Non-invasive stratification of hepatocellular carcinoma risk in non-alcoholic fatty liver using polygenic risk scores

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Supplementary methods

Study cohorts characterization

In the NAFLD cohort, the diagnosis was based on recently proposed criteria for MAFLD [1], in the absence of at-risk alcohol consumption (>30/20 g/day in males/females, respectively) and other liver diseases were excluded. Severe fibrosis was defined in the presence of histological fibrosis F3-F4 (when liver biopsy was available) or in presence of clinical, endoscopic or radiological signs of portal hypertension or cirrhosis, or liver stiffness ≥8.4 kPa evaluated by Fibroscan® [2]. Diagnosis of HCC was based on EASL-EORTC Clinical Practice Guidelines [3].

As controls, we considered a group of 865 individuals: 370 were healthy Italian blood donors without clinical and biochemical evidence of liver disease (ALT<30/19 IU/l in M/F and fatty liver index <30 [4]), 91 Italian patients, who underwent metabolic surgery due to severe obesity without histological evidence of steatosis at routine biopsy, and the 404 non-Finnish European healthy participants to the 1000 Genome project (https://www.internationalgenome.org/) [5].

The German NAFLD/MAFLD cohort (n=427, 72, 16.8% with HCC) was recruited by applying the same selection criteria in patients referred to the Dresden and Leipzig centres.

The demographic and clinical data of these individuals are shown in Table S1.

For the UK Biobank cohort (UKBB), we restricted our analysis to participants of European ancestry and further excluded individuals with withdrawn consent, excessive relatives, a mismatch between the self-reported and genetically inferred gender, putative sex chromosome aneuploidy, and those who were identified by the UKBB as outliers. Finally, we identified the maximal set of unrelated individuals (no 3rd degree or closer relatives) based on the pairwise kinship coefficients provided by UKBB. In UKBB, HCC was defined by combining *International Classification of Diseases, Tenth Revision* (ICD-10) code C22.0 from both UK cancer registry (data-field 40006),

and hospitalization records (data-field 41270). As controls, we considered individuals without HCC or any other type of liver and intrahepatic bile ducts cancers. Cirrhosis was defined as ICD-10 codes I85.0, I85.9, K70.3, K70.4, K72.1, K74.1, K74.2, K74.6, K76.6, K76.7 using hospitalization records (data-field 41270). Diabetes was defined as individuals having either of following criteria: 1) self-reported type 2 or unspecified diabetes (codes 1220 and 1223 in data-field 20002); 2) ICD-10 diagnoses codes E11 and E14 (data-field 41270); 3) insulin treatment or use of oral glucose lowering drugs (data-fields 6153, 6177 and 20003); 4) serum glucose level ≥11.1 mmol/L (200 mg/dL); 5) HbA1c ≥ 48 mmol/mol (6.5%).

Genotyping

NAFLD cohort participants were genotyped for the rs738409 (*PNPLA3* I148M variant), rs58542926 (*TM6SF2* E167K), rs641738 C>T variant at *MBOAT7*, rs1260326 (*GCKR* P446L) and rs72613567 (*HSD17B13*:TA), which encompass all validated genetic risk variant for NAFLD-HCC [6]. Genotyping was performed in duplicate by TaqMan 5'-nuclease assays at the Translational Medicine and Metabolic Liver Disease lab, University of Milan. For genotyping, Taqman assays C_7241_10 for rs738409 *PNPLA3*, C_89463510_10 for rs58542926 *TM6SF2*, C_8716820_10 for rs641738 *MBOAT7* and C_2862880_1_for rs1260326 *GCKR* were employed (all from ThermoFisher, Waltham, US). Taqman assay and probe for rs72613567 were designed as previously described (ThermoFisher, Waltham, US) [7].

UKBB participants were genotyped using two similar (>95% overlap) arrays: the UK BiLEVE or UK Biobank Axiom array. Genotyped data were then imputed based on the 1000 Genomes Phase 3, UK10K haplotype, and Haplotype Reference Consortium (HRC) reference panels1 [8]. *PNPLA3-TM6SF2-GCKR-HSD17B13* variants were directly genotyped, and rs641738 variant at *MBOAT7* was among imputed genotype data (imputation score = 0.99).

Mendelian randomization

Mendelian randomization is a framework exploiting human genetic variation to understand if a trait is a causally related risk factor for another trait of interest and is considered the most appropriate tool to assess causality when randomized controlled trials are not feasible. This analysis is based on the idea that because the assignment of alleles is random at conception independently of confounders, genetic variation influencing a trait can be used to assess causality against another trait of interest. Causal effect of hepatic fat on HCC were estimated by examining the PRS for association with FLD, as well as with HCC using a triangular approach:

- The observational association between hepatic fat / FLD and HCC was examined in a traditional cross-sectional study design. These observational associations can arise from both directions and can be biased due to confounding.
- PRS are confirmed to be associated with FLD
- The association between PRS and HCC is tested. The genetic effect on HCC is assumed to be mediated by hepatic fat. Since genetic variants are inherited randomly at conception, transmission of the effects may be assumed independent of confounders. Further, genetic variation cannot be modified by phenotype, therefore ruling out reverse causation. The instrumental variable (IV; causal effect) is the PRS association with HCC regressed on the PRS association with FLD

The causal effect of genetic predisposition to FLD on HCC was estimated by instrumental variable regression analysis in a two-sample Mendelian randomization approach by a two-stage least squares regression procedure (using the 'ivreg' command in the AER package in R), which was adjusted for age, sex, BMI and T2D. We assumed a lesser relevance or neutral impact of horizontal pleiotropic effects, that is an impact of the genetic variants on HCC risk independent of FLD, that can invalidate the Mendelian randomization framework. This was supported by the direct relationship between the risk conferred towards FLD and HCC independently of the specific

mechanism underlying the association with liver disease. The F statistics of the model was 107, thereby ruling out weak instrument bias. Wu-Hausman p=0.09, suggesting that the causal estimate was consistent with the observational association (the test examines the difference between the instrumental variant (PRS) and the observational (FLD) association with the outcome (HCC)).

To further account for the possible pleiotropy of the genetic variants considered, we also considered in sensitivity analyses robust Mendelian randomization approaches by the MendelianRandomization R package [9]. The inverse-variance weighted (IVW) method is the equivalent to the standard instrumental method using individual-level data (the two-stage least squares method reported above), but can be performed on summarized data. The *robust* option uses robust regression rather than standard regression in the analyses, and the *penalized* option downweights the contribution to the analyses of genetic variants with outlying (heterogeneous) causal estimates. The median- and mode-based methods calculate a median or mode of the variant-specific causal estimates from the ratio method for each genetic variant individually, respectively. The MR-Egger method is able to assess whether genetic variants have pleiotropic effects on the outcome that differ on average from zero (directional pleiotropy), as well as to provide a consistent estimate of the causal effect, under a weaker assumption—the InSIDE (INstrument Strength Independent of Direct Effect) assumption. The intercept from the MR-Egger analysis can be interpreted as the average pleiotropic effect of a genetic variant included in the analysis. The maximum likelihood involves maximizing a likelihood that has one parameter for each genetic variant, plus a causal effect parameter. The heterogeneity-penalized method uses the same consistency criterion as the mode-based estimation method, but evaluates the modal estimate by assessing weights for all subsets of genetic variants

Mediation analysis

Mediation analysis was conducted to estimate the fraction of the effect of hepatic fat accumulation – FLD on HCC predisposition, which is mediated through the development of severe fibrosis. Analyses were conducted by the "mediation" package in R (http://CRAN.R-project.org/package=mediation). We used model based causal mediation analysis ("mediate" function), calculating quasi-Bayesian confidence estimated with 1,000 simulations. In a Mendelian randomization framework, a positive PRS score, indicating increased genetic predisposition, was treated as active treatment/exposure. The analysis was adjusted for age, sex, BMI, and T2D.

Supplementary results

Study cohorts

The clinical features of the subjects included in the NAFLD cohort are presented in Table S1, upper panel. BMI was higher in patients with NAFLD than in controls. Patients with severe fibrosis and HCC were older ($p<0.0001$) and had higher prevalence of T2D ($p<0.0001$) compared to the other groups. Moreover, HCC patients were more frequently males ($p<0.0001$). PRS were influenced by the severity of liver disease ($p<0.0001$ for both), but while PRS-HFC increased progressively, PRS-5 was higher in patients with severe fibrosis than in those with HCC.

The clinical features of the individuals included in UKBB are shown in Table S1, bottom panel, and Table S2. Subjects with HCC were older ($p<0.0001$), more frequently men ($p<0.01$) and had a higher prevalence of T2D ($p<0.01$) compared to the other groups. BMI was higher in subjects with HCC than in non-cirrhotic individuals ($p<0.0001$), but not than in those with cirrhosis. PRS were higher in individuals with HCC than in controls ($p<0.01$ for both), even after exclusion of subjects with chronic viral hepatitis.

Mediation analysis

At mediation analysis, the impact of PRS on HCC risk was significantly mediated by severe fibrosis ($p<10^{-16}$ for both PRS-HFC and PRS-5). The impact of PRS-HFC on HCC was mediated by 56%, 95% c.i. 33-108% by severe fibrosis, while that of PRS-5 by 42%, 95% c.i. 23-106%.

Robust Mendelian randomization approaches in the NAFLD cohort

The causal estimate of the association between NAFLD and HCC in the NAFLD cohort (one-sample approach) by a range of robust Mendelian randomization methods that takes into

consideration and adjust for the potential pleiotropic effects of the genetic instruments, including robust and penalized approaches, is reported in Table S3 and summarized in Figure S1. Despite they highlighted a significant heterogeneity in the effect of the genetic instruments, justifying the application of robust approaches which aim to provide more accurate estimates taking into consideration this issue, all (including MR-Egger that allows that all considered variants have pleiotropic effects) gave uniform estimates consistent with a causal relationship between NAFLD and HCC. Importantly, evaluation of MR-Egger intercept estimates did not provide any evidence that the average pleiotropic effect of the variants considered differed from zero. By removing the *GCKR* variant that is known to have the most significant pleiotropic effect by reducing T2D risk, there reduced the heterogeneity in causality estimates, which was no longer significant, increasing the effect size.

Independent validation of PRS association with HCC in the German NAFLD cohort

In the German NAFLD cohorts, PRS were also associated with HCC (OR 8.68, 3.20-23.50, AUROC=0.64, $p=2*10^{-5}$, and OR 8.61, 3.31-22.37, AUROC=0.65, $p=1*10^{-5}$ for PRS-HFC and PRS-5, respectively). The association remained significant after adjustment for age, sex, BMI, and T2D (OR 6.56, 1.54-17.39, p=0.0072, and OR 6.36, 1.67-24.31, p=0.0068 for PRS-HFC and PRS-5, respectively). However, in this specific cohort the association between PRS and HCC was not independent of cirrhosis (not shown). In the German cohort, the best threshold for HCC identification were detected at 0.540 for PRS-HFC and 0.503 for PRS-5, also remarkably similar to those of the larger Italian/UK cohort.

By applying the thresholds identified in the Italian/UK cohort for validation (≥0.532/0.495 for PRS-HFC and PRS-5, respectively), high PRS were associated with HCC independently of age, sex, BMI, and T2D (OR 2.32, 1.16-4.66, p=0.016, and OR 2.40, 1.19-4.83, p=0.014 for PRS-HFC and PRS-5, respectively). In line with results obtained in the main cohort, PRS-HFC had 40.3%

sensitivity and 83.9% specificity, while PRS-5 had 40.3% sensitivity and 83.3% specificity for HCC.

Diagnostic accuracy of PRS in clinically relevant subgroups in UKBB

Positive PRS improved the detection of HCC in patients with T2D (OR 4.4, 2.7-6.9, $p=3.6*10^{-10}$ for PRS-HFC, and OR= 4.6, 2.9-7.3, $p=8.9*10^{-11}$ for PRS-5) and in obese ones in particular after exclusion of patients with viral hepatitis (OR=5.9, 3.8-9.2, $p=5.8*10^{-15}$, and OR=6.2, 4.0-9.7, $p=1.1*10^{-15}$). Positive PRS were associated with >5.5-fold higher risk of HCC ($p<10^{-7}$) and non-viral HCC ($p<10^{-8}$) also in patients with both obesity and T2D (Tables 3, S6 and S7). In individuals with metabolic risk factors, PRS had sensitivity between 35% and 40% and a specificity of 89-90%, being higher in patients with both obesity and T2D (Tables 3, S6 and S7), and the association between PRSs and HCC or non-viral HCC was independent of fibrosis severity ($p<10^{-3}$ for all; Table S5, middle and bottom panels).

When considering individuals over 50 years of age, PRS were able to improve the detection of both HCC (OR=3.4, 2.5-4.7, $P=3.2*10^{-14}$ for PRS-HFC, and OR=3.5, 2.6-4.8, $p=5.6*10^{-15}$ for PRS-5) and non-viral HCC (OR 3.8, 2.8-5.3, $p=8.0*10^{-16}$ for PRS-HFC, and OR=4.0, 2.9-5.6, $p=10.3*10^{-16}$ for PRS-5), independently of fibrosis severity ($p<10^{-3}$ after adjustment). In elderlies, PRS sensitivity was 27-30% and specificity 90% (Tables S4 middle and bottom panels, and Table S7).

Finally, positive scores conferred almost a 2-fold higher risk of HCC in non-obese individuals independently of fibrosis severity (OR=1.7, 1.1-2.7, $p=3.6*10^{-2}$ for PRS-HFC, and OR=1.8, 1.1-2.8, $p=2.4*10^{-2}$ for PRS-5), even after exclusion patients with chronic viral hepatitis (OR=1.9, 1.1-3.2, $p=1.5*10^{-2}$ for PRS-HFC, and OR=2.0, 1.2-3.3, $p=1.0*10^{-2}$).

SUPPLEMENTARY FIGURES

Figure S1

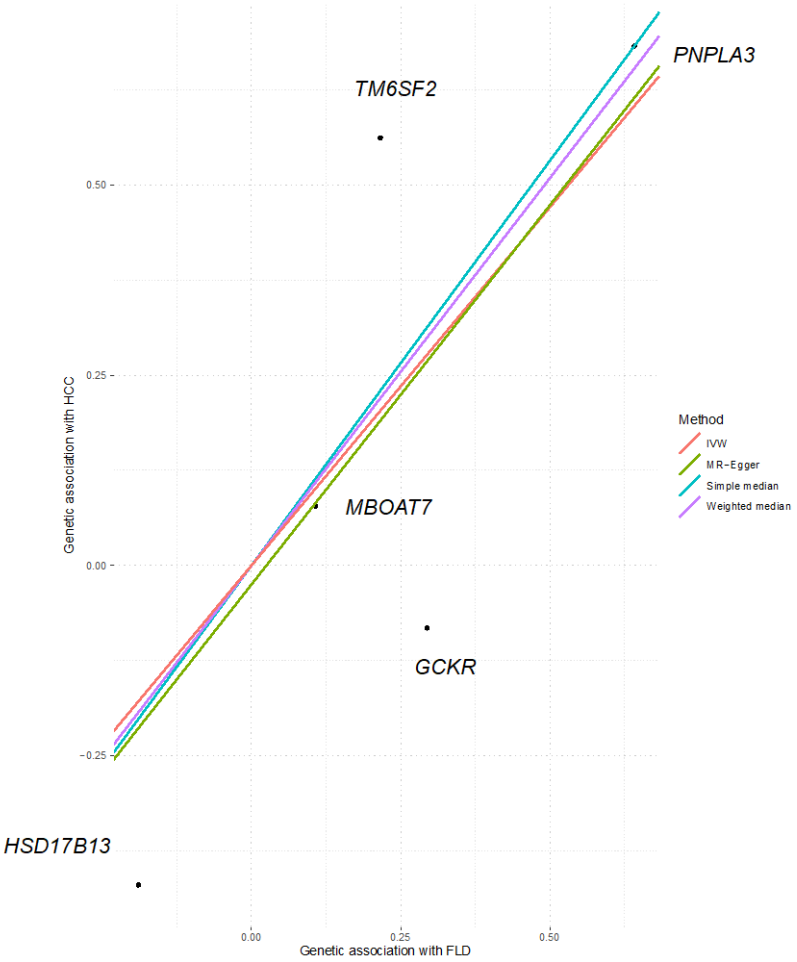

Fig. S1. **Graphical comparison of causality estimates of fatty liver disease on HCC by different Mendelian randomization approaches taking into consideration possible horizontal pleiotropic effects of the variants under consideration in the NAFLD cohort** [9]. MR: Mendelian randomization, IVW: inverse variance weighted. The coefficients to calculate the risk of FLD based on knowledge of the generic risk variants is the following ones: *PNPLA3*.rs738409.G +0.594, *TM6SF2*.rs58542926.T +0.166, *MBOAT7*.rs641738.T +0.073, *GCKR*.rs1260326.T +0.271 , *HSD17B13*.rs72613567.TA -0.216.

SUPPLEMENTARY TABLES

Table S1. Clinical features of the individuals from the NAFLD and in the UKBB cohorts stratified by the severity of liver disease.

NAFLD cohort n=2,564		No liver disease (n=865, 33.7%)	FLD F0-F2 (n=1,176, 45.8%)	FLD F3-F4 (n=297, 11.6%)	HCC (n=226, 8.9%)	p value*
	Age, years	44 ± 6	42 ± 16	58 ± 14	69 ± 9	$6.5*10^{-202}$
	Sex, M	455 (52.6)	677 (57.6)	171 (57.6)	178 (78.8)	$1.3*10^{-11}$
	BMI, Kg/m^2	25.3 ± 5.0	32.7 ± 8.6	30.7 ± 5.1	30.2 ± 5.6	$3.9*10^{-112}$
	T2D, yes	8 (0.9)	238 (20.2)	169 (56.9)	145 (64.2)	$1.1*10^{-154}$
	PRS-HFC	0.266 (0.128-0.402)	0.392 (0.13-0.522)	0.457 (0.329-0.631)	0.459 (0.329-0.662)	$1.3*10^{-40}$
	PRS-5	0.223 (0.065-0.394)	0.329 (0.128-0.459)	0.421 (0.256-0.597)	0.399 (0.266-0.660)	$3*10^{-44}$
German NAFLD cohort n=427		Non-cirrhosis (n=271)	Cirrhosis (n=72)	HCC (n=84)		
					p value^	p value§
	Age, years	45.3±13.3	60.3±10.9	65.8±10.4	$2*10^{-20}$	$9*10^{-31}$
	Sex, M	85 (31.7)	40 (47.6)	62 (86.1)	0.0087	$3*10^{-17}$
	BMI, Kg/m^2	36.7±11.0	31.1±6.5	30.1±4.7	$9*10^{-33}$	$8*10^{-34}$
	T2D, yes	68 (25.3)	49 (58.3)	52 (72.2)	$6*10^{-8}$	$7*10^{-13}$
	PRS-HFC	0.266 (0.126-0.394)	0.394 (0.191-0.605)	0.394 (0.191-0.710)	$3*10^{-7}$	$3*10^{-8}$
	PRS-5	0.193 (0.063-0.337)	0.392 (0.075-0.597)	0.343 (0.130-0.669)	$3*10^{-7}$	$9*10^{-9}$
UKBB cohort n=364,048		Non-cirrhosis (n=362,420, 99.55%)	Cirrhosis (n=1,426, 0.39%)	HCC (n=202, 0.06%)	p value°	p value§
	Age, years	56.8 ± 8.01	58.7 ± 7.16	61.7 ± 5.81	$2.4*10^{-8}$	$1.8*10^{-18}$
	Sex, M (%)	167,170 (46.1)	948 (66.5)	153 (75.7)	$8*10^{-3}$	$1.6*10^{-17}$
	BMI, Kg/m^2	27.4 ± 4.75	29.2 ± 5.73	29.4 ± 5.07	$4.6*10^{-1}$	$2.0*10^{-9}$
	T2D, yes	24,999 (6.9)	434 (30.4)	84 (41.6)	$2.1*10^{-3}$	$1.6*10^{-43}$
	PRS-HFC	0.193 (0.126-0.394)	0.329 (0.128-0.457)	0.337 (0.128-0.595)	$2.2*10^{-3}$	$4.7*10^{-10}$
	PRS-5	0.174 (0.063-0.337)	0.232 (0.065-0.394)	0.292 (0.126-0.524)	$3.5*10^{-3}$	$4.9*10^{-10}$

Values are reported as mean ± SD, number (%) or median (IQR) as appropriate. * Data were compared by generalized linear models (unadjusted). ^ Cirrhosis vs. non cirrhosis; ° HCC vs cirrhosis; § HCC vs. non-cirrhosis.

Abbreviations: NAFLD: nonalcoholic fatty liver disease, HCC: hepatocellular carcinoma, BMI: body mass index, T2D: type 2 diabetes, PRS-HFC: polygenic risk score of hepatic fat content, considering variants in *PNPLA3-TM6SF2-MBOAT7-GCKR*; PRS-5: polygenic risk score considering 5 risk variants, further adjusted for *HSD17B13* variation.

Table S2. Clinical features of the individuals from in the UKBB cohort without chronic viral hepatitis (n=363,513) stratified by the severity of liver disease.

	Non-cirrhosis (n=361,980, 99.58%)	Cirrhosis (n=1,355, 0.37%)	HCC (n=178, 0.05%)	p value°	p value§
Age, years	56.8 ± 8.01	58.9 ± 7.09	62.2 ± 5.53	$1.1*10^{-9}$	$1.8*10^{-20}$
Sex, M (%)	166,921 (46.1)	890 (65.7)	135 (75.8)	$6.7*10^{-3}$	$8.0*10^{-16}$
BMI, Kg/m^2	27.4 ± 4.75	29.3 ± 5.77	29.8 ± 5	$1.4*10^{-1}$	$8.4*10^{-12}$
T2D, yes	24,943 (6.89)	421 (31.1)	79 (44.4)	$4.8*10^{-4}$	$1.3*10^{-43}$
PRS-HFC	0.193 (0.126-0.394)	0.329 (0.128-0.457)	0.392 (0.191-0.597)	$1.9*10^{-4}$	$1.3*10^{-11}$
PRS-5	0.174 (0.063-0.337)	0.232 (0.065-0.394)	0.329 (0.128-0.570)	$2.6*10^{-4}$	$1.1*10^{-11}$

Values are reported as mean ± SD, number (%) or median (IQR) as appropriate. * Data were compared by generalized linear models (unadjusted). ° HCC vs cirrhosis; § HCC vs. non-cirrhosis.

Abbreviations: HCC: hepatocellular carcinoma, BMI: body mass index, T2D: type 2 diabetes, PRS-HFC: polygenic risk score of hepatic fat content, considering variants in *PNPLA3-TM6SF2-MBOAT7-GCKR*; PRS-5: polygenic risk score considering 5 risk variants, further adjusted for *HSD17B13* variation.

Table S3. **Comparison of causality estimates of fatty liver disease on HCC by different Mendelian randomization approaches taking into consideration possible horizontal pleiotropic effect of the variants under consideration in the NAFLD cohort** [9]. Results are reported for the whole panel of variants and after exclusion of the *GCKR* variant conferring direct protection against T2D development.

OVERALL: ***PNPLA3-TM6SF2-MBOAT7 GCKR-HSD17B13***				
Method	**Estimate**	**SE**	**95% c.i.**	**P-value**
Simple median	1.064	0.491	0.102 - 2.026	0.030
Weighted median	1.018	0.188	0.650 - 1.387	<0.001
Penalized weighted median	1.104	0.188	0.735 - 1.473	<0.001
IVW	0.942	0.310	0.334 - 1.549	0.002
heterogeneity				<0.001
Penalized IVW	1.159	0.187	0.793 - 1.526	<0.001
Robust IVW	0.969	0.226	0.526 - 1.412	<0.001
Penalized robust IVW	1.147	0.123	0.906 - 1.387	<0.001
MR-Egger	0.997	0.656	-0.289 - 2.283	0.129
heterogeneity				<0.001
intercept				0.92
Penalized MR-Egger	0.937	0.347	0.257 - 1.617	0.007
intercept				0.44
Robust MR-Egger	0.998	0.190	0.627 - 1.370	<0.001
intercept				0.90
Penalized robust MR-Egger	0.936	0.214	0.517 - 1.354	<0.001
intercept				0.42
Maximum likelihood	1.025	0.335	0.369 -1.682	0.002
Mode-based by Hartwig	0.966	0.212	0.550 - 1.382	<0.001
Heterogeneity penalized	1.07		0.70 - 0.46	
MODIFIED: **Without GCKR**				
Method	Estimate	SE	95% c.i.	P-value
Simple median	1.639	0.791	0.088 - 3.190	0.038
Weighted median	1.130	0.188	0.761 - 1.498	<0.001
Penalized weighted median	1.116	0.186	0.751 - 1.481	<0.001
IVW	1.161	0.214	0.741 - 1.581	<0.001
heterogeneity				0.073
Penalized IVW	1.932	0.435	1.079 - 2.075	<0.001
Robust IVW	1.154	0.113	0.933 - 1.375	<0.001
Penalized robust IVW	1.945	0.406	1.150 - 2.741	<0.001
MR-Egger	0.934	0.398	0.153 – 1.714	0.019
heterogeneity				0.067
intercept				0.48
Penalized MR-Egger	0.934	0.398	0.153 - 1.714	0.019
i*ntercept*				0.48
Robust MR-Egger	0.932	0.215	0.510 - 1.354	<0.001
intercept				0.39
Penalized robust MR-Egger	0.932	0.215	0.510 - 1.354	<0.001

intercept				0.39
Maximum likelihood	1.202	0.220	0.770 - 1.634	<0.001
Mode-based by Hartwig	1.063	0.181	0.708 - 1.419	<0.001
Heterogeneity penalized	1.07		0.74 - 1.46	

MR: Mendelian randomization, SE: standard error, 95% c.i.: 95% confidence interval, IVW: inverse variance weighted.

Table S4. **Comparison of polygenic risk scores (PRS) vs. single variants for the prediction of HCC in the NAFLD (left panel) and UKBB cohorts before (middle panel) and after (right panel) exclusion of individuals with viral hepatitis.**

	NAFLD cohort			Overall UKBB			UKBB (non-viral)		
	p-value*	OR	95% c.i.	p-value°	OR	95% c.i.	p-value°	OR	95% c.i.
PRS-HFC ≥0.532	$6.5*10^{-4}$	1.9	1.3-2.8	$1*10^{-13}$	3.3	2.4-4.5	$4*10^{-15}$	3.7	2.7-5.2
PRS-5 ≥0.495	$4.3*10^{-4}$	2.0	1.3-2.9	$3*10^{-14}$	3.4	2.5-4.7	$8*10^{-16}$	3.9	2.8-5.4
PNPLA3 I148M, carrier	$3.4*10^{-2}$	1.5	1.1-2.3	$4.6*10^{-4}$	1.6	1.2-2.2	$1.9*10^{-4}$	1.8	1.3-2.4
TM6SF2 E167K, carrier	$8.4*10^{-2}$	1.5	0.9-2.4	$4.7*10^{-5}$	1.9	1.4-2.7	$4.2*10^{-6}$	2.2	1.6-3.0
MBOAT7 rs641738 C>T, carrier	$8.6*10^{-1}$	1.0	0.7-1.5	$1.1*10^{-1}$	1.3	0.9-1.8	$1.0*10^{-1}$	1.3	0.9-1.9
GCKR P446L, carrier	$5.8*10^{-1}$	0.8	0.6-1.4	$6.3*10^{-2}$	1.3	0.9-1.8	$1.7*10^{-1}$	1.2	0.9-1.7
HSD17B13 rs72613567:TA, carrier	$3.8*10^{-2}$	0.5	0.2-1.0	$2.1*10^{-1}$	0.8	0.6-1.1	$1.9*10^{-1}$	0.8	0.6.1.1

* At logistic regression adjusted for age, sex, BMI, T2D.

° At logistic regression adjusted for age, sex, BMI, T2D, ethnicity (PC1:10), array batch, assessment center.

Abbreviations: OR: odds ratio, 95% c.i.: 95% confidence interval, PRS-HFC: genetic risk score of hepatic fat content, PRS-5: polygenic risk score considering 5 variants, HCC: hepatocellular carcinoma.

Table S5. **Association of PRS-HFC ≥0.532 and PRS-5 ≥0.495 with HCC in the NAFLD and UKBB cohorts stratified by the presence of the main risk factors (fibrosis severity, older age, BMI, T2D).**

	PRS-HFC ≥ 0.532			PRS-5 ≥ 0.495		
NAFLD cohort	p-value*	OR	95% c.i.	p-value*	OR	95% c.i.
Fibrosis F0-F2	$3.3*10^{-2}$	2.0	1.1-3.8	$1.2*10^{-2}$	2.3	1.2-4.5
Fibrosis F3-F4	$8.2*10^{-2}$	1.4	0.9-2.0	$2.3*10^{-1}$	1.3	0.9-1.8
Age ≥ 40 years	$1.9*10^{-14}$	3.1	2.3-4.1	$1.1*10^{-13}$	3.1	2.3-4.1
BMI <30 Kg/m^2	$2.0*10^{-8}$	3.2	2.1-4.9	$7.2*10^{-9}$	3.5	2.3-5.3
BMI ≥ 30 Kg/m^2	$1.0*10^{-6}$	2.7	1.8-4.0	$5.0*10^{-5}$	2.3	1.5-3.4
No T2D	$1.1*10^{-7}$	3.4	2.2-5.3	$5.3*10^{-7}$	3.3	2.1-5.3
T2D	$2.1*10^{-4}$	2.1	1.4-3.1	$3.2*10^{-4}$	2.1	1.4-3.2
Age ≥ 40 years – adjusted for severe fibrosis	$1.0*10^{-2}$	1.5	1.1-2.2	$2.4*10^{-2}$	1.5	1.1-2.1
BMI <30 Kg/m^2 - adjusted for severe fibrosis	$9.7*10^{-2}$	1.5	0.9-2.4	$4.2*10^{-2}$	1.7	1.1-2.7
BMI ≥ 30 Kg/m^2 – adjusted for severe fibrosis	$5.6*10^{-2}$	1.6	0.9-2.4	$2.7*10^{-1}$	1.3	0.8-2.0
No T2D – adjusted for severe fibrosis	$2.5*10^{-1}$	1.4	0.8-2.3	$3.7*10^{-1}$	1.3	0.7-2.2
T2D – adjusted for severe fibrosis	$2.4*10^{-2}$	1.6	1.1-2.5	$3.2*10^{-2}$	1.6	1.1-2.5
UKBB						
Overall	$1.0*10^{-13}$	3.3	2.4-4.5	$1.9*10^{-14}$	3.4	2.5-4.7
Overall – adjusted for cirrhosis	$4.9*10^{-7}$	2.3	1.7-3.2	$1.5*10^{-7}$	2.4	1.7-3.3
No cirrhosis	$2.7*10^{-2}$	1.8	1.1-3.14	$2.0*10^{-2}$	1.9	1.1-3.2
Cirrhosis	$4.0*10^{-6}$	2.7	1.8-4.2	$1.5*10^{-6}$	2.9	1.9-4.4
Age ≥ 50 years	$3.2*10^{-14}$	3.4	2.5-4.7	$5.6*10^{-15}$	3.5	2.6-4.8
Age ≥ 50 years – adjusted for cirrhosis	$2.5*10^{-7}$	2.4	1.7-3.3	$6.4*10^{-8}$	2.5	1.8-3.5
BMI <30 Kg/m^2	$1.9*10^{-3}$	2.1	1.3-3.4	$1.3*10^{-3}$	2.2	1.4-3.5
BMI <30 Kg/m2 - adjusted for cirrhosis	$3.6*10^{-2}$	1.7	1.1-2.7	$2.4*10^{-2}$	1.8	1.1-2.8
BMI ≥ 30 Kg/m^2	$8.3*10^{-14}$	5.2	3.4-8.1	$1.7*10^{-14}$	5.5	3.6-8.5
BMI ≥ 30 Kg/m2 – adjusted for cirrhosis	$2.1*10^{-6}$	3.1	1.9-4.9	$9.2*10^{-7}$	3.2	2.0-5.1
T2D	$3.6*10^{-10}$	4.4	2.7-6.9	$8.9*10^{-11}$	4.6	2.9-7.3
T2D – adjusted for cirrhosis	$3.4*10^{-4}$	2.5	1.5-4.0	$2.4*10^{-4}$	2.5	1.5-4.2
T2D and BMI ≥ 30 Kg/m2	$1.2*10^{-8}$	5.4	3.0-9.7	$4.6*10^{-9}$	5.8	3.2-10.3
T2D and BMI ≥ 30 Kg/m2 – adjusted for cirrhosis	$5.0*10^{-4}$	3.0	1.6-5.6	$4.5*10^{-4}$	3.1	1.6-5.8
UKBB (non-viral)						
Overall	$2.3*10^{-15}$	3.7	2.7-5.2	$3.9*10^{-16}$	3.9	2.8-5.4
Overall – adjusted for cirrhosis	$1.0*10^{-8}$	2.7	1.9-3.8	$2.3*10^{-9}$	2.8	2.0-3.9
No cirrhosis	$1.3*10^{-2}$	2.0	1.2-3.4	$9.6*10^{-3}$	2.0	1.2-3.5
Cirrhosis	$1.2*10^{-7}$	3.4	2.2-5.4	$3.8*10^{-8}$	3.6	2.3-5.7
Age ≥ 50 years	$8.0*10^{-16}$	3.8	2.8-5.3	$1.3*10^{-16}$	4.0	2.9-5.6
Age ≥ 50 years – adjusted for cirrhosis	$6.7*10^{-9}$	2.7	1.9-3.9	$1.3*10^{-9}$	2.9	2.0-4.1
BMI <30 Kg/m^2	$9.7*10^{-4}$	2.3	1.4-3.9	$6.5*10^{-4}$	2.4	1.4-4.0
BMI <30 Kg/m2 - adjusted for cirrhosis	$1.5*10^{-2}$	1.9	1.1-3.2	$1.0*10^{-2}$	2.0	1.2-3.3
BMI ≥ 30 Kg/m^2	$5.8*10^{-15}$	5.9	3.8-9.2	$1.1*10^{-15}$	6.2	4.0-9.7
BMI ≥ 30 Kg/m2 – adjusted for cirrhosis	$1.4*10^{-7}$	3.6	2.2-5.7	$5.0*10^{-8}$	3.7	2.3-6.0
T2D	$3.2*10^{-10}$	4.5	2.8-7.3	$8.0*10^{-11}$	4.8	3.0-7.7
T2D – adjusted for cirrhosis	$1.9*10^{-4}$	2.6	1.6-4.3	$1.2*10^{-4}$	2.7	1.6-4.5
T2D and BMI ≥ 30 Kg/m2	$7.5*10^{-9}$	5.6	3.1-10.1	$2.8*10^{-9}$	6.0	3.3-10.8
T2D and BMI ≥ 30 Kg/m2 – adjusted for cirrhosis	$2.6*10^{-4}$	3.2	1.7-6.0	$2.2*10^{-4}$	3.3	1.7-6.2

*At logistic regression analysis. Abbreviations: OR: odds ratio, 95% c.i.: 95% confidence interval, PRS-HFC: polygenic risk score of hepatic fat content, PRS-5: polygenic risk score considering 5 risk variants, HCC: hepatocellular carcinoma, BMI: body mass index, T2D: type 2 diabetes.

Table S6. Diagnostic accuracy of PRS-HFC-≥0.532 and PRS-5 ≥0.495 for HCC in UKBB cohort, after exclusion of chronic viral hepatitis, in non-cirrhotic individuals and in participants stratified by the presence of the main metabolic risk factors, namely obesity and T2D.

	Overall	No cirrhosis	BMI≥30	T2D
UKBB (non-viral)				
Cases N	174	89	82	76
PRS-HFC median (IQR) cases	0.392 (0.191-0.597)	0.274 (0.128-0.400)	0.402 (0.193-0.611)	0.397 (0.256-0.604)
Controls N	357,622	356,292	85,999	24,983
PRS-HFC median (IQR) controls	0.193 (0.126-0.394)	0.193 (0.126-0.394)	0.193 (0.126-0.394)	0.193 (0.126-0.394)
AUROC (PRS-HFC)	0.65	0.58	0.71	0.70
Positive PRS prevalence (%)	35,690 (11.1)	35,429 (11.0)	8,485 (10.9)	2,732 (12.2)
OR (95% c.i.)	3.7 (2.7-5.2)	2.0 (1.2-3.4)	5.9 (3.8-9.2)	4.5 (2.8-7.3)
p-value*	$2.3*10^{-15}$	$1.3*10^{-2}$	$5.8*10^{-15}$	$3.2*10^{-10}$
Sensitivity, %	29%	18%	39%	36%
Specificity, %	90%	90%	90%	89%
PPV	0.01	0.01	0.01	0.01
NPV	1.00	1.00	1.00	1.00
LR+	2.94	1.81	3.97	3.28
LR-	0.79	0.91	0.68	0.72
Cases N	173	89	81	75
PRS-5 median (IQR) cases	0.329 (0.128-0.570)	0.224 (0.065-0.395)	0.400 (0.174-0.599)	0.394 (0.193-0.596)
Controls N	356,244	354,923	85,687	24,892
PRS-5 median (IQR) controls	0.174 (0.063-0.337)	0.174 (0.063-0.337)	0.167 (0.063-0.337)	0.191 (0.063-0.337)
AUROC (PRS-5)	0.65	0.56	0.71	0.72
Positive PRS prevalence (%)	34,626 (10.8)	34,374 (10.7)	8,204 (10.6)	2,636 (11.8)
OR (95% c.i.)	3.9 (2.8-5.4)	2.0 (1.2-3.5)	6.2 (4.0-9.7)	4.8 (3.0-7.7)
p-value*	$3.9*10^{-16}$	$9.6*10^{-3}$	$1.1*10^{-15}$	$8.0*10^{-11}$
Sensitivity, %	29%	18%	40%	36%
Specificity, %	90%	90%	90%	90%
PPV	0.01	0.01	0.01	0.01
NPV	1.00	1.00	1.00	1.00
LR+	3.04	1.86	4.14	3.43
LR-	0.78	0.91	0.67	0.71

Genetic risk scores values are reported as median (IQR). *At logistic regression analysis.

Abbreviations: N: number, OR: odds ratio, 95% c.i.: 95% confidence interval, AUROC: area under the receiving operator characteristic curve, PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio, PRS-HFC: polygenic risk score of hepatic fat content, PRS-5: polygenic risk score considering 5 variants, BMI: body mass index, T2D: type 2 diabetes

Table S7. Diagnostic accuracy for HCC of PRS-HFC ≥0.532 and PRS-5 ≥0.495 in the overall UKBB cohort stratified by age, severity of hepatic fibrosis and BMI < 30, before (upper panel) and after (bottom panel) exclusion of individuals with viral hepatitis.

	Age ≥ 50	Cirrhosis	BMI ≥ 30 AND T2D	BMI < 30
UKBB				
Cases N	193	103	48	110
PRS-HFC median (IQR) cases	0.337 (0.128-0.595)	0.400 (0.193-0.609)	0.430 (0.224-0.604)	0.329 (0.128-0.402)
Controls N	278,558	1,401	14,114	272,010
PRS-HFC median (IQR) controls	0.193 (0.126-0.394)	0.329 (0.128-0.457)	0.193 (0.126-0.394)	0.193 (0.126-0.394)
AUROC (PRS-HFC)	0.63	0.64	0.73	0.58
Positive PRS prevalence (%)	27,929 (11.1)	276 (22.5)	1,543 (12.2)	27,237 (11.1)
OR (95% c.i.)	3.4 (2.5-4.7)	2.7 (1.8-4.2)	5.4 (3.0-9.7)	2.1 (1.3-3.4)
p-value*	$3.2*10^{-14}$	$4.0*10^{-6}$	$1.2*10^{-8}$	$1.9*10^{-3}$
Sensitivity, %	27%	36%	40%	19%
Specificity, %	90%	83%	89%	90%
PPV	0.01	0.13	0.01	0.01
NPV	1.00	0.95	1.00	1.00
LR+	2.74	2.11	3.67	1.91
LR-	0.81	0.77	0.68	0.90
Cases N	192	102	47	110
PRS-5 median (IQR) cases	0.311 (0.128-0.531)	0.394 (0.159-0.597)	0.402 (0.193-0.597)	0.27 (0.065-0.394)
Controls N	277,491	1,391	14,069	270,943
PRS-5 median (IQR) controls	0.174 (0.063-0.337)	0.232 (0.065-0.394)	0.191 (0.063-0.337)	0.174 (0.063-0.337)
AUROC (PRS-5)	0.64	0.65	0.73	0.58
Positive PRS prevalence (%)	27,077 (10.8)	268 (21.9)	1,504 (11.9)	26,456 (10.8)
OR (95% c.i.)	3.5 (2.6-4.8)	2.9 (1.9-4.4)	5.8 (3.2-10.3)	2.2 (1.4-3.5)
p-value*	$5.6*10^{-15}$	$1.5*10^{-6}$	$4.6*10^{-9}$	$1.3*10^{-3}$
Sensitivity, %	28%	36%	40%	19%
Specificity, %	90%	83%	89%	905
PPV	0.01	0.14	0.01	0.01
NPV	1.00	0.95	1.00	1.00
LR+	2.83	2.18	3.83	1.96
LR-	0.80	0.76	0.67	0.90
UKBB (non-viral))				
Cases N	170	85	47	92
PRS-HFC median (IQR) cases	0.392 (0.191-0.603)	0.404 (0.269-0.660)	0.457 (0.260-0.604)	0.329 (0.128-0.430)
Controls N	278,175	1,330	14,085	271,623
PRS-HFC median (IQR) controls	0.193 (0.126-0.394)	0.329 (0.128-0.457)	0.193 (0.126-0.394)	0.193 (0.126-0.394)
AUROC (PRS-HFC)	0.65	0.67	0.73	0.59
Positive PRS prevalence (%)	27,895 (11.1)	261 (22.6)	1,538 (12.2)	27,205 (11.1)
OR (95% c.i.)	3.8 (2.8-5.3)	3.4 (2.2-5.4)	5.6 (3.1-10.1)	2.3 (1.4-3.9)
p-value*	$8.0*10^{-16}$	$1.2*10^{-7}$	$7.5*10^{-9}$	$9.7*10^{-4}$
Sensitivity, %	30%	41%	40%	21%
Specificity, %	90%	83%	89%	90%
PPV	0.01	0.13	0.01	0.01
NPV	1.00	0.96	1.00	1.00
LR+	3.00	2.42	3.75	2.06
LR-	0.78	0.71	0.67	0.88
Cases N	169	84	46	92
PRS-5 median (IQR) cases	0.329 (0.128-0.576)	0.400 (0.208-0.628)	0.408 (0.193-0.597)	0.274 (0.087-0.408)
Controls N	277,110	1,321	14,041	270,557
PRS-5 median (IQR) controls	0.174 (0.063-0.337)	0.232 (0.065-0.394)	0.191 (0.063-0.337)	0.174 (0.063-0.337)
AUROC (PRS-5)	0.66	0.68	0.74	0.59
Prevalence (%)	27,041 (10.8)	252 (21.9)	1,499 (11.9)	26,422 (10.8)
OR (95% c.i.)	4.0 (2.9-5.6)	3.6 (2.3-5.7)	6.0 (3.3-10.8)	2.4 (1.4-4.0)
p-value*	$1.3*10^{-16}$	$3.2*10^{-8}$	$2.8*10^{-9}$	$6.5*10^{-4}$
Sensitivity, %	30%	42%	41%	21%
Specificity, %	90%	84%	89%	90%
PPV	0.01	0.14	0.01	0.01
NPV	1.00	0.96	1.00	1.00
LR+	3.10	2.54	3.92	2.12
LR-	0.77	0.70	0.66	0.88

Genetic risk scores values are reported as median (IQR). *At logistic regression analysis.

Abbreviations: N: number, OR: odds ratio, 95% c.i.: 95% confidence interval, AUROC: area under the receiving operator characteristic curve, PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio, PRS-HFC: polygenic risk score of hepatic fat content, PRS-5: polygenic risk score considering 5 variants, BMI: body mass index, T2D: type 2 diabetes.

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