentrations of non-P-Tau can be useful for an early diagnosis of MCI/AD.
Methods: First, a novel ELISA (AJ Roboscreen, Leipzig, Germany) has been technically validated taking into consideration its detection limit, linearity, precision, repeatability of the standard curves, and recovery. In the clinical part, the hypothesis was tested if non-P-Tau could discriminate patients with MCI and early AD whose clinical diagnoses were supported by 'classic' CSF biomarkers (Aβ1-42, Aβ42/40 Ratio, Tau, and pTau181) (n=58) from neuropsychiatric Controls without alterations in the CSF biomarkers (n=42).
Results: The method characterizes with very good inter- and intra-assay precision. The imprecision of the optical densities of the standards (six repetitions) was lower than 16% for all standards with the goodness of fit higher than 0.99. Patients with MCI/AD had statistically highly significantly increased non-P-Tau concentrations compared to the Controls (p
Conclusions: (a) the ELISA presented here characterizes with good analytical performance, including precision and repeatability; (b) for the first time we were able to measure non-P-Tau concentrations in human CSF; (c) our results suggest that CSF non-P-Tau could is worth consideration as a reliable and robust candidate biomarker of Alzheimer's Disease.

07c. Epidemiology, Risk Factors, Genetics & Epigenetics: metabolic

ADPD5-1166

PROTEIN EXPRESSION AND ACTIVITY OF MICROTUBULE AFFINITY-REGULATING KINASE (MARK2) CHANGE UPON ALTERED NUTRITION. A PROMISING LINK TO ALZHEIMER'S DISEASE?

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Objectives:

MARK2 is an important kinase regarding cell polarity, cytoskeletal structure and tau phosphorylation. Several upstream kinases of MARK2 are known, however regulation of MARK2 is not completely understood.

We aimed to investigate if nutritional factors can regulate MARK2.

Methods:

Adult mouse hypothalamic cells were treated with palmitate and subsequently analyzed for cell viability and MARK2 protein expression.

To investigate effects of a high fat diet (HFD) in animal models, we performed immunohistochemical analysis of active MARK (Thr208) in brains of mice treated with HFD over a period of 3 weeks. Furthermore hippocampal mRNA expression of MARK2 was analyzed by *in situ* hybridization with brains of wild-type (WT) and leptin deficient, obese $Lep^{ob/ob}$-mice.

Results:

After 12 h of palmitate treatment hypothalamic cells lost cellular processes. MARK2-proteinexpression and metabolic activity declined dramatically after 24 h of treatment and after 48 h cell death occurred. Observation of cytoskeletal structure is under progress.

Additional animal studies in WT-mice revealed a significant reduction of active MARK (Thr208) positive pyramidal cells in hippocampal CA2 region after 3 days of HFD. Furthermore $Lep^{ob/ob}$-mice, exhibited a significantly reduced hippocampal MARK2 mRNA expression compared to WT-mice.

Conclusion:

Our results strongly indicate that MARK2 is regulated by nutritional factors. Protein expression as well as protein activity is down regulated during a HFD in cellular and animal models. Based on these results we expect dramatic alterations in the cytoskeletal structure and propose that metabolic changes can trigger pathogenesis of neurodegeneration, like Alzheimer's disease via altered MARK2 protein expression and activity.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1019

TAU HAPLOTYPES AND THEIR ASSOCIATION WITH ALZHEIMER'S DISEASE ENDOPHENOTYPES IN THE AUSTRALIAN IMAGING, BIOMARKERS AND LIFESTYLE STUDY OF AGING

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Objectives: Aggregates of hyper-phosphorylated tau protein are one of the main neuropathological hallmarks of Alzheimer's disease (AD) and other neurodegenerative disorders.

The Microtubule Associated Protein Tau (MAPT) gene contains 2 haplotypes, H1 and H2. Studies show the H1 clade is diverse containing a number of sub-clades, compared to the H2 clade which has been shown to be a single non-recombining haplotype. The H1c haplotype, in particular, has been associated with an increased risk of AD and progressive supranuclear palsy, as well as increased levels of CSF tau in AD. H1 haplotypes have also been associated with an increased risk of PD and Frontotemporal dementia.

This study aimed to use endophenotypes available within the Australian Imaging, Biomarkers & Lifestyle (AIBL) Study of Aging to investigate the effect of MAPT genetic variation with regards to AD risk.

Methods: Seven SNPs within MAPT, namely rs1467967, rs242557, rs3785883, rs2471738, rs7521, rs9468 and rs1800547, were genotyped using the OpenArray® platform, in 1473 AIBL samples. Genotyping rs9468 and rs1800547 allowed for the differentiation of H1 and H2 haplotype, while the other 5 SNPs specifically tagged variation within the H1 clade.

Results and Conclusions: Cross-sectional analyses focused on associations with clinical classification of disease, levels of CSF and peripheral tau protein, neocortical amyloid burden as measured using PET imaging, hippocampal volume and cortical thickness. Further, longitudinal analyses were conducted to determine genetic influences on an individual's rate of cognitive decline.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1280

GENETIC VARIANTS OF GSK3B ARE ASSOCIATED WITH BIOMARKERS FOR ALZHEIMER'S DISEASE AND COGNITIVE FUNCTION

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Background: Glycogen synthase kinase 3 beta (GSK3B) is the major kinase phosphorylating tau protein. Hyperphosphorylated tau is one of the hallmarks of Alzheimer's disease (AD). Despite extensive research, the role of *GSK3B* in AD pathogenesis is not fully understood.
Objective: To evaluate possible associations between gene variants of *GSK3B* and risk of AD.
Methods: Twelve *GSK3B* tag single-nucleotide polymorphisms (SNPs), together with the previously AD-associated rs334558, were analyzed in 583 AD patients and 673 controls. Analyses on single marker and haplotype levels were done to relate to risk of AD, Mini Mental State Examination (MMSE) scores and cerebrospinal fluid (CSF) biomarker levels of total tau (T-tau), hyperphosphorylated tau (P-tau_{181}) and amyloid-β ($Aβ_{42}$).
Results: After correction for multiple testing, we found a number of associations of gene variants with CSF biomarker levels and cognitive function in the AD patients. Firstly, rs334558 was associated with elevated T-tau levels. Next, rs1154597 showed association with reduced $Aβ_{42}$ levels. Lastly, rs3107669 was associated with lower MMSE scores.
Conclusion: We found *GSK3B* gene variants associated with cognitive function and CSF biomarkers T-tau and $Aβ_{42}$. To our knowledge, this is the first time *GSK3B* has been associated with cognitive function or CSF biomarkers reflecting neuronal degeneration (T-tau) and brain amyloid load ($Aβ_{42}$). The regulation of *GSK3B* needs to be investigated further, to fully understand how these *GSK3B* gene variants are involved in AD pathogenesis.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1546

TAU PATHOLOGY AS QUANTITIATIVE TRAITS IN A GENOME-WIDE ASSOCIATION STUDY TO IDENTIFY GENETIC MODIFIERS IN PROGRESSIVE SUPRANUCLEAR PALSY

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Objectives: PSP arises from a combination of genetic and environmental factors which lead to intermediate pathological changes in the brain, eventually causing cell death and disease. We hypothesize that there are genetic variants associated with the PSP disease process that can be identified using neuropathologic features as phenotypes in a genome-wide association study.

Methods: Tau pathology scores were assessed in 970 pathologically-confirmed PSP cases for four tau lesion types: 1) neurofibrillary tangles 2) coiled bodies 3) tufted astrocytes, and 4) tau threads by one neuropathologist from 1998 – 2013. Tau lesions were scored (0,1, 2, or 3) in 18 brain regions. This dataset was converted into latent traits using the ltm R Package, which effectively reduces the dimensionality into a single, continuous variable for regression analyses. Linear regression was employed to test for association between latent traits using genome-wide genotyping in Stage 1 (498 PSP cases), and using age and sex as covariates. The top 35 SNPs from Stage 1 ($P < 10^{-5}$), were then genotyped in Stage 2 (406 PSP cases) using Sequenom MassArray platform, and association testing was performed.

Results: Preliminary results from a meta-analysis of Stage 1 and 2 identified a strong association located at chr3q27 within *ST6GAL1* (ST6 beta-galactosamide alpha-2,6-sialyltranferase 1) with variability of tau-immunoreactive coiled bodies and tufted astrocytes in hindbrain structures having the most significant associations.

Conclusions: We have identified genetic variants which associate with clinicopathologic heterogeneity of PSP, having the potential to reveal novel molecular pathways involved in the PSP disease process.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-1696

COPY NUMBER VARIATION ANALYSIS OF THE 17Q21.31 REGION AND ITS ROLE IN ALZHEIMER'S AND PD

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Objective: The 17q21.31 inversion polymorphism (leading to H1/H2 haplotypes) has been related to Alzheimer's (AD) and PD. Recent analyses have described several copy number variants (CNV) within 17q21.31 with a high diversity range in humans. We aim to analyze the structural variation of this chromosomal region through a high-resolution approach, and test whether differences in CNV could influence the risk of AD and PD.
Methods: We recruited 390 PD patients, 505 AD patients and 388 unrelated controls from Spain, previously genotyped for the H1/H2 haplotype. A new molecular approach based on Droplet-Digital PCR was used to determine the precise genomic dosage of the three known CNV that are present on the 17q21.31 chromosomal region.
Results: Our estimation of copy number duplications revealed a high degree of variation, ranging from two to eight copies. The PD group had a significant overrepresentation of the H1 allele; although the pattern of CNV distribution was similar to controls in the whole region. The H1/H2 haplotype was not associated to AD risk. However, our preliminary results indicated an excess of CNV in controls compared to AD patients.
Conclusions: Droplet-Digital PCR is a highly precise method to analyze CNV polymorphisms with multiple alleles. Copy number variation of the 17q21.31 region does not seem to meaningfully affect the PD risk. Our preliminary data indicates that AD patients have a distinct pattern of CNV compared to controls. Further analyses are in progress in order to elucidate the role of CNV within 17q21.31 and AD risk.

07o. Epidemiology, Risk Factors, Genetics & Epigenetics: histone modification, DNA methylation

ADPD5-2043

CBP/P300 ACETYLTRANSFERASE ACTIVATOR CSPTTK21 RESTORES MEMORY AND PLASTICITY DYSFUNCTIONS IN A TAU MOUSE MODEL

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Objectives: The underlying causes of memory loss associated with Alzheimer's disease (AD) are poorly understood and effective treatments are still under investigations. Several studies point to new therapeutic interventions by modulating brain acetylation levels with the use of histone deacetylase (HDAC) inhibitors. We recently described a new molecule (CSP-TTK21) that promotes CBP acetyltransferase activation in the brain. Herein, we used a transgenic mouse model of tauopathy (tg22) that develops neurofibrillary tangles and memory impairments, to assess the potential therapeutic effect of CSPTTK21.
Methods: we combined behavioral, biochemical and genome-wide molecular analyses.
Results: We found that CBP levels were decreased in the hippocampus of tg22 relative to WT mice. In addition, a global decrease of H2Bac acetylation (H2Bac), a CBP-targeted histone mark, was observed at a time where tg22 mice presented spatial memory impairments. ChIP-sequencing experiments confirmed that this mark was altered, showing a significant decrease of H2Bac at specific loci of genes important for synaptic plasticity and learning and memory. Remarkably, CSP-TTK21 treatment fully rescued long term retention of spatial memory performances and specifically increased H2Bac levels at learning and memory gene loci in the hippocampus of tg22 mice. How this translates on gene expression is currently being evaluated. Finally, we found that CBP levels were severely decreased in post-mortem brains from AD patients.
Conclusions: Our results strongly support that improving CBP histone acetyltransferase activity with molecules such as CSP-TTK21 could stand as a valuable epigenetic therapeutic option for the treatment of memory and plasticity dysfunctions associated with AD.

07p. Epidemiology, Risk Factors, Genetics & Epigenetics: other epigenetic factors

ADPD5-1316

ACUTE TRAUMATIC BRAIN INJURY INITIATES EARLIER AND MORE SEVERE TAU PATHOLOGY IN P301S TRANSGENIC MICE

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Background: History of traumatic brain injury (TBI) seems to be a strong epigenetic factor that can initiate molecular cascades increasing the risk of Alzheimer's disease (AD) or dementia. Following an acute TBI, individuals exhibit an elevation of A-beta and hyperphosphorylated tau (pTau) accumulation, suggesting a pathological link between TBI and AD. Furthermore, individuals with TBI experiences can develop chronic traumatic encephalopathy or dementia pugilistica having neurofibrillary tangles (NFTs) reminiscent of AD. ***Objective:*** To assess if a single, acute TBI increases the levels of pTau, which then form the first aggregates that can spread this pathology throughout the brain, resulting in early and more severe pathological changes. ***Methods:*** 3 month old P301S tau transgenic mice (n = 5/ time point) were subjected to TBI by controlled cortical impact (CCI) and sacrificed 1 day, 1 week, 1 month, and 6 months post-TBI. Coronal brain sections were immunohistochemically stained and levels of pTau and NFTs were quantified. Late stage post-TBI P301S were behaviorally characterized by Rotarod and Barnes maze tasks. ***Results:*** Injured P301S groups exhibited acceleration and augmentation of pTau brain deposition compared to aged-matched sham controls at the various time-points. TBI exacerbated the distribution of pTau as seen in different brain regions. ***Conclusion:*** Acute TBI may initiate accumulation of misfolded tau aggregates, resulting in early and exacerbated development of typical tau pathology. We are currently analyzing whether the data support the hypothesis that TBI leads to the formation of the initial tau aggregated seeds that can spread in brain by a prion-like mechanism.

08a. Animal Models: transgenic mice

ADPD5-0483

THE ULTRASTRUCTURAL CORTICAL PATHOLOGY OF ADULT P301S TAU TRANSGENIC MICE WITH NEUROFIBRILLARY PATHOLOGY

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A classic hallmark of Alzheimer's disease (AD) is the accumulation of neurofibrillary tangles in neurons. Tauopathies are a heterogeneous group of dementias sharing a common feature, aberrant tau metabolism and tau aggregation. The most prevalent tauopathy is AD. While many transgenic (tg) animals that develop Aβ deposition have been studied at the ultrastructural (EM) level, there are relatively few systematic and comparative EM studies on tg animals with aberrant tau metabolism. Here we present a detailed study on adult P301S heterozygous tg mice and compare our EM data to tg animal models with Aβ-related pathology and prototypic taopathy. The characteristic axonal pathology observed in the hippocampus, piriform/amygdala as well as in white matter tracts was similar to that seen in APP tg animals, single tg (tau) and double (APP/tau) tg mice. Specific 12-20 nm tangle like filaments were observed in neuronal somata, dendrites, myelinated, non-myelinated axons and postsynaptic sites in tau tg mice. Neurons showing signs of non-apoptotic (necrotic) cell degeneration were often encountered while apoptotic cell death was relatively rare. Nevertheless apoptotic cells presented classic EM features of programmed cell death similar to that observed in tg mice with Aβ deposition. Compared to other APP tg models a characteristic feature of astrogliosis in P301S tau tg animals was the abundance of intracytoplasmic intermediate filaments in hypertrophic cell processes, and was similar to results observed in P301L tg mice. However, in P301S tg animals astrocytic hypertrophy was accompanied by phagocytosis of axonal debris.

08a. Animal Models: transgenic mice

ADPD5-0826

EXPRESSION OF A TAU FRAGMENT ASSOCIATED WITH TAUOPATHY INDUCES MOTOR AND COGNITIVE DEFICITS IN TRANSGENIC MICE

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Objectives: Tauopathies are characterised by highly phosphorylated aggregated forms of tau, including truncated tau, associated with neuronal loss and cognitive decline. We have generated a transgenic mouse expressing a specific N-terminally cleaved fragment of human tau (Tau35). This form of tau was identified in tauopathies containing deposits of predominantly four repeat tau isoforms, but is absent from tauopathies containing mainly three repeat tau isoforms. We hypothesise that Tau35 triggers tau aggregation, leading to cellular dysfunction and cognitive impairment in transgenic mice.
Materials and Methods: *Tau35 e*xpression was assessed using RT-PCR and on western blots. The behaviour of the Tau35 mice was examined using motor and cognitive assessment tests. Pathology was identified by immunohistochemistry using a range of tau and conformational antibodies. Muscle morphology was assessed using hematoxylin and eosin staining.
Results: Tau35 mice express low levels of the transgenically expressed tau fragment, which comprises approximately 10% of total tau in these animals. Tau35 mice exhibit phenotypic abnormalities of clasping and kyphosis, reduced lifespan, and early motor deficits, followed by neuromuscular impairment and cognitive decline. Abnormal tau immuno-reactivity in the hippocampus is evident inTau35 mice from 8 months of age.
Conclusion: The relatively low expression of a disease-related tau species in Tau35 mice distinguishes these animals from over-expression of wild-type or mutant human tau in most existing tauopathy mice. Our results showcase Tau35 mice as a novel *in vivo* model in which a tauopathy-associated tau fragment causes motor, cognitive and pathological changes in the brain that parallel those seen in human disease.

08a. Animal Models: transgenic mice

ADPD5-0943

INVESTIGATING ABERRANT HYPERPHOSPHORYLATED TAU IN THE LOCUS COERULEUS

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Objective: Recent studies suggest that hyperphosphorylated tau aggregates (or pre-tangle tau) may develop first in the locus coeruleus (LC), the brainstem noradrenergic nucleus that supplies norepinephrine to the forebrain and degenerates in AD, long before other pathologies associated with AD begin to appear in the rest of the brain. In conjunction with studies demonstrating the prion-like ability of aberrant tau to spread from one cell or one brain region to another, these findings have motivated us to study the consequences of hyperphosphorylated tau in the LC. Utilizing a mutant form of human tau (P301S) that is prone to hyperphosphorylation and aggregation, we have developed tools to study both its impact on LC neuron function and survival, as well as its spread from the LC to the forebrain.
Methods: Primary cell culture. Bacterial artificial chromosome transgenic mice.
Results: By crossing mice expressing green fluorescent protein driven by the tyrosine hydroxylase promoter with mice expressing P301S tau, we have successfully isolated and cultured primary LC neurons expressing aberrant tau to study their susceptibility to toxic challenges associated with AD *in vitro*. In addition, we have created a transgenic mouse that expresses aberrant hyperphosphorylated tau selectively in the LC.Using a bacterial artificial chromosome harboring the noradrenergic-specific dopamine beta-hydroxylase gene to express P301S tau.
Conclusions: These *in vitro*/*in vivo* models will allow us to assess the effect of aberrant hyperphosphorylated tau on LC neuron function and survival, as well as its pathogenic spread from the LC to other areas of the brain.

08a. Animal Models: transgenic mice

ADPD5-1208

BEHAVIOURAL COMPARISON OF TAU TRANSGENIC MOUSE LINES DIFFERENTIATES ALZHEIMER AND FTD PHENOTYPES

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Tauopathy patients show different symptoms including a progressive cognitive decline frequently associated with Alzheimer's disease (AD) and motor and emotional/psychiatric abnormalities with Fronto-Temporal Dementia (FTD). Moreover, genetic mutations are only confirmed for FTD, but not for AD. This suggests that different genetic mouse lines could mimic different forms of tauopathies. We here compare 4 tau transgenic lines in tasks that interrogate core-features of tau-related dementias (cognition, anxiety, anhedonia, circadian activity). Included are the NMRI-derived Line1 and Line66 over-expressing a tau fragment or 301S mutated tau constructs under Thy1 control (Melis et al., 2014, CMLS in press) and C57/Bl6 derived $PLB2_{Tau}$ mice with a knock-in of a full-length 301L/406W mutant Tau gene, as well as $PLB1_{Triple}$ mice (Ryan et al., 2013, CLMS 70:2603) with mutant tau and APP and presenilin expressed under the CamKIIa regulatory element. Female mice were 5-7 months of age. Behavioural results were dependent on background strain and gene construct. Relative to controls expressing high preference for novel objects/social partners rather than familiar ones, Line 1 mice had no recognition bias and Line 66 showed signs of neophobia by preferring familiar objects. In addition, anxiety and sucrose preference was lower only in Line 66 mice, and $PLB1_{Triples}$ were hyperactive. No other phenotypes were significant. These data suggest that low and localised expression of mutant tau in PLB mice at 7 months did not lead to severe phenotypes; in contrast Line 1 showed deficits consistent with AD and Line 66 with symptoms of FTD.

08a. Animal Models: transgenic mice

ADPD5-1341

EXAMINATION OF AN IMMUNE DEFICIENT TAU TRANSGENIC MODEL IMPLICATES THE ADAPTIVE IMMUNE SYSTEM IN NEUROFIBRILLARY TANGLE PATHOGENESIS

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Objectives:

A number of studies have shown that tau pathology can be influenced by microglia- and astrocytes-derived inflammatory signals. While these studies implicate the innate immune system in neurofibrillary tangle (NFT) pathology, there remains little data regarding the potential role of the adaptive immune system in tauopathy. To study the influence of T-, B-, and NK-cells in tau pathogenesis, we generated and examined an immune-deficient tau transgenic model.

Methods:

A well-established tau transgenic model (Thy-Tau22) was backcrossed onto a Rag2-/- il2rgamma -/- double knockout background to produce tau transgenic that lack T-, B-, and NK-cells (Rag-Tau22 mice). These mice were then compared to immune-intact tau transgenics to determine the effects of peripheral immune cell depletion on pathogenesis and cognitive dysfunction. Given the growing interest in stem cell transplantation for neurodegenerative disorders, we used these mice to examine the effects of tau pathogenesis on human neural stem cell engraftment, migration, and differentiation.

Results:

Rag-Tau22 mice lack B-cells, T-cells, and NK-cells, but otherwise appear normal and healthy. Our results suggest however that the deletion of these peripheral immune cells dramatically increases tau hyper-phosphorylation and accumulation and exacerbates behavioral dysfunction. We have also found that Human neural stem cells transplanted into RagTau22 mice can survive for at least 3 months and exhibit distinct migration and differentiation phenotypes.

Conclusions:

Thus far our studies suggest that Rag-Tau22 mice could provide a promising new approach to examine the role of the peripheral immune system in tau pathogenesis and the long-term effects of human stem cell xenotransplantation.

08a. Animal Models: transgenic mice

ADPD5-1353

NEUROINFLAMMATION AND COGNITIVE DECLINE IN A NOVEL TRANSGENIC MOUSE MODEL EXPRESSING A RARE TAU MUTATION A152T.

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Objectives:

Various Tau-mutations are associated with neurodegenerative disorders. Recently, a rare Tau mutation (A152T) has been described as a novel risk factor for frontotemporal dementia spectrum disorders and Alzheimer's disease. Tau A152T shows a decreased binding to microtubules and reduced tendency to form abnormal fibers (Coppola et al., HumMolGen 2012).

Methods:

To investigate molecular mechanisms of genetically provoked tauopathies, we have generated a novel transgenic mouse model expressing human full-length Tau with a rare mutation A152T (hTau40/A152T) under the control of the neuron-specific Thy1.2-promoter.

Results:

Immunohistological analysis of young hTau40/A152T mice demonstrated the presence of pathological Tau conformation (MC1) and Tau hyperphosphorylation (PHF1, AT8) combined with Tau missorting. Tau-aggregates detected by Gallyas silver staining and sarcosyl-extraction indicate a progressive co-aggregation of endogenous mouse Tau and exogenous human Tau. Old hTau40/A152T mice show a prominent neuroinflammation in response to hTau40/A152T accumulation. To monitor and quantify neuroinflammatory changes in living Tau transgenic animals, we crossbred hTau40/A152T and GFAP-luciferase reporter mice and confirmed the age-related activation of astrocytes by in vivo bioluminescence imaging (BLI). In contrast to other Tau-transgenic models with reduced protein clearance, hTau40/A152T mice show a strong induction of autophagy. Although hTau40/A152T mice develop Tau pathology in spinal cord and motor cortex, their neuromotor performance was unaffected. Importantly, impaired spatial reference memory manifest in hTau40/A152T mice at the age of ~16 months and is accompanied by neuronal loss.

Conclusions:

The hTau40/A152T mouse model mimics pathological hallmarks of tauopathies and offers the potential to evaluate new therapeutic strategies against Tau-induced neurodegeneration.

08a. Animal Models: transgenic mice

ADPD5-1954

AMELIORATION OF SPATIAL PROBLEM-SOLVING COGNITIVE DEFICIT IN TAU TRANSGENIC MICE BY TREATMENT WITH THREE TAU-AGGREGATION INHIBITORS

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The abnormal folding and aggregation of the microtubule-binding protein tau is a key pathogenic hallmark in Alzheimer's disease (AD) and other neurodegenerative disorders, collectively named tauopathies. AD represents a major socioeconomic problem for which more effective therapies are required. In particular, compounds aimed to prevent and/or inhibit accumulation of tau aggregates hold considerable promise for the treatment of tau-related neurodegenerative diseases.

This study examined the effect of three tau-aggregation inhibitors (TAIs) in Line 1 mice, a novel model of tauopathy, in which a truncated fragment of human tau (AA 296-390) is overexpressed. Line 1 mice present with an age-dependent histopathological phenotype and are characterized by cognitive impairments revealed in a spatial problem-solving water maze (WM) task.

Methylthioninium (MT) acts as an inhibitor of tau aggregation in vitro and in vivo whether administered as chloride salt (MTC) or in the reduced leucoMT form (LMTX®). LMTX® is now been investigated in a phase 3 programme. The activity of MTC, LMTX® and a third diaminophenothiazine, ETN, have been compared in 5-months old line 1 mice.

Mice treated orally with one of the three compounds, were assessed in the spatial problem-solving WM test. All drugs were able to ameliorate the spatial deficits in Line 1 with ETN presenting efficacy already at the dose of 5 mg/kg. These findings support the use of TAIs other than MT as a disease-modifying treatment for AD and tauopathies.

08a. Animal Models: transgenic mice

ADPD5-2018

CHARACTERISATION OF A NOVEL P301S-TAU TRANSGENIC MOUSE MODEL OF FRONTOTEMPORAL LOBAR DEGENERATION

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Objectives

Frontotemporal Lobar Degeneration (FTLD) is one of the most common causes of early-onset dementia, and approximately 50% of cases are pathologically characterised by inclusions consisting of abnormally phosphorylated tau. Transgenic mice that express human tau carrying a mutation associated with FTLD are commonly used to investigate this disorder. In this study, we characterised a novel tau-transgenic mouse line, known as TAU58/2, which expresses human tau carrying the P301S mutation.

Methods

The motor phenotype of young and aged TAU58/2 mice was assessed using the Rota Rod and vertical pole test. Brain tissue was analysed with immunohistochemistry and silver staining. Tissue from various subtypes of FTLD, Alzheimer's disease and control patients was analysed for comparison.

Results

TAU58/2 mice showed early-onset and progressive motor deficits. Pathological hyperphosphorylation of tau was found throughout the brain resulting in increased neurofibrillary tangle formation with age. Gliosis was also observed in regions associated with pathological tau deposition, with activated microglia in close proximity to neurons harboring tau-positive inclusions. Interestingly, neurofilament-positive axonal swellings were seen in the TAU58/2 brain, prior to any substantial tangle formation, but coinciding with the onset of motor deficits. Similar neurofilament-positive pathology was also observed in human FTLD-tau and Alzheimer's disease brain tissue, but not FTLD with TDP-43 inclusions or controls. Together, this may suggest a role for neurofilament in mediating tau toxicity.

Conclusion

TAU58/2 mice recapitulate behavioural and pathological aspects of human FTLD-tau, making this an excellent model for studying the underlying pathological mechanisms, and for testing therapeutics in the future.

08b. Animal Models: transgenic rats

ADPD5-0827

NOVEL APPROACH FOR TESTING OF SENSORIMOTOR DYSFUNCTION IN TRANSGENIC RAT MODEL OF ALZHEIMER´S DISEASE BY FULLY AUTOMATED SYSTEM

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Objectives

Alzheimer´s disease (AD) is the most common neurodegenerative disorder characterized by memory impairment. The aim of the study was to set up the comprehensive testing method, which would provide quantitative reflection of sensorimotor alteration, would be sensitive enough to record early clinical symptoms and would have a high-throughput format.

Material and methods

In present study were used transgenic rat line SHR 72 and non-transgenic SHR age-matched controls. Testing was performed in two consecutive days in 3 timelines (3, 5, 6 months of the age of experimental animals). In each timeline three repetitions of correct trials were performed. For testing was used CatWalk XT A system for quantitative assessment of sensorimotor function in rodents.

Results

We observed measurable progressive decline of sensorimotor function in transgenic rats. From the total number of 63 variables we selected 26 in category „dynamic variables“, 4 in category „static variables“ and 5 in category „general run variables“. Early changed (in timeline – 3 months) were variables : Run duration, Stand, Stand Index, Contact area, Swing phase and Step cycle. Variables changed in late phase of experiment (timeline 5 and 6 months) were: Initial dual stance, Intensity and Regularity Index.

Conclusion

Hereby, we demonstrate novel approach for testing of sensorimotor deficits of animal models of AD, which is sensitive enough to detect even early changed symptoms without subjective influence of researcher. Acknowledgement This work was supported by research grant APVV-0200-11. ;

08c. Animal Models: primate models

ADPD5-1046

LONGITUDINAL BEHAVIOURAL CHARACTERIZATION OF TWO MODELS OF SPORADIC AND INHERITED TAUOPATHY IN NON-HUMAN PRIMATES

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Characterization of time-dependent changes in cognitive function observed in non-human primates (NHP) following hippocampal injection of adeno-associated viral (AAV) vectors overexpressing two forms of human Tau protein.
We recently demonstrated the ability of lentiviral vectors encoding Tau proteins to generate genetic models of tauopathies in rodents. These data stimulated the development of a similar model in NHP that allows for the preclinical exploration of functional deficits and offers additional opportunities for translational evaluation of innovative therapies.
Stereotaxic injections of AAVs were performed under preoperative MRI guidance in 12 adult males *Macaca fascicularis*. These NHPs were injected bilaterally into the hippocampal CA1 region with AAV2/9 vectors bearing a CBA promoter and overexpressing either the WT hTau46 (n=4), the hTau46 P301L mutated form of Tau (n=4) or a null GFP (n=4) where the reporter gene was not translated. All NHP were trained to perform a cognitive battery on tactile screens before and at 4, 8, and 12 months post-injection (p.i.) looking at performances in working memory, spatial memory and executive function.
Results suggest that overexpressing either WT hTau46 or hTau46 P301L in CA1 is sufficient to induce a cognitive deficit as early as 4 months p.i. that stabilizes at 8 months p.i. These NHPs showed no deficit in a visual discrimination task at 8 and 12 months p.i. suggesting the specificity of the impairment observed in spatial and recognition memory and executive functions.
These NHP models of sporadic or inherited tauopathies hold great promise for further investigation of novel therapeutic strategies.

08c. Animal Models: primate models

ADPD5-1048

SHORT AND LONG-TERM HISTOLOGICAL CHARACTERIZATION OF MODELS OF SPORADIC AND INHERITED TAUOPATHY IN NON-HUMAN PRIMATES

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Objectives: Histological characterization of non-human primate (NHP) models of sporadic and inherited tauopathies induced by hippocampal injections of various forms of human Tau proteins overexpressed by adeno-associated viral (AAV) vectors.

Background: We recently showed the feasibility of generating genetic models of tauopathies in rodents using lentiviral vector-mediated gene transfer. These data supported the development of a NHP model offering additional opportunities for the validation of imaging and biochemical biomarkers and the exploration of innovative therapies.

Methods: 14 adult male *Macaca fascicularis* received bilateral stereotaxic injections of AAV2/9 vectors bearing a CBA promoter and overexpressing either the WT hTau46 (n=5), the hTau46 P301L mutated form of Tau (n=5) or a null GFP (n=4) into the hippocampus (CA1). CSF and plasma samples were collected at 3, 5, 7 and 12 months post-injection (p.i.). Animals were sacrificed at 3 or 12 months p.i.

Results: Brain examination showed that both constructs led to tau hyperphosphorylation (AT8) and changes in tau conformation (MC1) in the hippocampus and connected cerebral regions as early as 3 months p.i. A further progression of the pathology (AT100 staining) was observed at 12 months p.i. in both WT hTau46 and hTau46 P301L injected NHPs.

Conclusions: This NHP model recapitulates the progression of lesions and the typical pathological features observed in various human tauopathies, holding great promise for further investigation of peripheral biomarkers as well as novel therapies.

08c. Animal Models: primate models

ADPD5-1099

CHARACTERIZATION OF TWO NON-HUMAN PRIMATE MODELS OF SPORADIC AND INHERITED TAUOPATHIES USING 18F-FDG AND 18F-DPA714 PET IMAGING

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PET imaging characterization of metabolic and inflammatory dysfunctions in two non-human primate models (NHP) of progressive tauopathies.

Tauopathy is correlated with progressive brain hypometabolism, and neuroinflammation has been identified as a key player in the pathology of the disease.

8 NHP, bilaterally injected into the CA1 hippocampal region with a viral vector (AAV2/9-CBA) overexpressing either WT hTau46 (n=4) or mutated hTau46 P301L (n=4), and 4 healthy controls underwent ^{18}F-FDG and ^{18}F-DPA714 PET imaging (FOCUS220, Siemens). Blood samples were withdrawn to measure the arterial input function and metabolite correction was performed for ^{18}F-DPA714. PET images were coregistered to corresponding MR-images and quantified using Patlak and Logan for ^{18}F-FDG and ^{18}F-DPA714 respectively. WT and mutated hTau46 groups were individually compared to healthy controls.

Segmentation of the hippocampus and its projection areas did not reveal any macroscopical atrophy as compared to controls. The mean CMRglu map of the mutated hTau46 group showed a strong pattern of hypometabolism in the parieto-temporal cortical region compared to controls, which did not reach significance due to one NHP. Hypometabolism was less present in the WT hTau46 group, and only in the parietal cortex. TSPO binding showed a global increase in parieto-temporal regions in the mutated hTau46 group ($p<0.05$), but not in the WT hTau46 model. The cerebellum remained unchanged in glucose metabolism and TSPO binding in both WT and mutated hTau46 groups.

These models of tauopathy will be used as a tool to screen in vivo new radioligands presumably specific for hyperphosphorylated tau and/or tau aggregates.

08c. Animal Models: primate models

ADPD5-2305

DETECTION OF SOLUBLE AMYLOID BETA AND TAU PROTEINS IN THE INTERSTITIAL FLUID OF NON-HUMAN PRIMATES BY IN VIVO MICRODIALYSIS

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Alzheimer's disease (AD) is the most common cause of age-related cognitive decline. Amyloid plaques of which amyloid is the main component and neurofibrillary tangles consisting of fibrillar tau aggregates are both hallmarks of Alzheimer's disease. Much attention has focused on the soluble forms of amyloid and tau proteins that are released by cells and can be found in the interstitial fluid (ISF). These soluble forms of the proteins are hypothesized to have a biological role on their own, and, in addition, contribute to the pathophysiology of AD. Previous work using in vivo microdialysis of the rodent brain has demonstrated the ability to detect the soluble forms of these proteins in mouse ISF as well as to modulate their levels in the CNS (Cirrito el al, 2003). In the current study we utilized push-pull microdialysis in the hippocampus and prefrontal cortex of 4-5 year old cynomologous monkeys to measure $A\beta_{42}$ and total-Tau protein in the ISF. In addition, $A\beta_{42}$ and total-Tau proteins were measured in the CSF and plasma of the same animals. We found that both $A\beta_{42}$ and total-Tau protein can be detected in the ISF from the cynomologous monkey. Studies are underway to further examine these proteins in response to pharmacological manipulation and to compare with the effects seen in rodents. Taken together with future pharmacological data in multiple species and compartments, this data may provide greater insight into the utility of in vivo microdialysis evaluation of pathogenic proteins as an impactful translational method in neurological disease drug discovery.

08d. Animal Models: drosophila

ADPD5-0835
IMPAIRED RETROGRADE TRANSPORT BY THE DYNEIN/DYNACTIN COMPLEX CONTRIBUTES TO TAU-INDUCED TOXICITY

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The R406W amino acid substitution in Tau is associated with Frontotemporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17) characterized by Tau-positive filamentous inclusions. These filamentous Tau-inclusions are present in a group of neurodegenerative diseases known as tauopathies, including Alzheimer's disease (AD). To gain more insight into the pathomechanism of tauopathies, we performed an RNAi-based large-scale screen in *Drosophila* to identify genetic modifiers of Tau[R406W]-induced toxicity. We screened a collection of fly lines, putatively silencing more than 7000 *Drosophila* genes using RNAi for their ability to modify Tau[R406W]-induced toxicity. This collection covers more than 50% of all protein coding fly genes and over 90% of all fly genes known to have a human ortholog. In summary, we identified 62 genes that, when silenced by RNAi, specifically modify Tau-induced toxicity. Among these modifiers were three subunits of the Dynein/Dynactin complex. Analysis on segmental nerves showed that pan-neuronal Tau[R406W] expression and concomitant silencing of Dynein/Dynactin complex member synergistically caused strong pathological changes within the axonal compartment, characterized by axonal inclusions, but only minor changes at synapses. These alterations did not cause locomotion deficits at larval stage, but manifested in adult flies. Thus our data suggest that Tau-induced detrimental effects originate from axonal rather than synaptic dysfunction. In conclusion, our findings suggest an important role of the Dynein/Dynactin complex in the pathomechanism in Tauopathies like FTDP-17 or AD.

08d. Animal Models: drosophila

ADPD5-1948

PHOSPHORYLATED TAU AFFECTS SUNDOWNING SLEEP BEHAVIOUR IN A DROSOPHILA TAUOPATHY MODEL

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Objectives

Sleep disturbance is often considered as a major risk factor in Alzheimer's and Parkinson's diseases (AD and PD); studies have linked poor sleep with both pathologies, monitoring the amyloid beta peptide (Aβ) levels in cerebrospinal fluid and brain deposits. The mechanisms underlying this possible linkage are not clear. *Drosophila* has been used to model toxicity of AD and PD aggregation. Recently, *Drosophila* has been used also to study sleep-like behaviours. Here we aimed to establish experiments to determine the effects of hyper-phosphorylated tau on sleep-like behaviours in the fly.

Methods

We expressed human 0N4R tau in combination with tau kinases in the circadian network of the fly brain. F1 progeny flies carrying the G1118GAL4 driver with or without both tau and BRSK2 were monitored for sleep-like behaviours over a seven-day period in Activity Monitors. PySolo and BeFly were used to analyse the amount, timing and duration of sleep-like episodes.

Results

Following birth, flies expressing hyper-phosphorylated tau showed sleep like defects at the beginning of evening sleep activity (zeitgeber time 13 to 14 hours). Aged flies further degenerated their sleep patterns during the daytime (zeitgeber time 0-3 hours). Additionally, aged flies showed increases in sleep-like episodes, but decreases in both the length of sleep bouts and their longest sleep-like bouts.

Conclusions

The *Drosophila* phenotypes are remarkably similar to those linking "sundowning" with dementia in humans, showing major sleep disturbances. We propose that *Drosophila* will be a good model to genetically analyse links between AD and PD toxicity and sleep defects.

08d. Animal Models: drosophila

ADPD5-2182

GENERATION OF TAU KNOCK-OUT AND HUMAN PATHOGENIC TAU KNOCK-IN DROSOPHILA MODELS USING MIMIC TRANSPOSONS AND CRISPR/CAS9

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Objectives: Abnormal accumulation of Tau has been implicated in Alzheimer's disease and more than twenty other neurodegenerative diseases. Despite its prevalence among neurodegenerative disorders, the functions of Tau in health and disease conditions remain widely elusive. We sought out to generate novel genetic models of *Drosophila melanogaster* to study the endogenous function of Tau as well as the molecular mechanisms underlying Tau-induced neuronal dysfunction and degeneration.
Methods: To generate a Tau loss-of-function model, we utilized a novel knock-out method whereby we took advantage of the vast collection of MiMIC transposon insertions in the *Drosophila* genome to insert a gene disrupting cassette. To study pathogenic Tau function, we knocked-in human wild-type and FTDP-17 clinical mutant coding sequences into the endogenous *Drosophila tau* locus. We first utilized the CRISPR/Cas9 system to replace the first exon of *Drosophila tau* with a cassette flanked by *attP* sites. In a second transformation step, we used PhiC31 integrase-mediated recombination to introduce cDNA sequences encoding human wild-type or mutant Tau, flanked by the endogenous 5' and 3' UTRs from the *Drosophila tau* transcript.
Results: Using MiMIC transposon and CRISPR/Cas9 technology, we successfully generated a Tau knock-out fly, as well as knock-in models expressing wild type or pathogenic mutant forms of human Tau. All transgenic flies were verified by sequencing, and gene expression was assessed by Western blot and immunohistochemical analysis.
Conclusions: The Tau knock-out and knock-in *Drosophila* models will serve as a powerful platform to study the physiological and pathological roles of Tau *in vivo*.

08g. Animal Models: natural & seminatural models

ADPD5-1170
HYPOTHERMIA IN VIVO – AN ACUTE MODEL OF TAU HYPERPHOSPHORYLATION
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Objectives
Enhanced and abnormal phosphorylation of the microtubule-associated protein Tau plays a major role in many neurodegenerative diseases, known as tauopathies. Recent evidence discloses the contribution of anesthesia-induced hypothermia in Tau hyperphosphorylation as novel, non-genetic and non-aging trigger [1]. Since Tau presents a promising therapeutic target, an acute, inducible model of Tau phosphorylation is a useful tool for studying central nervous system drug effects.
Methods
Wildtype C57BL6/J mice received either pentobarbital or vehicle under normo- or hypothermic conditions. Body temperature was evaluated every 5 minutes after treatment for a period of 30 minutes. Afterwards, brains were collected and histological and biochemical evaluations of total Tau and its phosphorylated species were performed. Additionally, cerebral pGSK3 beta, amyloid beta and alpha synuclein levels were investigated.
RESULTS
Pentobarbital induces hypothermia in mice and triggers hyperphosphorylation of different Tau sites which is attributable to enhanced GSK3beta activity. While total Tau levels remain unaltered under hypothermic conditions, amyloid beta levels in mice are significantly enhanced in contrast to unaltered alpha synuclein levels.
Conclusions
Pentobarbital induces hypothermia and Tau hyperphosphorylation in wildtype mice. Amyloid beta production is also induced under hypothermic conditions whereas alpha synuclein levels stay constant. Pentobarbital and hypothermia in wildtype mice are able to mimic pathogenic events of neurodegenerative diseases and are thus a useful non-genetic, acute model to study CNS drug mechanisms.
1. Maurin, H., et al., *Terminal hypothermic Tau.P301L mice have increased Tau phosphorylation independently of glycogen synthase kinase 3alpha/beta.* Eur J Neurosci, 2014. **40**(2): p. 2442-53.

08g. Animal Models: natural & seminatural models

ADPD5-1505

UNIQUE NEUROPROTECTIVE TAU ISOFORMS AND PROCESSING IN THE NAKED MOLE-RAT NERVOUS SYSTEM

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The microtubule associated protein tau is predominantly expressed in neuronal axons and functions to stabilize microtubules. Disregulated tau phosphorylation, folding, cleavage and/or cellular distribution coincide with neurodegeneration in numerous disorders including Alzheimer's disease. While such findings engendered the widely accepted dogma for pathogenic tau processing, commonly encountered environmental changes induce similar changes in tau but without toxic consequence.

Objective: Test the hypothesis that tau phenoplasticity is primarily a reversible neuroprotective response to homeostatic disruptions.

Methods: We exploit a rodent exceptionally suited to study physiological extremes, the naked mole-rat (NMR). These mammals naturally tolerate stressors known to alter tau metabolism including hypoxia, body temperature fluctuations (15°C - 40°C), reproductive/social status changes, development and aging (maximum lifespan 32 years). We conduct histological, biochemical and molecular assessments on neural tissue from NMRs collected during normal aging and altered environmental settings.

Results: NMRs express central and peripheral nervous system tau isoforms distinct from humans and mice, though highly similar in sequence (95% identical to human). NMR tau undergoes significant changes in expression (level and isoform), phosphorylation and subcellular distribution dependent on age, social status, body temperature and toxin exposure. Remarkably, these processes are generally reversible. Overall our data suggest that tau's exceptional sensitivity to perturbations in life stages and environmental situations are healthy physiological responses. NMRs may have naturally evolved neuroprotective mechanisms in tau metabolism to preserve neuronal function and stability in extreme living conditions, and similar processes may translate to other mammals and offer new molecular targets for neuroprotection especially in tau-associated diseases.

10b. Other: disease mechanisms

ADPD5-1443

HEPARAN SULFATE 3-O-SULFOTRANSFERASE-2 IS CRITICAL FOR THE ABNORMAL PHOSPHORYLATION OF TAU IN ALZHEIMER'S DISEASE-RELATED TAU PATHOLOGY

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Heparan sulfate 3-*O*-sulfotransferase-2 (HS3ST2 or 3OST2) is an enzyme predominantly expressed in neurons of some brain regions in where it generates rare 3-*O*-sulfated domains in heparan sulfates (HS). While HS persist at the cell membrane in normal brain, in Alzheimer's disease (AD) they accumulate at the intracellular level, in where they co-localize with the neurofibrillary pathology.

Objectives: We aimed to investigate whether 3OST2, and its 3-*O*-sulfated HS products, are involved in the mechanisms leading to the abnormal phosphorylation of tau.

Methods: We used two cells models and a zebrafish model of tauopathy (hTauP301L) combined with *in vitro* phosphorylation reactions, Western blot, FRET, ELISA assays, confocal microscopy, and gene silencing. Additionally, 3OST2 transcripts levels were analysed in AD brains.

Results: In cells, we show that under pathologic conditions 3-*O*-sulfated HS are internalized, co-localize with tau and favour its hyperphosphorylation, but not that of p38 or NF-kB(p65). *In vitro*, 3-*O*-sulfated HS bond to tau, but not to GSK3b, PKA or PP2A, inducing the tau abnormal phosphorylation. In zebrafish (hTauP301L), we demonstrated that inhibiting *3ost2* expression results in a strong inhibition of several abnormally phosphorylated tau epitopes in brain and in spinal cord, leading to a complete recovery of motor neuronal axons length and animal motor response to touching stimuli.

Conclusion: Upon binding to tau at the intracellular level, 3-*O*-sulfated HS produced by 3OST2 might act as tau molecular chaperons favouring its pathologic phosphorylation by different kinases. This opens a new route in the understanding of the disease mechanisms with forthcoming therapeutic possibilities.

10c. Other: preclinical research

ADPD5-1541

PROTEIN DEPOSITS IN THE BRAINSTEM AND OLFACTORY BULB IN PEOPLE OLDER THAN 50 YEARS OLD: A DESCRIPTIVE STUDY

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Objectives: We aimed to evaluate the presence and amount of protein deposit throughout the brainstem of donated brains from people with and without clinical dementia.

Methods: The samples were collected by convenience and with the informed consent of first-degree relatives. Fourteen brains were collected from individuals 50 years old or older who underwent death verification in the Forensics Department (FD) of Porto Alegre. We analyzed the brainstem and olfactory bulb by H&E stain and immunohistochemical techniques to AT8, beta-amyloid and alfa-synuclein.

Results: Of the 14 brains collected in this study, the tau protein was found in the brainstems of 10 (71.42%). Of the 7 individuals who had a final diagnosis of Alzheimer's disease (AD), 6 presented tau deposits in some region of the brainstem.

Conclusions: Protein deposits can pre-date the symptomatic phases of neurodegenerative diseases by years. The tau protein is particularly interesting: it might be found in the brainstem and olfactory bulb long before it reaches the limbic cortex, at which point symptoms occur.

10d. Other: diagnostics

ADPD5-0283
EVALUATION OF HIGHER CORTICAL FUNCTIONS OF SENILE DEMENTIA OF ALZHEIMER'S TYPE.
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Purpose. Evaluate the effectiveness of diagnostic disorders of higher cortical functions in patients with senile dementia of the Alzheimer's type using scales GDR (Global Deterioration Rating) and MMSE (Mini Mental Scale Examination).
Material and methods. Study involved 42 patients (22 men and 20 women) aged 65 to 77 years (mean age 70,8 ± 3,3 years) divided into 2 groups: group 1 (basic group) - senile dementia of Alzheimer's type - 35 patients, group 2 (comparison group) - 27 patients with chronic cerebral ischemia (II- IIIst.) with mild cognitive impairment. GDR and MMSEwereused for assessing the severity of cognitive impairment.
Results. These neuropsychological studies indicate the results of cognitive functions: in group I - GDR 5,0 ± 0,5 scores, MMSE 21,8 ± 4,05 scores. In group II - GDR 2,0 ± 0,5 scores, MMSE 27,0 ± 0,5 scores. Senile dementia develops in commonly characterized by a relatively sparse confabulation products. Confabulation shifted to a more or less distant past ideas about the environmental situation and the self (ekmnestic confabulation). At the stage of mild dementia clearly identified the most features of amnestic aphasia, amnestic disorder component of praxis, and in some cases, signs of constructive dyspraxia. There is a long preservation of motor component of praxis.
Conclusions. The total scores on the MMSE and the GDR is a sensitive indicator of cognitive deficits and higher cortical functions of mild to moderate dementia of the Alzheimer's type before, is effective in determining therapeutic approaches and tactics of early prevention in patients with dementia of the Alzheimer type.

10d. Other: diagnostics

ADPD5-0290

BEHAVIOURAL VARIANT OF FTD WITH TEMPORAL PREDOMINANT DEGENERATION: A UNIQUE CLINICOPATHOLOGICAL SYNDROME MOSTLY UNDERLINED BY TAU PATHOLOGY

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Objective: The behavioural variant of frontotemporal dementia (bvFTD) is currently defined by a specific behavioural and cognitive phenotype associated with imaging results showing frontal or anterior temporal atrophy or hypometabolism. We aimed at characterizing bvFTD patients with predominant atrophy or hypometabolism in the temporal lobes. **Methods**: We constituted two groups of longitudinally followed up patients diagnosed with bvFTD according to the FTD-consortium criteria: (i) patients with predominant atrophy or hypometabolism in the temporal lobe and (ii) patients with predominant atrophy or hypometabolism in the frontal lobe. We compared the behavioural, neuropsychological, genetic and neuropathological features across the two groups. **Results:** 62 patients were included in the study period from 2001 to 2014. The median age at onset was 57y (IQR 53-63). 27 patients were included in the temporal group. 35 patients were included in the frontal group. The temporal group was characterized by less frequent apathy and dysexecutive profile, but more impaired episodic memory and semantic knowledge at onset. A definite FTD pathology was obtained in 15 patients from the temporal group: argyrophilic grains disease (n=5), *MAPT* mutation (n=5), FTLD-TDP (n=3), corticobasal degeneration (n=1) and Pick's disease (n=1). **Conclusions:** Patients presenting with a clinical diagnosis of bvFTD and temporal predominant degeneration may represent a specific syndrome within the FTD spectrum. Moreover, this syndrome seems to be predictive of underlying Tau pathology, especially argyrophilic grains disease in patients with a late onset, and *MAPT* mutations in patients with significant family history of FTD.

10d. Other: diagnostics

ADPD5-1578

MOLECULAR DIVERSITY OF CEREBROSPINAL FLUID TAU INVESTIGATED USING QUANTITATIVE MASS SPECTROMETRY

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Microtubule-associated tau proteins are major actors in tauopathies, and specifically in Alzheimer's disease (AD) where hyperphosphorylated forms aggregate into neurofibrillary tangles. Understanding tau molecular profile with its multiple isoforms and post-translational modifications represent an important challenge. Mass spectrometry (MS) has recently allowed protein quantification in complex biological samples like the cerebrospinal fluid (CSF). To study tau which is found in femtomolar concentrations within the CSF, we first had to develop an innovative pre-fractionation strategy, which was not dependent on immuno-enrichment. We then relied on the sensitive multiplex peptide detection capability of targeted high-resolution MS to quantify 19 tau-specific peptides covering its entire sequence. This new method was used on a clinical cohort of patients with AD, frontotemporal lombar degeneration and progressive supranuclear palsy. Interestingly, CSF tau expression profile was characterized by a predominance of central core fragments and 1N/3R isoform presence. AD was associated to a unique profile, and very specific modifications of the tau peptide stoichiometry. Taken together these results provide remarkable information on tau modifications for future therapeutic interventions, and improved molecular diagnosis of tauopathies.

10d. Other: diagnostics

ADPD5-1745

CSF BIOMARKERS IN THE DIAGNOSIS OF SPORADIC AND GENETIC FORMS OF FRONTOTEMPORAL LOBAR DEGENERATION

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Objectives: CSF biomarkers are increasingly used in the differential diagnosis of neurodegenerative dementias, however the accuracy of these markers to distinguish Frontotemporal Lobar Degeneration (FTLD) from Alzheimer's Disease (AD) is still controversial. Therefore the advantage of investigating cohorts with a definitive diagnosis (mutation carriers or with neuropathology) has been highlighted. Our goal is to evaluate the contribution of the CSF biomarkers for the differential diagnosis of AD and FTLD, considering specifically the genetic forms of FTLD.

Methods: Two large sporadic dementia groups: AD (n=120) and FTLD (n=120), along with 20 carriers of FTLD mutations (C9orf72=9; progranuline=7; MAPTAU=2; SQSTM1=4; two patients harbouring two mutations) were investigated. CSF levels of t-Tau, p-Tau, Ab42 (Innogenetics) were determined by ELISA.

Results: In sporadic cases we identified Ab42 and t-Tau as the best biomarker subset to differentiate FTLD from AD (sensitivity=79%, specificity=84%). However, the diagnostic accuracy was still sub-optimal, with a significant percentage (24%) of FTLD patients showing a CSF-AD biomarkers profile. These FTLD patients were more likely to be women, ApoE-e4 carriers and of the PNFA clinical subtype. Considering the genetic forms of FTLD, two cases of familiar forms with progranuline mutations also presented a CSF-AD biomarker profile, although this pattern was absent in other affected members.

Conclusions: A CSF-AD biomarker profile can be seen in patients with a clinical phenotype of FTLD as well as in pathogenic mutation carriers (definitive diagnosis), reinforcing the need for the identification of new biomarkers, namely targeted at ubiquitinated-protein inclusions founded in neurons FTLD patients.

10d. Other: diagnostics

ADPD5-1915
A NEUROIMAGING RATING SCALE TO ENHANCE THE INTER-RATER RELIABILITY AND DIAGNOSTIC VALIDITY OF HUMMINGBIRD SIGN

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Although Hummingbird sign (HBS) is a distinctive feature of Progressive supranuclear palsy (PSP) vs. idiopathic Parkinson's disease (IPD) and other parkinsonian disorders, there are no consensus criteria. To enhance inter-rater reliability (IRR) and diagnostic validity, we developed a new radiologic rating scale for HBS (HBS-RS). Two raters blinded to the clinical diagnosis reviewed T1 midsagittal magnetic resonance images of 133 patients with IPD (n=93) or with PSP (n=40). The existence of HBS was assessed in two steps, separated by two weeks, first based on their own experience, and then according to the HBS-RS. The HBS-RS is comprised of 4 items (contour of third ventricle floor, shape of beak, shape of Hummingbird head, and midbrain atrophy), with weighted scores from 0 to 2.
IRRs of individual items in HBS-RS, and of composite scores, showed moderate to good agreement (κ, 0.479 - 0.766) and were observed to be highest in item #1. IRRs for HBS-RS total scores were better than HBSs (Cohen' κappa[κ], 0.666 VS. 0.596). Sensitivities and specificities varied depending upon the cut-off for each item or for composite scores. Sensitivities in each item were high (85.0 – 92.5) at low cut-off (0 VS. 1 or 2). Specificities reached higher than 80% by using composite scores of HBS-RS. Receiver operating characteristic curves for HBS-RS total score showed fair diagnostic accuracy for PSP (AUC, 0.76 and 0.73).
HBS-RS is a simple and measurable visual assessment tool to determine HBS with higher inter-rater agreement and adjustable diagnostic validity for PSP.

01a. Protein Misfolding & Aggregation: Tau

ADPD5-1247

THE PATTERN OF TAU PATHOLOGY IS DISTINCT IN CREUTZFELDT-JAKOB DISEASE

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Objective: To investigate whether the pattern of tau pathology in the hippocampus is influenced by the presence of pathological isoform of the prion protein.
Methods: We compared phospho-tau immunoreactivity (AT8) in 23 hippocampal regions in 26 cases of sporadic Creutzfeldt-Jakob disease (CJD) stratified into three molecular subgroups (MM1/MV1, MV2 and VV2). We compared AT8 immunoreactivity patterns between the molecular subgroups of CJD and other neurodegenerative diseases by mathematical analysis. Subsequently, we created a heat map of phospho-tau (AT8) immuoreactivity pattern to highlight the differences between the groups.
Results: We observed differences in the pattern of tau pathology between the CJD molecular subgroups as compared to Alzheimer-related neurofibrillary degeneration (Braak stages) and other neurodegenerative tauopathies. The tau pathology pattern in the VV2 subgroup was different not only form Braak stages 1 and 2 and but also from other CJD subgroups. Interestingly, the pattern of hippocampal pathology of MM1/MV1 group was comparable to that of progressive supranuclear palsy.
Conclusion: The pattern of phospho-tau pathology in CJD brain does not match the early stages of neurofibrillary degeneration according to Braak and Braak classification and shows differences between molecular subgroups of this disease.

01h. Protein Misfolding & Aggregation: PrP

ADPD5-0348

IATROGENIC CJD IN HUMAN GROWTH HORMONE RECIPIENTS IN THE UK

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Objectives: To date, 74 cases of accidental human-to-human transmission of Creutzfeldt-Jakob disease (CJD) have been reported in the United Kingdom (UK) in recipients of human growth hormone (hGH) derived from cadaveric pituitaries. Although hGH was withdrawn in 1985, cases of hGH-related iatrogenic CJD (hGH-iCJD) continue to occur. In this first comprehensive analysis, neuropathological features, prion protein (*PRNP*) genotype, and the biochemical properties of the disease associated prion protein (PrP^{Sc}) were determined in UK hGH-iCJD subjects. Results were compared with data from UK sporadic CJD (sCJD) cases.
Methods: Central nervous system (CNS) and peripheral tissues taken at autopsy from 38 UK hGH-iCJD patients were examined. Formalin fixed tissues were analysed by immunohistochemistry for PrP^{Sc} accumulation. Biochemical analysis of PrP^{Sc} in frozen tissue was performed by Western blot, following limited proteinase digestion.
Results: In hGH-iCJD patients, there was a strong bias towards the MV and VV genotypes at polymorphic codon-129 of *PRNP*, combined with type 2 PrP^{Sc} subtype (MV2 and VV2). In contrast, sCJD cases were predominantly of the MM1/MV1 subtype. Patterns of CNS neuropathology in hGH-iCJD showed similarities to sCJD VV2 and MV2 subtypes, but with more severe pathology in the cerebellum.
Conclusion: It has been proposed that human-to-human transmission of sCJD may be a function of host *PRNP* codon-129 genotype and the strain of agent involved. Our results suggest that in the UK hGH patients, iCJD has resulted from limited exposure to prion infectivity, most probably from hGH contaminated with either sCJD MV2 or VV2 tissue.

01h. Protein Misfolding & Aggregation: PrP

ADPD5-0795

FAMILIAL PRION PROTEIN MUTANTS INHIBIT HRD1-MEDIATED RETROTRANSLOCATION OF MISFOLDED PROTEINS BY DEPLETING MISFOLDED PROTEIN SENSOR BIP

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OBJECTIVES

Newly synthesized misfolded glycoproteins retrotranslocate from the endoplasmic reticulum (ER) to the cytosol for proteasomal degradation. Familial prion protein (PrP) mutants retrotranslocate abnormally. Here, we determined (1) PrP's retrotranslocation mechanism, (2) how familial PrP mutants avoid retrotranslocation, and (3) the effect of familial PrP mutant expression on heterogeneous misfolded ER protein retrotranslocation.

METHODS

The role of human E3 Ubiquitin ligase, Hrd1, on PrP retrotranslocation was assessed by overexpression or siRNA knockdown of Hrd1 and proteasome inhibition in human glioblastoma CR7 cells. Cells were submitted to subcellular fractionation to assess membrane-bound and cytosolic PrP levels. The effect of PrP mutants on PrP's and other Hrd1 substrates' retrotranslocation was determined by co-expression of PrP-V210I^{129V} and -M232R^{129V} in the presence or absence of Hrd1-dependent substrates, transthyretin^{D18G} or alpha-one-anti-trypsin^{NHK}. RT-PCR and western blotting evaluated misfolded protein sensor BiP levels in whole cell extracts.

RESULTS

We identified Hrd1, as a critical regulator of glycosylated PrP retrotranslocation in mammalian cells. PrP mutants not only failed to retrotranslocate, but prevented retrotranslocation of wild-type PrP, and Hrd1-dependent substrates, TTR^{D18G} and alpha-1-anti-trypsin^{NHK}. Mutant PrP decreased BiP levels by 50% and increased BiP turnover 10-fold. Over-expression of BiP rescued PrP-induced loss of retrotranslocation.

CONCLUSION

PrP mutants block a major pathway of misfolded protein degradation by down-regulating misfolded protein sensor, BiP. The accumulation of misfolded proteins could be most detrimental to terminally differentiated neurons, which, unlike dividing cell types, cannot cope with deregulated proteostasis by self-renewal. This provides a novel mechanism for familial PrP mutant-induced pathology.

01h. Protein Misfolding & Aggregation: PrP

ADPD5-1057

TRANSGENIC FATAL FAMILIAL INSOMNIA MICE INDICATE PRION-INDEPENDENT MECHANISMS OF PATHOGENESIS AND PHENOTYPIC EXPRESSION OF DISEASE

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Fatal familial insomnia (FFI) and a genetic form of Creutzfeldt-Jakob disease (CJD^{178}) are clinically distinct prion disorders linked to the D178N prion protein (PrP) mutation. The disease phenotype is determined by the 129 M/V polymorphism on the mutant allele, which is thought to influence spontaneous D178N PrP misfolding, leading to formation of distinctive prion strains with specific neurotoxic properties. Transgenic (Tg) mice expressing the mouse PrP homolog of the FFI mutation, develop a neurological illness with severe sleep disruption, different from that of analogously generated Tg(CJD) mice modeling CJD^{178}. No prion infectivity is detected in Tg(FFI) and Tg(CJD) brains by bioassay or protein misfolding cyclic amplification, indicating prion-independent mechanisms of neurotoxicity and phenotypic heterogeneity. Tg(FFI) and Tg(CJD) neurons accumulate mutant PrP in different compartments of the secretory pathway and show distinct morphological abnormalities of transport organelles, suggesting that mutant PrP-specific alterations of secretory transport may contribute to determine the disease phenotype.

01h. Protein Misfolding & Aggregation: PrP

ADPD5-1614

INTRAPERITONEAL INFECTION OF WILD-TYPE MICE WITH SYNTHETICALLY GENERATED MAMMALIAN PRION

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Prion hypothesis postulates that a protein conformation based agent is responsible for the infectivity of Transmissible spongiform encephalopathies (TSEs, also known as prion diseases). Synthetically generating prions *in vitro* with bacterially expressed recombinant prion protein strongly supports the prion hypothesis. However, it remains unclear whether the pathogenic properties of synthetically generated prion (sg-Prion) recapitulate those of naturally occurring prions, including the ability to transmit disease via routes other than the most efficient intra-cerebral inoculation, the neuroinvasion pathway, and the ability to induce specific pathological lesions of TSEs. Here we report intraperitoneal (i.p.) inoculation of wild-type mice with sg-Prion caused prion disease with an average survival time of 210 – 220 days post inoculation. Detailed pathological analyses together with a time course study revealed that the nature of sg-Prion induced lesions, including spongiform change, disease specific prion protein accumulation (PrP-d) and the PrP-d dissemination amongst lymphoid and peripheral nervous system tissues, the route and mechanisms of neuroinvasion were all typical of classical rodent prions. Moreover, the sg-Prion induced lesion profile confirmed that the sg-Prion is a unique murine prion strain, ruling out the possibility of inadvertent contamination. Our study revealed for the first time that, similar to naturally occurring prions, the sg-Prion is capable of causing prion disease via routes other than direct intra-cerebral challenge. Moreover, our results demonstrated that the *in vitro* generated sg-Prion caused neurodegenerative disorder recapitulates the pathological characteristics of naturally occurring TSEs.

01h. Protein Misfolding & Aggregation: PrP

ADPD5-2059

A NON-CLASSICAL ROLE OF ACETYLCHOLINESTERASE IN PRION DISEASES

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1 . Objectives

To investigate the influence of an *endogenous factor* on the conformational conversion of the cellular prion protein into the pathogenic isoform, we focused on acetylcholinesterase (AChE). There is compelling evidence that AChE is a widely distributed enzyme in mammalian tissues and fluids with a large spectrum of functions that go well beyond the hydrolysis of acetylcholine, such as the Aβ pro-aggregating activity that might contribute to Alzheimer's disease (AD) pathogenesis.

2. Methods

We addressed the hypothesis of a broader non-enzymatic function of AChE in prion diseases by combining biochemical, biophysical, cellular and *in vivo* approaches.

3. Results

We show that PrP can bind *in vitro* to AChE. This heterologous association induces aggregation of monomeric PrP and modifies the structural properties of PrP amyloid fibrils. We address the relationships between the formation of AChE/PrP hetero-assemblies and i) their enhanced cytotoxicity in primary neuronal cultures, as well as the extended survival of prion-infected $AChE^{+/-}$ mice; ii) the interference of dual-site binding AChE inhibitors with both PrP-AChE complex formation (*in vitro*) and prion accumulation (in cell cultures); and iii) the altered AChE homeostasis in both nervous and lymphoreticular systems of prion-infected mice and in chronically infected cell cultures

4. Conclusions

Our results indicate that AChE deserves consideration as a new actor in expanding pathologically relevant PrP morphotypes and as a therapeutic target for prion diseases. This could be also extended to AD, in which the functional involvement of both proteins has been previously described.

ADPD5-0369

EXTRANEURAL ROLE OF THE CELLULAR PRION PROTEIN IN COLORECTAL CANCER CARCINOGENESIS IN VITRO

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This study investigated the role of cellular prion protein (PrP^C) in colorectal cancer carcinogenesis, since PrP^C was found to aberrantly overexpressed in extraneural cancer tissues like breast, gastric, prostatic and pancreatic tumours. We revealed that overexpression of PrP^C by transfection correlates with the development of a more sustainable and chemotherapeutic drug-resistant LS 174T human colorectal adenocarcinoma cells. Endogenous and overexpression of PrP^C were assessed by immunoblotting and immunofluorescence microscopy. Cell growth in anchorage-dependent manner was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Overexpression of PrP^C was shown to increase viable cells in a time-dependent manner. Cell growth in anchorage-independent manner was evaluated by soft agar colony formation and anoikis assay. While LS 174T cells remained resistant to anoikis, overexpression of PrP^C further exacerbated the phenomenon. Overexpression of PrP^C also increased cell motility and invasiveness of LS 174T cells. Cell adhesion to extracellular matrix (ECM) using collagen type-I and fibronectin coated surfaces revealed increased cell attachment in LS 174T cells overexpressing PrP^C. Overexpression of PrP^C was found to mitigate doxorubicin-induced cell cytotoxicity in LS 174T cells. Analysis of apoptotic and necrotic cells with annexin V/PI-FITC staining showed that PrP^C overexpression attenuated apoptosis. Human apoptosis antibody array with 35 apoptosis-related proteins revealed that three inhibitors of apoptotic proteins (IAPs) - Survivin, XIAP, and cIAP-1 - were up-regulated in LS 174T cells overexpressing PrP^C when apoptosis is induced. In conclusion, the overexpression of PrP^C could enhance the invasiveness and survival of LS 174T colorectal cancer cells, indicating that PrP^C plays a role in colorectal carcinogenesis.

02k. Cell, Molecular & Systems Biology: PrP

ADPD5-0800

ER STRESS INDUCES PRION PROTEIN GENE EXPRESSION THROUGH RECRUITMENT OF SPLICED XBP1 AND CLEAVED LUMAN TO THE ERSE26 PROMOTER REGION.

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OBJECTIVES

The prion protein (PrP) is essential to prion disease and may contribute to Alzheimer's disease pathogenesis. Because these diseases exhibit perturbation of endoplasmic reticulum (ER) homeostasis, this study aimed at understanding the regulation of the prion gene (*PRNP*) expression by ER stress and its impact on neuronal survival.

METHODS

The impact of pharmacological ER stressors, and transcription factor overexpression on *PRNP* expression was assessed by western blot, qRT-PCR, and luciferase reporter assay. siRNA-mediated silencing highlighted the contribution of key transcription factors to ER stress-induced *PRNP* expression. *PRNP* promoter mutagenesis revealed ER stress regulatory elements (ERSE) involved in *PRNP* promoter regulation, and chromatin immunoprecipitation assessed transcription factor interaction with the *PRNP* promoter. The function of ER stress-induced *PRNP* expression on cellular survival was addressed by comparing condensed chromatin, and caspase activation levels in ER stressed PrP WT and KO hippocampal cells.

RESULTS

Pharmacological ER stressors, spliced XBP1 (sXBP1), and cleaved Luman (ΔLuman) overexpression increased *PRNP* promoter activity, PrP mRNA, and protein levels. XBP1 or Luman silencing decreased ER stress-induced PrP levels. Mutagenesis of the *PRNP* ERSE26 reduced pharmacologically-, sXBP1-, and ΔLuman-induced *PRNP* promoter activity. sXBP1 and ΔLuman interact with the *PRNP* ERSE26 promoter region, and ERSE26 homology is specifically conserved among primates. Prion knockout hippocampal cells are more vulnerable to ER stress-induced apoptosis.

CONCLUSIONS

Regulation of ER stress-induced *PRNP* expression by sXBP1 and ΔLuman constitutes an evolutionarily recent neuroprotective adaptation against ER stress-induced apoptosis that could collaterally exacerbate prion and Alzheimer's disease progression.

02q. Cell, Molecular & Systems Biology: protein degradation, proteasome & autophagy

ADPD5-0529

FLEXIBILITY OF THE OCTAREPEAT REGION IMPACTS PRP BETA-CLEAVAGE AND PRION DISEASE MANIFESTATION IN TG MICE

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Objectives: Alternative conformations of the PrP^{C} octarepeat region (OR), denoted components 1, 2 and 3, occur in the presence of different copper concentrations. We sought to test whether these different conformations cause different phenotypic properties.
Methods: Reiterative peptide mutagenesis yielded octarepeat variants with discrete component 1 or component 3 copper-binding geometries. We made transgenic (Tg) mice with component 1 PrP geometry (TgS1), component 3 geometry (TgS3) and also control Tg wt mice bearing identical 5' untranslated region leader sequences. Tg mice were phenotyped for the effects of mutant PrPs upon peripheral nerve myelination, spontaneous disease and response to prion infections.
Results: None of the Tg lines succumbed to spontaneous neurologic disease with observation periods of up to 600 days of age. S1 and S3 PrP resembled WT PrP in supporting peripheral nerve myelination. S1 and S3 Tg mice both accumulated PrP^{Sc} and infectious prion particles at levels lower than wt mice, but differed from each other in clinical presentation. Unexpectedly, S3 PrP expressed in the brain had an increased propensity to C2 (beta) cleavage adjacent to the main binding region for oligomeric forms of Abeta peptide. C2 overproduction occurred by a mechanism distinct from metal-catalyzed hydrolysis reported previously.
Conclusions: OR flexibility is concluded to impact diverse biological endpoints; it is a salient variable in infectious disease paradigms where levels of PrP^{Sc} and infectivity uncouple from the onset of clinical disease and is likely germane in familial prion disease where a single mutant OR produces multiple presentations within the same kindred.

ADPD5-0530

PRP WITH AN INFLEXIBLE OCTAREPEAT REGION HAS CONDITIONAL SENSITIVITY TO MISFOLDING

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Objectives: Alternative conformations of the PrP^C N-terminal octarepeat region (OR), denoted components 1, 2 and 3, occur in the presence of different copper concentrations. We set out to test the phenotypic impact of these different N-terminal PrP conformations.

Methods: Scanning of variant octarepeat peptides identified variants with discrete, component 1 or 3 copper-binding geometries. Corresponding alleles, S1 and S3, respectively, were engineered into a full-length mouse PrP to generate lines of transgenic mice and stable cell-lines.

Results: Striking divergence was noticed for the S1 and S3 alleles transfected into RK13 cells and challenged by *de novo* infection. Unlike S1 and wild type PrP, the S3 allele did not support replication of RML prions; instead S3 PrP produced a full-length protease-resistant PrP species in uninfected cells. Subsequent to deglycosylation the S3 PrP allele was characterized by simultaneous production of 17 and 21 kDa proteinase K-resistant fragments. Assessment of the S3 allele in RK13 cells grown in different conditions defined an important role for different culture media. Analyses to define the composition of media followed by reconstitution experiments allowed us to assign the crucial nutritional components conferring the ability of S3 PrP to form two protease-resistant species.

Conclusion: Our data establish that i) different conformations of the PrP OR can be associated with different phenotypic attributes, ii) the OR's are sensitive to nutritional factors and iii) that binding of nutritional factors to the OR's transduces effects in *cis* to affect folding of the PrP C-terminal domain *in vivo*.

02w. Cell, Molecular & Systems Biology: transcriptomics

ADPD5-1721

VALIDATION OF A NOVEL DISEASE GENE SIGNATURE IN CJD BRAINS

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Objectives. Prion diseases are fatal neurodegenerative disorders whose pathogenetic mechanisms are not fully understood. In this context, we conducted a microarray-based transcriptome analysis of brains from BSE-infected macaques and we identified a five-gene signature able to distinguish infected animals from controls: *HBB* and *HBA2* were down-regulated, whereas *TTR*, *APOC1* and *SERPINA3* were up-regulated. Our objective is validating this signature in CJD brain samples and eventually confirming the results at the protein level.

Methods. We collected about 100 mg of frontal cortex of vCJD, sCJD and fCJD affected patients. RNA was extracted using TRIzol and checked for quantity and integrity. Retrotranscription was carried out using Superscript III and RT-qPCR was performed on the five selected gene transcripts. Antibodies against the five encoded proteins were first checked on mouse tissues and then utilized to perform WB or IHC in human brain slices.

Results. The collection of the samples and the quality of the extracted RNA were a challenge, but the preliminary results are very encouraging and will be thoroughly discussed.

Conclusions. Some genes related to oxygen or lipid transport and to innate immunity were found to be dysregulated in prion infected macaques and are known to be involved also in Alzheimer's and PD. Validating this gene signature in brains of CJD affected patients would shed some light not only on prion disease molecular mechanisms, but also on neurodegeneration in general. The encoded proteins may become potential biomarkers for preclinical diagnosis and therapeutic purposes of many neurodegenerative diseases.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-1248

REDUCED CIRCULATING CHOLESTEROL LEVELS IN PLASMA PHOSPHOLIPID TRANSFER PROTEIN DEFICIENT MICE INCREASE SURVIVAL TIME FOLLOWING PRION INFECTION

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- Objectives : The various pathways of lipid/lipoprotein metabolism seem to play a key role in Alzheimer's (AD) and prion (PrD) diseases. Abnormal cholesterol metabolism is frequently observed in PrD, but whether cholesterol influences the neuroinvasion process is still unclear. Plasma phospholipid transfer protein (PLTP) affects the production of lipoproteins by the liver and is a major determinant of circulating cholesterol metabolism. In the present work, PLTP deficient mice ($PLTP^{-/-}$) displaying about 30% reduction in plasma cholesterol levels, were used as a model to study the influence of circulating cholesterol during prion neuroinvasion.
- Methods : C57Bl/6 WT and C57Bl/6 $PLTP^{-/-}$ mice were challenged with 22L prion strain by either intracerebral (i.c.) or intraperitoneal (i.p.) routes, and fed either with a standard chow diet or hypercholesterolizing diet. Blood samples were collected before prion inoculation for lipid assays. Mice were sacrificed at the terminal stage of the disease and tissues were collected.
- Results : A significant increase of the survival time and a decrease of spongiosis was observed in $PLTP^{-/-}$ mice challenged with prions by i.p. route and fed with standard chow diet. Upon feeding with the hypercholesterolizing diet, a significant increase of plasma cholesterol levels was observed in $PLTP^{-/-}$ mice, associated with a reduced survival time.
- Conclusion : The $PLTP^{-/-}$ mouse model led us to underline the implication of cholesterol during the neuroinvasion process. Therapeutic strategies targeting a decrease of cholesterol levels, associated with other targets could be a good way to slow down the disease progression.

04g. Therapeutic Targets & Mechanisms for Treatment: kinases

ADPD5-2323

THE KINASE PDK1, A COMMON THERAPEUTIC TARGET FOR PRION AND ALZHEIMER'S DISEASES TO RESCUE TACE ALPHA-SECRETASE NEUROPROTECTIVE ACTIVITY

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Objectives: Prion and Alzheimer's (AD) diseases make up a group of pathologies characterized by separate etiologies and distinct clinical manifestations but may share some common pathogenic cascades. The identification of such cascades would represent a biomedical challenge for therapies. How PrPSc in prion diseases or the amyloid Ab peptides in AD exert their toxicity remains elusive.
Methods: This study combines the use of prion-infected or "Alzheimer's" neurons and mouse models with prion disease or Alzheimer-like pathology (Tg2576, 3Tg-AD, 5Tg-AD).
Results: We show that PrPSc or Ab trigger the overactivation of the 3-phosphoinositide-dependent kinase-1 (PDK1) in prion-infected or "Alzheimer's" neurons, respectively. PDK1 then promotes the phosphorylation and internalization of TACE a-secretase. TACE internalization diverts TACE activity away from (i) the normal cellular prion protein, which amplifies the replication of PrPSc in prion diseases, (ii) the amyloid precursor protein, which favors the production of Ab in AD, and (iii) TNFa receptors, which accumulate at the plasma membrane and hypersensitize diseased neurons to TNFa. Inhibition of PDK1 is sufficient to target TACE back to the plasma membrane of diseased neurons, where it recovers its protective activity. Inhibition of PDK1 increases the lifespan and counteracts motor deficits in prion-infected mice and reduces memory and cognitive impairments in AD mouse models.
Conclusions: Deregulation of PDK1-dependent TACE activity emerges as a central neurodegenerative mechanism of both prion diseases and AD. Rescuing TACE a-secretase activity at the plasma membrane upon PDK1 inhibition represents a valuable entry point to combat these two diseases. Importantly, the therapeutic relevance of targeting PDK1 in AD is supported by a rise in PDK1 activity and reduced TACE a-secretase activity in the brain of AD subjects1.
1Pietri et al. PDK1 decreases TACE-mediated a-secretase activity and promotes disease progression in prion and Alzheimer's diseases. Nat. Med. (2013) 19:1224-35.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1961

CREUTZFELDT-JACOBS DISEASE; A CASE REPORT WITH RADIOLOGICAL AND CEREBROSPINAL FLUID FINDINGS

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Entrance:

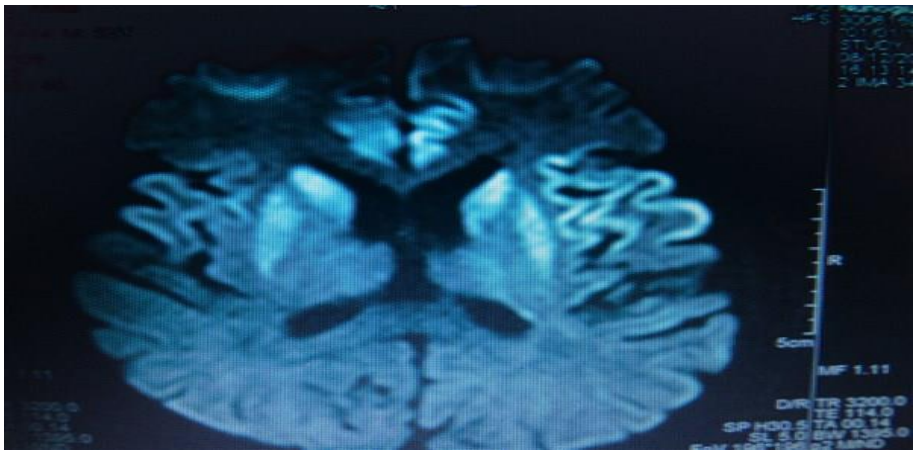
Crutzfeld-Jacob disease, a rare but fatal disease is a progressive neurological disruption.

Methods and Materials

52 years old man apply us with right hand tremor and speech disorder 1,5 months ago started. We found his neurological examination cognitive loss, pyramidal and exstrapyramidal signs. We were excluded metabolic and paraneoplastic disorders.
In MRI hyperintense kortikal ribbon marker in frontal area and head of caudate nucleus and anterior of basal ganglia in T2 and flair sequences .
Diffusion and ADC sequences show us hyperintense kortikal ribbon marker in frontal area and head of caudate nucleus and anterior of basal ganglia
Radiological findings were significant for this disease . Three months later myoclonic seizures began..
Clinical and radiological findings were consistent with sporadic Crutzfeld-Jacob disease .
We worked 14-3-3 protein in cerebrospinal fluid. It was negative. But neuron specific enolase was higher it is supported CJD.

Discussion

Definitive diagnosis of CJD is possible with tissue analysis..
Some types of CJD in cerebrospinal fluid 14.3.3.protein can be negative but tau and neuron specific enolase can be higher it is supported CJD.
In CJD clinical and radiological findings should be considered together.



10e. Other: imaging

ADPD5-0578
SERIAL MAGNETIC RESONANCE IMAGING CHANGES IN A PATIENT WITH GERSTMANN-STRÄUSSLER-SCHEINKER SYNDROME (P102L)

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We describe serial MRI findings of a patient with Gerstmann-Sträussler-Scheinker Syndrome (GSS) with a mutation in the prion protein gene at codon 102 (Proline to Leucine) in a Korean family. A 31-year-old woman began to experience gait unsteadiness 5 months before admission. Neurological examination revealed slow saccadic eye movement and bilateral limb ataxia. Brain MRI showed no abnormality. Her family members (mother, aunt, grandfather, and great grandfather) had a history of progressive gait disturbance and becoming bedridden that had been left undiagnosed. Over a period of next 3 months, the patient developed insomnia, voiding difficulty, and cognitive impairment. MRI of the brain showed hyper-intense signal changes in the cerebral cortex, predominantly in the parieto-temporal regions, caudate head, and ventral region of putamen. CSF 14-3-3 protein was found to be weakly positive. Analysis of the PRNP gene showed a proline-to-leucine substitution at codon 102. The neurological status had worsened progressively and she had reached akinetic mutism 9 months after onset. Follow-up brain MRI with an interval of 10 months showed areas of spreading diffusion restriction in entire cerebral cortex and marked aggravation of atrophy of cerebral and cerebellar hemispheres, and brainstem. It has been reported that MRI scans may be normal during the early stage of GSS. In this case, serial MRI show the progressive extension of the high signal intensity on DWI in accordance with the neurological status, which has been attributed to the severity of spongiform degeneration and to gliosis, and marked brain atrophy following the DWI changes.

01i. Protein Misfolding & Aggregation: huntingtin

ADPD5-0585

SELECTIVE DAMAGE OF STRIATAL PROJECTION CELLS OF THE DIRECT OR INDIRECT PATHWAY PRODUCES DYSREGULATION OF SLEEP AND CIRCADIAN RHYTHM IN MICE

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Neuropathology of Huntington's disease (HD) is associated with early and selective alteration of the indirect pathway originating from a subpopulation of GABAergic projection neurons in the striatum. This cell population expresses dopaminergic D2 receptor (D2R) and projects to the substantia nigra pars reticulata via the globus pallidus and subthalamic nucleus. On the contrary, neuropathology of PD is rather characterized by dysfunctioning of the direct pathway originating from the other class of striatal projection cells, which expresses preferentially dopaminergic D1 receptor (D1R) and projects directly to the substantia nigra pars reticulata.

To evaluate the impact of the abnormalities of the indirect or direct pathway on symptomatic changes observed in patients with HD or PD, respectively, we induced excitotoxicity of striatal D2R or D1R expressing cells in mice, by using Designer Receptors Exclusively Activated by Designer Drugs associated with Gq protein. Mice were then recorded for electroencephalogram during sleep and wake cycles over 24 h for potential changes of sleep physiology and circadian rhythm, phenotypic changes observed precociously in HD and PD patients and animal models of these diseases.

Our results show that the selective perturbation of the direct or indirect pathway produces significant changes of circadian rhythm and sleep architecture. The same manipulations, however, were not sufficient to produce changes in brain rhythms during waking and sleep states.

Our data suggest an important contribution of the striatal indirect and direct pathway to circadian rhythm regulation, and the alteration of these pathways to early sleep changes in HD and PD.

01i. Protein Misfolding & Aggregation: huntingtin

ADPD5-1679
DIFFERENTIAL EFFECT OF HDAC3 ON CYTOPLASMIC AND NUCLEAR HUNTINGTIN AGGREGATES

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Histone deacetylases (HDACs) are potential therapeutic targets of polyglutamine (pQ) diseases including Huntington's disease (HD) that may function to correct aberrant transcriptional deactivation caused by mutant pQ proteins. HDAC3 is a unique class 1 HDAC found in both the cytoplasm and in the nucleus. However, the precise functions of HDAC3 in the two cellular compartments are only vaguely known. HDAC3 directly binds to huntingtin (Htt) with short pQ and this interaction is important for suppressing neurotoxicity induced by HDAC3. With long pQ Htt, the interaction with HDAC3 is inhibited, and this supposedly promotes neuronal death, indicating that HDAC3 would be a good therapeutic target for HD. However, the knockout of one HDAC3 allele did not show any efficacy in reducing neurodegenerative symptoms in a mouse model of HD. Therefore, the role of HDAC3 in the pathogenesis of HD has yet to be fully elucidated. We attempted to resolve this issue by focusing on the different roles of HDAC3 on cytoplasmic and nuclear Htt aggregates. In addition to supporting the previous findings, we found that HDAC3 preferentially binds to nuclear Htt over cytoplasmic ones. Specific HDAC3 inhibitors increased the total amount of Htt aggregates by increasing the amount of nuclear aggregates. Both cytoplasmic and nuclear Htt aggregates were able to suppress endogenous HDAC3 activity, which led to decreased nuclear proteasome activity. Therefore, we concluded that Htt aggregates impair nuclear proteasome activity through the inhibition of HDAC3. Our findings provide new insights regarding cross-compartment proteasome regulation.

01j. Protein Misfolding & Aggregation: dipeptide repeat proteins

ADPD5-0696

POLY-GA DIPEPTIDE REPEAT PROTEINS OF THE C9ORF72 HEXANUCLEOTIDE REPEAT EXPANSION CAUSE UNC119 COAGGREGATION AND NEURONAL TOXICITY

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Amyotrophic lateral sclerosis (ALS) and Frontotemporal dementia (FTLD) are severe neurodegenerative diseases with overlapping clinical and pathological features. Recently, a hexanucleotide repeat expansion in the non-coding region of the *C9orf72* gene was discovered to be the most frequent pathogenic mutation in both diseases. Although lacking an ATG start codon, the repeat is translated in all reading frames into dipeptide repeat (DPR) proteins forming insoluble p62-positive, TDP-43-negative inclusions in patients. Their role in the disease, however, is still unclear.
To distinctively analyze the role of DPR proteins, we designed synthetic genes expressing the DPR sequence with an ATG start codon but no extensive GGGGCC repeats. Only poly-GA showed insoluble p62-positive aggregates and induced apoptosis in primary neurons. Using a mass spectrometry approach we identified poly-GA coaggregating proteins. We found a significant enrichment of proteins of the ubiquitin-proteasome system and additionally Unc119, a trafficking factor, previously linked to neuromuscular function and axon maintenance. The soluble levels of Unc119 in our system were strongly reduced. Furthermore, Unc119 knockdown inhibited dendritic branching and caused neurotoxicity similar to poly-GA overexpression and the addition of Unc119 partially rescued poly-GA toxicity. These findings indicate that poly-GA overexpression causes Unc119 loss of function. Whereas Unc119 is detectable in many poly-GA inclusions in the frontal cortex and the hippocampus of *C9orf72* patients, the abundant poly-GA inclusions in the cerebellum appear to be less affected by Unc119 coaggregation. Thus, poly-GA induced Unc119 loss of function might contribute to the selective vulnerability of neurons in the pathogenesis of *C9orf72* FTLD/ALS.

01m. Protein Misfolding & Aggregation: other

ADPD5-0230
CRYSTAL STRUCTURE OF THE ATAXIN-3 CARBOXY-TERMINAL REGION
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Machado-Joseph Disease, or Spinocerebellar Ataxia Type 3 (MJD/SCA3), is a conformational disease of the brain that is genetically associated with the CAG triplet expansion in the ATXN3 gene. MJD/SCA3 development is associated with polyglutamine elongation in Atx3 beyond pathogenic threshold of 55Q. Topologically Atx3 consists of N-terminally located Josephin domain (JD) with deubiquitination enzymatic activity (1-180 a.a.r.), intermediate flexible linker containing two ubiquitin-interacting motifs (182-291 a.a.r.) and C-terminal region encompassing polyglutamine repeat (polyQ) (begins at 292 a.a.r.). While JD is quite well structurally and functionally characterized, the remaining part of the protein lacks detailed information.
Here we report crystal structures of the C-terminal region (278-329 a.a.r.) of non-expanded ataxin-3 with 13 glutamine residues fused to maltose-binding protein. Our observations show that C-terminal domain is mainly disordered, solvent exposed region that likely adopts extended random conformation. Our findings suggest that disordered polyglutamine together with its flanking sequences can adopt structured alpha-helical conformation upon protein-protein interactions. It is tempting to speculate that these helix-to-loop transitions of polyglutamine can play an important role in normal functioning of ataxin-3 and other polyQ containing proteins while glutamine expansion can lead to disruption of these transitions leading to toxic conformational switch. The recent discoveries will be discussed on the meeting.

01m. Protein Misfolding & Aggregation: other

ADPD5-1556

DETECTION OF PROTEIN AGGREGATES IN BRAIN AND CEREBROSPINAL FLUID DERIVED FROM MULTIPLE SCLEROSIS PATIENTS

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Studies of the properties of soluble oligomers species of amyloidogenic proteins, derived from different proteins with little sequence homology, have indicated that they share a common structure and may share similar pathogenic mechanisms. Amyloid β, tau protein as well as amyloid precursor protein normally associated with Alzheimer's disease and Parkinson's disease were found in lesions and plaques of Multiple Sclerosis patients.

The Objective of the study is to investigate whether brain and CSF samples derived from Multiple Sclerosis patients demonstrate the presence of soluble oligomers normally associated with protein misfolding diseases such as Alzheimer's disease.

We have used anti-oligomer monoclonal antibodies to immunodetect soluble oligomers in CSF and brain tissues derived from Multiple Sclerosis patients.

In this report, we describe the presence of soluble oligomers in the brain tissue and cerebral spinal fluid of Multiple Sclerosis patients detected with our monoclonal anti-oligomer antibodies with Western blot.

These results might suggest that protein aggregation plays a role in Multiple Sclerosis pathogenesis although further and more refined studies are needed to confirm the role of soluble aggregates in Multiple Sclerosis.

01m. Protein Misfolding & Aggregation: other

ADPD5-1592

A NOVEL NON TETRAMERIC MUTANT OF TRANSTHYRETIN PROTEIN INVOLVED IN FAMILY CARDIAC AMYLOIDOSIS.

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Transthyretin (TTR) is one of many proteins that are capable of forming amyloid fibrils *in vivo*. This protein is associated with two distinct amyloidosis: Familial Cardiac Amyloidosis (FCA) that causes a restrictive cardiomyopathy and Familial Amyloid Polyneuropathy (FAP) that affects peripheral nerves, they are hereditary and caused by mutations in the TTR gene. The diagnosis was established at University Hospital since 2008 due to a collaboration between our group and the center of Amyloidosis CEPARM. Previously the only mutation found was V30M in 3 patients diagnosed in France. Since 2008 we discovered 5 new mutations in Brazil and a novel mutation not yet described in the world and until now 140 patients were diagnosed. The novel mutation A19D-TTR causes a severe restrictive cardiomyopathy that is certainly related to a higher profile of aggregation observed for this mutant if compared to others amyloidogenic mutants of TTR. The thermodynamic stability is lower in comparison with the WT TTR. Structural predictions using a bioinformatics tool showed that the insertion of the mutation caused an electrostatic clash that facilitates its dissociation and aggregation. Biophysical studies revealed that this protein is a dimer and not a tetramer as commonly found for the TTR. In addition the oligomers of A19D are toxic for primary culture of cardiomyocytes and fibroblast from murine heart. The recent CEPARM consolidation in our university hospital led to the identification of the rare A19D-TTR variant in a Brazilian patient, suggesting that other new, uncharacterized mutants could be identified in the coming years.

01m. Protein Misfolding & Aggregation: other

ADPD5-2108

ENDOGENOUS FUNCTION OF SERF AND ITS MOLECULAR ROLE IN NEURODEGENERATION

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Objectives The formation of amyloid-like fibrils through accumulation of aggregation-prone proteins in the human brain is an important hallmark in neurodegenerative diseases. Modifier of aggregation MOAG-4 was identified as a positive regulator of aggregate formation in C. *elegans*. MOAG-4 seems to act cell-autonomously and independent from classical cellular quality control mechanisms. The exact mechanisms in which MOAG-4 and its human orthologs SERF1A and SERF2 promote protein aggregation remains unknown[1,2]. In our study, we aim to investigate the endogenous role of SERF.

Methods A Yeast-two-hybrid screen was performed for the identification of possible SERF interactors. Possible SERF interactors were (over)expressed in Human Embryonic Kidney cells to investigate the possibility of these proteins to form insoluble inclusions.

Results Will be presented.

Conclusions Will be presented.

1. Van Ham, T. J., Holmberg, M. A. & van der Goot, A. T. Identification of MOAG-4/SERF as a Regulator of Age-Related Proteotoxicity. **142,** 601–612 (2010).
2. Falsone, S. F., Meyer, N. H. & Schrank, E. SERF Protein Is a Direct Modifier of Amyloid Fiber Assembly. **2,** 358–371 (2012).

ADPD5-1403

THREE VARIANTS OF LRRK2 EXHIBIT SIMILARITIES TO MULTIPLE PATHOGENIC MUTATIONS CAUSATIVE FOR PARKINSON'S DISEASE

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Objectives: We set out to determine whether three distinct variants in LRRK2, identified in Greek PD patients but not in controls, exhibited certain cellular phenotypes associated with multiple other common mutations in LRRK2 that are more clearly pathogenic.
Methods: Over-expressed wild type or mutant forms of LRRK2 were assessed for their ability to induce neuronal death, kinase activity, GTP binding, oligomerization, and interaction with the cell death protein FADD.
Results: When over-expressed in primary cultured neurons, A211V, K544E, and T1410M-LRRK2 induced apoptotic death to a similar extent as the pathogenic mutants G2019S or I2020T. However, only A211V-LRRK2 exhibited greater kinase activity compared to WT, but to a lesser extent than G2019S-LRRK2. Moreover, the interaction with FADD, and the effect of each variant on oligomerization, were not dramatically different from WT-LRRK2.
Conclusions: We find that some, but not all, of the phenotypes typically reported for pathogenic mutations in LRRK2 are also displayed by three selected variants found in PD patients of Greek origin. Importantly, each was able to induce neuronal death to a similar extent as the pathogenic mutations in a primary cellular model of LRRK2-PD, however the precise mechanism remains unclear.

ADPD5-0983

THERAPEUTIC AND DISEASE-MODIFYING EFFECTS OF GANGLIOSIDE GM1 IN MOUSE MODELS OF HUNTINGTON'S DISEASE

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BACKGROUND

Huntington disease (HD) is a neurodegenerative disorder due to the expansion of a polyglutamine stretch in the protein huntingtin. The resulting mutant protein acquires toxic conformations and aggregates within the cells, leading to neuronal dysfunction and death.

We previously showed that levels of ganglioside GM1, a lipid highly enriched in the brain, are decreased in HD models. Administration of exogenous GM1 resulted in normalization of motor behavior in a transgenic model of HD (YAC128 mice). These effects were accompanied by increased phosphorylation of mutant huntingtin at Ser13 and Ser16, a post-translational modification that was shown to decrease mutant huntingtin toxicity.

OBJECTIVES

The aim of this study was to further characterize the therapeutic effects of GM1 and to demonstrate that GM1 is a disease-modifying treatment for HD.

METHODS

We measured motor and non-motor behaviour in three different models of HD (R6/2, YAC128 and Q140 mice) during chronic intraventricular infusion of GM1 or vehicle. At the end of the treatment, filter-trap assay, immunoblotting and stereology analysis were performed to determine whether GM1 affects neurodegeneration and levels of mutant huntingtin aggregates in treated mouse brains.

RESULTS

GM1 treatment decreased levels of soluble and insoluble huntingtin in HD mouse brains, and dramatically improved both motor and non motor symptoms of the disease. In R6/2 mice, GM1 attenuated striatal neuron and white matter loss, increased body weight and prolonged survival.

CONCLUSIONS

Our data suggest that GM1 has disease-modifying properties across HD mouse models and could have therapeutic potential in HD patients.

ADPD5-2083

SYNAPTIC DYSFUNCTION IN AN IN VIVO CORTICO-STRIATAL MODEL SYSTEM OF HUNTINGTON'S DISEASE

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Huntington's Disease (HD) is caused by trinucleotide repeat expansion in the gene coding protein huntingtin. HD's pathology includes multiple motor and mental disorders. There are growing evidences that one of the manifestations of HD is abnormalities of synaptic activity between striatal and cortical neurons.

Objectives

The goal of the present study is to explore synaptic dysfunction during HD using *in vitro* model of co-cultured cortico-striatal neurons.

Methods

Cells of interest were obtained from the brain of mice YAC128 coding mutation causing HD and co-cultured. During *in vitro* cultivation cultures were characterized functionally by cell-attached patch-clamp and optogenetic stimulation and morphologically by immunocytochemistry.

Results

On the day 14 *in vitro* cultivation cortical neurons from YAC128 mice were characterized by spontaneous activity compared with wild type neuron culture. No difference in morphology of striatal dendritic spines between HD and wild type models was observed at that time.

On the day 19 *in vitro* cultivation the activity of YAC128 cortical neurons was reduced, while activity of wild type cortical neurons was increased. Spine elimination and changes in spine morphology were observed at that time.

Conclusions

Present work demonstrated synaptic dysfunction in the *in vitro* cortico-striatal model system of Huntington's Disease, which possibly resulted in reduced signal transduction activity between cortical and striatal neurons.

This work was supported by the contract with the Russian Ministry of Science 11.G34.31.0056 (Ilya Bezprozvanny).

02z. Cell, Molecular & Systems Biology: other

ADPD5-0387
EXTRANEURONAL ROLE OF GAMMA-SYNUCLEIN IN COLORECTAL CANCER CELL BIOLOGY

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γ-Synuclein, a predominantly neuronal protein of the Synucleins family, is associated with carcinogenesis and is proposed as a putative breast cancer marker. Its role in colorectal cancer carcinogenesis is still elusive. To investigate the role of γ-Synuclein in colorectal cancer carcinogenesis from invasion, metastasis and apoptosis evasion aspects, we overexpressed γ-Synuclein in the LS 174T colon adenocarcinoma cell line. When compared with untransfected and mock transfectants, LS 174T-γsyn had higher mobility in scratch wound assay, tend to scatter more in cell-scattering assay, and had enhanced lamellipodia and filopodia formation in cell-spreading assay. Enhanced adhesion of LS 174T-γsyn to fibronectin and collagen and significantly higher proliferation rate showed that γ-Synuclein was able to increase extracellular matrix interaction and promoted proliferation of LS 174T. Higher invasiveness of LS 174T-γsyn was evidenced by enhanced invasion to the bottom of the basement membrane in the Boyden chamber assay. However, LS 174T-γsyn cells were significantly more vulnerable to doxorubicin, vincristine and hydrogen peroxide insults, via apoptotic cell death. LS 174T-γsyn cells also had reduced anchorage-independent growth as shown by reduced colonies formation and reduced anoikis resistance. Higher ROS production also signified the pro-oxidant ability of γ-Synuclein. The pro-invasive property of γ-Synuclein was mediated through enhanced CREB phosphorylation. HGF down-regulation contributed to doxorubicin-mediated pro-apoptotic property of γ-Synuclein by suppressing cell survival and proliferation signalling pathways possibly involving ERK1/2, p38α, JNK pan and STATs. Taken together, the results suggest that γ-Synuclein may be possessing pro-invasive and doxorubicin-mediated pro-apoptotic properties at the same time.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-2273

REGULATION OF MITOCHONDRIAL BIOGENESIS IN NEURONS EXPRESSING N-TERMINAL MUTANT HUNTINGTIN

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Huntington's disease (HD) is an autosomal-dominant neurodegenerative disease characterized by abnormal movements and affective/cognitive difficulties. Typically, HD patients experience onset of symptoms at midlife. The genetic defect responsible for the disorder is a poly-glutamine expansion in the huntingtin protein. Expression of this mutant protein ultimately leads to neurodegeneration; striatal degeneration in particular underlies the resulting movement disorder. Numerous studies have indicated an important role of mitochondrial dysfunction in the evolution of neuronal dysfunction and death in HD.

Objective: Here, we are investigating modulation of key components of mitochondrial biogenesis in cultured neurons expressing N-terminal mutant huntingtin.

Methods: Neurons cultured from PND1 HD transgenic mice (line R6/2) and WT control littermates were treated with an activator of nuclear factor, erythroid 2-like 2 dependent (antioxidant response element-driven) transcription (tert-butyl hydroquinone). To assess mitochondrial biogenesis, neurons were fixed with paraformaldehyde, co-immunolabelled for mitochondrial protein NADH dehydrogenase 1 beta subcomplex-8 and the transcriptional co-activator peroxisome proliferator-activated receptor-gamma coactivator-1alpha. Quantitative immunofluorescence analysis was used to compare protein expression levels and subcellular localization in HD neurons compared to WT control neurons.

Results: Mitochondrial protein expression was comparable under basal untreated conditions in HD and WT neurons. Upon tert-butyl hydroquinone exposure, WT neurons increased mitochondrial protein expression while HD neurons were not responsive.

Conclusions: Basal levels of mitochondrial biogenesis are comparable in early post-natal HD and WT neurons, however, coupling of antioxidant-response element driven transcription activation to mitochondrial biogenesis is deficient in HD neurons, suggesting a fundamental deficit in this signalling pathway in neurons expressing mutant huntingtin.

03h. Pathophysiology & Disease Mechanisms: metabolism and insulin

ADPD5-0430

FIBRILLAR AND OLIGOMERIC AMYLIN DECREASES BRAIN PERICYTE GLUCOSE UPTAKE IN VITRO

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Background: Previous studies show degeneration of brain pericytes in patients with Alzheimer's disease (AD), a pathological event suggested to underlie the microvascular changes commonly seen in AD patients. The underlying causes to the pericyte loss are yet not defined. Interestingly, recent studies have shown depositions of aggregated hormone islets amyloid polypeptide (IAPP), also called amylin, in brain vessel walls of patients with AD. In normal conditions amylin moderates glucose metabolism, however, it is prone to aggregate and elevated secretion of the peptide leads to toxic depositions. To understand how amylin depositions may affect brain pericytes, we investigated pericyte glucose uptake in presence of different amylin aggregations forms.
Methods: Primary human brain vascular pericytes (HBVP) were exposed to fibrillar, oligomeric and monomeric preparations of amylin for 24 h. Immunocytoflourescence stainings with anti-GLUT-1 antibody were performed to assess alterations in GLUT-1 location and cell morphology. Glucose uptake, in the presence or absence of insulin, was evaluated by measuring the uptake of radiolabelled [^{14}C]-2-deoxyglucose.
Results: Exposure to fibrillar and oligomeric amylin, but not monomeric amylin, induced contracted pericyte morphology and significantly decreased glucose uptake. The decreased glucose uptake were counteracted by insulin supplement, but remained low compared to control cells.
Conclusion: Our study demonstrates an aggregation-dependent impact of amylin on pericyte glucose uptake. This finding suggests amylin depositions in the brain as a possible factor underlying the pericyte degeneration found in AD patients.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-1211

IGF-1 IS A POTENT INDUCER OF GDNF EXPRESSION, AND MAY CONTRIBUTE TO THE REGULATION OF GDNF VIA MT EXPRESSION.

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Parkinson disease, one of most common neurodegenerative disorders is elderly, is characterized massive loss of dopamine neuron. One of the neurotrophic factors that might protect neurons in harmful micro-environment is insulin growth factor-1 (IGF-1). Our previous study indicated that the induction of glial MT(metallothinein) as scavenger and/or regenerating factor lead to activation of IGF-1R neuron.Glial cell line-derived neurotrophic factor (GDNF) has significant neuroprotect potentials in PD. and, has been identified as a potent neurotrophic factor for a variety of neuronal cell populations. At present, it is still unknown whether human gliomas in vivo are also capable of producing GDNF.

In the present study, To determine factors that would enhance GDNF expression, we analyzed the effect of IGF-1 in C6 glioma cells under MPTP-neurotoxicity. Treatment of C6 cells with IGF-1 elicited an 8.5-fold increase in the level of GDNF content with expression of MT-mRNA, and elicited 18% of effective at recovery of C6 cell under MPTP. However, m-RNA expression of GDNF is not relative increased in course of IGF-1.

These data indicate that IGF-1 is a potent inducer of GDNF expression, and may contribute to the regulation of GDNF via MT expression.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-1640

IN VIVO CHARACTERIZATION OF THE BASAL GANGLIA DIRECT, INDIRECT, AND HYPERDIRECT PATHWAYS IN THE ZQ175 HETEROZYGOUS KNOCK-IN MOUSE MODEL OF HUNTINGTON'S DISEASE

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Objective: Huntington's disease (HD) is a lethal autosomal dominant neurodegenerative disorder caused by expansion of CAG repeats in the *Huntingtin (HTT)* gene. HD patient brains reveal a devastation of the caudate-putamen and cortico-striatal-thalamo-cortical circuits are thought to be particularly affected. Choreic symptoms correlate well with D2-containing medium spiny neuron (MSN) loss, which originate the indirect pathway. MSN dysfunction has been extensively studied *in-vitro* in models of HD, but the functional consequences on the immediate downstream nuclei of the indirect pathway, the external Globus Pallidus and subthalamic nucleus (STN), remains unknown.
Method: We characterized the properties of cortical to basal ganglia transmission in early symptomatic zQ175 heterozygous knock-in HD model mice (6-8 months old). Under urethane anesthesia, evoked responses of striatal MSNs and STN neurons were recorded following stimulation of primary motor cortex (M1).
Result: Evoked single unit recordings of MSNs demonstrated a reduced averaged number of spikes per stimulus but similar latency in male zQ175KI HET compared to WT. Furthermore, ~50% more spontaneously active MSNs were recorded in zQ175KI HET compared to WT. We also investigated the 'hyperdirect' cortico-STN pathway by recording evoked single unit activity in STN. Similarly to MSNs, male STN neurons in zQ175KI HET mice exhibited a reduced averaged number of spikes per stimulus compared to WT.
Conclusion: We have characterized a basal ganglia circuitry dysfunction relevant to the human HD condition. These functional *in-vivo* assays will allow for the assessment of novel compounds with potential disease modifying properties.
Study funded by CHDI Foundation, Inc.

04r. Therapeutic Targets & Mechanisms for Treatment: protein aggregation

ADPD5-1583

EVALUATION OF THE EFFICACY OF THE PEPTIDE P42B IN CELLULAR AND DROSOPHILA MODELS OF HUNTINGTON'S DISEASE

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Background: P42 is a 23aa peptide preventing aggregate formation in HeLa cells and in *Drosophila* HD models (Mugat, 2008; Arribat, 2013). Additionally, it reduces aggregate formation, weight loss, and brain atrophy, and ameliorates motor performances in R6/2 mice (Arribat, 2014). In P42TAT-containing brain extracts we identified a shorter 14aa fragment, P42B, still present after three hours. We tested whether P42B still harbours active properties.
Methods: P42 and P42B were tested alone, or combined with the transduction peptide TAT. HeLa cells were co-transfected with GFP-hHtt171aa-136Q and cherry-P42/P42B. Transgenic flies expressing polyQ-hHtt in salivary glands (*MS-1096*-Gal4; UAS-HA-hHtt^{171aa}-138Q/+) or eyes (*GMR*-Gal4; UAS-HA-hHtt^{67aa}-93Q/+) were crossed with flies expressing UAS-P42GFP/P42TATGFP/P42BGFP, or UAS-GFP as negative control. P42TAT or P42BTAT synthetic peptides were also tested in food of *MS-1096*-Gal4; UAS-HA-hHtt^{171aa}-138Q/+ flies. The aggregates were studied by immunostaining and quantified by filter retardation assays. The eye degeneration was studied in adult *Drosophila*.
Results: P42B prevents aggregate formation in HeLa cells and eye degeneration in adult flies. In the larval salivary glands P42B, either genetically or orally administered, does not significantly hamper aggregate formation; however, at immunostaining P42B co-localizes with the aggregates.
Conclusions: P42B harbors some of the active properties of the P42 peptide, but its efficacy varies. The entire peptide P42 may be required to fully hamper aggregate formation due to either a problem in peptide: i) stability, ii) expression or iii) cellular localization. Whereas P42B does not seem to fully prevent aggregation, its detection in aggregates further confirms a model whereby P42 might interact directly with polyQHtt.

10d. Other: diagnostics

ADPD5-1823
APPLICATION OF PROTEINOPATHY PLASMA BIOMARKERS IN NEURODEGENERATIVE DISEASES

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Protein aggregation is a common characteristic of many neurodegenerative disorders, and the interaction between pathological-toxic proteins to cause neurodegeneration is an important issue of current neuroscience research. Evidence increasingly indicates considerable overlap between synucleinopathies and tauopathies or other protein-misfolding diseases. New findings suggested synergistic effects of α-synuclein, hyperphosphorylated-tau, amyloid β, and other pathologic proteins including induction and spread of protein aggregates are possible underlying molecular pathogenic mechanisms.

We used an immunomagnetic reduction (IMR) assay to measure the plasma levels of Aβ40, Aβ42, tau proteins and α-synuclein in 20 older control participants, 10 participants who had either mild cognitive impairment due to Alzheimer's disease (AD) or early AD dementia; 5 patients with dementia of Lewy's bodies (DLB), 5 patients with Parkinson's disease dementia (PDD), 5 with Parkinson's disease and 5 with frontotemporal lobe dementia (FTLD). IMR assay works by determining the per cent of reduction in the magnetic field produced by an alternating current (ac). This reduction is due to a decrease in the magnetic susceptibility (c_{ac}) of a bio-functionalized magnetic nanoparticle reagent due to its association with target bio-molecules. All participants received ^{11}C-labeled Pittsburgh compound B PET scans to displays amyloid retention in the brain for correlation.

Results show that in comparison with normal elderly control, plasma Aβ42 (Aβ42/Aβ40) and plasma total tau proteins were both increased in early Alzheimer's disease; only plasma total tau proteins but not Aβ42 (Aβ42/Aβ40) were increased in FTLD. The results of plasma α-synuclein for patients with DLB and PDD will be also reported.

03e. Pathophysiology & Disease Mechanisms: proteasome and ubiquitin

ADPD5-0704

UBIQUITIN-MEDIATED PROTEOLYSIS IS AFFECTED IN BRAINS OF SMALL VESSEL DISEASE AND CADASIL PATIENTS.

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Objectives. Sporadic cerebral small vessel disease (SVD) and its hereditary form, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) lead to vascular cognitive impairment (VCI) and subcortical vascular dementia. This project was conducted in order to elucidate the molecular mechanisms underlying sporadic and hereditary SVD causing cerebral ischemia and consequently paresis, speech problems and cognitive decline.
Methods. We compared the gene expression in post-mortem brain specimen dissected from the frontal and occipital cortex and white matter of 5 SVD and 2 CADASIL patients with samples from 5 non-neurologically affected controls, using oligonucleotide-based microarray technology (Affymetrix® Genechips). Gene annotation and statistical analysis were performed using Partek® Genomics Suite. Genes with fold expression changes $F > 1.2$ in both directions, and $P < 0.05$ were considered significantly differently expressed between diseased and control groups. Biological functions enriched with these up- and down-regulated genes were identified using DAVID® bioinformatics resources.
Results. Functional classification revealed many cellular pathways affected in SVD and CADASIL. A common pathway involving many under-expressed genes in several brain regions of SVD and CADASIL patients was ubiquitin-mediated proteolysis. We confirmed these transcriptomic results by immunohistochemistry on brains sections, showing accumulation of mono-and poly-ubiquitinated proteins, including p62 or P-Tau.
Conclusions. These results demonstrated that, on a molecular level, SVD and CADASIL follow patterns of neurodegeneration with deregulation of the ubiquitin-mediated proteolysis, inducing formation of protein aggregates. These results suggest that activating the ubiquitin proteasome system may represent a new strategy to treat patients with cerebral small vessel diseases.

03h. Pathophysiology & Disease Mechanisms: metabolism and insulin

ADPD5-1276

DIABETES AUGUMENTS COGNITIVE DYSFUNCTION IN CHRONIC CEREBRAL HYPOPEFUSIO BY INCREASING NEURONAL CELL DEATH: IMPLICATION OF CILOSTAZOL FOR DIABETES MELLITUS-INDUCED DEMENTIA

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Background and purpose- Many patients with diabetes are at increased risk of cognitive dysfunction and dementia. We tested whether cognitive impairment could be exacerbated in combined injury using a rat model of chronic cerebral hypoperfusion with diabetes. We also determined whether a potent inhibitor of type III phosphodiesterase could prevent the cognitive decline caused by this combined injury.

Methods- We used OLETF rats as a model of type II diabetes (T2DM) and LETO rats as a control. Chronic cerebral hypoperfusion was modeled by permanent bilateral common carotid artery occlusion. At 24 weeks, the non-diabetic and T2DM rats were randomly assigned into groups for the following experiments: (1) sham non-diabetic ; (2) hypoperfused non-diabetic ; (3) sham T2DM ; (4) hypoperfused T2DM rats. Rats were orally administered cilostazol (50 mg/kg) or vehicle once a day for 2 weeks after 24 weeks. Morris water maze tasks were used to assess behavioral test., and neuronal cell death and neuroinflammation were investigated via Western blots and histological investigation.

Results- Spatial memory impairment was exacerbated synergistically in the hypoperfused T2DM group compared with the hypoperfused non-diabetic group and sham T2DBM group ($P < 0.05$). Compared with the control group, neuronal cell death was increased in the hippocampus of the hypoperfused T2DM group. Cilostazol, a PDE-3 inhibitor, improved the memory impairments through inhibition of neuronal cell death, activation of CREB phosphorylation and BDNF expression in hypoperfused T2DM group.

Conclusion- Our experimental results support the hypothesis that there are deleterious interactions between chronic cerebral hypoperfusion and T2DM

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0920

NEUROINFLAMMATION INCREASES IN A MOUSE MODEL OF ALZHEIMER'S DISEASE FEATURING VASCULAR COMORBIDITY

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Objectives: Obesity and type 2 diabetes mellitus are risk factors for developing Alzheimer's disease (AD) and vascular dementia. This disease pathology includes small strokes, cerebrovascular occlusions, and cerebral microhemorrhages. To examine this unique disease state we developed a db/AD mouse model: a cross between the db/db diabetic mouse and the APP(ΔNL)/PS1(P264L) double knock-in model of amyloid pathology. The diabetic phenotype causes unique vascular changes, including aneurysms, microhemorrhages, and gray matter microinfarcts.
Methods: Gene expression profiles were collected using microarray data analysis and array cards probing for neuroinflammatory markers. Microglial and astrocytic activation, astrocytic dysfunction, and neurovascular coupling were assessed via immunohistochemistry on fixed hemibrains.
Results: The db/AD mice had significant differences in metabolic pathways and a general increase in neuroinflammation, including astrocyte and microglial activation. Astrocyte activation was prominent near amyloid deposits, and around some blood vessels (vascular amyloid was not required for astrocyte activation). Additionally, we saw changes in the aquaporin-4 water channel, the calcium-dependent potassium channel Kir4.1, and several matrix metalloproteinases, indicating generalized astrocyte dysfunction, particularly at astrocytic end-feet.
Conclusions: We have created a mouse that models a mixed dementia disease state, with both AD and vascular pathologies that can be used to better understand how obesity and type 2 diabetes contribute to the development of dementia. Further elucidating the role of inflammation, and the specific role that astrocytes play in the development of pathology, will be important for improving our understanding of the disease, and for the design of potential therapies. Funded by NIH (ES024158, AG045809, NS083692).

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-2045

MARKERS OF INFLAMMATION IN ISCHEMIC STROKE PATIENTS:AN ADDITIONAL CRITERION OF LONG-TERM COGNITIVE IMPAIRMENT

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Limited data has shown that elevated levels of some inflammatory markers in the circulation of ischemic stroke patients indicate future cognitive deterioration.The objective of the present study is to establish important determinants of cognitive deficit that can be evaluated immediately after stroke onset and prove useful in predicting cognitive decline in the first year post-stroke. Methods: We examined 47 ischemic stroke patients admitted within 48 hours of ictus. Their neurological and cognitive status, biochemical parameters and microalbuminuria level were prospectively evaluated over a 1-year period post-stroke. Results: A more severe neurological deficit was found in the cognitively impaired then in the cognitively normal patients (p=0.003). A time-varying dynamics of the MMSE score was observed in both patient groups (p=0.000). Age (p=0.000), level of education (p=0.004), gender (p=0.041), history of diabetes (p=0.045) and serum high sensitive C-reactive protein (hs-CRP) on admission (p=0.003) were significant determinants of cognitive decline 1 year after stroke. The patients with cognitive decline had persistently high WBC and granulocyte counts. The albumine-to-creatinine ratio was high during the whole follow-up period in the cognitively impaired group even after adjusting for sex and age (p=0.010). The ordinal logistic regression analysis showed that hs-CRP (p=0.005) and age (p=0.000) were independent predictors of patients' cognitive status, represented as a three level ordinal scale. Conclusion: The level of inflammatory markers could be considered as an additional criterion of long-term post-stroke cognitive impairment and a rational approach in view of treatment strategies especially among female stroke patients.

03j. Pathophysiology & Disease Mechanisms: autoimmunity

ADPD5-1818
ROLE OF VON WILLEBRAND FACTOR IN EARLY DETECTION OF ACUTE ISCHEMIC CEREBROVASCULAR STROKE

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Role of Von Willebrand Factor In Early Detection Of Acute Ischemic Cerebroveascular Storke

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Background: von Willebrand factor is a blood glycoprotein involved in coagulation, Its primary function is to bind to factor VII to prevent it's degradation, also it is important for platelet adhesion activity becomes elevated in both the acute and subacute phases of ischemic stroke and it may be associated with increased mortality. Objective to asses the potential diagnostic utility of blood born protein biomarkers in predicting acute ischemic stroke. Material and methods: 40 patients of both sex admitted with acute ischemic stroke and ten control normal subject for each patient complete lab investigations and clinical investigations, Von Willebrand factor was done using ELIZA technique. Results: Von Willebrand factor showed significantly more positive results among patients 74.5% than control 10%. Conclusion: Von Willebrand factor become elevated in early stage of acute ischemic stroke and is related to the stroke severity.

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-0959

HIGH DISEASE LEVEL - HDL: DEPRESSION AND CARDIOVASCULAR DISEASE. THE LINK!

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Objective:
Assessement between the relation of 189 patients, 132 women (69.84%) with ages between 20-55 years, mean range 37,5 years and, 57 men (60.16%) with ages between 25-55 years, mean age 40 years, bearers of dyslipidemia and depressiv syndrome (DS) for at least 3 years, with familiar priors of metabolic and CV risk.
Design and method:
All patients involved in this study had a previous anthropometric, biochemical, clinical and vascular evaluation. Therapeutic response was initiated with serotonin reuptake inhibitors (SRI) and statin, associated a modification in lifestyle.
In 67 women (50.75%) between 20-30 years, an important correlation was found, between de clinical condition and hypotension. After 24 months an auto evaluation (15D and SF-36 surveys) was proposed to all patients.
Results:
The mean values of cholesterol in women´s group was HDLc-38 mg/dl, LDLc - 135.5 mg/dl and in men´s group was a HDLc - 33 mg/dl and LDLc-163 mg/dl
In 75 (39.68%) patients with ages comprises between between 35-55 years, augmented values of IMT were found during carotid vascular assessement (mean - 2.3 mm) without differentiation of sexes.
After 24 months 98% complying therapeutic proposed and changing lifestyle.
Subsequent biochemical evaluation of women´s group showed mean values of HDLc - 53 mg/dl and LDLc - 93 mg/dl, whereas the men´s group had a mean HDLc - 44 mg/dl and LDLc - 90.5 mg/dl.
In the group of 75 patients with ages comprised between 35-55 years, there was a significant decrease of the mean IMT - 1.75 mm.
The women´s group with ages between 20-30 years kept a hypotension profile.

03r. Pathophysiology & Disease Mechanisms: vasculature & neoangiogenesis

ADPD5-0412

PERLECAN DOMAIN V IS DIFFERENTIALLY ALTERED IN AN EXPERIMENTAL MODEL OF COGNITIVE IMPAIRMENT AND DEMENTIA

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Objectives: Although vascular contributions to cognitive impairment and dementia (VCID) are the second leading causes of dementia after Alzheimer's disease, they remain poorly understood due to their diverse etiology. The brain may attempt to compensate for vascular injury and resultant hypoperfusion via angiogenesis, the process of new blood vessel growth from pre-existing vasculature, and regulated by growth factors and the extracellular matrix (EM). We hypothesize that the bioactive domain V (DV) protein fragment of the EM component perlecan regulates angiogenesis in response to VCID pathology which is experimentally studied with the bilateral carotid artery stenosis (BCAS) model. Furthermore, we hypothesize that vascular injury and cognitive impairment is caused, in part, by gradual diminishment of DV levels.
Methods: Male C57/BL6 mice were subjected to BCAS by wrapping metal coils around the carotid arteries which remained in place for 30 days or 6 months. Brains were removed for qPCR, immunohistochemical, and Western analysis.
Results: qPCR analysis showed no change in perlecan gene expression in the thalamus of BCAS mice at 30 days or 6 months. However, there was a significant increase in gene expression in the cortex of BCAS mice at 30 days compared to sham. Immunohistochemistry also showed an increase in DV in the cortex after 30 days. Western blot analysis demonstrated a trend of decreased cortical DV protein expression in BCAS mice compared to sham mice after 6 months.
Conclusions: DV may play a role in regulating angiogenesis, and its gradual diminishment over time could contribute to VCID.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-0644
COGNITIVE CORRELATES OF CEREBROVASCULAR REACTIVITY IN OLDER ADULTS

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Introduction: Cerebrovascular dysfunctions play a role also in the pathogenesis of Alzheimer's disease (AD), and Transcranial Doppler sonography (TCD) can be used for the cerebral hemodynamic measures. The aim of this study was to explore the contribution of cerebral hemodynamic changes to the cognitive impairment in patients with AD.
Methods: A total of 194 subjects representing 52 controls, 75 patients with mild cognitive impairment (MCI) and 67 patients with AD were included. By using TCD, cerebrovascular reactivity (CVR) was evaluated by the breath-holding, in addition to the mean blood flow velocity (MFV) and pulsatility index (PI) of the middle cerebral artery. Analyses were adjusted for age, education, severity of white matter hyperintensities, and vascular risk factors.
Results: AD patients were older and low-educated (all, $p<0.001$). After adjusting for covariates, MFV and PI did not differ among three groups. However, CVR was significantly reduced in AD group (45.33±11.49 cm/sec), compared with other groups ($p<0.001$). MMSE score was correlated with PI ($r=-0.192$, $p=0.004$) and CVR ($r=0.263$, $p<0.001$). Multiple regression also showed CVR was associated with mini-mantal state examination (MMSE) score. CVR value was different according to clinical dementia rating (CDR) score ($p<0.001$)
Conclusion: Our finding that CVR is reduced in AD may be suggestive of underlying microangiopathic mechanism. Furthermore, there was an association between impaired cerebrovascular reactivity and cognitive impairment in older adults. Further research is needed to fully establish whether altered cerebral hemodynamics may be considered an independent factor of cognitive decline or an effect of pathological processes involved in AD.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1520
EFFECTS OF WHITE MATTER DISEASE ON DEMENTIA

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White Matter Disease (WMD) is a reflection of multiple microinsults to the brain over a person's lifetime. We aim to study the burden of white matter disease in an elderly urban population diagnosed with dementia. We hypothesize that White Matter Hyperintensity (WMH) volume and region-specific distribution may be associated with an increased risk of developing dementia and may serve as an MRI marker of risk of dementia. METHODS: We retrospectively selected the MR images of patients with a diagnosis of dementia and quantitatively measured the volume and distribution of WMH on MR fluid attenuated inversion recovery images using an import DICOM (Digital Imaging and Communications in Medicine) image tool of Analyze 10.0 software (AnalyzeDirect). The locations specified were Subcortical, Deep White Matter (DWM) and Periventricular parenchyma. We compared them to age matched controls. RESULTS: 877 patients were diagnosed with Alzheimer's dementia from 2000 to 2010 in an urban hospital. There were 398 patients identified with both dementia and stroke; from the remaining 479 patients with dementia only, 105 had MR imaging available for review (age: 69.2+10.4 years). The dementia group had a significantly higher total ((1.38%+1.30% vs. 0.55%+0.66%, $p<0.02$), deep (0.36%+0.38% vs. 0.14%+0.15%, $p<0.01$) and periventricular (0.10%+0.09% vs. 0.03%+0.04%, $p<0.04$) WMD compared to the controls. CONCLUSION: Dementia patients have significantly more WMD burden than age matched controls in total, deep and subcortical white matter disease. Patients with extensive WMH should be identified and aggressively treated to slow the progression of vascular insults that may affect the progression of dementia.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1571

BRAINS FOR DEMENTIA RESEARCH: CONCORDANCE OF VASCULAR FACTORS DURING LIFE WITH VASCULAR PATHOLOGY POST MORTEM

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Background:
Brains for dementia Research (BDR) is a programme of planned brain donation with structured assessments of cognition behaviour and function during life. There are now approximately 3,000 participants of which over 250 have died. Participants may or may not have cognitive impairment when they join. This cohort provides the opportunity to examine concordance between vascular factors recorded during life (Hachinski Ischemia score, CAMDEX past history) and evidence of vascular pathology at post-mortem.
Methods
Hachinski Ischemia score and CAMDEX past history were used to provide evidence of vascular factors and the neuropathological examination where details of any vascular pathology was recorded using established methods by BDR neuropathologists.
Results.
Preliminary examination of data showed that 47 of the deceased had Hachinski score calculated during life with 7 scoring 5 or above. Surprisingly only 4 of these cases had clear evidence of vascular pathology. Of the remaining 41, with score 4 or below evidence of vascular pathology was noted in 13 cases (3 as principle diagnosis). Examination of CAMDEX past history is ongoing.
Conclusions
At face value and consistent with some previous reports there is limited concordance between assessments of vascular factors during life and post-mortem vascular pathology. It may be that some recording of vascular pathology may relate to vascular events after the last assessment or that specific risk factors provide a better indication of subsequent vascular pathology. The BDR cohort (data and tissue) is available to researchers to answer questions relevant to people with dementia.

04a. Therapeutic Targets & Mechanisms for Treatment: immunotherapy

ADPD5-0974

EFFECTS OF IMMUNOTHERAPY ON STROKE PATHOLOGY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE AND VASCULAR DEMENTIA

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OBJECTIVES People with type 2 diabetes (T2DM) are at a higher risk for age-related dementias such as Alzheimer's disease (AD), and for vascular complications such as stroke and vascular de-mentia. We have created a novel mouse line which combines a diabetic phenotype with a model of amyloid deposition in order to study the confluence of T2DM and AD.

METHODS We crossed the leptin receptor deficient db/db mouse with the APP(ΔNL)/PS1(P264L) knock-in mouse to create a novel mouse line that displays vascular disturbances (stroke, microhemorrhage) and severe cognitive deficits – the db/AD mouse. To determine the effect of amyloid-beta (Aβ) on vascular pathology, we began active immunotherapy with fibrillar Aβ42 at ~2 months of age, with boosters at regular intervals for 10 months. Cognitive ability was then measured with Morris water maze and stroke pathology was measured via MRI.

RESULTS Preliminary results indicate a potentially small cognitive benefit due to active immunization independent of genotype, although the bulk of the study is still underway. However, the benefit was small, and unrelated to improving cerebrovascular pathology. In fact, as with other immunotherapy treatments, we have seen some increased severity of stroke pathology in a small number of animals.

CONCLUSIONS The results of this study imply that removal of Aβ alone will not necessarily be sufficient to treat AD with vascular comorbidity or vascular cognitive impairment. A secondary arm of this study will investigate the broader role of γ-secretase function in the development of pathology.

Funded by NIH (ES024158, AG045809, NS083692) and AHA (13IRG14330016).

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-0987

GLUTAMATE INDUCES NA+/H+ EXCHANGER-1 (NHE-1) ACTIVATION THROUGH SUSTAINED INTRACELLULAR ACIDOSIS, WHICH LEADS TO PHOSPHORYLATION OF NHE-1 THROUGH PKC-BETA PATHWAYS

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1. Objectives The Na^+/H^+ exchanger-1 (NHE-1) is a ubiquitously expressed pH-regulatory membrane protein that functions in the brain. It is increased by intracellular acidosis through the interaction of intracellular H^+ with an allosteric modifier site in the transport domain. In the previous study, we investigated that glutamate-induced NHE-1 phosphorylation mediated by activation of PKC-beta in cultured neuron cells via ERK/p90RSK pathways result in NHE-1 activation. However, whether glutamate stimulates NHE-1 activity solely by the allosteric mechanism remains elusive. **2. Methods** Cultured primary cortical neuronal cells were subjected to intracellular acidosis by exposure to 100 μM glutamate or 20 mM NH4Cl. After the desired duration of intracellular acidosis, the phosphorylation and activation of PKC-beta, ERK1/2 and p90RSK were determined by Western blotting. **3. Results** We investigated that duration of intracellular acidosis is controlled by glutamate exposure time. The NHE-1 activation increased while intracellular acidosis is sustained for > 3 min. To determine sustained intracellular acidosis induces NHE-1 phosphorylation, we examined the phosphorylation of NHE-1 induced by intracellular acidosis by transient exposure to NH_4Cl. Sustained intracellular acidosis activated and phosphorylated NHE-1. In addition, sustained intracellular acidosis also activated the PKC-beta, ERK1/2 and p90RSK in neuronal cells. **4. Conclusions** We conclude that glutamate stimulates NHE-1 activity through sustained intracellular acidosis which mediates NHE-1 phosphorylation regulated by PKC-beta/ERK1/2/p90RSK pathways in neuronal cells. This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology [Grant 2012R1A1A2044497].

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0381

THERAPEUTIC POTENTIAL OF WITHANIA SOMNIFERA SUPPLEMENTATION: IMPLICATIONS IN EXPERIMENTAL FOCAL CEREBRAL ISCHEMIA

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Objective:
To investigate the neuroprotective efficacy of *Withania somnifera (WS)* supplementation in preventing focal cerebral ischemia.

Methods:
Ischemic stroke was induced in male wistar rats using middle cerebral artery occlusion (MCAO) for 90 min, followed by reperfusion injury for 24 hrs. The progression of cerebral ischemia was assessed by single-photon emission computed tomography (SPECT) and the preventive effect of *WS* supplementation was analyzed via TTC staining including histopathological examination. Neurobehavioral tasks including, actophotom-eter, narrow beam walk, morris water maze and novel object recognition test were evaluated using Anymaze tracking software. Brain water content, blood-brain barrier integrity and matrix metalloproteinases (MMPs) levels were assessed.

Results:
MCAO rats showed increased neurobehavioral deficit score, significant motor impairments (assessed using narrow beam walk, actophotometer test) and cognitive deficits (assessed using Morris water maze test and novel object recognition test). A significant reduction in cerebral blood flow was observed in SPECT scanning of MCAO animals. MCAO animals also showed marked histopathological changes in terms of increased cerebral infarct volume, presence of pyknotic nuclei and astrogliosis in the cortex. Enhanced blood brain barrier leakage and brain edema were observed following ischemia reperfusion injury, as shown by increased Evan's blue staining, higher brain water content and increased MMPs levels. However, *WS* pre-treatment (300 mg/kg body weight) for 30 days to MCAO animals was able to efficiently improve behavioral functions, attenuate blood brain barrier alterations and reduced histological changes.

Conclusion:
The result suggests the propensity of *WS* supplementation in preventing ischemic stroke.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0719

POSSIBILITY OF METABOLIC THERAPY IN TREATMENT OF VASCULAR COGNITIVE IMPAIRMENT.

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Objectives. Neuropathological studies in most cases of dementia and cognitive impairment reveal a large load of vascular ischemic brain lesions. Therefore we have explored effect of antioxidant drug mildronat (meldonium) and neuroprotective citicoline (ceraxon) use in patients with vascular cognitive impairment. This drugs have demonstrated neuroprotective effects in acute stroke and has been shown to improve cognition in patients with vascular cognitive impairment and even in some patients with Alzheimer disease but with no clear consensus.

Methods . 120 patients (72 female , 48 male, average age 71,6 years) with vascular cognitive impairment received mildronat 1000 mg i/v and ceraxon 1000 mg i/v for ten days and then continued treatment orally for 6 months. Main clinical manifestations were impaired attention and forgetfulness, psychomotor slowing, impaired executive and visuospatial skills, change in personality, and emotional disturbance. The patient closely followed clinically, with repeated neuropsychological assessment. Results compared with a control group (N=135).

Results

The benefits of treatment began to be apparent within the first monthes.46 patients showed stable improvement in cognitive performance measures and in daily life. 40 patients trend back to their baseline. 34 patients had no improvement after treatment. There was no case of worsening disease or bad drug bearing. Statistically significant reduction in cognitive impairment was seen in the treated group in the domains of memory, attention and executive functions.

Conclusion The use of mention drugs has proved to be a valid treatment in patients with a vascular cognitive impairment and appears to be a safe.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-1632
COGNITIVE IMPAIRMENT AND HYPOTHYREOSIS
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Objectives. In the practice of neurology we often face to cognitive impairment due to is different couses. One of which is hypothyreosis; the last one is often unrecognised, becouse it proceeds under mask of other deasises. The timely recognition of patology promotes the benifits of treatment resalts.
Methods. We observed 70 patients with mild and moderate cognitive impairment, 51 female, 19 male, average 60±7,5. The patiens were observed in dynamic with clinical examination, neuropshichology tests, CT, MRI, labarator, inclusive hormonal tests. 43 patients detected clinical or subclinical hypothyreosis. 40 patents have accompanied anemia and bradycardia.
Results. 38 patients were get same improuvment after addition of hormonal therapy to neuroprotective treatment, benefits were seen in the domains of memory, attention, executive functions, bradycardia and anemia. 5 had no any improvement. There was no case of worsening disease or bad drug bearing.
Conclusion. Additional ivertigations should be done to understand in this cases the real couse of cognetive impairment (hypothyreosis, anemia, bradycardia). All patients with mild and moderate cognitive impairment should be screened for revealing hormonal disturbanses.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0285
NOOTROPIC TREATMENT OF COGNITIVE IMPAIRMENT IN EARLY REHABILITATION PERIOD OF ISCHEMIC STROKE

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Objectives. To determine cognitive status in poststroke patients and carry out pathogenetic therapy.
Methods. Ninety five poststroke patients (38–82 years, 61 males and 34 females) were randomly assigned to receive phenotropil (n=64) or placebo (n=31) in a double-blind study. Clinical and neuropsychological investigation was carried out at one and three months after the first hemispheric ischemic stroke with the use of NIHSS, Barthel index (BI), MMSE, FAB, Semantic and Phonetic Verbal Fluency test (SVFT and PVFT), attention test (AT), Clock Drawing Test (CDT). Background indices and their change during 2 months' treatment with phenotropil/placebo were evaluated. Control group consisted of 35 persons without stroke.
Results. The data differences between poststroke patients and control group were fixed in MMSE (p=0.0003), FAB (p=0.0000), SVFT (p=0.0001), PVFT (p=0.0033), AT (p=0.0001) one month after stroke. The decrease of neurological deficit and frontal dysfunction was found in both groups: phenotropil (p = 0.0031 and p = 0.0000) and placebo (p=0.0208 and p=0.0003). Only after treatment with phenotropil the indices MMSE (p = 0.0004) and CDT (p = 0.0064) were improved, differences with control group in MMSE, FAB and PVFT disappeared. The data differences between poststroke patients and control group in MMSE (p = 0.0043), FAB (p = 0.0094) and PVFT (p = 0.0383) remained after treatment with placebo.
Conclusion. The decrease of frontal functions, verbal fluency and attention is observed in poststroke patients. The use of phenotropil in rehabilitation period leads to normalization of existing disorders and regress of neurological deficiency.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0612

GLYCEMIC REGULATION THROUGH KETOGENIC DIET OR METFORMIN TREATMENT IN A NOVEL MURINE MODEL OF TYPE 2 DIABETES AND ALZHEIMER'S DISEASE

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OBJECTIVES. Our mice combine two disease models, Type 2 Diabetes and Alzheimer's disease (AD). They are morbidly obese, diabetic, and show both severe vascular and beta-amyloid (Aβ) pathology in the brain with increasing age. In this study, we investigated the outcome of treatment with either the anti-diabetic drug metformin or a short-term ketogenic diet in regards to weight, cholesterol, insulin and glucose levels, and the effects on neuropathology in this mouse line.
METHODS. For our diet study, we placed 4-5 month old mice of varying genotypes on a ketogenic diet (~80% calories from fat) for three months and evaluated weight, glucose, Aβ, phospho-tau, insulin, leptin and cholesterol levels. Metformin (200 mg/kg/day) was used to treat middle-aged diabetic mice, which were then subjected to a cognitive evaluation via Morris water maze, and subsequently imaged by MRI.
RESULTS. Endpoint measures of the ketogenic diet showed that, although the diet had no effect on weight, it lowered both plasma glucose and insulin levels, while cholesterol levels remained unchanged. Leptin levels increased significantly, whereas the presence of both Aβ and phospho-tau were decreased. The mice were surprisingly resistant to metformin, which resulted in only modest benefits.
CONCLUSIONS. As vascular or mixed dementia is an important area for the development of therapies, further exploration of these and other interventions will be important in the db/AD mice. We are particularly interested in how environmental exposures interact with the natural course of the disease to influence the development of neuropathology. Funded by NIH (ES024158, AG045809, NS083692).

ADPD5-2008

EFFECT OF INTENSIVE GLYCAEMIC CONTROL ON COGNITIVE DECLINE IN PATIENTS WITH TYPE 2 DIABETES: A META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS

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Abstract

Aim:

The aim of this meta-analysis was to compare the effect of intensive versus standard glycaemic control on cognitive decline in type 2 diabetic patients.

Methods:

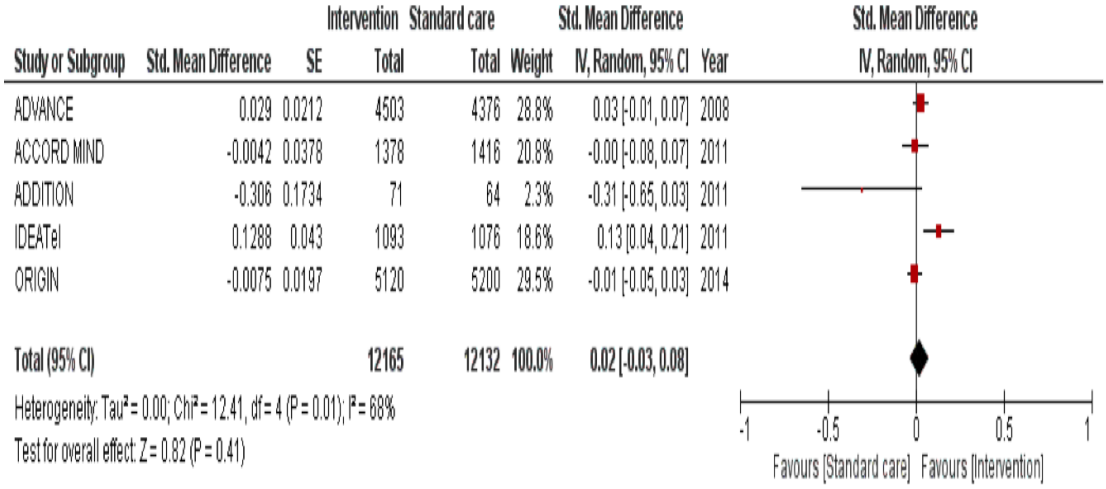
A systematic search of PubMed and ALOIS was conducted from inception up to October 30, 2014. Randomized controlled trials (RCTs) of type 2 diabetic patients comparing the rate of change in cognitive function among participants assigned to intensive versus standard glycaemic control were included. An inverse-variance weighted random-effects model was used to calculate standardized mean differences (SMDs) and 95% confidence intervals.

Results:

A total of 24, 297 patients from five RCTs were included in the meta-analysis. Follow-up ranged from 3·3 to 6·2 years. The result from the pooled analysis showed that intensive glycaemic control was not associated with slower rate of cognitive decline in patients with type 2 diabetes, compared to standard glycaemic control. (SMD=0·02; 95% confidence interval = -0·03 to 0·08) although there was some heterogeneity across individual studies (I^2=68%, P for heterogeneity=0·01).

Conclusions:

There are few diabetes control trials including cognitive endpoints and a small number of trials comparing intensive and standard treatment strategies. Currently, intensive glycaemic control should not be recommended for prevention of cognitive decline in patients with type 2 diabetes because there is no evidence of its effectiveness. Moreover, the use of intensive diabetes treatment results in an increase of risk of hypoglycaemia, which is linked to greater risk of poor cognition.



04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-2141

EFFECT OF A SPECIFIC MULTI-NUTRIENT DIET ON SYSTOLIC BLOOD PRESSURE AND CEREBRAL HEMODYNAMICS IN AGING APOE4 AND APOE-KO MICE

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Objectives
It is known that the cholesterol-transporter apolipoprotein ε (apoE) genotype is associated with the development of both cardiovascular and neurodegenerative diseases like Alzheimer's disease (AD).
We tested the effects of long-term consumption of a specific multi-nutrient diet on systolic blood pressure (SBP) and cerebral hemodynamics in two vascular risk models: the apolipoprotein ε4 (apoE4) and the apoE knockout (apoE-ko) mice.
Methods
We investigated the relationship between SBP monitored twice each month via tail cuff plethysmography, cerebral blood flow (CBF) measured with MR by FAIR-ASL, and a dietary intervention in 16-18 month old wild-type (WT) C57bl/6j controls, apoE-ko and apoE4-mice. At 2 months of age, the mice were put on the diets (Control + Experimental) for the remainder of the experiment.
Results
From 16-18 month of age, apoE-ko mice had an increased SBP compared to WT littermates, which was lowered by the Experimental diet. In addition, the Experimental diet increased also the cortical CBF in apoE-ko mice.
At 18 month of age, only Control fed apoE4 mice had a raised SBP compared to their WT littermates, being not visible in apoE4 mice fed the Experimental diet. The cortical and thalamic CBF was decreased at 18 month of age. All animals fed the Experimental diet had a higher CBF than Control fed animals.
Conclusions
We provide new evidence for a relationship between apoE and risk factors for AD. Our data suggest that this specific multi-nutrient diet has beneficial effects on early pathological consequences of hypercholesterolemia and vascular risk factors for AD.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0262

THE CORRELATION OF WMH AND COGNITION IN BINSWANGER TYPE VCIND AND VAD

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Objectives: To evaluate the correlation of white matter hyperintensities(WMH) and cognition in patients with Binswanger type vascular cognitive impairment without dementia(VCIND) and vascular dementia(VaD).

Backgrounds: Because of its heterogeneity, research of vascular dementia is very difficult to perform and design. Among them, Binswanger type subcortical abnormalities(i.e. white matter hyperintensities, WMH) is relatively happened homogenously to its pathophysiology and clinical features. Although we frequently notice WMH on advanced MRI, we don't yet understand the role of WMH on cognition.

Methods: Patients(N = 57) with Binswanger type VCIND(N=27, K-MMSE =22.67±3.85) and VaD(N=30, K-MMSE =14.53±5.99), according to neuroradiological working criteria, were selected. We excluded subjects with significant number of lacunes for more homogenous group. We evaluated the volume and location of WMH on MRI by visual rating. For detailed cognitive function assessment, all subjects were performed Seoul Neuropsychological Screening Battery-Dementia version (SNSB-D).

Results: The performance of general cognitive function is not correlated with total volume of WMH in Binswanger type VCIND and VaD. The performance of each cognitive subdomain is also not correlated with related area in MRI. The cognitive function is influenced by their educational level and sometimes age. After control the total volume of WMH, education and age factors, ratio of anterior to posterior WMH volume is correlated with general cognitive function.

Conclusions: The volume and location of WMH is not correlated with cognitive performance in Binswanger type VCIND and VaD. We suggest its possible role of WMH as auxiliary factor of cognitive reserve.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0291

NEUROIMAGING CHARACTERISTICS OF BRAIN STRUCTURE CHANGES IN VASCULAR PATIENTS WITH MILD COGNITIVE IMPAIRMENT

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The location and severity of brain changes are not included in neuroimaging of patients with mild cognitive impairment (MCI).

Objective. To evaluate brain structure changes in vascular patients with cognitive impairment. To determine of diagnostic capabilities of structural magnetic resonance imaging in evaluation of vascular cognitive impairment.

Methods. Forty two patients (16-males, 36-female) aged 40-81 with risk factors for cerebral vascular diseases. Clinical and neuropsychological testing (MMSE, FAB) were conducted. Neuroimaging data were evaluated according to presence of diffuse and focal changes and expansion of ventricular system.

Results. Twenty one patient had MCI. Eighteen of them had a history of cerebral stroke. Indicators of neuropsychological testing differ in groups with and without MCI (MMSE-26,35±0,85 and 28,70±0,82; p=0.0000; FAB-12,84±2,14 and 16,2±2,65; p=0.0000). Severity of atrophic process in patients with MCI was more defined than in patients without MCI (p <0.02). Periventricular and subcortical leukoaraiosis was observed more frequently in patients with MCI. Focal changes in the form of cystic glial ones and lacunar infarctions dominated in patients with post-stroke variants of MCI. No statistically significant differences of ventricular system expansion in groups were revealed.

Conclusion. Combination of periventricular and subcortical leukoaraiosis localized in frontal and parietal lobes with lacunas in underlying parts of grey and white substances and convexital atrophy is the most significant in cognitive deficiency. Leukoaraiosis and subcortical silent brain infarcts are the basis of vascular cognitive impairment. Relationship of cognitive deficiency with atrophic processes of cortical structures exists.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1154

WHITE MATTER MICROSTURCTURAL CHANGES IN PURE ALZHEIMER'S DISEASE AND SUBCORTICAL VASCULAR DEMENTIA

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Background & Objective : After eliminate confounding effects mixed Alzheimer and vascular pathology, there are different patterns of neuropsychologic and cortical thickness analysis between AD and SVaD. Therefore, we investigate white matter microstructural changes of Alzheimer disease and subcortical vascular dementia.
Methods : We prospectively recruited patients who were clinically diagnosed with AD and SVaD at Samsung Medical Center between September 2008 and May 2011. And we recruited individuals who had subjective memory impairment (SMI) as a control group. We used tract-based spatial statistics of diffuse tensor imaging (DTI) to compare patterns of reduced fractional anisotropy (FA).
Result : Compared with SMI, AD patients showed reduced FA in frontal and parietal area, while SVaD patients showed reduced FA in the whole white matter tract. Comparing between AD and SVaD patients directly showed that SVaD patients had reduced FA across the entire white matter tract skeleton.
Conclusions : SVaD patients showed more reduced FA compared with AD patients. It suggest that microstructural change of white matter is more progressed in SVaD patients than AD patients. Further studies may be needed to clarify microstructural changes of both dementias.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1694

INFLUENCE OF AMYLOID-BETA UPON COGNITIVE DECLINE IN VASCULAR COGNITIVE IMPAIRMENT – A 3-YEAR LONGITUDINAL STUDY

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Background:
We hypothesized that amyloid-beta (Aβ) deposits drive progressive cognitive decline in vascular cognitive impairment (VCI). We investigated the influence of Aβ deposits upon long-term cognitive decline in subjects with stroke/transient ischemic attack (TIA).
Methods:
We recruited 76 subjects with varying severity levels of cognitive impairment after stroke/TIA to receive Pittsburgh compound B (PiB) positron emission tomography (PET). Subjects with known Alzheimer's dementia prior the index event were excluded. We performed mini-mental state examination (MMSE) upon subjects at 3 month (m, baseline), 15 m (year 1), 27 m (year 2) and 39m (year 3) after the index event. We arbitrary defined stage 1, 2, and 3 as the period from baseline to year 1, year 1 to year 2, and year 2 to year 3, respectively. Patients with global PiB retention standardized uptake value ratio (SUVR) > and ≤ 1.5 were classified as mixed and pure VCI, respectively. We compared rate of cognitive decline between subjects having mixed VCI (mVCI) and pure VCI (pVCI), with age and education adjusted.
Results:
Sixty subjects completed 3 years follow-up. For mVCI subjects (n=13), MMSE declined at a similar rate throughout all stages (stage 1, -0.6± 4.5; stage 2, -1.6± 2.3; stage 3, -2.7± 2.5, *p*= 0.272). Cognition of pVCI subjects (n=47) declined initially in stage 1 (-1.8±3.6, *p*=0.005) and remained static at stage 2 (0.3± 3.2) and 3 (-0.3±2.7, *p*=1).
Conclusion:
Cognition of subjects with VCI harbouring Aβ deposits continues to decline in the long-term.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-0306

IMPACT OF MILD COGNITIVE IMPAIRMENT ON THE CHANGES IN BIOELECTRICAL BRAIN ACTIVITY IN PATIENTS UNDERGOING ON-PUMP CORONARY ARTERY BYPASS GRAFTING

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Background: Neurological complications after on-pump cardiac surgery are one of the largest unsolved problems of modern cardiac surgery. The patients suffering from mild cognitive impairment (MCI) are regarded as a high-risk group for ischemic changes in the brain and the progression of cognitive impairment induced by cardio-pulmonary bypass. Non-invasive neuromonitoring (electroencephalography (EEG)) can provide information on subclinical symptoms and topography of cerebral ischemia.
Purpose: To study the topography of the spectral EEG power in coronary artery disease (CAD) patients with or without MCI before and after on-pump coronary artery bypass grafting (CABG).
Materials and Methods: A total of 53 male patients (age 47-68 years) were assigned to two groups: with MCI (n = 19, MMSE score – 26.3±0.95) and without MCI (MMSE score – 28.5±0.79, n=34). Monopolar EEGs were recorded on 3–5 days before and 7–10 days after surgery in 62 sites of 10-20 system. The EEG power was calculated in frequency ranges of 4-30 Hz.
Results: All patients have demonstrated theta1 and beta1 power increase 7–10 days after CABG compared to preoperative values. The topographic distribution of EEG changes was different in MCI and non-MCI groups: the patients with MCI had more prominent cortical dysfunction in the frontal and central regions.
Conclusions: The cortical dysfunction in the frontal brain areas can be associated with the progression of cognitive deficits, worsening social and household maladjustment in CAD patients with MCI.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-0516
NEUROPHYSIOLOGICAL ASSESSMENT OF COGNITIVE IMPAIRMENT IN VASCULAR DEMENTIA.
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Background. Search neurophysiological markers of cognitive impairment in vascular dementia is still an unsolved problem.Cognitive evoked potentials (p300) used to assess cognitive impairment in various neurological diseases, including vascular dementia. However, it is interesting to study the level of short-term memory and the state of p300.
Material and methods. We studied 26 patients with a diagnosis of vascular dementia (17 female and 9 male, average age 63.7 y.). Average score of MOCA test was 21.5. To evaluate the short-term memory, we used the "Digit Span" scale. To study the auditory cognitive evoked potentials using the standard "10-20%" EEG montage and "odd-ball" paradigm. As a comparison group selected healthy of similar age and sex (n=18).
Results. When comparing groups of patients and healthy significant differences were observed in the test result memory ($p<0.01$) and index latency p300 ($p<0.05$).There was no physiological growth of p300 latency depending on the age of the patient. In addition, the level of p300 latency depended on the number of these numbers during the test for memory for patients.The more numbers were called, the less was the latency ($p<0.01$).
Conclusion. By our hypothesis, p300 indicator can be used to assess the state of short term memory in patients with vascular dementia. However, the study is limited by the small sample of patients and requires more subjects.

06p. Imaging & Biomarkers: near infrared spectroscopy

ADPD5-0813
ABSOLUTE NON-INVASIVE NIRS DETECTS DIET-INDUCED AND AGE-ASSOCIATED CHANGES IN BRAIN MICROVASCULAR CIRCULATION

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Objectives: Commonly occurring senescent and pathological changes to cerebral microvascular circulation may play an important role in the early and potentially preventable stages of cognitive decline. We and others have found a loss of capillaries to correlate with cognitive decrements and precede neurodegeneration in animals.
Methods: To understand the functional impact of such microvascular loss, we developed an innovative non-invasive near infrared spectroscopy (NIRS) instrument, which is particularly sensitive to the microvasculature and which allows us to measure *absolute* brain hemoglobin concentration (reflecting microvascular volume) and oxygen saturation at rest and in response to physiologic challenges. We used it to evaluate cerebral blood volume and vascular reactivity in several animal models as a function of age, hypertension and dietary vascular risk (hyperhomocysteinemia and NAFLD).
Results: We detected substantial decrements in absolute hemoglobin concentrations between young and old hypertensive animals indicating substantial structural microvascular loss, even when the relative vascular reactivity did not differ between groups (i.e. percent change from resting conditions). In the model of dietary vascular-risk, young but not old animals showed a reversible diet-induced reduction in brain hemoglobin concentration, indicating that vascular plasticity might be limited in older animals. In some respects, these results are consistent with cross-sectional findings comparing absolute NIRS measures in young adult and old humans.
Conclusions: Non-invasive absolute NIRS may be useful as a biomarker for the early detection and monitoring of brain microvascular disease in pre-clinical research and in humans. The most informative diagnostic parameters and their interpretation remain to be established.

07c. Epidemiology, Risk Factors, Genetics & Epigenetics: metabolic

ADPD5-1536

CEREBROVACULAR RISK FACTORS AND THEIR DIFFERENT EFFECT ON NEURODEGENERATIVE AND VASCULAR BIOMARKERS

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Introduction: It is well known that concomitant vascular pathology aggravates Alzheimer's dementia (AD). However vascular risk factors, particularly diabetes mellitus have shown to be related independently to hippocampal atrophy hence a marker of neurodegeneration. This retrospective analysis was dedicated to estimate the effect of the three most common cerebrovascular risk factors on vascular encephalopathy and AD biomarkers in a sample of subjects ranging from healthy controls to AD via mild cognitive impairment (MCI) in sense of a cross-sectional study for neurodegeneration and from AD to pure vascular dementia (VD) via mixed dementia in sense of different lots of vascular and AD pathology.

Material/Methods: A total of 120 subjects (28 controls, 22 MCI, 29 AD, 26 mixed dementia and 15 VD) were included. For the whole group of subjects an association of vascular risk factors (arterial hypertension, diabetes mellitus and hypercholesteraemia) and markers of vascular pathology (white matter hyperintensity, intima media thickness of the carotid artery) and neurodegeneration (cerebrospinal fluid markers, e.g. ptau, tau, Aβ42 and Aβ ratio and hippocampal atrophy), respectively, were investigated.

Results: Subjects with diabetes mellitus had smaller hippocampi (p=.05), reduced Abeta (p=.06) and elevated tau (p=.04) while there was no association to white matter hyperintensities. Arterial hypertension was related to white matter hyperintensities (p=.002) only and hypercholesterinaemia to a smaller hippocampus (p=.01).

Discussion: From these data evidence derives that different cerebrovascular risk factors have a different impact on vascular encephalopathy, respectively neurodegenerative markers.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-1601

NEUROPHYSIOLOGIC EVALUATION OF DEPRESSION AND DEMENTIA IN POST STROKE ELDERLY PATIENTS

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Objective: To evaluate the influence of brain lesion localization as a risk for depression and dementia in post stroke patients (PSP). Material and methods: The study was prospective, randomized, in duration of 1 year, and included post stroke patients diagnosed and treated at the Department for Cerebrovascular Diseases. The patients were evaluated by means of the NIH Stroke Scale, Mini-Mental State Examination, Barthel Index, modified Rankin Scale, and MOS-Short Form 36. The evaluation period was 1, 3, 6 and 12 months after stroke. Results: Sixty eighth stroke survivors were assessed (mean age: 61.2 years; 52.2% males). The m-RS score ≤2, had 51.2% of the PSP had an m-RS score ≤2. The prevalence of depression and vascular dementia was significantly higher in females than in males (24.6 vs. 14.4%; x^2, $p = 0.03$). Elderly patients had significant worst MMSE and Bartel changes. The evaluation period showed the biggest changing 6 months after stroke. Post stroke dementia and depression was significantly associated ($p < 0.0001$) with education level, lower social and cognitive functioning, dependence in the instrumental activities of daily living and presence of diabetes in the multivariable regression analysis (R adjusted = 0.34). The relative risk of depression after a left-hemisphere stroke, compared with a right-hemisphere stroke, was 0.94 (95% CI 0.73-1.11). Conclusions: Post stroke dementia and depression was highly prevalent in the chronic phase of stroke. Early detection and recognition of associated risk factors is important to treat and prevent depression and dementia, in a rehabilitation setting.

08a. Animal Models: transgenic mice

ADPD5-0783
MODULATING NEUROLOGICAL OUTCOMES IN A MURINE MODEL OF MIXED DEMENTIA
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OBJECTIVES. Obesity and type 2 diabetes mellitus (T2DM) confer an increased risk for neurological disorders, including Alzheimer's disease (AD), vascular dementia, and stroke, though the underlying mechanisms are currently unknown. To answer this question, we created a murine model that encapsulates features of these diseases.
METHODS. We crossed the obese, diabetic db/db mouse with the *APP*$^{deltaNL/deltaNL}$*/PS1*$^{P264L/P264L}$ knock-in model of AD. We characterized the resulting mice (*db/AD*) for metabolic dysfunction, cognitive impairment, and amyloid deposition. We also assessed vascular structure and health using various methods, including MRI, histology, and vascular corrosion casting.
RESULTS. The *db/AD* mice were morbidly obese, exhibited metabolic dysfunction, and showed severe cognitive deficits. These mice did not have increased parenchymal or vascular amyloid deposition compared with non-diabetic controls, though expression of PS1 was elevated. *db/AD* mice did have severe vascular abnormalities, including aneurysms and strokes, that are consistent with the pathology observed in human diabetics.
CONCLUSIONS. The presence of vascular dysfunction in the absence of excess amyloid deposition is a key feature of the *db/AD* mouse line making them a useful model in which to study the mixed dementia that typifies T2DM. We are currently using adeno-associated virus technology to investigate the molecular mechanisms of neurological dysfunction and the role of tau in these pathologies. Finally, we are now using the *db/AD* mice to investigate different potential therapies, including telmisartan, an angiotensin receptor antagonist with off-target effects on cerebrovascular function. Funded by CART Foundation, Alzheimer's Association (IIRG-10-172905), Bright Focus Foundation, NIH (ES024158, AG045809, NS083692), and AHA (13IRG14330016).

09a. Patient Care & Support: caregiver support

ADPD5-0426

FAMILY CAREGIVERS: A SUPPORT THAT NEEDS A SUPPORT (A STUDY WITH SOCIAL WORK INTERVENTION PERSPECTIVE)

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Objectives:

1. To understand care giving activities and processes of care giving in the select families of Vadodara taluka having Pateint with Dementia (PWD).
2. To find out the stress associated domains of caregivers and its management
3. To suggest and prepare guidelines for a training module with focus on - Coping strategies for caregivers

Methods:

It was both exploratory and descriptive study .Both qualitative and quantitative approaches are used. A non- probability, purposive sampling method was used to study 103 dementia patients clinically diagnosed (and available)

The above sample is supported with substantial number of case studies and Interviews with Health care professionals

3. Results:

For the informal support, data reveals that 67.7% family caregivers get help from the spouse and 60.2% family caregivers receive support from other family members.

68.9% caregivers receive direct help in maintaining personal hygiene (cleaning if patients soils, comb hair, wear clothes, nails trim, brush shaving etc).

16.5% caregivers reported that family members had discontinued support and 6.8% caregivers have reported that friends, relatives and maids have stopped giving support.

4.Conclusion:

The study helped to gather insights into care giving support. Caregivers are not able to recognize early symptoms,they take it as a part of normal Aging. Recognition of illness happens only when psychiatric symptoms are evident in the patient.Caregivers are not able to make proper arrangements due to lack of proper training.Lack of information related to course of the disease impacts the care giving arrangements. Registration of cases is low in PHCs, Government and Private Hospitals.

Figure no. 1 the Social Work Intervention Model for family caregivers of PWD

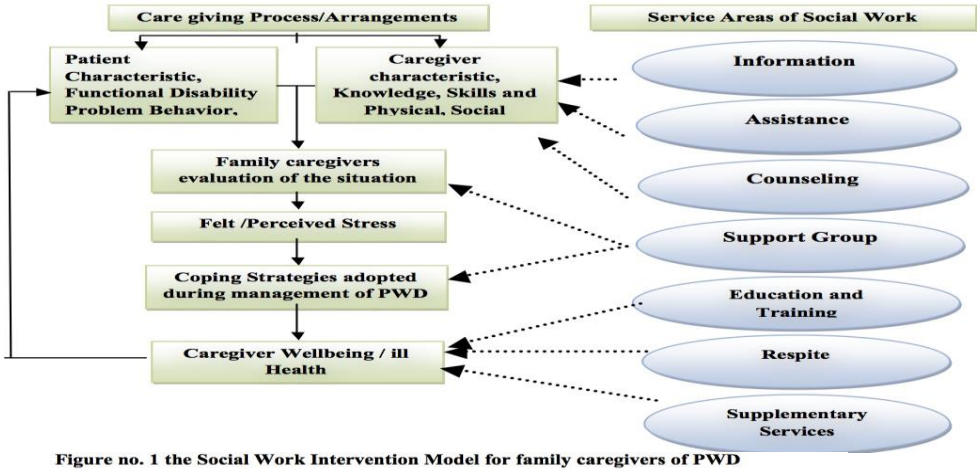


Figure no. 1 the Social Work Intervention Model for family caregivers of PWD

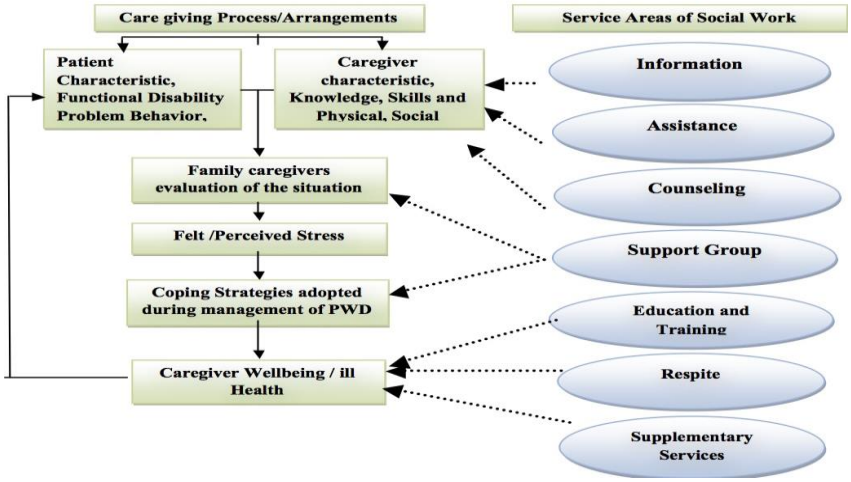
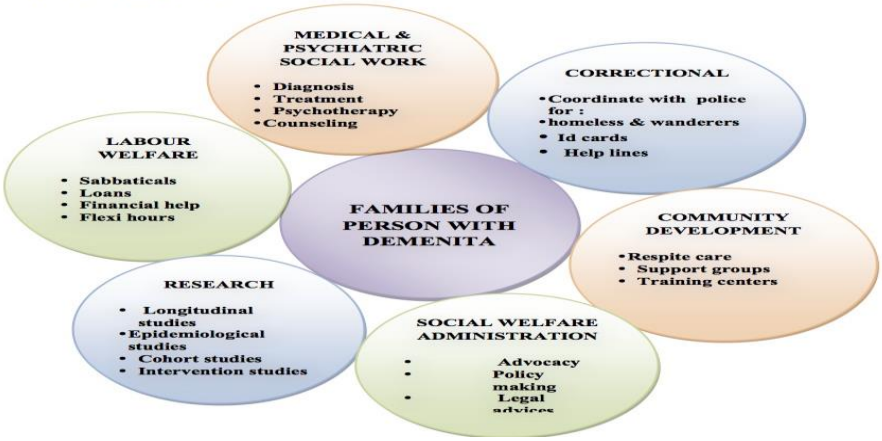


FIGURE NO. 2 : INTEGRATION WITH SOCIAL WORK PROFESSION FOR FAMILIES OF PWD



09f. Patient Care & Support: cognitive training

ADPD5-1693

EFFECTIVENESS OF TRAINING INFIRM ELDERLY PEOPLE USING A MACHINE WITH A COGNITIVE DYSFUNCTION IMPROVEMENT SYSTEM

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1. Objective: The decrease in balance control in the elderly is an important risk factor for falls and markedly influences their activities of daily living (ADL) and quality of life (QOL). In a previous study, we reported a correlation between balance control and mental function in the elderly.
In this study, we investigated the effectiveness of training using a machine with a cognitive dysfunction improvement system (mirgometer) for improving the physical and cognitive functions and mental state of infirm elderly individuals.
2. Methods : The subjects comprised infirm elderly individuals using elderly facilities. Mirgometer intervention was monitored for 6 months, and the subjects were evaluated for motor function (balance ability and flexibility), mental function (cognitive function and psychological state), ADL, and QOL.
Furthermore, the change of each function over time in the control group, which synchronized a condition, was compared with that in the intervention group.
3. Results: At 6 months, the mirgometer score and cognitive function were significantly improved. The meaningful drop of the balance function was recognized in the exercise function in the control group.
4. Conclusion: This intervention significantly improved cognitive and motor functions in the infirm elderly. Therefore, continual training could aid in improving and maintaining the physical and mental functions and QOL in infirm elderly people.

09f. Patient Care & Support: cognitive training

ADPD5-1725

COMPUTER COGNITIVE PROGRAMS AND ENTERTAINING COMPUTER GAMES: WHAT ARE DIFFERENCES?

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[1]Chair of Neurology and Medical Rehabilitation,
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Objectives: Comparing an effectiveness of using neuropsychological computer programs for cognitive correction with effects of using entertaining computer games for rehabilitation of patients with post stroke cognitive impairments.
Methods: Patients after hemispheric stroke in recovery period (up to 1 year) with cognitive impairments (N=47, age 40-65) were randomized into three groups. All patients received physiotherapy. Patients in the intervention group had 10 everyday training sessions with neuropsychological computer programs of 40 min duration. Participants in the active control group played entertaining games keeping the identical regimen. The passive control group patients received standard treatment.
Results: In the intervention group after training course we observed significant improvements on every cognitive and functional scales. In the active control group changes on FAB, Moca, Shulte's test, MMSE were also statistically significant. We found no significant changes on cognitive scales in the Passive control group after treatment. Significant improvements were observed in the intervention group comparing with the passive control group (MoCA, CDT, FAB, Shulte's test). Differences between groups where patients played neuropsychological and entertaining games were statistically insignificant. At the same time there were no significant differences between the active control and the passive control groups.
Conclusions: Additional using of the complex of neuropsychological computer programs is an effective and simple method of the correction cognitive impairments in post stroke patients comparing with the standard rehabilitation without special cognitive training. It is still necessary to clarify whether there is a difference between effectiveness of neuropsychological computer training and entertaining computer games.

09g. Patient Care & Support: art, music & life style

ADPD5-1669

A CASE STUDY OF THE RESPONSES OF A PERSON WITH SEMANTIC DEMENTIA TO THE USE OF MUSIC WITH ACTIVE VIDEO

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[2]Gerontology, previously with Univ of Western Ontario, London Ontario, Canada

Objectives: The question of the use of music for persons with dementia is an area of intense investigation. However, there is limited literature available regarding their responses to engaging with music (e.g., singing or humming to the music, clapping or tapping feet to the beat) while at the same time viewing themselves on a video of the session on a computer. The aim of our case study is to provide insights about this type of situation, which we term Active Video Musical Interaction (AVMI).
Method: A total of 61 short video clips were made,using Skype video component on a lap top computer with a 85 year old relative of the 2nd author who has been experiencing Semantic Dementia since 2009. These videos were shown to the relative during each informal music listening session over a 6 month period. Only her favorite music during her adult years were selected.
Results: The salient findings of this case study revealed that the older relative was more attentive and enjoyed the sessions as indicated by increased eye contact with the laptop screen and her smiles.
Conclusion:: The AVMI technique is an easy to use method that family caregivers could use to enhance interaction with their relative and the quality of life for the family unit. A formal large scale study is planned in order to measure specific affective changes using the Affect Rating Scale.

10d. Other: diagnostics

ADPD5-0453
NEUROMONITORING IN PATIENTS WITH FOCAL BRAIN LESIONS DURING REHABILITATION

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Objectives. Neuromonitoring is important not only for checking the effectiveness of rehabilitation, but also for timely correction of the rehabilitation program to prevent the exhaustion of functional reserves. The aim is to search for the reliable criteria for determining the functional prognosis and monitoring the condition of patients during rehabilitation.
Methods. We examined 100 patients with stroke and traumatic brain injury, mean age 67.5 years. All patients underwent clinical neurological examination, extended neuropsychological testing, neurophysiological examination in the dynamics, which included the evoked potentials of the brain.
Results. In the study of contingent negative variation (CNV), we found that low amplitude and short duration of the positive part of the wave correlated with cognitive decline, low social activity, and preceded the decompensation of the disease and exacerbation of already existing symptoms. We found that the elongation of the latent period of P300 potential and the violation of the ratio of amplitudes of positive and negative parts of CNV were typical mainly for patients with mild cognitive impairment, who had unsteadiness when walking with high risk of falls. In these patients, the improvement of motor functions during rehabilitation was less clear compared with others. The improvement of the parameters of cognitive evoked potentials in dynamics during rehabilitation correlated with the improvement of motor functions of hand and leg.
Conclusions. The evoked potentials of the brain in conjunction with the results of the extended neuropsychological testing may be the objective indicators for monitoring the condition of patients during rehabilitation.

10d. Other: diagnostics

ADPD5-0817
CHANGES ON BLESSED-DEMENTIA-INFORMATION-MEMORY-CONCENTRATION-TEST AFTER STROKE

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Objectives: Ischemic stroke is one of the leading causes for death and disabilities. Searching for clear and easier screening tests, suitable for everyday practice, remains an open question. One of them can be Blessed-dementia-information-memory-concentration test (BDIMCT). The aim of our study was to apply BDIMCT at subacute stroke stage and to find changes on it.
Methods: We examined 109 poststroke subjects (at 3rd month after stroke) on average age 66.67±9.03 years (67 males, 42 females) and 112 controls (aged 66.70± 9.13, 69 males, 43 females) with BDIMCT.
Results: Patients showed lower total points number than controls (23.80±5.87 vs 29.87±2.41, p=0.0001), lower 5-min recall results (1.98±1.46 vs 3.86±1.11, p=0.0001), lower information subscale results (11.51±2.66 vs 13.64±0.85), lower personal memory (6.50±1.01 vs 6.99±0.09, p=0.0001), lower nonpersonal memory (1.91±1.02 vs 2.81±0.65, p=0.0001) and lower concentration points (1.88±0.92 vs 2.64±0.58, p=0.0001). Patients with cortical strokes had better results on total BDIMCT points than those with subcortical strokes (27.61±3.26 vs 21.00±5.41, p=0.0001). Additional multifocal ischemic encephalopathy leaded to low total BDIMCT points (21.03±5.83 vs 27.13±3.89, p=0.0001). Severity of stroke (measured by NIHSS) was associated with decreasing of BDIMCT total points (r=-0.52, p=0.0001). Aging and low education level were also associated with lower test results. Some vascular risk factors (arterial hypertension, chronic ischemic heart disease and diabetes mellitus) were associated with lower BDIMCT results but only in control group.
Conclusions: On the basis of our results, we can conclude that BDIMCT may be a useful test for cognitive impairment after stroke in general practice.

10d. Other: diagnostics

ADPD5-1569

COMPARISON OF VERBAL AND NONVERBAL FLUENCY PERFORMANCE WITH MINI-MENTAL STATE EXAMINATION IN DETECTING COGNITIVE IMPAIRMENT IN DIFFUSE SUBCORTICAL WHITE MATTER DISEASE OF THE BRAIN

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Introduction: The use of Magnetic Resonance Imaging (MRI) recently have revealed changes in the cerebral white matter however, the clinical significance of these are incompletely understood. Leukoaraiosis was introduced to designate periventricular or subcortical areas of hyperintensity on MRI and could be the only finding in younger people with cognitive deficits secondary to stroke. Early identification of vascular dementia was emphasized in order to administer treatments earlier. Verbal fluency tests measures executive processes as it requires word retrieval, recall, self-monitoring, initiation, and inhibition of responses. The design fluency test measures the ability to generate series of novel abstract designs.

Objective: To compare the performance in verbal and nonverbal fluency test in detecting cognitive impairment in patients with diffuse subcortical white matter disease in a tertiary hospital.

Method: Subjects, atleast 30 years old underwent MRI of the brain from August 1 to September 30, 2013 were purposively recruited. Excluded were the patients with clinical stroke, Alzheimer's and Parkinson's Disease. MMSE, verbal and design fluency test were performed and scored.

Results: Sixty subjects were included in the study. T-test was used to compare two groups with numerical data and showed that there was a significant difference noted in phonemic (p=0.02), semantic (0.04), design fluency test (0.01) scores as proven by the p values all less than 0.05.

Conclusion: Patients with diffuse subcortical white matter disease has significantly lower performance on both the verbal and design fluency test with no significant difference on the MMSE.

10d. Other: diagnostics

ADPD5-1798

NEUROPSYCHOLOGICAL TESTS AND CSF BIOMARKERS PREDICT DEMENTIA AND DISTINGUISH BETWEEN VASCULAR DISORDERS AND AD AT THE MCI STAGE

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Objective: The objective was to study which neuropsychological tests and cerebrospinal fluid biomarkers in a Mild Cognitive Impairment (MCI) population strongest predict conversion to dementia in general, Alzheimer's disease (AD), and mixed/vascular dementia (MD/VaD).
Method: Three hundred and fifteen MCI subjects were followed up after two years. The baseline neuropsychological battery consisted of tests assessing speed and attention, learning and episodic memory, visuospatial, language and executive functions. The biomarkers were T-Tau, P-Tau Aβ1-42 and NFL.
Results: At follow up 63 MCI subjects had progressed to dementia; 29 to AD, 16 to MD, 13 to VaD and 5 to other forms of dementia. After correction for age, education and multiple comparisons there were highly significant differences between stationary MCI and incipient dementia patients on 19 out of 20 neuropsychological tests. Also on T-Tau, P-Tau and Aβ1-42 there were significant differences. The variables that best predicted dementia in general were memory, speed/attention, executive tests and T-Tau. The variable profiles of incipient AD and MD/VaD differed quite clearly. T-Tau, P-Tau, memory and visuospatial tests predicted AD; speed/attention, executive and language tests predicted MD/VaD. The sensitivity and specificity figures were quite good for both dementia in general and the specific dementia diagnoses, with areas under the curve of 0.91 for dementia, 0.92 for AD and 0.87 for MD/VaD.
Conclusions: The test battery and the biomarkers predicted dementia with good sensitivity and specificity. The combination of neuropsychological tests and CSF biomarkers distinguished quite convincingly between AD and MD/VaD two years before diagnosis.

10d. Other: diagnostics

ADPD5-1935
ROLE OF COMORBID RISK FACTORS ON POST-STROKE COGNITIVE PERFORMANCE
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Aim: The aim of the present investigation was to evaluate the role of some comorbid risk factors on post-stroke cognitive performance.
Contingent and methods: In the study were included 109 patients with first ever clinically verified ischemic stroke (mean age 66.67±9.03, 61% males) and 112 controls (mean age 66.70±9.03). Cognitive functions were examined with a neuropsychological battery as follows: Mini Mental State Examination (MMSE), Blessed Dementia Information Memory Concentration Test, 10 word test for short-term verbal memory and delayed recall, Benton visual retention test, Isaack's Verbal Fluency test (VF), Clock drawing test, Hamilton Depression Scale.
Results: Arterial hypertension was found associated with cognitive impairment (CI) of the controls and the visual spatial ability of the post-stroke survivors 3 months after the onset. Ischemic heart disease (IHD) was associated with lower MMSE and VF scores and higher depression levels of ischemic stroke patients and with lower global cognitive performance, verbal and visual memory changes, VF score and higher depression levels of the age-matched controls. Arrhythmias lead to higher depression levels in the acute stage of stroke. Diabetes mellitus (DM) was not found associated with additional cognitive impairment of the ischemic stroke patients. Controls with DM showed global CI, memory and VF changes and higher depression levels. Patients with chronic obstructive pulmonary disease (COPD) also had VF and visual memory impairments.
Key words: comorbidity, poststroke cognitive impairment

10h. Other: other

ADPD5-0755
RENAL FUNCTION, MRI BRAIN CHANGES AND POST-STROKE COGNITIVE IMPAIRMENT

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Background and Purpose: Limited data exist regarding the relationship between chronic kidney disease and post-stroke cognitive impairment. We aimed to evaluate the relationship between impaired renal function and markers of cerebral small vessel disease, brain pathology and cognitive decline in a longitudinal post-stroke cohort.
Methods: The TABASCO study is a prospective cohort of mild-moderate ischemic stroke/TIA patients, who underwent a 3T MRI, and were assessed for their cognitive function at hospital admission, 6, 12 and 24 months later, using the Montreal Cognitive Assessment (MoCA) and a computerized cognitive testing battery. Renal function was estimated at admission using the Cockcroft Gault creatinine clearance (CCl) equation. The volume and integrity of preexisting white matter hyperintensity (WMH), ischemic lesions and brain atrophy on MRI were measured.
Results: Baseline data were available for 462 subjects (mean age 67.4 years, 60.4% males). Participants with a CCl <60 ml/min (n=182) performed significantly worse in all cognitive tests over time ($p<0.001$) than those with a CCl $\geq$60 ml/min (n=280), had enlarged WMH volume ($p<0.001$), cortical atrophy ($p=0.002$) and smaller hippocampi ($p<0.001$).
After the 2-years, 16% of the participants developed cognitive impairment.
Multiple logistic regression analysis controlling for traditional risk factors, including cardiovascular, showed a significant association of CCl <60 ml/min with development of cognitive impairment at the end of follow-up [odds ratio: 2.99 (95% confidence interval: 1.08–8.27), $p = 0.035$].
Conclusions: Decreased renal function is associated with increased cortical atrophy and is a predictor of cognitive decline 2 years after stroke/TIA, possibly reflecting small vessel disease.

10h. Other: other

ADPD5-1150
SMALL VESSEL DISEASE MARKERS CORRELATE WITH MOTOR DYSFUNCTION IN PATIENTS WITH SUBCORTICAL VASCULAR IMPAIRMENT
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Background & Objective: Specific effects of small vessel disease markers on motor dysfunction of subcortical vascular cognitive impairment are less reported than cognitive dysfunction. Pyramidal and extrapyramidal scale (PEPS) is a scale for the assessment of motor impairment in vascular cognitive impairment associated with small vessel disease. We aim to evaluate the relationships between each small vessel disease markers and motor function using the PEPS in SVCI patients.
Methods: We prospectively recruited 137 participants with SVCI at Samsung Medical Center between September 2008 and May 2011. Among 137 patients with SVCI, we performed PEPS to 129 subjects. The association of microbleeds with motor impairment was estimated using linear regression models. Adjustment was made for age, gender, education and vascular risk factor, other small vessel markers and amyloid burden.
Result: Of the 129 patients with SVCI, overall volumes of WMHs were 38.9 ± 18.5 mL. Prevalence of lacunes and MBs in these subjects were 116 (89.9%) and 85 (65.9%), respectively. Total WMH volumes were associated with total, gait, EPS and pyramidal PEPS. This trend is more prominent in the relationships between periventricular WMH volumes and PEPS. Total lacunes were associated with total, gait and pyramidal PEPS. This trend is more prominent in the relationships between lacunes located white matter area and PEPS. Lobar MBs are associated with pyramidal symptoms.
Conclusions: WMHs especially located on the periventricular area and lacunes especially located on the white matter area are associated with motor function. Cerebral MBs are not associated with motor symptoms except lobar MBs.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0311

MITOCHONDRIAL AMYLOID BETA-BINDING ENZYME - THE BIOMARKER OF ALZHEIMER DISEASE OR MULTIPLE SCLEROSIS?

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Objectives:

Mitochondrial enzyme 17beta-hydroxysteroid dehydrogenase type 10 interacts with intracellular amyloid beta and is probably involved in the pathogenesis of Alzheimer disease via its overexpression. Our previous experiments revealed that enzyme levels in cerebrospinal fluid could be a prospective biomarker of Alzheimer disease (the changes start many years before the disease onset and the sensitivity is about 80%), unfortunately, the specificity is not very high (73% compared to controls, 53 – 59% compared to other types of dementia). Similar changes were observed in people with autoimmune neuroinflammatory multiple sclerosis, but this enzyme has not been evaluated as a biomarker.

Methods:

We estimated levels of enzyme (competitive ELISA) and of amyloid beta 1-42 (sandwich ELISA) in cerebrospinal fluid of 121 people (controls, people with clinically isolated syndrom due to multiple sclerosis and finally people with multiple sclerosis with 2 and more attacts).

Results:

We found the significant increase in enzyme levels (to 115%) only in people with multiple sclerosis with 2 and more attacts. The sensitivity was 64%, the specificity about 60% when compared to controls. No significant changes were observed in levels of amyloid beta 1-42.

Conclusions:

Levels of mitochondrial enzyme in cerebrospinal fluid seem to be the better biomarker of Alzheimer disease than of multiple sclerosis. Our results indicate that mitochondrial dysfunction plays an important role only in later stages of multiple sclerosis and that enzyme overexpression is not probably associated with effects of amyloid beta, in contrast to Alzheimer disease.

Supported by GACR P304/12/G069 and MHCR NT13890 projects.

03j. Pathophysiology & Disease Mechanisms: autoimmunity

ADPD5-1172

COGNITIVE IMPAIRMENTS PRECEDE MOTOR DYSFUNCTION DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) MODEL: POTENTIAL THERAPEUTIC ROLE OF KALLIKREIN–KININ SYSTEM

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Multiple sclerosis (MS) is a progressive, demyelinating and inflammatory disease of the central nervous system that still without an effective therapy, which about 50–70% of MS patients experience important cognitive deficits, although the underlying pathogenic mechanisms remain unknown. Kallikrein–kinins (KKS) are polypeptides mediator key in inflammation and pain processes, and exert a critical role in regulating the early development of experimental autoimmune encephalomyelitis (EAE). **Objectives and Methods:** we sought to investigate whether KKS exert a role in modulating the development of cognitive impairments during the motor pre-symptomatic phase of EAE. **Results:** we provide evidence that spatial reference memory impairments occur before the pre-symptomatic phase of EAE, and these effects are directly associated with a decrease in ChAT expression and up-regulation of inflammatory cytokines in the brain areas (hippocampus and prefrontal cortex) involved in acetylcholine control of memory acquisition and maintenance. Relevantly, a marked increase of kinin receptors, mainly, B_1R expression occurred selectively in the hippocampus, whereas protein level was upregulated in both brain areas. In addition, genetic deletion of kinin B_1R prevented cognitive deficits and cholinergic dysfunction, as well as blocked expression of both Th17 and Th1 cytokines in the prefrontal cortex, lymph node and spleen of mice subjected to EAE. **Conclusion:** the discovery of cognitive deficits and upregulation of B_1R expression during pre-symptomatic phase of EAE could give rise to temporal markers of the disease as well as open up fresh perspectives in the development of therapeutic approaches for autoimmune disorders, such as MS.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-1865

REMYELINIZATION AND OPC DIFFERENTIATION BY DUAL GSK-3/PDE7 INHIBITORS

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1. Objectives. Demyelinating diseases, as multiple sclerosis (MS), are unmet pathologies without no effective treatment up to date. We have shown that GSK-3 inhibitors and PDE7 inhibitors are useful MS drug candidates as they are effective in EAE mice model. Moreover we have shown how PDE7 inhibitors are able to promote oligodendrocyte precursors cells differentiation [1]. Our goal is to test if dual GSK-3 /PDE7 inhibitor may be a valuable drug candidate
2. Methods. We use a couple of dual GSK-3/PDE7 inhibitors belonging to the iminothiadiazole family to test remyelinization after treatment in in vitro and ex vivo models. We use cerebellum mice slices treated with lysolecitine and OPCs from newborn mice.
3. Results. A clear increase in OPCs differentiation was observed after drug candidates treatment. Remyelinization in cerebellum slices is recovered after three days treatment with our two dual GSK-3 /PDE7 inhibitors named VP1.15 and VP3.15
4. Conclusions. These two compounds, VP1.5 and VP3.15, that simultaneously inhibit GSK-3 and PDE7, are good drug candidates for the disease-modify pharmacological treatment of multiple sclerosis. These new drugs will show their efficacy in future clinical trials.

[1] Medina-Rodríguez EM, Arenzana FJ, Pastor J, Redondo M, Palomo V, García de Sola R, **Gil C**, Martínez A, Bribián A, de Castro F. Inhibition of endogenous phosphodiesterase 7 promotes oligodendrocyte precursor differentiation and survival. Cell Mol Life Sci. 2013 Sep;70(18):3449-62

04p. Therapeutic Targets & Mechanisms for Treatment: neurotrophic factors

ADPD5-1608
KLOTHO ENHANCES REMYELINATION FOLLOWING CUPRIZONE-INDUCED DEMYELINATION
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Objectives: The current study examined whether overexpression of Klotho in transgenic mice can enhance remyelination following cuprizone-induced demyelination, and improves the clinical outcome in experimental autoimmune encephalomyelitis (EAE).
Methods: Demyelination was achieved by feeding transgenic mice overexpressing the transmembrane form of Klotho (KL-OE) and wild type (WT) littermates cuprizone-containing chow for 6 weeks. The animals were then allowed to remyelinate for 3 weeks. Paraphenylenediamine staining and PDGFRα and GSTpi immunohistochemistry were performed on corpus callosum (CC) sections for quantification of myelin, and progenitor and mature oligodendrocytes, respectively. The EAE model was induced with the MOG35-55 peptide. The animals were scored daily for clinical symptoms for 30 days.
Results: Following 6 weeks of demyelination, both KL-OE mice and WT littermates demonstrated almost complete and comparable demyelination of the CC. However, the level of spontaneous remyelination was increased approximately two-fold in KL-OE mice. Although there were no significant differences in the numbers of PDGFRα and GSTpi positive cells in KL-OE or WT, the increase in myelinated axons in the KL-OE could potentially be due to individual oligodendrocytes myelinating more axons. Following EAE induction, Klotho overexpression did not affect the clinical scores, likely due to the different roles Klotho plays in the brain and spinal cord.
Conclusions: This is the first *in vivo* description of the striking beneficial effect of Klotho as a regulator of remyelination in the CNS white matter and, thus, increasing Klotho expression should be considered as a therapy for enhancing remyelination in individuals with MS and other demyelinating diseases.

05g. Drug Development & Clinical Trials: immunomodulators

ADPD5-0373

CANNABIDIOL EFFECTS ON MRNA LEVELS AND SIGNALING PATHWAYS IN MOG35-55 ACTIVATED ENCEPHALITOGENIC T CELLS

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Cannabinoids exert potent immunoregulatory activities. Our previous studies showed that the non-psychoactive cannabinoid, cannabidiol (CBD, a component of *Sativex*), ameliorated clinical symptoms in murine Myelin Oligodendrocyte Glycoprotein (MOG)35-55-induced Experimental Autoimmune Encephalomyelitis model of Multiple Sclerosis. Moreover, CBD decreased MOG-specific T cell proliferation and cytokine secretion including of IL-17. The mechanisms of these activities are poorly understood. Herein, we describe gene networks and intracellular pathways that are mediating the suppressing effects of CBD in an activated MOG35-55-specific T cell lineage. Encephalitogenic MOG35-55-specific T cells were stimulated with MOG35-55 in the presence of spleen-derived APCs with or without CBD, then separated using magnetic-bead CD4+ selection and subjected to microarray analysis of mRNA levels. Ingenuity Pathway Analysis and Gene Ontology identified IL-17 polarization, IL-6 and IL-10-signalling as top canonical pathways affected by the CBD treatment. Main upstream regulators affected by CBD were recognized as EGR2, STAT5A and NRF1. The effects of CBD on activated encephalitogenic T cell were linked to the regulation of selective T cell activation molecules (BTLA, CD40, CD69, IFNGR1), intracellular modulators of MAPK (DUSP6, DUSP2), PKA (CREM) and Jak/STAT (SOCS3, STAT5) pathways. Gene targets within cell cycle regulation pathways (PTPN6, SLC3A2, VAV3, DDR1) and modulators of oxidative stress (MT1A, HMOX1, SLC30A1) were also significantly affected by the CBD treatment. The microarray results were confirmed using qPCR on selected gene targets. Immunoblotting demonstrated that CBD reduces IL-17 by decreasing STAT3 phosphorylation and increasing that of STAT5. Our observations increase our understanding of the mechanisms of the anti-inflammatory activities of CBD.

05y. Drug Development & Clinical Trials: other

ADPD5-1594

DEVELOPMENT OF SCREENING SYSTEM FOR BLOCKERS OF NMO-IGG BINDING TO AQUAPORIN-4

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Neuromyelitis Optica (NMO) is an inflammatory demyelinating autoimmune disease of optic nerve and spinal cord caused by binding of NMO-specific Immunoglobulin-G autoantibodies (NMO-IgG) to astrocyte water channel aquaporin-4 (AQP4). We prepared human M23-AQP4-expressing U87MG stable cell lines and performed high-throughput small molecule inhibitor screening system by quantitative measurement of two immunofluorescence imaging on binding of NMO-IgG to AQP4 using high content screening (HCS). Furthermore, we set up a screening system for small molecule blockers against the binding of NMO-IgG to AQP4 measuring lactate dehydrogenase release for complement dependent cytotoxicity. Our development of screening system on small molecule blockers of NMO-IgG to AQP4 would lead to better understanding of further pathogenesis and therapeutic researches.

07g. Epidemiology, Risk Factors, Genetics & Epigenetics: autoimmune

ADPD5-0323

ALZHEIMER'S DISEASE RISKS IN AUTOIMMUNE DISORDERS PATIENTS

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OBJECTIVES: To investigate association between autoimmune disorders and hospitalization for Alzheimer's disease, and to study whether the risk is associated with follow-up time and age.
PATIENTS & METHODS: In this follow-up study, the nationwide database was used to identify 43 hospitalized conditions of autoimmune disorders in patients from the Hospital Discharge Register. Follow-up of 797 424 patients with autoimmune disorders was carried out from 1964 to 2010. This study includes separate follow-ups for shorter intervals. Standardized incidence ratios (SIRs) were calculated for depression in patients with autoimmune disorders by comparing them to subjects without autoimmune disorders.
RESULTS: Among total of 43 conditions of autoimmune disorders, 10040 Alzheimer's disease was identified with an SIR of 1.29 (95% CI 1.26-1.32). 17showed an increased risk. The remaining 14 conditions were still at risk when Alzheimer's disease diagnosed in the year of autoimmune disorders diagnosed was excluded. The risks depended on the age at hospitalization for Alzheimer's disease. The SIRs for Alzheimer's disease declined by age at hospitalization of patients with chronic rheumatic heart disease, Crohn disease, diabetes type 1, Grave disease, hashimoto thyroiditis, multiple sclerosis, pernicious anemia, Sjören syndrome, and system lupus erytematosus. The risk of Alzheimer's disease decreased with follow-up time. Chronic rheumatic heart disease, type 1 diabetes, Graves' disease, pernicious anemia, polymyalgia rheumatic, and Wegener granulomatosis showed significant risk of Alzheimer's disease after ten years follow-up of autoimmune disease.
CONCLUSIONS: This large study quantified the increased risks of Alzheimer's disease in patients with many types of autoimmune disorders.

10d. Other: diagnostics

ADPD5-1655

MEMORY IMPAIRMENT IS PRESENT REGARDLESS OF DEPRESSION AND ANXIETY IN PATIENTS AT RISK OF MULTIPLE SCLEROSIS

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Background: Multiple sclerosis (MS) is a chronic disease of young adults, where beside motor and visual deficits also cognitive impairment including memory and neuropsychiatric symptoms, especially depression and anxiety, are present. Recent studies indicated that cognitive impairment may be present also in patients who are at a high risk of MS, in patients with clinically isolated syndrome (CIS).
Objectives: To assess whether cognitive functions, especially memory, regardless of depression and anxiety are impaired in patients with CIS.
Methods: Patients with CIS on interferon-β (n=44; 8.1±9.5 months on medication; EDSS 1.6±0.7) underwent clinical examination, brain MRI, cerebrospinal fluid assessment and neuropsychological examination of all cognitive domains. Depression and anxiety were assessed with Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI), respectively. Results were compared to age, gender and education matched healthy controls (n=32).
Results: Patients with CIS and controls did not differ in basic demographic characteristics. General linear model analysis with BDI and BAI scores as covariates revealed that patients with CIS were impaired on learning and memory recall (ps=.009) compared to controls. Patients with CIS were further impaired on tests of executive functions (ps≤.038) and attention and working memory (ps≤.038). There was no impairment of language and visuo-spatial functions.
Conclusions: Our results suggest that mild cognitive impairment with predominant amnestic syndrome is present regardless of depression and anxiety in patients at a high risk of MS.

01i. Protein Misfolding & Aggregation: huntingtin

ADPD5-0276
PLASMA INFLAMMATORY BIOMARKERS FOR HUNTINGTON'S DISEASE PATIENTS AND MOUSE MODEL
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Huntington's disease (HD) is caused by expanded CAG repeats encoding a polyglutamine tract in the huntingtin (HTT) protein. Lines of evidence have observed neuroinflammation, particularly microglial activation, is involved in the pathogenesis of HD. By examining the expression levels of 13 microglia-derived inflammatory markers in the plasma of 5 PreHD carriers, 15 HD patients and 16 healthy controls, we found plasma levels of IL-6, MMP-9, VEGF and TGF-β1 were significantly increased in HD patients when compared with the controls, while plasma level of IL-18 were significantly reduced in HD patients compared with controls. Plasma level of IL-6 was reversely correlated with the UPDRS independence scale and functional capacity. The inflammatory markers were further measured in plasma from R6/2 mouse HD model. In rotarod test, R6/2 HD mice started to manifest HD phenotype at 7.5 weeks of age. Higher plasma VEGF levels of R6/2 mice than those of age-matched wild-type (WT) littermates were noted from 7 (presymptomatic stage) to 13 weeks of age (late symptomatic stage). The plasma IL-6 levels of R6/2 mice were higher than those of the WT littermates from 9 (early symptomatic stage) to 13 weeks of age. R6/2 mice demonstrated higher MMP-9 and TGF-β1 levels than their WT littermates from 11 (middle symptomatic stage) to 13 weeks of age. In contrast, the plasma IL-18 level was lower than those in WT littermates since 11 weeks of age. These altered expressions of inflammatory markers may serve as the potential biomarkers for HD onset and progression.

01k. Protein Misfolding & Aggregation: clearance of misfolded proteins

ADPD5-1827
NEUROPATHOLOGIC PHENOTYPE ASSOCIATED WITH A UBQLN2 P497L MUTATION IN 3 AFFECTED WOMEN FROM 3 GENERATIONS

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Objectives: Ubiquilin 2, encoded by the *UBQLN2* gene on the X chromosome, delivers ubiquitinated proteins to the proteasome for degradation. *UBQLN2* mutations have been linked to amyotrophic lateral sclerosis (ALS)-frontotemporal dementia.
Methods: A 63 year-old (63y) woman with progressive weakness, dysarthria, and dysphagia was diagnosed with ALS. Her 20y daughter developed spastic paralysis, dysarthria, dysphagia, and dementia, and this woman's 20y daughter developed ALS. Duration of clinical symptoms ranged from 4-17 years. Deaths occurred at ages 67y, 37y, and 24y, respectively. Brain and spinal cord autopsies followed. Formalin-fixed, paraffin embedded sections of brain and spinal cord were stained with hematoxylin and eosin-Luxol fast blue and Thioflavin S. Immunohistochemical stains included β-amyloid, tau, α-synuclein, UBQLN2, and TDP-43. DNA was isolated from frozen brain tissue for genetic analysis.
Results: Upper and lower motor neuron loss was variable among the 3 subjects. The 37y brain showed frontal lobe atrophy with neuronal loss and gliosis in cortex and caudate nucleus. Substantia nigra degeneration ranged from mild (24y) to moderate (67y) to severe (37y). The 67y brain contained Lewy bodies, sparse-moderate beta-amyloid immunopositive diffuse plaques, and sparse phospho-tau. UBQLN2-cytoplasmic and neuropil aggregates were present in frontal and temporal cortex, hippocampus, and inferior olivary nucleus. TDP43-immunoreactive inclusions were present in spinal cord and hippocampus. Genetic analyses confirmed a c.1490C>T in the *UBQLN2* gene in the 67y and 24y brains.
Conclusions: Neuropathologic studies of this X-linked disorder, hypothesized to result in perturbed protein clearance, may lead to new insights into the pathogenesis of misfolded protein diseases.

01l. Protein Misfolding & Aggregation: computer simulations

ADPD5-1134

IN SILICO MODELING OF RECENTLY DISCOVERED KOREAN PSEN1 MUTATIONS

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Three genes can be involved in early onset Alzheimer's disease: APP, PSEN1 and PSEN2. Majority of mutations were described in PSEN1 (almost 200 due to the recent updates). A few mutations were studied in cell models to confirm their pathogenic nature, but the functional data was not available for all mutations. Since the transfection, *in vivo* cell line studies of mutations are expensive, time consuming, we performed *in silico* modeling on the PSEN1 mutations.

We performed PolyPhen2 prediction for the variants. In addition, we tried to model the 3D structure of proteins with RAPTOR-X software, and compared the 3D structure of normal and mutant PS1 proteins. This software might be a promising approach to predict the pathogenic nature of variants.

In our studies, we discovered four novel and known variants in Korean EOAD patients, which might be involved in disease onset: H163P (novel), T116I (known), L226F (known), L232P (novel). 3D structure of PS1 with T116I showed differences in the N-terminal region, because of the hydrophobic isoleucine. PS1 with H163P looked similar like the wild PS1, but the different properties of histidine and prolie might support the pathogenic nature of this mutation. PS1 with L226F looked also similar to the normal PS1, but since phenylalanie is the biggest amino acid, it might result structural changes in the TM region. In L232P mutation, the rigid proline might result torsion in the helix structure.

This *in silico* analysis could be promising approach to predict the pathogenic nature if mutations, especially the novel ones.

01m. Protein Misfolding & Aggregation: other

ADPD5-1308
HIPPOCAMPAL EXPRESSION OF GABAA RECEPTOR SUBUNITS IN NEURODEGENERATIVE DEMENTIAS
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Objectives: The $GABA_A$ receptor family is a target for various clinically relevant drugs, including the benzodiazepines. They are pentameric chloride ion channels, which are composed of a total of 19 different subunits. The alpha5 subunit of the $GABA_A$ receptors has been implicated in cognition. Thus, our aim was to evaluate the presence of the alpha5 subunit and its most common assembling partner the gamma2 subunit in strategic regions involved in memory processing such as the dentate gyrus and the CA1 subregion of the hippocampus in various neurodegenerative dementing illnesses.
Methods: We included 46 cases with Alzheimer`s disease (AD)-related changes grouped according to Braak stages, 14 cases with AD-related changes and argyrophilic grain disease, 20 AD cases with limbic TDP-43 pathology, and 10 cases with dementia with Lewy bodies. We performed morphometric immunohistochemistry and compared the density of subunit expression with the amount of protein deposits (phospho-tau, phospho-TDP-43, a-synuclein) in the same subregions.
Results: $GABA_A$ receptor subunits are relatively preserved during the progression of AD-related changes. Moreover, this is not significantly influenced by concomitant neurodegenerative disorders. However, significant inter-individual variability of the expression of $GABA_A$ receptor subunits is associated with the load of protein deposition in the evaluated anatomical regions.
Conclusion: Our results suggest that $GABA_A$ receptor-based therapies in neurodegenerative dementias should be initiated on an individual basis rather than based on artificial disease categories.

ADPD5-0570

THE DEREGULATIONS OF GENE EXPRESSION IN LRRK2- ASSOCIATED PARKINSON'S

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BACKGROUND Leucine rich repeat kinase 2 (LRRK2) is one of the most important causative gene in Parkinson's disease (PD). The pathophysiological role of LRRK2 is presently
unclear.
OBJECTIVES To identify the deregulations caused by LRRK2 gene mutations that is associated with pathogenesis of PD.
METHODS LRRK2 gene stable transfected DA neuronal cell lines were generated containing wild type or variant LRRK2 gene cDNA (G2019S/G2385R). Total RNA was isolated from the three stable transfected cell lines. The GeneChip Human Gene 1.0 ST array (Affymetrix) was used for identification of gene expression profiling. Real- time PCR and Weston blotting were used to further verify the genes that showed deregulation results in Gene Chips.
RESULTS The results of genome wide gene expression profiling showed the differences in multiple genes of LRRK2 mutational cell lines compared with the wild type. In particular the dopamine metabolizing genes Dopamine Bata-Hydroxylase (DBH) and DOPA Decarboxylase (DDC) were significantly decreased. DBH and DDC genes code for two enzymes in the dopamine metabolism pathway. The decreases of DDC and DBH have been further confirmed by Real-time PCR and Western Blotting.
CONCLUSIONS The disorder of Dopamine metabolism is a significant pathogenesis of PD, dysregulated dopamine metabolism contributes to the pathogenesis of PD have been confirmed. To identify the Dopamine deregulators caused by LRRK2 gene mutations may provide the evidence of pathogenesis in PD.

02l. Cell, Molecular & Systems Biology: huntingtin

ADPD5-0781

A HUNTINGTIN BASED PEPTIDE INHIBITOR OF CASPASE-6 PROVIDES PROTECTION FROM MUTANT HUNTINGTIN INDUCED MOTOR AND BEHAVIORAL DEFICITS

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Over the past decade, increasing evidence has implied a significant connection between
caspase-6 activity and the pathogenesis of Huntington's disease. Consequently, inhibiting caspase-6 activity was suggested as a promising therapeutic strategy to reduce mutant Huntingtin toxicity, and to provide protection from mutant Huntingtin induced motor and behavioral deficits. Here we describe a novel caspase-6 inhibitor peptide based on the huntingtin caspase-6 cleavage site, fused with a cell penetrating sequence. The peptide reduces mutant Huntingtin proteolysis by caspase-6, and protects cells from mutant huntingtin toxicity. The continuous sub-cutaneous administration of peptide protected pre-symptomatic BACHD mice from motor deficits and behavioral abnormalities. Moreover, administration of the peptide in an advanced disease state resulted in the partial recovery of motor performance, and an alleviation of depression related behavior and cognitive deficits.
Our findings reveal the potential of substrate-based caspase inhibition as a therapeutic strategy, and present a promising agent for the treatment of Huntington's disease.

ADPD5-1225

REVERSAL OF APOE4 INDUCED BRAIN PATHOLOGY BY LENTIVIRUS-DRIVEN VEGF EXPRESSION

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Background: Alzheimer's disease (AD) is associated with neuronal and vascular dysfunction. Vascular endothelial growth factor (VEGF) has been shown to play an important role in both the neuronal and vascular systems. ApoE4, the main genetic risk factor for AD is associated with increased neurodegeneration and vascular impairments.
Objectives: To study the effects of apoE4 on brain VEGF levels and to examine the extent to which the brain and cognitive pathological effects of apoE4 can be reversed by over-expression of VEGF.
Methods: The levels of VEGF in hippocampus of naïve apoE3 and apoE4 young targeted replacement mice ware determined prior to and following injection of either VEGF nor GFP expressing lentivirus and the resulting effects on apoE4-driven brain and behavioral pathologies ware than measured.
Results: The levels of VEGF in the hippocampus of naïve apoE4 mice were lower than those of corresponding mice which express its AD benign isoform apoE3. Similar results were obtained with VEGF mRNA, suggesting that apoE4 down-regulates the expression of VEGF. These effects was associated with cognitive impairments; decreased levels of the pre-synaptic marker VGluT and of the ApoE receptor Apoer2 in hippocampal neurons; and corresponding accumulation of hyperphosphorylated tau and A-beta. Over-expression of VEGF utilazing lentivirus reversed cognitive improvement and reduced the isoform specific effects in various brain parameters between apoE4 and apoE3 mice.
Conclusions: VEGF play a role in mediating apoE4-driven cognitive and brain pathologies in TR-mice. This suggests that VEGF tailored treatment can be beneficial in AD and apoE4 carriers.

ADPD5-1237

VEGF MEDIATES APOE4-INDUCED NEOVASCULARIZATION AND SYNAPTIC PATHOLOGY IN THE CHOROID AND RETINA

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Background: Apolipoprotein E4 (apoE4), the most prevalent genetic risk factor for Alzheimer's disease (AD), is associated with neuronal and vascular impairments. Recent findings suggest that retina of apoE4 mice have synaptic and functional impairments.

Objectives: To investigate the extent to which the functional and synaptic effects of apoE4 in the retina are associated with parallel vascular effects. This was performed by studying the effects of apoE4 on the retinal and choroidal vascular systems in young targeted replacement mice under resting conditions and following laser-induced neovascularization (CNV).

Methods: Mice were subjected to laser-induced CNV and the extents to which this treatment effects the neuronal and vascular systems in the choroid and retina were measured prior to and following 3 and 14 days from treatment.

Results: There were no histological differences between the retinal and choroidal vasculatures of naïve apoE3 and apoE4 mice. In contrast, laser-driven choroidal injury induced higher levels of CNV in apoE4 than in apoE3 mice. VEGF levels of naïve apoE4 retina were lower than in apoE3 mice. In contrast, VEGF levels rose more pronouncedly in apoE4 than in apoE3 mice following laser injury. Similar effect was observed in apoE levels.

Conclusions: The findings obtained suggest that VEGF mediates the apoE4-induced neovascularization and synaptic pathology in the choroid and retina. These findings have important implications regarding the treatment of apoE4-related ophthalmologic pathologies (i.e. AMD), as well as of apoE4-related brain neuronal and vascular pathologies (i.e. AD) and suggest a novel therapeutic approach for treating these pathologies.

ADPD5-2295

APOLIPOPROTEIN E ISOFORM-SPECIFIC EFFECT ABOUT AUTOPHAGY AND SURVIVAL IN CAD CELLS

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ApolipoproteinE (ApoE) carries cholesterol and other lipids, within both, the plasma, and the central nervous system. There are three ApoE alleles in humans (ApoE2, 3 y 4). Although ApoE4 has been defined as a risk factor in Alzheimer's diseases, the molecular effects associated to ApoE4 have not been fully described. The main purpose of the research is to study the differential effects of ApoE isoforms in the survival and autophagy pathways under a neurotoxic model context.

Methods: the differentiated cells were incubated with the ApoE 2, 3 and 4. After 1 hour of pre-treatment with ApoE, ceramide was added for incubation process during 24 hours. Mitochondrial metabolism was measured by MTT assay and cell survivor by LDH assay. Protein's expression was evaluated by Western blot's technique and immunofluorescences, so to finally detect, autophagosomes.

The ApoE4 and ceramide treatment causes a cellular viability decrease and the ApoE2 and 3, does not. It decreases in the phosphorylation level of AKT and AMPK, compared to ApoE2-ceramide and ApoE3-ceramide treatments. The presences of the phosphorylation of ERK protein does not show a significant variation in the treatments; phosphorylation of p38 variation is mainly revealed in ApoE2 and 3-ceramide treatments.

Finally, these results suggest that ApoE4-ceramide treatment raises metabolism, and cellular stress response associated to activation of the p38 pathway (maybe associated to an increase production of ROS). Inhibition of autophagy is associated to cellular death whereas ApoE3 and ApoE2-ceramide treatment protects cells from the neurotoxic effect through autophagy's activation.

02q. Cell, Molecular & Systems Biology: protein degradation, proteasome & autophagy

ADPD5-1488

LACTULOSE AND MELICIOSE AS AGGREGATION REDUCERS TARGETING AUTOPHAGE IN CELL MODELS OF SPINOCEREBELLAR ATAXIA 3

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Trehalose, a chemical chaperone and mTOR-independent autophagy enhancer, has shown promise in models of huntington's disease, Parkinson's disaese and tauopathies. In this study, two trehalase-indigestible trehalose analogs, lactulose and melibiose, were examined for their potentials in spinocerebellar ataxia (SCA) treatment. Using ATXN3/Q_{75}-GFP 293 cells, a putative SCA3 cell model, we found the ATXN3/Q_{75} aggregation was significantly prohibited by lactulose and melibiose because of theirs abilities to influence ATXN3/Q_{75} protein folding and up-regulating of autophagy. Meanwhile, lactulose and melibiose reduced the production of reactive oxygen species (ROS) in ATXN3/Q75 cells. Both of them further inhibited the aggregation and promoted neurite outgrowth in neuronally differentiated SH-SY5Y ATXN3/Q_{75}-GFP cells. These findings suggest the therapeutic applications of trehalase-indigestible trehalose analogs in aggregation-associated neurodegenerative diseases.

ADPD5-1638

INFLAMMATORY CYTOKINES AND CHOLINERGIC SYSTEM DYS-FUNCTION IN MULTIPLE SCLEROSIS PATIENTS

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Objectives: To evaluate whether inflammatory state in MS patients may be related to non-neuronal cholinergic system dys-function we have measured ACh levels, AChE and BuChE activity and expression and the production of pro-inflammatory cytokines in PBMC and serum of MS patients and in healthy donors (HD).

Methods: MS patients were, matched for sex and age to HD. ACh cytokine levels in serum were measured by commercial kit and AChE and BuChE by Ellman test. Real-Time PCR were performed to determinate AChE, BuChE and cytokines mRNA expression in PBMC.

Results: In MS patients expression of AChE and BuChE was higher than in HD. In PHA-stimulated PBMC of MS patients, the expression of IL-4, TNFα , IFNg, IL-6 and IL-17 was significantly higher, while, IL-10 and IL-18BP were significantly lower compared with HD.

When PBMC isolated from MS patients were co-treated with PHA and nicotine, the expression and production of TNF-α and IFNg were significantly reduced, IL-4 and IL-18BP were increased, while IL-17, IL-6 and IL-10 not appeared significantly modified. Interestingly, the serum levels of IL-17, as well as AChE and BuChE enzymatic activity were higher in MS than HD, while levels of ACh were lower.

Conclusions: Our results suggest that decreased levels of ACh in MS may be related to increased activity of its hydrolytic enzymes and to higher pro-inflammatory cytokine levels. Stimulation of nicotinic receptors contribute to reduce the levels of pro-inflammatory cytokines suggesting that the re-stablishment of cholinergic function may contribute to reduce the inflammatory state in MS

02u. Cell, Molecular & Systems Biology: network biology

ADPD5-0730

5-HT3A RECEPTORS MODULATE HIPPOCAMPAL GAMMA OSCILLATIONS BY REGULATING SYNCHRONY OF PARVALBUMIN-POSITIVE INTERNEURONS

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Gamma-frequency oscillatory activity plays an important role in information integration across brain areas. Disruption in gamma oscillations is implicated in cognitive impairments in psychiatric disorders, and 5-HT3 receptors (5-HT3Rs) are suggested as therapeutic targets for cognitive dysfunction in psychiatric disorders. Using a 5-HT3aR-EGFP transgenic mouse line and inducing gamma oscillations by carbachol in hippocampal slices, we show that activation of 5-HT3aRs, which are exclusively expressed in cholecystokinin (CCK)-containing interneurons, selectively suppressed and desynchronized firings in these interneurons by enhancing spike-frequency accommodation in a small conductance potassium (SK)-channel-dependent manner. Parvalbumin-positive interneurons therefore received diminished inhibitory input leading to increased but desynchronized firings of PV cells. As a consequence, the firing of pyramidal neurons was desynchronized and gamma oscillations was impaired. These effects were independent of 5-HT3aR-mediated CCK release. Our results therefore revealed an important role of 5-HT3aRs in gamma oscillations and identified a novel cross-talk among different types of interneurons for regulation of network oscillations. The functional link between 5-HT3aR and gamma oscillations may have implications for understanding the cognitive impairments in psychiatric disorders.

02w. Cell, Molecular & Systems Biology: transcriptomics

ADPD5-1233

ALZHEIMER DISEASE SPECIFIC CODING AND NON-CODING GENE TRANSCRIPTION IN TEMPORAL CORTEX

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Objectives: Use RNASeq to characterize coding and non-coding RNA transcription in temporal pole tissues from Late-Onset Alzheimer's disease (LOAD) versus control and Dementia with Lewy Body (DLB).

Methods: RNA-Seq was performed on the HiSeq2000 using total RNA. Tissues were from matched temporal pole (BA 38) from 10 cases each of LOAD, DLB and controls. RNA was extracted using Qiagen's miRNeasy and libraries prepared using Epicentre's Script-Seq. GSNAP aligned ~75% of the reads to the genome.

Results: Each library generated 40-65 million reads. A total of 53,245 genes (19,207 protein-coding and 34,038 ncRNAs, based on ENCODEv15) having detectable levels of transcription were identified. 2,504 out of 53,245 genes had nominal differences in transcription ($p<0.05$) between the ten LOAD samples and ten normal controls. After correcting for multiple testing by False Discovery Rate (FDR<0.05), 16 (11 coding) of the 2,504 genes differed significantly between LOAD and normal controls. More ncRNA transcripts were observed than protein-coding transcripts, but the average read depths of ncRNAs were less than protein-coding genes. To study the processes disrupted in LOAD, we performed network analysis on the 2,504 genes that had nominal significance between LOAD and normal controls. Using Weighted Gene Co-transcription Network Analysis (*WGCNA*) on transcription values of the 2,504 genes revealed that these genes aggregated into seven GO networks, two of which, innate immune response and myelination were specific to LOAD, when compared to both normal and DLB transcription.

Conclusions: RNASeq demonstrates significant differences in transcription and affected networks between LOAD and DLB and controls.

02x. Cell, Molecular & Systems Biology: synaptic plastcity

ADPD5-1695

PHASIC DOPAMINE RELEASE PLAYS A CRITICAL ROLE FOR SPIKE-TIMING DEPENDENT PLASTICITY OF EXCITATORY SYNAPSES ONTO STRIATAL MEDIUM SPINY NEURONS.

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[Objectives] The striatum is the principal input nucleus of the basal ganglia, receiving glutamatergic afferents from the cerebral cortex. It has been suggested that activity-dependent synaptic plasticity in the striatum is a cellular mechanism for reinforcement-based motor learning and phasic dopamine (DA) release is associated with positive reinforcement. However, there has been little evidence showing the role of phasic DA in the striatal STDP. In this study, we tested that the hypothesis that phasic DA release in the striatum is critical for the induction of corticostriatal synaptic plasticity.
[Methods] Whole cell recording in slice preparations obtained from adult BAC transgenic mice that selectively express GFP in D1R-expressing cells was performed in this study. The rapid application techniques by caged-dopamine photolysis was used to investigate effects of phasic DA on the striatal STDP.
[Results] We found that spike-timing dependent long-term potentiation (t-LTP) was induced by prepost protocols in D1R-expressing projection neurons in the striatum. The photolysis was applied at 300 ms prior to, 0 msec, 2 sec, or 4 seconds after the pairing protocols. Of these timing conditions, the photolysis at 2 sec after the pairing significantly blocked the t-LTP. We suggest that the timing of DA release could be an important factor to modulate the striatal STDP.
[Conclusion] We conclude that the phasically, timing-dependent DA release was a key determinant for induction of striatal STDP. These results suggest the timing of striatal dopaminergic regulation is implicated in motivational processes and motor function, and also a therapeutic relevance of Parkinson’s disease.

02z. Cell, Molecular & Systems Biology: other

ADPD5-0461

DOPAMINERGIC CELL LOSS AND STRIATAL NEUROCHEMISTRY IN 14-3-3GAMMA KNOCKOUT MICE TREATED WITH MPTP

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Objectives: Dysregulation of 14-3-3 proteins has been observed in Parkinson's disease (PD) and overexpression of specific 14-3-3 isoforms, including 14-3-3gamma, in dopaminergic cells was protective in different PD models. Here, we investigated the effect of 14-3-3gamma deletion in the acute MPTP model of PD *in vivo*.
Methods: 14-3-3gamma knockout mice and wildtype controls were treated with MPTP (1-methyl-4-phenyl-1,2,3,6,-tetrahydropyridine, 4x10 mg/kg, 2h interval). The striatal concentrations of dopamine (DA) and serotonin (5-HT) and their metabolites were measured by HPLC and sections of the substantia nigra pars compacta (SNpc) were examined for tyrosine hydroxylase (TH)-positive cells to quantify neurodegeneration.
Results: Untreated and MPTP treated 14-3-3gamma knockout mice had higher concentrations of DA, DOPAC, and 3-MT, compared with untreated and MPTP treated wildtype mice, respectively. The DA turnover was comparable in untreated 14-3-3gamma knockout mice and wildtype controls. However, the MPTP-induced increase in DA turnover was attenuated in 14-3-3gamma knockout mice compared to wildtype. The number of TH positve neurons was reduced in MPTP treated WT and 14-3-3gamma knockout mice to a similar extent by about 60%.
Conclusion: Since 14-3-3s activate TH it could have been expected that 14-3-3gamma knockout leads to decreased DA synthesis and increased rate of cell death, but we observed the opposite effects regarding dopamine and did not find an influence on neurodegeneration in the SNpc in the acute MPTP model. This points to different roles of 14-3-3 isoforms *in vivo* and a more complex regulation of DA levels and cell death compared to *in vitro* models.

02z. Cell, Molecular & Systems Biology: other

ADPD5-0686

STRUCTURAL INVESTIGATIONS ON DISEASE-RELATED AMYLOID FIBRILS BY SOLID-STATE NMR SPECTROSCOPY

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Objectives

Elucidation of structure and dynamics of amyloid fibrils and their implication in disease progression is still a challenge due to the non-crystalline, insoluble nature of aggregated proteins [1]. Recently, solid-state NMR-spectroscopy has emerged as a promising tool for structure elucidation in aggregated proteins.

Methods

We applied MAS NMR Solid-state NMR-spectroscopy with and without DNP enhancement to the study of fibrils from recombinantly expressed [2] human IAPP and ovine PrP [3].

Results

For hIAPP, we could obtain site-selective resonance assignments for most of the residues and define the location of the core region. Strongly reduced flexibility throughout the peptide was confirmed. Residues of the N-terminal loop gave rise to extremely well-resolved signals. For ovine PrP we could recently obtain NMR suitable fibrils by seeding with PrPSc particles. A comparison with spontaneously generated fibrils from recombinant PrP revealed higher homogeneity in the seeded fibrils, and location of beta-strands could be proposed.

Conclusions

The location of beta-strands of fibrillar hIAPP and the well-ordered state of the N-terminal loop confirms its important role in the aggregation process. For ovine PrPSc we could show the effect of seeding with infectious particles on a molecular level.

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02z. Cell, Molecular & Systems Biology: other

ADPD5-0853

THE CEREBRAL SPINAL FLUID (CSF) CONCENTRATION OF VARIOUS BIOMARKERS FOR THE DIFFERENTIAL DIAGNOSIS IN IDIOPATHIC NORMAL PRESSURE HYDROCEPHALUS (INPH)

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Objectives:
New diagnostic criteria based on a guideline for iNPH was submitted. Although CSF tap test and MRI features are used for diagnosis, the utility of CSF biomarker has not been strictly evaluated for assistance of diagnosis. It was reported that the CSF concentration of Lipocalin-type prostaglandin D synthase (L-PGDS), secreted in the CSF as β-trace, was decreased in iNPH as compared to other diseases with dementia. In order to investigate whether the different expression of CSF biomarkers between iNPH and other diseases with dementia is useful for differential diagnosis, we determined the CSF levels of L-PGDS, p-tau (181), Aβ40 and Aβ42.
Methods:
CSF samples were obtained from 35 patients diagnosed with possible or probable iNPH and 26 patients, which included Alzheimer's disease (AD), other diseases showed dementia, autoimmune diseases and control subjects. Each patient provided informed consent. The CSF biomarkers were measured by enzyme-linked immunosorbent assay (ELISA).
Results:
L-PGDS was significantly decreased in iNPH (mean±SD: 6.57±4.20) compared to the other diseases group with dementia (13.37±3.35). Decrease of Aβ42 was more often observed in AD, but also found in a part of iNPH. There was no significant difference between AD and iNPH. A trend towards higher levels of p-tau (181) was observed in AD.
Conclusions:
The CSF L-PGDS level was significantly decreased in iNPH as compared with other demented patients. Recently iNPH is increased among the geriatric population, and often accompanied by AD. It may be useful for differential diagnosis to measure various CSF biomarkers correlated with dementia simultaneously.

02z. Cell, Molecular & Systems Biology: other

ADPD5-1255

REGULATION OF MEMORY FORMATION BY THE TRANSCRIPTION FACTOR XBP1

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Introduction: Contextual memory formation relies on induction of new genes in the hippocampus. A polymorphism in the promoter of the transcription factor XBP1 was identified as a risk factor for Alzheimer´s disease and bipolar disorders. Here we uncover a key role for XBP1 in higher cognitive function, unrelated to its function as a major regulator of the ER stress response.

Methods: We performed behavioral screening on *xbp1* conditional knockout mice. Additionally, we overexpressed XBP1s with a novel transgenic mouse and with localized adenoviral delivery into the hippocampus of wild-type animals. We evaluated LTP and gene expression related to memory formation.

Results: Mice lacking XBP1 in neurons showed ablated contextual memory formation and impaired LTP while overexpression of XBP1s improved performance in memory tasks. XBP1 regulates a cluster of genes involved in memory formation, including BDNF. The $XBP1^{Nes-/-}$ phenotype could be reversed by site-specific delivery of BDNF to the hippocampus.

Discussion: Our study reveals an unanticipated function of XBP1 in cognitive processes that operates via a bidirectional regulatory loop with BDNF. Strategies to enhance XBP1s activity in the brain may translate into beneficial effects for the treatment of memory disorders.

Support from FONDECYT 1140549, Millennium Institute P09-015-F, FONDEF D11I1007, Ring initiative ACT1109, Alzheimer´s association, (CH), Basal Grant Se(Conicyt-PFB 12/2007). CONICYT Capital Humano en la Academia 7912010006 (RV), FONDECYT 3140388 (PM). CONICYT PhD Felllowships (G.M.; P.V.)

02z. Cell, Molecular & Systems Biology: other

ADPD5-1531
SRY KNOCKDOWN REDUCED MOTOR DEFICITS IN RAT PD MODEL
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Whilst the cause of dopamine cell loss in Parkinson's disease (PD) is unknown, it is clear that the male-sex is a strong risk factor. The incidence and prevalence of PD is 2-fold higher and disease progression more rapid in males than females. Growing evidence suggests that sex-specific genes contribute to this male-bias in PD. We previously showed that the male-sex determining gene, SRY, co-localises with male dopamine neurons, where it regulates dopamine biosynthesis and motor function. Here, we investigated the regulation and function of nigral SRY in normal and 6-hydroxydopamine (6-OHDA) lesioned hemiparkinsonian rats. We assessed the effect of reducing nigral SRY levels, via repeated injection of SRY antisense oligonucleotide into the rat substantia nigra (SNc), on motor and dopaminergic function. In normal male rats, SRY antisense treatment significantly reduced motor function in the cylinder and rotarod tests, which was associated with a reduction in nigral TH mRNA and striatal dopamine content, compared to the sense-treated group. Conversely, SRY antisense treatment in female rats did not affect motor function. In 6-OHDA-lesioned male rats, nigral SRY mRNA was significantly up-regulated at 7 days following 6-OHDA injection. Remarkably, SRY antisense treatment significantly attenuated 6-OHDA-induced motor deficit and dopamine cell loss in male rats, compared to the sense-control, indicating a detrimental role for SRY in the injured male SNc. These data indicate a double-edged role for SRY in normal and injured dopamine neurons in the male SNc and that inhibition of SRY may be a novel therapeutic target for PD in males.

02z. Cell, Molecular & Systems Biology: other

ADPD5-1944

ER-A36, A NOVEL VARIANT OF ER-A, MEDIATES B-ESTRADIOL-DERIVED NEUROPROTECTION FROM MPP+-INDUCED DAMAGE IN PC12 CELLS

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Accumulating experimental evidence indicates that estrogen exerts dose-dependent neuroprotection at usual physiological levels and can improve damage from cerebral ischemia at supraphysiological concentrations. Extranuclear estrogen receptors mediate this beneficial effect. ER-α36, a novel subtype of estrogen receptor, functions differently from classical ER-α in response to membrane-initiated estrogenic signaling and recent findings have demonstrated that physiological β-estradiol exhibits a protective effect on PC12 cells (ER-negative) undergoing oxygen-glucose deprivation *in vitro*. We have identified expression and the subcellular location of ER-α36 in ER-negative PC12 cells. To investigate the potential involvement of ER-α36 in the beneficial effects of β-estradiol, an *ER-α36* gene knockdown cell model was developed in PC12 cells. It was found that β-estradiol attenuated MPP^+-induced cellular damage. Experiments with PC12-36KD cells revealed that β-estradiol failed to protect against cellular damage following *ER-α36* gene knockdown, suggesting that ER-α36 mediates the beneficial effects of β-estradiol against MPP^+-induced cellular damage in PC12 cells. Further studies are underway to understand the more comprehensive function and molecular mechanism of estrogenic signaling within the nervous system.

02z. Cell, Molecular & Systems Biology: other

ADPD5-2244

DEVELOPMENT AND CHARACTERIZATION OF SCALABLE HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED MIDBRAIN DOPAMINERGIC NEURONS FOR DRUG DISCOVERY AND DISEASE MODELING

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Objective: Since the discovery of human induced pluripotent stem cells (iPSCs), much excitement and interest has been created around this technology as a platform for generating pluripotent stem cell lines from a range of specific genetic backgrounds, both normal and diseased. We have developed highly consistent and scalable differentiation protocol for making various types of human neurons, specifically midbrain dopaminergic neurons. This protocol provides a consistent platform to study various aspects of midbrain dopaminergic neuron biology, including Parkinson's disease.
Methods: Using an optimized episomally-derived human iPSC platform, we developed a scalable method for the generation of differentiated, cryopreserved human midbrain dopaminergic neurons (iCell(R) DopaNeurons). Gene expression was analyzed by target-focused PCR arrays. Electrophysiological properties were measured using whole-cell patch clamp and the network-level activity was evaluated on multi-electrode array (MEA).
Results: Here, we present data characterizing gene expression for these floor plate-derived midbrain dopaminergic neurons with proper regional and neural subtype specifications. These cells displayed characteristic neuronal electrophysiological properties, including ion channel activity, evoked and spontaneous action potentials and excitatory post-synaptic currents. In addition, results from the MEA showed characteristic excitatory phenotypes with responses to known pharmacological agents and enhanced population bursts in an astrocyte co-culture environment.
Conclusions: Robust and reproducible methods to generate functional iCell DopaNeurons at high purity will enable the successful downstream production of panels of disease-specific samples derived from donor iPS cells for the study of neurodegenerative disorders such as Parkinson's disease.

03a. Pathophysiology & Disease Mechanisms: cell to cell transmission and spreading of pathology

ADPD5-1778

DEPRESSION ASSOCIATED TO ANIMAL MODELS OF PARKINSON'S DISEASE IS DIRECTLY CORRELATED WITH DEFICITS IN STRIATAL DOPAMINE AND HIPPOCAMPAL SEROTONIN

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The neurotransmitter deficiency hypothesis of PD considers that low serotonin levels in the brain of patients is a risk factor for depression. We investigated if the infusion of 6-hydroxydopamine (6-OHDA) was able to induce depressive-like behavior. Rats were infused bilaterally in the substantia nigra with 6-OHDA. The animals were randomly divided into SHAM (n=8-9) and 6-OHDA (n=8-9). The open field was performed 1, 7, 14 and 21 days after the stereotaxic surgery. The forced swimming test was carried out 7, 14 and 21 after the surgery, although those groups were submitted to the sucrose preference consumption test on days 7, 14 and 21 after neurotoxin. The striatum and hippocampus structures were rapidly dissected after FST. In the open field the rats of 6-OHDA groups presented a decrease in locomotion and rearing frequencies 1 day after surgery. The results of forced swimming test indicated that 6-OHDA rats exhibited reduction in the time of swimming and increased in the immobility time regarding the groups control and SHAM in the days 7, 14, 21 after surgery. After the motor recovery, 6-OHDA was able to produce anhedonia and behavioral despair in the days 7 and 21 after surgery. These behavioral responses were accompanied by reductions of striatal DA. Additionally, decreases in hippocampal 5-HT content were detected in the 6-OHDA group. Notably, correlations were found between 5-HT and DA levels and swimming, immobility, and sucrose preference. Our results indicate that 6-OHDA produced depressive-like behavior accompanied by striatal DA and hippocampal 5-HT reductions.

Sponsored: CNPq, CAPES, REUNI

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-0475

A TARGETED PROTEOMICS APPROACH FOR THE IDENTIFICATION AND QUANTIFICATION OF LAMP2 IN CEREBROSPINAL FLUID, A CANDIDATE BIOMARKER FOR NEURODEGENERATIVE DISEASES

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Objective
The objective was to identify candidate biomarkers in cerebrospinal fluid (CSF) to investigate the involvement of the endo- lysosomal system in neurodegenerative diseases, with focus on Alzheimer's disease. To achieve this, we developed a targeted proteomics method against Lysosome-associated membrane glycoprotein 2 (LAMP2), a protein which might reflect pathophysiological alterations in the endo- lysosomal system.
Methods
A targeted proteomics hybrid approach was used were immunoprecipitated LAMP2 protein was digested with trypsin and subjected to analysis with a nano liquid chromatography system coupled to a hybrid quadropole orbitrap mass analyzer. Western blotting was used for validation of the method. Using isotope labeled peptides a selected reaction monitoring (SRM) method was developed to monitor and quantify a set of identified endogenous peptides in CSF. The method was evaluated in a set of experiments exploring the variability.
Results
We have with confidence identified six proteotypic non-isoform specific LAMP2 peptides in CSF using mass spectrometry. By the combination of mass spectrometry and immunoprecipitation we have confirmed the presence of a LAMP2 fragment consisting of at least amino acids 46-351. Furthermore, we have developed an SRM method with the ability to quantitate the level of LAMP2 in CSF.
Conclusions
For the first time we have detected LAMP2 in human CSF using mass spectrometry. The SRM method, which has been developed for the quantitation of LAMP2 in CSF, is presently being used to explore the protein as a potential CSF biomarker for neurodegenerative diseases in a number of case-control cohorts.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-0907

PROBING MOLECULAR MECHANISMS UNDERLYING LYSOSOMAL CALCIUM DEFECTS IN ALZHEIMER'S DISEASE AND DYSTONIA

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In addition to the endoplasmic reticulum (ER) the lysosome is a major storage organelle for intracellular calcium ions. Calcium homeostasis in the lysosome is not well understood, and it is unclear how lysosomes acquire calcium. Interesting for Alzheimer's disease pathology, lysosomal calcium levels are significantly lowered in presenilin-1 deficient cells. As presenilin seems to be required for lysosomal calcium loading, it is likely that familiar Alzheimer's disease patients have altered lysosomal calcium levels. Using induced-pluripotent stem cell (IPSC) techniques we demonstrate that lysosomal calcium levels are indeed altered in fibroblast and IPSC derived neurons of presenilin mutant familiar Alzheimer's disease patients. Furthermore we find that lysosomes are "overloaded" with calcium in a newly identified dystonia type. Our data indicates that regulation of lysosomal calcium levels at the cellular levels is important for neuronal function.In addition, we have set up a candidate based genetic screen in patient and control fibroblasts and IPSC derived neurons to identify genes involved in lysosomal calcium loading. Identifications of such genes will increase our insight into the regulation of lysosomal calcium and possibly offer new (drugable) targets to tackle diseases in which lysosomal calcium homeostasis is affected.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-2153

LYSOSOMAL FUNCTIONS AND IRON IN NEUROFERRITINOPATHY AND ALZHEIMER'S DISEASE

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OBJECTIVES: Imbalance of iron homeostasis has been implicated in neurodegenerative disorders such as Alzheimer's disease (AD). It is unclear however whether the accumulation of iron in the brain is caused by nerve cell loss or whether it is involved in nerve cell death. Neuroferritinopathy (NFR), a rare adult onset movement disorder caused by mutations in the ferritin light chain (FTL), provides a direct link between iron dysmetabolism and neurodegeneration. Mutant FTL has a dominant negative effect on the function of ferritin, the major iron storage molecule. The aim of this study was to investigate the possible pathomechanisms related to brain iron accumulation.

METHODS: Post-mortem Frontal Cortex from 4 AD, 3 NFR and 5 age-matched controls was subjected to analysis using routine molecular biology techniques.

RESULTS: The levels of FTL were significantly higher in NFR compared to controls and the ratio of ferritin light/heavy chain was altered. A similar trend was observed in AD. A tendency towards abnormalities in lysosomal markers LAMP1 and LAMP2 in relation to FTL was characteristic for NFR and AD. Cathepsin D was significantly reduced in NFR compared to controls. Ongoing investigation will determine lysosomal activities in these cases. A trend towards downregulation of the transcription factor EB, which induces lysosomal biogenesis and regulates mitochondrial quality control, was observed in AD, with no changes in NFR. Mitochondrial protein levels were severely reduced in AD, however no significant abnormalities were observed in NFR.

CONCLUSIONS: Defect in iron homeostasis may lead to lysosomal pathology. Further research is warranted.

03d. Pathophysiology & Disease Mechanisms: autophagy and lysosomes

ADPD5-2166

IMPLICATIONS OF DISRUPTED AUTOPHAGY ON CHOLESTEROL TRAFFICKING, NEURONAL SURVIVAL AND STRATEGIES FOR DRUG DEVELOPMENT IN NIEMANN PICK TYPE C1

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Successful development of therapeutic interventions for Alzheimer's Disease (AD) and related neurodegenerative disorders will require a deeper understanding of mechanisms of disease initiation and progression. Niemann-Pick type C1 (NPC1) is a fatal pediatric dementia characterized by the accumulation of cholesterol in the lysosomal compartment. The disease shares intriguing clinical and histological similarities with AD, suggesting a common mechanism of onset and progression. We used reprogramming technology to develop sets of NPC1 and control human induced pluripotent stem cell (hIPSC) lines, and we generated patient-specific pure neuronal cultures using a standard differentiation protocol. We found that NPC1 neurons have disrupted mitochondrial turnover by autophagy that leads to mitochondrial depolarization and increased production of reactive oxygen species, all of which are likely to contribute to neuronal failure. Our data also raise the important and new possibility that NPC1 neurons initially survive cholesterol accumulation because they activate autophagy. We have evidence that in NPC1 mutant neurons, autophagy may function as a backup pathway that releases trapped cholesterol, albeit at lower efficiency, but sufficient to protect neuronal viability until birth and perhaps for a few additional years. Further mechanistic studies lead us to identify a potential new transporter that mediates autophagy-dependent cholesterol efflux from the lysosomal compartment. Our data highlight the central role that autophagy disruption plays in the selective neuronal failure observed in NPC1. Additionally, our approach establishes a cell-based platform for the high-throughput screening of therapeutic compounds that can revert mitochondrial dysfunction while preserving bulk autophagy in NPC1 and related neurodegenerative diseases.

03e. Pathophysiology & Disease Mechanisms: proteasome and ubiquitin

ADPD5-0579

UBIQUITIN/NGF INTERACTIONS ARE REGULATED BY METAL IONS

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Objectives. Ubiquitin-proteasome system (UPS), Nerve Growth Factor (NGF) trafficking, and metal homeostasis are crucial factors in neuronal survival. UPS is known to regulate the NGF sorting machinery; in addition metal ions bind NGF and Ub. However, only a coarse description of the molecular events lying at the root of these biological events is available. In this work we investigate the physical association between Ub and NGF and the role played by metal ions in regulating this interaction.

Methods. WB assays have been employed to investigate Ub-NGF association in the absence and in the presence of Cu(II) and Zn(II) metal ions. Next, we have also studied NGF(1-14), a peptide fragment encompassing the metal binding N-terminal domain of human NGF, to model NGF-Ub interactions by means of NMR, CD and ITC.

Results. Ubiquitin weakly associates with NGF. In particular, the flexible, metal binding N-terminal part of NGF (NGF1-14) interacts with residues 1-20, 52-61, 66-76 of Ub and undergoes a random-coil to Polyproline II conformational transition upon Ub binding. Metal ions, Cu(II) in particular, may turn off Ub-NGF association.

Conclusions. Altogether these evidences support a causative relationship between the age-related increase in the metal-ion levels, UPS malfunction, NGF signaling and neurodegeneration. Hopefully, these evidences will pave the way to studies addressing the role played by metal ions in driving Ub-mediated NGF signaling in vivo.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0495

IMPROVED MITOCHONDRIAL LIPID PROFILE AND COGNITIVE FUNCTIONS IN EXPERIMENTAL MODEL OF HUNTINGTON'S DISEASE: PROTECTION USING MITOCHONDRIA TARGETED THERAPY

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Objectives:
The study was designed to investigate the neuroprotective effect of mitochondrial modulators; alpha-lipoic acid (ALA) and acetyl-L-carnitine (ALCAR) in 3-NP induced alterations in mitochondrial lipid composition, mitochondrial structural appearance and memory functions.
Methods:
Experimental model of HD was developed by administering 3-NP at sub-chronic doses, twice daily for 17 days. The 3-NP treated animals were supplemented with combination of ALA+ALCAR for 21 days and their therapeutic effect was evaluated in terms of improving mitochondrial lipid composition and cognitive functions by using automated video tracking software (Anymaze^{TM}).
Results:
The levels of conjugated dienes, cholesterol and glycolipids were found to be significantly increased, whereas the levels of phospholipids (phophoethanolamine, phosphocholine, phosphoserine) including cardiolipin were found to be significantly decreased in the mitochondria isolated from the striatum of 3-NP treated animals. 3-NP induced increase in cholesterol to phospholipid ratio reflected decreased mitochondrial membrane fluidity. Mitochondrial membrane fluidity assessed using DPH and pyrene probes showed increased values for fluorescence polarization, anisotropy, anisotropy parameter, order parameter, microviscosity, annular fluidity, bulk fluidity and excimer/monomer ratio. The 3-NP administration also resulted in mitochondrial ultra-structural changes assessed using electron microscopy and spatial memory impairments (in elevated plus maze test). Combined supplementation with ALA+ALCAR to 3-NP treated animals for 21 days restored mitochondrial lipid composition, improved structural appearance and ameliorated memory impairments.
Conclusion:
The results suggest an imperative role of these two modulators in improving mitochondrial lipid profile and cognitive functions if given together and thus could be engaged in managing HD.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-0626
AUTOPHAGY AS A SCAVENGER OF REACTIVE SPECIES DAMAGE AND PROTECTOR AGAINST NEURODEGENERATION

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Objectives: Reactive damage contributes to age-dependent accumulation of dysfunctional mitochondria and protein aggregates, and are associated with neurodegeneration. Supplementation of reactive species scavengers to suppress the initiation of oxidative stress with molecules such as alpha-tocopherol, ascorbate and coenzyme Q has limited success in clinical trials. This may due to: 1) reactive species scavengers cannot reverse established damage to proteins and organelles; 2) reactive species scavengers are limited in action to specific radicals, and can only be effective if neurodegeneration specifically involves the reactive species to which they are targeted and 3) suppression of endogenous reactive species may perturb cell signaling, induce reductive stress and be deleterious. Alternative approaches that can circumvent these limitations are needed. We proposed that autophagy, while not previously considered a reactive species scavenging system, may serve this essential function by removing damaged or dysfunctional proteins and organelles particularly mitochondria which can generate reactive species. **Methods:** We tested this hypothesis using primary neurons exposed to lipid peroxidation product 4-hydroxynonenal (HNE), rotenone and nitric oxide. **Results:** We found that neurons exhibited concentration dependent bioenergetic dysfunction and cell death in response to HNE and rotenone in normoxia, and $NO^{\cdot}$ in hypoxia-reoxygenation conditions. Inhibition of autophagy exacerbated cell death observed in response to HNE, rotenone or $NO^{\cdot}$. Furthermore, cell metabolism plays an important role in regulating autophagy and survival. **Conclusions:** These studies provide new insight and a novel mechanism regarding the role of autophagy in scavenging reactive species damage and protecting against neurodegeneration.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-1764

P62/SQSTM1 DEFICIENCY IS ASSOCIATED TO MITOCHONDRIAL DISTURBANCES

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BACKGROUND: A growing body of published experimental evidences is showing abnormal mitochondrial function related to patients with frontotemporal dementia and amyotrophic lateral sclerosis (FTD/ALS). Mutations in the same disease-causing genes in both disorders have been reported and they support the idea of a FTD-ALS continuum. Interestingly, *VCP* and *p62*/*SQSTM1* mutations which have been found causing FTD/ALS are also identified causing Paget's Disease of the Bone. We have recently demonstrated that VCP (Valosin Containing Protein) deficiency is associated with profound mitochondrial uncoupling, resulting a significant reduction of cellular ATP production. Decreased ATP levels in VCP deficient cells lower their energy capacity, making them more vulnerable to high energy demanding processes.
OBJECTIVES: To study the mitochondrial pathophysiology associated to p62/SQSTM1 (p62) deficiency.
METHODS: Dynamic live-cell imaging techniques to explore the mitochondrial pathophysiology in a p62-knock-down (p62 KD) human dopaminergic neuroblastoma cell line (SH-SY5Y) and fibroblasts from patients carrying two independent pathogenic mutations in the *p62/SQSTM1* gene.
RESULTS: We confirm that p62 deficiency is associated to inhibition in the cell respiration inducing decreased mitochondrial membrane potential. This inhibition resulted in higher ROS production, compelling the cells to switch from glycolysis to the pentose phosphate pathway as reflects the elevated NADPH and GSH levels.
CONCLUSIONS: These findings highlight the pathophysiological events that may occur in FTD/ALS associated with p62 deficiency.

03g. Pathophysiology & Disease Mechanisms: mitochondrial dysfunction

ADPD5-1909

BEHAVIOURAL, BIOCHEMICAL, MITOCHONDRIAL ALTERATIONS OF PPAR-GAMMA AGONIST IN RAT MODEL OF CHRONIC PAIN ASSOCIATED COGNITIVE DEFICITS

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Objectives: Oxidative stress and mitochondrial dysfunction has been suggested to play key role in the development and maintenance of the neuropathic pain. The present study was designed to explore the role of oxidative stress in the spinal nerve ligation (SNL) induced neuropathic pain with the help of various behavioral, biochemical, mitochondrial and cellular alterations in rats.
Methods: In the present study, unilateral lumbar L5 and L6 spinal nerves were ligated to induce neuropathic pain in rats. Behavioral parameters were assessed on the day before ligation and successively on day 7th, 14th, 21st and 28th post ligation. Oxidative stress parameters and mitochondrial enzyme functions were assessed on day 28 after behavioral observations.
Results: SNL resulted in significant increase in mechanical allodynia, mechanical hyperalgesia, cold allodynia and heat hyperalgesia, transfer latency as assessed by Vonfrey, Randall Selitto, Acetone drop, Hot plate and elevated plus maze tests respectively. SNL also resulted in significant increase in oxidative stress parameters (increased lipid peroxidation, nitrite, reduced superoxide dismutase, catalase and glutathione) in lumbar spinal cord and brain. Mitochondrial enzyme complexes activities were significantly inhibited by SNL. Pioglitazone (a PPAR gamma agonist) treatment (10 and 20 mg/kg, i.p.) for 28 days significantly reversed the various behavioral, biochemical and mitochondrial alterations in SNL treated animals.
Conclusions: Results of the present study show that ameliorative potential of pioglitazone in SNL induced behavioral and mitochondrial alterations which may be further attributed to inhibition of oxidative stress and mitochondrial dysfunction in rats.

03h. Pathophysiology & Disease Mechanisms: metabolism and insulin

ADPD5-1867

THIOREDOXIN 80, A NOVEL PLAYER IN INSULIN SIGNALING: IMPLICATIONS FOR ALZHEIMER´S DISEASE

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Objectives

Despite being widely investigated, the mechanisms behind Alzheimer´s disease (AD) are not completely understood but increasing evidence suggests the implication of metabolic alterations related to glucose metabolism and insulin resistance in its development. Insulin is related with tau protein phosphorylation and autophagic clearance of Aβ, thus participating in the regulation of two major hallmarks in AD. We have previously demonstrated that Thioredoxin 80 (Trx80), which is dramatically decreased in AD brains, has an anti-amyloidogenic effect on Aβ and prevents its toxicity. Insulin has been described to form amyloid aggregates that lead to a loss of function. We believe that Trx80 could prevent insulin aggregation, and thus act as a key player in regulating insulin signaling in brain. For this reason, understanding Trx80 role in brain and its effects over metabolic regulation and insulin signaling could be essential for understanding the molecular mechanisms underlying AD.

Methods

Western Blot, MTT assay, Glucose uptake measurement, ThT assay, qPCR.

Results

We show that Trx80 prevents insulin aggregation *in vitro* and that aggregated insulin fails to activate the most common insulin pathways. Cell viability studies clearly demonstrate that aggregated insulin lacks its mitogenic effect. Interestingly, in all cases the lack of function of aggregated insulin is rescued when insulin is co-incubated with Trx80.

Conclusions

The results suggest a key role for Trx80 in regulating insulin signaling and glucose metabolism as well as Aβ clearance in brain, although the molecular mechanisms and the possible coordination between these two processes remain to be investigated.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-0380

PHYSICAL EXERCISES REDUCE PERIPHERAL LEVELS OF INFLAMMATORY CYTOKINE IN MCI ELDERLY WITH DIFFERENT APOE GENOTYPE.

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Inflammatory activity has been investigated as potential biological mechanism associated to neurodegenerative cascade affecting Alzheimer's disease (AD) and may be related to mild cognitive impairment (MCI) due this pathology. Apolipoprotein E e4 allele has been established as a potential risk factor to neurodegenerative condition linked to AD. Regular physical exercises can contribute to reduce pro-inflammatory cytokines. Therefore, the objective of this study was to analyze the effects of 16-week of physical exercises program on peripheral levels of tumor necrosis-alpha (TNF-a) and interleukin-6 (IL-6) in elderly with mild cognitive impairment (MCI). Participants were genotyped for APOE polymorphism. Sixty-seven participants were assigned into control (CG) and trained (TG) groups. The TG was composed by 35 participants, and 10 were genotyped as e4 allele carriers. All subjects included in the TG participated in a physical training for a 16-week period and attended at least 75% of the sessions. Considering the control group, for the APOE genotype, from 32 participants, 8 were identified as e4 allele carriers Results showed a significant between-subjects interaction ($p<0.05$) indicating the beneficial contribution of training on reductions of TNF-α and IL-6 levels, independently of the APOE genotype. Physical exercises may reduce low-grade chronic inflammation even under neurodegenerative conditions and APOE genotype seems not modulate these effects.

03i. Pathophysiology & Disease Mechanisms: inflammation

ADPD5-2132

EFFECTS OF CO-ADMINISTRATION OF GHRELIN AGONIST (GHRP-2) AND GH ON TNF-A, IL-6 AND INOS GENE EXPRESSION INDUCED BY LPS IN THE BRAIN

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The aim of this study was to examine the anti-inflammatory effects of co-administration of growth hormone-releasing peptide-2 (GHRP-2) and growth hormone (GH) on tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and inducible nitric oxide synthase (iNOS) gene expression induced by LPS in the mouse brain. Thirty-five male NMRI mice (25±5 g) were injected through the mouse tail vein with saline, GHRP-2 (100 µg/kg), GH (25 µg/kg) or GHRP-2 + GH, 30 min before the intraperitoneal injection of LPS (5 mg/kg). Then, inflammation was induced by the intraperitoneal injection of LPS. The control animals received sterile saline in the first and second injections. Changes in the expression level of TNF-α, IL-6 and iNOS genes were studied in the mouse brain by a semi quantitative RT-PCR method. The results of this study showed that GHRP-2 or GH significantly decreased the expression of TNF-α and IL-6 genes in brain 2 h after the injection of LPS. Co-administration of GHRP-2 and GH markedly reduced the expression of TNF-α and IL-6 genes. LPS had no effect on the expression of iNOS gene in brain. The data suggest that co-administration of GHRP-2 and GH has a protective effect in brain inflammation induced by LPS through inhibition of TNF-α and IL-6.

03j. Pathophysiology & Disease Mechanisms: autoimmunity

ADPD5-0981
ANTIBODY-DRIVEN MEMORY IMPAIRMENT

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Objectives: The role of antibodies in neuropsychiatric disorders remains little understood. Cognitive impairment is frequently observed in systemic lupus erythematosus (SLE), the prototypic autoimmune disease. Here we studied the pathogenic potential of autoantibodies against ribosomal phosphorylated proteins (anti-P), which cross-react with a neuronal surface P antigen (NSPA) protein and have long been considered a risk factor in diffuse brain dysfunctions.
Methods: Anti-P from SLE patients were analyzed in: 1) Passive transfer experiments and electrophysiology assays, assessing memory related hippocampal functions in mice; 2) Intracellular calcium variations (Fura 2-AM) and apoptosis (caspase-3 activation and Tunel); 3) Cognitive function in SLE patients using *Cambridge Neuropsychological Test Automated Battery* (CANTAB) and ANCOVA models.
Results: Anti-P directly injected by stereotaxis into the hippocampus or added to primary cultures induced neuronal apoptosis, likely due to calcium influx. However, intravenously injected anti-P impaired memory (water maze assays) without detectable signs of apoptosis in hippocampus. Anti-P enhanced excitatory post-synaptic transmission involving over-activation of both AMPA and NMDA receptors and leading to abrogation of long-term potentiation in hippocampal CA1. Cognitive deficit in SLE patients associated with anti-P in fronto-parietal cortex dysfunctions such as attention and spatial planning abilities.
Conclusions: Anti-P have the potential to perturb neuronal function in the brain inducing apoptosis and altering glutamatergic synaptic transmission and plasticity, which depending on the region can lead to memory impairment. Anti-P might contribute to the frequently observed cognitive impairment in SLE. (Financed by Conicyt Basal Project PFB12/2007).

03k. Pathophysiology & Disease Mechanisms: lipids, lipoproteins and membrane trafficking

ADPD5-0588

GOLGI APPARATUS FRAGMENTATION AND ACTIVATION OF ATF6 SIGNALING IN THE UNFOLDED PROTEIN RESPONSE IN HEREDITARY SPASTIC PARAPLEGIA TYPE 54

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Objectives: Hereditary Spastic Paraplegias (HSPs) are a group of genetic disorders characterized by limb spasticity and weakness, psychomotor delay and intellectual disability. Recently, DDHD domain-containing protein 2 (DDHD2) was identified as the causative gene for autosomal recessive HSP54. DDHD2 is an intracellular phospholipase A1 (iPLA1) implicated in organelle biogenesis and membrane trafficking. Here we examine a role for DDHD2 in endoplasmic reticulum (ER) and Golgi vesicle trafficking to determine if irregular protein accumulation and activation of the unfolded protein response (UPR) contributes to the pathogenesis of HSP54.
Methods: Human fibroblasts derived from an HSP54 patient with a compound DDHD2 heterozygous frameshift mutation [c.1386dupC (p.Ile463-Hisfs*6)] and a missense mutation [c.1978G>C (p.Asp660His)] were characterized via Western Blot Analysis, real-time PCR, and Immunofluorescence staining.
Results: We identified abnormalities in the UPR of HSP54 fibroblasts: BiP was induced, PERK was downregulated, XBP-1 splicing remained unchanged, and ATF6 protein levels were increased leading to induction of its proteolytic cleavage. These alterations resulted in induction of the UPR downstream effector CHOP. Fragmentation and dispersion of the Golgi apparatus was also observed. Finally, it was found that HSP54 patient cells have an increase in DDHD1, another iPLA1 protein with similar activity as DDHD2.
Conclusions: Our results clearly indicate that HSP54 patient cells have defects in protein processing in the ER, which triggers activation of the ATF6 branch of the UPR, and perhaps a compensatory increase in DDHD1 expression. These results indicate that the UPR likely plays a role in HSP54 pathogenesis and may serve as a therapeutic target.

03n. Pathophysiology & Disease Mechanisms: kinases and phosphatases

ADPD5-0248

INCREASED TAU PHOSPHORYLATION CORRELATES WITH PKA AND AKT ACTIVATION AFTER TRAUMATIC BRAIN INJURY IN 3XTG-AD MICE

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Traumatic brain injury (TBI) is a major environmental risk factor for Alzheimer's disease (AD). Tau pathology can be found in post-mortem brain many years after a single severe or repetitive mild TBI. Since Tau hyperphosphorylation is suggested to play a major role in AD pathogenesis, its induction after injury may play a role TBI pathology. There are reports of acute phospho-Tau accumulation after experimental TBI using a controlled cortical impact (CCI) model. Here, we examined Tau and phospho-Tau accumulation in the 3xTg-AD mouse model 2 and 4 weeks post-CCI to determine if these acute changes can persist chronically after injury in the mouse.

An increase in Tau phosphorylation was observed in the ipsilateral striatum of injured mice and persisted 4 weeks post-injury. In this side, a sustained PKA activation was observed concomitantly with an increase of the activated form of Akt at 4 weeks. In the contralateral striatum, TBI induced a transient increase in Tau phosphorylation. This Tau hyperphosphorylation was associated with a temporary activation of Akt 2 weeks after CCI. The injured mice did not show any changes in either Tau phosphatase levels or APP cleavage, as compared with sham mice. Our data suggest that the hyperphosphorylation of Tau found in the ipsilateral striatum could be linked to sustained PKA activation, while transient Tau hyperphosphorylation observed in the contralateral side could be related to Akt activation. Moreover, these results provide further evidence for the independent relationship between Tau hyperphosphorylation and APP processing in the TBI context

03n. Pathophysiology & Disease Mechanisms: kinases and phosphatases

ADPD5-1757

GSK3BETA HAS A ROLE IN BIDIRECTIONAL SYNAPTIC PLASTICITY: GENETIC AND PHARMACOLOGICAL EVIDENCE.

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Objectives: GSK3β, a proline-directed serine-threonine kinase, has multiple roles in neuronal energy homeostasis, protein translation, synaptogenesis, synaptic plasticity and cognition. Dysfunction of this enzyme has been proposed in neuropsychological and neurodegenerative disorders such as schizophrenia, bipolar depression, autism and Alzheimer's disease (AD). A common trait of these disorders is defective synaptic functioning, which manifests as perturbed synaptic plasticity. Current hypotheses position GSK3ß in a central role in long-term depression (LTD) but not in long-term potentiation (LTP).

LTD and LTP are thought to underlie different forms of memory at the cellular level.

Methods: Using long-term field recordings in the hippocampal CA1-region of mice, we re-evaluated the function of GSK3ß in protein-synthesis dependent late forms of LTD and LTP (<3 h, L-LTD and L-LTP) by using specific pharmacological inhibitors and neuron-specific GSK3ß KO mice (*Gsk3b$^{n-/-}$*).

Results: We found an impairment of L-LTD upon bath-application of the specific GSK3ß inhibitors SB216763 and indirubin-3-oxime thereby corroborating previous reports. Strikingly, the same compounds caused a severe deficit in L-LTP. These findings were confirmed by recordings in the intact brain from freely-moving rats. Furthermore, an independent genetic analysis of *Gsk3b$^{n-/-}$* mice revealed severely deteriorated L-LTD and L-LTP. In addition, we provide pharmacological evidence that upstream regulators of GSK3β (CDK5, AKT-PI3K pathways) are differentially involved in L-LTD and L-LTP.

Conclusions: The role of GSK3β in long-term synaptic plasticity has to be re-defined, which has major implications for its normal functions - and for its dysfunction in neurodegenerative disorders.

03o. Pathophysiology & Disease Mechanisms: cellular signalling

ADPD5-0462

GHRELIN ENHANCES NIGRAL DOPAMINERGIC NEURONAL EXCITABILITY BY INHIBITION OF IM AND IA

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Objectives: The gut-derived orexigenic peptide hormone ghrelin enhances neuronal firing in substantia nigra pars compacta (SNc) where excitability of dopaminergic neurons determines the function of nigrostriatal system for motor coordination. However, how ghrelin regulates the excitability of dopaminergic neurons remains largely unknown. Therefore, we sought to understand the molecular mechanism and signaling pathway of the excitatory effects of ghrelin on nigral dopaminergic neurons.Methods: Whole-cell and nystatin-perforated patch clamp recordings were used to record the neuronal firing and potassium current on rat brain slices. Dopamine release and turnover in the rat striatum were measured using HPLC-ECD and fast cyclic voltammetry (FCV). Postural and bar test were used to detect the effects of ghrelin on haloperidol-induced catalepsy.Results: Ghrelin (0.1-1000 nmol/L) directly stimulated the firing rate of dopaminergic neurons does-dependently, as it was not antagonized by excitatory and inhibitory synaptic inhibitors. In addition, ghrelin (100 nmol/L) reversibly and significantly decreased the amplitude of both Kv7/KCNQ/M-current (I_M) and transient potassium current (I_A) to 50% and 53% of control, respectively. This effect was abolished by selective inhibitors of GHS-R1a, PLC, and PKC. XE991 and 4-AP, the specific I_M and I_A inhibitors, abolished ghrelin-induced hyperexcitability. *In vivo*, intracerebroventricular ghrelin administration causes increased dopamine release and turnover in the striatum. Microinjection of ghrelin into SNc results in contralateral dystonic posturing, and attenuates the catalepsy elicited by haloperidol. Conclusions: Our findings reveal that ghrelin exerts its function by inhibiting I_M and I_A via GHS-R1a-PLC-PKC pathway, resulting in enhanced excitability of nigral dopaminergic neurons for improvement of motor impairment.

03q. Pathophysiology & Disease Mechanisms: blood-brain barrier & transport

ADPD5-0532

NANOTECHNOLOGY APPLICATIONS FOR ALZHEIMER'S DISEASE

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Objectives: Dementia of Alzheimer's type(AD) affects memory,thinking and behavior.Scientists believe that changes in the brain may begin 10-20 years before symptoms appear and AD is diagnosed.The need to diagnose and treat the devastating disease at an early stage is critical to manage and treat AD.Unfortunately,the lack of valided biomarkers limits the possibility of the earlier stages of Alzheimer's disease. The advance of nanotechnology could offer huge opportunities in early-stage diagnosis and well-treatment of AD.
Methods: This presentation discusses the challenges of current treatment and diagnosis of AD and the development on biocompatible nanoparticles, and provide the rational and potentials of using nanoparticles for both drug carrier and imaging contrast agent for diagnosis and treatment of AD.
Results: Biocompatible nanoparticles with diameter in the range of 1-100nm could be used as targetes delivery system for drugs(e.g. Rivastigmine) to overcome the blood-brain barrier(BBB), and to minimize the side effects caused by over-dosage.In addition, biocompatible nanomaterials with enhanced optical and magnetic properties, may allow them being excellent alternative contrast agents for early-stage diagnosis.
Conclusion: With more studies using nanomaterials and nanotechnology in complex biochemical environment of the central nervous system, it is most likely that nanomaterials and nanotechnology can be give significant impact on the early-stage diagnosis and treatment of AD

03s. Pathophysiology & Disease Mechanisms: neurogenesis and stem cells

ADPD5-1036

GENERATION OF PATIENT-SPECIFC HUMAN NEURAL STEM CELLS WITH THE ABILITY TO PRODUCE MATURE NEURONS AND ASTROCYTES FOR DISEASE-MODELLING

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Objectives

To develop a novel hiPSCs-based protocol which allows the generation of expandable hNSCs that can be either maintained under self-renewing conditions over high passages or differentiated into various neuronal sub-types.

Methods

hNSC were first generated from iPSCs by the chronological administration of media with defined compositions. After generating a stable hNSC culture, transition to either neurons or astrocytes was achieved after 2 and 8 weeks respectively. Cell identity was confirmed by immunofluorescence and quantitative real time PCR investigations.

Results

The generation and maintenance of hNSCs was robustly achieved. hNSCs maintained both self-renewing features as well as the potential of indefinite proliferation over numerous passages as demonstrated by the expression of marker as Nestin, SOX1, SOX2 and of the proliferation marker Ki67. Under neuronal differentiation media, hNSC differentiated into functional neurons expressing MAP2, GABA, vGlut1 and TH. hNSC were also differentiated into astrocytes expressing GFAP, S100ß and Vimentin. We were able to obtain a population of mature astrocytes both in a quiescent state with a protoplasmic morphology (not positive for GFAP) as well as in a reactive phenotype characterized by GFAP expression. The expression of glutamate transporter and water channel in all the cells strongly support the acquisition of mature functions.

Conclusions

The easy execution of the differentiating steps allows the generation of functional neurons and long expandable astrocyte cultures, which can be used for modeling disease-specific pathological traits. Moreover, human iPSCs bear the advantage of being derivable in a patient-specific manner. This makes them suitable for autologous engraftments.

03s. Pathophysiology & Disease Mechanisms: neurogenesis and stem cells

ADPD5-1189

PHYSICAL ACTIVITY INCREASES ADULT HIPPOCAMPAL NEUROGENESIS AND SPATIAL LEARNING ABILITIES IN A MOUSE MODEL OF PARKINSON'S DISEASE

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Objectives

Modulation of adult neurogenesis in neurodegenerative disorders has been investigated in animal models of Parkinson's disease (PD). PD is associated with continuous loss of dopaminergic neurons in the substantia nigra leading to dopamine depletion. In the clinic, physical exercise improved motor and cognitive functions in PD patients. Animal experiments have demonstrated the involvement of endogenous neural precursors correlating with better clinical outcome.

Methods

Here, we investigated the impact of different durations of voluntary physical exercise on hippocampal neurogenesis in the MPTP mouse model. Additionally, we measured dopamine levels in the striatum and hippocampus and determined the expression of genes that might link dopamine to neurogenesis. We also studied the association of neurogenesis with spatial learning and memory in the Morris Water Maze (MWM).

Results

MPTP transiently altered the differentiation process of newly generated neural precursor cells. Physical exercise accelerated the differentiation process and increased net neurogenesis. Exercise did not reversed the MPTP-induced dopamine depletion but increased the upregulation of dopamine receptor D1 (drD1) expression in the hippocampus. MPTP impaired spatial learning, whereas short-term exercise increased spatial learning in the MWM.

Conclusions

The results indicate that dopamine via its receptor drD1 modulates neurogenesis in the hippocampus and associated spatial learning and memory abilities. Physical exercise reversed the effects of dopamine depletion on neurogenesis and cognition probably through a mechanism that is independent of the dopamine level in the hippocampus. Hence, physical exercise might serve as a potential therapeutic for neurodegenerative disorders in humans by positively regulating adult neurogenesis and cognition.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-1689

MICROGLIA-SPECIFIC PROGRANULIN LOSS IN FRONTOTEMPORAL DEMENTIA

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Frontotemporal dementia (FTD) represents a clinically and pathologically heterogeneous group of neurodegenerative disorders that form the second-most prevalent cause of early-onset dementia. Mutations in the granulin gene (*GRN)* leading to progranulin (PGRN) haploinsufficiency account for 5-10% of all FTD cases and nearly 25% of familial FTD cases, but the mechanism through which progranulin deficiency leads to neurodegeneration is unknown. Progranulin, a pleiotropic growth factor, is expressed by both neurons and microglia cells in the brain. By depleting and exchanging the endogenous $PGRN^{+/+}$ (wild-type) microglia pool with PGRN-deficient myeloid cells in otherwise wild-type ($PGRN^{+/+}$) mice, we assessed the microglia-specific contribution of PGRN deficiency to FTD-like neuropathology in aged animals, while sparing neuronal PGRN expression. Histological, biochemical and cognitive analyses of FTD-related pathology in mice lacking PGRN exclusively in microglia or in all CNS resident cells allowed for the identification of the specific cellular source accountable for FTD-like changes. These data broaden our understanding about PGRN actions and pinpoint specific targets for future therapeutic approaches.

03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-2093

A SPECTRUM OF CHRONIC TRAUMATIC ENCEPHALOPATHY PATHOLOGY IN THE BRAINS OF BOXERS

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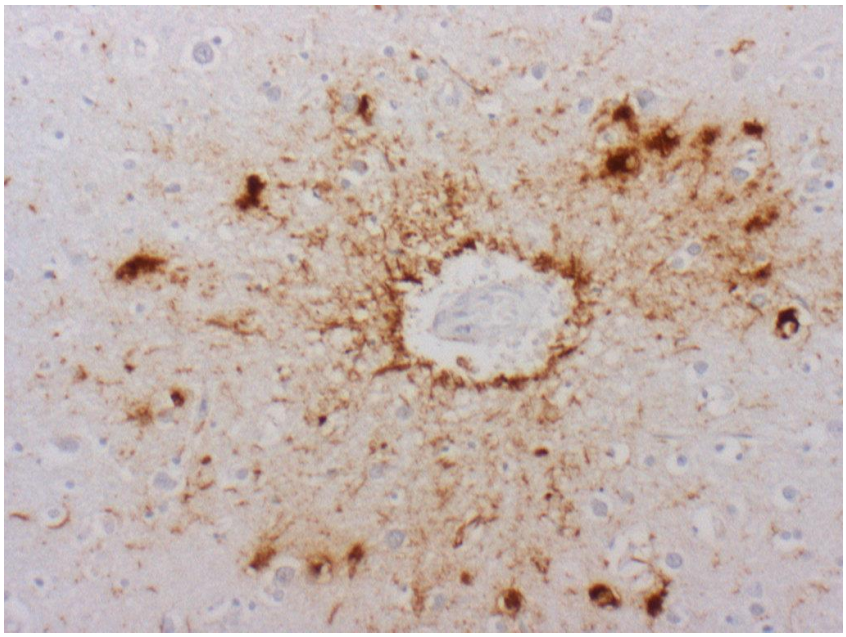
Chronic traumatic encephalopathy (CTE) is a progressive neurodegenerative condition associated with a history of repetitive mild traumatic brain injury as can be experienced in some contact sports. In recent years the clinical presentation of deficits in cognition, behaviour, mood and motor function associated with this condition have been linked to the presence of extensive glial and neuronal tau pathology in the post mortem brain. Although initially described in boxers as 'dementia pugilistica', the term CTE is now used to cover other sporting and non-sporting activities associated with repetitive brain injury. The aim of this study was to determine the extent and distribution of pathology in a seminal series of dementia pugilistica cases which first reported in the 1970s (Corsellis et al. Psychol Med 1973; 3: 270-303). Tissue sections from the brains of 25 boxers (amateur and professional) and 4 age-matched non-boxing controls from the Corsellis archive were immunostained for tau, A-beta and alpha-synuclein. Twelve of the seventeen professional boxers (70%) and, interestingly, one of the non-boxing controls displayed tau pathology consistent with CTE. Neurofibrillary tangles, neuropil threads and astrocytic tangles were seen located focally in the depths of the sulci, and in perivascular (see figure), subpial and periventricular locations. Two of the amateur boxers also showed some minor tau pathology but even within the professional boxer group there was a wide variation in the extent of the CTE pathology. Further work is underway to determine what factors may have determined the severity of the pathology in this cohort.



03t. Pathophysiology & Disease Mechanisms: glial cells

ADPD5-2152
DISEASE-DRIVEN CHANGES IN A DISCRETE POPULATION FROM THE CEREBELLAR CORTEX

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In mammals, adult neurogenesis is thought to be restricted to two regions in the brain, the lateral ventricle and the hippocampus. Recently, cells exhibiting some stem cell characteristics have been identified in the Purkinje cell layer (PCL) of the adult cerebellum. These cells identified in adult mouse as the Bergmann glia have also been reported in human cerebellum. Bergmann glia cells are characterised by long radial glia processes, and share co-expression of markers such as Sox1/2/9 with neural stem cells (NSCs) typically found in the subventricular and subgranular zones. When isolated in culture, these cells exhibit marker expression, proliferation and differentiation characteristics comparable to NSCs.

To further investigate the possible role of these cells in adult cerebellum, a disease model presenting gradual cerebellar degeneration was analysed and compared to tissue from asymptomatic controls. Immunohistochemical analysis confirmed the persistence of the Sox1/2/9^{+} cell population in the Purkinje cell layer even at stages where Purkinje cells had partially or totally disappeared. Tissue analysed at different time-points provided insight into the cellular processes associated with the ataxic phenotype, and the cellular features of the Bergmann glia evaluated across the different stages. The present study reveals the differential changes occurring in the 2 intercalated cell populations present in the PCL during cerebellum degeneration, which have implications for approaches to cerebellar pathologies.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-0465

PREFERENTIAL HEME OXYGENASE-1 ACTIVATION IN STRIATAL ASTROCYTES ANTAGONIZED DOPAMINERGIC NEURONS DEGENERATION IN MPTP-INTOXICATED MICE

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Objective: PD is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) with accompanying evidence of increased oxidative damage. Heme oxygenase-1 (HO-1) is crucial to the response to oxidative stress via the catabolism of heme into carbon monoxide, biliverdin and iron. The present study aims to investigate neuroprotective effects of HO-1 activation induced by cobalt protoporphyrin IX (CoPPIX) in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxicated mice. Methods: Double immunefluorescence staining and western blots were used to measure HO-1 levels in vivo and in vitro. High performance liquid chromatography was used to analyze the striatal dopamine levels. Flow cytometry was used to indicate the mitochondria function. Results: MPTP triggered a robust HO-1 activation in the astrocytes of striatum after 1d treatment, and then dropped dramatically. Intraventricular administration of CoppIX for 8 days could preferentially activate HO-1 in astrocytes in striatum rather than SNpc. The loss of dopaminergic neurons was blocked and striatal dopamine content were restored in the subacute MPTP models with CoPPIX administration. We then analyzed HO-1 response in primary cultured ventral mesencephalic astrocytes and neurons treated by 1-methyl-4-phenyl-pyridinium (MPP^+). The results showed HO-1 up-regulation in astrocytes appeared much earlier than that in neurons. Although HO-1 activation induced by CoppIX might be double-edged in neurons, it always showed cytoprotective effects in astrocytes. Conclusion: These results indicated that preferential HO-1 activation in striatal astrocytes might convey neuroprotective effects on dopaminergic neurons in PD. This also provided evidence for targeting HO-1 in striatal astrocytes for PD therapeutics.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-1585

PAK4 CONFER NEUROPROTECTION IN PD THROUGH CRTC1

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PD is an aging-related neurodegenerative disease caused by dopaminergic (DA) neuron loss in the substantia nigra (SN) in which decreased dopamine lead to motor dysfunctions. p21-activated kinase 4 (PAK4) plays a key role in cell survival and neuronal development. We therefore investigated a possible neuroprotective role of PAK4 in PD. We found that $pPAK4^{S474}$ levels, an index of PAK4 activation, were markedly decreased in DA neurons in human PD brain and a 6-hydroxydopamine (6-OHDA) Parkinson animal model. Intriguingly, the remaining pPAK4-positive DA neurons in PD brain were immunolabelled with anti-B-cell lymphoma 2 (Bcl-2) but not by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL). Knocking down PAK4 expression sensitised rats to 6-OHDA-induced PD development, including behavioural dysfunction. Conversely, constitutively active PAK4 (caPAK4) expression protected DA neurons from 6-OHDA insult, thereby preserving motor function. This neuroprotective effect of caPAK4 was mediated by phosphorylation of CREB-regulated transcription coactivator (CRTC1) at serine 215. $CRTC1^{S215A}$, a nonphosphorylated isoform, compromised the ability of caPAK4 to induce the expression of the neuroprotective CREB target genes Bcl-2, brain-derived neurotrophic factor (BDNF) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α). Our results support a neuroprotective role for PAK4 in PD and elucidate a downstream mechanism. These results identify this PAK4 pathway as a potential target for PD management.

03u. Pathophysiology & Disease Mechanisms: cell death

ADPD5-1955

MYOCARDIAL INFARCTION IN RAT INDUCES BRAIN ISCHEMIA UPREGULATING PS2 AND PS1 PROMOTING NEURONAL DEGENERATION DURING AGING

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Objective: Presenilin (PS) expression is regulated by several cellular factors with age. PS1 & PS2 is regulated by ischemic conditions increasing their expression levels promoting apoptosis during Myocardial Ischemia (MI). Therefore age associated MI may cause brain ischemia (BI) and thereby expected to influence PS overexpression in the brain. Present study thus aims at investigating MI as a possible cause for BI and neurodegeneration during aging.
Methodology: Young and aged male wistar rats induced with MI and BI through LAD ligation and MCAO respectively was utilized for present study, where young and aged rats treated as –ve control and BI rats treated as +ve control. To understand age associated neurodegeneration mediated by MI, PS1 & PS2 levels, Inflammatory and apoptotic markers were studied using Immunoblotting. Histopathology was done to confirm neurodegeneration.
Results: Aged rat heart ischemia had significantly raised brain ischemic pattern that was evident through elevated HIF-1α levels. Further, PS2 & PS1 levels were significantly upregulated rising inflammatory and apoptotic markers in aged rat brain induced with MI. Pathological observation further confirmed neurodegenerative pattern in aged rat brain induced with MI when compared to young and aged control rats. Interestingly, MI induced aged rats displayed similar observation as that of BI induced aged rats providing major link between heart ischemia and neurodegeneration.
Conclusion: Upregulation of AD determinants (PS2 & PS1) along with inflammatory and apoptotic markers provided a valuable information on age associated MI that can induce BI initiating neurodegeneration, and may thus shed light on age associated SAD.

03v. Pathophysiology & Disease Mechanisms: metal ions

ADPD5-0287

CONBINED SPECIATION TECHNIQUES PROOF CHANGES IN THE METALLOME AND METABOLOME AS A CAUSE FOR MANGANESE RELATED NEURODEGENERATION

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Chronic Manganese exposure leads to degeneration of dopaminergic neurons inducing a Parkinson-like complaint called Manganism. Deciphering the ongoing neurodegenerative mechanisms in the affected brains is still a major task for understanding the complex modes of action.
We analyzed and identified relevant Mn-carriers being responsible for a widely uncontrolled transport across neural barriers (NB): Mn speciation in paired serum/cerebrospinal fluid (CSF) samples was performed two-dimensionally by SEC-ICP-DRC-MS and CZE-ICP-DRC-MS. The most important Mn-carrier, Mn-citrate, was identified by ESI-FT-ICR-MS. Elevated Mn-citrate concentration in serum were shown to act as marker for increased Mn concentration in CSF (and brain), the latter elevating the risk of Mn-dependent neurological disorders.
To clarify molecular mechanisms of Mn-neurotoxicity we applied ESI-FT-ICR-MS and IC-ICP-OES to rat brain extracts after low-dose Mn-feeding, simulating chronic Mn-exposure. ESI-FT-ICR-MS-analysis of brain extracts revealed an increase in oxidative stress markers like glutathione-disulfide (GSSG), prostaglandins, and 15(S)-HETE, a marker for lipid peroxidation. Acetylcholinesterase activity and glutamate concentrations were also increased in brain samples of Mn-supplemented rats, indicating oxidative stress in brain, too. Furthermore, a shift in neuronal Fe(III) to Fe(II) was observed, promoting Fenton reaction and formation of chemical radicals. For the first time altered Fe-species distribution could be related to Mn-induced neuroinflammation, enlarging knowledge of this complex neurodegenerative condition. The combination of various speciation- and different mass spectrometry techniques provided information how Mn enters the brain without efficient control at NB and provided substantial evidence that Mn-induced neuroinflammation leads to oxidative stress triggered by multifactorial pathophysiological processes.

03v. Pathophysiology & Disease Mechanisms: metal ions

ADPD5-0389

LACTOFERRIN SYNTHESIZED BY MICROGLIA ANTAGONIZES MPP+-INDUCED NEUROTOXICITY TO THE DOPAMINERGIC NEURONS

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Objectives: Lactoferrin (Lf) can bind to lactoferrin receptor (LfR), leading to iron transport through the plasma membrane. Lf also has a wide variety of biological functions, for example, antioxidant, anti-carcinogenic and anti-inflammatory properties. In the brain, Lf is only synthesized and released by activated microglia, and LfR is mainly expressed in neurons. Lf mRNA levels and LfR expression were increased in Parkinson's disease (PD) patients and animal models which indicate a relevance of Lf/LfR and PD. The aim of the study was to investigate how iron influenced Lf release by microglia, as well as to clarify if Lf tended to transport iron to dopaminergic neurons leading to cell death or protect dopaminergic neuron from neurotoxin. Methods: enzyme-linked immunoabsorbent assay, real-time PCR, flow cytometry, western blots, laser confocal scanning microscopy and other methods were used in this experiment. Results: activated microglia could synthesize and release abundant Lf. This process was further enhanced by iron load. In ventral mesencephalon neurons, both forms of Lf: iron-free (apo-Lf) and iron-saturated (holo-Lf) exerted neuroprotective effects against MPP+-induced neurotoxicity by a mechanism, believed to enhance the mitochondrial transmembrane potential, improve the activity of SOD, increasing the expression of anti-apoptotic protein Bcl-2. Although apo-Lf but not holo-Lf chelated cellular iron, there were no difference between the two types of Lf for the protective effects. Conclusions: iron overload can increase the activated microglia releasing Lf. Both apo-Lf and holo-Lf play protective role on ventral mesencephalon neurons against MPP^+, which is independent on iron chelating ability.

03w. Pathophysiology & Disease Mechanisms: calcium homeostasis

ADPD5-1627

RC DEFECT-ALTERED CARDIOLIPIN REMODELING IN ASSOCIATION WITH MITOCHONDRIAL DYNAMICS IN NARP CYBRIDS AND ITS PARENTAL 143B AND P0 CELLS

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Mitochondrial (m) respiratory complexes (RC) are vital for electron transport and ATP generation. RC defects result in significant mitochondrial stress for an enhanced apoptosis. This study investigated RC defect-altered remodeling of cardiolipin (CL), a signature phospholipid in the mitochondrial inner membrane crucial for RC stability and ATP generation, in association with mitochondrial dynamics of mROS formation, mCa2+ overload, delta si depolarization in 143B osteosarcoma cells, NARP cybrids and its parental 143B and mtDNA less p0. We demonstrate that NARP cybrids, in contrast to 143B and p0 cells which are more sensitive to oxidative stress, are more vulnerable to Ca^{2+} stress possibly due to complex V inhibition-induced enhanced delta si, the main driving force for mCa2+ uptake. Confocal imaging also revealed that all RC inhibitions and cell stresses resulted in various degrees of mCa2+ overload, mROS formation, delta si depolarization and CL depletion. Compared to complexes I or II, inhibition of complex III, IV, or V enhanced more NARP toxicity due to more mROS dependent CL depletion. Interestingly, among these stresses, overloaded mCa2+ and inhibition of complex IV and V demonstrated clear CL-dependent threshold effects on cell viability particular in NARP cybrids. These results conclude a RC defect-altered CL remodeling associated with NARP toxicity.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-1198

INSIGHTS INTO THE PATHOPHYSIOLOGY OF NEURODEGENERATIVE FXTAS BY GENOMIC APPROACH

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Objectives: Fragile X-associated Tremor Ataxia Syndrome (FXTAS) is an adult-onset neurodegenerative disorder leading to a combination of tremor, gait imbalance, Parkinsonism and cognitive decline. It is due to the elevated level of *FMR1* mRNA (2-8 fold), whose absence causes the Fragile X Syndrome. At the cellular level, FXTAS is characterized by the presence of age-dependent nuclear inclusions, containing *FMR1* mRNA, which are considered the cause of neurodegeneration. Mouse models exist recapitulating the disorder. Interestingly, young people and mice not yet displaying FXTAS symptoms and/or nuclear inclusions, may suffer of neurological and psychiatric problems (e.g. poor memory, autism, bipolar disorders), probably because of the elevated *FMR1* mRNA.
Methods: We combined genomic and neuronal morphology approaches.
Results: We performed whole-genome analysis on mRNA obtained from total brain and synaptosomes from young (15 weeks old) and aged (72 weeks old) FXTAS mice. The synaptosomal analyses display alterations in pathways involved in learning and memory, synaptic plasticity, as well as neurodegeneration (increased SNCA) also in young animals. Abnormal gene expression in total brain is only relevant in old mice and the classes of genes that are altered are consistent with the neurodegenerative character of FXTAS. However, in these mice, also pathways involved in neuronal maturation and morphology have been highlighted by our analysis.
Conclusions: Our results strongly suggest a double nature of FXTAS: neurodegenerative and neurodevelopmental. For this reason we started a morphological analysis (dendritic arborization and spine morphology) of mouse FXTAS cultured cortical neurons (not showing nuclear inclusions) and will present recent results.

ADPD5-1404

HUMAN IPSC-DERIVED NEURONAL NETWORKS GROWING ON MICRO-ELECTRODE ARRAYS USED IN NOVEL PHENOTYPIC FUNCTIONAL IN VITRO SCREENING ASSAYS FOR PARKINSON'S AND ALZHEIMER'S DRUGS

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Phenotypic screening has led to the majority (6/7) of successfully launched CNS drugs in the last decade.

Objectives: our aim was to apply human iPSC-derived neuronal networks growing on multiwell micro electrode arrays (MEAs) for functional phenotypic in vitro screening of Alzheimer's (AD) and Parkinson's (PD) relevant compounds.

Methods: we cultured different commercially available cortex-like and dopaminergic neurons derived from human iPSCs and optimized culture conditions to obtain spontaneous activity showing network communication patterns. These networks were treated with low-concentrated Abeta42 and MPP+, respectively, to induce a functional pathophysiology but no systemic neurotoxicity and tested known neuroprotective substances and drugs to rescue and/or prevent the functional impairment. Using multi-parametric data analysis of spike trains we calculated a readout able to capture, both, compound-induced impairment and rescue.

Results: we show that the used commercial human neuronal cultures produce robust spontaneous activity within 2 weeks in vitro, that both, Abeta42 and MPP+ induce significant functional effects on network activity and plasticity/communication on cortex-like and dopaminergic cultures, respectively, which can be prevented and rescued by neuroprotective substances. We compare the results with those of our available assays using primary mouse neurons.

Conclusion: functional phenotypic in vitro screening using human iPSC-derived neuronal networks growing on multiwell MEAs provides a scalable platform to test novel leads or advanced drugs (repurposing) for future AD and PD therapies.

03x. Pathophysiology & Disease Mechanisms: neural networks & plasticity

ADPD5-1922

LATERAL HABENULA AS A LINK BETWEEN DOPAMINERGIC AND SEROTONERGIC SYSTEMS CONTRIBUTES TO DEPRESSIVE SYMPTOMS WITH PARKINSON'S DISEASE

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Objectives: Degeneration of substantia nigra dopaminergic neurons is a key pathological change of Parkinson's disease (PD), and its motor consequences have been widely recognized. Recently, mood disorders associated with PD have begun to attract a great deal of interest, however, their pathogenesis remains unclear. The lateral habenula (LHb) is closely related to the substantia nigra and raphe nuclei, which are associated with the pathogenesis of depression. In this study, we investigated the effect and its mechanism of the LHb lesions on depressive-like behavior in PD rats.
Methods: We screened rats with depressive-like behaviors from PD model animals by using forced swim test; the cytochrome coxidase (CCO) activity in the LHb of PD rats with depressive-like behaviors (PDD rats) was measured; The changes of depressive-like behaviors and 5-HT level in the raphe nuclei of PDD rats was observed after the LHb lesions respectively.
Results: We found that CCO activity in the LHb of PDD rats was twice that seen in the control rats. In the forced swim test, LHb lesions caused a decrease in depressive-like behavior of PDD rats as indexed by decreased immobility times and increased climbing times. LHb lesions also caused an enhance in 5-HT levels of the raphe nuclei.
Conclusions: It suggested that LHb lesions may improve depressive-like behavior in PD rats by increasing 5-HT levels in the raphe nuclei. Thus, LHb contributes to the depressive-like behavior in PD rats via mediating the effects of dopaminergic neurons in the substantia nigra on serotonergic neurons in the raphe nuclei.

03y. Pathophysiology & Disease Mechanisms: aging

ADPD5-0246

CURCUMIN SUPRESSESS EXPRESSION OF COX-2 AND FORMATION OF MITOCHONDRIA MEDIATED FREE RADICALS IN D-GALACTOSE INDUCED AGEING IN RATS

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Objectives: Aging alters the configuration of neurons often leading to neurodegenerative disorders. During aging, antioxidant defenses are compromised producing mitochondrial damage and apoptosis of neurons. Neuroinflammation and accumulation of abnormal proteins also promote neuron damage. The aim was to delineate the role of curcumin in minimizing neuron damage induced by D-Galactose; by inhibiting the expression of inflammatory & apoptotic proteins and evaluating its impact on memory in rats. **Methods:** Rats were categorized into naïve control group, D-galactose (150 mg/kg; s.c; 56 days) exposed groups, and curcumin (50 & 100 mg/kg; orally; 63 days) treated groups. Cognitive studies were undertaken. Mitochondrial complexes, oxidation of lipids & proteins and antioxidant enzymes were determined in the brain mitochondrial fraction. Western blot was performed to detect COX-2 protein and cleaved caspase-3. Histological assessment of the CA2 region of hippocampus was done. Behavioral studies were analyzed by Repeated Measures ANOVA and other parameters by one way ANOVA followed by Post Hoc Dunnett test using SPSS version 16.0 at $p<0.05$ level of significance**. Results:** D-galactose induced significant cognitive deficits owing to mitochondrial dysfunction, neuroinflammation and apoptosis. Treatment with curcumin down regulated the expression of COX-2 protein, reduced apoptosis, increased the activity of complex-I & III and improved antioxidant defense compared with D-galactose treated group ($p<0.05$). Curcumin protected the CA2 region of the hippocampus from the deleterious impact of D-gal. **Conclusion:** By abating the over-expression of COX-2 and cleaved caspase-3, curcumin exerted significant protection of neurons and improved memory thereby serving as a prospective neuroprotective agent.

03y. Pathophysiology & Disease Mechanisms: aging

ADPD5-0670

MICE DEFICIENT IN COLLAPSIN RESPONSE MEDIATOR PROTEIN-1 EXHIBIT AGE-RELATED COGNITIVE DECLINE

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Background: Collapsin response mediator protein-1 (CRMP-1) was initially identified as a molecule that mediates the extracellular signals from semaphorin 3A and involved in neuronal development. However, the impact of CRMP-1 in cognitive deficits with aging remained unknown. *Objective*: We aimed to study whether the absence of CRMP-1 accelerated aging-related cognitive impairment. *Methods*: The study examined behavioral performance in Morris water maze (MWM) of wild-type (WT) and age-matched *crmp1* knock-out (KO) mice at different ages: adult (9-10-month-old), middle-aged (14-15-month-old) and aged (19-20-month-old). Then, the level of phosphorylated tau in cortex and hippocampus was compared to WT and age-matched *crmp1* KO mice in different ages after sacrificed. *Results*: In MWM, the *crmp1* KO mice had longer mean daily escape latencies than WT mice. We also analyzed search strategies using MWM. *crmp1* KO mice used less spatial strategies and more nonspatial systematic strategies, whereas WT mice used more spatial strategies. Both in escape latencies and search strategies, the elder mice had the worse performance than younger mice, especially in *crmp1* KO mice. Moreover, deletion of CRMP-1 in hippocampus resulted in the increase of phosphorylated tau level at serine202 in middle-aged mice and at serine202 and serine396 in aged mice when compared to age-matched WT mice. These data were consistent with previous studies that tau is hyperphosphorylated in its pathological forms. *Conclusions*: Our results implied CRMP-1 deficits was associated with poor escape latencies and search strategies, and led to the hyperphosphorylation of tau at serine202 and serine396 with aging.

03y. Pathophysiology & Disease Mechanisms: aging

ADPD5-1631

CHANGES OF PROTEOMIC PROFILES IN THE AGING HIPPOCAMPUS FROM POSTMORTEM HUMAN BRAINS

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Neurodegenerative diseases such as Alzheimer's Disease (AD) are highly correlated with aging. With the newly founded human brain bank in the Neuroscience Center, Chinese Academy of Medical Sciences & Peking Union Medical College, we performed a proteomic study in the hippocampus of postmortem human brains. The ages of death ranged from 22-98, and were grouped into 4 aging groups: 20-50, 50-70, 70-90, and over 90 (n=4 each). None of the donors was diagnosed with neurodegenerative diseases according to their medical history. Our studies identified 4582 proteins, among which 99 were upregulated and 43 were downregulated. Three of these changed proteins, glial fibrillary acidic protein (GFAP), Vimentin and annexin A1 were used to verify the proteomics results by western blot. Combined bioinformatics analysis shows that some proteins (such as tau and APOE) seriously associated with the AD pathogenesis showed no change in aged group compare with the younger groups, but a number of proteins involved in the electron transport chain were downregulated, which was also found in previous studies of AD. Therefore the regulatory process involved in people with normal aging process is different with the AD patients. The results demonstrated the feasibility of brain tissue research on the basis of standardized Chinese human brain bank, and suggested a series of changes in proteomic profile in human hippocampus that may relate to aging and age-related neurodegenerative disorders.

Supported by: CAMS Neuroscience Center Fund #2014C01 (CM), PUMC&CAMS/IBMS Dean's Fund #2011RC01 (CM); and the Chinese Human Brain Banking Consortium.

03y. Pathophysiology & Disease Mechanisms: aging

ADPD5-1809

THE STUDY OF VASOREACTIVITY OF CEREBRAL VESSELS IN PATIENTS WITH PARKINSON DISEASE

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Background: The frequent orthostatic intolerance in PD could be the consequences of cardiovascular-autonomic failure and/or damaged cerebral auto regulation **Objective**: The purpose of this study is to evaluate cerebral vasoreactivity in patients with Parkinson disease and 15 healthy controls were included in the study. Methods: Transcranial Doppler study was done to measure the mean velocities of the middle cerebral artery on either side at rest and after breath-holding to see the effect of hypercapnia on the blood velocities. This is done twice (once after the intake of antiparkinsonian drug and the other time 12 hours after the last dose) [group 2 and 1 respectively). **Results:** There was statistical significant difference between the patients (whether in group 1 or 2) and the control group regarding the breath holding index(P=0.003 and 0.022respectively).while the difference between the two patient groups was not statistically significant (p=0.477).Conclusion patients with Parkinson disease have poor vasoreactivity of cerebral blood vessels that may share in pathogenesis of some symptoms. This is independent on drug therapy.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-0250

NEURAL CORRELATES OF PROGRESSIVE REDUCTION OF BRADYKINESIA IN DE NOVO PD

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Background: A progressive reduction in the speed and amplitude of repetitive action is an essential component of bradykinesia, which is called sequence effect (SE). Because SE is specific to Parkinson's disease (PD) and is suggested to be associated with motor arrest, its features are of great interest. The aim of this study is to find the neural correlates of SE and to demonstrate whether dopaminergic deficit is correlated with SE, which has never been studied.

Methods: We enrolled 12 patients with de novo PD in an academic tertiary referral hospital. Correlations between SE severity and alterations in gray and white matter were studied. The association between severity of the SE and striatal dopaminergic deficits was also analyzed.

Results: The volumetric changes of the anterior cingulate cortex (ACC) and the inferior semilunar lobule of the cerebellum were significantly negatively correlated with the degree of SE. The long association fibers connecting the frontal lobes to the temporal, parietal, and occipital lobes were significantly associated with SE. There was a significant correlation between SE in the more affected hand and the caudate dopamine transporter binding in the more affected hemisphere.

Conclusions: Our results suggest that the ACC and the cerebellum (inferior semilunar lobule) are associated with the severity of SE. Taken together with DTI findings, the present study proposes that ACC may have an important role. Our data show that the caudate dopaminergic activity may be related to SE.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-0382

DYSFUNCTION OF THE VEGETATIVE NERVOUS SYSTEM AS A FACTOR OF FORMATION OF COGNITIVE IMPAIRMENT

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Objective. To identify the presence and the factors contributing to the emergence of cognitive impairment in patients with dysfunction of the vegetative nervous system.
Methods. We examined 24 patients with dysfunction of the vegetative nervous system, including 4 men and 20 women age 39,0±13,5. All patients underwent examination neurological of status, MOCA, verbal descriptive scale of pain scores, the questionnaire of neurotic disorders, the Hamilton depression rating scale, the scale of Taylor, the questionnaire against non-adaptable traits, MRT of the brain.
Results. All patients had complaints about headaches, poor memory and attention. The presence of white demographism in an atypical plase was revealed in 7 patients, the presence of simpathicotonia after the test Asner Dannii in 19 patients. MRT of the brain pathology was not revealed. The presence of cognitive impairment confirmed by MOCA totaled 25,0±0,4 in 83,3%. Verbal descriptive scale of pain scores showed a level 2,5±1,6, that corresponded to weak pain. The questionnaire of neurotic disorders revealed in all patients the presence of neurotic disorders. The Hamilton depression rating scale revealed the presence of a mild depressive episode in 18 patients. The scale of Taylor showed average level of anxiety (17,9±2,6). The questionnaire against non-adaptable traits revealed a tendency to frequent mood swings (56,0±4,1).
Conclusions. Patients with dysfunction of the vegetative nervous system with predominance of simpaticotonia have cognitive impairment in the presence of weak pain, the neurotic, the average level of anxiety in the presence of against non-adaptable traits that may be considered when prescribing therapy.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-0540
CARPAL TUNNEL SYNDROME IN TREMOR DOMINENT PD
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Unilateral hand tremor is one of cardinal symptoms in Parkinson's disease. Also, mechanical traumatic hand movement is one of risk factors in carpal tunnel syndrome. Our objectives were to examine the hypothesis that repetitive mechanical movement may be related to the development of carpal tunnel syndrome in Parkinson's disease with unilateral hand tremor by using neurophysiological study. Study participants comprised 33 de novo Parkinson's disease patients with unilateral hand tremor, and compared between tremor hand and no tremor hand in the same patients. Seven patients (21.2%) of 33 patients had carpal tunnel syndrome. All CTS patients showed neurophysiological abnormalities in hand without tremor, instead of the hand without tremor. In addition, in patients with no carpal tunnel syndrome, sensory nerve action potential was lower in the hand without tremor than the hand without tremor, although there were no significant differences statistically. We concluded that hand tremor in de novo Parkinson's disease patients was not related to the development of carpal tunnel syndrome directly. In contrast, more frequent use of the normal hand may induce mechanical loading and may be associated with CTS in hand without tremor. Early diagnosis of Parkinson's disease and the proper education of hand use may be essential for the prevention of carpal tunnel syndrome in Parkinson's disease tremor patients.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-0883

REPRODUCTIVE EXPERIENCE IMPACTS ON PARENTAL BEHAVIOR AND BRAIN DEVELOPMENT IN MICE.

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For all species of mammals motherhood is associated with as significant changes at the behavioral and cellular levels in combination with the accumulated experience of social interactions that attracting more and more attention of scientists to study the molecular mechanisms of parental behavior in different contexts by using animal models. Reproduction, with its attendant natural endocrine and postpartum sensory experiences, may facilitate lifelong learning and memory, and may mitigate markers of neural aging, in the rat. Combining natural hormonal exposure with subsequent substantial experience with stimuli from the offspring may preserve the aged parous female brain relative to that of null females. For a small number of mammals, including humans, fatherhood and paternal behavior play an equally important role in the nurturing offspring. Much less is known about the neural and hormonal mechanisms of paternal behavior, but emerging knowledge in this area indicate that the processes of paternal behavior formation involve similar hormones and neuromodulators, and similar regions of the brain as is established in maternal brain. Critical effects of parental behavior may be considered as a consequence of structural and functional changes in the male and female brain associated with parental behavior from rodents to humans. Searching for new molecular and cellular markers for the development of targeted control of neuroplasticity induced by reproductive experiences and parental behavior open up a new opportunity for the diagnosis, prevention and treatment of neurological dysfunction associated with neurodegeneration.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1616

CATATONIA-LIKE SYNDROME DUE TO HIPOXIC ISCHEMIC ENCEPHALOPATHY: A REPORT FROM A PULMONARY THROMBECTOMY SURGERY CASE

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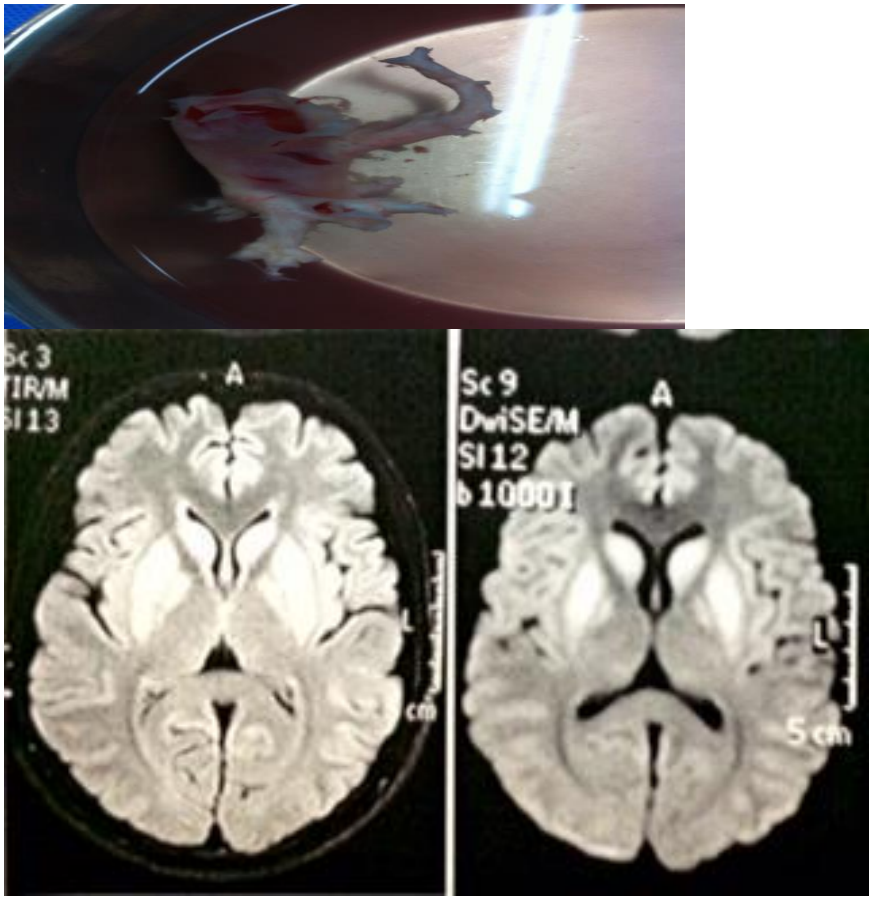
Objective: To present a patient with pulmonary thromboembolism who underwent surgical thrombectomy and presented a catatonia-like syndrome secundary to hypoxic ishemic bilateral basal ganglia lesion.

Methods: 28 yom history of idiopathic pulmonary thromboembolism ans pulmonary hypertension admitted for pulmonary thrombectomy (Figure 1). Cardiopulmonary bypass and several controlled cardiorespiratory arrest with hypothermia were done. After patient began to decrease verbal fluency, fixed gaze, decreased blink rate and movements, altered mental state, sadness and crying. At postsurgical day 3 mutism and not voluntarily movement, IC Neurology suspecting CVD. He was alert, poor eye contact and marked decrease in verbal fluency with insistence emitted words; opposed to evaluation and crying episodes, tendency to keep fixed posture and mild bilateral cogwheel rigidity. A catatonia-like syndrome diagnsois was done, paraclinical to rule out organic etiology. In NCCT no lesions (Figure 2), normal EEG, CPK, no elevation in acute phase reactants. AngioMR ruled out vessel lesion, hyperintense bilateral lesions in the basal ganglia suggestive of hypoxic ischemic encephalopathy were seen (Figure 3).

Results: Dramatic improvement with Lorazepam 2 mg tid, increased verbal fluency, voluntary movements an ambulation. He began to be anxious, difficulties to articulate. Psychiatric evaluation: abnormality in abstraction, concrete thinking, impaired phonological semantic verbal fluency, difficulty in tracking sequences TMT-A. No sensoperception disturbances, delusions, depressive symptoms; anxiety in relation to pulmonary pathology He continued to evolve successfully on Escitalopram for anxiety disorder but was stopped because maniac symptoms.

Conclusion: Patient had secondary catatonia-like síndrome due to hypoxic ischemic encephalopathy with bilateral basal ganglia lesions.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03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1707

EVALUATING THE MICROTUBULES AND TAU IN CORTICOSTERONE-INDUCED DEPRESSION, SIMILAR PATHOLOGICAL PROCESSES AS IN BETA-AMYLOID PEPTIDE NEUROTOXICITY

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Objectives: Among different risk factors, it has been implicated that depression can be a risk factor for developing Alzheimer's disease (AD). Cytoskeleton plays important role in stabilize both axonal transport and even the spines. It has been reported for the perturbation of cytoskeleton in AD and different psychiatric disorders. Therefore, we aim to evaluate cytoskeletal protein microtubules, phosphorylation of tau and actin in experimental models of depression and AD.

Methods: We have used corticosterone as a model agent for depression and oligomeric ß-amyloid (Aß) peptide as a toxin agent for AD. We employed cultured hippocampal neurons for as our experimental model. We used live-cell imaging, Western-blot analysis and immunofluorescent staining to evaluate the stability of microtubules, phosphorylation of tau and even the abundant of postsynaptic density protein PSD-95.

Results: After exposure to Aß or corticosterone, aggregation of microtubules was found in neurons expressing GFP-tubulin. The level of acetylated tubulin was reduced, suggesting instable microtubules. On the other hand, increased phosphorylation of tau occurred. The effect was not limited to microtubule. By expressing mCherry-actin in cultured hippocampal neurons, Aß or corticosterone also induced actin rod formation and reduction of PSD95 protein. To maintain the stability of microtubules by taxol, all the above pathological changes could be attenuated.

Conclusion: Our results have proved that corticosterone in depression induces similar pathological changes of microtubules and actin as if Aß in AD, which may explain why depression can be a risk factor for promoting cognitive impairment in AD.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1787

STIMULATION-INDUCED THETA OSCILLATIONS IN GPI OF PATIENTS WITH PD

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Tremor is a common symptom of movement disorders including PD (PD). Current theories tend to focus on central movement control circuits, such as the basal ganglia and the cerebello-thalamic-cortical loop as being key components in PD tremor. Until recently, the globus pallidus internus (GPi) was thought to be a passive relay nucleus in a tremor-generating circuit. However, we have discovered neurons in the GPi during microelectrode mapping which can be induced into a theta oscillation following stimulation.

Objectives: The purpose of this study was to characterize the firing patterns of these neurons.

Methods: Electrical stimulation trains were delivered through a microelectrode recording neuronal activity with the following parameters: 1 sec duration; 3, 5, and 7.5 uA intensity; 200 Hz frequency.

Results: Preliminary data was collected from 37 neurons across 7 patients with either PD or dystonia. This data was analyzed for firing rate and pattern using a burst index prior to, and following stimulation. Firing frequency decreased following stimulation: 3uA baseline - 73.6Hz, decreased by 13.7Hz; 5uA baseline- 71Hz, decreased by 4.6Hz; 7.5uA baseline- 68.7 Hz, decreased by 16.9 Hz. Stimulation at 3uA increased the burst index by 0.34 and converted three cells to a bursty rhythm; at 5ua, the burst increased by 0.16 and changed the firing pattern of one neuron from random to bursty; and at 7.5uA, stimulation increased the burst index by 0.25 but no cells were converted to a bursty rhythm.

Conclusions: These results could implicate the GPi as an important component of tremorgenesis.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-1920

DECIPHERING THE ROLE OF GLUTAMATERGIC RECEPTORS AND ASSOCIATED SIGNALING IN ARSENIC INDUCED NEUROTOXICITY AND PROTECTIVE EFFICACY OF CURCUMIN IN RAT HIPPOCAMPUS

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Background: Involvement of cholinergic dysfunctions in arsenic induced neurotoxicity and protective effect of curcumin has been demonstrated recently by us. **Purpose:** Studies have been carried out to investigate involvement of NMDA receptors and post synaptic signalling proteins in arsenic induced neurotoxicity and protective efficacy of curcumin. **Methods:** Rats were exposed to arsenic (20 mg/kg b.w, p.o) or curcumin (100 mg/kg b.w, p.o) or simultaneously with arsenic and curcumin for 28 days. Assay of NMDA receptors by radioligand receptor binding and expression of NMDA receptor subunit (NR2A, NR2B, and NR1) and post synaptic signalling proteins (CAMKIIα, PSD-95, SynGAp, ERK ½) was carried out by Western blotting. mRNA expression of NMDAR1, NR2B and NR2A was studied by RT- PCR. TEM was utilized to count the number of synapse in hippocampus. **Results:** Arsenic exposure caused significant decrease in learning and memory associated with marked decrease in NMDA receptors, reduced NR2A mRNA levels, decreased expression of NR2A, p-CaMKIIα, PSD-95, increased expression of SynGAP in hippocampus, compared to control rats. Arsenic exposure also resulted to reduce number of synapses in hippocampus Simultaneous treatment with arsenic and curcumin was found to improve learning and memory and protect arsenic induced changes in post synaptic signalling proteins. Increased number of synapse in hippocampus was also observed in rats simultaneously treated with arsenic and curcumin. **Conclusion:** The results exhibit involvement of NMDA receptors and post synaptic signalling proteins in arsenic induced neurotoxicity and suggest that such changes could be protected curcumin.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-2027
UNDERSTANDING THE RELEVANCE OF THE BODY MASS INDEX IN ALZHEIMER'S DISEASE

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Background
The occurrence of obesity, commonly estimated using body mass index (BMI), and the most common late-onset dementia, Alzheimer disease (AD), are increasing globally.
Methods
Thus, in the present report we will describe the fundamental importance of this topic in public health, given the global epidemic of high adiposity and its consequences.
Results
In this way, both low and high BMI has been associated with cognitive impairment and dementia risk, including AD. Moreover, studies investigating the association between midlife BMI and risk for dementia demonstrated in generally an increased risk among overweight and obese adults. Also, a high BMI in middle-age or a decrease in BMI at late-age has been considered a predictor for the development of AD. Still, very few aspects are known about the BMI changes close to or after AD onset.
Conclusions
Thus, the possibility that high adiposity increases Alzheimer's disease risk is alarming given global trends of overweight and obesity in the general population. However, prevention and manipulation of adiposity may also provide away to prevent Alzheimer disease. In this way, further research evaluating BMI and dementia is required.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-2030

THE RELEVANCE OF SOME MEMORY DEFICITS IN A RAT MODEL OF AUTISM

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Background:
Autism is a complex disorder characterized by repetitive behavior and impaired social communication. Still, apart from these main manifestations, a significant number of cases display impaired emotional learning and memory functions.
Methods:
We tried to better understand the memory functions in an environmentally induced rat animal model of autism, based on the administration of valproic acid (VPA) during gestation (500 mg/kg or saline on day 12.5 of gestation) and examined the resultant progeny on specific memory tests, such as the Y maze task and the 8-arms radial maze.
Results:
Our data indicated that animals perinatally exposed to VPA are showing, besides specific social interaction deficiencies, significant behavioral alterations in Y maze task, as expressed in decreased spontaneous alternations percentage, suggesting affected immediate working memory and in the radial arm maze, as expressed to an increased number of both reference and working memory errors.
Conclusions:
In conclusion, we showed significant memory deficits in a VPA-induced rat model of autism, demonstrating also the relevance of the memory processes in autism, apart from the social deficiencies.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-2034

STUDYING THE MEMORY DEFICITS IN A KETAMINE-INDUCED RAT MODEL OF SCHIZOPHRENIA

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Background

Significant cognitive impairment is common in schizophrenia, affecting up to 75% of patients. In this way, it seems that a wide range of cognitive functions are affected, and particularly memory. Moreover, it seems that the cognitive impairment often pre-dates the illness onset.

Also, it is now generally accepted that a subchronic administration of 30 mg/kg ketamine induces reliable changes in behaviour of rat and parameters of dopaminergic, glutamatergic, and serotonergic neurotransmissions, which could resemble to schizophrenia manifestations.

Methods:

In this way, in the present experiment, we want it to test if there are any memory deficits in a ketamine-induced rat model of schizophrenia, as tested in the Y maze and radial arm maze tasks. To test this, rats were injected with 30 mg/kg ip ketamine or saline daily for seven consecutive days, while the behavioral experiments were performed 2 weeks after ketamine treatment.

Results:

Our data suggested significant memory deficits in this ketamine-induced rat model of schizophrenia in rat, as demonstrated by an increased number of reference memory errors in 8-radial arm maze. Also, the time necessary to finish this test was increased in the ketamine group, as compared to saline. Moreover, the spontaneous alternation percentage was significantly decreased, suggesting deficiencies in the immediate working memory.

Conclusions:

Our results presented here suggest that subchronic treatment with subanaesthetic doses of ketamine are inducing significant memory deficits, as tested in the Y maze and radial arm maze tasks.

03z. Pathophysiology & Disease Mechanisms: other

ADPD5-2213

FUNCTIONALITY OF ENERGY AND CALCIUM REGULATION IN ASTROCYTES AND MITOCHONDRIA IN BRAIN FROM ADRENOLEUKODYSTROPHY (X-ALD) MODEL ANIMALS

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1-Objectives

Malfunctions of peroxisomal ABCD1 transporter proteins are responsible for clinical symptoms in the severe neurodegenerative disease X-ALD (X-linked adrenoleukodystrophy). ABCD1 mediates uptake of free very-long-chain fatty acids (VLCFA) and their CoA-esters into peroxisomes. ABCD1 dysfunctions result in VLCFA accumulation in body tissues of X-ALD patients. We aim to understand the still largely elusive underlying molecular mechanism of X-ALD pathogenesis.

2-Methods

Abcd1-knockout mouse, contributed by Aurora Pujol, displays biochemical abnormalities, like reduced VLCFA ß-oxidation and accumulation of VLCFA, as seen in X-ALD patients.

3-Results

We exposed astrocytes from wild-type and *Abcd1*$^{-/-}$ mice to supraphysiological VLCFA concentrations (C22:0, C24:0 and C26:0). Important for elucidating the X-ALD pathogenic mechanism is the severely diminished capability to revert oxidized pyridine nucleotides to NAD(P)H in *Abcd1*$^{-/-}$ astrocytes. Long-term exposure to VLCFA induces enhanced ROS generation, cellular *in situ* depolarization of mitochondria. The VLCFA-induced intracellular Ca^{2+} response is diminished in Abcd1$^{-/-}$ astrocytes. In isolated brain mitochondria from *Abcd1*$^{-/-}$ and wild-type mice, VLCFA similarly cause increased ROS generation, impaired oxidative ATP synthesis and diminished Ca^{2+} uptake capacity. VLCFA exacerbate cell death in astrocytes.

4-Conclusions

We found multiple impairments of energy metabolism. The differences in responses of mitochondria and astrocytes, observed for the hydrocarbon chain length of VLCFA suggest that detrimental activities of VLCFA in astrocytes involve defective cellular functions besides mitochondria. Astrocytes from *Abcd1*$^{-/-}$ mice respond more sensitively to VLCFA than those derived from wild-type mice. VLCFA increase the vulnerability of *Abcd1*$^{-/-}$ astrocytes, and finally, we suggest that their antioxidative defense is diminished in *Abcd1*$^{-/-}$ conditions.

04i. Therapeutic Targets & Mechanisms for Treatment: other enzymes

ADPD5-0511

7-MEOTA-DONEPEZIL LIKE COMPOUNDS – NOVEL AGENTS TO COMBAT ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a multifactorial progressive neurodegenerative disorder that manifests as memory loss, spatial disorientation, and gradual deterioration of intellectual capacity. Nowadays, there are no effective preventative measures or curative therapy for AD, its precise etiology remains unknown as well. To date, the pharmacotherapy of AD has relied on acetylcholinesterase (AChE) inhibitors – tacrine, donepezil, rivastigmine and galantamine – and, more recently, on an NMDA receptor antagonist – memantine. Multi-target-directed ligands (MTDLs) have a great potential for treating complex diseases such as neurodegenerative disorders because they interact with multiple targets. Since one of the two currently approved drug-groups in AD therapy are cholinesterase inhibitors, they are one of the most used starting compounds in MTDLs design strategy. In our contribution we will introduce 7-MEOTA-donepezil like compounds as MTDLs combining 7-MEOTA unit, representing less toxic tacrine (THA) derivative, with analogues of *N*-benzylpiperazine moieties mimicking *N*-benzylpiperidine fragment of donepezil. 7-MEOTA-donepezil like compounds exerted mostly non-selective profile in inhibiting cholinesetrases of different origin with IC_{50} values ranging from micromolar to sub-micromolar concentration scale. Kinetic analysis revealed mixed-type mode of inhibition presuming that these inhibitors are capable to simultaneously bind peripheral anionic site (PAS) as well as catalytic anionic site (CAS) of AChE. Molecular modeling studies and QSAR studies were performed to rationalize *in vitro* studies.

Supported by the Post-doctoral project (No. CZ.1.07/2.3.00/30.0044), Long Term Development Plan – 1011 and by the Grant Agency of the Czech Republic (No. P303/11/1907)

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-0452

LOW DOSE CHOLINESTERASE INHIBITOR AND SEROTONIN 5HT2A ANTAGONISM REDUCE COGNITIVE DEFICITS DUE TO 6-HYDROXYDOPAMINE LESION IN RATS

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Objective: While Parkinson's disease (PD) is primarily associated with motor impairment, non-motor symptoms, including behavioral and cognitive disturbances, are prevalent and greatly impact daily functioning and quality of life. Current treatments for PD dementia include administration of cholinesterase inhibitors which can be accompanied by undesirable side effects. Lowering the required dose of cholinesterase inhibitor could reduce the prevalence or severity of these side effects. The current work examined the potential of using low-dose cholinesterase inhibitors in conjunction with a $5HT_{2A}$ antagonist to improve cognition in an animal model of PD.
Methods: Sprague Dawley rats received bilateral sham or 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra (SN). Following recovery, rats were administered the $5HT_{2A}$ antagonist (M100,907), a cholinesterase inhibitor (donepezil or rivastigmine), or a combination of the two prior to assessment of novel object recognition (NOR) or working memory performance in the Morris water maze (MWM).
Results: Rats with a 6-OHDA lesion had impaired performance in the NOR and MWM compared with sham controls. Treatment with a cholinesterase inhibitor attenuated cognitive deficits of 6-OHDA lesioned rats. In addition, combined low and ineffective doses of M100,907 and a cholinesterase inhibitor also reversed deficits in both of these tests.
Conclusions: The current work suggests that rats with 6-OHDA lesion of the SN display cogntitive deficits. Further, combined administration of low dose cholinesterase inhibitors and low dose $5HT_{2A}$ antagonists, while ineffective alone, could be efficacious in treating cognitive deficits associated with dopaminergic cell death, potentially reducing the incidence of side effects.

04j. Therapeutic Targets & Mechanisms for Treatment: neurotransmitter-based targets

ADPD5-0617

STRUCTURAL ANALYSIS OF RGS4 INHIBITION: IMPLICATIONS FOR THERAPEUTIC DEVELOPMENT FOR PD

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Regulator of G protein signaling (RGS) proteins modulate G-protein-coupled receptors, whose proper regulation is essential for diverse physiologic processes. Recent findings support a critical role of RGS4 in the etiology of Parkinson's disease (PD). Pathological RGS4 over-expression brought on by decreased dopaminergic signaling in the striatum causes motor deficits by blunting cortico-striatial synaptic plasticity. RGS4 knockout mice are free from motor impediments in a mouse model (6-OHDA lesion) of PD, positioning RGS4 as a therapeutic target downstream of dopamine loss such that RGS4-targeted therapeutics would retain efficacy long after patients become refractory to L-DOPA therapy. Small molecule screens for RGS4 inhibitors have yielded a few hit compounds, and we have investigated their mechanisms using biophysical approaches in order to develop a framework to enable the rational design of small molecule RGS4 inhibitors for the treatment of PD. Our findings show that current RGS4-specific allosteric inhibitors exploit unique intrinsic protein dynamics of RGS4 to drive selectivity over homologs, e.g. RGS8. Our ongoing efforts seek to quantitatively measure RGS4 dynamics over broad timescales (ps-ms) to guide *in silico* molecular dynamics modeling of the inhibited conformational states of RGS4 for virtual docking.

04k. Therapeutic Targets & Mechanisms for Treatment: nicotinic & other ionotropic receptors

ADPD5-0580

CONCENTRATION-RESPONSE RELATIONSHIP OF AN ENCENICLINE ANALOGUE FRM-0017874 ACROSS MULTIPLE IN VITRO AND IN VIVO ASSAYS

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Pharmacological activation of nicotinic alpha7 receptors (nAChRs) may improve cognition in schizophrenia and Alzheimer's disease. The present study describes effects of FRM-0017874, an analogue of encenicline, on alpha7 receptors in vitro, and in behavioral and neurophysiological assays of cognitive function. FRM-0017874 demonstrated high affinity binding to rat brain alpha7 nAChRs, displacing [^{125}I]-alpha7-bungarotoxin (K_i = 18.1 ± 7.8 nM). In *Xenopus* oocytes expressing human alpha7 nAChRs, FRM-0017874 acted as a partial agonist, evoking inward currents with an EC_{50} of 0.42 µM. Lower concentrations of FRM-0017874 (0.04-1 nM) elicited no detectable current, but primed receptors to respond to sub-maximal concentrations of acetylcholine, potentiating these currents up to 1.9-fold. Higher concentrations induced receptor desensitization. FRM-0017874 improved novel object recognition in rats, and enhanced memory acquisition and reversal learning in mice in a water T-maze assay. Modulation of hippocampal theta oscillation is considered a neurophysiological correlate of the cognitive effects of drug treatment. FRM-0017874 showed a dose-dependent facilitation of the power of stimulation-induced hippocampal theta oscillation in both mice and rats. The brain unbound drug concentration-response relationship for increased theta synchrony was similar in both species, exhibited a biphasic pattern peaking around 3 nM, and overlapped with active doses and exposures observed in cognition assays. In summary, behavioral and electrophysiological assays indicate an "inverted-U"-shaped effective concentration range, underscoring the critical importance of clinical dose selection and pharmacokinetics for alpha7 nAChRs agonists.

04k. Therapeutic Targets & Mechanisms for Treatment: nicotinic & other ionotropic receptors

ADPD5-0611

IN VITRO PHARMACOLOGICAL AND ELECTROPHYSIOLOGICAL PROFILE OF S 47445, A NOVEL POSITIVE ALLOSTERIC MODULATOR OF AMPA TYPE GLUTAMATE RECEPTORS

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S47445 is a novel positive allosteric modulator of glutamate AMPA-type receptors which possesses neuroprotective, antidepressant and cognitive enhancing properties in the absence of side effects in rodents after acute administration (see abstract on in vivo properties). The aim of the present study was to characterize its mechanism of action and effects on neuronal plasticity.

S47445 did not present affinity towards orthosteric binding sites of AMPA receptors but increased AMPA-mediated depolarization measured by fluorescent membrane potential dyes on rat primary cell cultures. Similarly, on oocytes injected with either rat cortex or human hippocampal mRNA, S47445 increased AMPA-evoked current and did not affect NMDA and kainate-evoked currents. Differential sensitivity of S47445 was examined at the human AMPA receptors subtypes.

On rat hippocampal slices, S47445 alone did not induced presynaptic noradrenaline release but enhanced AMPA-mediated release.

No toxicity was observed even at high concentrations (30-100µM) on rat cortical neurons.

S47445 both increased the expression of Brain Derived Neurotrophic Factor (BDNF) protein alone and was able to stimulate AMPA-induced BDNF expression on rat primary cortical cell cultures.

In vivo, S47445 (10-30 mg/kg ip) increased both the induction and the maintenance of the Long-Term Potentiation induced by 4 bursts tetanus in the dentate gyrus of the hippocampus in anaesthetized Wistar rats.

Taken together, these results indicate that S47445 via a selective positive allosteric modulation of AMPA receptors enhances neuronal and synaptic plasticity (LTP, BDNF expression), phenomenon that could be of interest for the treatment of various neurodegenerative diseases and mood disorders.

04k. Therapeutic Targets & Mechanisms for Treatment: nicotinic & other ionotropic receptors

ADPD5-0614

IN VIVO PHARMACOLOGICAL PROFILE OF S 47445, A NOVEL POSITIVE ALLOSTERIC MODULATOR OF AMPA-TYPE GLUTAMATE RECEPTORS

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S47445 is a novel positive allosteric modulator of glutamate AMPA-type receptors, with a high permeability of the Blood-Brain-Barrier. It was shown to possess neurotrophic and antidepressant properties and to enhance synaptic plasticity. The present study describes S47445 in vivo pharmacological properties in rodents.

S47445 showed robust pro-cognitive effects in various models of episodic and spatial working memory in rodents at low doses (0.3-3 mg/kg p.o.). S47445 improved episodic-like memory in a novel object recognition test and partially counteracted scopolamine-induced amnesia. In mice, S47445 improved object recognition performance and counteracted alprazolam-induced memory deficits in delayed spatial discrimination task. Furthermore, S47445 was able to reverse age-induced deficits in contextual memory performances in mice. S47445 improved spatial working memory assessed by spontaneous alternation behavior on a T-maze in young mice and on Y-maze in old mice.

Besides its potent procognitive and neurotrophic action, S47445 also displayed neuroprotective activity in a model of hippocampal vulnerability in rats (30 mg/kg i.p.). S47445 did not induce wake-promoting effect as assessed by cortical EEG recordings in freely moving rats nor modify pentobarbital induced sleep duration in rats (30 mg/kg i.p.). No effect on general behaviour, body temperature, motor coordination and spontaneous locomotor activity nor occurrence of epileptic seizures were noticed after acute administration in mice (10-100 mg/kg p.o.) and rats (10-1000 mg/kg p.o.). All these results indicate that S47445 displays cognitive-enhancing properties without detectable CNS side-effects in the tested dose ranges and could have promising therapeutic potential for the treatment of cognitive disorders in Alzheimer's disease.

04k. Therapeutic Targets & Mechanisms for Treatment: nicotinic & other ionotropic receptors

ADPD5-0903

POTENTIAL NEUROPROTECTIVE EFFECTS ASSESSMENT OF THE ALPHA-7 NICOTINIC RECEPTOR AGONIST PHA 543613 IN A MODEL OF EXCITOTOXIC LESION IN RATS

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Objectives:

New compounds targeting the neuroinflammation through the alpha7 nicotinic acetylcholine receptors generate major interest. We aimed to evaluate the effects of an agonist of these receptors, PHA 543613, in an *in vivo* model of excitotoxicity induced by intrastriatal injection of quinolinic acid (QA).

Methods:

Two groups of 6 rats each were exposed to surgery (150 nM QA in the right striatum). They received either four i.p. injections of PHA 543613 (6mg/kg 2h before QA lesion and at 2, 4 and 6 days post-lesion, dpl) or vehicle (control group). At 7 dpl, several neuronal and neuroinflammatory markers were evaluated, i.e. NeuN and GFAP by western blotting and the translocator protein 18 kDa (TSPO) by autoradiography. These parameters were also studied by immunofluorescence staining.

Results:

In the cortex and striatum, we observed an upward trend of NeuN expression in the lesioned side of PHA vs control group. In the striatum, the percentage increase of GFAP expression between ipsi- and controlateral sides was lower in PHA than in control group (59% vs 158%). Moreover, the increased accumulation of the TSPO ligand [^{3}H]PK-11195 was significantly smaller in the PHA than in control group (361% vs 450%, $p<0.05$). The positive impact of PHA 543613 treatment on inflammation was supported by the morphologic cells observation showing delayed microglial activation with mainly branched microglia in PHA-treated rats.

Conclusions:

In our experimental model of excitotoxicity, we showed that the administration of PHA 543613 lead to decrease neuroinflammation. Current studies are aimed at studying the consequences on neurodegeneration.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0257

ANTIOXIDANT EFFECTS OF LOW MOLECULAR WEIGHT FRACTION OF THE VENOM OF BOTHROPOIDES JARARACA AGAINST H2O2-INDUCED APPOPTOSIS IN HIPPOCAMPAL CELLS

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Objective: The present study rated the neuroprotective property of low molecular weight fraction (LMWF) of the venom of *Bothropoides jararaca (Bj)* in cultured hippocampal cells

Materials and methods: Crude venom (CV) of *Bj* was filtered through a 10 kDa MW cutoff membrane (Amicon®) to obtain the LMWF. To confirm the absence of proteolytic enzymes and other proteins (>10kDa), the LMWF was analyzed by electrophoresis in SDS-PAGE and mass spectrometry. The primary hippocampal culture was obtained of Wistar neonate rats. The culture was characterized by immunocytochemistry assays. Cell stress was promoted using 50 µM of H_2O_2 for 20 hours. Neuroprotection was evaluated by concomitant treatment with LMWF (0.1µg/mL) and H_2O_2 (50 µM). Cell viability was determined using an assay based on MTT reduction. The expression of cleaved caspase-3 enzyme was performed by western blot. Quantitative values were analyzed by one-way ANOVA (Tukey´s post-test, respectively; $p<0.05$).

Results: The results indicate that the LMWF filtrate contained no high-molecular weight contaminants. After the treatment with 100 µM of H_2O_2 we could observe a decrease of cell viability of 32,84± 8,06% ($p <0,001$), while the previous treatment in culture cells with 0,1 µg/mL LMWF following by 100 µM of H_2O_2, it was observed 77,46 ± 5,35% ($p<0,05$) of cell viability. Evidence has shown that cells treated with LMWF 0.1 µg/mL and then with H_2O_2 (50µM) show lower expression of cleaved caspase-3 cells than the cells treated just with H_2O_2.

Conclusion: The results suggest that LMWF has cytoprotective activity against oxidative stress caused by H_2O_2.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0272

EFFECT OF VITAMIN E ON PASSIVE AVOIDANCE LEARNING IN ANIMAL MODEL OF ALZHEIMER'S DISEASE

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Objective :Free radicals and the oxidative agent are important in aging and age-related neurodegenerative disease such as Alzheimer. Vitamin E is an antioxidant compound that play a beneficial role in maintaining neural integrity and preventing cell death. In this research the effect of intracerebroventricular (ICV)administration of vitamin E on inhibitory avoidance(IA) memory in rats with Alzheimer's disease(AD)was investigated.
Material and method: 42 aged Wistar male rats (350-400 g,18-20 months old)were divided into 6 groups : n-L+Veh group: non-lesioned rats with vehicle treatment; L group: NBM-lesioned rats; L+Veh group: NBM-lesioned rats with vehicle injection and L+vit.E groups(10 or 25 or 50µg/rat): NBM-lesioned rats with vitamin E injection.
A guide cannula was implanted stereotaxically in the right lateral ventricle for injection of vitamin E, AD-like cognitive deficiency was induced by injection of ibotenic acid into Nucleus Basalis of Meynert (NBM) bilaterally (5µg/µl in each side). A step-through inhibitory avoidance task was used for memory assessment.
Result :Our results showed that vitamin E(25 and50µg/rat) caused a significant increase in consolidation and memory retention in AD.
Conclusion: These results suggest that vitamin E could improve memory retention in aged rats with dementia type of AD (with NBM lesioned).
Key words: Alzheimer's disease , Nucleus Basalis of Meynert (NBM),vitamin E, passive avoidance learning, rat.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0362

ORIENTIN PROTECTS NEURONAL CELL LINE FROM HYDROGEN PEROXIDE INDUCED OXIDATIVE DAMAGE VIA UP-REGULATING NRF2/KEAP1 REDOX SIGNALLING PATHWAYS

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Introduction: Orientin is a water-soluble flavonoid C-glycoside which is isolable from *Ocimum sanctum*. It has been suggested to have antioxidant, antiviral, vasodilatation, cardioprotective, radiation protective effect, neuroprotective effect and to be able to cross blood-brain-barrier. Thus, it is a potential treatment or prevention of neurodegenerative diseases (ND). Furthermore, oxidative stress has been highly implicated in the progression of ND, hence, this leads to the study on Nrf2/Keap1 redox signalling pathway for the targeted effective therapies of ND in the future.
Objectives: To determine the effects of orientin on nitric oxide levels, intracellular calcium levels and the mechanism by which orientin protects SH-SY5Y neuroblastoma cells from H_2O_2-induced oxidative damage.
Methods: Orientin at pre-determined maximum non-toxic dose (MNTD), 20 µM and half MNTD (1/2 MNTD), 10µM were subjected to SH-SY5Y cells for 24 h, followed by 150 µM of H_2O_2 oxidative induction for 24 h at 37ºC. The Griess assay and Fluo-4 NW calcium assay were then performed to measure the NO levels and intracellular Ca^{2+} levels by a microplate reader respectively. The regulation of Nrf2/Keap1 redox signalling pathway was analysed by Western blotting.
Results: MNTD and 1/2 MNTD significantly reduced NO levels and significantly reduced intracellular Ca^{2+} levels by 33.49% and 28.20% when compare to H_2O_2 treatment alone. The regulation of Nrf2/Keap1 redox signalling pathway was confirmed by Western blotting.
Conclusions: Orientin protects SH-SY5Y cells from H_2O_2-induced oxidative damage by reducing NO levels and intracellular Ca^{2+} levels while up-regulating Nrf2/Keap1 redox signalling pathway.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0364

MECHANISM ACTION OF METHANOL EXTRACT OF OCIMUM SANCTUM ON HYDROGEN PEROXIDE-INDUCED SH-SY5Y CELLS IN NRF2/KEAP1 SIGNALLING PATHWAY REGULATION

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Introduction: Neurodegenerative disorders are incurable disorder associated with the functional defect on the neuronal cells evoked by various machineries. Among these, oxidative stress is one of the main pathogenic machineries causing neuronal cell impairment and lead to neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. It occurred due to the imbalance of reactive oxygen species or reactive nitrative species (ROS/ RNS) and antioxidant in the neuronal cells. Nrf2/KEAP1 is the antioxidative pathway which is triggered by oxidative stress, leads to the expression of antioxidative enzymes for neuroprotection.
Objectives: Our preliminary studies suggested that methanol extracts of *Ocimum santum* (OcSME) potentially protect the human neuroblastoma cells from hydrogen peroxide-induced neurodegeneration. Thus, this study aims to investigate the mechanism action of neuroprotective effects of OcSME on hydrogen peroxide (H_2O_2)-induced SH-SY5Y cells.
Methods: The effects of OcSME on nitrate oxide level was determined by Griess reagent assay. Intracellular calcium level, mitochondria membrane potential (MMP) activities and western-blot analysis in H_2O_2-induced SHSH5Y cells were evaluated.
Results: Studies demonstrated that maximum non-toxic dose (MNTD) of OcSME could reduce the nitric oxide level while the intracellular calcium level was not significantly decrease upon H_2O_2 exposure. Meanwhile, exposure of SHSY5Y cells on 150 µM H_2O_2 showed the effects on the MMP. The potential of OcSME on Nrf2/KEAP1 signalling pathway regulation was further confirmed by western blot.
Conclusions: OcSME suggested to possess the potential to protect SH-SY5Y cells from H_2O_2 induced oxidative stress. This present study suggested the mechanism action of OcSME in Nrf2/Keap1 signalling pathway regulation.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0388

ORIENTIN PROTECTS NEURONAL CELL LINE FROM HYDROGEN PEROXIDE INDUCED OXIDATIVE DAMAGE VIA UP-REGULATING PI3K/AKT SURVIVAL WHILE DOWN-REGULATING MAPK/ERK APOPTOSIS PATHWAY

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Introduction: The current prescribed medications for neurodegenerative diseases (ND) only treat the symptom rather than the cause while possess various adverse effects to the patients. Therefore, natural products or plants derivatives might be a better alternative to prevent or to treat ND. This study targets on one of the flavonoid compounds, orientin, in which it has been reported to show anti-apoptotic effect on SH-SY5Y neuroblastoma cells. Nevertheless, the basic mechanisms underlying are yet to be elucidated.

Objectives: To study the mechanism by which orientin protects SH-SY5Y neuroblastoma cells from H_2O_2-induced oxidative damage.

Methods: The cells were treated with 20µM and 10µM of orientin, which are the pre-determined maximum non-toxic dose (MNTD) and half MNTD (1/2 MNTD) for 24 h at 37ºC and subsequently with 150µM of H_2O_2 oxidative induction. Cells were then subjected to the measurement of mitochondrial membrane potential (MMP) and Annexin V/propidium iodide apoptosis assay by flow cytometry whereas the regulation of PI3K/Akt survival pathway and MAPK/ERK apoptosis pathway was analysed by Western blotting.

Results: Studies showed that MNTD and 1/2 MNTD of orientin restored the loss of MMP by 30.66% and 34.21% and reduced 27.85% and 43.98% of early apoptotic cells when compared to H_2O_2 treatment alone. The regulation of PI3K/AKT survival and MAPK/ERK apoptosis pathways was further confirmed through Western blot.

Conclusions: Orientin protects SH-SY5Y cells from H_2O_2-induced oxidative damage by restoring the loss of MMP, preventing early apoptosis of cells and up-regulating PI3K/Akt survival pathway while down-regulating MAPK/Erk apoptosis pathway.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0504

CHRYSIN SUPPLEMENTATION IMPROVES ACQUISITION AND RETENTION BEHAVIOR IN ANIMAL MODEL OF MEMORY IMPAIRMENT

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Chrysin (5,7-dihydroxyflavone) is a naturally occurring flavone, obtained from passion flower *Passiflora incarnata* Linn. (Passifloraceae). The present study aimed to explore the putative role of Chrysin in the modulation of memory processes in mice. Swiss albino mice (either sex; 6-8 weeks; 20-30 g) were used in this study. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC). Chrysin was administered in two doses (30 and 60 mg/kg; *p.o.*) to mice daily for 28 successive days. Memory impairment was induced by two methods- Lipopolysaccharide (LPS; 1 mg/kg; *i.p.*) induced amnesia and Alprazolam (0.5 mg/kg; *i.p.*) induced amnesia. Morris water maze (MWM) and Elevated plus maze (EPM) served as exteroceptive behavioral models to evaluate the memory. After behavioral studies, the brain ACHE) activity, TBARS, GSH, nitrite, SOD and catalase levels were measured. Administration of chrysin (60 mg/kg; *p.o.*) for 28 days to mice not only prevented the LPS- as well as alprazolam-induced enhancement of transfer latency of mice in EPM test but reduced the escape latency time on 4th day and increased the time spent in target quadrant on 5th day in MWM test as compared to control group. Further, Chrysin treatment caused the reduction in brain ACHE activity and also reversed the enhancement of brain TBARS and nitrite levels. Furthermore, GSH, SOD & Catalase activity also raised in chrysin treated group. Thus, Chrysin may prove to be useful remedy for the management of memory owing to its possible neuroprotective and its antioxidant properties.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-0671

ANTI-OXIDATIVE PROPERTY OF EDIBLE BIRD NEST AND ITS POTENTIAL IN ATTENUATING INTRANEURONAL REACTIVE OXYGEN SPECIES FORMATION INDUCED BY NEUROTOXIN

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Objectives: This study aims to investigate the anti-oxidant activities of edible bird nest (EBN) and evaluate its potential in reducing neurotoxin-induced oxidative stress in neuronal cells.
Methods: EBN was cleaned and oven-dried. Part of the EBN was ground into powder and digested with pancreatin (sample S1). The remaining EBN was boiled with water. Filtrate of the extract was freeze-dried followed with pancreatin digestion (sample S2). DPPH (1-diphenyl-2-picryl hydrazyl) radical scavenging, ferric ion reducing antioxidant power (FRAP) and metal chelating assays were carried out for the determination of EBN's anti-oxidant activities. For evaluation of EBN's effect in reducing oxidative stress, SH-SY5Y cells were cultured and pre-treated with EBN. The cells were then challenged with 6-hydroxydopamine hydrochloride (6-OHDA) and intracellular reactive oxygen species (ROS) level was assessed with the dichlorofluorescein-diacetate (DCFH-DA) assay.
Results: S1 displayed higher DPPH radical scavenging activity than S2. S1 recorded highest activity of 16.70% at 0.0625 mg/ml whilst S2 achieved comparable results (12.21%) at 4 mg/ml. At 4 mg/ml, S1 showed ferric ion reducing antioxidant power of 32.93% whereas S2 showed slightly lower activity of 26.25%. Generally, S1 displayed higher reducing power than S2. As for metal chelating ability, S2 displayed better effect compared to S1. S1 showed 23.69% of activity whereas S2's activity was 46.47% at 4 mg/ml. Results from the DCFH-DA assay showed that S2 reduced 6-OHDA-induced ROS formation while S1 showed no significant reductive effect.
Conclusions: S1 and S2 displayed moderate antioxidant activity and S2 was able to inhibit ROS formation in neuronal cells.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-1424

THE TRANSCRIPTION FACTOR NRF2 AS A POTENTIAL TARGET FOR NEUROPROTECTION AND REDUCTION LEVODOPA-INDUCED TOXICITY IN PD

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Levodopa, an precursor of dopamine, is a drug for relieving the symptoms of PD. The progressive decrease in drug response and occurrence of motor complications are frequent problems in the management of patients with PD. Moreover, the levodopa fails to prevent the progression of the degenerative processes. Various studies show that oxidant formation, following levodopa metabolism, could cause dopaminergic neuronal death. Recent studies demonstrate the ability of Nrf2 inducers to reduce the neuronal death occurring in neurodenegerative diseases. The Nrf2 regulates the expression of a wide array of redox status, cytoprotective and anti-inflammatory genes. Among Nrf2 inducers, we studied the ability of sulforaphane (SF) isothiocyanate to prevent and/or counteract the redox status impairment and cell death induced by a high concentration of levodopa in *in vitro* model of dopaminergic neurons. We found that the pre-treatment of neurons with SF showed inhibitory effects of levodopa-induced neuronal death through the translocation of Nrf2 into the nucleus and subsequent antioxidant endogenous molecule induction, such as glutathione. The ability of SF to exert neuroprotective effects was also recorded during or after the treatment with levodopa, suggesting that Nrf2-controlled target genes are involved in neuronal survival. Moreover, SF reduced levodopa-induced dyskinesia in a rat model of PD. Synergistic neuroprotective effects are also very interesting, especially for the combination of low concentrations of levodopa and SF. Taken together, these findings suggest that Nrf2 pathway inducers may be a promising class of drug to ameliorate the ratio risk/benefit associated with levodopa therapy.

04o. Therapeutic Targets & Mechanisms for Treatment: anti-oxidants

ADPD5-2001

ASSOCİATİON OF SERUM URİC ACİD LEVELS WİTH THE L-DOPA TREATMENT AND DİSEASE PROGRESSİON İN PARKİNSON'S DİSEASE

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Background: This study aimed to evaluate whether the serum UA level was associated with the disease progression and L-Dopa treatment of Parkinson' Disease.
Methods: 80 İdiopatic Parkinson's Disease patients age and sexually similar 80 healthy people Serum Uric Acid levels were measured. Parkinson's Disease group was divided into 2 subgroups according to L Dopa treatment. 1st group was İdiopatic Parkinson's disease Patients who did not use as pharmaceutical treatment L-Dopa and 2nd group Parkinson's disease Patients who used L-Dopa.The disease progression was scored by Hoehn and Yahr (H&Y) scales and disease durations; PD group was divided into 2 subgroups according to H&Y scales. 1st group was considered as the early stage of İdiopatic Parkinson's Disease and it included 1st stage patients. 2nd group included İdiopatic Parkinson's Disease Patients in other upper stages.
Results: İdiopatic Parkinson's Disease patients were found to have significantly lower levels of serum Uric Acid than controls. Differences between İdiopatic Parkinson's disease groups according to pharmaceutical treatment, the serum UA levels were gradually reduced in group which used L-Dopa treatment.As the disease progression, the serum UA levels were gradually reduced. There was significantly inverse correlation of UA levels with H&Y scales.
Conclusion: Association of serum Uric Acid levels and İdiopatic Parkinson's disease progression was found. Serum Uric Acid levels may lead to endogen antioxidantcapacity which could decrease the oxidative stress in patogenesis of Parkinson Disease. As the result, Uric acid could play a neuro-protective role in Parkinson's Disease.

04r. Therapeutic Targets & Mechanisms for Treatment: protein aggregation

ADPD5-1738

DECREASED O-LINKED GLCNACYLATION PROTECTS FROM CYTOTOXICITY MEDIATED BY HUNTINGTIN EXON1 PROTEIN FRAGMENT

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O-GlcNAcylation is an important post-translational modification of proteins and is known to regulate a number of pathways involved in cellular homeostasis. This involves dynamic and reversible modification of serine/threonine residues of different cellular proteins catalyzed by O-linked N-acetylglucosaminyltransferase and O-linked N-acetylglucosaminidase in an antagonistic manner. We present here our findings that decreasing O-GlcNAcylation enhances the viability of neuronal cells expressing polyglutamine-expanded huntingtin exon 1 protein fragment (mHtt). We also show that O-GlcNAcylation regulates the basal autophagic process and that suppression of O-GlcNAcylation significantly increases autophagic flux by enhancing the fusion of autophagosome with lysosome. This regulation considerably reduces toxic mHtt aggregates in eye imaginal discs and partially restores rhabdomere morphology and vision in a fly model for Huntington disease. This study is significant in unraveling O-GlcNAcylation-dependent regulation of an autophagic process in mediating mHtt toxicity. We would discuss our attempts to target the autophagic process through the suppression of O-GlcNAcylation as a therapeutic approach in treating Huntington disease.

04r. Therapeutic Targets & Mechanisms for Treatment: protein aggregation

ADPD5-2029
FLAVONES AS INHIBITORS OF AMYLOID-LIKE FIBRIL FORMATION
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Several natural and synthetic flavone derivatives have been reported to inhibit formation of amyloid fibrils or to remodel existing fibrils. In many studies the primary method for determining the effectiveness of inhibition is measuring Thioflavin T (ThT) fluorescence. This method demonstrably results in a number of false positives for inhibition. First we studied the effects of 265 commercially available flavone derivatives on insulin fibril formation. We enhanced the effectiveness of ThT fluorescence measurements by fitting kinetic curves to obtain halftime of aggregation. We showed that without a change in an assay, but just by observing complete kinetic curves it is possible to eliminate numbers of false positive and sometimes even false negative results. Five flavones proved to be outstanding inhibitors of insulin amyloid-like aggregation.
To check if these five derivatives could be universal inhibitors of amyloid-like fibril formation we tested their effect on lysozyme, amyloid-beta, alpha-synuclein, and prion protein aggregation. All of the tested flavones decrease ThT fluorescence intensities in case of all tested polypeptides; however the picture is different when halftimes of aggregation are compared. Preliminary results suggest all five flavones to inhibit alpha-synuclein fibrillation, at least one to work on lysozyme and prion protein and none in case of amyloid-beta.

04t. Therapeutic Targets & Mechanisms for Treatment: gene therapy

ADPD5-0804

GENE THERAPY WITH NEUROTROPHIC FACTORS IMPROVED RECOVERY AFTER SCIATIC NERVE INJURY IN MICE

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Background: Damage to the sciatic nerve (Sciatica) is a common medical condition causing great morbidity. The usual causes include direct trauma, prolonged external pressure on the nerve, and pressure from disk herniation. Possible complications include loss of sensation in the leg and loss of motor control. In mild cases, conservative treatment is feasible but following a severe injury to the nerve, recovery may not be possible. Previously we have shown that transplantation of bone marrow derived stem cells or muscle progenitor cells that ectopically secrete neurotrophic factors improve and accelerate recovery of sciatic nerve damage. The aim of our current study was to evaluate the effect of neurotrophic factors in a sciatic nerve crush injury mouse model facilitated via viral vectors.

Results: Mice injured at the sciatic nerve showed motor deficits and reduction in compound muscle action potential (cMAP) amplitude as well as reduced nerve conduction velocity. Recovery was monitored up to 40 days after injury and a significantly faster recovery was demonstrated in animals injected with viral vectors containing BDNF, GDNF, IGF-1, and VEGF genes as well with a mixture of these genes, compared to those injected with GFP gene or saline as controls.

Conclusion: Our preliminary results indicate an enhaced recovery after sciatic nerve injury in mice transfected with one or all four neurotrophic factor genes. This indicate for further investigations and possible future clinical studies using direct transfection of neurotrophic factor genes to enhance recovery after nerve injury and prevent long term disability.

04w. Therapeutic Targets & Mechanisms for Treatment: adult neurogenesis

ADPD5-0467

ROLE OF VASCULAR TROPHIC FACTORS IN NEURAL STEM CELL NICHE

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Micro-environment of neurogenic region contains specialized cells such as micro-vascular endothelium in addition to neural precursor cells (NPCs) and astrocytes. We found ErbB4, a receptor for heregulin, was expressed in the early NPCs (type1) and the proliferating NPCs (type2a) which express Sox2 and GFAP, but not in the mature NPCs. Heregulin isoforms were, however, produced by brain vascular cells of endothelium and pericytes in neurogenic niche of the sgz of the adult rat brain. Ki-67 positive proliferating NPCs were mainly found nearby vascular ECs labeled with CD31 and pericytes expressing NG2 and α-SMA, where micro-vessels branch out. When co-cultured with EC or pericytes, the Ki-67 positive proliferating NPCs prepared from E16 rat hippocampus were markedly increased. When we knocked down the expression of ErbB4 by siRNA injection into the adult mice hippocampus, proliferating NPCs were significantly decreased. We next investigated the factors released from brain vascular cells by RNA sequencing method and protein array. We found that many genes including heregulin isoforms were up- or down-regulated when NSCs were co-cultured with vascular cells. This result proposes that factors released from brain ECs and pericytes mediate the interaction between NPCs and niche cells and the neurogenesis is largely influenced by these factors in the NPC niche of the adult hippocampus.

This study was supported by a grant from Korea Science and Engineering Research Foundation (KOSEF; Stem cell research project, 2011-0019354) and a grant (2012M3A9C6049937) funded by the Ministry of Education, Science and Technology, the Republic of Korea.

04x. Therapeutic Targets & Mechanisms for Treatment: deep brain stimulation

ADPD5-0466

PROBE PIN DEVICE EMBEDDING WIRELESS POWER TRANSMISSION TECHNOLOGY FOR ADAPTIVE DEEP BRAIN STIMULATION

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Current DBS technology is limited to provide autonomic parameter adjustment depending on patient's symptoms with Parkinson's disease. Various adaptive DBS technologies are proposed and under development to continuously and precisely respond to clinical conditions using a closed loop feedback system and enhance therapeutic effectiveness. In this research, we present a Probe Pin Device (PPD) for integrative neural sensing which is based on optical sensing technology and continuously and selectively measure neurotransmitter levels in targeted brain area along with temperature and pressure sensing. Furthermore, in this research, a wireless power transmission technology is integrated with the pin probe device for long term operation without the need of post-surgical operation for battery change.

In this research, a proto-typed PPD which encompasses optical neurochemical sensing and wireless power transfer technologies has been developed. It is clearly demonstrated that intraoperative and post-operative neurochemical monitoring in real time and long term operation is possible by the miniaturized PPD. The novel PPD sensing system we developed will allow autonomous feedback control of DBS and significantly enhance the treatment efficacy for Parkinson's patients.

04x. Therapeutic Targets & Mechanisms for Treatment: deep brain stimulation

ADPD5-1582

SYNERGETIC EFFECT OF INTRATHECAL BACLOFEN AND DEEP BRAIN STIMULATION IN TREATING DYSTONIA

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Abstract
Dystonia is a syndrome of sustained muscular contractions of opposing muscles with various etiologies. The currently available symptomatic treatment strategies are quite effective for some of various types of dystonia. They help in decreasing involuntary movements, correcting abnormal posture, preventing contractures, reducing pain, and improving function and quality of life. Intrathecal Baclofen and Deep Brain Stimulation were proved to be fairly effective in controlling dystonia when used separately. We are reporting a synergetic effect of ITB and DBS when used simultaneously in two cases of primary generalized dystonia with excellent control of dystonia.
Key Words: Dystonia, Intrathecal Baclofen, Deep Brain Stimulation.

04x. Therapeutic Targets & Mechanisms for Treatment: deep brain stimulation

ADPD5-1633

ROLE OF PREOPTIC AREA THERMO TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE I (TRPV1) AND TYPE IV (TRPV4) CHANNEL IN SLEEP & THERMOREGULATION IN RATS

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Method: The study was conducted in 12 male Wistar rats. Under thiopentone sodium anesthesia (40 mg/kg BW) a bilateral guide cannula (24G) with indwelling styli was implanted with their tips aimed at 2 mm above the preoptic area (POA) as per De Groot's atlas. A radio transmitter TA10TAF-40 (Data Science International, USA) for the telemetric recording of body temperature (Tb) was implanted in the abdomen. A K- type thermocouple wire was inserted near the hypothalamus to measure the brain temperature (Tbr). The study was conducted into two groups. In first group (n=6) TRPV1 agonist, capsaicin (0.2µg/0.2µl) injection was given. While in second group (n=6) TRPV4 agonist, GSK1016790A injection was given. The site of injection was confirmed histologically.

Result:TRPV1 agonist and TRPV4 agonist decrease body and brain temperature at different time interval from twelve PM to four PM after microinjection and increase REM sleep.

Conclusion: The TRPV1 channel agonist and the TRPV4 agonist injection in the preoptic area (POA) brings about fall in body and brain temperature and increasing REM sleep.

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04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0214
PISA SYNDROME, BONSAI SYNDROME AND DROPPED HEAD SYNDROME IN PD: TREATMENT WITH BOTULINUM TOXIN INJECTION.

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Objectives: Lateral axial dystonia (LAD) has been described in patients with Parkinson's disease (PD) as Pisa syndrome (PS). In patients with PS, the trunk is pulled by the paraspinous muscles to the lateralized side. In these cases, botulinum toxin (BTX) should be injected into the paraspinous muscles of the lateralized side. However, in some cases of LAD, the enlarged paraspinous muscles push contralaterally. When this happens, BTX should be injected into the paraspinous muscles opposite the lateralized side. The leaning tower of Pisa is pulled by gravity, but Bonsai trees are tightened by wires. Similarly, in Bonsai syndrome (BS), LAD is a result of tension created by enlarged contralateral paraspinous muscles. In cases with dropped head syndrome (DHS), BTX should be injected into the sternocleidomastoid muscles or scalene muscles.
Methods: We experienced three cases of PS, two cases of BS and four cases of DHS. BTX was injected into the paraspinous muscles of the lateralized side in PS, opposite of the lateralized side in BS and into the sternocleidomastoid muscles or scalene muscles in DHS.
Results: All cases were improved.
Conclusions: Treatment with BTX is useful for PS, BS and DHS in PD. When treating LAD in PD, PS or BS should first be identified.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0239

EFFECT OF PIOGLITAZONE AGAINST STREPTOZOTOCIN INDUCED MEMORY DYSFUNCTION: POSSIBLE ROLE OF NITRIC OXIDE MODULATORY MECHANISM

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Objective of the study- The present study was carried out to elucidate the neuroprotective effect of pioglitazone against the intracerebroventricular infusion of streptozotocin (ICV STZ) induced cognitive impairment and mito-oxidative damage in rats.

Materials and Methods- Male adult Wistar rats were injected with intracerebroventricular streptozotocin (ICV-STZ) bilaterally (3 mg/kg) in first day and 3 days later. Pioglitazone was administrated for three weeks in post surgery. The rats were sacrificed on the 21nd day following the last behavioral test, followed by biochemical estimations (oxidative stress parameter, mitochondrial enzymes).

Results- ICV STZ resulted in poor retention of memory in Morris water maze task and caused marked mito-oxidative damage as compared to sham group. It also caused a significant increase in the oxidative damage, alteration of mitochondrial enzyme complex activity (I to IV), increased acetylcholinesterase enzyme activity in hippocampus and cortex as compared to sham animals. Chronic treatment pioglitazone (15, 30 mg/kg) treatment significantly improved memory retention and attenuated mito-oxidative damage parameters, restored mitochondrial enzyme complex activity and acetylcholinesterase activity in streptozotocin treated rats. L-arginine (50 mg/kg) pretreatment significantly reversed the protective effect of pioglitazone in ICV treated animals. Further, L-NAME (5 mg/kg) pretreatment with pioglitazone (15 mg/kg) significantly potentiated their protective effect which was significant as compared to their effect per se.

Conclusion- The study results demonstrate the nitric oxide modulatory mechanism could be involved in the neuroprotective effect of pioglitazone in preventing the cognitive impairment as well as the mito-oxidative stress caused by ICV STZ in rats.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0366

EFFECTS OF THE PAIN RECOGNITION AND TREATMENT PROTOCOL ON REDUCING EXPRESSION OF PAIN AMONG INSTITUTIONALIZED RESIDENTS WITH DEMENTIA

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Background: Many strategies were used to improve pain management in institutionalized residents with dementia, but the effects of these methods are controversial.
Objectives: The study purpose was to compare the effect of PRT protocol coupled with basic pain education (Experimental group) versus the effect of basic pain education alone (Control group) in improving pain management performance of registered nurses (RNs) and reducing pain related expressions of residents with dementia at post-intervention and 3-month follow-up.
Design: A double-blind cluster randomized controlled trial was conducted in 4 dementia special care units with 195 residents.
Methods: The RNs' weekly pain management performance (eg, use of pharmacologic and non-pharmacologic strategies, use of referral) was recorded by a structure sheet, and the Verbal Descriptor Scale (VDS), Mobilisation-Observation-Behaviour-Intensity-Dementia Pain Scale (MOBID) and the Cohen-Mansfield Agitation Inventory (CMAI) were used to assess residents' pain-related expressions.
Results: The generalized linear mixed model (GLMM) analysis showed that after intervention, the experimental group's number of weekly non-pharmacologic pain relief strategies and number of weekly referral for pain management were significantly increased than the control group. And residents' verbal and behavioral expressions of pain in the experimental group were significantly decreased than those in the control group. However, no significant differences between groups were found in the use of prescribed pain medications and the agitated behaviors expressed by residents.
Conclusions: The study findings support the effectiveness of performance of the PRT protocol and it is recommended for routine use in residents with dementia to improve the quality of pain care.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0375
TRANSFECTING CELLS WITH LASER LIGHT.
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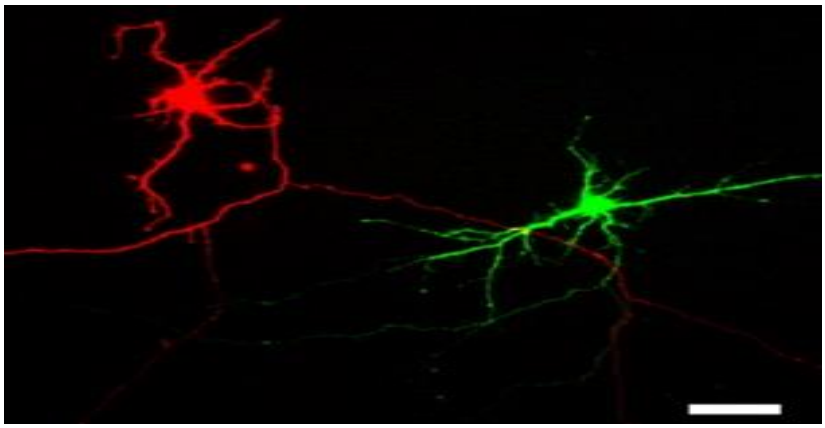
Objectives
To be able to transfect cells in the mammalian nervous system neurons has always relied on a variety of different methods for example microinjection, electroporation, lipophilic chemicals, or the production of viral constructs. All of these techniques have their advantages but none of them can provide fast cell specific or a sub-cellular manipulation. Here we have developed this ability by utilising laser light in adapted commercially available optical set-ups.
Methods
We are able to shape and control exactly laser light, as such we have developed, and are continuing to develop, a series of different optical setups which provide the capability of using laser light to introduce biological material into cells including mammalian neurons. Our work is with the end-user in mind and so we have developed this ability using continuous wave blue diode lasers through to the use of femtosecond pulsed titanium-sapphire lasers, and have ported these through either standard optical microscopes, optical fibers and have also developed a touch-screen interface.
Results
We describe some of the optical set ups we have developed. Specifically we show our touch-screen interface system for the selective transfection of primary neurons with optogenetics based constructs. These light induced proteins allowed us to show that the transfected neurons are electrophysiologically normal.

Conclusions
We have developed a suite of optical based tools that can be adapted to transfect cells of the nervous system either in 2D or 3D cultures but also hold the potential of transfecting cells in situ potentially within a living brain.



04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0486

PAIN IS UNDERESTIMATED IN THE MANAGEMENT OF CASES WITH SEVERE DEMENTIA.

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Objectives

Many older people experience pain, usually related to medical conditions such as arthrtis, cancer, vascular diseases or wounds. However, the rate of analgesic use in cases with dementia is far lower than cognitively healthy elderly, indicating a high likelihood of untreated pain in dementia. Pain may remain unrecognized and untreated due to dementia specific manifestations such as limited language skills, impaired memory or altered ability for reflection.

Pain can manifest in other presentations in dementia where behavioral and psychological symtoms in dementia, BPSD, are common. Different examples are resistance of aid in ADL, aggression, anxiety etc. There is even a risk of overtreatment with psychotropic drugs instead of appropriate pain management.

The Swedish BPSD-registry has a mandatory question about pain and recommends the Abbey Pain Scale for assessment.

Method

Extracted data from the BPSD-registry including information on presence of pain and prescription of analgesic drugs.

Results

In November 2011, 50 % (n=502) of the registred persons with dementia had **no** pain manifestations. In September 2014 there was an increase to 70 % (n= 18 858) of the registred persons. During the same time period the use of analgesics was also increasing.

Conclusions

Through the Swedish BPSD-registry we show that management of pain is underestimated in persons with dementia. By the use of of a mandatory question, pain management can be improved.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0709

THE BENEFICIAL EFFECTS OF COMBINING NOVEL IRON CHELATING/MAO INHIBITORY COMPOUND WITH FORTIFIED HIGH CALORIE/ENERGY DIET IN SOD1-G93A TRANSGENIC ALS MICE

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Objectives: Based on a novel therapy approach that consider a simultaneous manipulation of multiple targets in Amyotrophic Lateral Sclerosis (ALS) treatment, we demonstrated that the multi-target, brain permeable, iron chelator-monoamine oxidase (MAO) inhibitory compounds, M30 series conferred a significant improvement in survival time and motor performance of SOD1-G93A transgenic ALS (mSOD1) mice. The current study examined the prospect of strengthening the basal energy status of mSOD1 mice, by combined treatment of another multifunctional-iron chelator member of this series, VAR with high calorie-energy supplemented diet (CED).
Methods: mSOD1 mice treated with VAR (0.5 and 2.5 mg/kg, p.o. 3 times weekly) +CED cocktail treatment, starting at disease symptomatic stage of 88 days after birth and continuing until time of death. Total neurological deficits were assessed during the experiment and histological, enzymatic and biochemical analyses were determined at the symptomatic stage of 121 days old in all treated-mice.
Results: We demonstrated that VAR+CED treatment, significantly delayed the onset of motor dysfunction and showed superiority in extending the lifespan of mSOD1 mice, compared to the effects of each individual components. In addition, VAR+CED treatment significantly up-regulated the mitochondrial master regulator biogenesis pathway, PPARγ/PGC-1α, increased the mitochondrial respiratory chain enzymes, NADH and succinate dehydrogenases and attenuated neuromuscular junction denervation and motoneuron loss, compared to the individual treatments, in mSOD1 mice.
Conclusions: Our findings provide significant evidence that our multifunctional iron chelating drug VAR may improve mitochondrial function and bioenergetic deficits and thus, can be suggested as novel effective ALS therapeutic approach.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-0791

ISOPRENOID CASCADE AS A NOVEL THERAPEUTIC TARGET FOR THE TREATMENT OF COMMON ALZHEIMER´S DISEASE.

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Farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) are precursor to numerous human metabolites and responsible for the post-translational prenylation of approximately 2% of the total mammalian proteome. Nitrogen-containing bisphosphonate (*N*-BP) inhibitors are currently used in the treatment of osteoporosis, osteolytic metastases from breast, prostate cancer and multiple myeloma. The drugs risedronate and zoledronate typify the molecular architectures of current *N*-BP drugs. However, the very poor cell-membrane permeability and tissue distribution of these compounds, as well as their modest selectivity for hFPPS *versus* other related enzymes of the mevalonate pathway, renders these compounds unsuitable for investigating CNS disorders.. Recently, we found that specific genetic polymorphisms in the hFPPS and hGGPPS genes correlate significantly with aberrant accumulation of Tau and phospho-Tau in human autopsy-confirmed AD brains, but not with AB42 concentrations.

Our efforts towards the design of molecules that have an optimized size, shape and electrostatic surface complimentarily with the active site cavities of hFPPS and hGGPPS, and exhibit lower affinity for bone and higher cell-membrane permeability, will be described. Our medicinal chemistry efforts are guided by compound profiling *in vitro* using the recombinant hFPP and hGGPP synthases, the latter being the gateway enzyme to the prenylation of the small GTPase protein RhoA-cdc42, associated with the accumulation of phospho-Tau protein in the brain. We believe that this specific pathway is implicated either in the response to neuronal damage or, as part of the neurodegenerative process that characterises progression in Alzheimer’s disease and related tauopathies.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1086

EXENDIN-4-INDUCED GLP-1 RECEPTOR SIGNALING PATHWAYS RESTORE AUTOPHAGY AND COGNITIVE FUNCTION AND AVOID ALZHEIMER DISEASE LIKE-HALLMARKS

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1. Objectives: The neuroprotection exerted by the anti-type 2 diabetes (T2D) drug exendin-4 (Ex-4) suggests that it may prevent and/or attenuate T2D-associated neuronal/cognitive deficits and ultimately neurodegenerative diseases. We hypothesized that chronic peripheral Ex-4 administration is neuroprotective and decreases AD-like neuropathology in middle-aged T2D male rats.
2. Methods: 8-month-old male Goto-Kakizaki (GK) and Wistar control rats were given Ex-4 (5 ug/kg/day, 2.5 ul/h, 28 days) or saline subcutaneously. Spatial memory was evaluated by Morris water maze (MWM) test. GLP-1 receptor (GLP-1R) signaling, autophagy, synaptic integrity, amyloid-beta (Abeta) and phosphorylated tau (P-Tau) protein were given by immunoblotting and ELISA in blood and brain homogenates.
3. Results: Peripheral Ex-4 decreased fasting blood glucose levels, glycated hemoglobin (HbA1C) and insulin resistance (HOMA-IR) in GK rats. Accordingly, Ex-4 also lowered brain glucose levels and stimulated GLP-1R-mediated signaling (as given by cAMP, Akt and PI3K levels and PKA activity). Consequently, Ex-4 was able to stimulate autophagy in GK rat brains (as given by mTor, LC3, p62, Beclin, Atg7, Parkin, LAMP-1). Strikingly, the Ex-4-induced decrement in brain Abeta1-42 levels and P-Tau (Thr181 residue) was accompanied by improved synapse function/integrity (synapthopysin and PSD-95) and spatial memory in T2D rats.
4. Conclusion: Ex-4 therapy may enhance GLP-1R-mediated signaling, thereby improving glucose homeostasis, insulin sensitivity and 'protective' autophagy, preventing the development of AD-like hallmarks and ultimately restoring synaptic and cognitive function.

Supported by FEDER (Programa Operacional Factores de Competitividade – COMPETE) and Portuguese funds via FCT (projects: PTDC/SAU-TOX/117481/2010; Pest/SAU/LA0001/2011; fellowships: SFRH/BD/90036/2012, PTDC/SAU-TOX/117481/2010 and SFRH/BPD/84473/2012).

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1490

SHORT-TERM TREATMENT OF TRIPLE TRANSGENIC MICE (3XTG-AD) WITH LIVER X RECEPTOR (LXR) AGONISTS INDUCED CHANGES IN DNA METHYLATION PATTERN AND COGNITIVE IMPROVEMENT.

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Grupo de Neurociencias de Antioquia Universidad de Antioquia., Medellin, Colombia
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[5]Autors, contributed equally to this work., Bogotá, Colombia

Introduction: Changes in DNA methylation were recently described to play a role in the progression of Alzheimer's disease (AD). Nuclear receptors, in particular LXRs, have been shown to improve cognitive function in murine models of AD and to regulate epigenetic modifications of DNA inducing transcriptional de-repression.

Objectives: To evaluate the effect of a short-term treatment (ST) of GW3965 (LXR agonist) in cognitive function and DNA methylation pattern in the hippocampus of 3xTg-AD and WT mice.

Methods: 24 months-old 3xtg-AD mice were treated with GW3965 (50mg/kg/day – 6days). Cognitive performance was evaluated by Morris Water Maze; Aβ-burden by thioflavin-S; methylation pattern of DNA from hippocampus was evaluated via modified bisulfite conversion and subsequent hybridization on Infinium Methylation BeadChip 450K.

Results: GW3965 improves cognition without a significant change on Aβ burden. These changes were associated to restoration of the DNA methylation pattern in promoter regions of *LMOD3, DLGAP3,* and *TUBB2A* genes, similar to WT mice. *LMOD3* is highly expressed in the murine brain during development and is associated with smooth muscle cells, which are reduced in AD; *DLGAP3* is a postsynaptic scaffolding protein that is highly expressed in glutamatergic synapses, and is downregulated in AD patients; *TUBB2A* is a tubulin protein critical for neuronal development proliferation and migration.

Conclusion: GW3965 treatment improves cognition in the 3xtg-AD associated with recovery of methylation pattern of genes that may be important for improvement of synaptic and cognitive function.

Acknowledgements: We thank Dr. Jon Collins (GSK, Stevenage, UK) for providing the GW683965A.

Keywords: Alzheimer, LXR, LMOD3, DLGAP3, TUBB2A.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1547

COGNITIVE EFFECTS OF BENZODIAZEPINE-USE IN SUBJECTS WITH MILD TO SEVERE ALZHEIMER'S DISEASE AND RELATED DEMENTIA

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BACKGROUND:
Long-term benzodiazepine (BDZ) use affect cognition adversely and may be partly irreparable. The degree of cognitive retrieval after withdrawal, if any, that is preserved is also unclear. Psychiatrists use benzodiazepines to treat anxiety, sleep disorders, and acute agitation associated with AD. The aim of the study was to investigate the link in core cognitive functions for subjects who are treated with BDZ.

METHODS:
A naturalistic longitudinal dataset of 2859 subjects (134 on BZD) followed for over 30 years was examined. Categorical analysis was completed for BZD and non- BZD subjects. Transformations were computed to correct for normality and group imbalances.

RESULTS:
Subjects' scores in the BDZ group on most tests remained at the lower percentiles of normative scores. The following cognitive items showed significant differences at the $p \leq 0.001$ level indicating more impairment in BZD group: Finding way around the house (Chi-Square=9.564), around neighborhood (Chi Square=14.302), remembering things (Chi-Square=11.483), MMSE What city are we in (Chi-Square=11.743), MMSE What borough? (Chi-Square=44.678), MMSE Name 3 objects (Chi-Square=44.056) and MMSE Naming (Chi-Square 55.524). Long-term benzodiazepine users were consistently more impaired than non-BDZ across cognitive categories, with effect sizes ranging in magnitude from –1.07 to –0.40. Mean weighted effect size –0.66 (SD±0.21).

CONCLUSIONS:
The observation that BDZ use has several implications for the informed decision to include subjects on BDZ or subjects with long term use. Emerging evidence may suggest that some medications might be a safe and efficacious treatment of long-term BDZ use should be considered for subjects with AD.

04z. Therapeutic Targets & Mechanisms for Treatment: other

ADPD5-1993

A NEW TREATMENT FOR CLINICAL STABILIZATION IN PARKINSON´S DISEASE.

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It is mandatory to have a safe and effective treatment to stop Parkinson´s disease (PD) progression. Previous clinical trials designed to stop PD´s progression have failed to demonstrate significant clinical stabilization. We administrated to PD patients a new oral treatment designed to stop the disease progression called Cervô. It contains four substances that have a synergic effect in controlling the most important known mechanism of disease progression as: aberrant apoptosis, oxidative damage, mitochondrial degeneration, caspases activation, Mitogen-Activated Protein-Kinases (MAPK) activation. We previously demonstrated that it is safe to use Cervô in humans and that has no collateral effects. **Results**: In the Cervô clinical stabilization clinical trial, we included 42 patients with Parkinson´s disease. Age: 42 to 89 years old (mean 66.12 years), 23 female (54.8%), 19 male (54.2%). Initial United Parkinson´s Disease Rating Scale subscale 3 (UPDRS) score: 1-15 (mean 5). Maximum follow-up period: 72 months, mean 29.6 (+/- 22.28 SD). We included only patients with more than 6 months of follow-up for the clinical stabilization analysis (n=37). No disease progression (no increase in the UPDRS score) in 34 (91.8%) patients, and 31 (83.78%) patients improved their basal UPDRS score. There were 2 clinical remissions (5.4 %) and the disease progressed in 1 (2.7 %). **Conclusions:** Until today, there is no medication that had proven to be effective in controlling PD´s progression. Cervô is a new and promising compound that may stop PD´s progression.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-0213

THERAPEUTIC POTENCY OF ESMIRTAZAPINE IN MPTP INDUCED PARKINSON'S DISEASE MODEL

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Objective: 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-is reported neurotoxin to develop Parkinson's disease (PD) Model. Esmirtazapine, a noradrenergic and specific serotonergic antidepressant (NaSSA) was also reported to increase dopamine release in the cortical neurons with 5-HT dependent manner. Hence, this study investigates the therapeutic potency of Esmirtazapine in MPTP-treated rat model of PD.

Methods: SD rats, 8 weeks of age, were used in this study. Rats were subjected to MPTP treatment (20mg/kg, intraperitoneal) to establish a PD model. Esmirtazapine (4mg/kg, 8mg/kg or 61mg/kg, intraperitoneal) was administered once a day for 3 days after MPTP treatment. Beam-walking and rota rod tests were used for behavior assessment. Dopamine (DA) and its metabolites activity were quantified by validated LC-MS/MS methods and Western blot analysis.

Results: MPTP-induced motor dysfunction, assessed by beam-walking and rota-rod tests, was significantly improved by administration of esmirtazapine . Biochemical examinations by LC-MS/MS and western blot analysis suggested esmirtazapine facilitated utilization of dopamine by increasing turnover and protein expression of transporters, without affecting on neurodegenerative process by MPTP. These therapeutic effects of esmirtazapine were reduced by administration of clonidine, a selective agonist for α2-NAR, or of prazosin, an inhibitor for α1-NAR, respectively.

Conclusion: These results showed esmirtazapine had a therapeutic potency against PD in a rat model. Because PD patients sometimes show depression together, it will be a useful drug for a future PD treatment.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-0438

EFFECTS OF COFFEE ON BDNF ACTIVITY IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS.

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Objectives

Coffee is one of the most world widely consumed beverage on a daily basis. Recent epidemiological studies have suggested that daily coffee consumption reduces the risk of several neurodegenerative diseases. However, the precise molecular mechanisms of the effects of coffee are yet uncertain. Neurotrophins, such as BDNF, have effects on various neurodegenerative diseases in development of symptoms and prolongation of life. In this study, we analyzed the effects of coffee on BDNF activity in human neuroblastoma cells SH-SY5Y.

Methods

SH-SY5Y cells were induced to differentiate by *all-trans* retinoic acid treatment for 6 days. On day 6, cells were exposed to coffee (cf.) or decaffeinated coffee (dcf.) at 0, 2.5% (v/v) for two hours followed by BDNF treatment. After 0-120 minutes, whole cell lysates were isolated and phosphorylation of TrkB, ERK, and Akt was measured by immunoblots. Total RNA was isolated from the cells treated with BDNF plus cf. or dcf. for 4 hours and real-time PCR was performed to measure BDNF-induced gene expression.

Results

Cf. and dcf. inhibited BDNF activity by inhibiting TrkB and Akt phosphorylation. Furthermore, cf. and dcf. abolished the induction of BDNF gene expression mediated by TrkB signaling. Major components of coffee including caffeine did not show any effect on TrkB signaling.

Conclusion

Coffee inhibited BDNF-mediated TrkB signaling by interfering phosphorylation of TrkB and Akt. The active component of coffee is not major constituents. Further study to elucidate the mechanism of the effect of coffee on BDNF function in neuronal cells should be carried out.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-0736

CXXC PEPTIDE MOTIF FROM MESENCEPHALIC ASTROCYTE-DERIVED NEUROTROPHIC FACTOR (MANF) ANTAGONIZES APOPTOTIC AND NECROPTOTIC DEATH AND HAS ANTI-OXIDANT ACTIVITY

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Objective: To study the cytoprotective effect of MANF-derived CXXC motif containing peptide CKGC against different types of programmed cell death (PCD).
Background: Novel factors MANF and CDNF are good candidates for a drug against neurodegeneration. However, they raise the problem of their administration to the brain of a patient, as they cannot penetrate the blood-brain barrier. Small molecules that could substitute neurotrophic factors would help to overcome this difficulty. One such molecule could be CKGC, a MANF-derived neuroprotective peptide.
Methods: Human T lymphocyte-derived Jurkat cells were exposed to the conditions inducing intrinsic apoptosis, extrinsic apoptosis or necroptosis. CKGC peptide at different concentrations was added to growth medium to reveal the cytoprotective capacity of the peptide against different types of PCD. SKGS control peptide with cysteines substituted to serines was used to reveal the importance of cysteine residues in the function of the peptide. The ability of CKGC to decrease reactive oxygen species (ROS) and preserve mitochondrial membrane potential, as well as its cell membrane permeability were measured.
Results: CKGC peptide protects Jurkat cells against all three PCD types, although high concentrations are needed. It effectively decreases ROS and maintains the integrity of mitochondria, which explains its anti-apoptotic effect. The peptide passively penetrates plasma membrane, although not very effectively.
Conclusions: MANF-derived peptide with CXXC motif could theoretically be an alternative for MANF protein as a drug against neurodegenerative diseases, as it can counteract different modes of programmed cell death, although some improvements in efficiency and cell permeability are still needed.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-1029

SULFURETIN PREVENTS INFLAMMATORY RESPONSES AND NEURONAL CELL DEATH BY BLOCKING THE NF-κB SIGNALING PATHWAYS IN NEURONAL CELLS

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We investigated the mechanisms of sulfuretin protection of neuronal cells from apoptotic cell death induced by the Parkinson's disease (PD)-related neurotoxin, 6-hydroxydopamine (6-OHDA). We examined whether sulfuretin acts as an anti-oxidant to reduce oxidative stress and mitochondrial-mediated apoptotic cascade events in 6-OHDA-induced neurotoxicity in SH-SY5Y cells. We also investigated whether sulfuretin specifically acts by inhibiting phosphorylation of mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)/Akt, and glycogen synthase kinase-3beta (GSK-3β) as well as activation of the nuclear factor-kappa B (NF-κB) pathway. Sulfuretin significantly inhibited neuronal cell death, neurotoxicity, apoptosis, and reactive oxygen species (ROS) production. Sulfuretin also strikingly attenuated 6-OHDA-induced mitochondrial dysfunction. Moreover, sulfuretin significantly attenuated 6-OHDA-induced phosphorylation of c-Jun N-terminal kinase (JNK), p38, extracellular signal-regulated kinase 1/2 (ERK 1/2) MAPKs, PI3K/Akt, and GSK-3β. Eventually, sulfuretin inhibited 6-OHDA-induced NF-κB translocation to the nucleus induced by 6-OHDA. The results of the current study provide the first evidence that sulfuretin protects SH-SY5Y cells against 6-OHDA-induced neuronal cell death, possibly through inhibition of phosphorylation of MAPK, PI3K/Akt, and GSK-3β, which leads to mitochondrial protection, NF-κB modulations and subsequent suppression of apoptosis via ROS-dependent pathways. Thus, we conclude that sulfuretin may have a potential role for neuroprotection and, therefore, may be used as a therapeutic agent for PD.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-1530

ANTICHOLINESTERASE ACTIVITY OF ISORHAMNETIN 3-O-BETA-D-(6''-ACETYL)-GALACTOPYRANOSIDE ISOLATED OF ETHANOLIC EXTRACT OF BELLIS PERENNIS L. (ASTERACEAE)

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Objectives: Evaluate acetylcholinesterase (AChE) activity of an isolated compound of ethanolic extract of flowers of *Bellis perennis* L. **Methods:** Flowers were collected in Pacoti, Ceará and deposited at herbarium Graziella Barroso of Federal University of Piauí, with register number 27,276. *In vitro* activity of AChE was evaluated by Ellman and colleagues method (1961). All the *ex vivo* experiments performed in this study got approved by the Animal Experimentation Ethics Committee of the Federal University of Piaui (# 077/10). **Results:** The analysis of IR and NMR spectrums allowed to conclude that the isolated fraction is isorhamnetin 3-O-β-D-(6''-acetyl)-galactopyranoside. There was an inhibition in AChE activity *in vitro* of 83.85 and 17.22 when rivastigmine was used (positive control) at the concentrations of 0.2 e 0.0125%, respectively. Isolated fraction at concentrations 0.0125; 0.00652; 0.003125 and 0.001563% produced an inhibition of 64.30; 55.83; 43.87 and 38.13% in AChE activity, respectively. In the *ex vivo* studies, we verified a decrease of 91% in the AChE activity among mice treated with 10 mg/kg (0.89 ± 0.21) of fraction when compared with negative control (10.03 ± 0.16; $p<0.05$). In comparison with the group treated with rivastigmine (5.69 ± 1.20), we verified a decrease of 84.4% in AChE activity among mice treated with at dose 10 mg/kg (0.89 ± 0.21; $p<0.05$) of isolated compound. **Conclusion:** The isolated compound is able to more effectively increase cholinergic stimulation, suggesting its possible use in treatment of neurodegenerative diseases such as Alzheimer's disease.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-1974

MONOAMINE OXIDASE INHIBITORS ISOLATED FROM CRATAEGUS PINNATIFIDA BGE. VAR. MAJOR FRUITS

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OBJECTIVES - In previous experiments, it was found that the fruits extract of *Crataegus pinnatifida* Bge. inhibit monoamine oxidase (MAO) activity. This study addresses the isolation of well known-but not previously reported in this plant-organic acid methylesters that show inhibitory activity against MAO from methanol extract of fruits.

METHODS - MAO activities was determined according to the method previously reported by Ryu *et al*.(A) and Han *et al*.(B). The powered sample of the fruits was extracted with MeOH at room temperature for one month. Repeated chromatography on silica gel and reverse phase silica gel afforded compound 1 and 2 from ethyl acetate fraction.

RESULTS – The ethyl acetate extract of the hawthorn fruits showed higher inhibition and on both of MAO-A and MAO-B. Compound 1 and 2 were not positive Liebermann-Buchard reaction, ninhydrin test or Pauly reaction. IC_{50} values Compound1 against MAO-A and MAO-B were determined 0.12 mM and 0.068mM, respectively. IC_{50} values of compound2 against MAO-A and MAO-B were determined 0.01 mM and 0.025mM. Spectral data indicated that Compound 1 was citric acid dimethylester and Compound 2 was citric acid monomethylester.

CONCLUSION - Two citric acid methylesters were major components in MAO activities found in the fruit of *Crataegus pinnatifida* . These compounds have been reported in other plants but have not been reported in *Crataegus pinnatifida* Bge. var. major. The present study indicates that these molecules including ursolic acid and quercetin have MAO inhibitory activities and other biological activities or industrial usages of these molecules may be found through futher investigation.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-1976

ADAMANTYL-GROUP CONTAINING 1,4-DIHYDROPYRIDINE DERIVATIVE PROTECTS AGAINST STRESS-INDUCED CHANGES IN BRAIN PROTEIN EXPRESSION IN RATS

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Objectives: Neurotrophins, adhesion molecules and enzymes related to cholinergic and glutamatergic systems are considered critical molecules that maintain synaptic plasticity and memory formation. Stress is a significant factor that can alter brain cell properties leading to impairments in synaptic plasticity and further to neurodegeneration. We assessed the ability of adamantyl-containing 1,4-dihydropyridine (DHP) derivative (compound AV-6-93) to protect against stress-induced alterations in synaptic plasticity proteins in the striatum and the hippocampus in rats. Previously adamantane molecule has been used in the design of neuroprotective drugs (Kornhuber et al., 1991), while DHP ring serves as the carrier moiety.
Methods: Male Wistar rats (8-10 per group) were treated ip for two weeks with AV-6-93 at the dose 1 mg/kg or saline (control). One hour after the last drug administration, rats were exposed to 2 h immobilization stress. The expression of the hippocampal and striatal BDNF, AChE, GAD67 and NCAM were analysed by Western blot and immunohistochemically.
Results: Our data showed that 2h immobilization stress caused a considerable (about 2-fold) induction of striatal and hippocampal BDNF and striatal AChE, NCAM, but did not influence GAD67 expression. The compound AV-6-93 normalized the expression of BDNF, AChE and NCAM in the striatum and hippocampus close to control levels, while did not influence GAD67 expression.
Conclusion: The data obtained indicate that the compound AV-6-93 regulates the stress-induced overexpression of brain synaptic plasticity proteins, such as BDNF, AChE and NCAM. We suggest that adamantyl- and DHP moieties can be useful for the design of neuroprotective drugs.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-2023

GREEN TEA: A NEW DAWN IN THE ERA OF NEURODEGENERATIVE

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A few cups of tea in a day keep doctor away. This proverb is proving its magical impact in the in the era of neurological disorders. Neurodegeneration in Parkinson's, Alzheimer's, or other neurodegenerative diseases emerges due to various reasons, due to composite set of toxic reactions, counting oxidative stress (OS), inflammation, and amassing of protein aggregates, results in the failure of neurons. Green Tea Flavonoides (catechins), poly phenols, and their antioxidants screening promising antioxidant, anti-inflammatory and divalent metal chelating actions, to infiltrate the brain barrier and to shield neuronal death in a broad display of neurological bugs. By this study we endeavour the new dawn on the multi-pharmacological neuro-protective activities of green tea with exceptional accent on brain-permeable, biodegradable ,patient compliance, transitional metal (iron and copper)-cheatable/radical forager nontoxic possessions.

05i. Drug Development & Clinical Trials: vitamins, anti-oxidants & neuroprotective compounds

ADPD5-2036

THE PROTECTIVE EFFECT OF CURCUMIN ON TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN BOTH SUBSTANTIA NIGRA AND THE VENTRAL TEGMENTAL AREA, AFTER ACUTE ALUMINUM INTOXICATION.

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Aluminum (Al) is a potent neurotoxic metal wich has been associated to several neurodegenerative diseases, it has no known function in human; however, this metal is accumulated in substantia nigra, and considered by some authors as a risk factor for developing Parkinson's Diseases. Moreover, some few studies demonstrated that Al can include changes in many neurotransmitter levels including the dopaminergic system. Among several medicinal plants having a beneficial effect on health; curcumin is extracted from *Curcuma longa* rhizomes, and well known as a multi-functional drug with antioxidative activities. Using the immunohistochemistry procedure, with tyrosine hydroxylase antibody (TH: the key enzyme of dopamine synthesis), the present study evaluates the possible protective effect of curcumin on Al intoxicated rats (animals receiving Al acute treatment). Thus, experiments were carried out on male wistar rats intoxicated acutely with an intraperitoneal injection of Al (25mg/Kg B.W.), and (100mg/Kg B.W.), the two Al intoxicated groups (25mg/Kg B.W.) and (100mg/Kg B.W.) received concomitantly Curcumin by oral gavage (30mg/kg B.W.). Our results showed, a significant and gradual increase of TH immureactivity in both the substantia nigra (SN) and the ventral tegmental area (VTA) in acute Al intoxicated rats, this enhanced immunolabelling concerns both somata and their related fibers. This increased TH immunoreactivity has been remedied with daily curcumin administration in all Al intoxicated groups. We suggested that Al causes alterations on dopamine neurotransmission, this inconvenience might be treated by curcumin exhibiting a potential protective effect.

05j. Drug Development & Clinical Trials: neurotransmitter modulators

ADPD5-1534

ANTICHOLINESTERASE AND ANTIOXIDANT ACTIVITIES OF APIGENIN-7-O-GLUCOPYRANOSIDE ISOLATED OF BELLIS PERENNIS L.

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Objectives: This study describes the isolation and identi?cation of apigenin-7-O-glucopyranoside (ApG), a ?avonoid isolated from ?owers of *Bellis perennis* L. (Asteraceae). The *in vitro* antioxidant activity and the inhibition against enzyme acetylcholinesterase (AChE) were evaluated. **Methods:** The chemical structure of the substance isolated was de?ned based on the analysis of ^{1}H NMR and ultraviolet spectroscopic data, and comparison of data obtained with those reported in literature. Quantitative activity AChE was evaluated by Ellman and colleagues spectrophotometric method adapted (1961) and Moyo and colleagues (2010). Evaluation of antioxidant activity *in vitro* for formation of hydroxyl radical (OH•) from the Fenton reaction and TBARS and nitric oxide (NO). **Results:** The flavonoid showed strong *in vitro* antioxidant potential, because of the capacity of removal of hydroxyl radicals and nitric oxide, and also prevented the formation of thiobarbituric acid-reactive substances. These parameters were inhibited at the highest concentration of ApG at rates of 77.7%, 72% and 73.4%, respectively, in addition to inhibiting acetylcholinesterase, suggesting potential use in the treatment of neurodegenerative diseases. In vitro studies indicated that there was 92.02% inhibition of AChE activity when Neostigmine (Exelon®) was used as positive control at a concentration of 0.1%. The 50% inhibitory concentration (IC_{50}) was also determined, which corresponded to 1.91 µmol/l, with 95% con?dence intervals of 0.53 and 3.42 µmol/l (r^2 = 0.8332). **Conclusion:** If these e?ects are established, it is possible to justify the use of this ?avonoid in preclinical and pharmacological models that simulate neurodegenerative diseases.

05n. Drug Development & Clinical Trials: mitochondrial drugs

ADPD5-1388

FOLIC ACID IS NEUROPROTECTIVE IN A PARKIN KNOCKDOWN DROSOPHILA MODEL OF PD

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Objectives: Loss-of-function mutation(s) in *parkin* is commonly associated with familial Autosomal recessive juvenile Parkinsonism (AR-JP). Mutations in *the Drosophila parkin* (a human *parkin* ortholog) have deciphered important insights to AR-JP etiopathology. In this context, the therapeutic potential of folic acid (FA) in the alleviation of parkin loss of function in dopaminergic (DA) neuron associated discrepancies in *Drosophila* transgenic model of AR-JP was explored.
Methods: *parkin* knockdown in dopaminergic neuron was carried out using UAS *parkin*^{RNAi}-DA GAL4 genetic approach. Mortality, climbing assay, ATP levels, and oxidative stress were used to assess the effect of FA supplementation to UAS *parkin*^{RNAi}-DA GAL4 flies. qRT-PCR for relative analysis of mitochondrial biogenesis and mitophagy was carried to study any possible FA potential in regulating mitochondrial functioning.
Results: *parkin* knockdown in DA neurons leads to high mortality rate, reduced climbing ability, elevated oxidative stress, low ATP level and altered mitochondrial functioning. Folic acid supplementation reverses high mortality, significantly improves motor ability and reduces oxidative stress. ATP levels improve in FA supplemented flies with *parkin* knockdown background. qRT-PCR analysis depicted improved mitochondrial biogenesis.
Conclusions: Folate is an essential cofactor known for its importance in neurogenesis, and its deficiency is linked with Alzheimer's and Parkinson's. In this context, we looked for the effect of FA supplementation in flies with a specific parkin knockdown background in DA neurons. Our result shows that FA improves behaviour response, reduces stress, and improves the ATP level, suggesting FA therapeutic potential in context to discrepancies linked with *parkin* associated AR-JP in Drosophila model.

05s. Drug Development & Clinical Trials: nanotechnology

ADPD5-1064

IMPACT OF NANOTECHNOLOGY BASED DRUG DELIVERY SYSTEM IN THE ARENA OF NEURODEGENERATIVE DISORDERS

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Nanotechnology in 21st centaury unlock new-fangled eon in the meadow of medicine and healthcare. The impact of Nanotechnology is incredibly profound in the treatment of many ailments especially in cancer, Neurodegenerative disorders, Skin diseases etc. Nanotechnology based drug delivery like liposomes, Nanoparticles, Nanogels, Niosome, Phytosome etc create a novel avenue for the management of world most exaggerated neurodegenerative disorders like Alzheimer etc. Nanotechnology in simple term implies the engineering, mechanization and the manufacturing of materials and substances at the atomic and the molecular level that provides an aid for combating of Neurogenerative disorders. Herein, we discuss the important aspects of Nanotechnology based drug delivery and tissue engineering, revealing the supremacy and the challenges we are currently facing, in dealing with variety of diseases especially Neurogenerative disorders.

Keywords: Nanotechnology, Neurogenerative disorders, drug delivery, Diseases, Cancer: Engineering: Manufacturing: atomic:

05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-0743

A NOVEL IN VIVO ACTIVE ANTI-AMYOTROPHIC LATERAL SCLEROSIS (ALS) CHEMICAL CHAPERON BASED DRUG CANDIDATE

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Amyotrophic Lateral Sclerosis (ALS) also known as Lou Gehrig's disease is a fatal neurodegenerative disease, characterized by the selective degeneration of motor neurons in the brain and spinal cord, which leads to progressive paralysis and death. The majority of ALS is acquired spontaneously (sALS), with inherited disease accounting for only 10-15% of all cases. Recent studies provide compelling evidence that aggregates of mutated or misfolded proteins underlies both types of ALS.
Chemical chaperones that include polyols, trimethyl N-oxid (TMAO), phenylbutiric acid and different amino acid derivatives, have been shown to reverse the mislocalization and aggregation of proteins associated with many human diseases. However, using chemical chaperones as drugs is limited by their very high active concentration (mM range). We propose to overcome this obstacle by coupling known chemical chaperons to organelle targeting moieties in order to increase their concentrations at specific sites, in particular in cellular organelles where aggregation takes place (lysosomes, ER, Golgi, mitochondria). Refolding by chemical chaperons enabled proteolytic enzymes and proteasome system to cleave the misfolded proteins properly.
We have synthesized several ester and amide based chemical chaperon prodrugs. The lead compound, GZ-23, in the micromolar concentration range, has shown both neuronal and astrocyte protective effects *in vitro* and in daily doses of 10 mg/kg has dramatically improved the neurological functions and delayed body weight loss in ALS mice. Thus, this novel class of chemical chaperones is a strong candidate for developing new anti-ALS drugs.

05x. Drug Development & Clinical Trials: non-pharmacological interventions

ADPD5-1637

EFFECT OF AEROBIC TRAINING ON BDNF LEVELS AND AEROBIC CAPACITY IN ALZHEIMER'S DISEASE

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Objective: To investigate the effects of aerobic training on BDNF plasma levels and aerobic capacity of older adults with AD. Also, there were verified possible relations between BDNF plasma levels, metabolic variables, and cognitive functions. Method: Twenty-nine older adults with AD participated in the study, which was a nonrandomized, controlled, blind trial. Sample of were assigned to a training group (n = 16 - Moderate aerobic training on the treadmill, three times a week, for twelve weeks) and a control group (n = 13 - without systematic physical activity). We determined aerobic capacity at incremental test, using VO_2, treadmill grade, treadmill time and maximum lactate. Blood collection to obtain BDNF plasma levels, metabolic variables and lactate levels. Cognitive function was evaluated using Frontal Assessment Battery, Clock Drawing Test, Verbal Fluency and Symbol Search. Results: Training group and control group showed a significant increase on BDNF levels ($p = 0,02$; $F= 6,07$), however, the effect size was moderate for the training group (0,42) and small for the control group (0,14). Training group improve aerobic capacity, VO2 ($p < 0,001$), treadmill time ($p < 0,001$), and treadmill grade ($p < 0,001$) and decrease levels of LDL cholesterol ($p= 0,04$; $F= 4,28$) when compared to the control group. Conclusion: The moderate aerobic training was also effective for increased BDNF levels, improving aerobic capacity and reduced LDL cholesterol of older adults with AD.

05y. Drug Development & Clinical Trials: other

ADPD5-0405

IS THERE THE PREVENTIVE EFFECT OF COMT-INHIBITOR ON PARKINSON'S DISEASE ASSOCIATED WITH DEMENTIA?

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Background: Elevated homocysteine (hcy) levels are associated with dementia, which is a frequent non-motor symptom of Parkinson's disease (PD). High levels of hcy in PD patients treated with levodopa are thought to result from increased synthesis during the metabolism of levodopa by COMT, and that use of a COMT-inhibitor may reduce hcy levels. In this study, we sought to clarify the effects of COMT-inhibitors on dementia in PD patients.
Methods: Thirty-eight PD patients without dementia (PDwoD), 35 PD patients with dementia (PDD), and 48 controls were enrolled in this study. All subjects underwent neuropsychological testing and a neurological examination. The hcy levels were measured in all subjects, and the relationship between hcy levels and dementia was evaluated in two PD groups (those that underwent treatment with levodopa-alone versus treatment with levodopa plus a COMT-inhibitor).
Results: Patients in the PDD group showed higher hcy levels than patients in the PDwoD group, though there was no significant difference in the hcy level between PDwoD patients and healthy controls. Regarding the effects of a COMT-inhibitor, there was no correlation between hcy levels in the 2 PD subgroups, indicating that there were no significant effects of the COMT-inhibitor on PDD. In addition, the odds ratio for PDD with the use of a COMT-inhibitor was 0.864 (95% CI=0.342-2.180).
Conclusions: These results are in agreement with previous studies in that levodopa treatment in PD patients leads to elevated hcy concentrations. COMT-inhibitors, on the other hand, had no preventive effect on cognitive impairment in PD patients.

05y. Drug Development & Clinical Trials: other

ADPD5-1075

EFFICACY AND SAFETY OF OPICAPONE, A NEW COMT-INHIBITOR, IN PD PATIENTS WITH MOTOR-FLUCTUATIONS: PHASE-III, RANDOMIZED, DOUBLE-BLIND, ACTIVE- AND PLACEBO-CONTROLLED, PARALLEL-GROUP STUDY (BIPARK I)

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Aims: Investigate the efficacy, safety and tolerability of 3 different doses (5, 25 and 50mg) of opicapone (OPC) administered once-daily, compared with 200mg entacapone (ENT) and placebo, in patients with PD on levodopa treatment and with motor fluctuations.
Methods: Multinational, multicentre, double-blind (DB), placebo- and active-controlled, parallel-group study. Subjects were randomized for the DB period to placebo (n=121), 5mg-OPC (n=122), 25mg-OPC (n=119), 50mg-OPC (n=116) or 200mg-ENT (n=122). Treatment duration was of 14-15 weeks, where subjects were instructed to keep ON/OFF diaries. The primary efficacy variable was the change from baseline in absolute OFF-time by an analysis of covariance (ANCOVA). Key secondary endpoints were the proportion of responders. Other secondary endpoints include Investigators'/Subjects' Global Assessment of Change (IGAC/SGAC), UPDRS, quality-of-life (PDQ-39, NMSS, PDSS, UPDRS), tolerability and safety (including mMIDI, C-SSRS and clinical laboratory tests) assessments.
Results: The 50mg-OPC group was significantly better than placebo (p=0.0005). The 200mg-ENT group also achieved statistical significance versus placebo (p=0.0141). Significantly more patients receiving either 25 or 50mg-OPC achieved the OFF-time responder endpoint (60.3% [p=0.0464] and 69.6% [p=0.0011], respectively), with both 5mg-OPC and 200mg-ENT not reaching statistical significance compared to placebo. Only patients receiving either 25 or 50-mg OPC reported significant improvements in IGAC and SGAC compared to placebo. The 50mg-OPC tended to be the most efficacious treatment tested, followed by all other active treatments.
Conclusion: Opicapone was safe, well tolerated, and effective in reducing the OFF-time in patients with PD on levodopa treatment and with motor fluctuations.

05y. Drug Development & Clinical Trials: other

ADPD5-1748

PHOSPHOLIPIDS AND PD: NEW OPPORTUNITIES FOR THERAPY.

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Objective: to evaluate the effectiveness of treatments of ion-reflex fonoforeza phospholipids hypothalamus in correcting motor and cognitive impairments in patients with PD.
Materials and Methods: 39 patients studied: 25 men and 14 women; age: 63 to 69; disease duration: 8,1±2,2 years; Hoehn and Yahr scale points - 2,4±0,4. In determining motor impairment degree Tinetti scale was used; cognitive disorders – mini-mental state examination (MMSE), scales of Wechsler (WMS, WAIS) and Matisse; life quality - measured by McDowell index. Patients received an intramuscular injection of phospholipids hypothalamus, then after 1.5-2 hours sessions held ion-reflex fonoforeza phospholipids hypothalamus using techniques longitudinal fronto-occipital head galvanization.15 patients treated by protocol composed control group.Results: Motor activity's improvements and decrease in rigidity, tremor and hypokinesia were noted in patients receiving sessions of ion-reflex fonoforeza phospholipids hypothalamus. Patients'static and dynamic balance abilities (the Tinetti test) improved significantly by 25%. McDowell's disability index fell to 36. Neurodynamic and operational functions improved firmly. MMSE cognitive impairment degree showed positive trend with 23,3±1,5 reaching 27,1±1,5; Matisse scale indexes increased to 7,1% ($p < 0.01$). Logical, visual memory, account and thinking tests' results improved. Conclusions: research's dynamic demonstrates the sessions ion-reflex fonoforeza phospholipids hypothalamus using techniques longitudinal fronto-occipital head galvanization is positive impact on motor and cognitive function in patients with Parkinson's disease.

05y. Drug Development & Clinical Trials: other

ADPD5-1803

IMPACT OF CURRENT ANTIPSYCHOTIC MEDICATIONS ON COMPARATIVE MORTALITY AND ADVERSE EVENTS IN PEOPLE WITH PD PSYCHOSIS (PDP)

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Over 50% of people with PD will develop psychosis during the course of their disease. The symptoms of hallucinations and delusions are distressing and challenging for the individuals and their caregivers. PDP is commonly linked to institutionalization and increased risk of mortality. Atypical antipsychotics are frequently prescribed to address PDP despite limited evidence to support their efficacy and concerns about their safety in this generally elderly population. Limitations in the scope and design of published trials prevent a clear interpretation of mortality risk and adverse events, particularly for treatment periods exceeding 6 weeks. A post-hoc analysis was performed on data from 423 participants with PDP in an ongoing multicenter, open-label extension study of pimavanserin. Safety assessments were conducted at two weeks, one, three, six, nine and twelve months, and every six months thereafter, including comprehensive recording of adverse events and mortality. Data were analyzed according to whether participants received a concurrent antipsychotic drug (APD) versus those receiving pimavanserin alone. Participants who received a concurrent APD were significantly more likely to experience SAEs, including cognitive decline and infection. Increases in sedation, cardiovascular and stroke-related events, and thromboembolic events were also observed. In addition, there was a significant increase in mortality with concurrent APD use. These results highlight safety concerns with the use of current antipsychotics in PD, which appear similar to those reported previously in people with AD, and underscore the importance of identifying safer therapies for PDP that provide an effective alternative to current antipsychotic medications.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0265
PARKINSONISM-DEMENTIA
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28 patients (18 M,10 F) cared for Parkinsonism who presented memory decline evaluated by MMSE (mean 15) and respectively confirmed brain atrophy, aged from 24-76 (mean 58,57) years, were selected.
At age distribution were observed 2 picks of occurrence with 43% (50-60), 39% (60-70), succeed in elderly with 8% (>70 years), and youngest < 30 years of 10%. At social occupation; 36% retired, 10% professionals, 54% workers or unemployed.
Geographically they come from; center 43%, capital 29% and 28% from north and south of Albania equally dispersed.
Their isolated or combined ATCD; 21% vascular, cranial trauma or SRLS 8%, 10% smoking or alcoholics, 4% hepatitis, 43% nothing. Only 14% against 86% showed a familiar history. Disease duration was in 54% about 5 years, 25% of one year, 21% > 10 years. Parkinsonism was 54% hypertonic/akinetic and 46% of tremor form.
Among all 29% showed also spasticitet or 18% psychosis.
At MRI; in 82% brain atrophy in 14% of which,bi-Temporal DA-like atrophy was observed. As to diagnosis; idiopathic or vascular parkinsonism (25%, 21%), DA-like and MSA equally stated with 14%, Levy body disease 11%, CBD, neuroacantocitosis, MELAS and FTD with 4% respectively.
After treatment the half one had unchanged clinical state while others experienced clinical improvement.
Conclusion; parkinsonism patients with clinic of dementia as well were aged mostly between 50-70 years old, and presented more the akinetic/hypertonic form followed by the tremor form. The common diagnosis was the idiopathic and vascular parkinsonism, succeed less by MSA and other neurodegenerative diseases.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0598

MILD COGNITIVE IMPAIRMENT – CORRELATIONS OF IMAGISTIC AND CLINICAL DATA

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BACKGROUND: Mild cognitive impairment(MCI) is the clinical state between normal cognition and dementia in elderly people which does not interfere notably with activities of daily life; subjects perform more poorly on a variety of cognitive, functional and behavioral measures than normal persons of the same age.

MATERIAL AND METHOD: The study comprises 141 subjects between 60 and 92 years, diagnosed with MCI with Mini Mental State Evaluation (MMSE), clock drawing test (CDT) and computer tomography (CT).

MMSE score between 28-21 points is considered mild cognitive impairment.

RESULTS: The CT imaging showed specific types of cortical and subcortical atrophies. CT multislides was performed with 2.5 mm axial sections of brain and axial and coronary reconstruction with sections at 1mm, presenting accurate diagnosis criteria, highlighting the group studied the following:

-53.1% (75 patients) specific lesions to vascular dementia : moderate atrophy of the temporal lobe, hippocampus atrophy, frontal diffuse ischemia and subcortical hipodense ischemic lesions ;

-12.1% specific changes to Alzheimer's disease, as: obvious atrophy of hippocampus, ipsilaterale enhancement of temporal cone of lateral ventricle, temporal lobes atrophy, diffuse cerebral atrophy (lateral ventricles less extended), widening perimezencefalics cisterns;

-specific pathological changes due to age (34.8%): widening external and internal fluid spaces (lateral ventricles, ventricle III) enlargement of fronto-temporal fluid space, heterodense millimeter images on basal nuclei.

CONCLUSIONS: The study emphasizes the importance of imagistic examination to increase the accuracy of clinical diagnosis allowing early diagnosis of Alzheimer and vascular dementia, in order to recommend specific therapy from the early stages of cognitive dysfunctions.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0641
DIFFUSION TENSOR IMAGING OF IDIOPATHIC NORMAL-PRESSURE HYDROCEPHALUS AND THE CEREBROSPINAL FLUID TAP TEST

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Objectives: Damaged neural tracts contribute to the symptomatology of idiopathic normal-pressure hydrocephalus (INPH). Diffusion tensor imaging (DTI) is sensitive to microstructural changes in white matter not always detectable with ordinary MRI. We evaluated relationships between DTI parameters and clinical profiles in INPH patients, along with differences in these DTI measures between CSF tap test (CSFTT) responders and non-responders.
Methods: Fifty-four INPH patients (26 CSFTT responders and 28 CSFTT non-responders) constituted the final group for analysis. Fractional anisotropy (FA) and mean diffusivity (MD) were assessed using atlas-based tract mapping methods for 12 different fiber tracts.
Results: CSFTT non-responders, when compared to responders, showed lower FA values in the left anterior thalamic radiation (ATR) and left cingulum–hippocampus (CgH). The associations between clinical symptoms of INPH and DTI measures were most commonly found in the CgH. Higher MD in the CgH correlated with poorer cognitive performance. Higher MD in the right CgH also correlated with gait or motor dysfunction. Lower FA in the right CgH was correlated with urinary disturbance and cognitive impairment. Lower FA values in the corticospinal tract (CST) and right ATR significantly correlated with poorer cognitive performance, and correlated with gait dysfunction. Higher MD in left CST correlated with cognitive impairment.
Conclusions: Our findings may suggest a possibility for considering microstructural changes in white matter in ventriculomegaly patients as potential imaging markers for the prediction of CSFTT responders. We suppose that DTI parameters may help to assess cognitive dysfunction, gait, and motor deterioration in INPH, along with urinary disturbance.

06a. Imaging & Biomarkers: structural MRI

ADPD5-0901

SHAPE CHANGES OF HIPPOCAMPUS IN FRONTOTEMPORAL DEMENTIA AND ALZHEIMER'S DISEASE

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Objectives: To investigated hippocampus shape and cortical thickness patterns in subjects with behavioral variant frontotemporal dementia (bvFTD), progressive non-fluent aphasia (PNFA), and semantic dementia (SD) to understand the clinical implication of hippocampal shape changes.
Methods: From the Clinical Research Center for Dementia of South Korea–FTD Registry, 64 patients who fulfilled the diagnostic criteria by Knopman were recruited. All the participants completed thorough neuropsychological tests and 3-tesla brain MRI. Finally, MR data from 29 bvFTD, 11 PNFA, 24 SD, 17 subjects with normal cognition (NC), and 48 Alzheimer's disease (AD) were analyzed. The analyses were performed using methods previously described by authors. Institutional review board of the Asan Medical Center approved the study protocol.
Results: The hippocampal atrophy patterns were observed in the left hippocampal head (HH) and both hippocampal bodies (HB) in bvFTD, right HH and HB in PNFA, and almost all hippocampi in SD. Compared to bvFTD subjects, SD patients showed significant atrophy in the left HH and HB, which was more prominent than AD patients. Cortical thinning was observed in both frontal and temporal gyri in bvFTD, bilateral superior and middle frontal gyri and left inferior frontal gyrus in PNFA, and bilateral anterior temporal lobes in SD.
Conclusions: In conclusion, shape changes in the hippocampus possibly serve as additional biomarkers to differentiate the FTD subtypes. A relationship between the cortical thickness and the hippocampal shape will further denote the implication of hippocampal shape changes in patients with FTD.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1623

IMPAIRED LONG-TERM MEMORY FOR NEUTRAL BUT NOT EMOTIONALLY NEGATIVE STIMULI IN SUBJECTS AT RISK OF PD

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Objectives
Substantia nigra hyperechogenicity (SN^+) has been proposed as a risk marker for PD, Berg 2006). However, it currently remains unclear how SN^+ relates to dopaminergic function particularly with respect to learning and memory. Recent findings have supported a role for dopamine in human episodic long-term memory consolidation (Chowdhury et al., 2012). Here we investigated whether individuals with SN^+ demonstrate impaired long-term memory for novel events with respect to age-matched controls.

Methods
Eight healthy young (20-25), elderly (SN^-, 65-75) and SN^+ individuals (all groups M:F, 7:1) performed an incidental encoding task using emotional negative and neutral stimuli in which subjects were asked to discriminate between indoor/outdoor stimuli. Ten min and 6 hours after encoding subjects were asked to rate their recognition memory for the stimuli on a 5-point confidence scale.

Results
Healthy young individuals demonstrated a significant improvement in recollection of emotionally negative compared to neutral stimuli at 6 hours post encoding (corrected hit-rate emo vs neu 87.2% ± 2.4% vs 66.9% ± 8.5%, p = 0.03). This bias was not observed in SN^- individuals (emo vs neu 70.6% ± 8.0% vs 60.6% ± 6.1%) owing to a reduction in recollection of emotionally negative stimuli. SN^+ individuals demonstrated comparable recollection of emotionally negative stimuli with age-matched controls yet a significant decline in recollection of neutral stimuli (emo vs neu 72.2% ± 7.8% vs 51.6% ± 9.1%, p = 0.003).

Conclusions
Our preliminary findings suggest that preclinical SN pathology is associated with impaired long-term memory.

06a. Imaging & Biomarkers: structural MRI

ADPD5-1706

DISRUPTED WHITE MATTER INTEGRITY IN PARKINSON'S DISEASE WITH VISUAL HALLUCINATIONS

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Visual hallucinations carry poor prognosis in Parkinson's disease (PD). The integrity of structural connections in this condition has not been examined.

Magnetic resonance imaging was used to evaluate 12 PD patients with visual hallucinations (PDVH), 15 with PD without hallucinations (PDnonVH), and 14 healthy controls (HC). Diffusion tensor imaging (DTI) was used to calculate mean diffusivity (MD) and fractional anisotropy (FA) with tract-based spatial statistics (TBSS).

The PDVH had lower FA in regions coinciding with the left cortico-spinal tract, left superior longitudinal tract, right forceps major and right posterior inferior fronto-occipital fasciculus relative to PDnonVH and HC groups. FA in the right medial inferior fronto-occipital fasciculus and posterior inferior longitudinal fasciculus was however greater in PDVH than other groups. PDVH had lower FA in the right anterior thalamic radiation and right uncinate fasciculus, but higher FA in left uncinate fasciculus, bilateral posterior corpus callosum and bilateral medial inferior fronto-occipital fasciculus compared to HC. PDnonVH and HC had no difference in white matter indices.

In conclusion, PDVH had widespread abnormalities in the microstructure of visual pathways. Alongside degeneration reflected by lowered FA, there might be a potentially aberrant compensatory response signaled by higher FA, and these collectively contribute to visual hallucinations in PD.

06a. Imaging & Biomarkers: structural MRI

ADPD5-2082
JUVENILE, SYMPTOMATIC AND REVERSIBLE HEMIPARKINSON
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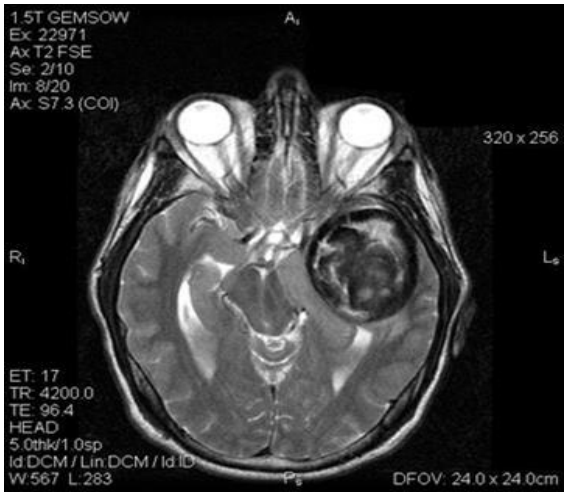
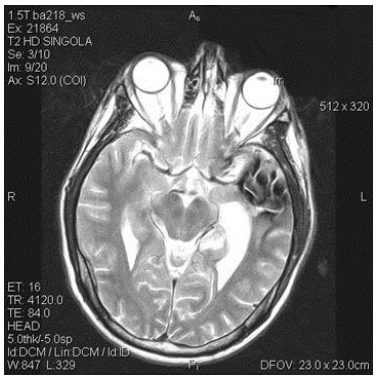
***Objectives**:* We show brain MRI and clinical features (video) of a peculiar and unilateral parkinsonism due to a giant aneurysm with good outcome after neurosurgery, widening the spectrum of conditions causing symptomatic parkinsonism. ***Case report**:* a 40-year-old man came to our attention with a 6-months history of tremor limited to his right arm. The tremor (4 Hz, middle amplitude) was present at rest during emotional activation or during drinking, shaving and writing. Neurological examination disclosed mild rigidity of the right arm and impairment of fine movements. Swing of the right arm was reduced during walking. Brain MRI revealed a left middle cerebral artery bifurcation giant aneurysm with almost complete thrombosis. The lesion resembled a space occupying process with compression on the left basal ganglia (F1). The patient underwent a complete aneurysm excision (F2). After surgery, the patient showed an almost complete clinical recovery indicating a still reversible impairment of nigro-striatal functions. ***Conclusions**:* A pure and strictly unilateral extra-pyramidal syndrome characterized this case suggesting a focal lesion in basal ganglia rather than an idiopathic Parkinson's disease. MRI disclosed an extremely rare cause of symptomatic hemiparkinsonism and allowed an effective treatment.



F1



F2

06a. Imaging & Biomarkers: structural MRI

ADPD5-2180

MAGNETIC RESONANCE IMAGING OF HIPPOCAMPAL SUBFIELDS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Objectives
Hippocampal dysfunction and alteration of hippocampal anatomy have been reported in several neuropsychiatric diseases. The hippocampus consists of anatomically distinct subfields. There are several data available that describe neuropsychiatric symptoms, focusing on cognitive impairments in systemic lupus erythematosus (SLE). We investigated whether in vivo volumes of hippocampal subfields differ between SLE patients and healthy control volunteers.

Methods
We recruited 18 SLE patients who meet the criteria of American College of Rheumatology 1997 (ACR 1997). Clinical examination and magnetic resonance imaging were performed in patients with SLE (n=18; mean age: 49.4 years (SD=12.0); 100% female) and in an age, gender, and education-matched healthy control group (n=20). We used FreeSurfer and manual segmentation techniques for labeling hippocampal subfields and cortical/subcortical regions in a high-resolution MRI setting at 3T (T1-weighted images with 1.0x1.0x1.0 mm resolution).

Results
SLE was associated with 15.6 % (SD=1.8) (P = 0.01) smaller mean Cornu Ammonis 1 and 3 (CA1/CA3) subfield volumes, whereas other subfields were spared. We found no evidence for smaller caudate nucleus, thalamus, or reduced neocortical volume in patients with SLE as compared with the control individuals. Cortical and subcortical volumes did not correlate with age and education.

Conclusions
Hippocampal subfield volume reductions were found in patients with SLE relative to matched healthy controls. The magnitude of reduction was significant only in CA1/CA3, which may be related to impaired cognition or affective disturbances. Further studies are warranted to elucidate the clinical correlates and pathophysiological mechanism of hippocampal subregion loss in SLE.

Keywords: Hippocampal subfields, magnetic resonance imaging

06a. Imaging & Biomarkers: structural MRI

ADPD5-2206

MRI IN THE RHINELAND STUDY: A PROTOCOL FOR MAPPING BRAIN STRUCTURE AND FUNCTION IN A PROSPECTIVE COHORT OF 30.000 ADULTS

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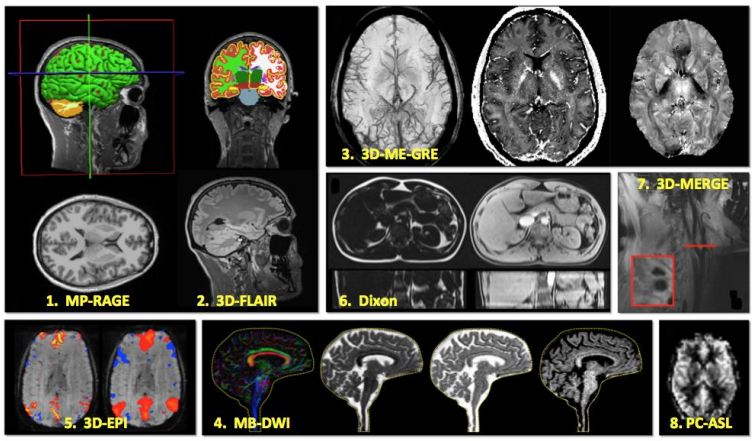
Introduction: The Rhineland Study is a prospective population study among 30,000 participants aged ≥30 years and investigates the aging human brain and related neurological disorders. This longitudinal study will include quantification of brain structure and function over the adult lifespan, using magnetic resonance imaging (MRI). Therefore, we are developing an advanced MRI protocol that enables state-of-the-art data collection at high-throughput (150-200 adults weekly)**.**

Methods: MRI data will be acquired on 3T Prisma scanners (Siemens, Erlangen), with high performance gradient system and 64 channel head-neck coils. We generally utilize whole-brain 3D sequences with 2D parallel imaging and elliptical k-space coverage. The MRI protocol (>1 hour scan time) is shown in Table 1. The Free Protocol will allow specific data acquisition in subsets of the Rhineland cohort.

Results: Preliminary results indicate that accelerated sequences provide excellent image quality for further analysis. For instance, the MP-RAGE at 0.8 mm yields accurate cortical surface reconstruction, whereas the 3D-FLAIR and 3D-ME-GRE provide detail for white-matter lesions and microbleed detection. The MB-DWI and 3D-EPI provide excellent data to quantify the connectome. Figure 1 depicts a representative single-subject dataset.

Conclusion: It is feasible to obtain high-quality MRI data in limited acquisition time. In-house development of MRI sequences guarantees long-term support. The versatile MRI protocol provides a multitude of quantitative biophysical information of brain tissue. The Rhineland study also includes clinical, lifestyle and neuropsychological exams and collection of biomaterials, which together with this MRI protocol will enable discovery and validation of novel biomarkers for neurodegeneration

Figure	Protocol	Sequence	Res. [mm^3]	Time	Modality / Purpose
0	Core	Scout	1.6x1.6x1.6	0:20	Auto Align
1	Core	MP-RAGE	0.8x0.8x0.8	7:00	Anatomy (T_1-w), grey/white matter volume and thickness
2	Core	3D-FLAIR	0.8x0.8x0.8	5:20	Anatomy (T_2-w), white-matter hyperintensities, segmentation
3	Core	3D-ME-GRE	1.0x1.0x1.0	4:45	Anatomy (T_2*-w), microbleeds, iron, calcification
4	Core	MB-DWI	1.6x1.6x1.6	10:00	Diffussion-weighted, white-matter connectome
5	Core	3D-EPI	2.4x2.4x2.4	10:00	Resting-state (BOLD), functional connectome
total scan time core protocol [min]				37:42	
6	Free	Dixon	5.0x5.0x10.0	0:05	Body fat
7	Free	3D-MERGE	0.8x0.8x0.8	4:04	Carotid artery MRI, plague build-up
8	Free	PC-ASL	3.2x3.2x3.2	6:00	Perfusion (CBF), bloodflow
0	Free	3D-EPI	2.4x2.4x2.4	10:00	Task-evoked fMRI (BOLD), functional localization, memory-related
0	Free	other		10:00	CSI, SVS, MT, qMRI, regions of interest
total scan time [min]				55:00	



06b. Imaging & Biomarkers: functional MRI

ADPD5-0336

METABOLIC PECULIARITIES AND DYSFUNCTION OF THE RESTING STATE NETWORK IN PATIENTS WITH PARKINSON'S DISEASE AND DIFFERENT LEVEL OF COGNITIVE IMPAIRMENT: MRS AND FMRI STUDY

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Purpose: We study integrity of RS DMN connectivity and local metabolic state in patients with PD and different level of CI.
Methods: Three groups of PD-patients (57-73y) are studied with 1.5T SIGNA EXCITE (GE). The 1st group (DPDG)-13 PD-patients with dementia. The 2nd group (CIPDG)-15 PD-patients with mild CI. The 3rd group (NPDG)-12 PD-patients with normal cognitive function. EPI BOLD scans were acquired using pulse sequence:TR/TE=3000/71ms. ICA analysis was used to identify 20 networks of RS-activity. Spectra are in anterior (APCG) and in posterior portion of cingulate gyrus (PPCG):TR/TE=1500/144ms.
Results: The analysis of the DMN revealed a gradual reduction in functional connectivity for the patients of DPDG in the cuneus, precuneus and PPCG, which correlate with severity of CI. RSNs involving areas of the cerebellum and frontal lobe, that could be interpreted as a potential compensatory mechanism to functional disorders caused by the CI. In NPDG connections between the APCG and PPCG, and inferior parietal gyrus bilaterally were found, activation of APCG decreased, but connectivity patterns persisted. In CIPDG activated clusters were found precuneally, and in PPCG, however no connection to the parietal lobe or APCG. In APCG the NAA/Cr in DPDG, CIPDG, NPDG:(1.68+-0.02),(2.04+-0.03),(2.32+-0.05), Cho/Cr:(0.84+-0.02),(0.81+-0.05),(0.53+-0.03). In PPCG NAA/Cr in DPDG, CIPDG, NPDG:(1.14+-0.12),(1.81+-0.02),(1.98+-0.04), Cho/Cr:(0.96+-0.02),(0.77+-0.03),(0.68+-0.03).Progressive decreasing NAA/Cr in PPCG and increasing of Cho/Cr for the patient of NPDG, CIPDG, DPDG were found.
Conclusions:Metabolic alterations in PPCG are indicators of neuronal loss and dysfunction, and may be marker of CI in PD-patients. fMRI, MRS give new approach for understanding pathophysiological changes in PD-patients associated with CI.

06b. Imaging & Biomarkers: functional MRI

ADPD5-0636

HIPPOCAMPAL RESTING-STATE CONNECTIVITY CHANGES IN EARLY ONSET VERSUS LATE ONSET ALZHEIMER'S DISEASE

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Objectives
Different clinical manifestations have been reported according to early onset Alzheimer's disease (EOAD) and late onset Alzheimer's disease (LOAD). Furthermore, the hippocampal atrophy is frequently not seen in mild cases of EOAD. In this study, we investigated the changes in functional connectivity of the hippocampus using resting state functional MRI (fMRI) between EOAD and LOAD to estimate the clinical usefulness of hippocampal connectivity in EOAD with relatively sparing hippocampal structure.
Methods
Total 30 mild AD patients (≤ CDR 1) and 31 age matched control subjects were recruited. According to age onset, patients were further categorized by EOAD (<65 years old) and LOAD (>70 years old). For the resting fMRI, 2D echo planar images were acquired from 3.0T MRI and Data Processing Assistant for Resting-State fMRI (DPARSF, V2.3) was used for analysis.
Results
The left inferior parietal cortex of LOAD was significantly ($p<0.001$) reduced in the functional connectivity with the hippocampus compared to the EOAD. Bilateral middle orbitofrontal, inferior orbitofrontal, and right superior temporal cortex as well as left inferior parietal cortex showed relatively reduced connectivity. On the contrary, left hippocampus in EOAD showed more reduced connectivity with left middle frontal, right superior parietal, left inferior frontal cortex, and bilateral middle cingulate gyrus than LOAD.
Conclusions
EOAD revealed more connectivity reduction of hippocampus with frontal and parietal areas compared with LOAD. This result is consistent with differences of clinical symptoms of previous reports. Therefore, hippocampal connectivity may consider one of early diagnostic markers in EOAD with relatively mild hippocampal atrophy.

06b. Imaging & Biomarkers: functional MRI

ADPD5-1466

COMBINED USE OF VOXEL-BASED MORPHOMETRY AND FUNCTIONAL MRI IN PATIENTS WITH MEMORY IMPAIRMENT AFTER TRAUMATIC BRAIN INJURY.

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Objectives. The purpose of this study was to evaluate brain activation by visual memory task in patients with posttraumatic memory impairment and to determine correlation between memory impairment and atrophy of memory specific brain regions.
Methods. To investigate the organization of memory and localize cortical areas activated by visual memory task we used functional MRI and to evaluate brain atrophy we used voxel-based morphometry (VBM). We studied 25 patients (age 29,6±8,9 years), 22 matched by age volunteers as a control group. For test stimuli we used series of 12 not related images for "baseline" and 12 images with for "active". 6 images in “active” period have been already presented in “baseline”. Stimuli were presented 3 times with reduction of repeated images to 4 and 2. For VBM and functional data post-processing we used SPM8.
Results. FMRI showed less activation in hippocampal formation and parahippocampal gyri comparing to controls group. The study also showed reduced activation in posterior cingulate gyrus. VBM showed general atrophy of grey matter, especially of both temporal lobes (fusiform and parahippocampal gyri), frontal lobes (superior frontal gyri), parietal lobes and cingulate gyrus. The most significant changes were found in mediobasal temporal lobe (up to 3.6 cm^3 at $p<0,01$) and thalamuses (up to 4.5 cm^3 at $p=0,01$).
Conclusions. Combined application of fMRI and VBM allows to assess brain atrophy along with functional component of memory impairment and can help to detect effects of traumatic brain injury before they may be revealed by means of conventional MRI study.

06b. Imaging & Biomarkers: functional MRI

ADPD5-1555

EMOTIONAL PROCESSING DYSFUNCTIONS IN MILD COGNITIVE IMPAIRMENT ARE THE RESULT OF LACKING NEURONAL INHIBITION

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Objectives
The recognition of emotional stimuli depends on the neuronal interplay of brain regions involved in memory- and emotional processing. Individuals with mild cognitive impairment (MCI) have problems to judge about the emotional content of images. We examined whether this is the result of functional impairments in the memory- or emotional system.
Methods
In 21 participants (10 MCI), we applied functional magnetic resonance imaging (fMRI) during a sustained emotional attention task. Participants should rate the valence of images (i.e. neutral or negative). We also recorded GABA-sensitive MR spectroscopy in the posterior cingulate cortex. Significant fMRI group differences are reported at $p < 0.001$ (uncorrected). Interactions between fMRI and GABA will be assessed by Spearman's correlations ($p < 0.01$).
Results
Accuracy was comparable between groups ($p > 0.1$). In participants with MCI, the contrast "negative - neutral stimuli" revealed lower fMRI signal responses in the hippocampus but larger responses posterior cingulate cortex relative to controls. In this region, GABA negatively correlated with the fMRI signal strength for negative stimuli ($p < 0.01$, $r^2 = 0.75$).
Conclusions
Our results suggest: First, participants with MCI need to engage a brain region (the posterior cingulate cortex) classically associated with resting-state activity. Second, the lack of hippocampal activity indicates a disturbed memory processing in MCI. Third, the observed coupling between lower levels of GABA and higher fMRI signal responses indicates that the control of (negative) emotional stimuli depends on local inhibitory control.

06m. Imaging & Biomarkers: SPECT imaging

ADPD5-0406

BRAIN PERFUSION SPECTCAN DIFFERENTIATE CLINICAL SUBTYPES OF PD

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Backgrounds: The clinical heterogeneity of Parkinson's disease (PD) is well recognized. It is possible to distinguish two main clinical subgroups, a tremor dominant PD (TP) and non-tremor dominant PD (NTP). However studies compared the regional cerebral blood flow between subtypes of PD had rarely been performed yet. Therefore, we conducted this study to investigate difference of perfusion SPECT in clinical subtypes of PD (TP and NTP) using SPM program. **Methods**: We recruited 43 patients with PD (21 TP and 22 NTP). All patients with PD underwent brain perfusion SPECT and evaluated motor severity by using the Hoehn and Yahr stage and Part III of the Unified Parkinson's Disease Rating Scale (UPDRS). We also compared tremor and non-tremor symptoms with motor phenotype scores between two subtypes of PD. **Results**: Brain perfusion SPECT showed significant hypoperfusion in TP compared with NTP in the left occipital lobe and middle occipital gyrus, left frontal lobe and superior frontal gyrus, cerebellar hemisphere, left lentiform nucleus. On the other hand, brain perfusion SPECT of NTP compared with TP showed hypoperfusion in right parietal lobe and precuneus, left temporal lobe and inferior temporal gyrus, both frontal lobe and inferior frontal gyrus. **Conclusion**: The present study indicates that pathophysiology of TP compared to NTP has more closely association with neuronal circuits including cerebellar pathway, such as CTC circuit. Therefore, we cautiously assert that neuronal systems other than the nigrostriatal dopaminergic system are likely involved in the generation of tremor in PD.

06n. Imaging & Biomarkers : multimodal imaging

ADPD5-1581

DISEASE-SPECIFIC STRUCTURAL AND FUNCTIONAL CHANGES IN THALAMUS AND DENTATORUBROTHALAMIC TRACT IN PSP

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Objectives
To identify disease-specific changes of the thalamus, the basal ganglia, pons and midbrain in progressive supranuclear palsy (PSP) using diffusion tensor imaging, resting state fMRI (rs-fMRI) and volumetric analysis.
Material and methods
Two cohorts were used. In cohort A, MRI diffusion and volumetric data were acquired in controls (n=21), PSP (n=27), Parkinson's disease (PD) and multiple-system atrophy (MSA-P) (n=11). In cohort B, comprising 30 controls and 8 patients with PSP, rs-fMRI data and clinical measures of motor performance and balance were available in addition to MRI diffusion and volumetric data. ROI-based analysis and tractography of diffusion data and seed-based analysis of rs-fMRI data were performed.
Results
In cohort A, we observed changes in mean diffusivity (MD) in the thalamus, red nucleus, superior cerebellar peduncle and midbrain in patients with PSP; most of these, including thalamic abnormalities, were not found in patients with PD or MSA-P. These changes were validated in cohort B. Further, MD of the dentatorubrothalamic tract was increased in PSP patients from both cohorts. Increased MD in the thalamus and along the dentatorubrothalamic tract correlated with impaired motor function or balance in patients with PSP. Volumetric analysis showed reduced thalamic volumes in PSP. The connectivity between the thalamus and anterior cingulate gyrus, caudate nucleus as well as frontal regions was reduced in PSP compared to controls.
Conclusions
Patients with PSP, but not PD or MSA-P, exhibit signs of structural and functional abnormalities in thalamus and in the dentatorubrothalamic tract. These changes might be associated with impaired balance.

06o. Imaging & Biomarkers: EEG & brain mapping

ADPD5-0345
QUANTITATIVE EEG AND MEDIAL TEMPORAL LOBE ATROPHY IN ALZHEIMER'S DEMENTIA

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The electroencephalogram (EEG) abnormalities in Alzheimer's disease (AD) have been widely reported and medial temporal lobe atrophy (MTLA) is one of the hallmarks in early stage of AD. We aimed to assess the relationship between EEG abnormalities and MTLA, and its clinical validity. A total of 18 patients with AD were recruited (the mean age: 77.83 years). Baseline EEGs were analyzed with quantitative spectral analysis. MTLA was assessed by a T1-axial visual rating scale. In relative power spectrum analysis according to the right MTLA severity, the power of theta waves in C4, T4, F4, F8, and T5 increased significantly and the power of beta waves in T6, C4, T4, F8, T5, P3, T3, F7 decreased significantly in severe atrophy group. In relative power spectrum analysis according to the left MTLA severity, the power of theta waves in T3 increased significantly and that of beta waves in P4, T6, C4, F4, F8, T5, P3, C3, T3, F3, F7 decreased significantly in severe atrophy group. The severe MTLA group, regardless of laterality, showed more severe quantitative EEG alterations. These results suggest that quantitative EEG abnormalities are correlated with the MTLA, which may play a important role in AD process.

06r. Imaging & Biomarkers: other

ADPD5-1782
VALIDATION OF GAIT ANALYSIS USING THE SINGLE-POINT TRIAXIAL ACCELEROMETRY.

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Objectives
We investigate the validity of the single-point triaxial accelerometry in gait analysis.
Methods
82 healthy elderly (48 men and 34 women, age : 68.9 ± 6.5) participants were recruited. The triaxial accelerometer was attached on L3 spine level as they walked. Subjects were given instructions to stand up from a chair, walk 20m at their comfortable speeds, turn around, walk back and sit down. *GAITRite*™ walkway was placed on the middle of walking distance. This protocol was carried out 3 times including one practice trial. MATLAB R2014a was used for signal processing and gait parameters estimating. Gait analysis was done using acceleration scores of each axis (mediolateral,vertical,anteroposterior) independently. The level of agreement with *GAITRite*™ was determined using intraclass correlation coefficients [ICC(3,1)] via SPSS 19.
Results
Table 1. The level of agreement between triaxial accelerometer and GAITRite™ (n=82). $\mu \pm \sigma$ = mean ± standard deviation.

Parameter	GAITRite® (μ±σ)	Intraclass Correlation (3,1)			Accelerometor (μ±σ)		
		Mediolateral	Vertical	Anteroposterior	Mediolateral	Vertical	Anteroposterior
Cadence (steps/min)	120.21±8.05	0.96 [0.93,0.97]	0.94 [0.90,0.96]	0.95 [0.93,0.97]	119.22±8.40	119.27±7.73	119.35±8.41
Velocity (m/s)	1.27±0.14		0.91 [0.86,0.94]			1.29±0.16	
Step time (s)	0.50±0.03	0.96 [9.32,0.97]	0.94 [0.91,0.96]	0.96 [0.94,0.97]	0.51±0.04	0.51±0.03	0.51±0.04
Stride time(s)	1.00±0.07	0.95 [0.92,0.97]	0.96 [0.94,0.97]	0.95 [0.93,0.97]	1.01±0.07	1.01±0.07	1.01±0.07
Step length (cm)	63.23±5.29	0.91 [0.86,0.94]	0.88 [0.82,0.92]	0.92 [0.88,0.95]	64.88±5.98	63.77±5.43	64.81±5.92
Stride length (cm)	126.64±10.59	0.90 [0.85,0.94]	0.91 [0.87,0.94]	0.91 [0.87,0.94]	129.97±12.00	129.51±11.90	129.74±11.88

Acceleration-based gait analysis showed excellent agreement with GAITRite™ over three axes; Cadence (ICC=0.94~0.96), Velocity (ICC=0.91), Step time (ICC=0.94~0.96), Step length (ICC=0.88~0.92), Stride time (ICC=0.95~0.96), Stride length (ICC=0.90~0.91). Right-left foot discrimination could be done from mediolateral acceleration data. Gait analysis using mediolateral and anteroposterior acceleration data showed higher agreement in step time and step length than vertical one.
Conclusions
Triaxial accelerometer based gait analysis gave accurate measurements over each of the three axes in healthy elderly subjects. Using tri-directional acceleration data simultaneously would be more powerful for analyzing gait.

06r. Imaging & Biomarkers: other

ADPD5-1853
IMPACTS OF AGE AND GENDER ON GAIT IN THE ELDERLY.
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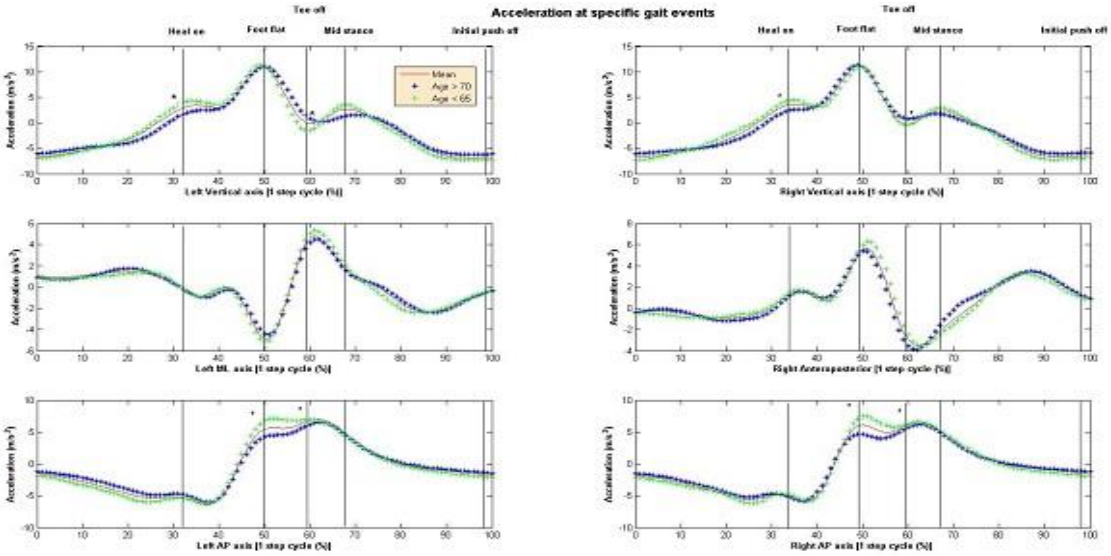
Objectives
To explore the impact of age and gender on gait parameters derived from triaxial accelerometer in healthy seniors.
Methods
82 healthy elderly participants were recruited. The triaxial accelerometer was attached on L3 spine level. Subjects underwent three trials of 40m walking. MATLAB R2014a was used for signal processing and gait parameters estimating. Within-subject ensemble-average patterns were generated from at least 40 strides for each subject. Position of specific gait events and magnitude of acceleration at those events were determined from the pattern.
Results
There was no difference in the average ages of the women and men. Height, leg length and weight were bigger in men while those were not different by age. Step length, proportion of swing phase and single support phase was larger in men. (after correction of height, leg length and age). Walking speed and step length were smaller in older group. That decrease of walking speed was contributed by shorter step length, while the cadence was maintained. Subjects among younger age group had bigger acceleration scores at several gait events. Mediolateral acceleration showed definite difference by sex.

Conclusions
Age was related to changes in velocity and step length. Gender affected the proportion of gait phases. Gender impact on acceleration was observed most dramatically on mediolateral axis while age impact was biggest on vertical and anteroposterior axis.



06r. Imaging & Biomarkers: other

ADPD5-1854
VALIDATION OF GAIT ANALYSIS USING THE SINGLE-POINT TRIAXIAL ACCELEROMETRY.
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Objectives
We investigated the validity of the single-point triaxial accelerometry in gait analysis.
Methods
82 healthy elderly (48 men and 34 women, age : 68.9 ± 6.5) participants were recruited. The triaxial accelerometer was attached on L3 spine level as they walked. Subjects were given instructions to stand up from a chair, walk 20m at their comfortable speeds, turn around, walk back and sit down. *GAITRite*™ walkway, which was employed as a gold standard, was placed on the middle of walking distance. This protocol was carried out 3 times including one practice trial. MATLAB R2014a was used for signal processing and gait parameters estimating. Gait analysis was done using acceleration scores of each axis (mediolateral,vertical,anteroposterior) independently. The level of agreement with *GAITRite*™ was determined using intraclass correlation coefficients [ICC(3,1)] via SPSS 19.
Results

Acceleration-based gait analysis showed excellent agreement with GAITRite™ over three axes; cadence (ICC=0.94~0.96), velocity (ICC=0.91), step time (ICC=0.94~0.96), step length (ICC=0.88~0.92), stride time (ICC=0.95~0.96), and stride length (ICC=0.90~0.91). Right-left foot discrimination could be done from mediolateral acceleration data. Gait analysis using mediolateral and anteroposterior acceleration data showed higher agreement in step time and step length than vertical one.
Conclusions
Triaxial accelerometer may be a cost-effective and convenient alternative in gait analysis to the gait analyzers using pressure or video sensors.

Table 1. The level of agreement between triaxial accelerometer and GAITRite™ (n=82). $\mu \pm \varepsilon$ = mean ± standard deviation.

Parameter	GAITRite® ($\mu \pm \varepsilon$)	Intraclass Correlation (3,1)			Accelerometor ($\mu \pm \varepsilon$)		
		Mediolateral	Vertical	Anteroposterior	Mediolateral	Vertical	Anteroposterior
Cadence (steps/min)	120.21±8.05	0.96 [0.93,0.97]	0.94 [0.90,0.96]	0.95 [0.93,0.97]	119.22±8.40	119.27±7.73	119.35±8.41
Velocity (m/s)	1.27±0.14		0.91 [0.86,0.94]			1.28±0.16	
Step time (s)	0.50±0.03	0.96 [9.32,0.97]	0.94 [0.91,0.96]	0.96 [0.94,0.97]	0.51±0.04	0.51±0.03	0.51±0.04
Stride time(s)	1.00±0.07	0.95 [0.92,0.97]	0.96 [0.94,0.97]	0.95 [0.93,0.97]	1.01±0.07	1.01±0.07	1.01±0.07
Step length (cm)	63.23±5.29	0.91 [0.86,0.94]	0.88 [0.82,0.92]	0.92 [0.88,0.95]	64.88±5.98	63.77±5.43	64.81±5.92
Stride length (cm)	126.64±10.59	0.90 [0.85,0.94]	0.91 [0.87,0.94]	0.91 [0.87,0.94]	129.97±12.00	129.51±11.90	129.74±11.88

06r. Imaging & Biomarkers: other

ADPD5-1912
EVALUATION OF BIOMARKERS TROPONIN-I AND CK-MB IN SERUM OF MYOCARDIAL INFARCTION PATIENTS

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Abstract—Myocardial infarction (MI), commonly known as a heart attack, results from the interruption of blood supply to a part of the heart, causing heart cells to die. The most often used blood markers for myocardial infarction are the creatine kinase-MB (CK-MB) fraction and the troponin levels. Troponin is a highly sensitive and specific marker of myocardial Infarction. Complete study was designed by drew sample of 30 patients and compare them with 30 normal individuals age and gender wise respectively.
Results of complete study shows that significantly increased levels of cardiac biomarkers were found in age of 30-60 & 60-80 in cardiac patients as compare to normal one of same age group. The results of this study also show that levels of these biomarkers also indicate variation gender wise. The level of both biomarkers increases in female patients as compared to male patients.

Table I:

		Cardiac patients			Normal individuals			P values
Biomarkers	Age (years)	Mean ±SD	Median	Range	Mean± SD	Median	Range	
CK- MB	30-60	39.28±16.81	34.5	53	10.30±4.43	10	14	0.011
	60-80	44.13±29.75	31	114	14.85±5.97	13	17	0.024
Troponin – I	30-60	8.24±11.99	3.52	37.91	0.13±0.10	0.11	0.29	0.000
	60-80	9.34±14.89	2.39	49.59	0.18±0.11	0.18	0.34	0.001

Table II:

		Cardiac patients			Normal individuals			P values
Biomarkers	Gender	Mean ±SD	Median	Range	Mean ± SD	Median	Range	
CK- MB	Male	36.24±13	31	43	13.07±5.50	11	17	0.031
	Female	47.92±32.38	35	114	11.47±5.68	10	19	0.023
Troponin – I	Male	5.70±9.27	2.36	34.5	0.12±0.10	0.08	0.31	0.041
	Female	12.27±16.69	4.01	49.58	0.18±0.10	0.19	0.33	0.030

06r. Imaging & Biomarkers: other

ADPD5-2055

DOES THE RIGHT INFERIOR PARIETAL LOBULE CORRELATE WITH TASK-UNRELATED THOUGHTS? : A TRANSCRANIAL DIRECT CURRENT STIMULATION STUDY

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Background: Task-unrelated thought (TUT) is associated with various impairments and excessive TUTs or rumination are reported in both normal and pathological conditions such as attention deficit/hyperactivity disorder (ADHD), alzheimer's desease, and mild cognitive impairment. Although TUTs have been associated with activity in the right inferior parietal lobule (IPL), which is a core region of the default mode network (DMN), the causal relationship remains unclear.
Methods: We investigated the relationship between the right IPL and TUT by applying transcranial direct current stimulation (tDCS), which efficacy was proved in psychiatric diseases such as depression, to the right IPL/left supraorbital forehead prior to a perceptually demanding flanker task, and TUTs during the task were compared among different tDCS conditions.
Results: Compared to the sham condition, the TUT index was increased and decreased by cathodal and anodal tDCS, respectively. The observed correlation of the TUT index with task performance supported the validity of the index. Moreover, anodal and cathodal stimulation induced the flanker effect (i.e., response delay caused by peripheral conflicting stimuli) in a more perceptually demanding condition, confirming the stimulation effect, and reduced it in a less perceptually demanding condition. These results support our hypothesis that tDCS to the right IPL modulates TUT frequency in a following attention-demanding flanker task, indicating that the right IPL plays causal role in regulating TUTs.
Conclusions: Our findings contribute to understanding the neural underpinnings of TUT and demonstrate that tDCS is a non-invasive way to modulate TUT occurrence that could be an efficient intervention in MCI and alzheimer's desease.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-2170

TYPICAL AGING VERSUS COGNITIVE "SUPER-AGING" IN COMMUNITY-DWELLING OLDER ADULTS: LONGITUDINAL TRAJECTORIES IN CORTICAL THICKNESS AND VOLUME OVER SIX YEARS

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Objectives: Typical aging is usually accompanied by memory and executive function declines. Previous research has identified a group of 'super-agers" (SA) that retain cognitive abilities comparable to peers 20-30 years younger. In SAs, greater whole-brain cortical thickness and volume have been reported compared with typical-agers (TA). However, longitudinal data is lacking. This study evaluated baseline and longitudinal brain morphology trajectories in these groups.
Methods: Cognitively healthy (based on Clinical Dementia Rating/MMSE) participants (mean age = 71.08, SD= 6.28) were identified from the Australian Imaging Biomarker and Lifestyle (AIBL) study. Cognitive testing and brain Magnetic Resonance Imaging were completed every 18 months for six years. SAs (n=15) scored at/above normative mean memory and executive scores for individuals aged 30-44. Conversely, TAs (n=14) scored in the average range for same-aged peers. Groups were similar in age, education, gender, and *APOE* status (*p*s > 0.30).
Results: A mixed-model 2 (Group) x 5 (time) ANOVA revealed a group x time interaction for overall cortical thickness ($p < 0.05$), whereby groups did not differ at the first three time points, but SAs had greater cortical thickness at 54 and 72 months (*p*s < 0.05) compared to TAs. No differences in volume were found.
Conclusions: SAs and TAs displayed similar cortical thickness at baseline, but differing trajectories. The attenuated cortical thinning seen in SA could be a morphological effect of compensatory cognitive activity. This may involve preferential allocation of cognitive resources to monitoring and control, with subsequent implications for differential thickness in related neural regions.

07a. Epidemiology, Risk Factors, Genetics & Epigenetics: aging

ADPD5-2171

20 YEARS OF CUMULATIVE RISK AFFECTS VERBAL MEMORY PERFORMANCE IN AGEING: DATA FROM THE WOMEN'S HEALTHY AGEING PROJECT.

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Background: It is estimated that targeting modifiable risk factors has the potential to prevent millions of cases of dementia worldwide [1] but this analysis assumes causation. To date no consensus exists on what the modifiable risk factors are for Alzheimer's Disease or when to target [2]. Few studies have longitudinal data stretching over multiple decades which assess all of these risks to determine the optimum targets and timing for intervention to improve cognition in ageing. In this paper we examine the timing and exposure to predictive factors on verbal memory performance in late life.
Methods: 389 participants had complete neuropsychiatric assessment and clinical information, physical measures and biomarkers collected over at least 2 time-points in the Women's Healthy Ageing Project. Mixed Linear models were conducted to assess the significance of main and interaction effects of risk factors on later life verbal memory. We explore the influence of latent, contemporaneous and cumulative exposures.
Results: Age and education were associated with baseline memory testing. Over the 20 years of study follow-up cumulative mid to late life physical activity had the strongest effect on later life memory, with cumulative hypertension and suboptimal HDL being the next most likely contributors to poor memory in later life.
Conclusion: Earlier modification appears ideal for disease prevention, with intervention after 65 missing two decades of significant cumulative risk. Further work will determine if irreversible neuropathological changes are associated with this risk exposure.

1 - Barnes et al , 2011
2- Di Marco et al, 2014

07c. Epidemiology, Risk Factors, Genetics & Epigenetics: metabolic

ADPD5-1540
REVERSIBLE METABOLIC ENCEPHALOPATHY ASSOCIATED WITH FOLATE DEFICIENCY AFTER SELF STARVATION FOR DIET
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Background: Folate is essential for the formation of biogenic amines and pterins in the central nervous system. Folate deficiency may lead to various neurological symptoms and disorders, including cognitive impairment, dementia, depression and movement disorders. Low folate and raised homocysteine levels are risk factors for cardiovascular disease and dementia. But, reversible metabolic encephalopathy associated with folate deficiency was rarely reported and folate deficiency after self starvation was not reported.
Case : A 43-year-old female presented with dizziness and tilting tendency. She was poor oral intake and had fasted intermittently for diet. Her neurological examinations revealed not only abulic symptoms diminished the number of words, but also recently aggravated memory impairment. Brain MRI demonstrated asymmetric and bilateral hyperintense lesions in the both corona radiata, lentiform nuclei, thalamus, midbrain and pons on T2, FLAIR and DWI. Laboratory blood test showed normal range of thyroid function, thiamine (95.9nmol/L), vitamine B12 (682.31pg/mL) except Folic acid (2.82ng/mL). Further evaluation revealed increased homocysteine (10.3umol/L) and abnormal activity of methylenetetrahydrofolate reductase (MTHFR) (60% of normal activity). Her neurological symptoms and the abnormalities in the brain MRI showed rapid improvement after administration of folic acid.
Conclusions: To our knowledge, this is the first case report of reversible metabolic encephalopathy associated with folate deficiency after self starvation for diet. As like this case, if there are neurological symptoms with folate deficiency after self starvation or diet, it should be considered diagnosis of metabolic encephalopathy.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-0346

BILATERAL STRIATAL NECROSIS IN A CHINESE FAMILY, A NEW TYPE?

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Objective

To report the clinical characteristics, gene mutation of bilateral striatal necrosis in a Chinese family.

Material and Methods

A family with BSN in the remote northwest mountain areas was collected. The proband was a 20 year old woman with dystonia, the elder and younger sister had the similar symptoms(Figure 1). The head MRI was performed for two patients and CT for one patient, what's more, muscle biopsy was performed for the proband. The NUP62,SLC19A3,SLC25A19,PANK2 gene and mitochondrial gene were examined.

Results

Three patients manifested generalized dystonia and the MRI or CT image showed bilateral striatal necrosis(Figure 2). There were no evident pathologic findings in the skeletal muscle. In the NUP62 gene, we found a cDNA337G-A heterozygous mutation(pG113S mutation). The same mutation was found in the other two patients, the normal sister, the father and the grandmother. The other genes(PANK2,SLC25A19,SLC19A3 and mitochondrial gene) were normal.

Conclusion

The clinical features of BSN in this family were summarized. The correlation between BSN and some known genes was ruled out. We deduce that the disease may be a new type and further study need to be done.

[key words]: bilateral striatal necrosis, gene, mutation, pedigree.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-0778

HMGCR IS A GENETIC MODIFIER FOR RISK, AGE OF ONSET AND MCI CONVERSION TO ALZHEIMER'S DISEASE IN A THREE COHORTS STUDY.

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Several retrospective epidemiological studies report that utilization of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) inhibitors called statins at mid-life can reduce the risk of developing sporadic Alzheimer's disease (AD) by as much as 70%. Conversely, administration of these inhibitors in clinically diagnosed subjects with AD confers little or no benefits over time. Here, we investigated the association between AD and *HMGCR* rs3846662, a polymorphism known to be involved in regulation of *HMGCR* exon 13 skipping, in a founder population and in two distinct mixed North American populations of converting mild cognitively impaired (MCI) subjects [ADCS and ADNI cohorts]. Targeting more specifically women, the G allele negative (G-) AD subjects exhibit delayed age of onset of AD [***P* = 0.017**] and significantly reduced risk of AD [**O.R.: 0.521; *P* = 0.0028**], matching the effect size reported by the *APOE2* variant. Stratification for *APOE4* in a large sample of MCI patients from the ADCS cohort revealed a significant protective effect of G negative carriers on AD conversion three years after MCI diagnosis [**O.R.: 0.554; *P* = 0.041**]. Conversion rate among *APOE4* carriers with the *HMGCR*'s G negative allele was markedly reduced [**from 76% to 26.97%**] to levels similar to *APOE4* non-carriers [27.14%], which strongly indicate protection. Conversion data from the independent ADNI cohort also showed significantly reduced MCI or AD conversion among *APOE4* carriers with the protective A allele [***P* = 0.005**]. In conclusion, *HMGCR* rs3846662 act as potent genetic modifier for AD risk, age of onset and conversion.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-0796

UNUSUAL AD/ALS PHENOTYPE IN A PATIENT CARRYING THE C9ORF72 EXPANSION AND A SQSTM1 MUTATION

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Objectives: A genotype-phenotype correlation in a patient with late-onset Amyotrophic lateral sclerosis (ALS) with dementia carrying the *C9orf72* repeat expansion and the exon 1 (c. 47C>T, p. A16V) mutation in the sequestosome 1 (*SQSTM1)* gene [1].

Methods: DNA alteration was sequenced by ABI PRISM 310 sequencer. Genetic testing for repeats expansions in *C9orf72* gene was performed with microsatellite analysis on ABI 3730 DNA Analyzer

Results: Initially, the patient showed a progressive cognitive and behavioral impairment at 71 years. Memory difficulties, language disturbances and severe anxiety and agitation were described. After one year, he became aware of weakness of two fingers of the right hand. A first Electromyography (EMG) examination directed towards a possible carpal tunnel syndrome, but a second EMG revealed signs of motoneuron disease and he was diagnosed as ALS with spinal onset.

Brain magnetic resonance images showed moderate atrophy of the temporal structures (temporal lobes, hippocampus) suggesting Alzheimer's disease (AD) features.

The patient died of head injury from an accidental fall at age 74.

Conclusion: Combining clinical and genetic data, our findings suggest that the co-existence of *C9orf72* and SQSTM1 mutations may be implicated in the pathogenesis of AD/ALS unusual phenotype.

1. van der Zee J, et al., Rare mutations in SQSTM1 modify susceptibility to frontotemporal lobar degeneration. Acta Neuropathol. 2014;128(3):397-410.

07k. Epidemiology, Risk Factors, Genetics & Epigenetics: disease-causing mutations

ADPD5-2005

TARGETED EXOME CAPTURE AND SEQUENCING IDENTIFIES NOVEL SPG 11 MUTATION IN HEREDITARY SPASTIC PARAPLEGIA WITH THIN CORPUS CALLOSUM IN A CHINESE FAMILY

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Introduction: Hereditary spastic paraplegia (HSP) is a neurodegenerative disease characterized by progressive weakness and spasticity of the lower extremities, in complicated forms with additional neurological signs.

Material and methods: Nine subjects from the family were examined through detailed clinical evaluations, physical examinations and genetic tests. Targeted exome capture technology was utilized in identifying gene mutation.

Results: A novel compound heterozygous mutations in the *SPG11* gene were identified in this family, consisting of c.4001_4002insATAAC and c.4057C>G.

Conclusion: Our findings broaden the spectrum of *SPG11* mutations causing HSP and the phenotypic spectrum of the disease in Chinese patients. The targeted exome capture technology is an efficient method for molecular diagnosis in HSP patients.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0212

EVALUATION THE RELATIONSHIP OF EDUCATION LEVEL AND COGNITIVE IMPAIRMENT WITH MOCA TEST

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Objective:Mild cognitive impairment (MCI) is defined as “A cognitive decline greater than that expected for an individual’s age and education level but that does not interfere notably with activities of daily life.The Montreal Cognitive Assessment is a screening test for MCI.
Methods:We investigated the performance of MoCA in detecting MCI among elderly persons, a majority of whom have low level of education. Educational level was divided into three categories: group 1: Less than primary (<5 years) group 2: Primary (5 years), group 3: More than primary (more than 5 years). We evaluated effect of education in theMoCA scores and compared their test performance among group 1, 2 and 3.
Results:A total of 50 patients with a MCI (mean age 70.74±7.87, men) met the inclusion criteria.There was no differences by education in the total scores and the subscores for visuospatial/executive function, naming, attention, abstraction, delayed recall. Language was the only domain that showed significant differences between groups. In post hoc anlaysis, differences were found between group 1 and 3, and group 1 and 2. Group 1 had significantly lower scores for language.Repeat subscore of language was significantly lower in group 1 than group 2. In fluency, there were significant differences between group 2 and 3, and group 1 and 3.
Conclusion: Our results emphasized the need to adapt this test in only language section, thus it can be easily used in population with low education.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0335

DECLINE IN COGNITIVE FUNCTION AND ELDER MISTREATMENT: FINDINGS FROM THE CHICAGO HEALTH AND AGING PROJECT

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Background: This study aimed to examine the longitudinal association between decline in cognitive function and elder mistreatment (EM).
Method: Longitudinal study of a geographically-defined community in Chicago. Chicago Health and Aging Project (CHAP) is an epidemiological study conducted in a geographically-defined community (N=6,159). We identified 143 CHAP participants who had longitudinal cognitive data and EM reported to social services agency. The primary predictor was cognitive function, which was assessed using the Mini-Mental State Examination (MMSE), the Symbol Digit Modalities Test (Perceptual Speed), and both immediate and delayed recall of the East Boston Memory Test (Episodic Memory). An index of global cognitive function scores was derived by averaging z-scores of all tests. Logistic regression models were used to assess the association of cognitive function domains and risk for EM.
Results: After adjusting for potential confounders, every 1 point decline in global cognitive function (OR, 1.57(1.21-2.03)), MMSE (OR, 1.07(1.03-1.10)), episodic memory (OR, 1.46(1.14-1.86) and perceptual speed (OR, 1.05(1.02-1.07)) scores were associated with increased risk for EM. Lowest tertiles in global cognitive function (OR, 2.71 (1.49-4.88), MMSE (OR, 2.02 (1.07-3.80)), episodic memory (OR, 2.70(1.41-5.16)) and perceptual speed (OR, 4.41 (2.22 -8.76)) scores were associated with increased risk for EM.
Conclusion: Decline in global cognitive function, MMSE, and perceptual speed scores were associated with increased risk for EM.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0497

RELIABILITY AND VALIDITY OF A NEW BEHAVIORAL SCALE TO MEASURE BEHAVIORAL AND PSYCHOLOGICAL SYMPTOMS IN DEMENTIAS (BPSD): LUTHRA'S BEHAVIORAL ASSESSMENT AND INTERVENTION RESPONSE (LUBAIR) SCALE

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OBJECTIVES: Establish reliability and validity of LuBAIR Scale. It is hypothesized LuBAIR will be less labour intensive, more comprehensive and improve categorization of behaviors into clinically meaningful categories.

METHODS Seven Long Term Care Facilities in Ontario, Canada, were selected for the study. 120 residents with a dementia diagnosis were recruited. Sixty residents exhibiting BPSDs were included in the study group and sixty participants not displaying BPSDs in the control group. Pittsburg Agitation Scale was used to screen for presence of BPSDs. Two registered nurses (RN) completed LuBAIR scale, BEHAVE-AD, and Cohen-Mansfield Agitation Inventory (CMAI) for each study group participant. This was done to establish inter-rater, Construct and Criteria Validity. Fourteen days later, the same RN completed LuBAIR Scale again for each participant for intra-rater reliability. A Clinical Utility Survey (CUS) was developed to evaluate nurse viewpoints on the usefulness of LuBAIR on three variables: less labor intensive, more comprehensive and better categorization of behaviors in clinical meaningful categories.

RESULTS: Intra-rater reliability was established for 8 of the 12 behavioral categories. Inter-rater reliability was established for 10 of the 12 behavioral categories. LuBAIR scale had comparable Construct and Criteria Validity. CUS findings showed 23% of nurses found LuBAIR to be less labor intensive, 77% found LuBAIR to be more comprehensive and an overwhelming majority, 98%, agreed LuBAIR helps understand behaviors in a clinically meaningful way.

CONCLUSIONS: LuBAIR has acceptable inter- and intra-rater reliability and Construct and Criteria Validity. It is more comprehensive and better at categorizing behaviors in clinically meaningful categories.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0542
PRESENILE DEMENTIA IN A COMMUNITY BASED DEMENTIA CLINIC
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Objective: Dementia most commonly occurs in late life, but a small percentage of patients begins in an early age. The most frequent diagnosis in early onset dementia is Alzheimer's disease, vascular dementia and frontotemporal dementia. There are a few studies in characterizing presenile dementia compared to an older onset dementia. The purpose of this study is to review and analyse the characterization of presenile dementia patients in our community based dementia clinic.
Methods: We reviewed presenile dementia patients, whose clinical onset of dementia has begun before 65 years of age, consecutively seen in our clinic, from January to November, 2013. We excluded vascular dementia and traumatic brain injury.
Results: The number of presenile dementia patients were 15. Among them, thirteen patients were AD and 2 were frontotemporal dementia. The mean age of patients was 64.7years and the mean onset age was 58.3 years old. The mean score of initial K-MMSE was 18.3±6.2 and the mean score of follow up K-MMSE in 2013 was 4.2±6.1, which showed statistically significant difference. The mean duration of first evaluation from onset of cognitive impairment was 27.8 months.
Conclusion: Our results showed that Alzheimer's disease is the major cause of presenile dementia. Even though presenile demenita patients were relatively uncommon and were a minority, we should pay attention to this diagnosis , especially to young people with cognitive complaints.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-0654

SCREENING FOR THE PRESENCE OF FMR1 PREMUTATION ALLELES IN PATIENTS WITH VARIOUS MOVEMENT DISORDERS IN TAIWAN

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Objective: To investigate the presence of *FMR1* premutation alleles in patients with various movement disorders.
Background: Fragile X-associated tremor/ataxia syndrome is a neurodegenerative disorder characterized by cerebellar ataxia, intention tremor and less common Parkinsonism, polyneuropathy, executive function deficits and cognitive impairment. It might be caused by premutation CGG repeat expansion (range: 55~200) in the fragile X mental retardation 1 (*FMR1)* gene.
Methods: We enrolled 771 patients with various movement disorders including 649 Parkinsonism; 63 progressive supranuclear palsy (PSP), 105 multiple system atrophy (MSA), 30 corticobasal syndrome (CBS), and 417 atypical Parkinsonism (aPM), 15 chorea excluding Huntington's disease, 107 spinocerebellar ataxia (SCA) without known genetic causes. The control group included 1,407 women (mean age at test: 34.0±0.1 years) who had received prenatal tests from Ko’s obstetrics and gynecology clinic. Genotyping of CGG repeated length in *FMR1* was performed with a recently developed highly sensitive PCR method.
Results: The mean age at onset was 63.8±8.3, 57.0±20.3, and 43.8±18.2 years in respective parkinsonism, chorea and SCA groups. Three premutation carriers of 80, 55, 92 CGGs were detected in respective MSA, SCA and aPM patients. Seven premutation carriers were detected in 1407 controls. Gray-zone alleles (range: 45-54 CGG repeats) was found in 5 patients (1 PSP, 1 MSA, 2 aPM, and 1 SCA) and 16 controls. There was no difference in frequency of premutation carriers between patient (0.4%) and control groups (0.5%) (p=0.503).
Conclusions: *FMR1* premutation alleles were not correlated with various movement disorders in Taiwan.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-1872
PSP AND MSA BOTH IN THE SAME FAMILY
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INTRODUCTION: Different neurodegenerative diseases can be seen in the same family,Regular follw ups are important to diagnose those patients and their relatives.
CASE 1: 69 year old male patient came to our hospital suffering from falls. He also had a slowdown in movements,mild tremor,postural instability,rough voice and limited eye movements.His routine laboratuary findings and brain tomography showed no specific sign.He had no response to increased levodopa doses..He was then hospitalized his inspiratuary stridor, REM sleep disorder and spontan falls were observed. With these symptoms he was referred to a university with the diganose of possible supranuclear palsy. The diagnosis was confirmed at the university.
CASE 2: 61 year old male patient came to our hospital with vertigo, amnesia and sudden hypotension symptoms. His brother was diagnosed as progressive supranuclear palsy 1 year ago and he was afraid that he would be like him in the end. In his examination orthostathic hypotension, mild postural instability and tremor was found. His cranial MRi and routine laboratuary finding seemed to be normal except low vitamin B12 level (196 pg/ml).He was treated for B12 deficiency but the symptoms still remained. He was referred to the university where he was diagnosed as possible multysystem atrofy.
CONCLUSION: Neurodegenerative disorders are common especially in older patients. Rarely different neurodegenerative diseases can be seen in the same family. Sometimes at the small hospitals the diagnosis for neurodegenerative disorders can be skipped. More attention and regular follow ups will be helpful for both the patient and the doctor.

07q. Epidemiology, Risk Factors, Genetics & Epigenetics: other

ADPD5-2107

COGNITIVE FUNCTION IN SUBJECTS WITH PHYSICAL FRAILTY BUT WITHOUT DEMENTIA OR COGNITIVE COMPLAINTS: RESULTS FROM THE I-LAN LONGITUDINAL AGING STUDY

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Background
Previous evidence has shown frailty may associate or predict dementia. However, the impact of frailty on different cognitive domains in subjects with physical frailty but without dementia or cognitive complaints have not been well investigated
Methods
Community residents aged 50 years and older in the I-Lan Longitudinal Aging Study were evaluated. All participants received frail status and cognitive function assessments. Frail status was diagnosed by the Cardiovascular Health Study criteria. Cognitive function was examined by a standard battery of neuropsychological tests, including the Mini-Mental State Examination (MMSE), memory, language, visuospatial and executive function tests. The effects of frailty on different cognitive domains were examined.
Results
Totally 1686 persons aged 50-92.2 years (mean 63.4±8.9) were enrolled. There were 678 persons (40.2%) recognized as pre-frail and 82 persons (4.9%) as frail. The pre-frail and frail persons had significant poorer performance in the MMSE and all cognitive domains. Slowness, weakness and exhaustion were the most significant frailty components associated with cognitive impairment. Frail subjects had greater risk for ≥1 cognitive domain impairments than prefrail subjects (OR 1.31 in pre-frail subjects versus OR 2.24 in frail subjects). Non-memory domain was first affected in pre-frail state and memory domain was significantly affected in becoming frail.
Conclusion
Cognitive impairment is presented in frail subjects without dementia and even in subjects without subjective cognitive complaint. The incremental impact of frailty on cognition and the susceptibility of non-memory domain may provide a new view in evaluating the pathogenesis of both frailty and cognitive impairment.

08a. Animal Models: transgenic mice

ADPD5-1743

CORTICAL STRUCTURE ALTERATIONS IN NF-KAPPAB P50 SUBUNIT LACKING MICE

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Objectives: Structural remodelling of cortical neurites is a dynamic process underlying neuronal network formation and functional brain plasticity, both in development and in adulthood. We previously demonstrated that NF-kappaB and Notch pathways cross-talk acts as neurites structural plasticity modulator. Considering the key role of Notch and NF-kappaB pathways during neurodevelopment, we investigated if $p50^{-/-}$ mice cortex present structural and neurochemical abnormalities.

Methods: NF-kappaB p50 subunit-deficient ($p50^{-/-}$) and wild type mice were analyzed in terms of cortical neuron morphology, cortical structure and behavioural abnormalities.

Results: We observed altered distribution of Notch1 immunoreactivity in the cortex of $p50^{-/-}$ mice compared to wild type littermates at first postnatal day 1 (P1). We found that P2 $p50^{-/-}$ mice present an increase in radial glial cells, Reelin levels and abnormalities in cortical layers. In adult $p50^{-/-}$ mice, we observed an abnormal columnar organization and a disorganization of brain connectivity network in the cortical somato-sensory area. Concerning neurotransmission, we found an imbalance in glutamate/GABA signallings. Finally, $p50^{-/-}$ mice reported a significant reduction in social interaction compared to the wild-type mice.

Conclusions: The structural plasticity alterations previously observed in the brain of $p50^{-/-}$ mice are followed by cortex cytoarchitecture, neurotransmission and behavioural defects. A better understanding of the molecular events participating in neurite remodelling and neuron connectivity may provide relevant information for innovative therapeutic approaches in a variety of neurological disorders, including Alzheimer's disease, depression, autism, and schizophrenia, which have been associated with dysregulated neuroplasticity.

08a. Animal Models: transgenic mice

ADPD5-2115
DELETION OF RCN2 CAUSES EARLY-ONSET DYSTONIA IN MICE
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Primary dystonia is a common but poorly understood movement disorder characterized by involuntary muscle contractions leading to abnormal movements and postures. Here, we report that deletion of mouse *Rcn2* results in abnormal movements and postures typical of early-onset **dystonia**. A fraction of *Rcn2* knockout mice manifested curly tails, twisting movements and abnormal postures. Abnormalities started from the tail and hind legs and progressed gradually to involve the front legs and trunk. Symptoms became more evident with increased or prolonged activity. Dystonic mice also manifested increased anxiety-like behaviors and markedly reduced body weight. Their lifespans varied from 7 months to over a year. Subtle histological changes, including a reduction in neuronal cells and an increase in apoptosis, were observed in the brain of 2-month-old dystonic mice, but apparent cell loss and formation of gliosis were observed in the cerebrum, basal ganglia, and cerebellum of 5-month-old dystonic mice. Thus, we have provided the first direct evidence that loss of *Rcn2* expression causes early-onset dystonia in mice partially due to increased neuron death.

08d. Animal Models: drosophila

ADPD5-0759

PROTECTIVE EFFICACY OF FERULIC ACID AGAINST PB-INDUCED OXIDATIVE DYSFUNCTIONS AND NEUROTOXICITY IN DROSOPHILA MELANOGASTER

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Objective: Lead (Pb) toxicity is a persistent public health problem throughout the world. Epidemiological studies have shown that Pb exposure is associated with significant deficits in cognitive functions among children. Approaches such as chelation, antioxidants and their combination can alleviate Pb-associated neurotoxicity. We examined whether ferulic acid (FA), enrichment can attenuate Pb-induced phenotype, oxidative stress and neurotoxic effects employing *Drosophila melanogaster* as the model.

Methods: Employing a co-exposure paradigm, we determined the lethality response and locomotor phenotype (speed) among two age groups (young/ adult) of flies exposed (7d) to Pb acetate (5-20mM, 7 d) in the medium. Subsequently, we studied the propensity of FA (25-50µM) - enrichment to offset Pb-induced (5mM) - oxidative dysfunctions and neurotoxicity in young flies.

Results: A concentration -dependent mortality ensued with Pb exposure, while young flies were more susceptible. Hyperactivity was discernible among both age groups. Pb exposure caused a robust global oxidative stress (as evidenced by elevated ROS/protein carbonyl levels in head/body regions, perturbations in antioxidant enzyme activities) and significant increase in the activities of complex I-III, despite elevated levels of total thiols/ GSH. Further, flies exhibited elevated activity of acetylcholinesterase and dopamine levels in head region. Interestingly, FA enrichment although had no significant effect on Pb-induced hyperactivity, offered marked protection against Pb-induced oxidative impairments and restored the activity levels of AChE and dopamine levels.

Conclusions: Employing this model, we are investigating the interactive effects of low levels of Pb exposure and neurotoxins (eg. rotenone and Paraquat) and the protective effects of selected phytoconstituents.

08d. Animal Models: drosophila

ADPD5-0772
PROTECTIVE EFFICACY OF CREATINE IN DROSOPHILA MODEL OF NEUROTOXICITY: RELEVANCE TO PD

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Objectives: To elucidate the efficacy of Creatine (Cr) to abrogate locomotor deficits, lethality and oxidative stress in *Drosophila melanogaster* induced by model neurotoxins such as Acrylamide (ACR) and 1-methyl-4-phenylpyridine (MPP^+).
Methods: The status of endogenous levels of oxidative stress markers, activities of antioxidant enzymes and acetylcholinesterase (AChE) were determined in adult flies maintained in Creatine (5-10mM) -enriched medium for 7d. Further, employing a co-exposure paradigm, the efficacy of Cr to abrogate ACR-induced locomotor phenotype, lethality, oxidative dysfunctions and neurotoxicity were investigated in adult flies. In another experiment, flies provided with Cr prophylaxis (for 10 d) were challenged with MPP^+ for 48 h in order to determine the resistance rendered against toxin insult.
Results: *D. melanogaster* exposed to Cr-enriched diet for 7 days exhibited significant diminution in the levels of reactive oxygen species (ROS), hydroperoxides (HP), nitric oxide with concomitant elevation in the levels total thiols, GSH and reduced AChE activity. At the higher concentration, Cr (10mM) significantly offset the incidence of ACR -induced mortality and improved the locomotor phenotype. Further, Cr-enrichment alleviated ACR-induced oxidative stress, (restoration of ROS, HP, NO levels and activity levels of antioxidant enzymes) and cholinergic deficit. Interestingly, Cr prophylaxis offered robust protection (29-57%) against MPP^+ exposure as evidenced by the incidence of lethality and improvement in locomotor phenotype.
Conclusion: Our experimental findings suggest the neuroprotective efficacy of Cr against neurotoxin exposure and its propensity to modulate oxidative dysfunctions in the Drosophila system.

08f. Animal Models: pharmacological & lesion models

ADPD5-1278

COGNITIVE AND MOTOR IMPAIRMENTS INDUCED BY A BILATERAL 6-OHDA LESION INTO THE STRIATUM OF THE RAT

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A number of non-motor symptoms such as depression, anxiety, and cognitive decline are observed at an early stage of Parkinson's disease (PD). The present study investigated the effects of a bilateral 6-hydroxydopamine (6-OHDA) lesion into the striatum on motor and cognitive end-points in the rat.
Animals were bilaterally lesioned with 6-OHDA at 4 µg/µL (two infusions per striatum). Three weeks after lesion, motor coordination was evaluated in the accelerating rotarod test. Animals were then tested in the operant delayed alternation (DA) and Morris water maze (MWM) tests.
In bilateral 6-OHDA lesioned rats, the drop-off time was significantly decreased in the accelerating rotarod test, as compared with sham-operated animals ($p < 0.05$). In the DA test, 6-OHDA-lesioned rats showed a clear increase in the number of sessions required to reach the criteria of 90% correct responses during the acquisition period. In the MWM test, the escape latency and the distance swum were significantly increased on Day 1 ($p < 0.001$ and $p < 0.01$, respectively) and on Day 3 ($p < 0.05$, for both parameters).
These results suggest that a bilateral 6-OHDA lesion into the rat striatum induced modest deficits on motor coordination in the accelerating rotarod test. Learning and working memory impairments were also observed on the acquisition of a DA task.
The bilateral 6-OHDA lesion into the striatum in the rat represents a partial lesion model of the nigro-striatal pathway with quantifiable motor and cognitive deficits mimicking an early stage of PD.

08f. Animal Models: pharmacological & lesion models

ADPD5-1610

OLFACTORY IMPAIRMENT IS MODULATED BY BULBAR DOPAMINERGIC D2 ACTIVITY IN THE ROTENONE MODEL OF PD AFTER REM SLEEP DEPRIVATION.

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[1]Physiology, Federal University of Paraná, Curitiba, Brazil

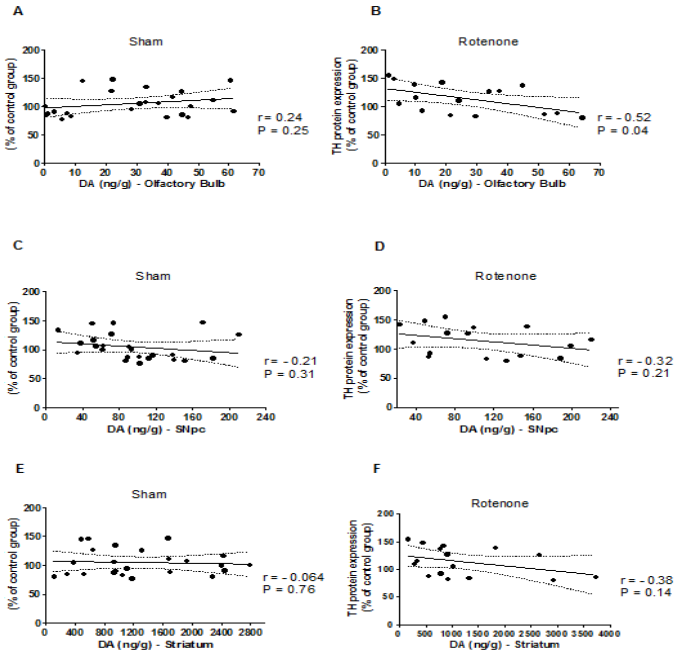
Objectives: We have led to the hypothesis that a modulation of the dopaminergic D2 receptors in the olfactory bulb could provide a more comprehensive understanding of the olfactory deficits in PD and after a short period of rapid eye movement (REM) sleep deprivation (REMSD).

Methods: We decided to investigate the olfactory, neurochemical and histological alterations generated by the administration of piribedil (a selective D2 agonist) or raclopride (a selective D2 antagonist), within the glomerular layer of the olfactory bulb, in rats submitted to intranigral rotenone and REMSD.

Results: Our findings provided an evidence of the occurrence of a negative correlation (r = - 0.52, P = 0.04) between the number of periglomerular TH-ir neurons and the bulbar levels of dopamine (DA) in the rotenone, but not sham groups. A significant positive correlation (r = 0.34, P = 0.03) was observed between nigral DA and olfactory discrimination index (DI), for the sham groups, indicating that increased DA levels in the substantia nigra pars compacta (SNpc) are associated to enhanced olfactory discrimination performance.

REMSD supposedly generates supersensitivity of D2, promoting a restorative effect to DA in different investigated areas.

Conclusion: The present evidence reinforce the idea that DA produced by periglomerular neurons, particularly the bulbar dopaminergic D2 receptors, is an essential participant in olfactory discrimination processes, the SNpc, and the striatum.



08f. Animal Models: pharmacological & lesion models

ADPD5-1653

STRIATAL MT2 MELATONIN RECEPTORS: REGULATION OF ANXIETY AND DEPRESSIVE-LIKE BEHAVIORS IN THE ROTENONE MODEL OF PARKINSON'S DISEASE

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[1]Physiology, Federal University of Paraná, Curitiba, Brazil

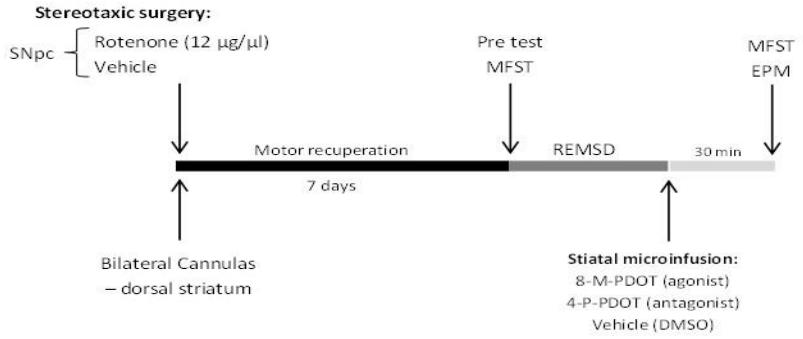
Objectives: The present study investigated the potential role of striatum MT2 melatonin receptor on anxiety and depressive-like behaviors after REM sleep deprivation (REMSD), in the rotenone model of Parkinson`s disease.

Methods

Results: We observed substantial anxiolytic-like effects promoted by activation of striatal MT2 receptors (P<0.01). The 4-P-PDOT group did not show an anxiogenic-like behavior, however, after 24h of REMSD rats spend more time on open arms (P<0.05). An anxiolytic-like behavior was observed in rotenone REMSD group, treated with MT2 agonist (P<0.01), antagonist (P<0.05) or vehicle (P<0.01).

The swimming, immobility and climbing parameters evidenced that 4-P-PDOT promoted antidepressant-like effects that were observed even in the rotenone REMSD 4-P-PDOT group (P<0.01).

Conclusions: These results indicated that striatal MT2 melatonin receptors exhibited important roles in anxiety and depressive-like behaviors in the rotenone PD model. Additionally, REMSD modulated these effects even after rotenone-induced lesion. Furthermore, we observed substantial antidepressant-like effect promoted by the blockade of striatal MT2 receptors.



Legend: Elevated plus maze (EPM); Modified forced swimming test (MFST); Substantia nigra pars compacta (SNpc);

Figure 1

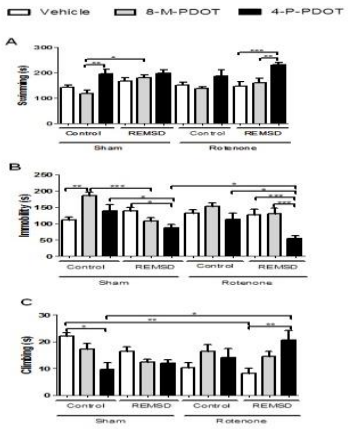


Figure 3

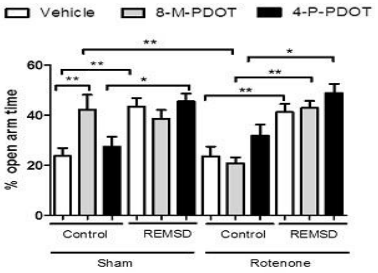


Figure 2

08f. Animal Models: pharmacological & lesion models

ADPD5-1657

PEDUNCULOPONTINE TEGMENTAL NUCLEUS AND DOPAMINERGIC SYSTEM: POSSIBLE ASSOCIATION BETWEEN REM SLEEP REGULATION AND MEMORY CONSOLIDATION

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Objective: The aim of this study was to investigate a possible association between the pedunculopontine tegmental nucleus (PPT) and the nigrostriatal dopaminergic system in REM sleep regulation and, consequently, in memory consolidation.

Methods: Rats underwent stereotaxic surgery for direct infusion of ibotenic acid (24 µg/µL) within the PPT. After seven days, the animals were exposed to REM sleep deprivation (REMSD), followed by intrastriatal: D2 receptor agonist injection (piribedil, 3 µg/µl), antagonist (raclopride, 10 µg/µl) or vehicle (DMSO). The animals were subjected to the object recognition test (ORT) after the REMSD and after the sleep rebound period (REB) and the sleep recording occurred during the REB period for 24 hours.

Results: The piribedil PPT-lesioned REMSD group exhibited an increase in REM sleep compared to the raclopride group ($P<0.01$) and this augment did not corresponded to the memory consolidation since there was not a difference between this groups in the time exploring the novel object. However, it was possible to observe a similarity between time spent in REM sleep and memory consolidation since the raclopride PPT-lesioned REMSD group spent less time in REM sleep compared to its control ($P<0.01$) and was not able to recognize the novel object.

Conclusion: We suggest a mutual involvement of the nigrostriatal dopaminergic system with the PPT, both contributing in sleep regulation and memory consolidation. Thus, we propose that REM sleep is probably the key player in modulating such events triggered by this PPT-nigrostriatal circuitry.

08f. Animal Models: pharmacological & lesion models

ADPD5-1783

OLFACTORY DEFICIT PRODUCED BY PARADOXICAL SLEEP DEPRIVATION AND INTRANIGRAL ROTENONE LESION IN RATS

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[1]Physiology, Federal University of Paraná, Curitiba, Brazil

Objectives

We Investigated the occurrence of olfactory deficits produced by paradoxical sleep deprivation (PSD) associated to a dopaminergic nigrostriatal lesion induced by intranigral rotenone administration in rats.

Methods

Animals were distributed into two groups: rotenone that received a bilateral intranigral infusion of rotenone (12 µg/µL) by stereotaxic surgery; other group received only dimethylsulfoxide (DMSO) as a vehicle. After 7 days the olfactory (social and non-social odor) and open field tests were performed. Subsequently rats underwent PSD protocol for 24 h and were re-tested. After PSD, they were allowed to perform the rebound sleep in the next 24 h, followed by another re-test. Finally, the animals were decapitated for the quantification of tyrosine hydroxylase immunoreactivity (TH-ir) neurons within the substantia nigra.

Results

The densities of the TH-ir neurons showed a significant decrease of about 30% ($p<0.0001$) in the rotenone group compared to the sham. We found that the rotenone group demonstrated an olfactory impairment ($p<0.05$), which remained after the PSD ($p<0.01$), that was counteracted by the rebound. Besides the lesion and PSD generated impairment in the discrimination of non-social odors ($p<0.05$) without motor bias.

Conclusions

Both the decrease of dopaminergic neurons in the substantia nigra and PSD were able to generate a similar olfactory loss.

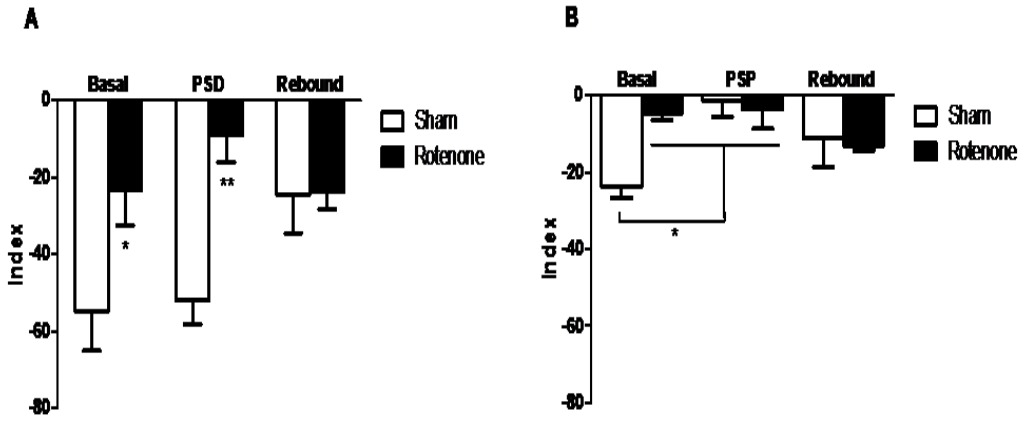

Figure 1. Olfactory Tests. (A) Social odor; (B) Non-social odor. Sham n=10 and Rotenone n=7

08f. Animal Models: pharmacological & lesion models

ADPD5-1962

LEVODOPA ATTENUATES FATIGUE IN RESERPINE-TREATED MICE – AN ANIMAL MODEL OF PARKINSON'S DISEASE

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There is a great demand for knowledge of perceived exertion during physical activity. Decreased ability to work or exercise until failure is known as fatigue, which has central and peripheral components, and is also a common symptom in neurology. In order to understand the mechanisms of fatigue, it is important to distinguish symptoms of peripheral neuromuscular fatigue from the symptoms of central fatigue characteristic of basal ganglia disorders, such as Parkinson's disease. Some evidence suggests the role of increased serotonin/dopamine (5-HT/DA) ratio in the pathophysiology of this central fatigue. We depleted striatal DA in mice (Swiss, 10-12 weeks age, 30-40 g) by systemic treatment with reserpine (1 mg/kg, i.p.), an animal model of Parkinsonism. This increased the 5-HT/DA ratio in the striatum of animals. The reserpinized animals also showed fatigue intolerance in the treadmill test, characterized by reduced mechanical work and muscular recruitment. Levodopa (100 mg/kg, i.p) plus Benserazide (50 mg/kg, i.p) partially reversed DA depletion reversed, Parkinsonism and fatigue tolerance. We consider that increased striatal 5-HT/DA ratio played a crucial role in the development of central fatigue because reserpine did not alter mitochondrial physiology and anatomy, and glucose uptake in muscle tissue. The results suggest that striatal DA may be involved in the development of central fatigue. Furthermore, the effectiveness of levodopa suggests an important motor component of this symptom in the Parkinsonism, which does not discard non-motor components, such as motivation and attention.

08f. Animal Models: pharmacological & lesion models

ADPD5-2025

THE INTERACTION BETWEEN DECREASED SHORT-TERM SPATIAL MEMORY AND INCREASED OXIDATIVE STRESS IN A SCOPOLAMINE-INDUCED RAT MODEL OF ALZHEIMER'S DISEASE

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· Objectives

Scopolamine is a well known muscarinic cholinergic competitive antagonist involved in human and animal memory processes, particularly in the processes of learning acquisition and short-term memory and it has been one of the most used drugs to induce animal models of Alzheimer disease (AD). Also, there is an increased awareness regarding the relevance of the oxidative stress in the progression of AD.

· Methods

In this context, we were interested in studying the effects that scopolamine induction of a rat model of AD has on oxidative stress, as expressed by the Total Antioxidant Status (TAS) from the temporal lobe, the most sensitive brain area to the effects of the oxidative stress status.

· Results

The cognitive deficits of scopolamine were confirmed in the Y maze task, as expressed through a significant decrease of the spontaneous alternation. Also, our data indicated that the administration of scopolamine has a significant prooxidant effect, which is manifested by a decrease in the TAS of the temporal lobe, as compared to the controls. Moreover, a significant Pearson correlation was observed between the levels of the behavioural tasks and the values of the TAs in the temporal lobe.

· Conclusions

In this study we have demonstrated the presence of increased oxidative stress in a rat model of Alzheimer's disease obtained through the administration of scopolamine. Moreover, there is a significant correlation between the behavioral markers in the Y maze and the levels of TAS, as a result of scopolamine administration.

08f. Animal Models: pharmacological & lesion models

ADPD5-2069

ONE SINGLE ADMINISTRATION OF MPTP IS ENOUGH TO PRODUCE MEMORY DEFICITS IN A RAT MODEL OF PARKINSON'S DISEASE

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Background:
Besides the well known locomotory aspects, the various neuropsychological investigations of patients with Parkinson's disease (PD) have shown specific cognitive impairments, ranging from minor disturbances in memory to intellectual function or even dementia.
Also, one of the most used animal models of PD in rats in referring to the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).
Methods:
In this way, while most of the administration patterns are including several different intraperitoneally (i.p.) injections of MPTP (e.g. 4 injections X·20mg/kg, 2 h apart; 1–2 daily injections of MPTP, 20–30mg/kg, 5 days), here we were interested, for the first time in our best of knowledge, to see if just one acute administration of a single injection of MPTP 20mg/kg i.p. will result in any cognitive deficits in rats, as studies in the Y maze task. The behavioral testing was performed one week after the MPTP administration, while the control group received saline.
Results:
In this way, the administration of single i.p. MPTP dose resulted in a significant decrease of the spontaneous alternation percentage in the Y maze task (77.5± 6.2% in controls vs. 52.2± 4.1% in MPTP group), suggesting deficits in the immediate working memory. Moreover, these results were not generated by some locomotor deficiencies, considering that there was no significant difference in the number of arm entries between the two groups of rats.
Conclusions:
One single i.p. administration of MPTP 20 mg/kg is enough to produce memory deficits in a rat model of PD, as studies in the Y maze task.

08f. Animal Models: pharmacological & lesion models

ADPD5-2190

INFLUENCE OF A PARTIAL LESION OF DOPAMINERGIC SYSTEM AND CHRONIC ANTIDEPRESSANTS ON SERT AND DAT IN BRAIN STRUCTURES IN RATS

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Binding to serotonin (SERT) and dopamine (DAT) transporters is used by imaging techniques in Parkinson's disease (PD). However, no studies are available concerning SERT and DAT binding and its possible relationship with depression of preclinical phase of PD.

The aim of the present study was to investigate the influence of a moderate dopaminergic lesion and chronic administration of pramipexole and imipramine on the "depressive-like" behavior, as well as binding of radioligands to DAT and SERT in limbic and nigrostriatal structures.

Rats were bilaterally injected with 6-OHDA into the caudate-putamen (CP). Imipramine was injected (10 mg/kg) once a day and pramipexole (1 mg/kg) twice a day for 2 weeks. 24 h after the last drug injection rats were tested in the forced swimming test (FST) and actimeters. After decapitation dopamine and serotonin levels (HPLC), as well as radioligand binding to DAT and SERT were investigated (autoradiography).

6-OHDA administration increased immobility in FST, without influencing locomotion of rats. 6-OHDA decreased dopamine but did not influence serotonin levels in CP, nucleus accumbens (NAC) and frontal cortex. 6-OHDA reduced DAT in CP and NAC, and SERT in these structures, as well as in hippocampus, amygdala, habenula, prefrontal cortex, substantia nigra and VTA. Pramipexole but not imipramine reversed immobility prolongation in lesioned animals. Both pramipexole and imipramine decreased SERT in mesolimbic and nigrostriatal systems but had no influence on DAT.

These results seem to indicate that 6-OHDA-induced partial dopaminergic but not serotonergic lesion, as well as chronic antidepressants, evoked adaptive changes in SERT binding.

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08h. Animal Models: other

ADPD5-1448
CANINE MODEL FOR HUMAN NEURODEGENERATIVE DISORDERS
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Objectives: The study of the pathogenesis of human neurodegenerative disorders including Alzheimer´s disease (AD) requires the use of proper animal models that develop age-related neurodegenerative process in the brain. Previously it has been shown that aged canines suffered from cognitive dysfunction (CCD) and developed characteristic features of Alzheimer´s disease brain pathology. The scope of this study is to diagnose CCD by differential exclusion, physical examination, diagnostic testing and the screening checklist with owner cooperation.
Methods: We were studying fifteen aged dogs (from 9 to 17 years) with severe behavioural and cognitive deficits. The testing includes clinical and neurological examination, hematology, blood biochemistry, serology, autopsy as well as histopathology, immunohistochemical and proteomic analyses of dog brain. Moreover we analyzed proteome of cerebrospinal fluid (CSF) to identify potential biomarkers for disease progression.
Results: Test of cognition by CCD questionnaire showed marked deficits in two dogs. We found increase senile plaques in neocortex and hippocampus only in two investigated brains of the 15 aged dogs, this pathology does not correlate with cognitive impairment. On the other hand, we found in majority of tested brain tissue samples increased number of activated microglia in white matter.
Conclusion: Our findings indicate that neuroinflammation rather that senile plaques pathology may correlate with cognitive impairment of aged dogs. Results of the study could lead to use of the dog as an animal model in research of human brain ageing and neurodegenerative disorders.
Acknowledgement: This work was supported by the grant APVV-0206-11.

08h. Animal Models: other

ADPD5-1551
PHENOTYPIC CHARACTERIZATION OF THE CAENORHABDITIS ELEGANS STRAIN N2 WITH ETHANOLIC EXTRACTS OF OF WITHERINGIA COCCOLOBOIDES

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Neurodegenerative diseases have a high prevalence, causing a major *public health problem.* The lack of treatments has allowed the use of alternative therapies in the pharmaceutical industry and the use of plant secondary metabolites (*Witheringia coccoloboides* native of Colombia) to prevent and control the disease. Using a biological model as *Caenorhabditis elegans (Ce)*, which has been used in different studies of neuroscience, allowing to will evaluate in vivo the ethanol extract of the plant, as a promising treatment for these diseases. **Aim:** Analyze the phenotypic characteristics of the strain N2 with the aim to be used as a model for screening the extract of plant.
Materials and Methods: The nematode was cultured and grown in NGM medium with E. coli OP50. Strain was synchronized from eggs to L1 larvae. The extract was performed and standardized for phenotypic characterization of the strain N2 using the length, longevity, reproduction thermal stress trials.
Results and Conclusion: the longevity of the control strain N2 was 16 to 22 days; however using the extract with a concentration of 1 mg was 28 days. The offspring reproduction was 225, when used extract the reproduction was affected at the highest concentration where there was a decreasing the number of offspring. The nematode length was approximately 1100 +/- 50 µm using extract where there was on length increment of 1400µm. Also it was showed that the extract does not affect nematode thermo tolerance and it is not toxic to the N2 Strain.

08h. Animal Models: other

ADPD5-2051
STUDYING PAIN MANIFESTATIONS IN AN MPTP-INDUCED RAT MODEL OF PARKINSON'S DISEASE

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Background
Generally, Parkinson's disease (PD) is less widely appreciated as a disease causing pain syndromes, although pain is found in 40-80 % of PD patients, as described by the very few reports in this area of research. Moreover, in some PD patients, pain is so severe and intractable that it overshadows the motor symptoms of the disorder. Still, pain in PD frequently goes underacknowledged and undertreated. Also, the studies regarding pain perception in the existing animal models of PD are very few.
Methods
We experimentally induced the PD model in rats by injecting subcutaneously one dose of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 20mg/kg, while the control group received saline. The behavioral testing for pain included the hot-plate task and was performed 7 days after MPTP injection.
Results
In this way, our rat model resulted from the acute treatment with a low dose of MPTP, exhibited an increased sensitivity to pain perception, as demonstrated by the significant decrease in the values of the latency time in hot-plate for rats treated with MPTP, as compared to the controls. The latency time is expressed in seconds and is referring to the reaction time to two different types of behavior: licking the paw and jumping (11.33 ± 2.1 in controls vs. 6.8 ± 0.9 in MPTP group).
Conclusions
Our data is suggesting, for the first time in our best of knowledge, an increased sensitivity to pain in a MPTP-induced rat model of PD. In this way, further studies in this area of research seem warranted.

09a. Patient Care & Support: caregiver support

ADPD5-0939

MALE CAREGIVERS TO PERSONS WITH LEWY BODY DEMENTIA HAVE HIGHER STRESS LEVEL THAN MALE CAREGIVERS TO PERSONS WITH ALZHEIMERS DEMENTIA.

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Title: Male caregivers to persons with Lewy Body Dementia have higher stress level than male caregivers to persons with Alzheimers Dementia.

Authors: Ellen Svendsbø, Toril Terum, Ingelin Testad, Dag Aarsland & Arvid Rongve.

Objectives: To identify distress for male caregivers to persons with Dementia with Lewy bodies (DLB) compared to male caregivers to persons with Alzheimers dementia.

Method: The study sample comprised 126 caregivers for persons with AD and 90 caregivers for persons with DLB, recruited for a longitudinal dementia study (DEMVEST) and from a register (HUKLI), both from Norway. To get the gender differences in this study we selected the persons that were married to or lived together (opposite gender).The caregivers answered the Relative Stress Scale (RSS). RSS is divided into three groups, Emotional distress, Social distress and negative feelings. DLB were diagnosed according to the revised consensus criteria. Patients were home-dwelling outpatients referred to memory clinics, and had standardized comprehensive assessment.

Result:16 male DLB caregivers together with 29 male AD caregivers answered the RSS form. Mean score from male DLB caregivers (22.47) differed significantly from mean score from male AD caregivers (11.07, p=0.002). Sub items like RSS emotional distress was 10.67 for male DLB caregivers and 3.64 for male AD caregivers (p=0.001) Social distress score was for male caregivers 9.44 DLB and 5.07 AD (p=0.032) and negative feeling 3.78 for male DLB and 2.34 for male AD (p=0.234).

Conclusion: Male caregiver to persons diagnosed with DLB reports significantly higher caregiver distress, as compared to male AD caregivers.

09a. Patient Care & Support: caregiver support

ADPD5-1069

SHOULD WE ASSESS FAMILY CAREGIVERS' DEPRESSIVE SYMPTOMS AT THE EARLY PHASE OF CAREGIVING?

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Objectives: Family caregivers' Sense of coherence (SOC) is associated with the capacity to cope with caregiving. SOC express the inner trust that life will be understandable, manageable and meaningful despite changes in life situation. The aim was to investigate the trajectory of SOC during 3 year follow-up time.
Methods: Kuopio ALSOVA study is a prospective follow-up Alzheimer Disease (AD) study. Spousal caregivers (n=170) were followed three years after their spouses had been diagnosed with AD. Both person with AD- (CDR-SOB, MMSE, NPI, ADCS-ADL) and caregiver (BDI, SOC) were evaluated annually. Linear mixed models were used to analyze relationship between SOC and potential clinical parameters.
Results: Mean drop-out adjusted SOC score (at baseline 148.5) decreased by -4.56 points (p=0.002) during follow-up. Caregivers' depression at baseline predicted the significant decrease of SOC (every + 1 BDI point decreases 2.181 points in SOC, p=0.0001). When compared to non- depressive caregivers (BDI≤9, n= 95, 56%), minor (BDI 10-18, n= 54, 32%), moderate to severe (BDI 19-30, n= 21, 12%), depressive symptoms decreased (adjusted for length of follow-up) the level of the SOC score by an average of -19.0 (p=0.0001), -38.3 (p=0.0001) points during follow-ups.
Conclusions: The average SOC decreased in all caregivers. Paying attention to family caregivers' health as well as health protecting factors and recourses may help to identify the most vulnerable caregivers at the early stage. Spousal caregivers who suffer even minor depressive symptoms at the beginning of caregiving should be recognized and individualized support and care should be provided for them.

09a. Patient Care & Support: caregiver support

ADPD5-1658
GPS, A SOLUTION FOR THE PATIENT AND THE CAREGIVER
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Target:analyse the experience of Alzheimer patients' caregivers who use GPS.
Analysethe main causes which overload the caregiver.
Design:interpretative and qualitative investigation. Phenomenological perspective.
Participants:caregivers of GPS users and caregivers of non GPS users.
Method:biographical story technique through an interview between the caregiver and theinvestigator,
auto-recorded and transcribed.
Result:analysis of seven stories. The use of the GPS is "releasing" (freedom, distressing,calming),
related to "continuity" the user can stay more time at his own home,keeping on doing his "routines",
playing card games with friends, going for awalk, doing a little bit of shopping.
Conclusions:the caregivers match the GPS with the concept of freedom for themselves and therelease
from stress which is caused by the anxiety of having to close all thedoors and not being able to let the patients walk alone on the street becauseof the fear that they can get lost easily.

09a. Patient Care & Support: caregiver support

ADPD5-2078

INITIAL MEDICAL CONSULTATION OF DEMENTIA PATIENTS DEPENDS ON THEIR CAREGIVERS

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Objectives
To maintain daily and social activities ofdementia patients, supports by caregivers are essential. Moreover, since patientsoften do not aware of their illness and do not consult to hospitals voluntarily,medical consultation tends to be late without caregivers' help. We aim toevaluate care environments and their influence on medical consultation ofdementia patients.
Methods
We surveyed dementia patients who havevisited hospitals for consultation. Patients were stratified in three groups,first, patients who live by themselves, second, patients who live with theirelderly spouse, and third, patients who live with their sons and/or daughters.Items investigated included motivations to consult medical care, the severityof cognitive impairment, BPSD, living conditions, care environments, andcare-giving burdens.
Results
Reasons for seeking treatmentswere mostly core conditions such as: 1) Amnesia, 2) Disorder in Comprehension and Judgment,3) Disorientation, 4) Disability in performing daily routines. The peripheralsymptoms were: 1) Hallucination and Delusion 2) Abnormal Behavior 3) PersonalityChange 4) Lack of Initiative 4. Complaints on physical conditions wererelatively infrequent. Dementia patients visited to hospital without caregiverswere none. Patients who came to theclinic on their own volition for the first time diagnosed as not beingdementia.
Conclusion
On comparison between those living aloneand with other family members, there were differences in such symptoms and thetime of their recognition. Furthermore, in those living with elderly familymembers, the time needed to undergo the initial consultation was longer.

09c. Patient Care & Support: web-based care

ADPD5-0561
THE EFFECT OF TELEMEDICINE ON THE DURATION OF TREATMENT IN DEMENTIA PATIENTS

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Objectives
The aim of the present study was to evaluate the effectiveness of telemedicine for long-term follow-up of dementia patients in rural South Korea and to identify the factors predicting long-term treatment
Methods
We studied 442 patients who met the DSM criteria for dementia and whose treatment was initiated at the Kangwon National University Hospital (KNUH) in Chuncheon. Over a five-year period, there were 259 patients who regularly visited the KNUH dementia clinic in person, and 168 patients who received medical services from the dementia clinic via telemedicine. The telemedicine patients attended a public health centre in Hongcheon, a facility located in a rural area about 50 km south east of the KNUH.
Results
The mean treatment duration was significantly longer for the telemedicine group than for the clinical visit group (P<0.001), with durations of 26.6 and 14.6 months, respectively. Low Clinical Dementia Rating scores (hazard ratio =1.47, 95% confidence interval=1.26-1.71) and use of telemedicine (hazard ratio=0.55, 95% confidence interval=0.42-0.72) were found to be independent predictive factors of increased treatment duration.

	Hazard ratio (95% CI)	P-value
Age	1.15 (0.95-1.41)	0.15
Clinical Dementia Rating	1.47 (1.26-1.71)	<0.001
Experimental group	0.55 (0.42-0.72)	<0.001

Conclusions
These findings suggest that telemedicine may be useful in slowing disease progression in dementia patients in rural areas

09i. Patient Care & Support: other

ADPD5-0445
AGITATION PREDICTS MORTALITY IN SEVERE DEMENTIA.
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Background:
In dementia, increased severity of cognitive impairment is strongly associated with presence of more severe behavioural and psychological symptoms of dementia (BPSD). BPSD result in distress for the patients, the families, and for the caregivers but is also associated with mortality. The presence and severity of BPSD can be estimated with the Neuropsychiatric Inventory (NPI). The 12-item version of NPI measures different domains such as hallucination, delusion, agitation, depression, anxiety, and apathy. The Swedish BPSD-registry includes repeated NPI-measurements and provides a working tool for the management of BPSD at nursing homes. We investigated the association between different BPSD and mortality in severe dementia.
Method:
The data originate from an 18-month longitudinal study performed at five nursing homes. 95 cases were included in the study. Information on age, gender and dementia diagnosis was registered. Repeated NPI measurements were performed to follow the course of BPSD. 28 cases (29.5%) deceased during the study period.
Results:
There was no significant difference in gender, NPI-score or number of drugs at baseline between deceased and survived cases. The deceased had higher NPI-scores on the items delusion, apathy, and agitation ($p<0.05$). Multivariate logistic regression analysis showed that higher age (OR 1.20 [95% CI 0.07-1.35] per year, $p<0.001$) and higher agitation score (OR 1.24 [95% CI 0.08-1.24] per point, $p<0.005$) increased the risk of mortality.
Conclusions:
Agitation predicted mortality among patients with severe dementia, independent of age, total BPSD burden and other investigated factors. This indicates that special attention should be paid to the presence of agitation.

09i. Patient Care & Support: other

ADPD5-0477

PREVALENCE, COURSE AND TREATMENT OF NEUROPSYCHIATRIC SYMPTOMS IN CASES WITH SEVERE DEMENTIA - A 4-YEAR UPDATE OF THE SWEDISH BPSD-REGISTRY

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Objectives:
Management and optimal care of patients with behavioural and psychological symptoms of dementia (BPSD) is challenging. Guidelines recommend non-pharmacological interventions as first-line treatment and consider pharmacological treatment as second-line. Based on national guidelines, the Swedish BPSD-registry was initiated in November 2010 to improve dementia care. This quality registry uses the Neuropsychiatric Inventory (NPI) for repeated measurements of the incidence and severity of BPSD at dementia care units throughout the country. The registry provides a systematic working tool for the staff resulting mainly in non-pharmacological interventions. The aim of this study was to describe the prevalence, development, and treatment of neuropsychiatric symptoms in patients at dementia care units in Sweden.
Method:
We extracted data on more than 20 000 cases from the BPSD-registry over a four-year period between November 2010 and November 2014.
Results:
The presentation will include data on more than 20 000 cases included in the registry during four years. Preliminary 3-year data showed a significant reduction of the NPI-score at patients with more than 50 points at baseline and a subsequent stabilization over time and a significant increase in the proportion of tailor-made care-plans from 40% in 2011 to 80% in 2014.
Conclusions:
The Swedish BPSD-registry is a working tool for the multidisciplinary team to reduce prevalence and severity of BPSD through non-pharmacological interventions in cases with severe dementia.

09i. Patient Care & Support: other

ADPD5-0667
THE RELATIONSHIP BETWEEN GDS STRUCTURE AND COGNITIVE-BEHAVIORAL ASPECTS IN AD

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Background: Although 15-item of the Geriatric Depression Scale(GDS15) is a widely used depression screening questionnaire, the implications of GDS15 in AD may be questioned. We designed this study to explore the factor structure of GDS15 and the relation between these factors and cognitive-behavioral aspects.
Methods : GDS15, cognitive function tests, Korean-Neuropsychiatry Inventory(K-NPI) were administered to 310 patients with probable AD, who were not medicated before visiting the hospital. Using Principal component analysis (PCA), three factors were identified. To determine the relationship between factors and neurocognitive, behavior symptoms, bivariate correlation was used.
Results : The factor 2 was correlated with K-BNT(Korean Boston Naming Test), calculation, Go-no-go test, COWAT(Controlled Oral Word Association Test), CWST(Color Word Stroop Test; word and color), aggression, depression, and apathy. The factor 3 was correlated with calculation, SVLT(Seoul Verbal Learning Test) immediate recall, RCFT(Rey-Osterrieth Complex Figure Test) copy, RCFT delayed recall, contrasting, COWAT, CWST word, and delusion. Our study identified three factors and revealed that the GDS15 may be comprised of a heterogeneous scale.
Conclusions : These results suggest that the GDS15 may be comprised of a heterogeneous scale and suggested multi-dimensionality properties of GDS15 in AD.

09i. Patient Care & Support: other

ADPD5-0699

PRESCRIBING STATUS OF PSYCHOTROPIC DRUGS FOR THE TREATMENT OF BPSD; A SURVEY FOR PRIMARY CARE PHYSICIANS AND DEMENTIA SPECIALISTS IN JAPAN

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Background/Objectives; Currently, there are no anti-psychotics officially approved for the treatment of BPSD in Japan. However many physicians often prescribe psychotropic drugs including anti-psychotics for patients with BPSD. This survey aimed to investigate the prescribing status of psychotropic drugs by primary care physicians, and to assess the differences in the prescribing practices between primary care physicians and dementia specialists in Japan.

Methods; A survey was mailed to 3,098 family physicians extracted from active members of the Japan medical association (response rate 19.5%) and to 3,147 dementia specialists affiliated with the Japanese Psychogeriatric Society, the Japan Society for Dementia Research, the Japan Geriatrics Society, or the Japan Psychiatric Medical Conference (response rate 20.3%).

Results; The demographic profiles of the primary care physicians who responded were similar to that of dementia specialists for age, type of clinical practice, and clinical experience. Almost 90% in both primary care physicians and dementia specialists see patients with dementia and prescribe psychotropic drugs for patients with dementia in daily clinical practice. The two groups reported a wide range of psychotropic medication for a great variety of BPSD and similar prescribing practices for the treatment of BPSD. A proportion of physicians who obtained informed consent, when they prescribe anti-psychotics, was approximately as low as 20 %.

Conclusions; The results of the present survey highlight the psychotropic drug prescription status in the management of BPSD. A majority of Japanese clinicians prescribe miscellaneous psychotropic drugs including anti-psychotics for patients with a wide variety of BPSD in daily practice.

09i. Patient Care & Support: other

ADPD5-1574
CLASSIFICATION OF BEHAVIORS IN DEMENTIA BASED ON THEORIES OF INFORMATION PROCESSING

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Objectives: There is vast heterogeneity in use of terminology and classification of behaviors in dementia with no universally accepted classification system.
Methods: Criteria proposed by Davis, Buckwalter and Burgio (1997) were identified as the basis for classification of behaviors in dementia. A review of the literature was done to identify the "Specification of the Theoretical Construct" (STC) to justify aggregation of similar behavioral symptoms into clinically meaningful categories.
Results: STC identified for these behavioral categories are those based in theories on information processing (TIP). Two behavioral categories emanating from pathological changes in TIP are: Disorganized Behaviors (DOB), and Misidentification Behaviors (MiB).
Discussion: DOB is the result of an alteration in the physiological status of the patient. This result in changes in arousal and attentiveness and this, in turn, leads to impairment of the sequential organization of information processing thereby giving way to fragmentation of the process at many different levels of the brain. MiB are the result of a specific breakdown in two specific steps of TIP; schema identification and pattern recognition. This result in the failure of the usual pairing of old and new information with an altered sense of relatedness between self and persons, places, objects and events.
Keywords: Dementia, Behavioral Symptoms, Classification, Information Processing, Disorganized Behaviors, Misidentification Behaviors

09i. Patient Care & Support: other

ADPD5-1575
CLASSIFICATION OF BEHAVIORS IN DEMENTIA BASED IN "MOTIVATIONAL" AND "NEEDS BASED" THEORIES

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Objectives: There is vast heterogeneity in use of terminology and classification of behaviors in dementia with no universally accepted classification system.

Methods: Criteria proposed by Davis, Buckwalter and Burgio (1997) were identified as the basis for classification of behaviors in dementia. A review of literature was done with a view to identify the "Specification of the Theoretical Construct" to justify aggregation of similar behavioral symptoms into clinically meaningful categories.

Results: "Specification of the Theoretical Construct" identified for these behavioral categories are motivational and needs based theories. Behavioral categories emanating from these constructs are: Apathy Behaviors (AB), Goal Directed Behaviors (GDB), Motor Behaviors (MB), and Importuning Behaviors (IB).

Discussion: Apathy behaviors are the result of a decrease in the motivational drives with an absence of any need fulfillment. Goal-Directed Behaviors are the result of an increase in motivational drives with increase in detection and fulfillment of "belongingness" needs. Motor behaviors are the result of varying degrees of changes in motivational drives and are concomitants to other behavioral categories. Importuning behaviors are the result of preserved motivational drives in detection and fulfillment of "physiological needs".

Keywords: Dementia, Behavioral Symptoms, Motivational Theories, Needs-Based Theories

09i. Patient Care & Support: other

ADPD5-1576
CLASSIFICATION OF BEHAVIORS IN DEMENTIAS BASED UPON THEORIES OF REGULATION OF EMOTIONS

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Objectives: There is vast heterogeneity in use of terminology and classification of behaviors in dementia with no universally accepted classification system.
Methods: Criteria proposed by Davis, Buckwalter and Burgio (1997) were identified as the basis for classification of behaviors in dementia. A review of literature was done with a view to identify the "Specification of the Theoretical Construct" (STC) to justify aggregation of similar behavioral symptoms into clinically meaningful categories.
Results: STC identified for these behavioral categories are theories on regulation of emotions. Behavioral categories emanating from this construct are; Emotional Behaviors (EB), Fretful/Trepidated Behaviors (FTB) and Vocal Behaviors (VB).
Discussion: EB are based in expression of the emotion of melancholy and discontentment. EB based in melancholy provide the patient with a measured catharsis to allow for decompression from pain. EB based in discontentment provide the patient with protection from pain. FTB are based in the expression of emotion of fear. FTB make caregivers aware of the insecurity needs of the patient. VB are based in the expression of emotions of anger and joy. They highlight the 'out of proportion' nature of responses in patients. This response may or may not be associated with functional motor activity (FMA).
Keywords: Dementia, Behavior, Classification, Vocal Behaviors, Fretful/Trepidated Behaviors, Emotional Behaviors

09i. Patient Care & Support: other

ADPD5-1577
CLASSIFICATION OF BEHAVIORS IN DEMENTIAS BASED ON PRINCIPLES OF COMPLIANCE AND AGGRESSION
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Objectives: There is vast heterogeneity in use of terminology and classification of behaviors in dementia with no universally accepted classification system.
Methods: Criteria proposed by Davis, Buckwalter and Burgio (1997) were identified as the basis for classification of behaviors in dementia. A review of literature was done with a view to identify the "Specification of the Theoretical Construct" (STC) to justify aggregation of similar behavioral symptoms into clinically meaningful categories.
Results: STC identified for these behavioral categories are theories on compliance and aggression. Behavioral categories emanating from this construct are; *Oppositional Behaviors* (OB) and *Physically Aggressive Behaviors* (PAB).
Discussion: OB is the result of non-compliance to the directions being given by the care provider. The types of OB are determined by the level of developmental sophistication or conversely by the degree of cognitive impairment in patients with dementia. PAB are the result of perceived impediment by the patient in goal attainment. This results in the emergence of negative emotions. These emotions are 'out of proportion' to the stimulus. The purpose of this behaviour is to warn the care provider of the noxious nature of their involvement in the present situation.
Keywords: Dementia, Behaviors, Opposition, Aggression, Classification

09i. Patient Care & Support: other

ADPD5-2076

INTEGRATED ONE-STOP COGNITIVE ASSESSMENT CLINIC FOR DEMENTIA PATIENTS

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Introduction
Patient-centred care planning is vital to dementia patients. Multidisciplinary approach ensures continuity of care and unification of care goals. In a district hospital in Hong Kong, an integrated cognitive assessment clinic was established in 2013, operating with a novel collaborative model of providing an one-stop service.
Objective
To launch an integrated one-stop service for dementia patients through collaboration of an interdisciplinary team
Methodology
An interdisciplinary team comprising geriatricians, nurses, occupational therapists and physiotherapists was established in 2013. During the first consultation patients are assessed on various domains by members of the team. Related investigations including structural imaging are performed beforehand. Geriatricians are responsible to establish the clinical diagnosis. In the subsequent case conference, care planning and goals are aligned and formulated. Anti-dementia medications were prescribed to suitable patients. Cognitive rehabilitation including mind-body exercise was arranged whenever appropriate. Community resources were introduced and recommended so that patients and caregivers could receive maximal community support.
Results
In the recent 12 months, a total of 124 cases were attended. 71 (57.2%) were diagnosed dementia and 47 (37.9%) were mild cognitive impairment. Among those with dementia, 47 (37.9%), 17 (13.7%) and 7 patients (5.6%) were respectively graded as mild, moderate and severe stage of dementia. 31 patients (43.7%) received anti-dementia medications, 62 patients (50%) completed cognitive rehabilitative training, and 35 patients were recruited for SmartMove training (a sort of Mind-Body exercise programme).
Conclusion
An integrated one-stop cognitive assessment clinic could provide a holistic, people-centred framework in assessing and managing patients with dementia.

10b. Other: disease mechanisms

ADPD5-1119

ANTIPSYCHOTIC INDUCED CATALEPSY – POSSIBLE NEURODEGENERATIVE MARKER IN SCHIZOPHRENIA (ANIMAL MODEL STUDY)

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Objectives
Highlighting on animal model of potentially histological cerebral changes induced by haloperidol (dopamine blocker) and aripiprazole (partially dopamine agonist) associated with catalepsy. This model may be a clinical predictive indicator for the neurodegenerative-type course of schizophrenia.
Methods
Animal study on three samples of Wistar rats (8 per group), male adults, and weight between 200-250 grams, which were kept during the study in standard parameters imposed by ethical norms for animal study (study approved by Ethical Committee). Antipsychotics were intraperitoneal administered in maximum dosage: N1 – haloperidol (0.25mg/kg), N2 – aripiprazole (0.80mg/kg), N0 (control) – saline solution. Were assessed catalepsy (the homolateral limbs of the rats were crossed by the experimenter; animals which maintained this imposed abnormal position for more than 10 seconds were considered to be cataleptic) and abnormal behavior. Rats were sacrificed in day 11, after 12 hours from last injection. Biological material (frontal cortex and hippocampus) was histological prepared.
Results
On N1 sample, all rats presented catalepsy and abnormal behavior (apathy and aggression), while in N2 sample was not identified this phenomena. Histological assessment highlighted neuronal changes in N1 sample: apoptosis, vacuolization, pinocytosis, necrosis and microhemorrhages in frontal cortex and hippocampus. In N2 sample, these changes were minimal.
Conclusions
Haloperidol lead to catalepsy and important histological changes in frontal cortex and hippocampus, compared to aripiprazole, according to the neurodegenerative model.

10b. Other: disease mechanisms

ADPD5-1331
ASSESSMENT OF BASELINE AND FOLLOW-UP SERUM URIC ACID LEVELS IN ESSENTIAL TREMOR

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Objective: Decreased serum uric acid has been associated with neurodegenerative diseases such as Parkinson's disease (PD) in the elderly. Several studies suggest that there may be a link between PD and Essential tremor (ET) which is thought to be a neurodegenerative disease. Serum uric acid level (UA) and its relationship with prognosis in ET patients have not been addressed.
Method: Subjects with ET were evaluated. We collected serum samples to determine biochemical indicators including UA, glucose, blood lipids, liver function, and renal function. All the patients with vascular risk factors, dementia, depression or other neurodegenerative disorders were excluded, as were subjects on uric acid-lowering therapy or with serious illnesses such as severe anemia, chronic renal failure, hepatic disease or active or ongoing cardiovascular or cerebral vascular disease. One hundred and sixteen subjects (52 isolated ET patients and 64 healthy controls well matched in comparison of age and sex) were enrolled.
Results: UA level was similar between the groups. Follow-up UA levels of the patients was similar to controls, too. UA level correlated to age, ET starting age, cholesterol level and creatinine level ($p<0.05$).
Conclusion: There were reasonable epidemiological evidences to support a link between ET and UA level, but we did not find any difference between serum UA levels of ET patients and controls in follow-up. Age was one of the factors contributing to the increased content of UA. These findings also supported the knowledge about isolated form of ET which was stable and benign.

10c. Other: preclinical research

ADPD5-0744
THE MCCUSKER SUBJECTIVE COGNITIVE IMPAIRMENT INVENTORY: A NEW SELF-REPORT MEASURE OF COGNITIVE CONCERNS

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Subjective Cognitive Impairment (SCI) has recently been acknowledged as an early preclinical stage of dementia. It refers to an individual's concerns about their own cognitive functions. There is a shortage of reliable and valid measures to assess ones concerns in relation to their current cognitive functions as compared to their previous functioning. The currently available measures do not provide information with clinical and research applications. We report on our newly developed 46-item measure, namely the McCusker Subjective Cognitive Impairment Inventory (McSCI). The McSCI captures ones' complaints about the changes they have noticed in their cognitive functions. It measures concerns associated with six cognitive domains including language, orientation, attention and concentration, executive function, memory, and visuoconstructive abilities. Each question is scored on a five point *Likert scale* ranging from four (almost always true) to zero (almost never true). The McSCI has patient and informant versions and provides scores for concerns associated with each cognitive domain in addition to a total SCI score. The McSCI has acceptable reliability and validity and has been examined against objective and subjective cognitive and memory measures. As a short and quick measure, the McSCI provides enough information to be used in research and in clinical settings.

10c. Other: preclinical research

ADPD5-0761

ANTICHOLINESTERASE ACTIVITY OF AN ACTIVE FRACTION OF THE EXTRACT OF LEAVES OF CITRUS LIMON (L.) BURM

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Objectives: The aim of this study was the qualitative and quantitative evaluation *in vitro* and *ex vivo* of a mix of compounds (5,8-Dimethoxypsoralen and 5,7-Dimethoxycoumarin) isolated from leaves of *Citrus limon* (L.) Burm to verify potential inhibition of acetylcholinesterase. **Methods:** Qualitative inhibitory activity was performed according to Ellman's method (1961) and quantitative activity was evaluated by Ellman and colleagues spectrophotometric method adapted (1961) and Moyo and colleagues (2010). All the *ex vivo* experiments performed in this study got approved by the Animal Experimentation Ethics Committee of the Federal University of Piaui (CEEA / UFPI # 44/09). **Results:** The compound presented positive result in the qualitative inhibition of acetylcholinesterase enzyme, observed through the thin-layer chromatography plate, which presented yellow colored with white spaces. In the qualitative study we noticed an inhibition of 95.9% when neostigmine (positive control) was used at a concentration of 0.1 mg/mL. With the isolated compound at concentrations of 0.1, 0.05, 0.025, 0.0125 and 0.00625 mg/mL we have detected an inhibition of 57.75, 49.89, 35.03, 23.78 and 8.71% in the *in vitro* activity of AChE, respectively. Based on these results, the EC_{50} was also determined as 0.061 mg/mL with confidence interval of 95% (0.033 a 0.18 mg/mL; $r^2 = 0.9935$). *Ex vivo* studies (10 and 25 mg/kg) revealed inhibition of 30.09 and 30.06% of AChE activity compared to neostigmine. **Conclusion:** Compared to results of other studies, our data agree with the hypothesis that coumarinic compounds present anticholinesterase potential.

10c. Other: preclinical research

ADPD5-1014

COPPER CHELATION EFFICACY OF D-PENICILLAMINE NANOPARTICLES IN EXPERIMENTAL RAT MODEL FOR NON-WILSONIAN BRAIN COPPER TOXICOSIS

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Objective: D-penicillamine is unable to mitigate the brain copper overload and neurological manifestations in Alzheimer's/Wilson's disease patients. The aim of this study was to assess the therapeutic efficacy of orally administered D-penicillamine loaded nanoparticles to that of conventional D-penicillamine for 90 days in Wistar rat model for non-Wilsonian brain copper toxicosis.
Methods: High performance liquid chromatography, atomic absorption spectrophotometry, neurobehavioral and histopathological studies, and nanoparticles preparation/physicochemical characterization were carried out.
Results: D-penicillamine nanoparticles exhibited mean 274.09 nm size and less than 29.32% of D-penicillamine release under in vitro conditions. Pharmacokinetics studies showed augmented levels of D-penicillamine in brain of nanoparticles based D-penicillamine delivery group compared to conventional D-penicillamine delivery group. Conventional and nanoparticles based D-penicillamine therapy resulted in significantly improved neuromuscular coordination and memory along with concomitant increase in urinary copper levels, and acetylcholinesterase activity in rat model of copper toxicosis. Conventional D-penicillamine therapy resulted in negative rhodanine staining of liver and brain sections corroborated by 60.1%, and16.4% reduction in hepatic and brain copper content, respectively compared to non-treated copper-intoxicated group. However, liver and brain sections of nanoparticles based D-penicillamine therapy group demonstrated grade 1 copper and no copper depositions substantiated by 47.2% and 32.8% reduction in hepatic and brain copper content, respectively in comparison to non-treated copper-intoxicated group.
Conclusions: Taken together, the present study reveals the first *in vivo* evidence for therapeutic efficacy of nanoparticles based D-penicillamine therapy in chelating more brain copper and alleviating neurological deficits even at half the dose as given in conventional D-penicillamine therapy.

10c. Other: preclinical research

ADPD5-1713
MODELIZING TOPOGRAPHICAL DISORIENTION, AN ALERT SIGN IN ALZHEIMER'S DISEASE, IN MICE USING A NOVEL COMPLEX MAZE: THE HAMLET TEST®

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One of the alert signs that Alzheimer's disease in the elderly is disorientation in familiar environment. We designed an apparatus mimicking a small village to examine topographical memory and disorientation in complex environment. The Hamlet Test® comprises a central agora and 20 streets expanding from it in a 5-branch shape, leading to functionalized houses. Five physiological needs are modelized in the different houses : Eat, Drink, Play, Run and Interact. Animals were trained in groups, spending 4h/day during 1d, 1, 2 or 4 weeks in the Hamlet Test and their ability to orientate was tested after different conditions and timing. Topographical memory was assessed in water- or food-deprived animals by measuring the latency and number of errors spent to reach the Drink or Eat house. Animals explored all houses, but differentially, according to their rewarding impact, suggesting that they efficiently integrated the spatial complexity. Deprived animals showed significantly decreased latencies and numbers of errors, particularly after 1 day, 2 or 4 weeks habituation period, showing that topographical memory is activated in the test. Finally, animals trained during 2 weeks were intracerebroventricularly injected with oligomeric amyloid ß25-35 peptide or scrambled control peptide 3 days after training. When retested in the Hamlet Test after 7 days, Aß25-35-treated animals showed memory impairment. We are currently testing transgenic mouse lines. The Hamlet Test appears as a novel behavioral test that can address not only environmental enrichment, but also, more specifically, topographical disorientation observed in Alzheimer's pathology.

10c. Other: preclinical research

ADPD5-2091

PHYSICAL EXERCISING IS REDUCING ANXIETY, DEPRESSION AND MEMORY DEFICITS ASSOCIATED WITH A MPTP-INDUCED RAT MODEL OF PARKINSON'S DISEASE

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Background
It is well known that in Parkinson's disease (PD), individuals have greater reduction in physical activity levels. Also, inactivity is considered an important factor in accelerating the degenerative process of PD. In addition, PD is known for its cognitive impairments, as well as for depression and anxiety disorders, which may be important causes of morbidity (40% prevalence in PD).
Also, one of the most used animal models of PD is generated by the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).
Methods:
We want it to see if induced physical exercising in an MPTP-induced rat model of PD (20 mg/kg i.p.), will result in any changes in memory (as tested in Y maze), anxiety (as tested in elevated-plus-maze) and depression-like behaviour (forced-swim-test), as compared to a non-exercised control group of rats which also received MPTP.
The exercising was performed on an adapted treadmill, for 2 weeks (3 series of 5 minutes/day).
Results
In the group of exercised MPTP group we could observe an increased time spent by the rats in the open arms of the elevated-plus-maze, together with a significant decrease of stretching behaviour and increased head dipping, as compared to non-exercised MPTP group, factors with are suggesting an anxiolytic-like manifestation. In addition, spontaneous alternation in Y maze (index for immediate memory), and swim time (anti-depressive index) in forced swim test were increased in the exercised rats with an MPTP-induced model of PD.
Conclusions
Physical exercising seems to reduce anxiety, depression and memory deficits associated with a MPTP-induced rat model of PD.

10d. Other: diagnostics

ADPD5-0695

THE TIME OF THE ONSET OF URINARY SYMPTOMS IN MULTIPLE SYSTEM ATROPHY

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Objective

We aimed to clarify the time of the onset of urinary symptoms with respect to the clinical phenotype.

Methods

We have retrospectively reviewed the medical records of the 100 patients with MSA who were diagnosed with probable or possible MSA according to the Gilman's second consensus criteria over the past 5 years to identify the initial symptoms, the time of the onset of urinary symptoms and the post-void residuals (PVR).

Results

Our results included 59 patients with MSA-C and 41 patients with MSA-P. Mean disease duration was 3.1 years in both groups. The initial symptoms of MSA-C were as follows: Autonomic symptoms n=12, Cerebellar symptoms n=47.(Fig 1-a) In MSA-P, the initial symptoms were as follows: Autonomic symptoms: n=14, Extrapyramidal: n=27.(Fig 1-b) The time of the onset of urinary symptoms in patients whose initial symptoms were motor dysfunction was 2.2±1.3 years in MSA-C and 2.1±1.2 years in MSA-P without significant difference.(Fig 1-c) The maximum durations between the onset of urinary symptoms and initial motor symptoms were 6.0 years in MSA-C and 4.7 years in MSA-P. The mean PVR at the time of examination was 148±119 ml in MSA-C and 152±111ml in MSA-P without significant difference.

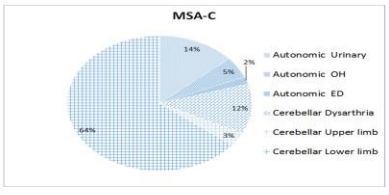
Conclusions

The time of the onset of urinary symptoms were various and seemed to be unrelated to the clinical phenotypes.

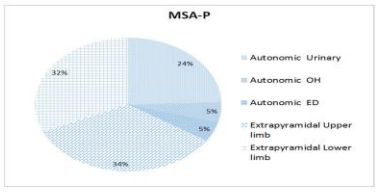
A long term examinations of urinary symptoms are necessary for the correct diagnosis of MSA.

Figure 1

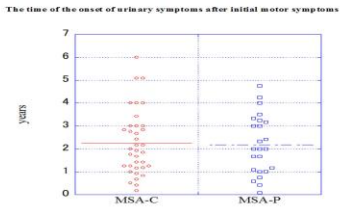
(a)



(b)



(c)



10d. Other: diagnostics

ADPD5-0769

JOINT ANALYSIS OF ELECTROENCEPHALOGRAM, ELECTROMYOGRAM, AND TREMOR IN THE EARLY STAGE OF PARKINSON'S DISEASE

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· Objectives

Electroencephalogram, electromyogram and tremor of the non-treated early stage Parkinson's disease (PD) patients were investigated jointly. The control group and the 1st stage of Hoehn-Yahr scale patients group were consisted of 16 and 25 people respectively.

· Methods

The time-frequency distribution of extrema points of the electroencephalogram, envelope of electromyogram and tremor wavelet spectrograms were analyzed to extract quantitative PD features. Frequency synchronization of electroencephalogram, electromyogram and tremor were found out.

· Results

Figures illustrate the interhemispheric asymmetry of the motor cortex shown by time-frequency extrema points distribution, and the connectivity of 4-6 Hz electroencephalogram rhythm in one cortex area with the contralateral electromyogram and tremor. C3, C4, EMG_RH, EMG_LH, RH, and LH mark the EEG and EMG electrodes, right and left hand accelerometers respectively.

The coincidences with the clinical diagnosis for joint electroencephalogram, electromyogram and tremor investigations is equal to 92 percent's for the 1st stage PD patients and 87 percent's for the control group.

· Conclusions

Joint electroencephalogram, electromyogram, and tremor investigation gives reliable quantitative diagnosis of the 1st stage Parkinson's disease in comparison with the clinical diagnosis.

10d. Other: diagnostics

ADPD5-0799

VALIDATION OF THE KOREAN LEWY BODY COMPOSITE RISK SCORE FOR DISCRIMINATION OF DEMENTIA WITH LEWY BODIES IN THE KOREAN ELDERLY

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The Lewy Body Composite Risk Score (LBCRS) was developed as an useful tool to enhance the accuracy of the cognitive diagnosis for patients affected by Lewy Bodies' pathological conditions. This study aimed to assess the diagnostic accuracy of the Korean version of the LBCRS (K-LBCRS) among Korean population with Alzheimer's dementia (AD) and Dementia with Lewy bodies (DLB). The 49 subjects who participated in this study have (32 with AD, 17 with DLB) visited the neurology outpatient clinic of KUMC. The subjects' demographic data and administered K-MMSE, Clinical Dementia Rating sum of boxes (CDR-SB), K-LBCRS, NPI, Mayo Fluctuation Scale (MFS), Mayo Sleep Questionnaire (MSQ), Epworth Sleepiness Scale (ESS), and mini Physical Performance Test (PPT) were collected. The K-LBCRS was created through translation and back-translation of the LBCRS. The sensitivity, the specificity, and the area under the curve were evaluated by receiver operator characteristics (ROC) analysis. An ROC curve was used to determine the optimal cut-off values for discrimination of DLB against AD. The ROC analysis showed that the optimal cut-off point of the K-LBCRS for identification of DLB was 2/3, which gave the balance between sensitivity (94%) and specificity (75%). The K-LBCRS was significantly correlated with CDR-SB (r=0.40), MFQ (r=0.75) in AD group, whereas ESS (r=0.71), MFQ (r=0.82) was significant in DLB group. The K-LBCRS has important clinical characteristics of DLB that may differentiate it from AD, and as a result may enable the K-LBCRS as a clinically useful screening tool to discriminate the two groups.

10d. Other: diagnostics

ADPD5-1032

DO REHABILITATION STAFF RECOGNIZE THE DIFFERENCE BETWEEN LATE PARAPHRENIA AND DEMENTIA? A QUESTIONNAIRE SURVEY

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Objectives: Medical support staff in Japan are generally unfamiliar with late paraphrenia, and it is easy to misdiagnose elderly patients with paraphrenia as suffering from dementia. Therefore, it is predicted that many patients with late paraphrenia in Japan have been misdiagnosed in this way. A pilot study is reported here that aimed to develop a checklist for judging late paraphrenia and dementia. Our initial purpose was to investigate the knowledge rehabilitation staff have regarding late paraphrenia.
Methods: A questionnaire was sent to 300 occupational therapists working within elder-care institutes in Japan. Names were sampled randomly from an applicable association list. Questionnaires were returned anonymously by mail.
Results: There was a response rate of 36% (108 questionnaires returned), of which most responses were from women over the age of 30. Only 12% of respondents were familiar with late paraphrenia. Three respondents (2.8% of the sample) had encountered patients with late paraphrenia. In contrast, 40% of the therapists had examined a patient in a condition consistent with late paraphrenia. The content of the patient's delusions were what led the therapist to suspect that the patient might have a condition other than dementia.
Conclusion: In Japan, most rehabilitation staff do not know of late paraphrenia, but it is clear that they recognize when a patient might be suffering from a disease inconsistent with their diagnosis of dementia. The content of delusions might help discriminate dementia from late paraphrenia.

10d. Other: diagnostics

ADPD5-1167
WHAT IS THE DIFFERENCE BETWEEN THE COGNITIVE PROFILES OF HUNTINGTON'S AND PD?
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Aim: Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disease, characterized by motor, cognitive and behavioral problems. Here, our aim is to define the cognitive profile specific to HD with comparison to PD and to search for its relationship with CAG repeat numbers.
Materials and Methods: Demographics and disease features of non-demented patients with HD (n=24), PD (n=20), control (n=16) groups were noted. For detailed cognitive evaluation, minimental state examination, geriatric/Beck's depression scales, enhanced cued recall (ECR), semantic and phonemic fluency, digit-span forward and backward, trail making test part A-B, reciting months backward, Stroop, clock drawing, Benton's line orientation, Benton's facial recognition and Hooper tests were administered. The relationship between the test results and CAG repeat numbers and CAP scores (product of CAG repeat number and age) for HD was also evaluated.
Results: Age was similar for all groups; disease duration and education were similar for HD and PD. All test results of HD group were significantly worse than those of controls; the results for ECR, semantic fluency, digit-span forward, reciting months backward and Benton's facial recognition were significantly worse than PD. CAP score, which determines the duration and severity of exposure to the mutant gene product, is found to be significantly correlated with the results of digit-span forward, Stroop and trail making test part B.
Conclusion: This study showed that HD has a cognitive profile with certain particular features, which differentiates it from PD. Also executive functions were found to have correlation with CAP score.

10d. Other: diagnostics

ADPD5-1618
IDENTIFICATION OF VERY MILD DEMENTIA BY ACTIVITIES OF DAILY LIVING: A CREDOS STUDY
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Background: We aimed to select more sensitive items in instrumental ADL questionnaires and make a identification system of very mild dementia.
Methods: We recruited 8779 patients who were diagnosed with healthy subject, subjective memory impairment (SMI), mild cognitive impairment (MCI), and dementia from the Clinical Research Center for Dementia of Korea (CREDOS) registry. All subjects were classified into three patient groups by clinical diagnosis and Clinical Dementia Rating (CDR) score: predementia, very mild dementia (CDR 0.5), and remaining dementia. Multivariate logistic regression were used for identifying discriminative items in Seoul instrumental ADL (SIADL) in men or women, respectively. Prediction model was made with equations including discriminative items and age.
Results: At first, we compared dementia patients with predementia patients. In male, SIADL questionnaire excepting 2 items (outgoing for a short distance and securing a door) had same discrimination ability as original questionnaire with 15 items (area under the curve, 0.903 vs 0.903). In female, SIADL questionnaire excepting 5 items (outgoing for a short distance, grooming, using household appliances, managing belongings, and securing a door) presented almost same results (area under the curve , 0.914 vs 0.913). With the same method, we compared very mild dementia patients with remaining dementia patients. SIADL questionnaire excepting 9 items in male and excepting 5 items in female showed good discrimination ability (area under the curve, 0.834 and 0.833, respectively).
Conclusions: We expect that this result could be helpful for primary physician examining suspicious dementia patients in Korea.

10d. Other: diagnostics

ADPD5-1656

CRYPTOGENIC TEMPORAL LOBE EPILEPSY: RESTING STATE FMRI RESULTS

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Cryptogenic temporal lobe epilepsy (CTLE). has no evidence of morphological changes in brain structure, that can be revealed using conventional neurovisualisation tools. Patients with CTLE usually suffer memory dysfunction. It is considered that verbal memory is affected by left-sided Hippocampal sclerosis (HS), whereas visuo-spatial memory is more affected by right HS. But what if there's no HS take place along with functional abnormality mentioned above. Some of these impairments may be related to breakdown of the network in which hippocampus may take part. Functional connectivity establishment can lead to understanding how the hippocampi interact with other brain areas. It can be estimated with the help of resting state fMRI by evaluating patterns of functional networks. In this study we investigated functional connectivity patterns of 19 controls, 31 patients with right CTLE and 25 with left-sided CTLE.

We found differences in functional connectivity within and between hippocampi in patients with unilateral CTLE. Functional connectivity turned out to be more impaired ipsilateral to the seizure focus in both patient groups when compared to control subjects. This effect was even more obvious in the left-sided CTLE patient group. These results suggest that left-sided CTLE meets more reduction of functional connectivity than right-sided one in subjects with left hemisphere language dominance. Still morphological study must take place to dicover if there are any brain matter volume changes, especially in critical for brain network functional areas.

10d. Other: diagnostics

ADPD5-1716

OBJECTIVE MEASUREMENT OF GAIT PARAMETERS IN HEALTHY AND COGNITIVELY IMPAIRED ELDERLY USING THE DUAL TASK PARADIGM

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Background
Motor function involves the integration of various cognitive functions including visuospatial perception, attention, and planning. Deficits in these cognitive functions can affect motor performance. Subtle changes in motor function can therefore be an early indicator of cognitive decline. Attentional deficits are of particular interest because motor function requires attentional resources and these decline in very early stages of dementia. Research has only recently started to look into ways to objectively measure subtle changes in attention.
Methods
A study including 60 participants is currently conducted to explore the effect of attentional deficits on gait parameters. The sample consists of MCI and AD patients and healthy age-matched controls. Neuropsychological measures include the MMSE and the Trail Making Test. All participants perform a single walking task and a dual task. The dual task involves walking while counting backwards. During these tasks, participants wear a wrist-worn accelerometer from which objective measures for gait speed, variability and cadence are derived. We hypothesize that walking speed is slower, cadence is lower and variability is greater in participants with cognitive impairment under the single task condition compared to healthy controls, and that these differences are more pronounced under the dual task condition.
Results
The study is ongoing and results will be presented at the conference.
Conclusions
The findings will add to the growing body of research on the relation between cognitive functions and motor performance and are relevant for clinical practice. An assessment using the dual task paradigm can contribute to an improvement of clinical decision-making.

10d. Other: diagnostics

ADPD5-1802

SPIRITUAL WELL-BEING QUESTIONNAIRE SHALOM, CZECH VERSION – A VALIDATION STUDY. DATA FROM THE CZECH BRAIN AGING STUDY

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Objectives: The Czech Republic is one of most atheistic countries in the world. Interestingly one third of population identifies themselves in sociological researches as a 'spiritual' in some way, but they do not want to be associated with any form of institutionalized religion. Spirituality was identified in few studies as a protective factor in neurodegenerative diseases. To verify this finding we need reliable tool for measuring spirituality. The aim of this study is to examin validity of Czech version of Spiritual well-being questionnaire SHALOM and to examine limits of this questionnaire for subjects with cognitive impairment.

Methods: 107 elderly subjects devided into three groups: mild cognitive impairment (MCI), subjective memory complaints (SMC), and healthly eldrely (HE), completed SHALOM questionnaires. All subjests also underwent complete neuropsychological examination.

Results: Reliability was determined using Cronbach's alpha for internal consistency. The results indicate that the Cronbach's alpha coefficients showed acceptable and satisfactory internal consistencies, standardized Cronbach Coefficient Alpha was 0.951. Results also suggested that the SHALOM questionnaire is reliable tool to measure spirituality also in population with mild cognitive impairment.

Conclusion: The results showed that SHALOM questionnaire is reliable tool for measuring spirituality in Czech elderly population, including subjects with mild cognitive impairment.

10d. Other: diagnostics

ADPD5-1911
PARKINSONIAN MOVEMENT DISORDER FROM PONTINE INFACRTIONS
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BackGround:
61 year old female was treated for Parkinsonian Disorder with Co Carledopa and Amantadine 11 years ago. Until a few years ago she was managing life fairly Independently with some help from the family.In January she noticed multiple falls And distinctive movment disorder set in which was not akin to levo-dopa induced dyskinesia. She was ataxic and off balance with Lying BP 130/70mmHg and Standing BP of 130/70mmHg Sinus rhythm of 78 bpm.
Hospitalisation
In January, she was admitted after a fall, found to have nystagmus, ataxic quadriparesis with lateropulsion and left to right sway. In addition she had athetoid head , neck, hands,pelvis and leg movement disorder. She had grade 3 motor deficit but no sensory impairment. No Horner,s or internuclear opthalmoplegia were noted.
Pursuit movement was positively saccadic. Her initial dysphonia and dysphasia resolved after a few days. Vascular screen was negative.
Radiology
MR scan confirmed 2 lacunar infarcts in the right pons and one in the left pons. Carotid dopplers showed minimal stenosis at the origin of the carotid arteries.
Diagnosis:
Bilateral Pontine Infacrtions with movement disorder mimicking worsening of Parkinsonian Symptoms.
Management:
She improved with slight reduction of Levodopa dosage and introduction of Procyclidine. Intensive rehabilitation helped get her back to mobility and vastly
improved movement disorder.
References: Movement disorder after strokes, Age &Ageing 2009,38.3:260-266
Clinical Spectrum of Pontine Infarctions, E Kumral, G Bayulkem, D Evyapan, Journal of Neurology, Vol 249,12 Dec 2002 , 1659-70.

10d. Other: diagnostics

ADPD5-1937
SHOULD DSM-5 HAVE RECOGNIZED "NEUROCOGNITIVE DISORDER DUE TO SCHIZOPHRENIA" AS A SEPARATE DIAGNOSIS?

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Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) introduced the term Neurocognitive disorder (NCD) and subsumed dementia into it. While it is known that Schizophrenia can lead to cognitive impairment it's exclusion from NCD is unclear. Through this presentation, the speaker will discuss the reasons why the diagnosis "Neurocognitive disorder due to Schizophrenia" should have been included. While mental illnesses are excluded with the consideration that cognitive deficits are not a core feature, NCDs due to Parkinson's disease and stroke are included which are primarily non-cognitive in nature. Functional decline is an expected feature in the proposed "NCD due to Schizophrenia" like all the other NCDs. Age of presentation of cognitive problems may vary from young-old just as may do with NCD due to traumatic brain injury and HIV. While it may be debated whether cognitive impairment in Schizophrenia takes a progressively deteriorating course or remains static, this becomes irrelevant considering that Substance induced and HIV related NCDs have a static course or may even improve.

The above explanation suggests that "NCD due to Schizophrenia" should be considered for inclusion in the future revision of DSM and should be kept in mind by authors of other classification systems. This is also important considering that the pathology and resultant profile of affected cognitive domains are less likely to match those of other NCDs. Recognizing this as a separate entity can potentially help with focused research, improved access to care and treatment, and better support for patients and carers.

10d. Other: diagnostics

ADPD5-2049

GOAL-DIRECTED MOTOR CONTROL FUNCTION ESTIMATION IN A MAN-MACHINE INTERACTION SET-UP FOR THE OBJECTIVE EVALUATION OF MOVEMENT DISORDERS.

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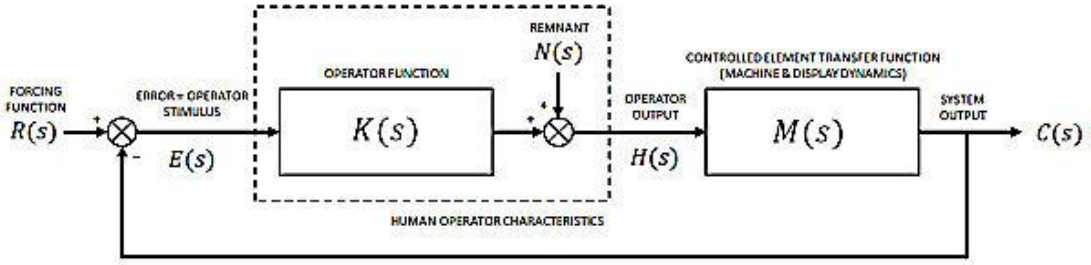
This study concerns the application of control-theory to humans for the modelling and the numerical estimation of goal-directed motor control and learning. Control-theory is a concept largely used in engineering for conceiving automatic systems (like automatic pilot in aircrafts). In our case we were interested in modelling the process implemented by the brain in controlling by hand an external machine. We use a black-box numerical identification of the human operator performances in a man-machine control-loop, where the human is asked to impose to the machine a given behaviour.

In the standard experiment set-up, a pursuit task is proposed to the subject under test. On the screen a target spot (yellow) is presented which the subject has to follow with a joystick controlled spot (red). In the figure the position of the yellow spot is the forcing function or reference while the red spot position is the system output. The task of the human under test is that of minimizing the error, i.e. the difference in position of the two spots. The machine under control is the couple of the joystick and the red spot whose transfer function can be modified by software. A system identification software (MATLAB) evaluates the parameters of the transfer function implemented by the subject providing a "finger-print" numerical assessment of the operator performance. Being the behaviour of the subject simultaneously affected by the cortex (goal-directed) and basal ganglia (habitual control), the proposed method should in principle show a high sensibility and sensitivity to central movement disorders.



10d. Other: diagnostics

ADPD5-2128

CORTICAL THICKNESS IN ALZHEIMER'S DISEASE WITH POSTURAL INSTALBILITY MEASURED BY COMPUTERIZED DYNAMIC POSTUROGRAPHY

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Background

Postural instability in the elderly may be affected by cognitive functions such as attention, executive and visuospatial functions besides underlying physical conditions. The aim of our study is to view the association between dynamic postural instability, cognitive dysfunction and cortical thinning in Alzheimer's disease (AD).

Methods

We investigated 107 AD and 37 controls. All of the participants were taken neuropsychological evaluation, brain MRI, and computerized dynamic posturography (CDP). Image analyses were done by multiple regression under measuring cortical thickness using freesurfer. The demographic features and CDP performances were compared between groups and within AD.

Results

Control showed better performance of CDP and neuropsychological evaluation, and less cortical thinning than AD group. The performance of the unilateral stance in AD, such as swaying distances, velocities, the frequencies of falling, were poorer than controls after adjusting for age and gender. Within AD, falling frequency was correlated with visual memory and COWAT test. However, motor latency and sensory organization test were not significant. Imaging analyses revealed that cortical thinning in the right temporal and frontal area and bilateral precuneus, was correlated with CDP parameters.

Conclusion

Our study represents dysequilibrium in AD is associated with localized cortical thinning of the prefrontal and the temporal cortex, and cognitive dysfunction such as frontal executive functions, visual memory and naming ability. Imbalance might be the epiphenomenon of AD.

10d. Other: diagnostics

ADPD5-2178

STANDARD MOLECULES IN OLIGOMER-BASED DIAGNOSIS FOR ALZHEIMER'S DISEASE

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Objectives:

The sFIDA (surface-based fluorescence intensity distribution analysis) technology for early diagnosis of Alzheimer's disease is based on highly specific counting of amyloid beta (Aβ) oligomers in body fluids. For assay calibration and absolute quantification of Aβ oligomers, internal standards with defined numbers of Aβ molecules are required.

Methods:

Standard molecules were synthesized and serially diluted in CSF and plasma and applied to the sFIDA assay.

Results:

TEM micrographs show standard molecules of defined size. Analysis by FTIR spectroscopy demonstrates the efficiency of the synthesis.
By sFIDA we could successfully detect and quantify the standard molecules down to a femto-molar range.

Conclusion:

We observed reliable and biochemically stable standard molecules of known size and defined number of binding sites for applications in diagnostic assays and for spiking experiments in Alzheimer's disease diagnosis assay development.

10e. Other: imaging

ADPD5-1522

EFFECTS OF SCOPOLAMINE ON WORKING MEMORY TASK AND RESTING FUNCTIONAL CONNECTIVITY USING FMRI IN HEALTHY KOREAN SUBJECTS: A MODEL SYSTEM TO EVALUATE PRO-COGNITIVE COMPOUNDS IN AD.

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OBJECTIVE: To evaluate the effects of scopolamine on circuitry mediating cognitive performance using fMRI to evaluate pro-cognitive compounds in AD.
METHODS: Ten healthy Korean subjects participated in a randomized, double-blind, placebo-controlled, cross-over, single-dose, study to investigate the effects of scopolamine on working memory load (2-back vs 0-back) and resting state functional connectivity using fMRI. Subjects were randomized to receive scopolamine (0.5 mg) or placebo administered subcutaneously under fasted conditions 90-min prior to initiating fMRI scanning protocol. A Siemens 3T-Verio scanner, with an 18-channel head coil, was used to capture EPI-BOLD sequences during resting state and cognitive challenges using an established version of the n-back paradigm. Dosing and fMRI scanning procedures were conducted over 2 separate clinic visits for each subject separated by a 2-4 day washout.
RESULTS: Subcutaneous scopolamine during increased WM load resulted in decreased accuracy (p=0.08) and increased errors (p=0.09), with a moderate effect size in decreased accuracy in the 2-back condition (d=-0.5). Scopolamine significantly decreased activity in cholinergically-relevant regions involved in WM including the vmPFc, basal forebrain and cuneus/precuneus. Scopolamine reduced functional connectivity between bilateral DLPFc, the PCC and other regions involved in WM.
CONCLUSIONS: Our results demonstrate the utility of scopolamine disruption of WM task in an fMRI paradigm that may aid in evaluation of novel pro-cognitive compounds for neurodegenerative disorders. We suggest that neurocircuitry derived measures from fMRI may allow a more sensitive assessment of the reversal of scopolamine effects by potential pro-cognitive treaments than achieved with traditional cognitive testing

10e. Other: imaging

ADPD5-1828
STEREOLOGICAL EVALUATION OF THE OPTIC NERVE VOLUME IN ALZHEIMER'S DISEASE

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Background: Alzheimer Disease (AD) is the most common cause of dementia. As previous studies indicate optic nerve damage occurs in AD occurs related to the loss of the retinal ganglion cells that contribute fibers to the optic nerve and reduction of the density of optic nerve.
Objective: To evaluate weather the patients with AD show optic nerve atrophy or not and evaluate the relation between the cerebrum volume and optic nerve volume in AD patients.
Materials and Methods: The study evaluated the volumetric measurements of optic nerve by applying stereo logical method on magnetic resonance images (MRI). It included age matched study and control groups of which were composed of 20 patients with probable AD and 20 healthy subjects respectively. MRI images were analyzed by using point counting approach holding the Cavalieri's principle.
Results: There were statistically significant optic nerve and cerebral atrophy in AD patients compared with the age matched health subjects ($p=0.013$, $p<0.001$, respectively) but there was no correlation between the optic nerve and cerebral volumes in AD patients ($r=0.326$, $p=0.160$).
Conclusions: There was significant atrophy in optic nerves between the AD patients and control subjects. The stereo logical evaluation of optic nerve volume is of importance for both clinicians and anatomists and it can provide valuable information in the evaluation of morphological changes of AD in vivo.

10e. Other: imaging

ADPD5-2279

HYPOGLYCEMIA AS A TRIGGER FOR THE SYNDROME OF ACUTE BILATERAL BASAL GANGLIA LESIONS IN PATIENTS TREATED WITH REGULAR DIALYSIS: TWO CASE REPORTS

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Acute movement disorder associated with symmetrical basal ganglia lesions occurring on the background of diabetic end stage renal disease (ESRD) is a recently described condition with distinct clinico-radiological features: Reversible symmetrical lesions located in basal ganglia on MRI are hallmarks of this syndrome. Parkinsonism and/or involuntary movements are the prominent clinical presentations. Pathophysiology is still unclear, but metabolic and vascular factors are thought to play an important role. We aimed to draw attention to this clinical picture with two cases of diabetic ESRD, showing common clinical and radiological features following episodes of hypoglycemia. Bilateral hypointense lesions on T1-weighted and hyperintense lesions on T2-weighted images at basal ganglia level were detected in both patients (Figures A_1-B_1). Repeated scans in a period ranging from 3 weeks to 2 months after initial insult showed the vanishing of the lesions (Figures A_2-B_2). As previously reported, we also consider hypoglycemia as a candidate trigger factor for the syndrome of acute bilateral basal ganglia lesions, especially in diabetic ESRD patients. We would like to highlight these two cases to raise awareness of this syndrome and to emphasize the importance of adequate dialysis, prevention and correction of hypotension, acid-base and electrolyte imbalances and strict glucose control in uremic patients.

10f. Other: clinical trials

ADPD5-1238
PREVENTION OF FALLS IN PD: EFFECTS OF STOCHASTIC WHOLE BODY VIBRATION
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Objective: Postural instability confers a high risk of falls and shows insufficient response or even aggravation to dopaminergic therapy in Parkinson's disease (PD) patients. We performed a double-blind randomized controlled study to test the effects of stochastic whole body vibration (sWBV).
Methods: 56 PD patients were allocated either to the experimental or sham group. The experimental group received 4 cycles of sWBV on four days, each cycle consisting of 6 stimulus trains of 60 seconds duration (frequency 6-7 Hz) and 30 seconds resting time in between stimuli using the SRT zeptor system. Patients allocated to the control group received a sham treatment with 1 Hz. United Parkinson's Disease Rating Scale ($UPDRS_{III}$), Tinetti Balance-and-Gait, 8-Meter-Walk (8MW), Timed-Up-and-Go-Test and posturography were performed prior to and after treatment (days 1 and 4). The reduction of subscores relative to baseline served as primary outcome measure.
Results: In the experimental-group several items improved significantly after the 4 day course: rigor 41.6% (P = 0.001), bradykinesia 23.7% (P = 0.001), tremor 30.8% (P = 0.006), pull-test 17.9% (P = 0.032), $UPDRS_{III-}$ sum-score 23.9% (P = 0,000), 8MW 8.75% (P = 0.011), posturography 17.5% (P = 0.005). When patients were subdivided based on fall frequency, fallers showed a more pronounced improvement in posturography 19.9% (P = 0.019).
Conclusion: SWBV alleviated motor symptoms and significantly enhanced postural stability in PD-patients even in patients with increased risk of falling. SWBV thus offers a supplementation to canonical physical treatments of PD.

10f. Other: clinical trials

ADPD5-1474

THE BRAINHEALTHREGISTRY.ORG: THE USE OF UNSUPERVISED ONLINE TESTING TO IDENTIFY OLDER ADULTS IN THE EARLY STAGE OF COGNITIVE DECLINE FOR PARTICIPATION IN CLINICAL TRIALS

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Objective: The identification and recruitment of older adults in the earliest stage of cognitive decline represents the most challenging inclusion criteria for many clinical trials for Alzheimer's disease. The BrainHealthRegistry.org (BHR) is an online research registry developed to facilitate neuroscience clinical trials. The purpose of this study is to evaluate the large scale use of online neuropsychological tests as a mechanism to identify older adults with early stage cognitive decline for participation in clinical trials.
Methods: The BHR is IRB approved. After participants register at the BHR website they can complete a series of health questionnaires and an online neuropsychological test battery including tests of attention, working memory, learning, and psychomotor speed. Evidence of cognitive dysfunction was identified for individuals over the age of 55 scoring $\geq$ 1.5 standard deviations below mean age adjusted performance for each neuropsychological test.
Results: In the past 7 months more than 9500 participants have registered with the BHR and 2798 individuals over the age of 55 have completed neuropsychological tests. Approximately 7% of older adults showed evidence of cognitive dysfunction on each cognitive test. Cognitive dysfunction is strongly associated with diagnosis of memory problem and participant report of memory complaint ($p<0.001$ for both). Results of ongoing data collection will be presented including validation with traditional neuropsychological measures and clinical characteristics of the sample.
Conclusions: Preliminary results suggest online neuropsychological tests may be very useful for the identification of individuals who are in the earliest stage of cognitive decline for participation in clinical trials.

10f. Other: clinical trials

ADPD5-1801
RELATIONSHIP BETWEEN FATIGUE AND DEPRESSION IN PATIENTS WITH IDIOPATHIC PD

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Relationship between fatigue and depression in patients with idiopathic PD
Introduction: Idiopathic Parkinson's disease (IPD) is a progressive and debilitating. Among non motor symptoms fatigue is common and disabling.Study deals with fatigue as a feeling of exhaustion, with different phenomenology of depression or muscle weakness.
Objective: Scale fatigue and depression as a major symptom in DPI, through appropriate scales.
Material and Methods: This study was conducted using two groups, experimental control with 40 subjects each, both sexes and varying ages. Scales of UPDRS, Hoehn and Yahr, Schwab and England, Hamilton, Beck, Mini-mental fatigue scale of Chalder, Inventory Fatigue Severity Scale and Fatigue Severity were applied.
Results: The 40 patients with ILD 22.5 were at stage 1.The, 45% and 32.5 stages stages 1.5 and 2.0 in the control subject were all at zero stage according to Hoehn and Yahr. Noted in the Schwab and England scale of the patients with ILD
to zero according to Hoehn and Yahr.Obervou in the range Schwab and England patients with ILD 5% of subjects had ADL performance of 60%, 5% were 70%, 65% had 80% of subjects had depression and through the Hamilton Rating Scale 90%. On the scale of Chalder demonstrated presence of fatigue in 80% of subjects with DPI in the control group none of the subjects had such symptoms.
Conclusion. Observed association between depression and fatigue in 90% of patients with ILD. No association between cognitive impairment and fatigue.

10g. Other: alternative hypotheses

ADPD5-0715

PERSONALITY TRAITS MAY MEDIATE LIFESTYLE FACTORS RESULTING IN DIFFERENTIAL COGNITIVE TRAJECTORY LATER IN LIFE

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There is a consensus that interplay between genetic, environmental and behavioural factors may account for the increasing number of late onset Alzheimer's disease (AD) patients. Lifestyle has been proposed as a significant factor involved in cognitive decline and dementia. Interestingly, personality as the underlying construct controlling our lifestyle and behaviour has been spared. Personality is a relatively stable, but dynamic psychological construct affecting a person's cognitive functions, motivation, and behaviour. Research has indicated significant relationship between personality traits, brain volume, and specific brain regions. Apparently, only in severe brain pathology, personality changes in mid to late adulthood can be expected. AD is one of the main neurodegenerative diseases resulting in personality changes. There are evidence that neuropathological changes due to AD may begin >20 years prior to any diagnosable signs and symptoms. These changes may be delayed or stopped given a healthy lifestyle is followed. In a study of personality traits and genetic, imaging and biological markers associated with AD, we examined personality characteristics in 150 individuals aged 60 years and over using the Neuroticism-Extroversion-Openness Five Factor Inventory (NEO-FFI). This is the first study to examine the relationship between personality traits and various markers associated with pathological cognitive decline and will further our understanding of the relationship between these factors. If personality modulates neurodegenerative processes through lifestyle choices, then psychological intervention may prove useful in promoting healthy lifestyle that may prevent or delay AD.

10g. Other: alternative hypotheses

ADPD5-1846

SUCCESSFUL INITIAL ZINC THERAPY IN WILSON'S DISEASE WITH ACUTE LIVER FAILURE AND COPPER INTOXICATION: WONDER DRUG EXISTS

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Objectives: To evaluate initial zinc monotherapy on copper levels and hepatic improvement.

Background: Accumulation of copper in brain and liver is regarded as the cause of Wilson's disease. However, there is evidence suggesting the free copper intoxication as the origin.

Methods: Retrospectively, a 10-year-old girl with Wilson's disease presented with diffuse jaundice, acute liver failure, hemolytic anemia, and high urine copper, liver function and copper values with a positive family history was evaluated before and after initial zinc monotherapy. The patient was treated with 125 mg elemental zinc per day, and liver transplantation was considered in case of nonresponsiveness.

Results: The patient improved biochemically and clinically in 10 days after zinc therapy, which were in accordance with 86% urinary copper decrease. The revised Wilson's disease prognostic index improved from 13 to 5, and the patient was removed from the transplant list. After 2, 4, 8 weeks on zinc therapy, AST, ALT, bilirubin, urine copper showed constant improvements of up to 62, -26, 82, 77%, respectively by the eighth week,. The results of the second 8-week period indicated a further decrease of 25, 41, 48, 86, respectively, and 33% for serum free copper, compared to the previous results. No side effects, or clinical deterioration has been observed.

Conclusions: The results suggest zinc therapy as a promising, fast-acting, safe and affordable choice for the treatment of copper intoxication in Wilson's disease with acute liver failure. It also supports the hypothesis of normalization of raised serum free copper as a rational targeted-therapeutic strategy.

10g. Other: alternative hypotheses

ADPD5-1849
ZINC THERAPY REVERSES NEURODEGENERATION IN WILSON'S DISEASE PATIENTS WITH PARKINSONISM
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Objective: To evaluate how effective zinc therapy can reverse parkinsonism in Wilson's disease patients who were initially treated with penicillamine.
Background: For years, the treatment of Wilson's disease has been aiming at stimulating the excretion of accumulated copper by chelating agents. The dilemma arises when patients paradoxically deteriorate, which is because chelators increase free copper levels. Conversely, zinc therapy normalizes the raised serum and urine free copper by inducing metallothionein production.
Methods: In a retrospective observational study on four Wilson's disease patients with severe parkinsonism being initially treated with penicillamine with or without zinc, we compared the subjective and objective clinical improvement before and one year after shifting to zinc monotherapy. Global Assessment Scale (GAS) was used for clinical evaluations. Global disability were measured in four domains: liver, cognition and behavior, motor, and osseomuscular, and the neurological dysfunction were assessed in 14 main categories.
Results: Three of the four patients who had been deteriorated on penicillamine (0.5-1 g/d), have improved significantly to a normal biochemical and clinical state on zinc (150 mg/d). The fourth patient on 250 mg/d penicillamine plus 200 mg/d zinc slightly improved in lower extremities after one month, while she considerably improved to a symptom-free state in the following months on zinc monotherapy. Their global disability improved by about 73%, and neurological assessments revealed a 100% amelioration.
Conclusions: The results suggest zinc monotherapy as the safe and promising treatment for copper intoxication, and support normalizing the increased serum and urine copper values as a rational therapeutic strategy.

10g. Other: alternative hypotheses

ADPD5-2125

DISTANT ANTAGONISM BETWEEN PATHOGEN FUNGAL COMMUNICATIVE BODY AND MULTISPOT-ORGANIZED SYNERGISTIC SYNBIOTIC GLYCOCONJUGATES-RECOGNIZING SYSTEMS: ONE OF FACTORS INVOLVING BRAIN MIXED FUNGAL DISSEMINATION

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Invasive fungal pathogen dissemination to the central nervous system and low effectiveness of antibiotics again brain diseases are important problems. We proposed how human biotope fungal communicative body (FCB) is organized and reply to the presence or absence of antifungals [1-3].

The aim is application of our advanced conception of predicted distant biotope FCB behavior under stress for further understanding brain multifungal distribution (as direct brain degeneration).

Proposals: Development of strategies using synbiotics (glycoconugates-recognized synergistic antimicrobial systems based on bifidobacterial and lactobacillar consortia; their delivery as mosaics in localization and time). Conversion of internal (increased multilayers) resistant (to antimicrobials and other stress factors) areas of FCB into sensitive/sensor border/peripheral areas (landscape-depended sensitization). Limitation of multifungal cascade biofilms (*Candida albicans—Aspergillus spp.—Penicillium spp.*) by anti-*C.albicans* combinative antimicrobial synergistic systems (early disruption of mixed-FCB relationships). Monitoring conversion of “early active one-few-center FCB (antimycotic sensitive)” into “late conservative multi-center FCB (locally disseminated resistant residuals in degenerated brain)”.

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10h. Other: other

ADPD5-1091

NEUROPSYCHOLOGICAL PROFILE OF COTARD'S SYNDROME: REVIEW OF THE LITERATURE AND PRESENTATION OF A SINGLE CASE

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Objectives: Cotard syndrome (CS) is a rare delusional disorder characterized by the conviction of being dead (Berrios et al, 1995). As pointed out by Colthearth (Colthearth et al. 2010), in every type of delusion a neuropsychological explanation is possible. The aim of this study is twofold: (1) reviewing all cases of CS described in Italian, English and Spanish literature, focusing on neuropsychological impairment, and (2) describing a single case of CS recently evaluated in our clinic (E.M.).
Methods: we reviewed single case publications available until 2014 and we administered a neuropsychological battery, investigating memory, attention, frontal function, visuo-praxic abilities and language to a patient showing nihilistic delusions.
Results: we analyzed 89 CS cases. Thirty-two contain a reference to patients' cognitive status, but only ten provide a detailed description of the neuropsychological evaluation. Results however, appear heterogeneous and do not make any clear neuropsychological explanation possible. The psychometric profile of E.M. showed a neuropsychological impairment limited to Cognitive Estimation Test (CET- Wagner et al, 2011). This result appears interesting because it seems to support recent neuropsychological explanations of delusion (Colthearth et al. 2011).
Conclusions: Neuropsychology of CS has received little attention in literature. The impairments showed by E.M., support Coltheart's model, postulating the existence of two factors responsible for the onset of misidentification delusions (Coltheart et al. 2011): a distorted perception of reality and a difficulty in making inference. This last cognitive ability is exactly what is measured during the CET. Further analysis is needed to explore other possibilities of interpretation.

10h. Other: other

ADPD5-1367

AN ISOLATED FORM OF RESTLESS ARM WITHOUT LEG RESTLESSNESS

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Background Upper extremity symptoms can develop in restless legs syndrome as an initial symptom., but it is seen very rarely in isolated form.
Objectives To report a case of restless arm syndrome without any leg symptom during 20 year follow-up and to review the literature.
Patient A 61-year-old man had involuntary movements of arm and restlessness which caused sleep disturbance.He reported "twitching and wiggling" sensations in his right arm that was worse at rest, and felt the need to move his right arm. There were extension like movements of fingers and rotatory movements of upper arm. He could not control these involuntary movements. He had not been diagnosed during previous 20 year period. His EMG exam, cranial and cervical MRI exams were normal. He was given pramipexol (3 mg/day), which reduced his symptoms and improved his sleep.
Results The pathogenesis of restless leg or arm syndromes is not known. Compared with those with isolated leg restlessness, subjects with arm restlessness had more severe leg restlessness and worse sleep efficiency. Isolated forms of restless arm problems usually are followed by leg restlessness.
Conclusion Only restless arm syndrome is very rare and our case is the first report without any leg symptom during 20 years.

10h. Other: other

ADPD5-1843
CADASIL NOVEL MUTATION WITH ATYPICAL MRI FINDINGS
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More than hundred different mutations in 18 exons have been already described. We present a Korean family with CADASIL with a novel NOTCH3 mutation who presents atypical MRI findings. **Case** 46-year-old woman developed dysarthria for 2 weeks. She did not have vascular risk factors but with positive family history of stroke at a young age. She also manifested headaches over 20 years. On a neurologic examination, right hemiparesis were shown but cognitive function and deep tendon reflexes were normal, and pathologic reflex was absent. In a complex neuropsychological test, her cognitive function was normal. woman's Her brother had repeated stroke for three times, and her father and two aunts were dead due to recurrent strokes. MRI of her brain showed confluent microbleeds in the basal pons and high–signal intensities with inherent microbleeds in the bilateral periventricular white matter, basal ganglia, and thalamus. Genetic testing confirmed a novel Pro1008Ser mutation in exon 19 of NOTCH3 gene. In a familial genetic analysis, her 55-year-old brother (II-2) also carried the same mutation, showing symptoms of stroke and the proband's 45-year-old sister (II-4) was an asymptomatic carrier of the P1008S mutation. This report illustrates a novel mutation in NOTCH3 from Korean CADASIL family with atypical MRI features. Until now, there is no relation between mutation site and clinical or radiological features but further investigations are needed whether atypical findings of massive confluent microbleeds in the patient could be ascribed to chance or whether the P1008S mutation of the gene is responsible.

10h. Other: other

ADPD5-1953

MEMORY AND QUALITY OF LIFE IN PATIENTS OLDER THAN 50 YEARS WITH EPILEPSY

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Objectives: To study memory and its relationships with the clinical aspects and quality of life of patients with epilepsy (PWE) older than 50 years.
Methods: Ninety-eight PWE, 63 with epileptic seizure (ES) onset before age 50 (G<50) and 35 with ES onset after age 50 (G≥50) were assessed for epilepsy-related aspects and submitted to the Memory Complaint Questionnaire (MAC-Q), Mini-Mental State Examination (MMS), Brief cognitive battery (BCB), and Quality of Life in Epilepsy Inventory (QOLIE–31). The relationship between MAC-Q data and the other variables was investigated and compared with those of 42 individuals without chronic diseases (CG). The significance level was set at 5%.
Results:In PWE the mean MAC-Q and QOLIE-31 scores were 24.8(±5.4) and 58.7(±17.5), respectively. Forty-seven percent of the PWE complained of loss of memory (MAC-Q≥25), which did not differ significantly from the CG. The MAC-Q of the G<50 and G≥50 groups did not differ significantly. PWE with uncontrolled ES and those with epilepsy of vascular etiology had higher MAC-Q scores. MAC-Q scores were negatively associated with MMS scores, education level, age at ES onset, epilepsy duration, verbal fluency, immediate and incidental memory, and QOLIE-31 scores (p<0.05). Linear regression showed that the MAC-Q score of the study sample was significantly associated with education level and epilepsy duration (p=0.001).
Conclusions: Higher MAC-Q scores reflect appropriate perception of low performance in memory tests, and the most important clinical factor was epilepsy duration. Higher quality of life was associated with lower MAC-Q scores.
Sponsored by FAPESP,SP.

10h. Other: other

ADPD5-2032
THE IMPACT OF NOCTURNAL DISTURBANCES ON QUALITY OF LIFE IN PATIENTS WITH PARKINSON'S DISEASE
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Objective: The aims of the present study were to explore the sleep disturbances in Taiwanese PD patients and their impact on quality of life (QoL).
Methods: A cross-sectional study was conducted consisting of 211 patients with PD. The participants were interviewed using instruments, including the PD Sleep Scale (PDSS-2), Pittsburgh Sleep Quality Index (PSQI), PD QoL Questionnaire (PDQ39), Epworth Sleepiness Scale (ESS), Hoehn and Yahr staging (H&Y), and Unified Parkinson's Disease Rating Scale (UPDRS). Multiple regression analyses were performed to determine the contribution of predictive variables to quality of life.
Results: There were 54.7% male (age: 63.8 ± 8.9 years, disease duration: 5.9 ± 4.3 years). The H&Y was 2.3 ± 0.85 and the UPDRS was 35.5 ± 16.6. The actual sleep time was 6.1 ± 1.3 hours; average sleep efficiency was 85.1 ± 13.2%. Up to 56% were classified as 'poor" sleeper (PSQI > 5), and 14.4% suffered from daytime sleepiness. The disease duration, stage, UPDRS, and PDSS-2, as well as PSQI were significantly correlated to the quality of life. The final stepwise regression model revealed that daytime dysfunction, PD symptoms at night, and UPDRS-I as significant predictors for the total score of PDQ39 scores (R^2 = 56, F = 27.06, df = 4, p = .000).
Conclusion: Most patients have sleep problem and some have abnormal daytime somnolence. Daytime dysfunction, nocturnal symptoms, and non-motor symptoms are important contributors leading to a worse QoL. More extensive research is needed to explore the possible management programs in the future.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-2019

FRUCTUS MUME ALLEVIATES CHRONIC CEREBRAL HYPOPERFUSION-INDUCED HIPPOCAMPAL DAMAGE VIA INHIBITION OF INFLAMMATION AND DOWNREGULATION OF TLR4 AND P38 MAPK SIGNALING

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Objective: We investigated the effects of Fructus mume (FM), a processed unripe fruit of Prunus mume, on hippocampal damage induced by chronic cerebral hypoperfusion.

Methods: Permanent bilateral common carotid artery occlusion (BCCAo) was performed on male Wistar rats to induce chronic cerebral hypoperfusion. Daily administration of FM (200 mg/kg) was started on day 21 after post-BCCAo and continued for 42 days. The experimental groups were divided into three groups: a sham-operated group, a BCCAo group, and a BCCAo group that was administered with the FM extract. The activation of glial cells, including microglia and astrocytes, and the levels of myelin basic protein (MBP), inflammatory mediators, Toll-like receptor 4 (TLR4), myeloid differentiation factor 88 (MyD88), and p38 mitogen-activated protein kinase (MAPK) phosphorylation were measured in the hippocampus from rats subjected to chronic BCCAo.

Results: Our results revealed that FM alleviates the reduction in MBP expression caused by chronic BCCAo in the hippocampus and significantly attenuates microglial and astrocytic activation. In addition, FM treatment reduced the increased expression of cyclooxygenase-2, interleukin-1β and interleukin-6, as well as the activation of TLR4/MyD88 and p38 MAPK signaling, in the hippocampus of rats subjected to chronic BCCAo.

Conclusion: Taken together, our findings demonstrate that brain injury induced by chronic BCCAo is ameliorated by the anti-inflammatory effects of FM via inhibition of MBP degradation, microglial and astrocytic activation, increased inflammatory mediator expression, and activated intracellular signalings, including TLR4 and p38 MAPK, implying that FM is potentially an effective therapeutics for the treatment of vascular dementia.

06b. Imaging & Biomarkers: functional MRI

ADPD5-2135

CHANGES OF WHITE MATTER AND ASSOCIATION FIBER TRACTS IN AMCI AND AD: A DTI STUDY

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Objective : To assess DTI differential diagnosis characteristic between aMCI and AD.

Materials and Methods : Routine MRI and DTI scanning were performed to 20 NC volunteers, 20 aMCI patients and 20 AD patients at Philips 3.0T supraconduction MR scanner. The regions were set in the anterior frontal, temperal, parietal, occipitals, hippocampus, inferiorfronto-occipitalfascicles, corpus callosum (genu was set right, splenium was set left), superior longitudinal fascicles Ⅱ (SLF Ⅱ) and cingulated bundles in the postprocessing workstation after scan. Bilateralis FA and ADC values were measured, pair t test was respectively compared in the bilateralis ROIs every group.

Results : In NC, aMCI and AD groups, the FA values of the genu and splennium of corpus callusum had significantly difference(P<0.05). In NC and aMCI groups, the FA values of inferior fronto-occipital fascicles and cingulate bundles had significantly difference(P<0.05). In aMCI and AD groups, the FA values of cingulate bundles had significantly difference(P<0.05). In NC and AD groups, the FA values of anterior frontal lobe, temporal lobe, hippocampus, inferior fronto-occipital fascicles , genu of corpus callusumand and cingulate bundles had significantly difference(P<0.05). In NC and AD groups, ADC values of temporal lobe and hippocampus had significantly difference(P<0.05).

Conclusion : Abnormal change of inferior fronto-occipital fascicles and cingulate bundles can imply that DTI could be as an imaging diagnostic method for aMCI patients. The FA values of cingulate bundles show significantly difference in aMCI and AD patients. Abnormal FA values of anterior frontal lobe, temporal lobe, hippocampus, inferior fronto-occipital fascicles, genu of corpus callusum, cingulated bundles could help us to understand AD patients.

07d. Epidemiology, Risk Factors, Genetics & Epigenetics: cardiovascular

ADPD5-2236

TIME TO TARGET SMALL VASCULAR LESIONS IN AGING BRAINS?

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Objective: Although neurodegenerative disorders are still considered the most common cause of cognitive impairment in the elderly, recent evidence suggests that small and microvascular pathology are a lot more frequent than previously thought. Our aim was to ascertain the contribution of small and microvascular lesions on cognitive function in aged individuals

Methods: clinicopathological and radiopathological correlation studies were performed and reviewed to determine the consequences of location, type, size and multiplicity of vascular lesions on cognition with special attention to microscopic vascular damage. Capillary density, length and diameter and amyloid angiopathy were also assessed

Results: microinfarcts and thalamic and basal ganglia lacunes are strong vascular correlates of cognitive decline accounting for over one third of the clinical variability. These lesions are common and invisible on MRI suggesting that many cases of pure neurodegenerative dementia may in fact include a clinically essential undiagnosed vascular component.

Conclusion: Small and microvascular pathology are key determinants of cognitive function in older people and may represent important therapeutic targets for the prevention of dementia in the elderly.

10b. Other: disease mechanisms

ADPD5-2160

DIABETES DECREASES EXPRESSION OF METABOLISM-RELATED PROTEINS IN THE BRAIN AFTER FOCAL CEREBRAL ISCHEMIA

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Stroke is a major cause of disability and death in adults. Diabetes mellitus is a metabolic disorder that strongly increases the risk of severe vascular diseases. This study compared changes in proteins in the cerebral cortex during ischemic brain injury between diabetic and non-diabetic animals. Adult male rats were injected with streptozotocin (40 mg/kg) via the intraperitoneal route to induce diabetes and underwent surgical middle cerebral artery occlusion (MCAO) 4 weeks after streptozotocin treatment. Cerebral cortex tissues were collected 24 h after MCAO and cerebral cortex proteins were analyzed by two-dimensional gel electrophoresis and mass spectrometry. Among the identified proteins, we focused on the following metabolism-related enzymes: glucose-6-phosphate isomerase (neuroleukin), pyruvate kinase, glyceraldehyde-3-phosphate dehydrogenase, isocitrate dehydrogenase, and adenosylhomocysteinase. Expression of these proteins was decreased in animals that underwent MCAO. Moreover, protein expression was reduced to a greater extent in diabetic animals than in non-diabetic animals. Reverse transcription-PCR analysis confirmed that the diabetic condition exacerbates the decrease in expression of metabolism-related proteins after MCAO. Impairment of energy metabolism in brain ischemia causes pathological changes. Thus, these results suggest that the diabetic condition may exacerbate brain damage during focal cerebral ischemia through down-regulation of metabolism-related proteins.

10c. Other: preclinical research

ADPD5-2149

EFFECT OF ANGII-INDUCED HYPERTENSION AND ANTIHYPERTENSIVE TREATMENT ON PATHOLOGICAL CHANGES IN A MOUSE MODEL FOR ALZHEIMER'S DISEASE

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Objectives
Hypertension is a risk factor for Alzheimer's disease (AD). It is a treatable condition which opens important avenues for prevention of AD. Elevated angiotensin II (AngII) is an important cause of essential hypertension and has deleterious effects on endothelial function and cerebral blood flow (CBF). In this study we therefore investigated the interaction between AngII, systolic blood pressure (SBP), and MRI-measurements in the $APP_{swe}/PS1_{[Delta]E9}$ (APP/PS1) mouse model of AD.
Methods
We studied the effect of 2 months of induced hypertension (AngII-infusion using osmotic micropumps, vs saline (sal) as control) and, subsequently (after 1 month of induced hypertension) the effect of treatment (vs placebo) with antihypertensive (eprosartan mesylate (EM), 0.35mg/Kg vs water) on SBP and metabolite levels, functional and neuronal connectivity and CBF in 10 months-old wildtype C57bl6/j (WT) and APP/PS1 mice. SBP was monitored twice a month via tail cuff plethysmography. RsfMRI, DTI, MRS, FAIR-ASL were measured on the 11.7T magnet (Bruker BioSpec).
Results
In this study, chronic AngII-infusion increased BP in both transgenic and WT mice, while at 12-Month under AngII-infusion APP/PS1 mice had a higher SBP than WT mice. Furthermore, only in hypertensive AD mice cortical CBF was lowered compared to hypertensive WT mice. Additional data will be presented on the impact of AngII-induced hypertension and subsequent treatment with EM on Aβ-pathology, cognition, metabolite levels, structural and functional connectivity.
Conclusions
Together, these data suggest an interaction between APP/PS1 induced pathologies, SBP, and antihypertensive treatment. Our results also reveal an association between hypertension (AngII), APP/PS1 and CBF.

10f. Other: clinical trials

ADPD5-2283

THE CLINICAL AND IMAGERY FINDINGS IN PATIENTS WITH POST STROKE DEMENTIA

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Aim:
The assessment of poststroke dementia and its correlation with clinical and imaging data.
Methods:
We studied 249 stroke patients who followed up at the neurology unit, during the last four years. They were presented at least three months after the first or recurrent stroke. Diagnosis of dementia was made according to the DSMIV criteria. All patients underwent a detailed neurological examination, imaging of the brain. HIS score was applied. Patients who had previous history of dementia were excluded from the study. SPSS 17.0 program was applied for data analyze.
Results:
There are 71 females, 178 males. The mean age is 68, 5 years old. We analyzed the data of 69 patients who met the criteria for dementia. 41 patients had 4-7 in HIS score (MixD). 28 patients had <7 in HIS score (VaD), 0 patients had > 4 in HIS score)AD(. There is a statistically significant correlation between poststroke dementia and the age of the patients, level of education, cardiovascular diseases, diabetes (p value< 0, 01). There is a statistically significant correlation between poststroke dementia and subcortical vascular lesions, multiple lesions, periventricular white matter lesions. No significant correlation between poststroke dementia and type of stroke, carotid stenosis, hypertension, hypercholesterolemia was found. No significant correlation between types of poststroke dementia and vascular risk factor was found.
Conclusion:
This study suggests that poststroke dementia is most frequent in stroke patients with multiple and subcortical vascular,periventricular white matter lesions. Multiple vascular risk factors are more predictive for post stroke dementia

05t. Drug Development & Clinical Trials: medicinal chemistry approaches

ADPD5-2062

FUSED ISOQUINOLINE-HYDANTOIN, A SELECTIVE SIGMA-1 LIGAND AS A DRUG CANDIDATE FOR THE TREATMENT OF MULTIPLE SCLEROSIS

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Objectives.
Selective agonists of sigma-1 protein (S1P) are reported to protect against neuronal damage and modulate oligodendrocyte differentiation. Lymphocytes possess saturable, high-affinity binding sites for sigma ligands and potential immunomodulatory properties have been described for S1P ligands. Fused tetrahydroisoquinoline-hydantoin (TicH) were designed, synthesized and evaluated. Experimental auto-immune encephalomyelitis (EAE) has unequivocal value as a model of the inflammatory aspects of MS. The objective was to evaluate the role of our most efficient S1P ligands in EAE.
Methods.
Fused TicH were optimized thanks to litterature pharmacophoric model. Affinity for S1P, S2P, cytotoxicity and ADME properties were measured. EAE was induced in SJL/J female mice by active immunization with myelin proteolipid protein. Best TicH compound was injected i.p. at immunization time. Disease severity was monitored by clinical and histopathological evaluation in the CNS, cytokine production by ELISA assay and the phenotype of B- and T-cell subsets by flow cytometry.
Results.
Most efficient TicH compounds showed nanomolar S1R affinity, selectivity versus S2P, very low cytotoxicity and ADME properties compatible with therapeutic development. Treatment of EAE-susceptible mice with TicH agonist prevented mononuclear cell infiltration and demyelination in brain and spinal cord and increased T2 B cells in spleen, resulting in an overall reduction in the clinical progression of disease.
Conclusions.
TicH S1P agonist could modulate B cells and most likely their effector functions in inflammatory processes in EAE, suggesting the involvement of S1P in the immunological response. Targeting S1P could thus provide new therapeutic opportunities for MS.

07i. Epidemiology, Risk Factors, Genetics & Epigenetics: genetic associations & susceptibility genes

ADPD5-2159

MULTIPLE SCLEROSIS POLYGENIC RISK SCORE IS ASSOCIATED WITH WHITE MATTER INTEGRITY IN 398 HEALTHY YOUNG ADULTS.

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Genetic risk is thought to play a substantial part in the pathogenesis of multiple sclerosis (MS). Recent GWAS have identified risk variants associated with the disease, building on heritability studies that suggest a polygenic effect. Because MS is primarily a white-matter disease, we tested the association between polygenic MS risk scores and white-matter integrity in healthy young adults. Diffusion tensor imaging (DTI) and genotyping data were obtained from 398 healthy young Australians (mean age 23.6 ± 2.2 years) as part of the Queensland Twin Imaging Study. After LD pruning, 76 remaining SNPs from the top 102 significant variants from in the largest MS GWAS to date [1] were used to estimate individual polygenic scores, based on number of risk alleles an individual possessed weighted by the standardized SNP effect observed in the original GWAS. Final scores are normalized and account for missing SNPs. Associations with scores and FA were tested using mixed-model regression at each white matter voxel in FA maps, which controlled for age, sex and accounted for kinship. FA in several regions was significantly associated ($q < 0.05$) with polygenic risk for MS, including Corpus Callosum, L Inferior longitudinal fasciculus, and R Anterior Thalamic radiation. These results give functional validation to GWAS findings and suggest that an increased genetic risk for MS may have influence over white-matter microstructure before disease onset; highlighting a possible biomarker to be monitored for early intervention.

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08h. Animal Models: other

ADPD5-2172
CHARACTERIZATION OF EXPERIMENTAL ANIMAL MODEL FOR NEUROMYELITIS OPTICA

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Neuromyelitis Optica (NMO), also known as Devic's disease, is an inflammatory demyelinating disease of the central nervous system that often produces paralysis and blindness. NMO is more common in Asians than Caucasians. Most NMO patients are seropositive for immunoglobulin G autoantibodies (NMO-IgG) against the astrocyte water channel aquaporin-4 (AQP4). NMO-IgG banded to AQP4 results in complement-dependent cytotoxicity and inflammation, leading to secondary demyelination and neuronal injury. Despite a defining feature of NMO, appropriate animal models of NMO are lacking. Some studies reported that immunoglobulin G from NMO patients has the potential to damage the central nervous system in the presence of human complement. Here, we show that intra-cerebral injection of IgG obtained from NMO patient serum with human complement might produce the characteristic histological features of NMO including inflammatory cell infiltration, loss of myelin, and AQP4 expression.

10a. Other: cell, molecular & systems biology

ADPD5-1939
IMMUNOHISTOCHEMICAL CHARACTERIZATION OF MULTIPLE SCLEROSIS PLAQUES IN THE HUMAN BRAIN

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Multiple Sclerosis (MS) is a demyelinating disease with a complex pathological profile that includes myelin degeneration, neuronal damage, and immune cell infiltration in the areas containing plaques. We evaluated the pathology associated with MS in the brain of a 39 year old female whose cause of death was unrelated to the disease. In acute plaques the amino cupric silver method (de Olmos) revealed a dense core of degenerating nerve cells and fibers. Chronic lesions had little staining of cells or fibers and were devoid of staining by the Nissl counterstain, Neutral Red. Another silver stain, the silver nucleolar stain (AgNOR) was developed to reveal the nucleolar organizing regions in cancerous cells. We utilized the stain here to reveal the differences in interior cellularity between acute and chronic plaques. This is useful in getting accurate counts of the cell populations present in brain regions undergoing demyelination, and has proven to be a useful tool for stereological purposes. Weil-Myelin staining revealed roughly spherical plaques devoid of myelin staining. Nissl staining with Thionine distinguished acute and chronic lesions.. Chronic lesions were surrounded by iron positive cells, some of which appeared to be phagocytic and filled with debris. Iba-1 immunoreactivity in acute plaques was observed both in the center of the plaque and in a dense ring of immunoreactive microglia surrounding the plaque. In chronic lesions the central immunoreactivity was diminished, but the ring of cells surrounding the plaque appeared thicker and more dense.

10h. Other: other

ADPD5-2116
DIAGNOSING MULTIPLE SYSTEMS ATROPHY AND MULTIPLE SCLEROSIS IN THE SAME PATIENT: A CASE REPORT

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Objective: To describe a patient with multiple-sclerosis(MS) and multiple-systems-atrophy-cerebellar(MSA-C) type concurrently.
Methods: We present a case of a 54yo-African-American female with symptoms of autonomic-dysfunction and cerebellar-ataxia for 2yrs, diagnosed with MS on copaxone without any relapses but with continued progression of her symptoms since diagnosis. Her symptoms included orthostatic-hypotension with multiple fainting episodes, urinary-retention, alternating diarrhea and constipation, gait instability, new-onset sleep apnea and tremors. She was treated by a cardiologist with midodrine/hiprex and by a urologist who recommended frequent self-catheterizing. However she continued to have severe orthostasis with near-syncopal attacks particularly when self-catheterizing and needed a cane and walker due to worsening ataxia. The patient also reported an episode of paraparesis 20years ago that was treated with steroids with complete resolution. On exam, she had cerebellar-deficits including nystagmus, dysmetria and impaired tandem-walking and positive orthostatics. On MRI-brain, she had symmetrical, non-enhancing, demyelinating lesions in bilateral cerebellar-peduncles and prominent ponto-cerebellar-atrophy consistent with MSA-C. She also had 3periventricular non-enhancing demyelinating lesions and a faint non-enhancing lesion in the C2-3level on cervical-spine-MRI consistent with MS. Her cervical lesion could represent transverse-myelitis from her episode 20yrs-ago.
Results: There are only 2reported cases of MS and MSA in the same patient but in those cases the patients were know to have MSA and incidentally diagnosed with MS with no significant change in management. However in our case it altered treatment course and prognostication significantly.
Conclusions: This case highlights the complexity of diagnosing white matter diseases and the need for a broad differential diagnosis.

02f. Cell, Molecular & Systems Biology: parkin

ADPD5-1934
EARLY ONSET PARKINSONISM
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95 early onset Parkinsonism patients among 335 MD selected during 5 last years youngest than 50 years old,
58 % M and 52% F, were studied retrospectively . Social occupation; 41% of patients were ordinary workers, 20% unemployed , 17% trades, 12% were intellectuals. 65% of patients had no familiar history of MD, 22% had positive familiar history (+ Parkinsonism, AVC (3), Psychotic Disorder (1), 13% have HTA familiar history or without familiar data. Diagnosis 63% had PD; 37% had parkinsonism . **PD :** 42% were unclassified, or with adversal DOPA-effects 30% had the tremorial form 25% the akinetic –hypertonic form, 3% had PD juvenil **PARKINSONISM:** 65% unclassified, 14% probable MSA, 6% vascular, 6% medicamentous, 3% neuroacantocitozis, 3% O-P-C, 3% somatoform. As to the chronicity, pts were divided
: **<1 Year** (8 pt), **≥ 1 y** (11 pt), **< 5 y** (30 pt), **≥ 5 y** (22 pt), **< 10 Y** (5 pt), **≥ 10 Y** (11 pt), **15-20 Y** (7 pt). **NEUROIMAGING :** Not indicated in 47% , performed in 53%; where normal in 50%, Gliozis of GB 12%, cerebral or cerebellar atrophy 8%, calcification of GB 2%, cavernoma 2%, on other 26% patients not available.. **Treatment**; 57% on Madopar only or + Amantadin, anticolinergic/antidepresiv, 24% on dopamine agonist only, 10% in combination with levodopa derivates, 9% with Amantadin or Parkopan combination.
Conclusion;
Among all; 63% had PD; 37% parkinsonism. PD; 30% had the tremorial 25% the akinetic –hypertonic form. **Parkinsonism;** 14% probable MSA, 6% vascular, 6% medicamentous, 3% neuroacantocitoza, 3% O-P-C, 3% somatoform disorder.

04n. Therapeutic Targets & Mechanisms for Treatment: anti-inflammatory targets

ADPD5-1978

IMMUNONEUTRALIZATION OF ENDOGENOUS AMINOPROCALCITONIN ATTENUATES SEPSIS-INDUCED NEUROINFLAMMATION

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Background: A systemic inflammatory reaction may have detrimental effects on the organism, including the central nervous system. During severe sepsis several immunological defense mechanisms initiate a cascade of inflammatory events leading to multi-organ failure (MOF) including septic encephalopathy. Previous studies have demonstrated that systemic lipopolysaccharide (LPS) causes chronic neuroinflammation and progressive neurodegeneration. The hippocampus is the most vulnerable brain region during experimental sepsis. Recently, we have shown that aminoprocalcitonin (NPCT), a neuroendocrine peptide ubiquitously produced during systemic inflammation, plays an important role in the development of sepsis and MOF.
Objective: To investigate the role of NPCT in a rodent model of LPS-induced neuroinflammation. We focused on the reaction and participation of parenchymal *cells in* the *hippocampus*.
Method: Male Wistar rats received 15 mg/kg LPS or vehicle intraperitoneally and were sacrificed for brain collection at 24h after induction of sepsis. One group of animals received 100 µg/kg of a *neutralizing* NPCT-*specific* monoclonal *antibody* or murine *IgG1a isotype control* intraperitoneally 1h before the experiment. Changes in CALCA gene, inducible nitric oxide (iNOS), microglia (OX-42) and astrocyte (GFAP) expression with the pattern of apoptosis (TUNEL) were analyzed.
Results: Immunohistochemical analysis revealed patterns of increased NPCT, iNOS, GFAP and OX-42 expression in hipoccampal cells, paralleled by an increase of apoptotic cells following sepsis. Immunoneutralization of NPCT prevents LPS-induced microglial activation, microglial iNOS expression and neuronal apoptosis.
Conclusions: Our results support a central role of NPCT in the neuroinflammatory responses, and suggest that NPCT might contribute to the uncontrolled neurotoxic glial activation under excessive inflammatory conditions.

04q. Therapeutic Targets & Mechanisms for Treatment: cell transplantation

ADPD5-2086

MESENCHYMAL STEM CELLS AND THEIR SECRETED EXOSOMES DELIVER EXOGENOUS MIRNAS AND SIRNAS TO NEURAL CELLS: THERAPEUTIC IMPACT IN MOUSE AND IN VITRO MODELS OF ALS

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miRNAs and siRNAs emerge as potential therapeutic targets in a variety of pathological conditions in the brain; however, their clinical application is hampered by lack of efficient delivery modes. Mesenchymal stem cells (MSCs) migrate to sites of injury and inflammation and exert therapeutic effects in various pathological conditions. Here, we examined the ability of MSCs to deliver exogenous miRNA mimics and siRNAs to human neural cells and characterized the functional impact of this delivery in ALS models. MSCs efficiently delivered miR-124 mimic to co-cultured NPCs and astrocytes as evident using Cy3-miR-124 and novel reporter plasmids which enable analysis of miRNA delivery in living cells. The delivered miR-124 significantly increased the expression of the glutamate transporters, EAAT1 on NPCs and EAAT2 in both NPCs and astrocytes. The miRNA delivery was mediated by MSC-derived exosomes and their administration exerted similar effects. Moreover, we generated exosomes targeted to neural and muscle cells which preferentially delivered the exogenous miRNA to these cells. Both MSCs and their secreted exosomes delivered mutant SOD1 siRNA which selectively decreased the expression of the mutant protein. We further demonstrated beneficial therapeutic impact of MSCs transduced with pre-miR-124 or the mSOD1 siRNA in motor neurons, astrocytes and microglia cells expressing mSOD1 and in a transgenic ALS mouse model. These results suggest that MSCs can functionally deliver exogenous miRNAs and specific siRNAs to neural cells and provide an efficient route of therapeutic small RNA delivery to the CNS in pathological conditions including ALS with clinical implications for regenerative medicine.

04y. Therapeutic Targets & Mechanisms for Treatment: neurosurgery

ADPD5-2282

MOTOR CORTEX STIMLATION IN PARKINSONIAN PATIENTS WITH REFRACTORY FREEZING OF GAIT AND BALANCE DISORDERS RESPONSIVE TO TRANSCRANIAL DIRECT CURRENT STIMULATION

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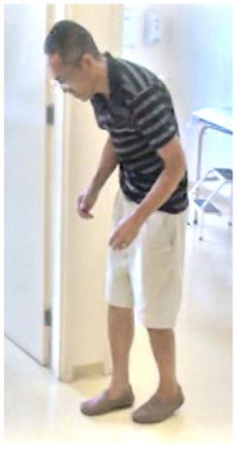
Background: Severe gait disturbances (SGD) in idiopathic Parkinson's disease are observed in up to 50% of the patients in advanced disease stages with important impact on quality of life. Few reports suggested improvement of bradykinesia and gait after motor cortex stimulation (MCS). Lately, the possibility of cortex modulation with transcranial magnetic stimulation and transcranial direct cortex stimulation (tDCS) have leaded to new perspectives and non-invasive approaches. Many authors reported changes in gait and motor skills after these modalities, always with short responses.
Methods: In this Open-Study, we selected PD patients, who presented motor fluctuations, SGD, and who showed insufficient improvement of gait after levodopa trial (<30%). All patients underwent *Motor Cortex Stimulation (*EMCS) of the left hemisphere, after positive response, with weekly transcranial direct cortex stimulation (TDCS). Outcome measures included: medication dosage change, Unified Parkinson's Disease Rating Scale (UPDRSII.3), and Gait and Balance Scale (GABS).
Results: All patients showed expressive gait changes, in GABS and UPDRS, following EMCS, in a follow-up of 4-18 mo. However, there was no reduction of mean daily medication intake.
Conclusions: Transcranial Direct Current Stimulation (TDCS) is a promising method of non-invasive brain stimulation to modulate cortical activity. It has been studied in many areas as a cognition and behaviour enhancement. This study suggests that MCS improves gait disturbances in PD and could be considered a safer alternative approach, in selected and refractory patients, in the treatment of Freezing of Gait and Balance disorders.

Case 5(after tDCS): Patient showing normal posture and balance improvement

Case 5 (6mo after MCS): Patient showing one foot stance



Case 5(before tDCS): Patient with severe stooped posture, loss of arm swing and small steps

Case 5(after tDCS): Patient showing normal posture, an increase of arm swing and step length

05m. Drug Development & Clinical Trials: antiepileptics

ADPD5-2007

IMPACT STUDY OF TREATMENT ANTIEPILEPTIC AT A POPULATION OF THE ALGERIAN EAST.

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Background: The epilepsy is a chronic neurological disease which relates to all the countries of the world,The main objectives are to give an epidemiologic approach of the epilepsy in the area of the East-Algerian and Antiepileptic determination of the impact of the treatment among these patients. **Material and Method:** We carried out a descriptive retrospective study on the treatment antiepileptic starting from an investigation in 49 patient epileptics on the Algerian East followed to the service of neurology CHU Annaba – Algeria including 33 adults with a 36,48 years median age during the time from March to May 2013. **Results:** We noted a prevalence of the cognitive disorders in the epileptics; whose disorders of memory (57, 14%) were at the head, the emotional disorders (44, 89%) and mental health disorders (42, 86%) in second position, others turbid of nature neurological, cardiovascular and gastro-intestinal were obtained with a less frequency.**Conclusion:** The nature of the developed disorders and the repercussion caused by the epilepsy suggest the need for medical ans psychosocial assumption of responsability by the improvement of the therapeutic follow-up like by the clarification and the programming of psycho-behavorial and cognitive therapy for the epileptics.**Keywords:**epilepsy, cognition, turbid mnemic,antiepileptic,neuropsychology.

06c. Imaging & Biomarkers: PET - amyloid

ADPD5-1950

PET-FLORBETAPIR FINDINGS IN PRIMARY CEREBRAL AMYLOIDOMA

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Objectives: Amyloidoma is a type of localized amyloidosis that can rarely affect the brain. To our knowledge, this is the first report of PET-Florbetapir findings in cerebral amyloidoma.

Methods: Case report

Results: A 51 year-old man was admitted after presenting with two complex partial seizures. Examination demonstrated an impairment of executive funtion and ideomotor apraxia. A CT scan showed multifocal hyperdense lesions. On MRI, these lesions were isointense with the white matter on T1-weighted images, hyperintense on T2-weighted images, and showed intense contrast enhancement. PET-Florbetapir revealed strong radioligand binding in the same pathological areas, without uptake of normal appearing tissue. Comprehensive evaluation including CSF examination did not reveal any other abnormalities. Stereotactic biopsy of the left parietal white matter showed nodular, confluent amyloid masses, that showed apple-green birefringence with Congo-red staining and intense fluorescence *with Thioflavin*. Amyloid deposits were also present in the wall of parenchymal vessels. Immunohistochemical staining showed strong staining for lambda light chain. There was also weaker staining (probably cross-reactive) for kappa chain and against different types of amyoloid proteins (AA, *beta-amyloid, transthyretin)*. These findings were consistent with the diagnosis of primary cerebral amyloidoma. No evidence of systemic disease was found. A trial of empiric radiotherapy or chemotherapy was discussed with the patient but was finally declined.

Conclusions. The clinical manifestations of cerebral amyloidomas are protean, but the neuroimaging characteristics are quite typical. PET with florbetapir can offer useful information for the diagnosis of patients with presumed cerebral amyloidosis.

07p. Epidemiology, Risk Factors, Genetics & Epigenetics: other epigenetic factors

ADPD5-2127

REGIONAL FEATURES OF PARKINSON'S DISEASE IN KAZAKHSTAN.

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Heterogeneous regional data on the incidence and prevalence of Parkinson's disease in the population, reflect differences in life expectancy in different countries, differences in the organization of the health system, differences in access to primary health care, as well as genetic and environmental features, together with differences in the reporting of cases of the disease.

Objectives: conducting the clinical and epidemiological research of patients with Parkinson's disease.

Methods: On the example of Almaty we conducted a population-based research and get an objective picture of primary and general morbidity of Parkinson's disease, as well as evaluated the age and gender structure the incidence and features of the disease.

Results: One of the major problems of our patients suffering from Parkinson's disease, is a late diagnosis. Beginning of the disease in almost all patients developed gradually, they did not seek medical attention for a long time. More than 80% of patients go to the doctor, already having a late stage of the disease. The prevalence of Parkinson's disease in the adult population in Almaty was 62 per 100 thousand population. Parkinson's disease is 1.5 times more prevalent in women than in men, and more frequently detected in the age groups over 70 years.

Conclusions: The data obtained allow justify the need to improve treatment and diagnostic, medical and social care to patients with Parkinson's disease.

08g. Animal Models: natural & seminatural models

ADPD5-1946

BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF AGE IN THE MOUSE

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Age-related cognitive deficits are widely studied in rodents. Nevertheless, the performances in tests evaluating cognition may be influenced by other effects of age. We compared adult (6 - 13 weeks) and aged (102-109 weeks) C57BL6 mice in behavioral tests evaluating motor, sensory and psychological endpoints and in tests evaluating respiratory, cardiovascular, renal and gastro-intestinal functions.
When compared with adult mice, aged mice displayed piloerection and hypothermia as reflected in the Irwin test and motor deficits in locomotion, beam walking and rotarod tests. Sensory functions were relatively preserved with normal vision and olfaction but deficits in pain sensitivity were observed following tactile and to a lower extent to thermal stimulations. The evaluation of psychological characteristics did not reveal major changes and the slight effects observed in the elevated-plus maze, stress-induced hyperthermia, sucrose preference and forced swimming tests may be partly explained by lowered locomotion. Some aged mice were unable to perform tests of cognitive functions, but the remaining testable aged mice displayed adult-like performances.
At the physiological level, in aged mice the low glycemia and urinary volume may be explained by a reduced food and water intake. The colonic transit was accelerated without modification of the small intestine transit or gastric emptying. The bronchoconstriction index was increased and the aorta phenylephrine-induced constriction was enhanced.
Our data suggest that age-related deficits in tests evaluating cognitive functions should be interpreted with caution and that additional effects of aging, mainly related to motor functions, should be taken into consideration when interpreting the data obtained.

09g. Patient Care & Support: art, music & life style

ADPD5-2175
EVALUATION OF THE STANDARDIZED MUSIC CARE© APP IN THE TREATMENT OF PAIN AND NEURODEGENERATIVE DISEASE"

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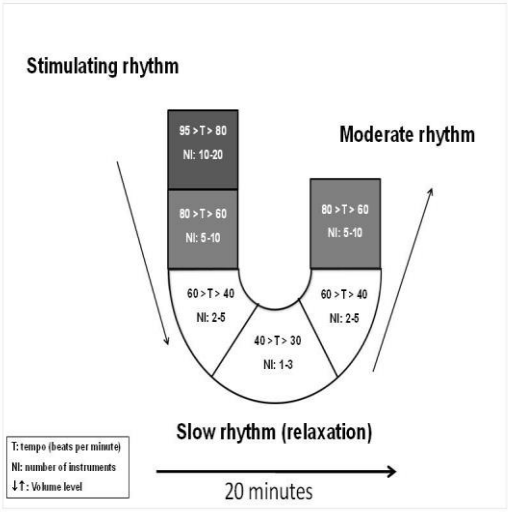
Objectives: Numerous studies emphasize the application of music therapy and music medicine in the treatment of pain. The MUSIC CARE© app that was designed at the University Hospital of Montpellier applies the U-shape music composing technique taking into account the available evidence of the literature on relaxation paradigms (Fig.1). The main objective of this article is to summarize recent research on the standardization and evaluation of this new app of music therapy in the treatment of pain and neurodegenerative disease (Fig.2).
Methods: Following a comprehensive review of the literature, a series of controlled, randomized, multi-centered studies were conducted including patients seeking care in such diverse setting as neurology, rheumatology, functional rehabilitation, oncology, geriatrics, and general pain treatment.
Results: The effect of the MUSIC CARE© app has been evaluated on different types of pain and neurodegenerative disease. Physiological effects on hemodynamic and respiratory markers as well as psychological outcomes, including the relationship between care-provider and patient have been emphasized within multiple trials.
Conclusions: The MUSIC CARE© app reduces pain, anxiety and depression to a significant degree and decreases the need for anxiolytics and antidepressants. Our first randomized controlled trials demonstrate the benefit of using MUSIC CARE© application in the management of pain and neurodegenerative disease. Future directions for the use of the app in various settings are discussed.

Fig. 1. New music listening technique: the "U" sequence



10a. Other: cell, molecular & systems biology

ADPD5-2020

RAPID DIFFERENTIATION OF ASTROCYTES FROM HUMAN EMBRYONIC STEM CELLS

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Astrocytes are the most abundant cells of the human brain and have the role of biochemical support of endothelial cell, supply of nutrients to the nervous tissue and maintenance of extracellular ion balance etc. In development of nervous tissue, the differentiation of astrocytes is later than neurons. In vitro, it takes more time and more techniques to obtain mature and pure astrocytes.

In this study, we developed the protocol to gain mature and pure astrocytes from human embryonic stem cells. To keep the quality of the differentiated astrocytes and to decrease the variations of the cell properties between batch to batch, we first tried to get neural progenitor cells (NPCs) and expand the cell number. The first step of astrocyte differentiation from neural progenitor cells were performed in astrocyte induction media containing EGF and heparin. Next, the cells were attached to coated plate in astrocyte defined media containing CNTF for 23 days. And then we checked the cell property with immunocytochemistry and western blot using antibodies for astrocyte specific marker proteins. To validate the functional property of the astrocyte, IL-6 release from astrocytes was tested. By FACS analysis, we found that the percentage of astrocytes among the cells differentiated from NPCs was over 90%. Taken together, with our protocol, we can obtain the mature and pure astrocytes within 4 weeks from NPCs and we have the plan to make micro-environment with neurons, astrocyte and endothelial cells.

10h. Other: other

ADPD5-2222

NIGROSTRIATAL PATHOLOGY CAUSED BY TOXIC AND GENETIC STRESSORS IS EXACERBATED IN PINK1-DEFICIENT MICE

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Objectives:
Loss-of-function mutations of PINK1 are associated with familial parkinsonism. A relatively mild phenotype characterizes PINK1 knock-out mice (PINK1 KO). The aim of this work was to investigate the role of PINK1 deficiency in neurodegenerative processes and, in particular, to determine if more overt Parkinson's disease (PD)-like pathology could be triggered in PINK1 KO by toxic or genetic stressors.
Methods:
To model interactions between PINK1 and toxic insults, PINK1 KO mice were injected intraperitoneally with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a mitochondrial toxin. To simulate interactions between PINK1 and other PD-related genes, adeno-associated viral vectors (AAV) carrying either wild-type or mutated (A53T) alpha-synuclein DNA were injected into the substantia nigra of PINK1 KO mice.
Results:
Levels of striatal dopamine and counts of nigral neurons were unaffected in PINK KO as compared to control mice. In the former, MPTP administration induced a more pronounced (20% vs. 10%) degeneration of nigral neurons. Similarly, AAV-induced alpha-synuclein overexpression was associated with greater nigrostriatal toxicity in PINK1-deficient animals. Following overexpression of wild-type alpha-synuclein, striatal dopamine and nigral neurons were decreased by 25% and 33%, respectively, in PINK1 KO mice; in control animals, corresponding values were 12% and 25%. The extent of nigral cell loss caused by overexpression of A53T alpha-synuclein was 40% in KO mice and 28% in controls.
Conclusions:
On the background of PINK1 deficiency, MPTP- and alpha-synuclein-induced pathology was exacerbated. Interestingly, overexpression of mutated alpha-synuclein was associated with greater toxicity than the wild-type protein only in the absence of PINK1.