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## No evidence for an increased liver uptake of SARS-CoV-2 in metabolic-associated fatty liver disease

To the Editor:

We read with interest the research article published by Ji and colleagues, in the *Journal of Hepatology*, showing that patients with metabolic-associated fatty liver disease (MAFLD) have a higher risk of COVID-19 disease progression and higher likelihood of abnormal liver blood tests from admission to discharge than patients without MAFLD.<sup>1</sup> Given the absence of data on medical history of these patients, this persistence of liver blood test abnormalities could be either a mere reflection of pre-existing abnormalities related to MAFLD or could alternatively be due to a higher susceptibility of the fatty liver to SARS-CoV-2 infection.

We therefore investigated whether MAFLD is associated with altered liver expression of SARS-CoV-2 critical entry proteins. SARS-CoV-2 attaches to cells by binding to angiotensin-converting enzyme 2 (ACE2). The cellular protease transmembrane protease serine 2 (TMPRSS2) cleaves the SARS-CoV-2 spike protein, allowing fusion of cellular and viral membranes.<sup>2,3</sup> Moreover, in the HEK293 cell line, overexpressing human ACE2, SARS-CoV-2 enters through endocytosis with critical roles played by endocytosis-regulating protein phosphatidylinositol 3-phosphate 5-kinase (PIKFYVE).<sup>4</sup> Finally, as described for SARS-CoV and MERS-CoV, cathepsin L is also critical for priming of the SARS-CoV-2 spike protein in lysosomes following entry through endocytosis.<sup>4</sup>

We analysed the influence of MAFLD on liver gene expression of these 4 proteins implicated in SARS-CoV-2 infection by analysing public data from patients and from mice with MAFLD. In 2013, Ahrens and colleagues published microarray data obtained on human liver biopsies.<sup>5,6</sup> They made available transcriptomic data from 12 lean patients without MAFLD, 16 obese patients without MAFLD, 9 patients with simple steatosis and 17 patients with biopsy proven non-alcoholic steatohepatitis (NASH). Using these datasets, we observed that none of the genes necessary for SARS-CoV-2 infection was differentially expressed between lean or obese controls and patients with simple steatosis or with NASH (Table 1).

We performed the same analysis in a mouse dataset published by Xiong and colleagues.<sup>8</sup> Similarly, we observed no increase in liver gene expression of the 4 proteins implicated in SARS-CoV-2 infection between MAFLD mice and control mice (data not shown).

In conclusion, MAFLD is not associated with changes in liver expression of genes implicated in SARS-CoV-2 infection. The observed persistence of liver blood test abnormalities reported by Ji and colleagues is thus likely not explained by increased hepatic SARS-CoV-2 uptake.

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**Table 1. mRNA expression of SARS-CoV-2 infection critical genes in human liver biopsy.**

Gene name	Lean without MAFLD (n = 12) vs. NASH (n = 17)		Lean without MAFLD (n = 12) vs. simple steatosis (n = 9)		Obese without MAFLD (n = 16) vs. simple steatosis (n = 9)		Obese without MAFLD (n = 16) vs. NASH (n = 17)		Lean and obese without MAFLD (n = 28) vs. simple steatosis or NASH (n = 26)	
	Fold-change	adj. p value	Fold-change	adj. p value	Fold-change	adj. p value	Fold-change	adj. p value	Fold-change	adj. p value
ACE2	1.41	0.39	1.00	0.99	0.99	0.97	1.39	0.14	1.24	0.24
CTSL	0.98	0.96	1.10	0.69	1.04	0.73	0.92	0.67	0.99	0.95
TMPRSS2	0.85	0.72	0.78	0.57	0.87	0.64	0.94	0.95	0.88	0.60
PIKFYVE	1.03	0.93	0.77	0.94	0.85	0.11	0.92	0.64	0.94	0.53

Human microarray data<sup>6</sup> was made available by Ahrens and colleagues<sup>5</sup> and reanalysed by us using Geo2R<sup>7</sup> default settings. Geo2R is based on the “Linear Models for Microarray Data” R package that computes a moderated t-statistic for each gene and corresponding p value. Adjustment for multiple testing was performed using Benjamini and Hochberg’s correction. *CTSL* gene encodes cathepsin L protein. Human transcriptomics data is available on GEO Dataset under the accession number GSE48452. MAFLD, metabolic-associated fatty liver disease; NASH, non-alcoholic steatohepatitis.

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## Conflict of interest

The authors declare no conflict of interest.

Please refer to the accompanying [ICMJE disclosure](#) forms for further details.

## Authors' contributions

L.B. performed the bioinformatic analysis; L.B. and P.E.R. wrote the manuscript; D.V. provided guidance and proof-read the manuscript; all authors revised and approved the final version.

## Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2020.04.035>.

## References

Author names in bold designate shared co-first authorship

- [1] **Ji D, Qin E**, Xu J, Zhang D, Cheng G, Wang Y, et al. Non-alcoholic fatty liver diseases in patients with COVID-19: A retrospective study. *J Hepatol* 2020;73:451–453.
- [2] **Hoffmann M, Kleine-Weber H**, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;181:271–280.e8.
- [3] **Zhou P, Yang X**, Wang X, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;579:270–273.
- [4] **Ou X, Liu Y, Lei X**, Li P, Mi D, Ren L, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun* 2020;11:1620.
- [5] **Ahrens M, Ammerpohl O, von Schönfels W**, Kolarova J, Bens S, Itzel T, et al. DNA methylation analysis in nonalcoholic fatty liver disease suggests distinct disease-specific and remodeling signatures after bariatric surgery. *Cell Metab* 2013;18:296–302.
- [6] Jochen H, Andreas T, Timo I. Human liver biopsy of different phases from control to NASH, access number GSE48452. GEO Dataset. 2013. Available at: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48452>; 2013.
- [7] Barrett T, Wilhite S, Ledoux P, Evangelista C, Kim I, Tomashevsky M, et al. NCBI GEO: archive for functional genomics data sets—update. *Nucleic Acids Res* 2013;41:D991–D995.
- [8] Xiong X, Wang Q, Wang S, Zhang J, Liu T, Guo L, et al. Mapping the molecular signatures of diet-induced NASH and its regulation by the hepatic cytokine Tsukushi. *Mol Metab* 2019;20:128–137.

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## Reply to: 'No evidence for an increased liver uptake of SARS-CoV-2 in metabolic-associated fatty liver disease'

To the Editor:

We read with interest the letter by Biquard *et al.*<sup>1</sup> In their study, mRNA expression of SARS-CoV-2 infection critical genes, such as angiotensin-converting enzyme 2 (ACE2), transmembrane protease serine 2 (TMPRSS2), phosphatidylinositol 3-phosphate 5-kinase (PIKFYVE) and cathepsin L were found not to be enhanced in patients with metabolic-associated fatty liver disease (MAFLD, previously called non-alcoholic fatty liver disease [NAFLD]) or obesity. This finding is of potential added value to our observation that patients with COVID-19 had worse outcomes if they had underlying MAFLD. Firstly, persistent liver injury observed in our patients was unlikely to be related to the direct cytopathic effects of the virus. Though ideally, one should compare hepatocyte expression of these 4 genes in patients with/without MAFLD that do not have COVID-19. Secondly, this is in keeping with our hypothesis that dysregulated hepatic innate immunity in patients with MAFLD contributes to the pathogenesis of COVID-19. Apart from lung alveolar epithelial cells,

the enterocytes of the small intestine also have abundant expression of ACE2 receptors and thus could be another portal of entry for SARS-CoV-2. In keeping with this, gastrointestinal manifestations, such as diarrhoea and abdominal pain occurred in up to one-quarter of patients with COVID-19, without cough. Overall about half of the patients with COVID-19 tested positive for SARS-CoV-2 RNA in faecal and respiratory specimens concomitantly.<sup>2</sup> The liver is enriched with innate immune cells (such as macrophages, natural killer, natural killer T, and  $\gamma\delta$  T cells)<sup>3</sup> and due to its rich blood supply from the small bowel, circulation of the virus via the hepatic reticular system is expected. Hepatic innate immunity populations are potent cytokine producers and there are reports that obesity and NAFLD were associated with increased production of pro-inflammatory cytokines like tumour necrosis factor- $\alpha$  by adipose cells and Kupffer cells.<sup>4,5</sup> This may lead to an increased likelihood of symptomatic SARS-CoV-2 infections and the high prevalence of NAFLD in our study populations. Further studies are required to enhance our understanding of the link between the dysregulated hepatic innate immunity and COVID-19. This could be the missing link between the well-recognized risk factors of diabetes mellitus,

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