(fibrosis stages 0–2) and advanced disease (fibrosis stages 3–4) to investigate significance. We discovered 26 candidates to be differentially expressed at both the transcriptomic and proteomic levels, 9 of which were amongst the most correlated, with an additional 17 proteo-transcriptomic markers significantly changing in advanced MASLD.
Conclusion: The current study provides new insights into the changing proteo-transcriptomic profile of the liver throughout the pathophysiological evolution of MASLD. It suggests optimum treatment targets/drug mechanisms of action may change as the disease progresses and has clinically relevant implications that will help guide future drug development.

FRI-367
Pathophysiological mechanisms involved in liver steatosis in metabolic dysfunction-associated steatotohepatitis over time : a bulk RNAseq data analysis
Guillaume Henin[1,2], Alvy Lamotte[1], Maxime Nachit[1], Axelle Loriot[3], Isabelle Leclercq[1], Nicolas Lanthier[1,2]. [1]*Laboratory of Hepato-Gastroenterology (GAEN), Institut de Recherche Expérimentale et Clinique (IREC), UCLouvain, Brussels, Belgium;* [2]*Department of Hepato-Gastroenterology, Cliniques universitaires Saint-Luc, UCLouvain, Brussels, Belgium;* [3]*BIOINFO Core Facility, de Duve Institute, UCLouvain, Brussels, Belgium*
Email: ghenin1993@gmail.com

Background and aims: Liver steatosis results from metabolic dysregulations of lipid homeostasis. Drugs targeting lipid metabolism are being evaluated in clinical trials with inconsistent results. We suggest that steatosis pathophysiology evolves with MASLD progression resulting in a mismatch between the deregulated mechanisms and the drug used. Our aim is to analyze the evolution of gene expressions of key lipid metabolism genes during MASH progression in mice.
Method: *Foz/foz* mice (FOZ) were fed normal diet (ND) (3.39 kcal/g, 16% of lipids) or high-fat diet (HFD) (5.24 kcal/g, 60% of lipids). Liver tissue was harvested at baseline (control group, N = 5) and after 4 (HFD = 4), 12 (HFD = 8) or 32 (HFD = 6) weeks. Liver samples were scored histologically (SAF score) using hematoxylin & eosin staining. Total RNA from liver samples was extracted using Qiagen® mini kits prior to bulk RNA sequencing. Sequencing data were normalized according to timepoint and diet (RStudio). Differentially expressed genes (DEG) and enrichment analysis reported by the Normalized Enrichment Score (NES) of FOZ HFD mice were compared at each timepoint with the control group.
Results: Maximal liver weight to body weight ratio was reached at 32 weeks ($p < 0.001$). Insulin resistance assessed by the HOMA-IR index was present at 4 weeks of HFD ($p < 0.01$). Liver histology showed the whole MASH spectrum with transcriptomics changes over time. At week 4, steatosis was observed with 284 DEG notably involved in fatty acid biosynthetic process (NES = 13.9, padj = 1.2e-4), lipid transport (NES = 9.7, padj = 8.0e-8), lipid storage (FES = 21.5, padj = 3.0e-6) and fatty acid oxidation (FAO) (NES = 9.4, padj = 0.02). At week 12, steatohepatitis was observed with 469 DEG involved in 504 enriched pathways including lipid transport (NES = 14.7, padj = 3.6e-15), lipid storage (NES = 24.2, padj = 1.66e-7) and FAO (NES = 22.2, padj = 2.7e-7). At week 32, fibrosing steatohepatitis was observed with 750 DEG involved in 1167 enriched pathways including lipid transport (NES = 11.3, padj = 6.3e-21), lipid storage (NES = 24, padj = 1.4e-15) and FAO (NES = 11, padj = 7.4e-7). At the gene level, the lipid metabolism key regulator Pparɣ was downregulated in a time-dependent manner: Log2FC = – 1.6, padj = 9e-5 at week 4; Log2FC = –1.8, padj = 9e-6 at week 12 and Log2FC = –2.35, padj = 6.6e-9 at week 32. Fatty acid oxidation related genes are all downregulated at week 12, such as Obp2a (Log2FC = –3.17, padj = 0.003) and Acaa1b (Log2FC = –1.64, padj = 9.9e-9). Lipid storage genes expression also greatly change over time. Both Cidec and Fitm1involved in lipid droplet homeostasis are significantly downregulated at week 12 (Log2FC = –2.13, padj = 3e-6; Log2FC = –1.37, padj = 1.68e-7 respectively) and at week 32 (Log2FC = – 2.7, padj = 2e-5; Log2FC = –1.02, padj = 2e-4 respectively).
Conclusion: Gene expression of key-lipid metabolism genes significantly change over time during MASH occurence. These shifts in gene expression of potential therapeutic targets may significantly influence the response to MASLD treatments.

FRI-368
Harnessing mouse genetic diversity to identify novel genetic determinants of MASLD to MASH transition
Giorgia Benegiamo[1], Wenyu Liu[1], Jean-David Morel[1], Maroun Bou Sleiman[1], Johan Auwerx[1]. [1]*École Polytechnique Fédérale de Lausanne, Laboratory of Integrative Systems Physiology, Lausanne, Switzerland*
Email: giorgia.benegiamo@epfl.ch

Background and aims: Metabolic dysfunction associated steatotic liver disease (MASLD), and its more severe form, metabolic dysfunction associated steatohepatitis (MASH), are a public health burden with high unmet therapeutic need. About one quarter of the general population has MASLD, but only about 20% of these patients will progress to develop MASH. The specific pathways involved in the MASLD-to-MASH transition are not fully elucidated. In a previous study we found large variability in MASLD/MASH susceptibility between genetically different mouse strains and identified highly sensitive and completely resistant strains. We used this information to design a 4-way F2 genetic cross to map novel quantitative trait loci (QTL) involved in disease susceptibility and find novel potential therapeutic targets.
Method: We performed a 4-way intercross of the strains with the highest (PWK/PhJ), intermediary (C57BL/6J and 129S1/SvlmJ) and lowest (CAST/EiJ) susceptibility to MASLD/MASH. 500 genetically unique F2 mice (250 males and 250 females) from this cross were fed a "western" style diet (WD) and were housed at thermoneutrality (30°C) for 17 weeks to induce metabolic dysfunction and MASLD/MASH. We measured activity, food intake, energy expenditure and respiratory exchange ratio (by the CLAMS system at week 0 and 13), body composition (by EchoMRI at week 0, 7 and 15) and body weight (bi-weekly). Plasma and urine were collected at week 0, 7 and 15. We quantified several disease biomarkers in the plasma (including ALT, AST, cholesterol and TIMP-1), performed histological analysis of the livers to quantify steatosis and fibrosis, as well as liver RNA-seq and proteomics.
Results: Analysis of the evolution of the body weight and body composition revealed a large variation in body weight and body fat gain in the F2 population, with a subset of mice being completely resistant to body fat gain. Unbiased hierarchical clustering of liver disease phenotypes identified 4 different clusters covering the whole spectrum of MASLD/MASH severity. Analysis of the transcriptomes allowed to identify distinct gene expression signatures that characterize different stages of MASLD and MASH severity. We found several genetic loci associated with obesity phenotypes and liver disease. By combining QTL analysis on clinical phenotypes (cQTL), transcriptome (eQTL) and proteome (pQTL), and validating the results in large human cohorts, we are now refining a list of novel candidate genes involved in MASLD and MASH susceptibility.
Conclusion: The F2 mouse population that we generated covers a very wide range of genetic susceptibility to resistance to MASH. In combination with human genetic data our data is an ideal tool to discover causative genes of both susceptibility to MASLD and the transition to MASH that are generalizable to humans.