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A Polygenic Risk Score to Refine Risk Stratification and Prediction for Severe Liver Disease by Clinical Fibrosis Scores

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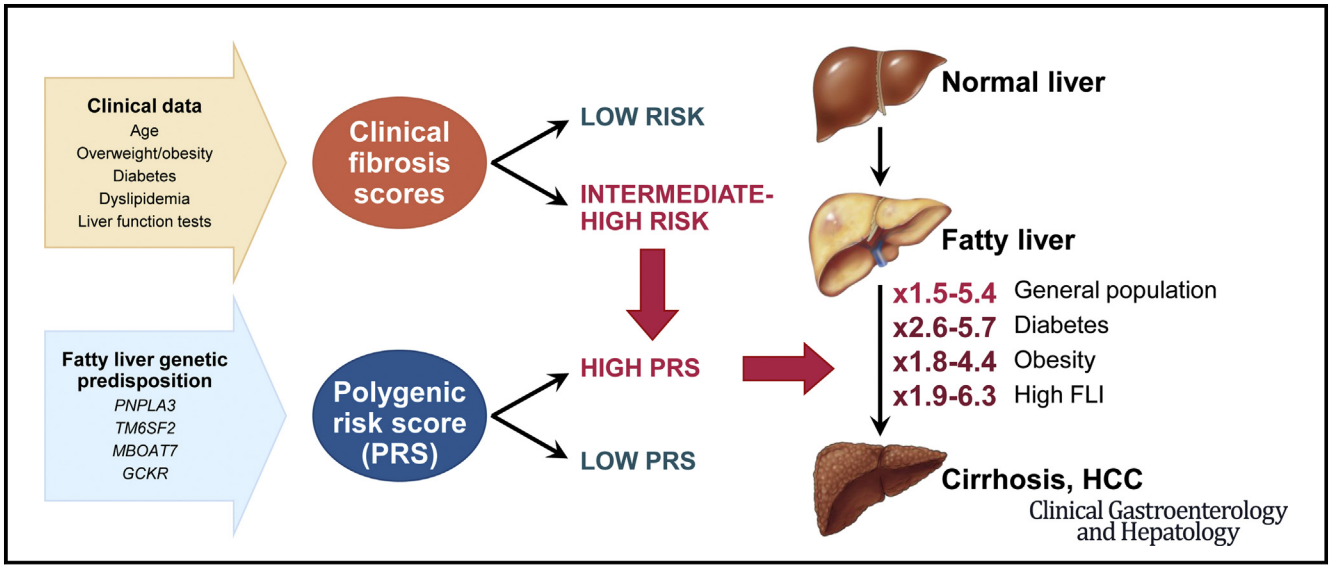

BACKGROUND & AIMS: A polygenic risk score based on well-known genetic variants in *PNPLA3*, *TM6SF2*, *MBOAT7*, and *GCKR* predicts hepatic fat content (polygenic risk score-hepatic fat content [PRS-HFC]). Here, we hypothesized that the addition of PRS-HFC to clinical fibrosis scores may improve risk stratification and prediction of severe liver disease (SLD).

METHODS: We used data from 266,687 individuals in the UK Biobank, evaluating the incidence of cirrhosis, decompensated liver disease, hepatocellular carcinoma, and/or liver transplantation during a median follow-up period of 9 years. Nonalcoholic fatty liver disease fibrosis score, Fibrosis-4, aspartate aminotransferase–to–platelet ratio, BARD, and Forns scores, and PRS-HFC, were computed. All analyses were stratified according to the presence of diabetes, obesity, and a positive fatty liver index (≥60).

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Abbreviations used in this paper: aHR, adjusted hazard ratio; APRI, aspartate aminotransferase–to–platelet ratio; AUROC, area under the receiver operating characteristic; BMI, body mass index; FIB-4, Fibrosis-4; FLI, fatty liver index; HCC, hepatocellular carcinoma; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; NFS, nonalcoholic fatty liver disease fibrosis score; PRS-HFC, polygenic risk score-hepatic fat content; PRS2, simplified version of the polygenic risk score-hepatic fat content; SLD, severe liver disease.

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RESULTS: **Unfavorable genetics (PRS-HFC, ≥0.396) further stratified the risk of SLD in subjects in intermediate-/high-risk classes of fibrosis scores, with a higher effect in those with metabolic risk factors, and the prediction was improved by integrating PRS-HFC (areas under the receiver operating characteristic increased for all scores with a *P* value of approximately 10^{-2} to 10^{-4}, except for the aspartate aminotransferase–to–platelet ratio in the overall population and in subjects with obesity). PRS-HFC improved diagnostic accuracies and positive predictive values for SLD in intermediate–high clinical score risk classes. Risk stratification and prediction were not affected or were poorly affected by unfavorable genetics in subjects without metabolic risk factors.**

CONCLUSIONS: **Integration of genetics with clinical fibrosis scores refines individual risk and prediction for SLD, mainly in individuals at risk for nonalcoholic fatty liver disease. These data provide evidence from a prospective cohort that common genetic variants capture additional prognostic insights not conveyed by validated clinical/biochemical parameters.**

Keywords: Nonalcoholic Fatty Liver Disease (NAFLD); UK Biobank; PNPLA3; Genetics.

Nonalcoholic fatty liver disease (NAFLD) is increasing in Western countries,[1,2] where it is rapidly evolving as the first cause of liver disease, hepatocellular carcinoma (HCC), liver transplantation, and liver-related death.[3] Although the histologic finding of steatohepatitis is an important indicator of disease severity, the degree of liver fibrosis is clearly the most relevant prognostic factor in NAFLD.[4] Several clinical scoring systems have been implemented for assessing liver fibrosis, either borrowed from other chronic liver diseases (eg, aspartate aminotransferase-to-platelet ratio index [APRI], Fibrosis-4 [FIB-4] score, and the Forns score[5–7]), or tailored specifically for individuals with NAFLD (eg, NAFLD fibrosis score [NFS] and BARD score[8,9]). These scores have been validated in cross-sectional studies in subjects with chronic liver disease (as such, with a high pretest probability of fibrosis) with respect to their capability to identify advanced fibrosis.[5–9] Consistently, in individuals with NAFLD, some of these scores also predict liver-related complications and all-cause mortality.[10] Recently, given the increasing burden of NAFLD and the need for risk assessment in primary care, some of these scores have been tested in the general population to predict overall and liver-related mortality and liver-related events.[11–14] These studies have shown good performances in risk stratification,[10–12] but a modest predictive ability for liver-related events and death, which worsened with the duration of follow-up evaluation.[11]

NAFLD heritability is estimated between 20% and 70%, and a body of evidence highlights the contribution of common genetic variants to the onset and progression of the entire spectrum of NAFLD.[15–17] We previously developed a weighted polygenic risk score (polygenic risk score-hepatic fat content [PRS-HFC]), based on variants primarily increasing liver fat content, and showed that an increase in liver fat content is causally related to inflammation, ballooning, and fibrosis in patients with NAFLD.[18] More recently, a genetic risk score combining 3 genetic variants was associated with the risk of cirrhosis and HCC in Northern Europeans.[19]

In this study, we hypothesized that PRS-HFC may improve the accuracy of clinical fibrosis scores in the prediction of future liver events. Therefore, we combined the PRS-HFC with fibrosis scoring systems in the UK Biobank and show an improvement in risk stratification and prediction of severe liver disease (SLD), especially in subjects at high-risk for NAFLD.[20]

Methods

Data Source and Sample Selection

We used data from the UK Biobank, a large prospective cohort including more than 500,000 participants (ages, 40–69 y) recruited between 2006 and 2010 from 22 assessment centers throughout the United Kingdom[21] (see the Supplementary Methods section for more detail). Details of baseline exclusion criteria are provided in the Supplementary Methods section and in Supplementary Tables 1 and 2. A total of 266,687 participants were included for the final analyses.

Baseline Covariates, Genetic Variants, and Outcomes

Variables assessed at baseline, and the formula applied to calculate liver fibrosis scores and the Fatty Liver Index (FLI),[20] a validated algorithm to predict ultrasonographic liver steatosis, are specified in the Supplementary Methods section.

Genetic variants associated with fatty liver disease also were retrieved: *PNPLA3* rs738409 C>G (p.I148M),

What You Need to Know

Background

Intermediate/high classes of clinical fibrosis scores are associated with an increased risk of severe liver disease (SLD) at the general population level, but the predictive performances are rather poor. Previous attempts to combine genetics with clinical scores to refine prediction for SLD have been unsuccessful.

Findings

A polygenic risk score (Polygenic Risk Score-Hepatic Fat Content [PRS-HFC]) based on variants in *PNPLA3*, *TM6SF2*, *MBOAT7*, and *GCKR* was causally related to liver injury, and was found here to be strongly associated with the occurrence of SLD. When added to clinical fibrosis scores, PRS-HFC improved risk stratification and prediction for SLD, especially in subjects with metabolic risk factors.

Implications for patient care

These data provide evidence from a prospective cohort that common genetic variants capture additional prognostic insights not conveyed by validated clinical/biochemical parameters. Well-designed polygenic risk scores seem the most suitable way to candidate genetics for its contribution in identifying subjects at risk for SLD at the general population level.

TM6SF2 rs58542926 C>T (p.E167K), *MBOAT7* rs641738 C>T, *GCKR* rs1260326 C>T (p.P446L), and *HSD17B13* rs72613567:TA. To summarize the impact of genetic predisposition to fatty liver, we exploited the PRS-HFC (including *PNPLA3*, *TM6SF2*, *MBOAT7*, and *GCKR*), which we recently developed to predict the inherited predisposition to hepatic fat accumulation quantified by the gold standard proton magnetic resonance spectroscopy (H1-MRS) in the general population.[18] A simplified version of the PRS-HFC (PRS2), including only *PNPLA3* and *TM6SF2* variants, also was calculated. For further details on genotyping and the PRS-HFC/PRS2 calculation, see the Supplementary Methods section.

Follow-up data on health-related events and mortality were obtained through linkage of the National Health Service records, including in-hospital admissions, death register, and cancer register. The outcome of interest was incident SLD, defined as a composite diagnosis of cirrhosis, decompensated liver disease, HCC, and/or liver transplantation in any of the aforementioned records. Further details on outcome ascertainment are provided in the Supplementary Methods section, and a list of all the diagnoses used to define SLD is presented in Supplementary Table 3.

Statistical Analysis

Data were presented according to descriptive statistics. Cox proportional hazard regression models were fitted to investigate the association of liver clinical fibrosis scores and of the genetic predisposition to fatty liver (expressed by single-risk variants and PRSs) with the occurrence of SLD. To this purpose, genetic risk variants were modeled according to both additive, dominant, recessive, or categoric inheritance, whereas the PRSs were first analyzed on a linear scale, and then categorized according to quartiles (PRS-HFC) or tertiles (PRS2). The proportional hazard assumption was verified through the inspection of the Schoenfeld residuals. Analyses were adjusted for age and sex, and stratified according to the presence of diabetes, obesity, and a FLI of 60 or higher.

To evaluate the predictive abilities, PRSs were divided into 3 categories (favorable, intermediate, or unfavorable), based on the observed trend of risk increase across quartiles or tertiles of PRS-HFC or PRS2, respectively. The discriminative performances of each clinical score (alone and combined with PRS-HFC/PRS2 categories) for the development of SLD were estimated, calculating the area under the receiver operating characteristic (AUROC) with 95% CIs of Cox models. The ability of the models including also PRS-HFC/PRS2 to reclassify subjects correctly was expressed by the category-free continuous net reclassification index.[21] Model calibration was evaluated by plotting the observed vs the predicted survival probability. In addition, we calculated general accuracy, sensitivity, specificity, positive predictive value, and negative predictive value for incident SLD at 5 years.

All analyses were conducted with R statistics 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline Characteristics and Incidence of Severe Liver Disease

A total of 266,687 participants of European descent from the UK Biobank were included in the analyses. The baseline characteristics of the study participants stratified by incident SLD status are shown in Table 1. In the overall cohort, the mean age was 56.5 years (SD, 8.1 y), more than half of the participants were men, and the mean body mass index (BMI) was in the range of overweight (27.4 kg/m^2; SD, 4.8 kg/m^2), and the prevalence of diabetes, hypertension, and dyslipidemia was 5%, 29%, and 22%, respectively.

During a median follow-up period of 9.0 years (IQR, 8.3–9.7 y), 543 (0.2%) subjects developed SLD, 457 developed cirrhosis with or without complications, and 86 developed HCC, 100 individuals died of liver disease and 62 died of HCC. Patients with SLD during the follow-

Table 1. Baseline Characteristics of the Study Population According to the Incidence of Severe Liver Disease During Follow-up Evaluation

		Incident severe liver disease		
	All	No	Yes	*P*
N	266,687	266,144 (99.8%)	543 (0.2%)	
Demographics				
Age, *y*	56.5 (8.1)	56.5 (8.1)	60 (6.9)	$2.4*10^{-22}$
Sex, female	148,008 (55%)	147,811 (56%)	197 (36%)	$6.1*10^{-4}$
Cardiometabolic factors				
BMI, kg/m^2	27.4 (4.8)	27.4 (4.8)	30 (6.1)	$8.0*10^{-34}$
Obesity	64,655 (24%)	64,417 (24%)	238 (44%)	$9.2*10^{-43}$
Waist circumference, *cm*	89.9 (13.5)	89.9 (13.5)	99.8 (15.7)	$2.3*10^{-44}$
Smokers	23,551 (9%)	23,465 (9%)	86 (16%)	$4.3*10^{-9}$
Diabetes mellitus	13,268 (5%)	13,143 (5%)	125 (23%)	$1.2*10^{-44}$
Hypertension	76,550 (29%)	76,254 (29%)	296 (55%)	$1.1*10^{-20}$
Dyslipidemia	57,390 (22%)	57,155 (21%)	235 (43%)	$4.5*10^{-14}$
Cardiovascular disease	15,521 (6%)	15,417 (6%)	104 (19%)	$4.3*10^{-16}$
Clinical biochemistry				
ALT level, *U/L*	19.9 (15.2–26.8)	19.9 (15.2–26.8)	26.4 (19–40.2)	$2.5*10^{-70}$
AST level, *U/L*	24.1 (20.8–28.4)	24.1 (20.8–28.4)	29.7 (23.9–43.3)	$6.2*10^{-162}$
GGT level, *U/L*	24.9 (17.9–37.7)	24.9 (17.9–37.7)	53.4 (27.9–120.2)	$6.7*10^{-154}$
Alkaline phosphatase level, *U/L*	80.5 (67.5–96)	80.5 (67.5–95.9)	93.3 (76.4–115.2)	$3.0*10^{-52}$
Total bilirubin level, *mg/dL*	0.5 (0.4–0.6)	0.5 (0.4–0.6)	0.5 (0.4–0.7)	$8.5*10^{-4}$
Albumin level, *mg/dL*	4.5 (0.3)	4.5 (0.3)	4.4 (0.3)	$5.6*10^{-20}$
Platelet count, 10^9 cells/L	253.9 (58.8)	253.9 (58.7)	229.6 (81)	$1.6*10^{-14}$
Total cholesterol level, *mg/dL*	221.4 (44.5)	221.4 (44.5)	197.4 (50)	$1.2*10^{-25}$
LDL level, *mg/dL*	139.2 (33.9)	139.2 (33.9)	122.4 (36.7)	$1.1*10^{-23}$
HDL level, *mg/dL*	55.5 (14.3)	55.5 (14.3)	49.7 (16)	$2.9*10^{-10}$
Triglyceride level, *mg/dL*	132.1 (93.5–190.2)	132.1 (93.5–190.1)	146 (100.1–203.5)	$1.6*10^{-1}$
HbA1c, %	5.4 (5.2–5.6)	5.4 (5.2–5.6)	5.6 (5.3–6.1)	$4.8*10^{-19}$
Genetic variants				
PNPLA3 rs738409 C>G				$6.7*10^{-6}$
CC	163,746 (61%)	163,452 (61%)	294 (54%)	
CG	90,240 (34%)	90,038 (34%)	202 (37%)	
GG	12,701 (5%)	12,654 (5%)	47 (9%)	
TM6SF2 rs58542926 C>T				$1.6*10^{-3}$
CC	228,122 (86%)	227,681 (86%)	441 (81%)	
CT	37,123 (14%)	37,028 (14%)	95 (17%)	
TT	1442 (1%)	1435 (1%)	7 (1%)	
MBOAT7 rs641738 C>T				$8.3*10^{-2}$
CC	83,814 (31%)	83,663 (31%)	151 (28%)	
CT	131,698 (49%)	131,419 (49%)	279 (51%)	
TT	51,175 (19%)	51,062 (19%)	113 (21%)	
GCKR rs1260326 C>T				$3.3*10^{-1}$
CC	96,384 (36%)	96,192 (36%)	192 (35%)	
CT	127,919 (48%)	127,665 (48%)	254 (47%)	
TT	42,384 (16%)	42,287 (16%)	97 (18%)	
HSD17B13 rs72613567:TA				$7.0*10^{-1}$
TT	139,629 (52%)	139,341 (52%)	288 (53%)	
TAT	106,778 (40%)	106,563 (40%)	215 (40%)	
TATA	20,280 (8%)	20,240 (8%)	40 (7%)	
Noninvasive scores				
Fatty liver index				$1.4*10^{-27}$
Low	98,476 (37%)	96,319 (37.4%)	2157 (25%)	
Intermediate	69,830 (26.2%)	67,653 (26.2%)	2177 (25.2%)	
High	98,160 (36.8%)	93,850 (36.4%)	4310 (49.9%)	
NFS				$6.9*10^{-59}$
Low	189,472 (71%)	189,256(71.1%)	216 (39.8%)	
Intermediate	74,145(27.8%)	73,891 (27.8%)	254 (46.8%)	
High	3070 (1.2%)	2997 (1.1%)	73 (13.4%)	
FIB-4				$4.0*10^{-48}$
Low	151,356 (56.8%)	151,182 (56.8%)	174 (32%)	

Table 1. Continued

		Incident severe liver disease		
	All	No	Yes	*P*
Intermediate	110,363 (41.4%)	110,102 (41.4%)	261 (48.1%)	
High	4968 (1.9%)	4860 (1.8%)	108 (19.9%)	
APRI				
Low	254,644 (95.5%)	254,270 (95.5%)	374 (68.9%)	$2.0*10^{-150}$
Intermediate	11,798 (4.4%)	11,655 (4.4%)	143 (26.3%)	
High	245 (0.1%)	219 (0.1%)	26 (4.8%)	
BARD				$3.6*10^{-33}$
Low	23,925 (9%)	23,892 (9%)	33 (6.1%)	
Intermediate	235,574 (88.3%)	235,158 (88.4%)	416 (76.6%)	
High	7188 (2.7%)	7094 (2.7%)	94 (17.3%)	
Forns				$1.2*10^{-103}$
Low	156,960 (58.9%)	156,837 (58.9%)	123 (22.7%)	
Intermediate	105,414 (39.5%)	105,131 (39.5%)	283 (52.1%)	
High	4313 (1.6%)	4176 (1.6%)	137 (25.2%)	
Follow-up time, *y*	9 (8.3–9.7)	9 (8.3–9.7)	5.6 (3.4–7)	$<10^{-300}$

NOTE. Continuous variables are shown as mean (SD) or median (interquartile range), if normally or not normally distributed, respectively. Categoric variables are shown as absolute numbers (percentage). *P* values are from generalized linear models corrected for age, sex, and assessment center.
ALT, alanine aminotransferase; APRI, AST to platelets ratio index; AST, aspartate aminotransferase; BMI, body mass index; FIB-4, Fibrosis-4; GGT, γ glutamyl transferase; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NFS, nonalcoholic fatty liver disease fibrosis score.

up evaluation were older; more frequently men; had a higher BMI and waist circumference; showed a higher prevalence of diabetes, hypertension, dyslipidemia and cardiovascular disease; and had higher liver enzyme levels and lower albumin and platelet levels. In those with SLD, there was an enrichment of *PNPLA3* rs738409 and *TM6SF2* rs58542926 variants, whereas no differences were observed for *MBOAT7* rs641738, *GCKR* rs1260326, and *HSD17B13* rs72613567. As expected, these individuals were more likely in the intermediate-/high-risk classes according to the NFS, FIB-4, APRI, BARD, and Forns scores.

Fibrosis Scores and the Risk of Severe Liver Disease

In the overall cohort, as compared with the relative low-risk classes, those in the intermediate- or high-risk classes of NFS, FIB-4, APRI, or Forns scores had a higher risk for SLD, ranging from a 2-fold (intermediate class of FIB-4) up to an 80-fold (upper class of APRI) (Table 2). In individuals with diabetes, obesity, and a FLI of 60 or higher, intermediate- and high-risk classes of FIB-4, APRI, and Forns showed higher hazard ratios (HRs) for SLD compared with those observed in the overall population, whereas risk stratification by NFS was comparable or worse. Concerning the BARD score, it performed worse in subjects with diabetes, but not in those with obesity or a FLI of 60 or higher. Finally, in individuals without metabolic risk factors, the overall performance was reduced for all clinical scores.

Polygenic Risk Score-Hepatic Fat Content and the Risk of Severe Liver Disease

Irrespective of the genetic model (additive, dominant, recessive, or categoric), *PNPLA3* rs738409 and *TM6SF2* rs58542926 variants were associated with an increased risk of SLD, while the *MBOAT7* rs641738, *GCKR* rs1260326, and *HSD17B13* rs72613567 variants were not (Supplementary Table 4).

When analyzed as a continuous trait, the PRS-HFC was highly associated with the risk of SLD in the overall population (age-sex adjusted HR [aHR] for a 1-SD increase: 1.25; 95% CI, 1.16–1.35; $P = 8.9*10^{-9}$). Notably, the risk effect size was larger in individuals with metabolic risk factors (age-sex aHRs: 1.55; 95% CI, 1.33–1.80; 1.40; 95% CI, 1.25–1.57; and 1.34; 95% CI, 1.23–1.47, with $P = .2*10^{-8}$, $4.8*10^{-9}$, and $2.6*10^{-10}$, for subjects with diabetes, obesity, and FLI ≥60, respectively), while the PRS-HFC did not associate with SLD in those without a metabolic risk factor (age-sex aHR: 1.07; 95% CI, 0.92–1.24, $P = 4.0*10^{-1}$).

When analyzed as a categoric trait, an increasing risk was present across quartiles of PRS-HFC (Figure 1). Age-sex aHRs for SLD in the highest PRS-HFC quartile with respect to the lowest quartile were higher in individuals with diabetes, obesity, and a FLI of 60 or higher (3.24; 95% CI, 1.93–5.43; $P = 7.8*10^{-6}$; 2.29; 95% CI, 1.60–3.29; $P = 6.9*10^{-6}$; and 2.13, 95% CI, 1.57–2.89; $P = 1.2*10^{-6}$, respectively) than in the overall population (1.63, 95% CI, 1.28–2.08; $P = 8.3*10^{-5}$). In contrast, in individuals without metabolic risk factors there was no significant difference in the risk for SLD across the different quartiles of PRS-HFC.

Table 2. Associations of Clinical Scores With Incident Severe Liver Disease in the Overall Population and in the Subgroups of Different Baseline Risk

Clinical score		Events/total	IR ($\times 10^4$ py)	HR (95% CI)	*P*
Overall					
NFS					
	Low	216/189,472	1.3	1 (1–1)	–
	Intermediate	254/74,145	3.9	3.1 (2.6–3.7)	$5.5*10^{-34}$
	High	73/3070	28.6	22.6 (17.3–29.5)	$2.8*10^{-117}$
FIB-4					
	Low	174/151,356	1.3	1 (1–1)	–
	Intermediate	261/110,363	2.7	2.1 (1.7–2.5)	$3.1*10^{-14}$
	High	108/4968	25.6	20.1 (15.8–25.5)	$2.6*10^{-132}$
APRI					
	Low	374/254,644	1.6	1 (1–1)	–
	Intermediate	143/11,798	13.9	8.5 (7–10.3)	$1.5*10^{-104}$
	High	26/245	129.8	79.6 (53.5–118.4)	$2.9*10^{-103}$
BARD					
	Low	33/23,925	1.5	1 (1–1)	–
	Intermediate	416/235,574	2	1.3 (0.9–1.8)	$1.6*10^{-1}$
	High	94/7188	15.2	9.9 (6.7–14.7)	$8.4*10^{-30}$
Forns					
	Low	123/156,960	0.9	1 (1–1)	–
	Intermediate	283/105,414	3	3.5 (2.8–4.3)	$3.9*10^{-31}$
	High	137/4313	37.9	43.7 (34.3–55.8)	$4.3*10^{-203}$
Diabetes mellitus					
NFS					
	Low	11/2434	5.1	1 (1–1)	–
	Intermediate	59/9096	7.5	1.5 (0.8–2.8)	$2.5*10^{-1}$
	High	55/1738	38.4	7.6 (4–14.5)	$9.1*10^{-10}$
FIB-4					
	Low	28/7184	4.4	1 (1–1)	–
	Intermediate	56/5679	11.5	2.6 (1.6–4.1)	$3.9*10^{-5}$
	High	41/405	130.4	29.8 (18.4–48.2)	$1.6*10^{-43}$
APRI					
	Low	64/12,045	6.1	1 (1–1)	–
	Intermediate	51/1181	51.1	8.4 (5.8–12.1)	$1.0*10^{-29}$
	High	10/42	339.5	57.5 (29.5–112.2)	$1.1*10^{-32}$
BARD					
	Low	4/540	8.4	1 (1–1)	–
	Intermediate	27/5540	5.6	0.7 (0.2–1.9)	$4.5*10^{-1}$
	High	94/7188	15.2	1.8 (0.7–4.9)	$2.5*10^{-1}$
Forns					
	Low	7/2916	2.7	1 (1–1)	–
	Intermediate	54/9268	6.7	2.5 (1.1–5.5)	$2.3*10^{-2}$
	High	64/1084	72.9	27.2 (12.5–59.4)	$1.1*10^{-16}$

Table 2. Continued

Clinical score		Events/total	IR ($\times 10^4$ py)	HR (95% CI)	*P*
Obesity					
NFS					
	Low	48/33,351	1.6	1 (1–1)	–
	Intermediate	137/29,100	5.4	3.4 (2.4–4.7)	$4.6*10^{-13}$
	High	53/2204	28.8	18.2 (12.3–26.9)	$5.1*10^{-48}$
FIB-4					
	Low	68/40,459	1.9	1 (1–1)	–
	Intermediate	116/22,996	5.8	3.1 (2.3–4.2)	$1.5*10^{-13}$
	High	54/1200	54.1	29.1 (20.3–41.6)	$3.0*10^{-76}$
APRI					
	Low	145/60,367	2.7	1 (1–1)	–
	Intermediate	76/4177	21	7.8 (5.9–10.3)	$1.8*10^{-47}$
	High	17/111	190.6	71.8 (43.4–118.6)	$2.4*10^{-62}$
BARD					
	Low	16/9944	1.8	1 (1–1)	–
	Intermediate	145/49,114	3.3	1.9 (1.1–3.1)	$1.9*10^{-2}$
	High	77/5597	16	9 (5.2–15.4)	$1.4*10^{-15}$
Forns					
	Low	35/33,077	1.2	1 (1–1)	–
	Intermediate	123/29,844	4.7	4 (2.7–5.8)	$4.6*10^{-13}$
	High	80/1734	55.2	47.5 (31.9–70.7)	$7.2*10^{-81}$
FLI, ≥60					
NFS					
	Low	107/58,944	2	1 (1–1)	–
	Intermediate	192/36,869	6	3 (2.3–3.7)	$2.7*10^{-19}$
	High	67/2347	34.4	17.2 (12.7–23.3)	$2.4*10^{-74}$
FIB-4					
	Low	105/59,076	2	1 (1–1)	–
	Intermediate	167/37,174	5.1	2.6 (2–3.3)	$1.6*10^{-14}$
	High	94/1910	59.3	30.2 (22.9–39.9)	$4.0*10^{-127}$
APRI					
	Low	222/91,348	2.7	1 (1–1)	–
	Intermediate	119/6660	20.6	7.5 (6–9.4)	$1.0*10^{-70}$
	High	25/152	207.1	77.1 (51–116.6)	$3.2*10^{-94}$
BARD					
	Low	28/17,842	1.7	1 (1–1)	–
	Intermediate	248/73,811	3.8	2.2 (1.5–3.2)	$1.0*10^{-4}$
	High	90/6507	16.1	9.3 (6.1–14.1)	$8.4*10^{-25}$
Forns					
	Low	53/46,199	1.3	1 (1–1)	–
	Intermediate	192/49,035	4.5	3.5 (2.6–4.7)	$6.8*10^{-16}$
	High	121/2926	49.5	39.2 (28.4–54.2)	$6.7*10^{-110}$

Table 2. Continued

Clinical score		Events/total	IR ($\times 10^4$ py)	HR (95% CI)	*P*
No metabolic risk factors					
NFS					
	Low	102/125,378	0.9	1 (1–1)	–
	Intermediate	53/32,294	1.9	2.1 (1.5–2.9)	$2.1*10^{-5}$
	High	3/496	7.2	7.9 (2.5–24.8)	$4.3*10^{-4}$
FIB-4					
	Low	62/86,079	0.8	1 (1–1)	–
	Intermediate	85/69,206	1.4	1.7 (1.3–2.4)	$9.7*10^{-4}$
	High	11/2883	4.4	5.5 (2.9–10.5)	$1.8*10^{-7}$
APRI					
	Low	138/153,270	1	1 (1–1)	–
	Intermediate	19/4815	4.5	4.5 (2.8–7.2)	$9.8*10^{-10}$
	High	1/83	14.1	14 (2–100.1)	$8.5*10^{-3}$
BARD					
	Low	4/5588	0.8	1 (1–1)	–
	Intermediate	154/152,580	1.1	1.4 (0.5–3.8)	$4.9*10^{-1}$
	High	–/–		–	–
Forns					
	Low	65/104,741	0.7	1 (1–1)	–
	Intermediate	82/52,258	1.8	2.6 (1.9–3.6)	$1.3*10^{-8}$
	High	11/1169	11.1	16 (8.5–30.4)	$1.8*10^{-17}$

NOTE. No metabolic risk factors means an absence of diabetes mellitus, obesity, and a FLI less than 60.
APRI, aspartate aminotransferase-to-platelets ratio index; FIB-4, fibrosis-4; FLI, fatty liver index; HR, hazard ratio; IR, incidence rate; NFS, nonalcoholic fatty liver disease fibrosis score; py, person-year.

Polygenic Risk Score-Hepatic Fat Content Class to Improve Risk Stratification and Diagnostic Accuracy of Fibrosis Scoring Systems

Finally, we examined if the PRS-HFC refines the risk conferred by the different clinical fibrosis scores. To this aim, PRS-HFC was stratified into 3 categories: namely, favorable (PRS-HFC <0.128, first quartile), intermediate ($0.128 \leq$ PRS-HFC < 0.396, second and third quartiles), or unfavorable (PRS-HFC ≥ 0.396, fourth quartile).

In the overall population, the PRS-HFC allowed restratification of the risk of SLD in the intermediate-risk and high-risk classes of clinical fibrosis scores, although it did not impact the risk of SLD in the lowest-risk classes (Table 3). Similar results were observed in patients with diabetes, obesity, and a FLI of 60 or higher, with the difference that unfavorable PRS-HFC did not affect the risk for SLD in the high-risk class of APRI, but in subjects with diabetes it increased the risk in its low-risk class. Notably, the relative increase of the risk conferred by unfavorable genetics in intermediate-risk classes of all scoring systems was higher in individuals with diabetes, obesity, and a FLI of 60 or higher than in the overall population. Conversely, in subjects without metabolic risk factors, an unfavorable PRS-HFC did not influence the risk in any risk class of any clinical score.

Altogether, the cumulative incidence of SLD progressively increased from low- to high-risk classes (log-rank *P* for trend $< 2*10^{-16}$ for all clinical scores), and the combination of unfavorable PRS-HFC identified, within those in intermediate or high clinical risk classes, subsets of individuals with a significantly more increased incidence of SLD ($P < 5*10^{-2}$ for NFS, FIB-4, APRI, and Forns after adjustment for Holm's correction for multiple testing) (Figure 2).

AUROCs for the development of SLD, without or with the addition of the PRS-HFC and both in the overall population and in the different subsets, are provided in Table 4. Generally, all scores performed better in subjects with diabetes, obesity, or a FLI of 60 or higher. Forns and FIB-4 emerged as the best-performing scores in individuals at risk for NAFLD (AUROCs for Forns 0.75, 0.75, 0.74, and for FIB-4 0.72, 0.71, 0.70 in subjects with diabetes, obesity, and FLI of 60 or higher, respectively). After including the PRS-HFC, the discriminative performance of all scores improved in the overall population and in individuals with diabetes, obesity, and a FLI of 60 or higher, except for APRI in the overall population and in subjects with

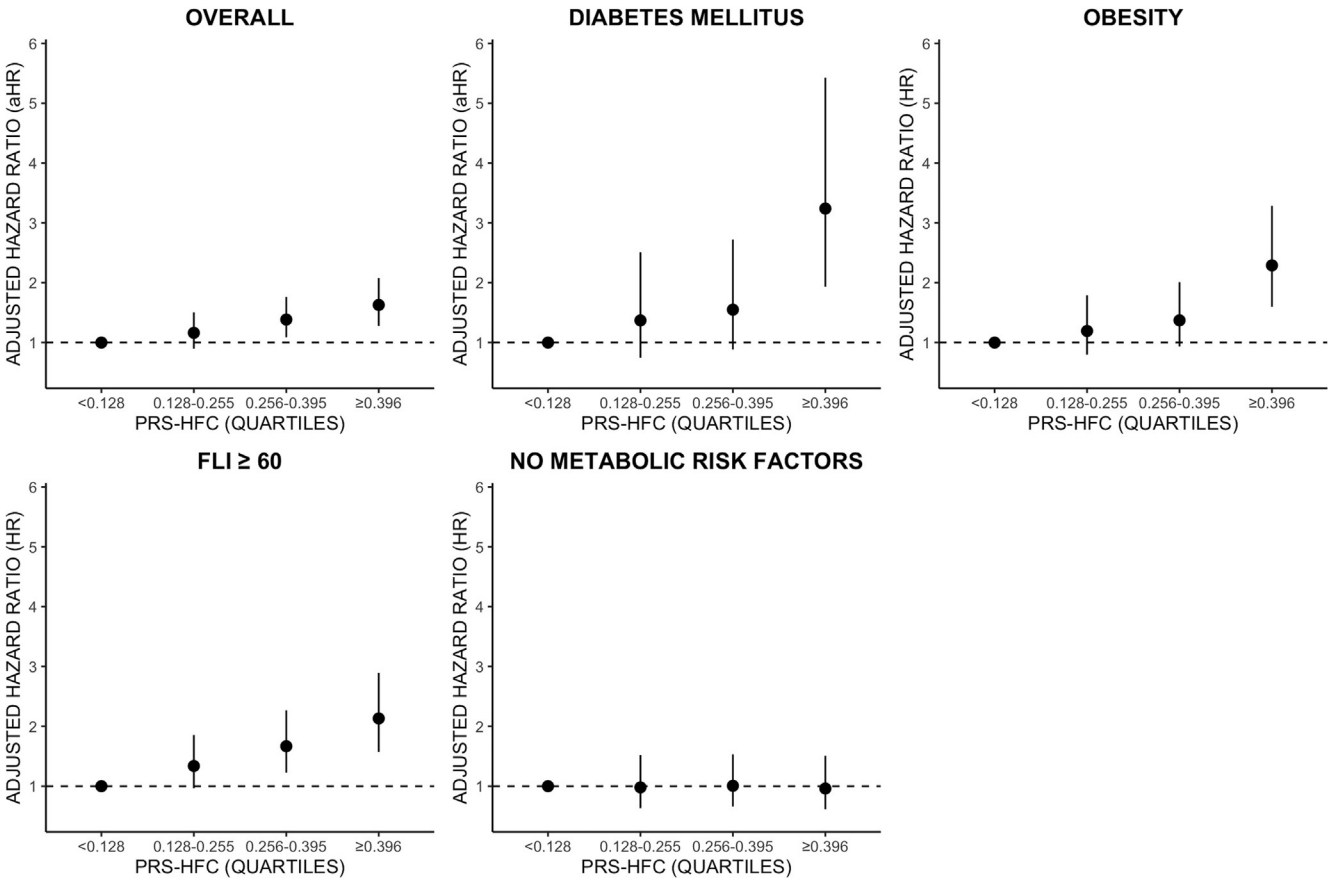

Figure 1. Association of polygenic risk score-hepatic fat content (PRS-HFC) quartiles with incident severe liver disease in the overall population and in subgroups with different baseline risk. Models are adjusted for age and sex, and stratified according to the presence of diabetes mellitus, obesity, and a Fatty Liver Index (FLI) of 60 or higher. No metabolic risk factors: absence of diabetes mellitus, obesity, and FLI les than 60.

obesity. Of note, the most consistent increase was observed in individuals with metabolic risk factors (*P* value of approximately 10^{-3}–10^{-4}), with a net reclassification index peaking at 0.39 in subjects with diabetes. Model calibration generally was good, and tended to improve after PRS-HFC consideration, exception in individuals without metabolic risk factors (Supplementary Figure 1).

The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of clinical fibrosis scores for the development of SLD, combined or not with unfavorable PRS-HFC, are provided in Supplementary Table 5, and comments can be found in the Supplementary Results section.

Sensitivity Analysis: Polygenic Risk Score 2

Lastly, because the *PNPLA3* and *TM6SF2* variants were associated more strongly with SLD than the other two (GCKR and MBOAT7) (Supplementary Table 4), we evaluated if the performance of a reduced PRS including only these 2 SNPs (PRS2) was comparable with that of PRS. Results, which are provided in the Supplementary Methods section, Supplementary Figure 2, and Supplementary Tables 6–8, are substantially in agreement with this hypothesis.

Discussion

The main finding of this work was that combining genetic risk with clinical fibrosis scores allowed us to refine the risk and prediction of SLD in the general population and in individuals at high risk for NAFLD. This shows that common genetic variants capture additional prognostic information not conveyed by validated clinical and biochemical parameters.

Clinical fibrosis scores were derived from high-risk individuals[5–9] based on fibrosis assessed by liver biopsies, and have not been calibrated in the general population, in whom the risk of SLD is much lower. However, because of their ease of calculation and the lack of alternatives, they have been used to predict liver events in the general population and in subsets at high risk for NAFLD by 2 previous studies.[11,12] Intermediate-

Table 3. Risk Stratification for Incident Severe Liver Disease of Clinical Scores Combined With PRS-HFC Classes in the Overall Population and in Subgroups With Different Baseline Risk

	N events/ total N	IR, $\times 10^4$ py	aHR (95% CI)	*P*	N events/total N	IR, $\times 10^4$ py	aHR (95% CI)	*P*	N events/total N	IR, $\times 10^4$ py	aHR (95% CI)	*P*
Clinical score, PRS-HFC	Overall				Diabetes mellitus				Obesity			
NFS												
Low												
<0.128	52/50,007	1.2	1 (1–1)	–	4/645	7	1 (1–1)	–	13/8881	1.6	1 (1–1)	–
0.128–0.395	114/97,140	1.3	1.1 (0.8–1.6)	$4.8*10^{-1}$	5/1269	4.5	0.6 (0.2–2.3)	$4.8*10^{-1}$	27/17,196	1.7	1.1 (0.6–2.1)	$8.3*10^{-1}$
≥0.396	50/42,325	1.3	1.1 (0.8–1.7)	$5.6*10^{-1}$	2/520	4.4	0.6 (0.1–3.3)	$5.5*10^{-1}$	8/7274	1.2	0.8 (0.3–1.8)	$5.3*10^{-1}$
Intermediate												
<0.128	52/20,863	2.8	1 (1–1)	–	9/2545	4	1 (1–1)	–	26/8254	3.6	1 (1–1)	–
0.128–0.395	131/37,628	4	1.4 (1–1.9)	$4.1*10^{-2}$	21/4498	5.4	1.3 (0.6–2.9)	$4.9*10^{-1}$	60/14,655	4.7	1.3 (0.8–2.1)	$2.6*10^{-1}$
≥0.396	71/15,654	5.2	1.8 (1.3–2.6)	$1.0*10^{-3}$	29/2053	16.4	4 (1.9–8.4)	$3.0*10^{-4}$	51/6191	9.4	2.6 (1.6–4.2)	$5.6*10^{-5}$
High												
<0.128	10/814	14.7	1 (1–1)	–	7/470	17.8	1 (1–1)	–	7/583	14.3	1 (1–1)	–
0.128–0.395	32/1578	24.2	1.6 (0.8–3.3)	$1.7*10^{-1}$	27/879	36.9	2.1 (0.9–4.8)	$8.4*10^{-2}$	23/1144	23.9	1.7 (0.7–3.9)	$2.3*10^{-1}$
≥0.396	31/678	56.1	3.8 (1.9–7.7)	$2.5*10^{-4}$	21/389	67.8	3.8 (1.6–8.9)	$2.2*10^{-3}$	23/477	59.4	4.2 (1.8–9.7)	$9.3*10^{-4}$
FIB-4												
Low												
<0.128	42/40,575	1.1	1 (1–1)	–	7/2085	3.8	1 (1–1)	–	15/11,254	1.5	1 (1–1)	–
0.128–0.395	95/77,688	1.4	1.2 (0.8–1.7)	$3.6*10^{-1}$	11/3615	3.5	0.9 (0.4–2.4)	$8.6*10^{-1}$	36/20,815	1.9	1.3 (0.7–2.4)	$3.9*10^{-1}$
≥0.396	37/33,093	1.2	1.1 (0.7–1.7)	$7.3*10^{-1}$	10/1484	7.7	2 (0.8–5.3)	$1.5*10^{-1}$	17/8390	2.3	1.5 (0.8–3.1)	$2.2*10^{-1}$
Intermediate												
<0.128	57/29,796	2.2	1 (1–1)	–	10/1475	7.9	1 (1–1)	–	25/6189	4.6	1 (1–1)	–
0.128–0.395	133/56,179	2.7	1.2 (0.9–1.7)	$1.8*10^{-1}$	22/2841	9	1.1 (0.5–2.4)	$7.2*10^{-1}$	54/11,566	5.3	1.2 (0.7–1.9)	$5.4*10^{-1}$
≥0.396	71/24,388	3.3	1.5 (1.1–2.2)	$1.8*10^{-2}$	24/1363	20.7	2.6 (1.3–5.5)	$1.0*10^{-2}$	37/5241	8.1	1.8 (1.1–2.9)	$2.9*10^{-2}$
High												
<0.128	15/1313	13.3	1 (1–1)	–	3/100	35.8	1 (1–1)	–	6/275	25.5	1 (1–1)	–
0.128–0.395	49/2479	23.3	1.7 (1–3.1)	$6.3*10^{-2}$	20/190	137.8	3.9 (1.2–13.2)	$2.7*10^{-2}$	20/614	39.2	1.5 (0.6–3.8)	$3.6*10^{-1}$
≥0.396	44/1176	44.5	3.3 (1.9–6)	$5.7*10^{-5}$	18/115	210.7	5.7 (1.7–19.4)	$5.4*10^{-3}$	28/311	110.6	4.4 (1.8–10.5)	$1.1*10^{-3}$
APRI												
Low												
<0.128	87/68,881	1.4	1 (1–1)	–	14/3402	4.7	1 (1–1)	–	36/16,844	2.4	1 (1–1)	–
0.128–0.395	201/130,464	1.7	1.2 (1–1.6)	$1.2*10^{-1}$	27/6035	5.2	1.1 (0.6–2.1)	$7.9*10^{-1}$	72/30,901	2.6	1.1 (0.7–1.6)	$6.5*10^{-1}$
≥0.396	86/55,299	1.7	1.2 (0.9–1.7)	$1.7*10^{-1}$	23/2608	10.2	2.1 (1.1–4.2)	$2.5*10^{-2}$	37/12,622	3.3	1.4 (0.9–2.2)	$1.6*10^{-1}$
Intermediate												
<0.128	25/2753	10.4	1 (1–1)	–	6/251	27.7	1 (1–1)	–	9/858	11.9	1 (1–1)	–
0.128–0.395	66/5764	13.2	1.3 (0.8–2)	$3.3*10^{-1}$	23/596	45.7	1.7 (0.7–4.1)	$2.6*10^{-1}$	32/2043	18	1.5 (0.7–3.1)	$2.9*10^{-1}$
≥0.396	52/3281	18.3	1.7 (1.1–2.8)	$2.4*10^{-2}$	22/334	79.1	2.9 (1.2–7.2)	$2.0*10^{-2}$	35/1276	31.8	2.7 (1.3–5.6)	$8.2*10^{-3}$
High												
<0.128	2/50	46.6	1 (1–1)	–	0/7	0	–	–	1/16	75.3	1 (1–1)	–
0.128–0.395	10/118	102.6	1.9 (0.4–8.8)	$4.0*10^{-1}$	3/15	276.5	–	–	6/51	143.6	1.7 (0.2–14.3)	$6.4*10^{-1}$
≥0.396	14/77	233.8	5.4 (1.2–23.8)	$2.6*10^{-2}$	7/20	546.9	–	–	10/44	292.9	3.9 (0.5–31.2)	$2.0*10^{-1}$

Table 3. Continued

Clinical score, PRS-HFC	N events/ total N	IR, $\times 10^4$ py	aHR (95% CI)	*P*	N events/total N	IR, $\times 10^4$ py	aHR (95% CI)	*P*	N events/total N	IR, $\times 10^4$ py	aHR (95% CI)	*P*
	Overall				Diabetes mellitus				Obesity			
BARD												
Low												
<0.128	10/5883	1.9	1 (1–1)	–	2/150	14.9	1 (1–1)	–	4/2444	1.8	1 (1–1)	–
0.128–0.395	18/11,738	1.7	0.9 (0.4–1.9)	$7.9*10^{-1}$	2/252	9.1	0.6 (0.1–4.6)	$6.5*10^{-1}$	11/4909	2.5	1.4 (0.4–4.3)	$5.9*10^{-1}$
≥0.396	5/6304	0.9	0.5 (0.2–1.4)	$1.6*10^{-1}$	0/138	0	–	–	1/2591	0.4	0.2 (0–2.2)	$2.0*10^{-1}$
Intermediate												
<0.128	91/63,795	1.6	1 (1–1)	–	5/1504	3.8	1 (1–1)	–	30/13,697	2.5	1 (1–1)	–
0.128–0.395	219/120,982	2	1.3 (1–1.6)	$5.4*10^{-2}$	11/2768	4.6	1.2 (0.4–3.4)	$7.4*10^{-1}$	69/25,277	3.1	1.3 (0.8–1.9)	$3.0*10^{-1}$
≥0.396	106/50,797	2.3	1.5 (1.1–1.9)	$7.9*10^{-3}$	11/1268	10.1	2.6 (0.9–7.4)	$8.0*10^{-2}$	46/10,140	5.1	2.1 (1.3–3.3)	$1.6*10^{-3}$
High												
<0.128	13/2006	7.4	1 (1–1)	–	13/2006	7.4	1 (1–1)	–	12/1577	8.8	1 (1–1)	–
0.128–0.395	40/3626	12.8	1.7 (0.9–3.2)	$8.9*10^{-2}$	40/3626	12.8	1.7 (0.9–3.2)	$8.9*10^{-2}$	30/2809	12.4	1.4 (0.7–2.8)	$3.1*10^{-1}$
≥0.396	41/1556	30.9	4.2 (2.2–7.8)	$7.2*10^{-6}$	41/1556	30.9	4.2 (2.2–7.8)	$7.2*10^{-6}$	35/1211	33.9	3.9 (2–7.5)	$5.0*10^{-5}$
Forns												
Low												
<0.128	28/41,986	0.7	1 (1–1)	–	2/840	2.7	1 (1–1)	–	10/9109	1.2	1 (1–1)	–
0.128–0.395	74/81,013	1	1.4 (0.9–2.1)	$1.6*10^{-1}$	4/1494	3	1.1 (0.2–6.2)	$8.9*10^{-1}$	19/17,125	1.2	1 (0.5–2.2)	$9.7*10^{-1}$
≥0.396	21/33,961	0.7	0.9 (0.5–1.6)	$8.0*10^{-1}$	1/582	1.9	0.7 (0.1–8)	$7.9*10^{-1}$	6/6843	1	0.8 (0.3–2.2)	$6.7*10^{-1}$
Intermediate												
<0.128	64/28,511	2.5	1 (1–1)	–	8/2526	3.6	1 (1–1)	–	23/8132	3.2	1 (1–1)	–
0.128–0.395	139/53,254	3	1.2 (0.9–1.6)	$3.2*10^{-1}$	20/4650	5	1.4 (0.6–3.1)	$4.5*10^{-1}$	59/15,035	4.5	1.4 (0.9–2.2)	$1.8*10^{-1}$
≥0.396	80/23,649	3.8	1.5 (1.1–2.1)	$1.4*10^{-2}$	26/2092	14.5	4 (1.8–8.8)	$6.5*10^{-4}$	41/6677	7	2.2 (1.3–3.6)	$2.9*10^{-3}$
High												
<0.128	22/1187	21.8	1 (1–1)	–	10/294	40.9	1 (1–1)	–	13/477	31.8	1 (1–1)	–
0.128–0.395	64/2079	36.8	1.7 (1–2.7)	$3.9*10^{-2}$	29/502	71.8	1.7 (0.9–3.6)	$1.3*10^{-1}$	32/835	45.9	1.4 (0.8–2.7)	$2.7*10^{-1}$
≥0.396	51/1047	58.4	2.6 (1.6–4.3)	$1.7*10^{-4}$	25/288	109	2.6 (1.2–5.4)	$1.1*10^{-2}$	35/422	101.7	3.2 (1.7–6)	$3.9*10^{-4}$

	FLI, ≥60				No metabolic risk factors			
NFS								
Low								
<0.128	25/15,348	1.8	1 (1–1)	–	25/34,375	0.8	1 (1–1)	–
0.128–0.395	60/30,415	2.2	1.2 (0.8–1.9)	$4.4*10^{-1}$	52/66,174	0.9	1.1 (0.7–1.8)	$6.4*10^{-1}$
≥0.396	22/13,181	1.9	1 (0.6–1.8)	$9.7*10^{-1}$	26/28,923	1	1.3 (0.7–2.2)	$3.9*10^{-1}$
Intermediate								
<0.128	34/10,318	3.8	1 (1–1)	–	18/9878	2.1	1 (1–1)	–
0.128–0.395	97/18,733	5.9	1.6 (1.1–2.3)	$2.2*10^{-2}$	29/17,679	1.9	0.9 (0.5–1.7)	$8.6*10^{-1}$
≥0.396	61/7818	9	2.4 (1.6–3.6)	$5.1*10^{-5}$	8/7288	1.3	0.6 (0.2–1.3)	$1.9*10^{-1}$

Table 3. Continued

	FLI, ≥60				No metabolic risk factors			
High								
<0.128	6/621	11.5	1 (1–1)	–	2/143	16.7	1 (1–1)	–
0.128–0.395	31/1215	30.5	2.7 (1.1–6.4)	$2.8*10^{-2}$	0/280	0	–	–
≥0.396	30/511	72.6	6.3 (2.6–15.2)	$3.7*10^{-5}$	1/126	9.5	0.5 (0–5.4)	$5.6*10^{-1}$
FIB-4								
Low								
<0.128	23/16,044	1.6	1 (1–1)	–	18/24,025	0.8	1 (1–1)	–
0.128–0.395	60/30,428	2.2	1.4 (0.9–2.2)	$1.9*10^{-1}$	32/46,319	0.8	1 (0.5–1.8)	$9.3*10^{-1}$
≥0.396	22/12,604	1.9	1.2 (0.7–2.2)	$4.9*10^{-1}$	13/20,090	0.7	0.9 (0.4–1.9)	$7.8*10^{-1}$
Intermediate								
<0.128	31/9799	3.6	1 (1–1)	–	23/19,531	1.3	1 (1–1)	–
0.128–0.395	85/18,970	5.1	1.4 (0.9–2.1)	$9.6*10^{-2}$	45/36,346	1.4	1.1 (0.7–1.8)	$7.3*10^{-1}$
≥0.396	51/8405	7	1.9 (1.2–3)	$3.9*10^{-3}$	19/15,593	1.4	1 (0.5–1.9)	$9.6*10^{-1}$
High								
<0.128	11/444	29.3	1 (1–1)	–	4/840	5.5	1 (1–1)	–
0.128–0.395	43/965	53.9	1.8 (0.9–3.6)	$7.4*10^{-2}$	4/1468	3.2	0.6 (0.1–2.3)	$4.2*10^{-1}$
≥0.396	40/501	97.2	3.3 (1.7–6.4)	$4.7*10^{-4}$	3/654	5.3	1 (0.2–4.3)	$9.6*10^{-1}$
APRI								
Low								
<0.128	48/24,888	2.2	1 (1–1)	–	37/43,047	1	1 (1–1)	–
0.128–0.395	121/47,060	2.9	1.3 (1–1.9)	$9.2*10^{-2}$	74/81,642	1	1.1 (0.7–1.7)	$5.9*10^{-1}$
≥0.396	53/19,400	3.1	1.4 (1–2.1)	$7.7*10^{-2}$	30/35,132	1	1 (0.6–1.7)	$9.7*10^{-1}$
Intermediate								
<0.128	15/1373	12.5	1 (1–1)	–	8/1327	6.9	1 (1–1)	–
0.128–0.395	57/3231	20.3	1.6 (0.9–2.9)	$9.3*10^{-2}$	7/2448	3.3	0.5 (0.2–1.3)	$1.5*10^{-1}$
≥0.396	47/2056	26.5	2.1 (1.2–3.8)	$1.1*10^{-2}$	4/1183	3.9	0.6 (0.2–1.8)	$3.3*10^{-1}$
High								
<0.128	2/26	93.6	1 (1–1)	–	0/22	0	–	–
0.128–0.395	10/72	173.4	1.9 (0.4–8.7)	$4.1*10^{-1}$	0/43	0	–	–
≥0.396	13/54	311.8	4.1 (0.9–18.4)	$6.6*10^{-2}$	1/22	56.3	–	–
BARD								
Low								
<0.128	6/4344	1.5	1 (1–1)	–	3/1468	2.3	1 (1–1)	–
0.128–0.395	18/8810	2.3	1.5 (0.6–3.7)	$4.1*10^{-1}$	0/2798	0	–	–
≥0.396	4/4688	1	0.6 (0.2–2.2)	$4.5*10^{-1}$	1/1551	0.7	0.3 (0–3)	$3.1*10^{-1}$
Intermediate								
<0.128	47/20,126	2.6	1 (1–1)	–	42/42,928	1.1	1 (1–1)	–
0.128–0.395	132/38,269	3.9	1.5 (1.1–2.1)	$2.2*10^{-2}$	81/81,335	1.1	1.1 (0.7–1.6)	$7.3*10^{-1}$
≥0.396	69/15,416	5.1	1.9 (1.3–2.8)	$6.2*10^{-4}$	34/34,786	1.1	1 (0.6–1.6)	$9.6*10^{-1}$
High								
<0.128	12/1817	7.6	1 (1–1)	–	–	–	–	–
0.128–0.395	38/3284	13.5	1.8 (0.9–3.4)	$8.7*10^{-2}$	–	–	–	–
≥0.396	40/1406	33.4	4.4 (2.3–8.4)	$6.7*10^{-6}$	–	–	–	–

Table 3. Continued

	FLI, ≥60				No metabolic risk factors			
Forns								
Low								
<0.128	13/12,371	1.2	1 (1–1)	–	15/29,310	0.6	1 (1–1)	–
0.128–0.395	32/24,099	1.5	1.3 (0.7–2.4)	$4.9*10^{-1}$	39/56,337	0.8	1.4 (0.8–2.7)	$2.3*10^{-1}$
≥0.396	8/9729	0.9	0.8 (0.3–1.9)	$5.9*10^{-1}$	12/24,008	0.6	1 (0.5–2.3)	$9.2*10^{-1}$
Intermediate								
<0.128	36/13,122	3.1	1 (1–1)	–	26/14,752	2	1 (1–1)	–
0.128–0.395	98/24,867	4.5	1.4 (1–2.1)	$6.2*10^{-2}$	38/27,198	1.6	0.8 (0.5–1.4)	$4.4*10^{-1}$
≥0.396	58/11,046	6	1.9 (1.3–2.9)	$2.1*10^{-3}$	20/12,057	1.9	0.9 (0.5–1.7)	$8.0*10^{-1}$
High								
<0.128	16/794	23.6	1 (1–1)	–	4/334	14.1	1 (1–1)	–
0.128–0.395	58/1397	50	2.1 (1.2–3.6)	$9.1*10^{-3}$	4/598	7.9	0.5 (0.1–2)	$3.2*10^{-1}$
≥0.396	47/735	77.5	3.2 (1.8–5.6)	$6.7*10^{-5}$	3/272	12.9	0.8 (0.2–3.4)	$7.2*10^{-1}$

NOTE. No metabolic risk factors means an absence of diabetes mellitus, obesity, and FLI less than 60. Hazard ratios are adjusted for age and sex.
aHR, adjusted hazard ratio; APRI, aspartate aminotransferase-to-platelets ratio index; FIB-4, fibrosis-4; FLI, fatty liver index; IR, incidence rate; NFS, NAFLD fibrosis score; PRS-HFC, polygenic risk score-hepatic fat content; py, person-year.

and high-risk classes of fibrosis scores were associated with an increased risk for SLD. The prediction was highly reduced by the low specificities resulting from the low number of events.[11] Notably, and somehow unexpectedly, sensitivities also were rather low, with the majority of subjects evolving to SLD classified in the low-risk classes.[11]

NAFLD is a multifactorial disease in which genetic and environmental factors interact to determine disease onset, progression, and outcomes. Based on this interaction, there have been several previous attempts to combine the genetic risk, mainly that conveyed by the *PNPLA3* rs738409, with clinical and laboratory data to predict NAFLD.[22] However, the heritability of NAFLD derives from many common genetic variants with various degrees of effect size and, therefore, gathering numerous variants in a PRS is the most sensible approach to maximize the contribution of genetics. Consistent with this, different genetic risk scores have been associated with an increased risk of SLD.[23–26] We recently showed that the PRS-HFC improves the accuracy in detecting HCC and may help stratify the risk of HCC in individuals with dysmetabolism, including those without advanced liver fibrosis.[27]

Our results show that the risk predicted by clinical fibrosis scores is a stronger determinant of SLD by several fold compared with the genetic risk defined by PRS-HFC, at least in the general population. Among the different scoring systems, APRI showed the highest increases in risk in the intermediate-/high-risk classes compared with the low-risk class, whereas BARD showed the lowest. Notably, as previously reported,[28] risk stratification by the NFS was worse in patients with diabetes, most likely owing to the presence of diabetes status in the formula of the score.

Overall, as reported by previous studies,[11,12] the predictive abilities of all the scores were rather modest, and generally better in subgroups at higher risk for NAFLD. This was justified by the low incidence of SLD in the general population, which was slightly higher in at-risk subgroups, because the prevalence of a disease highly affects the performance of tests that aim to diagnose it.

In general, unfavorable genetics did not affect the risk profile in the lower-risk fibrosis classes, although it did modulate the risk in the intermediate-risk and high-risk fibrosis scoring classes. Similarly, the effect on risk stratification in intermediate-risk and high-risk classes was generally higher for individuals at risk and virtually absent in those without diabetes, obesity, and with a FLI less than 60. This is consistent with the gene–environment interactions observed in NAFLD, in which the impact of the *PNPLA3* rs738409, *TM6SF2* rs58542926, and *GCKR* rs1260326 variants on steatosis accumulation increases as a function of the BMI,[29] the main risk factor for NAFLD. In line with this, the genetic risk score provided no additional

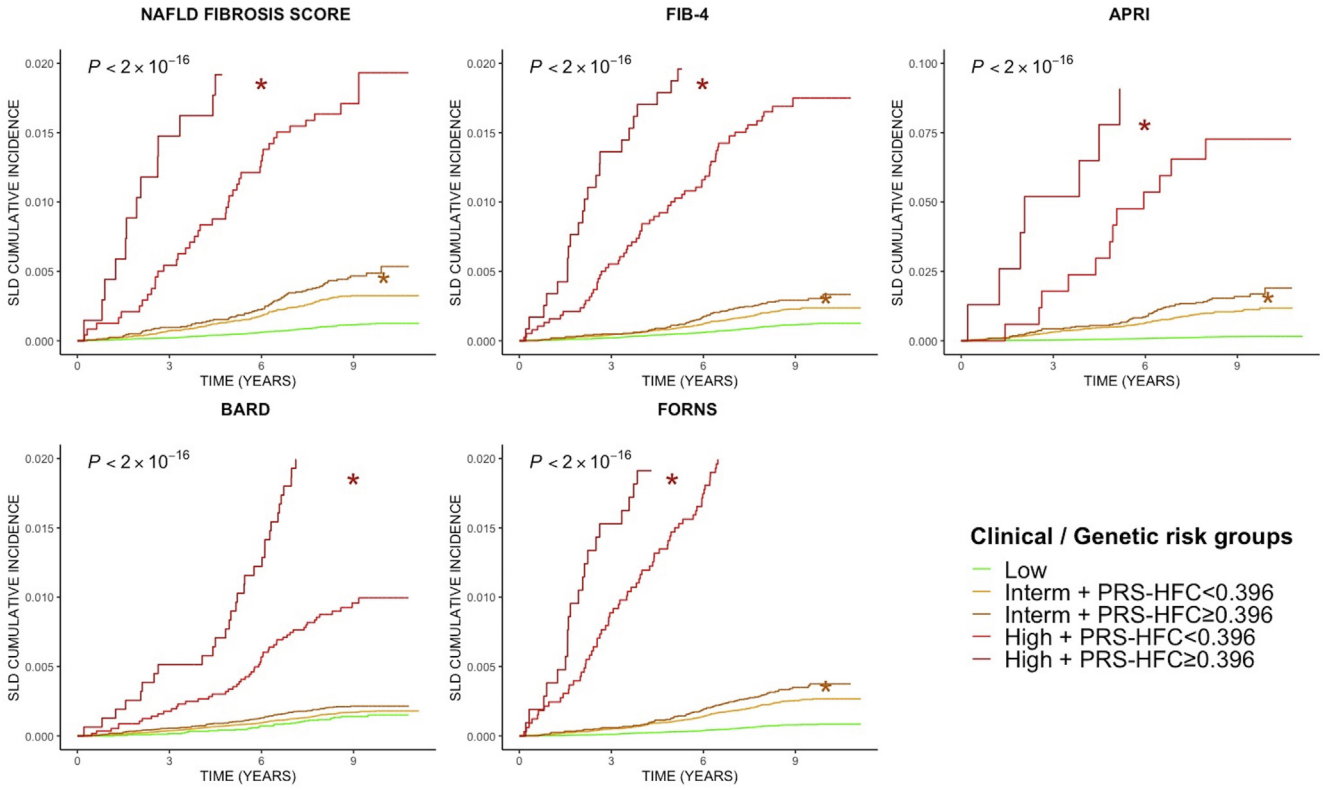

Figure 2. Cumulative incidence of severe liver disease (SLD) for the different clinical risk groups, combined with unfavorable polygenic risk score-hepatic fat content (PRS-HFC). *Green line*, *orange line*, and *red line* are low-, intermediate-, and high-risk subjects according to clinical scores. *Dark orange* and *dark red* are intermediate and high-risk subjects with unfavorable genetics (PRS-HFC≥0.396). Low-risk subjects (*green line*) were not stratified according to PRS-HFC since no significant increase of SLD was found. *P* value is from log-rank test for trend. *$P < 5*10^{-2}$ for the comparisons of subjects with unfavorable vs favorable genetics within intermediate- and high-risk groups, with Holm's correction for multiple testing. APRI, aspartate aminotransferase–to–platelet ratio; FIB-4, Fibrosis-4; NAFLD, nonalcoholic fatty liver disease.

information in subjects with a low risk of NAFLD. Finally, the capability of unfavorable genetics to modulate the risk in intermediate-risk and high-risk risk classes was paralleled by the increase in specificities and diagnostic accuracies of these same fibrosis scoring systems in a scenario with few events.

Strengths of this study were as follows: the prospective estimation of the risk in a well-characterized cohort, the large sample from the UK Biobank, with all fibrosis scoring systems and genetic variants evaluated concurrently in all subjects, and in which all procedures were standardized and centrally validated. The present study also had some limitations. First, it cannot be excluded that a proportion of individuals had undiagnosed cirrhosis at the time of the baseline assessment. However, the capability to recognize undiagnosed advanced liver fibrosis allows identification of high-risk subjects from the general population deserving further investigation to hamper the progression to clinically evident hepatic outcomes. Second, we evaluated fibrosis scoring systems, which were not designed to assess liver-related prognosis in the general population, and the PRS-HFC, whose genetic components have not been weighed for liver-related outcomes. However, fibrosis scoring systems are used in clinical practice and already have been validated in different populations, therefore maximizing the possibility to export the results obtained. Moreover, as already discussed in the introduction, we chose the PRS-HFC because genetically driven hepatic fat accumulation determined by this score already has been causally related to liver damage.[18] However, in a sensitivity analysis, we showed similar results by using a PRS composed of the 2 variants with the strongest effect: namely, *PNPLA3* and *TM6SF2*. Therefore, one may argue that an easier PRS with only 2 variants may be enough to restratify individuals with fibrosis at the general population level. However, further studies are needed to confirm these findings. Finally, the vast majority of the UK Biobank comprises individuals of European descent so future studies are needed in different ethnic groups.

In conclusion, our results show that PRS refines risk stratification and diagnostic performance for SLD of clinical fibrosis scores in the general population and in subjects at risk for NAFLD. This study showed that human genetics of common variants captures additional prognostic insights that are not conveyed by

Table 4. Discriminative Capacities of Clinical Scores Without or With the Addition of PRS-HFC Classes, in the Overall Population and in Subgroups With Different Baseline Risk

	AUROC (95% CI)			
	Alone	With PRS-HFC	*P**	NRI
Overall				
NFS	0.673 (0.651–0.695)	0.683 (0.659–0.708)	$4.9*10^{-2}$	0.118
FIB-4	0.661 (0.638–0.683)	0.670 (0.645–0.695)	$4.5*10^{-2}$	0.118
APRI	0.634 (0.615–0.654)	0.648 (0.622–0.673)	$1.2*10^{-1}$	0.118
BARD	0.581 (0.562–0.599)	0.603 (0.578–0.628)	$1.4*10^{-2}$	0.118
Forns	0.727 (0.706–0.748)	0.735 (0.712–0.758)	$5.0*10^{-2}$	0.118
Diabetes mellitus				
NFS	0.669 (0.623–0.716)	0.722 (0.672–0.772)	$1.1*10^{-3}$	0.389
FIB-4	0.724 (0.678–0.770)	0.753 (0.705–0.802)	$2.5*10^{-2}$	0.389
APRI	0.703 (0.658–0.747)	0.742 (0.691–0.793)	$1.9*10^{-2}$	0.389
BARD	0.608 (0.570–0.647)	0.669 (0.621–0.716)	$2.8*10^{-4}$	0.389
Forns	0.745 (0.702–0.788)	0.791 (0.745–0.836)	$4.0*10^{-4}$	0.389
Obesity				
NFS	0.698 (0.667–0.729)	0.721 (0.686–0.756)	$1.3*10^{-3}$	0.259
FIB-4	0.707 (0.674–0.740)	0.723 (0.688–0.759)	$1.9*10^{-2}$	0.259
APRI	0.665 (0.633–0.696)	0.683 (0.644–0.722)	$1.5*10^{-1}$	0.259
BARD	0.640 (0.609–0.672)	0.671 (0.633–0.709)	$6.4*10^{-3}$	0.259
Forns	0.754 (0.724–0.784)	0.771 (0.738–0.804)	$9.8*10^{-3}$	0.259
FLI, ≥60				
NFS	0.683 (0.657–0.709)	0.700 (0.671–0.729)	$3.1*10^{-3}$	0.181
FIB-4	0.702 (0.675–0.730)	0.717 (0.687–0.746)	$9.7*10^{-3}$	0.181
APRI	0.665 (0.639–0.690)	0.684 (0.653–0.715)	$4.7*10^{-2}$	0.181
BARD	0.623 (0.599–0.647)	0.655 (0.626–0.683)	$1.8*10^{-4}$	0.181
Forns	0.739 (0.714–0.763)	0.754 (0.727–0.781)	$4.1*10^{-3}$	0.181
No metabolic risk factors				
NFS	0.575 (0.537–0.613)	0.566 (0.518–0.614)	$5.1*10^{-1}$	0.011
FIB-4	0.586 (0.546–0.627)	0.587 (0.543–0.632)	$9.2*10^{-1}$	0.011
APRI	0.548 (0.522–0.574)	0.546 (0.501–0.591)	$9.2*10^{-1}$	0.011
BARD	0.505 (0.493–0.517)	0.503 (0.463–0.544)	$9.3*10^{-1}$	0.011
Forns	0.658 (0.610–0.707)	0.657 (0.609–0.706)	$5.7*10^{-1}$	0.011

NOTE. No metabolic risk factors: absence of diabetes mellitus, obesity, and a FLI less than 60. Discriminative capacity was evaluated by computing the AUROC with 95% CIs of Cox proportional hazard models including clinical scores (categorized as low-, intermediate-, and high-risk classes), alone or together with PRS-HFC (categorized into favorable, <0.128; or intermediate and unfavorable, ≥0.396).

APRI, aspartate aminotransferase-to-platelets ratio index; AUROC, area under the receiver operating characteristic; FIB-4, Fibrosis-4; FLI, fatty liver index; NFS, nonalcoholic fatty liver disease fibrosis score; NRI, net reclassification index; PRS-HFC, polygenic risk score-hepatic fat content.

**P* value is for the Delong test, comparing the difference between the AUROC of the models without or with the addition of PRS-HFC. The ability of the models including PRS-HFC, compared with those with clinical scores alone, to reclassify subjects correctly along the linear predictor scale of Cox models, was expressed by the category-free continuous NRI.

well-validated clinical and biochemical parameters. More studies in the general population and in individuals at risk are warranted to confirm this finding.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2021.05.056.

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Federica Tavaglione (Conceptualization: Equal; Data curation: Equal; Formal analysis: Equal; Methodology: Equal; Writing – review & editing: Equal)

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Stefano Romeo (Conceptualization: Equal; Data curation: Equal; Funding acquisition: Lead; Investigation: Equal; Methodology: Equal; Project administration: Equal; Supervision: Equal; Writing – review & editing: Equal)

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Conflicts of interest

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