External validation of a genetic risk score that predicts development of alcohol-related cirrhosis

To the Editor:

We read with great interest the article by Whitfield *et al.* on the development of a score that combines genetic risk variants in 3 genes (*PNPLA3, TM6SF2, HSD17B13*) and presence of diabetes to stratify heavy drinkers based on their risk of cirrhosis.[1] The authors evaluated the risk score in cohorts of patients with an alcohol consumption exceeding 80 g/day for men or 50 g/day for women for 10 years or more. We sought to validate their results by evaluating the score in an independent Danish cohort of 439 patients with a history of excessive alcohol use, but at lower levels of heavy drinking than in the 3 cohorts from Whitfield *et al.*'s study and thereby more similar to the average person with a history of alcohol overuse, but not dependence.[2] In our cohort, patients had a median of 15.5 years of excessive drinking (defined as >36 g/day for men or >24 g/day for women). Forty-two percent were abstinent at inclusion but the majority of them (74%) had been so for less than a year. For ongoing drinkers, the median alcohol intake was 48 g/day for men and 36 g/day for women in the week leading up to inclusion.

In our study, we found an AUC of 0.66 for diagnosing advanced fibrosis (≥F3), comparable to the AUC of 0.61-0.67 reported by Whitfield and colleagues in the 3 cohorts. The diagnostic accuracy was however mostly carried by PNPLA3 which alone had an AUC of 0.62 (Fig. 1). Together, all 3 single nucleotide polymorphisms (SNPs) explained 6.6% of the variance in presence of advanced fibrosis, while PNPLA3 alone explained 4.7% of the variance (genetic score: pseudo R^2 = 0.066. PNPLA3: pseudo R^2 = 0.047). The authors further defined cut-offs for risk stratification based on the distribution of the 3-SNP scores, categorising patients into 3 groups (low-, intermediate-, and high-risk) based on their risk of cirrhosis. We found an almost 4-fold higher risk of advanced fibrosis when comparing the low-risk and high-risk groups (odds ratio 3.78; 95% CI 2.17–6.62; p <0.001). This is in line with Whitfield *et al.*'s study, which reported odds ratios ranging from 2.66 to 4.96. The authors argue that the genetic risk and diabetes status cannot be combined into one risk score, in this case, and patients should be further subdivided by their diabetes status. Due to a low number of patients with diabetes in our cohort, we were not able to test the diagnostic accuracy after stratifying patients according to both their genetic risk score and diabetes status.

In addition, we tested the prognostic performance of the genetic risk score and found that the score was able to predict decompensation (overt ascites, overt hepatic encephalopathy, or variceal bleeding as recommended by Baveno VII[3]) with a hazard ratio of 1.58 (95% CI 1.07-2.36; p = 0.022).

Whitfield *et al.* observed the highest mean values, above 0.70, for the genetic score in patients who developed hepatocellular carcinoma. In our cohort, 10 patients developed hepatocellular carcinoma during 4.5 years of follow-up, with a mean genetic score value of 0.72 ± 0.4.

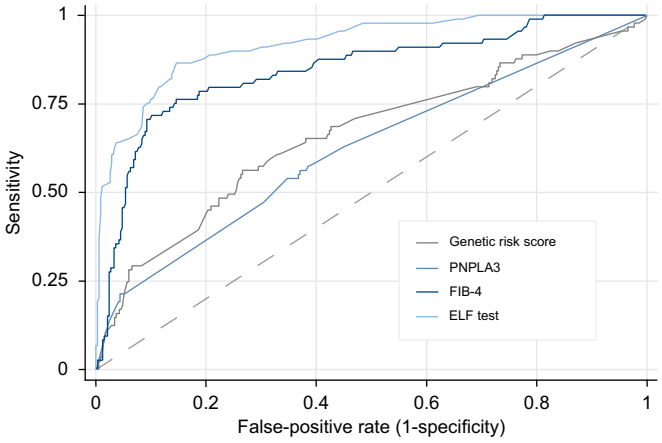

Fig. 1. Receiver operating characteristic curves. Diagnostic accuracy for advanced fibrosis (≥F3) of the genetic risk score, PNPLA3, FIB-4 and ELF test in 439 patients with alcohol-related liver disease using area under the receiver operating characteristics curves. FIB-4 and ELF test were chosen for comparison as they are potentially available in primary care. ELF, enhanced liver fibrosis; FIB-4, fibrosis-4.

This is a good effort to move genetics closer to the clinic with the advantages a genetic score holds, such as the possibility of performing an assessment the first time a patient presents with an alcohol problem or even at birth. But the closer the patients come to clinical disease, the less powerful a genetic score is compared to other non-invasive tests. The authors combine the genetic risk score with presence of diabetes for further risk stratification. One might argue that if the patient has diabetes, he has potentially progressed to a point in the natural history of liver disease where a genetic score holds less clinical value compared to non-invasive tests for advanced fibrosis, like the ELF (enhanced liver fibrosis) test and FIB-4 (fibrosis-4) index, which have far better diagnostic accuracy (AUC_{ELF} = 0.92 & AUC_{FIB-4} = 0.84 for diagnosis of advanced fibrosis in our cohort (Fig. 1)).[4,5]

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

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Authors' contributions

SJ: Analysis and interpretation of data; drafting of the manuscript; statistical analysis. MT: Study concept and design; obtained funding; acquisition of data; analysis and interpretation of data; critical revision of the manuscript for important intellectual content. HBJ: Acquisition of data; critical revision of the manuscript for important intellectual content. TH: Study concept and design; acquisition of data; obtained funding; critical revision of the manuscript for important intellectual content. AK: Study concept and design; analysis and interpretation of data; obtained funding; study supervision; critical revision of the manuscript for important intellectual content.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.06.006.

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Reply to: "External validation of a genetic risk score that predicts development of alcohol-related cirrhosis"

To the Editor:

Thank you for the opportunity to respond to the letter from Johansen *et al.*,[1] extending our earlier findings reported in the *Journal*.[2]

By applying our 3-SNP (single nucleotide polymorphism) score, Johansen *et al.* were able to stratify alcohol-related liver disease risk in a cohort with less extreme (but still elevated-risk) alcohol intake. The AUCs and odds ratios in this Danish cohort were very similar to those which we reported.

Johansen *et al.* comment that '… the closer the patients come to clinical disease, the less powerful a genetic score is compared to other non-invasive tests.' This is true, and important, and applies to all risk stratification protocols – not only genetic risk scores. A patient with clinical symptoms needs tests which evaluate their current situation. It is patients who are at high risk of conditions which have not yet occurred, or in some cases people from the general population, who may benefit from risk stratification through genetic scores. Specifically, patients with normal results for "… non-invasive tests for advanced fibrosis like ELF (enhanced liver fibrosis) test and FIB-4 (fibrosis-4) index …" may still develop significant fibrosis, cirrhosis and hepatocellular carcinoma in the future. Genetic tests can give a predictive measure of their underlying risk, as opposed to tests for already existing liver fibrosis (*e.g.* FIB-4 and ELF) that are intermediate tests diagnosing 'early disease' usually without symptoms.

The distinction between 'trait' and 'state' markers has been explored for a number of psychiatric conditions, including alcohol dependence and alcohol-related disease. For a long time no trait markers (able to assess future risk of disease) could be confirmed but genetic risk scores now offer this possibility for many conditions. The effectiveness of such scores in improving outcomes, and the ways to integrate them with current patterns of investigation, are being explored[3–5] and although the predictive value of a genetic score will always depend on the heritability of the condition, results so far are promising.

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Conflict of interest

JBW and DS have no conflict of interests. TRM has conducted clinical research with AbbVie, Genfit, Gilead, and Merck but none of these are related to this manuscript.