grade systemic inflammation in the development of cognitive dysfunction in NAFLD.

**Method:** Using a well-established model of NAFLD representing “western diet,” 20 male Sprague Dawley rats were fed either a high-fat, high-cholesterol (HFHC) diet for 16 weeks or standard diet (10 per group). These animals were assessed for behavioural changes using previously validated neuropsychological tests, and inflammation studied through a broad panel of cytokines. Liver histology was assessed to stage NAFLD severity.

**Results:** The HFHC diet resulted in significant behavioural changes compared to standard diet: HFHC rats manifest a depressive affect evidenced by a significant reduction in survival behaviour (p = 0.031) and increased immobility (p = 0.011) in Porsolt’s Swim Test. Moreover, the Novel Object Recognition test showed that HFHC rats displayed cognitive impairment, with impaired memory of previously encountered objects (p = 0.047). Chronic low-grade inflammation was confirmed in HFHC rats with significant increases in IFN-γ, GRO/KC, IL-1α, IL-2, IL-6, IL-10, IL-13, MIP-1α, RANTES and MCP-1 (all p < 0.05). Histopathological assessment confirmed early NAFLD with extensive steatosis and lobular inflammation but no fibrosis in HFHC rats.

**Conclusion:** This study shows that cognitive impairment and depression-like behaviour is present in a preclinical model of early NAFLD. These neurobehavioural changes were accompanied by chronic low-grade inflammation which may contribute to neuro-inflammation, as is observed in many neurodegenerative diseases. Our observations, if extrapolated to humans, suggest a substantial disease burden and potential socio-economic impact of cognitive dysfunc-
tion in early NAFLD, even before fibrosis progression. These findings require further validation in patients.

**SAT034**

Thr β-selective agonist MGL-3196 improves NAS score and plasma lipoprotein profile in the liver-humanized FRGN KO NASH model

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**Background and Aims:** Current preclinical rodent models of NAFLD and NASH are inadequate at predicting human clinical responses to pharmaceutical intervention because they do not show the full pathology of human disease and are incapable of modeling the role of specific genotypes known to influence disease progression within the context of human cellular signaling pathways. Building on the liver-humanized FRGN mouse platform, we previously established an in vivo model, that recapitulates NAFLD/NASH with only high-fat diet fed to animals repopulated hepatocytes from a PNPLA3-148M homozygous donor. This 1148M variant is significantly associated with a high risk of NASH development.

**Method:** FRGN KO mice were transplanted with human hepatocytes from a donor homozygous for the G allele on the minus strand of rs738409 in PNPLA3. After near complete repopulation of the liver with human hepatocytes, animals received a diet with 40 kcal% Fat, 20 kcal% fructose, and 2% cholesterol. As a proof-of-concept, liver-humanized animals on HFD were treated during eight weeks with MGL-3196, a first-in-class, orally-administered, small molecule, liver-directed, thyroid hormone receptor (THR) β-selective agonist.

**Results:** Both control and NASH-induced liver-humanized animals remained highly repopulated over the course of the study. Body weight and liver to body weight ratios increased in humanized mice on HFD compared to controls. Liver-humanized mice show a lipid chemistry profile similar to humans (LDL/HDL ratio of ~1.6). After four weeks, the humanized animals on HFD had cholesterol levels significantly higher than control humanized animals. From week 8 to week 24, key NAS characteristics were increasingly detectable in the HFD fed animals: steatosis, hepatocyte ballooning, Mallory bodies, collagen deposition, and bridging fibrosis.

During the intervention study, liver-humanized FRGN KO mice were dosed with MGL-3196 for 8 weeks after inducing NASH for 8 weeks. The animals continued to receive HFD during the study. Animals dosed with MGL-3196 showed a smaller liver compared to controls. Blood LDL was reduced by 14.3%. A 2-point reduction in NAS (NAFLD activity score) of 2.17 vs 4.25 was observed. This was mainly due to a reduction in steatosis. Fibrosis was not affected by the treatment and ranged from mild to perivenular/pericellular (score 1 to 2).

**Conclusion:** FRGN KO mice repopulated with human PNPLA3-148M hepatocytes and fed HFD developed key aspects of NASH. Unlike current rodent models, the blood lipid profiles resembled those in humans with NAFLD. Histological analysis showed time-dependent progressive accumulation of typical NASH features. As a proof-of-concept, FRGN KO mice were dosed with MGL-3196. Steatosis was reduced as shown by a significant reduction in the NAS score.

These results suggest that liver-humanized FRG KO mice fed an HFD may be a superior model for NASH.

**SAT035**

TM6SF2/PNPLA3/MBOAT7 loss-of-function genetic variants impact on NAFLD development and progression both in patients and in vitro models

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**Background and Aims:** The I148M PNPLA3 and E167K TM6SF2 variants alongside the rs641738 polymorphism in MBOAT7/TM6SF2 locus represent the main genetic risk factors for non-alcoholic fatty liver disease (NAFLD). We previously generated a full knock-out (KO) of MBOAT7 in HepG2 cells, homozygous for the I1148M PNPLA3 (I1148M MBOAT7−/−). We aimed to 1) investigate the synergic impact of the 3 risk variants on liver injury and hepatocellular carcinoma (HCC) in NAFLD patients 2) create in vitro models of genetic NAFLD by silencing TM6SF2 in I1148M and I1148M MBOAT7−/− cells.

**Method:** NAFLD patients (n = 1194) of whom 72 had HCC were stratified according to the presence of PNPLA3, TM6SF2 and MBOAT7 at risk variants as follows: 0 (none), 1 (1 variant in PNPLA3, TM6SF2 or MBOAT7), 2 (2 variants) and 3 (patients carrying all the 3 variants). The additive weight of the mutations was correlated with liver disease severity. Finally, we silenced TM6SF2 in HepG2 (I1148M TM6SF2−/− and I1148M MBOAT7−/−) through CRISPR/Cas9.

**Results:** At bivariate analysis, the co-presence of the 3 risk variants correlated with the grade of steatosis (p < 0.0001), lobular inflammation (p = 0.009), ballooning (p = 0.004) and fibrosis (p < 0.0001). At nominal logistic regression analysis adjusted for age, sex, BMI and T2D, patients carrying the 3 variants showed a 4-fold higher risk of fibrosis2> (p = 0.002), cirrhosis (p = 0.02) and HCC (p = 0.09). In I1148M TM6SF2−/− and I1148M MBOAT7−/− TM6SF2−/− cells, intracellular lipid droplets and TG content were higher than in I1148M HepG2 cells (p < 0.01), and the expression of genes involved in de novo lipogenesis, cholesterol biosynthesis and β-oxidation was altered (p
<0.05). Moreover, markers of endoplasmic reticulum (XBP1, GRP78) and oxidative stress, as well as lipid peroxidation and DNA damage were increased (p < 0.05). Cell injury was greater in I148M MBOAT7 and oxidative stress, as well as lipid peroxidation and DNA damage were increased (p < 0.05). Cell injury was greater in I148M MBOAT7 and oxidative stress, as well as lipid peroxidation and DNA damage were increased (p < 0.05). Cell injury was greater in I148M MBOAT7 and oxidative stress, as well as lipid peroxidation and DNA damage were increased (p < 0.05). Cell injury was greater in I148M MBOAT7 and oxidative stress, as well as lipid peroxidation and DNA damage were increased (p < 0.05).

Conclusion: We firstly generated an in vitro stable compound knockout of genetic NAFLD. The co-presence of the 3 risk variants impacts on NAFLD development and progression, in both human patients and experimental models. In particular, TM6SF2 silencing alone or combined with I148M PNPLA3 and MBOAT7 KO contributes to hepatocellular damage and cell proliferation.

SAT036
Enhancing autophagy improves and slows the progression of non-alcoholic steatohepatitis disease

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Background and Aims: Loss of endothelial cell (LSEC) phenotype, or endothelial dysfunction (ED), has been associated with the progression from non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH). NASH is associated with defects in endothelial autophagy, which maintains LSEC phenotype. Spermidine (SPD) is a polyamine that activates autophagy and has beneficial effects on cardiovascular endothelium. Our hypothesis is that enhancement of autophagy with SPD could alleviate NAFLD progression.

Method: The impact of SPD treatment on autophagy and endothelial phenotype were evaluated in vitro in mouse LSEC (TSEC), Wild-type mice were pretreated with SPD (in drinking water) for two weeks and continued in a concomitant fashion with a 60% kcal fat-deficient choline diet (CDAAH, Br J Pharmaco. 2018) during 9 weeks. We evaluated autophagy levels and its impact on liver damage, ED, oxidative stress, inflammatory response and liver fibrosis.

Results: SPD activated autophagy in TSEC in vitro and in whole liver in vivo. NASH mice treated with SPD showed a decrease in adipose tissue mass as well as an improvement in basal glucose, consistent with amelioration of the metabolic phenotype. SPD had a hepatoprotective effect with an improvement of ED and reduction of fibrosis degree. Changes in hepatocyte ballooning degree were observed but not in steatosis. SPD treatment reduced mitochondrial oxidative stress and activated the selective degradation of dysfunctional mitochondria (mitophagy), leading to an improvement in mitochondrial phenotype. Interestingly, SPD had also an anti-inflammatory effect in NASH mice, characterized by a deactivation of the NLRP3 inflammasome and a reduction of proinflammatory macrophage phenotype. No toxicity was observed. Finally, enhancement of autophagy by SPD improved endothelial response to oxidative stress by means of increased viability, reduced ROS production and improved mitochondrial phenotype.

Conclusion: Autophagy inducement by SPD promotes endothelial response to oxidative stress by eliminating dysfunctional mitochondria, improving ED, reducing inflammation and attenuating liver fibrosis during the progression of NASH disease and may be a new and attractive antifibrotic strategy.

SAT037
The PSRC1 rs599839 A > G variant disentangles the risk of coronary artery disease and hepatocellular carcinoma in Italian NAFLD patients

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Background and Aims: Several inherited variants that regulate hepatic lipid handling increase the susceptibility to develop nonalcoholic fatty liver disease (NAFLD) and progress to nonalcoholic steatohepatitis (NASH), fibrosis and hepatocellular carcinoma (HCC). Dyslipidemia and enhanced cardiovascular risk are typical features of NAFLD. The rs599839 A > G variant in the 3’ UTR region of PSRC1, located in the CELSR2-PSRC1-SORT1 locus, has been previously associated with coronary artery disease (CAD) and reduced circulating lipids. Aim was to examine the impact of the rs599839 variant on metabolic traits and liver damage in a large histologically characterized cohort of patients at risk of NASH.

Method: We studied the impact of the rs599839 variant in 1224 Italian NAFLD patients (Liver Biopsy Cohort (LBC)), in 500,000 individuals (UK Biobank Cohort (UKBBC)) and in 366 HCC (The Cancer Genome Atlas (TCA)). Hepatic expressions of PSRC1, SORT1 and CELSR2 genes were evaluated by RNAsesq in a subset of patients (n = 125).

Results: The rs599839 G allele was associated with lower circulating LDL (beta: -0.19; 95%c.i. -0.3–0.09; p = 0.0003), higher HDL (beta: 0.07; 95%c.i. 0.03–0.10; p = 0.0008), reduced intima-media thickness (beta: -0.06; 95%c.i.-0.1–0.01; p = 0.008), decreased carotid plaques (OR: 0.23; 95%c.i. 0.4–1.3; p = 0.09) and hypertension (OR: 0.43; 95%c.i. 0.18–0.98; p = 0.04) in LBC and with the protection from dyslipidemia in UKBBC. Concerning the liver damage, G allele was associated with ballooning (beta: 0.26; 95%c.i. 0.01–0.51; p = 0.03) and HCC risk (OR: 1.92; 95%c.i. 1.06–3.50; p = 0.03; N = 72 cases) in LBC, but not in UKBBC. Carriers of G allele showed increased hepatic expression of PSRC1, SORT1 and CELSR2 (p < 0.001). PSRC1 mRNA levels negatively correlated with those of genes involved in lipoprotein release (APOB and DGAT2) (p < 0.0001), while positively with those of genes implicated in cell proliferation (PCNA and TP53) (p < 0.0001). In TCA, PSRC1 expression was associated with tumor stage worsening (beta: 0.40; 95%c.i. 0.05–0.27; p = 0.006), advanced histological grade (beta: 0.17; 95%c.i. 0.05–0.29; p = 0.005) and tumor extension (beta: 0.17; 95%c.i. 0.06–0.28; p = 0.003). As in LBC, PSRC1 expression negatively correlated with that of APOB and DGAT2, and positively with SORT1, CELSR2 and PCNA (p < 0.01).

Conclusion: The PSRC1 rs599839 A > G variant disentangles the risk of CAD and HCC in Italian NAFLD patients, likely by modulating PSRC1, SORT1 and CELSR2 expressions.