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Obese patients with NASH have increased hepatic expression of SARS-CoV-2 critical entry points

To the Editor:

We read with great interest the article published by Biquard and colleagues showing that, according to public transcriptomic data, the hepatic expression of angiotensin converting enzyme 2 (ACE2) and the cellular transmembrane protease serine 2 (TMPRSS2) remains unchanged in patients with metabolic-associated fatty liver disease (MAFLD).[1] SARS-CoV-2 attaches to cells by binding to its receptor ACE2. TMPRSS2 then cleaves the SARS-CoV-2 spike protein, allowing fusion of cellular and viral membranes.[2] Despite this retrospective study, there is growing evidence that patients with MAFLD are at higher risk of COVID-19 disease progression.[3–5]

Given the ongoing discussion, we have assessed the expression of SARS-CoV-2 cell entry molecules in the liver of obese patients with non-alcoholic fatty liver disease (NAFLD) and/or type 2 diabetes T2D (see Table S1 for detailed characteristics), since this information seems crucial to understand and prevent cell infection. Considering that T2D has been associated with a worse prognosis in patients with COVID-19 and that well-controlled glycemia was associated with a markedly improved outcome,[6] we first focused on patients with T2D. Liver mRNA expression of *ACE2* was significantly lower in patients with T2D while *TMPRSS2* also tended to decrease but was not statistically significant (Fig. 1A). Then, we analysed separately men and women. In men, hepatic *ACE2* and *TMPRSS2* expression remained unchanged between the 2 groups (Fig. 1B). However, in women with T2D, *ACE2* was significantly lower while *TMPRSS2* gene expression tended to decrease compared to women without T2D (Fig. 1B). These results indicate that while the cell entry machinery of SARS-CoV-2 is not majorly altered in the liver of obese men with T2D, its downregulation in women might indicate a lower susceptibility to liver injury. These findings are in consonance with the well-established protective role of estrogens in dysmetabolism.[7]

Next, we measured the expression of these genes in the liver according to the presence of NAFLD. Liver mRNA expression of both *ACE2* and *TMPRSS2* did not show differences between individuals without liver injury and patients with only steatosis, but these genes were upregulated in obese patients with non-alcoholic steatohepatitis (NASH) (Fig. 1C). Moreover, *ACE2* and *TMPRSS2* were positively correlated with NAFLD activity score (Fig. 1D). Of note, *TMPRSS2* was also positively correlated with weight, BMI and cholesterol (data not shown). These results are apparently different to those described in a previous study performing transcriptomics in individuals with and without MAFLD[1,8] and also in animal models of diet-induced NASH,[8] where no changes were detected for either *ACE2* or *TMPRSS2*. However, it is important to highlight that there are important differences in the characteristics of the cohorts. In contrast to the previous report that analyzed lean and obese patients with MAFLD or NASH,[1] our cohort is exclusively composed of obese patients. Moreover, whereas in the previous analysis T2D is not mentioned, in our cohort a significant percentage of patients had T2D, which commonly coexists with MAFLD.[9] Lastly, methodological differences might also explain the discrepant results, since we used real-time PCR to specifically measure gene expression of *ACE2* and *TMPRSS2*, while in the previous reports[8] results were obtained by less quantitative techniques namely microarray or RNA sequencing. Further studies using larger cohorts of patients with liver damage need to be meticulously evaluated to understand whether the SARS-CoV-2 receptor *ACE2* and the serine protease *TMPRSS2* are indeed affected in advanced stages of NAFLD and to what extent their expression affects the incidence of complications, severity and mortality.

In summary, our results indicate that in the livers of obese patients, SARS-CoV-2 entry factors are differently affected by T2D and NAFLD. While obese women with T2D have unexpectedly lower levels of *ACE2* and *TMPRSS2* than obese normoglycemic women, obese patients with NASH show markedly higher expression of these genes, suggesting that advanced stages of NAFLD might predispose individuals to COVID-19.

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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

MFF, MMG, AR, MJGR, PI, VV, JE, MS, VP, CD, JC, GF, MLMC, RN contributed to conception and design, acquisition of data, analysis and interpretation of data. PI, MS, VP, CD, JC, GF, MLMC, RN

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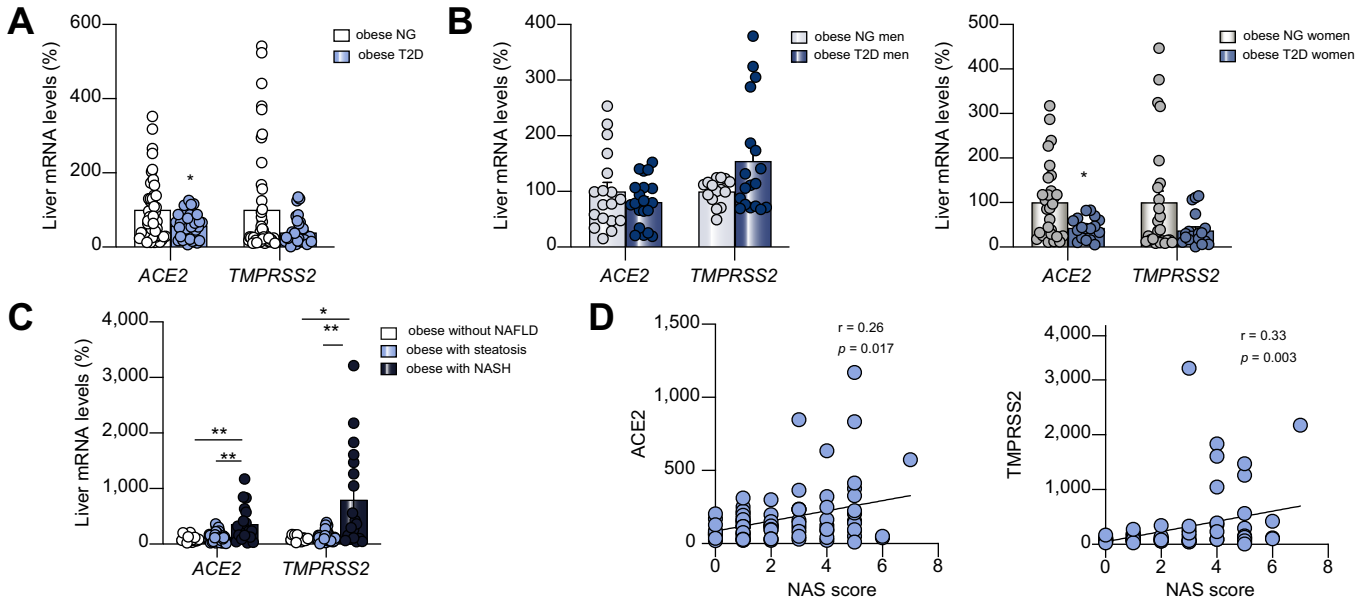

Fig. 1. *ACE2* and *TMPRSS2* hepatic mRNA levels in patients with T2D or NAFLD. (A) *ACE2* and *TMPRSS2* expression in obese patients with T2D (n = 43) or NG (n = 51); and (B) separately by men and women with T2D or NG. (C) *ACE2* and *TMPRSS2* expression in obese patients without NAFLD (n = 17), steatosis (n = 57), NASH (n = 20). (D) Correlation between *ACE2* and *TMPRSS2* with NAS score. **p* <0.05, ***p* <0.01, Mann-Whitney *U* test (A, B), Krustal-Wallis followed by Dunn *post-hoc* test (C). ACE2, angiotensin converting enzyme 2; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NG, normoglycemia; T2D, type 2 diabetes; TMPRSS2, transmembrane protease serine 2.

contributed drafting the article and revising it critically for important intellectual content.

Data availability statement

All the data used to support the findings of this study are included within the article. Reagents, resources and protocols are included in Supplementary methods.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2020.09.027.

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Tobacco, cannabis, and liquorice: Hidden players altering albendazole metabolism in patients with hepatic alveolar echinococcosis

To the Editor:

Alveolar echinococcosis (AE) due to the tumour-like hepatic development of the metacestode *Echinococcus (E.) multilocularis* is lethal if untreated. AE is highly prevalent in Western China[1,2] and its incidence is increasing in nearly all European countries.[1,2] Complete surgical resection (including liver transplantation or *ex vivo* hepatic resection and auto-transplantation in life-threatening cases) is the treatment gold-standard, feasible in one-third of patients.[1–4] The parasitostatic drug albendazole (ABZ) is administered either for 2 years after hepatectomy or lifelong in inoperable cases.[1–3] ABZ is a high-clearance inactive pro-drug; its liver metabolism is primarily through cytochrome P450 (CYP) 1A2 and CYP3A4[5] into the active form ABZ-sulfoxide (ASOX) then into non-active ABZ sulfone.[2,5] There are considerable inter-individual variations and no correlation between ABZ dosage and ASOX plasma concentration. Based on ASOX plasma levels (recommended range: 1 to 3 μmol/L, 4 hours after morning drug intake) therapeutic drug monitoring (TDM) allows dose adjustments for higher efficacy and less hepatic toxicity.[2]

We alert readers to the risk that lifestyle habits can interfere with ABZ metabolism.

Case 1 (Fig. 1A-C): A 23-year-old woman was diagnosed in 1993 with two asymptomatic AE lesions invading hepatic veins and making curative surgery impossible. During 21-years follow-up the lesions remained stable under ABZ 400 mg b.i.d. with optimal ASOX plasma levels. In 2014, low ASOX levels were observed. There were neither co-medications nor poor adherence to treatment. ABZ dosage was increased to 600 mg b.i.d. but ASOX levels remained inexplicably low for 2 years. In 2016 MRI detected microcysts newly appeared between the two unchanged initial lesions. The patient eventually mentioned regular cannabis consumption since 2014. Cannabis interruption was rapidly followed by increased ASOX levels and stable lesions.

Case 2: A 51-year-old man was diagnosed with AE in 2009. He was a heavy smoker (45 packs a year). Under ABZ 400mg b.i.d., ASOX levels were constantly low (<1 μmol/L) and dosage was increased under TDM. Finally, 700 mg b.i.d. were necessary to reach the recommended ASOX levels. In 2017, transient tobacco consumption reduction led to increased ASOX levels (4.02 μmol/L) without any clinical or biological adverse events. ABZ dosage was kept unchanged as the patient went back to his previous heavy consumption rapidly, with plasma ASOX levels similar to those observed previously and stable AE lesions.

Case 3 (Fig. 1D-F): A 64-year-old woman was diagnosed with AE in 2016. Under ABZ 400 mg b.i.d., ASOX levels were above the therapeutic range, leading to dose reduction. Under ABZ 200mg b.i.d., ASOX levels remained constantly high, above 3 μmol/L but without drug adverse effect. There was no co-medication. In 2019, regular liquorice ingestion through a popular French non-alcoholic drink (*Antésite*®) was eventually reported by the patient (1 to 1.5 L/day) and she was asked to stop. Two weeks later, ASOX levels were within the therapeutic range. Interestingly, this exposure to high ASOX levels for 2 years led to an excellent control of AE: the initially positive Em18 ELISA serology regularly decreased to become negative in October 2018 and in February 2019 PET showed a complete disappearance of the lesion metabolic activity.

Cytochrome P450 inducers (ritonavir, phenytoin, phenobarbital, or carbamazepine) are associated with low ASOX levels; conversely, inhibitors such as cimetidine may increase exposure to the active drug.[5–7] Our observations demonstrate that life habits may also interfere with ABZ. Both cannabis and tobacco smoking are known to induce CYP1A2,[8] which was responsible for low ASOX levels, a situation which led to AE progression in Case 1. In Case 2, tobacco consumption was known at time of diagnosis; TDM was very helpful to gradually increase ABZ dosage to a higher level than usually recommended.[2,3] Then, the patient remained asymptomatic for 10 years. On the contrary, in Case 3 we observed constantly high and unexplained ASOX levels. Querying the patient's life habits allowed us to identify liquorice consumption as the cause of the pharmacological disturbance. Liquorice's main constituent, glycyrrhizic acid and its gut-derived metabolite 18β-glycyrrhetinic acid inhibit several CYP450

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