

In a next step we also analyzed MAIT cell frequencies in 7 patients longitudinally before and after HCV therapy. In analogy to our previous results in HIV patients who were started on antiretroviral therapy (ART) we also did not see a recovery of the MAIT cell frequencies upon initiation of HCV therapy (Fig. 1C). Further studies will need to evaluate whether these cells need longer time to recover after successful HCV therapy [9].

To our knowledge, an analysis of MAIT cell frequencies in HCV mono-infection in parallel with HCV/HIV co-infection has not been performed before. While we see a slight deterioration of MAIT cells in HCV, not surprisingly we see a trend of even more profound depletion in ART-treated HIV mono-infected and ART-treated HIV/HCV co-infected patients. One hypothesis is that immune activation due to microbial translocation [8,10] is further aggravated by global lack of MAIT cells which might be a fundamental mechanism by which HIV accelerates progression of chronic liver disease and HCV infection [7].

Further phenotypic and functional studies are required to confirm our results. Most importantly, we note that studies of intrahepatic MAIT cell phenotype and frequency in different liver diseases, HIV mono-infection or sepsis are largely missing. These future studies should then also correlate MAIT cell frequencies with clinical parameters, liver histology (grading and staging) and the stage of the liver disease as well as with laboratory markers of microbial translocation [9].

#### Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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#### References

- [1] Eberhard JM, Hartjen P, Kummer S, Schmidt RE, Bockhorn M, Lehmann C, et al. CD161+ MAIT cells are severely reduced in peripheral blood and lymph nodes of HIV-infected individuals independently of disease progression. *PLoS One* 2014;9 e111323.

- [2] Wong EB, Akilimali NA, Govender P, Sullivan ZA, Cosgrove C, Pillay M, et al. Low levels of peripheral CD161++CD8+ mucosal associated invariant T (MAIT) cells are found in HIV and HIV/TB co-infection. *PLoS One* 2013;8 e83474.
- [3] Tang XZ, Jo J, Tan AT, Sandalova E, Chia A, Tan KC, et al. IL-7 licenses activation of human liver intrasinusoidal mucosal-associated invariant T cells. *J Immunol* 2013;190:3142–3152.
- [4] Jo J, Tan AT, Ussher JE, Sandalova E, Tang XZ, Tan-Garcia A, et al. Toll-like receptor 8 agonist and bacteria trigger potent activation of innate immune cells in human liver. *PLoS Pathog* 2014;10 e1004210.
- [5] Jeffery HC, van Wilgenburg B, Kurioka A, Parekh K, Stirling K, Roberts S, et al. Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. *J Hepatol* 2016;64:1118–1127.
- [6] Barathan M, Mohamed R, Vadivelu J, Chang LY, Saeidi A, Yong YK, et al. Peripheral loss of CD8(+) CD161(++) TCRValpha7.2(+) mucosal-associated invariant T cells in chronic hepatitis C virus-infected patients. *Eur J Clin Invest* 2016;46:170–180.
- [7] Hernandez MD, Sherman KE. HIV/hepatitis C coinfection natural history and disease progression. *Curr Opin HIV AIDS* 2011;6:478–482.
- [8] Balagopal A, Philp FH, Astemborski J, Block TM, Mehta A, Long R, et al. Human immunodeficiency virus-related microbial translocation and progression of hepatitis C. *Gastroenterology* 2008;135:226–233.
- [9] Greathhead L, Metcalf R, Gazzard B, Gotch F, Steel A, Kelleher P. CD8+/CD161+ mucosal-associated invariant T-cell levels in the colon are restored on long-term antiretroviral therapy and correlate with CD8+ T-cell immune activation. *AIDS* 2014;28:1690–1692.
- [10] Shmagel KV, Saidakova EV, Shmagel NG, Korolevskaya LB, Chereshev VA, Robinson J, et al. Systemic inflammation and liver damage in HIV/hepatitis C virus coinfection. *HIV Med* 2016;17:581–589.

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## The *MBOAT7* variant rs641738 alters hepatic phosphatidylinositols and increases severity of non-alcoholic fatty liver disease in humans

To the Editor:

We have recently shown in 125 subjects that insulin resistance and the *PNPLA3* I148M gene variant, two common risk factors of NAFLD, are characterized with markedly different content

and composition of lipids in the human liver [1]. In 2015, a variant in membrane bound O-acyltransferase domain containing 7 (*MBOAT7*) at rs641738 was discovered to increase the risk of alcohol-related cirrhosis [2]. This variant was also shown to increase

## Letters to the Editor

the risk of steatosis and histologic liver damage in NAFLD, independent of obesity [3]. The variant allele was common with a population prevalence of 58–67% and characterized by decreased hepatic gene and protein expression of MBOAT7 [3]. MBOAT7 is also known as lysophosphatidylinositol acyltransferase 1 (LPIAT1), which catalyzes acyl-chain remodeling of phosphatidylinositols (PIs) [4]. Consistent with this function, plasma lipidomics analyses showed that amongst various lipid classes (triglycerides, cholesteryl esters, phospholipids, ceramides and sphingomyelins), only concentrations of PIs were altered [3]. Specifically, plasma concentrations of PI (36:4), PI (38:3) and PI (38:5) were decreased in proportion to the number of MBOAT7 variant alleles, while most other PIs were increased [3].

To study effects of genetic variation in MBOAT7 on human liver histology and lipidome, we genotyped the subjects in our previous study at rs641738 [1]. The subjects were consecutive patients undergoing bariatric surgery recruited using the inclusion and exclusion criteria described in [1]. The liver lipidome was analyzed using ultra-high performance liquid and gas chromatography combined with mass spectrometry and histology as described [1]. DNA was available from 115 subjects (age  $48.0 \pm 0.8$  years, BMI  $45.4 \pm 0.5$  kg/m<sup>2</sup>, 67 % women), who were divided into three groups based on their MBOAT7 genotype at rs641738 (n = 35 for CC, n = 60 for CT, n = 20 for TT).

The MBOAT7 genotype groups were similar with respect to age, gender, BMI, waist circumference, PNPLA3 I148M and TM6SF2 E167K genotypes (data not shown).

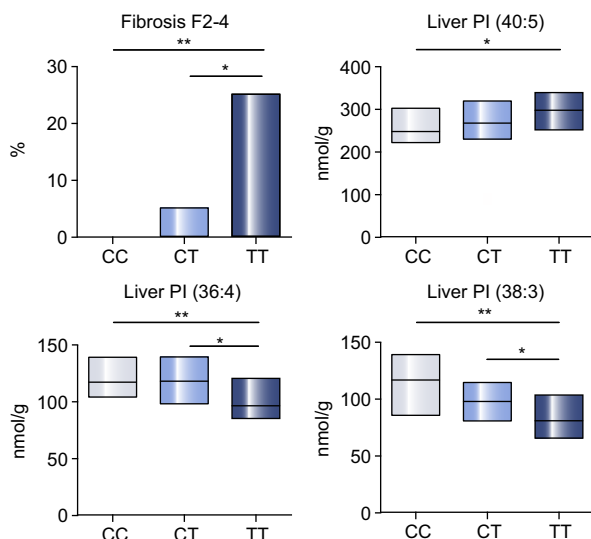
Steatosis (% of grades 0/1/2/3 were 23/60/3/14, 25/62/12/2 and 20/55/25/0,  $p = 0.03$  in CC, CT and TT groups) and necroinflammatory (% of grades 0/1/2/3 were 74/26/0/0, 87/13/0/0 and 60/35/0/5,  $p = 0.04$ ) grades differed significantly between the MBOAT7 groups. The prevalence of significant fibrosis (F2–4) increased with number of MBOAT7 variant alleles (0 vs. 5 vs. 25 %,  $p = 0.001$ , Fig. 1). Of 7 different PIs in the human liver, PI

(36:4) and PI (38:3), i.e., the same PIs as in the plasma in the study of Mancina and Dongiovanni *et al.* [3], decreased significantly as a function of the number of MBOAT7 variant alleles, while the concentration of PI (40:5) increased (Fig. 1). All other lipid classes in the human liver (triglycerides, cholesterol esters, ceramides, sphingomyelins, hexosylceramides, phospholipids, and free fatty acids) were similar between the groups (data not shown). Fasting insulin (13.7 [8.4–17.1], 11.2 [6.5–18.3] and 12.3 [7.0–18.8] mU/L in CC, CT and TT groups), glucose (5.9 [5.0–6.6], 5.8 [5.4–6.6] and 5.7 [5.1–6.1] mmol/L), triglycerides (1.24 [1.06–1.55], 1.29 [0.91–1.69] and 1.08 [1.00–1.59] mmol/L), HDL (1.15 [0.98–1.33], 1.09 [0.93–1.38] and 0.98 [0.86–1.13] mmol/L) and low density lipoprotein (2.5 [1.9–3.4], 2.3 [1.7–2.9] and 2.4 [1.5–3.5] mmol/L) cholesterol concentrations were similar between the groups.

We thus replicate effects of the MBOAT7 variant rs641738 on human liver histology with respect to steatosis and necroinflammation, and an increased prevalence of significant fibrosis [3]. The latter is the key predictor of overall mortality, liver transplantation, and liver-related events [5,6].

PIs are lipids, which regulate membrane dynamics and signal transduction pathways [4]. They consist of a glycerol backbone and two variable fatty acyl-chains, of which one is predominantly saturated and the other polyunsaturated [4]. MBOAT7 participates in acyl-chain remodeling of PIs in the Lands' cycle, in which it incorporates a polyunsaturated fatty acyl-chain into a PI [4]. In mice, knockout of LPIAT1, i.e. MBOAT7, affects concentrations of hepatic polyunsaturated PIs [7]. Another enzyme of the MBOAT family, MBOAT5, participates in the acyl-chain remodeling of phosphatidylcholines [8]. Knockout of MBOAT5 in mice decreases arachidonic acid-containing phosphatidylcholines in the liver and increases the risk of hepatic steatosis and inflammation [8]. MBOAT7 deficiency is thus predicted to increase free polyunsaturated fatty acids [9] and their proinflammatory metabolites, which are increased in plasma of subjects with non-alcoholic steatohepatitis [10]. Detailed understanding of the mechanisms via which the altered hepatic phosphatidylinositol metabolism leads to liver fibrosis are thus of considerable interest.

In conclusion, we confirm that the common variant in MBOAT7 rs641738 associates with histologic liver damage, particularly significant fibrosis. We extend previous data by showing that altered polyunsaturated PI metabolism characterizes the human liver in carriers of the MBOAT7 variant. These data are consistent with recent data in plasma and a role for MBOAT7 in hepatic phosphatidylinositol remodeling [3].



**Fig. 1. Prevalence of significant fibrosis and hepatic concentrations of phosphatidylinositols PI (40:5), PI (36:4), and PI (38:3) in groups according to the MBOAT7 genotype at rs641738.** Data are in % and median (25th–75th percentile), and were tested using Pearson's  $\chi^2$  test, Kolmogorov-Smirnov test and Mann-Whitney U test, as appropriate. \* $p < 0.05$ , \*\* $p < 0.01$ .

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**Authors' contributions**

PL – study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis. YZ, TH, ML, JA, MOM, MO – acquisition of data; critical revision of the manuscript for important intellectual content. HY – study concept and design; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; obtained funding; study supervision.

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**References**

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[1] **Luukkonen PK, Zhou Y**, Sädevirta S, Leivonen M, Arola J, Orešič M, et al. Hepatic ceramides dissociate steatosis and insulin resistance in patients with non-alcoholic fatty liver disease. *J Hepatol* 2016;64:1167–1175.  
 [2] Buch S, Stickel F, Trépo E, Way M, Herrmann A, Nischalke HD, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. *Nat Genet* 2015;47:1443–1448.  
 [3] **Mancina RM, Dongiovanni P**, Petta S, Pingitore P, Meroni M, Rametta R, et al. The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of european descent. *Gastroenterology* 2016;150:1219–1230 e6.  
 [4] D'Souza K, Epanand RM. Enrichment of phosphatidylinositols with specific acyl chains. *Biochim Biophys Acta* 2014;1838:1501–1508.  
 [5] Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Björnsson ES, Charatcharoen-witthaya P, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology* 2015;149:389–397 e10.  
 [6] Ekstedt M, Hagström H, Nasr P, Fredrikson M, Stål P, Kechagias S, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015;61:1547–1554.

[7] Anderson KE, Kielkowska A, Durrant TN, Juvin V, Clark J, Stephens LR, et al. Lysophosphatidylinositol-acyltransferase-1 (LPIAT1) is required to maintain physiological levels of PtdIns and PtdInsP(2) in the mouse. *PLoS One* 2013;8:e58425.  
 [8] Rong X, Wang B, Dunham MM, Hedde PN, Wong JS, Gratton E, et al. Lpcat3-dependent production of arachidonoyl phospholipids is a key determinant of triglyceride secretion. *Elife* 2015;4.  
 [9] Pérez-Chacón G, Astudillo AM, Balgoma D, Balboa MA, Balsinde J. Control of free arachidonic acid levels by phospholipases A2 and lysophospholipid acyltransferases. *Biochim Biophys Acta* 2009;1791:1103–1113.  
 [10] Loomba R, Quehenberger O, Armando A, Dennis EA. Polyunsaturated fatty acid metabolites as novel lipidomic biomarkers for noninvasive diagnosis of nonalcoholic steatohepatitis. *J Lipid Res* 2015;56:185–192.

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## Establishing the independence and clinical importance of non-alcoholic fatty liver disease as a risk factor for cardiovascular disease

*To the Editor:*

The evaluation of the nature of the association between non-alcoholic fatty liver disease (NAFLD) and cardiovascular risk has been the topic of a number of reports. There is emerging consensus that NAFLD is positively correlated with increased cardiovascular risk and several groups have indicated that this is independent of known risk factors [1]. The importance of this

association is underlined by the observation that cardiovascular disease is a leading cause of death in individuals with NAFLD [2,3].

To further illuminate this topic two recent papers have been published in the *Journal of Hepatology*. The first of these comes from the LIDO Study Group and assesses the impact of hepatic steatosis on the incidence and development of carotid