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Hepatic and Biliary Cancer

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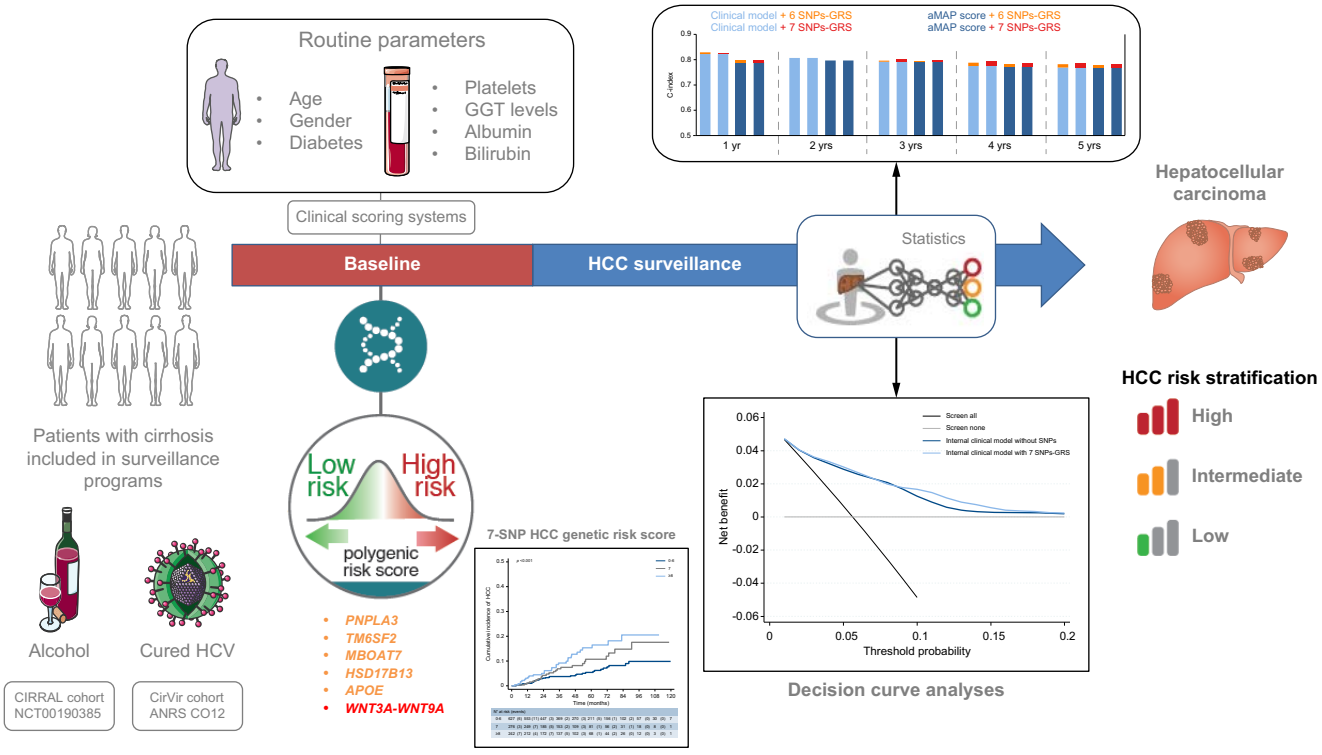
Graphical abstract



Highlights

- HCC risk stratification will ultimately enable refinement of surveillance strategies in patients with cirrhosis.
- Universal scoring systems based on routine parameters may currently be applied regardless of the cause of liver disease.
- Seven genetic variants can be combined into a genetic risk score for HCC in patients in surveillance programs.
- The addition of this genetic information to clinical scoring systems modestly improves their performance for risk stratification.

Impact and implications

The identification of patients at higher risk of developing liver cancer is pivotal to improve the performance of surveillance. Risk assessment can be achieved by combining several clinical and biological parameters used in routine practice. The addition of patients' genetic characteristics can modestly improve this prediction and will ultimately pave the way for precision medicine in patients eligible for HCC surveillance, allowing physicians to trigger personalised screening strategies.

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Integrating genetic variants into clinical models for hepatocellular carcinoma risk stratification in cirrhosis

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Background & Aims: Identifying individuals at higher risk of developing hepatocellular carcinoma (HCC) is pivotal to improve the performance of surveillance strategies. Herein, we aimed to evaluate the ability of single nucleotide polymorphisms (SNPs) to refine HCC risk stratification.

Methods: Six SNPs in *PNPLA3, TM6SF2, HSD17B13, APOE,* and *MBOAT7* affecting lipid turnover and one variant involved in the Wnt–β-catenin pathway (*WNT3A-WNT9A* rs708113) were assessed in patients with alcohol-related and/or HCV-cured cirrhosis included in HCC surveillance programmes (prospective CirVir and CIRRAL cohorts). Their prognostic value for HCC occurrence was assessed using Fine-Gray models combined into a 7-SNP genetic risk score (GRS). The predictive ability of two clinical scores (a routine non-genetic model determined by multivariate analysis and the external aMAP score) with/without the GRS was evaluated by C-indices. The standardised net benefit was derived from decision curves.

Results: Among 1,145 patients, 86 (7.5%) developed HCC after 43.7 months. *PNPLA3* and *WNT3A-WNT9A* variants were independently associated with HCC occurrence. The GRS stratified the population into three groups with progressively increased 5-year HCC incidence (Group 1 [n = 627, 5.4%], Group 2 [n = 276, 10.7%], and Group 3 [n = 242, 15.3%]; p <0.001). The multivariate model identified age, male sex, diabetes, platelet count, gamma-glutamyltransferase levels, albuminemia and the GRS as independent risk factors. The clinical model performance for 5-year HCC prediction was similar to that of the aMAP score (C-Index 0.769). The addition of the GRS to both scores modestly improved their performance (C-Indices of 0.786 and 0.783, respectively). This finding was confirmed by decision curve analyses showing only fair clinical net benefit.

Conclusions: Patients with cirrhosis can be stratified into HCC risk classes by variants affecting lipid turnover and the Wnt–β-catenin pathway. The incorporation of this genetic information modestly improves the performance of clinical scores.

Introduction

The risk of hepatocellular carcinoma (HCC) development in patients with advanced chronic liver diseases (ACLD) may be influenced by genetic factors.[1] Several single nucleotide polymorphisms (SNPs) have been reported as susceptibility loci of HCC, in particular rs738409 (*PNPLA3*),[2] rs58542926 (*TM6SF2*),[3] rs187429064 (*TM6SF2)*[4], rs72613567 (*HSD17B13*),[5] rs429358 (*APOE*)[4] and rs641738 (*MBOAT7*).[6] All of them were initially identified through genome-wide association studies (GWAS) exploring non-alcoholic fatty liver disease (NAFLD)[7,8] and/or alcohol-related liver disease (ALD).[9] They were subsequently tested, alone or combined into genetic risk scores (GRSs), in case-control studies encompassing patients with ACLD without active viral replication that was complicated or not by HCC.[4,10–12] Although the biological consequences of these SNPs are not fully understood, they seem to affect lipid metabolism without a demonstrated direct effect on hepatocarcinogenesis.[13] More recently, the first GWAS dedicated to HCC in individuals of European ancestry was performed,[14] and identified an additional variant modulating the Wnt-β-catenin pathway, *WNT3A-WNT9A*, specifically associated with liver cancer in individuals with ALD. Nevertheless, beyond these associations, the ability of this genetic information to predict the development of HCC and refine liver cancer risk stratification in patients with ACLD is currently unknown.

Semi-annual HCC surveillance using liver ultrasound examination in patients with cirrhosis is endorsed by all international societies.[15] However, this monitoring is affected by the low sensitivity of ultrasound to detect small HCC.[16] Improving the efficacy of HCC surveillance implies the use of more sophisticated tools, but this strategy faces cost-effectiveness issues.[15] HCC risk stratification will play a pivotal role in justifying the implementation of these costly procedures;[17] for instance, it has been shown that early HCC detection using MRI was cost-effective in patients with a yearly cancer

Keywords: cirrhosis; hepatocellular carcinoma; single nucleotide polymorphisms; surveillance; liver cancer risk; risk stratification.
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incidence above 3%.[18,19] This risk stratification can be easily performed using simple features encompassing routine parameters; this is particularly the case in the era of widespread use of antivirals,[20] as "universal" scoring systems have been developed in patients with ACLD regardless of the cause.[19,21] Routine clinical scores are already used for research purposes to stratify at-risk populations in the setting of clinical trials testing new performant but costly early HCC detection procedures. In this setting, the addition of genetic information to these already performant models needs to be assessed before considering their utility for clinical practice. The aim of this longitudinal study was to evaluate the added prognostic value to HCC prediction of these 5 SNPs, as well as their ability to refine HCC stratification based on clinical models.

Patients and methods

Patients

In this study, we used data from two French prospective cohorts of patients with biopsy-proven compensated cirrhosis without detectable focal liver lesions at inclusion; these cohorts have already been extensively described: the ANRS CO12 CirVir[22] and CIRRAL cohorts.[23] Each study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and French laws for biomedical research and was approved by the local ethics committees. They are both reported according to the STROBE statement. All patients gave written informed consent to participate. None of the patients from these cohorts were included in the previously published French GWAS;[14] unlike the CIRRAL or CirVir cohorts, which considered patients regularly screened for HCC and in whom follow-up was monitored according to pre-defined protocols, this two-stage case-control GWAS only selected patients who were referred for chronic liver disease and/or HCC management and does not comprise any recorded longitudinal follow-up for research purposes.

All patients enrolled in these cohorts underwent periodic liver ultrasound surveillance according to international and French guidelines, with or without serum alpha-fetoprotein measurement. In the case of detected focal liver lesions, a recalled diagnostic procedure using contrast-enhanced imaging (computed tomography scan or MRI) and/or guided biopsy was performed according to the 2005 AASLD guidelines updated in 2011.[24,25] A diagnosis of HCC was thus established by either histological examination or based on probabilistic non-invasive criteria (mainly dynamic imaging revealing early arterial hyperenhancement and washout on portal venous or delayed phases) according to the different time periods (before and after 2011). When HCC diagnosis was established, treatment was determined using a multidisciplinary approach according to AASLD[24,25] and the EASL–EORTC[26] guidelines.

In addition to HCC occurrence,[27,28] which was the primary endpoint of both cohorts, all events that occurred during follow-up (*i.e.*, death, liver decompensation, bacterial infection,[27] extrahepatic malignancies[29] and cardiovascular diseases[30]) were recorded using information obtained from the medical records of patients held by each centre.[31] Moreover, likely cause(s) of death were established. All recorded information during follow-up was secondarily monitored by clinical research associates localised in institution 1, 3 and 7. All medical diagnoses of events occurring during follow-up were confirmed by two senior hepatologists (authors N.G-C and P.N.).

Patients who underwent liver transplantation were censored for analysis at the date of transplantation. All treatments, including antiviral therapies, were recorded at inclusion, and patients were notified of any modifications during follow-up.[32] A single database encompassing clinical data from the two cohorts was built on November 18, 2019.[31] Among all included patients, only those with ALD or who achieved HCV eradication during follow-up were considered for the present analyses, the date of viral eradication being set as index time (see Fig. S1).

ANRS CO12 CirVir cohort

The ANRS CO12 CirVir cohort, sponsored and funded by the ANRS (France REcherche Nord & Sud Sida-HIV Hépatites), is a multicentre observational cohort that aims to characterise the incidence of complications occurring in biopsy-proven compensated cirrhosis and to identify the associated risk factors using competing risks analysis.[22] The full CirVir protocol is available on the ANRS website (http://anrs.fr). Specific additional inclusion criteria were i) cause of cirrhosis related to either chronic infection with HCV and/or HBV regardless of the levels of replication and alcohol consumption, ii) patients belonging to Child–Pugh A at enrolment, iii) absence of previous hepatic complications (particularly ascites, gastrointestinal haemorrhage, or HCC), and iv) absence of severe uncontrolled extrahepatic disease resulting in an estimated life expectancy of less than 1 year.

Among the 1,822 patients recruited in 35 French clinical centres between March 2006 and July 2012, 151 were subsequently excluded from analysis after reviewing individual data due to either non-compliance with inclusion criteria (n = 142) or consent withdrawal (n = 9), leading to a total of 1,671 patients selected for further analysis, including the present one.

CIRRAL cohort

CIRRAL is a multicentre cohort study being conducted in 22 French and 2 Belgian tertiary liver centres, with the aim of capturing the whole spectrum of complications occurring in compensated alcohol-related cirrhosis using competing risk analyses.[23] The promoter was APHP. The cohort was funded by the French National Institute of Cancer (INCa), the French Association for Research in Cancer and the ANRS (PAIR CHC 2009) and was registered on ClinicalTrials.gov (NCT00190385). Specific additional inclusion criteria were i) cause of cirrhosis related to chronic alcohol abuse according to the World Health Organization criteria (more than 21 glasses per week for females and more than 28 glasses per week for males) for at least 10 years, ii) absence of chronic infection with HCV or HBV, and iii) patients belonging to Child–Pugh A at enrolment. The follow-up of patients was strictly superposed on the ANRS CO12 Cirvir cohort design.

Among the 706 patients included between October 2010 and April 2016, 54 were subsequently excluded after reviewing individual data because of violations of the inclusion criteria (n = 48) or consent withdrawal (n = 6); ultimately, 652 patients were selected for further analysis, including the present one.

DNA storage, extraction and genotyping

DNA samples were prepared from blood samples collected in all participating centres and then centralised by the liver biobank of the Plateforme de Ressources Biologiques des Hôpitaux Universitaires Paris Seine-Saint-Denis (BB-0033-00027), Assistance Publique Hôpitaux de Paris, Bobigny, France. All patients gave written consent for blood sampling and genotyping. This study was approved by the Comité de Protection des Personnes d'Aulnay-sous-Bois, France. Genomic DNA was extracted from each patient's peripheral blood mononuclear cells using a MagNA Pure Compact Instrument (Roche Diagnostics).

Patients were genotyped for rs738409 (*PNPLA3* I148M variant), rs58542926 (*TM6SF2* E167 K), rs187429064 (*TM6SF2)*, rs641738 (C>T *MBOAT7*), rs72613567 (*HSD17B13*:TA), rs429358 (*APOE)* and rs708113 (*WNT3A-WNT9A*). *MBOAT7*, *TM6SF2*, *WNT3A-WNT9A* and *PNPLA3* SNPs were genotyped by allelic discrimination using fluorogenic probes and appropriate TaqMan assays (rs641738: C___8716820_10; rs738409: C_____7241_10; rs187429064: C_183043355_10; rs58542926: C__89463510_10; rs708113: C__11576791_10, rs429358: C___3084793_20, Thermo Fisher). *HSD17B13* rs72613567 genotyping was performed using custom primers and probes (forward primer: GCT CTA TTG GTG TTT TAG TAT TTG GGT GTT, reverse primer: TGT TCC ATC GTA TAT CAA TAT CTT TCT GAG ACT, qHSD17B13-A: CTG TGC TGT ACT TAC TTC T, qHSD17B13-AA: TGC TGT ACT TAA CTT CT.

PCRs (25 μl) consisted of 1x TaqMan Universal PCR master mix (Applied Biosystems), 1X assay mix, and 10 ng of genomic DNA. Real-time PCR was carried out on a Step One Plus PCR system (Applied Biosystems) using a protocol consisting of incubation at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of denaturation at 92 °C for 15 s and annealing/extension at 60 °C for 1 min. The FAM and VIC fluorescence levels of the PCR products were measured at 60 °C for 1 min, resulting in the clear identification of all genotypes of each SNP on a two-dimensional graph.

Ethnicity was defined by a predictive panel of 26 SNPs assessed on peripheral DNA. Samples were classified as European, sub-Saharan African, or East Asian based on the closest 1,000 Genomes population in a principal component analysis.[33]

Statistical analyses

The baseline was defined as the date of inclusion in the corresponding cohort for patients with alcohol-related cirrhosis and the date of sustained virologic response (SVR) achievement for patients with HCV-related cirrhosis.

Descriptive results are presented as medians (IQR) for continuous variables and as numbers (percentages) for categorical data. The characteristics of patients at the baseline date were compared between the two subsets of the cohort using *t* tests or Mann–Whitney rank-sum tests for continuous variables and the chi-squared test or Fisher's exact test for categorical variables.

The cumulative incidence of HCC was estimated in a competing risk framework, considering non-HCC death as a competing event. All eligible patients were analysed in the competing risk analysis, until death, HCC diagnosis, date of extraction or last known status for patients who were lost to follow-up. Unadjusted comparisons of incidence curves were performed using the Gray test. Fine-Gray regression modelling was used to determine independent baseline features associated with HCC occurrence to compute subhazard ratios (SHRs) along with their 95% CIs. To do so, clinical and routine biological (non-SNP) variables associated with HCC risk at the p <0.20 level in univariate analysis were entered in multivariate analysis, and we applied a backwards stepwise approach to retain significant factors at the p <0.05 level until reaching a final model, thereafter called the "routine basis model". The combined influence of the studied SNPs was analysed in the whole population by creating specific GRSs coding as 0, 1, and 2 for non-carriers and heterozygous and homozygous carriers of the HCC risk-increasing allele of each variant, respectively. Then, the GRSs were added to the routine basis model to assess their independent contribution to HCC prediction, in addition to the clinical features. The same method was secondarily applied with an external HCC clinical score applicable to patients with ACLD regardless of its cause, the aMAP score, encompassing older age, male sex, albumin-bilirubin and platelet count.[21]

The prognostic value of the models (without and with adjustment with SNP parameters) was assessed through three approaches. First, we calculated the Wolber's concordance index (C-index) for prognostic models with competing risks.[34] Second, we created two risk groups "high" and "low" by dichotomising the predictive risk score from each multivariate model at three cut-off points: a) 70th percentile; b) 80th percentile; c) 90th percentile. The 5-year cumulative incidence of HCC was calculated in each of the created groups. The more discriminative the predictive risk score, the more the cumulative incidence of the two groups are separated. Third, we estimated the standardised 'net benefit' derived from decision curve analysis (DCA).[35,36] DCA is an increasingly used method for evaluating alternative diagnostic or prognostic strategies, helping to identify the one with the highest clinical utility or 'net benefit'. The framework of HCC surveillance programmes represents an opt-out setting where the standard is to screen everyone biannually and a given risk model could then be used to opt low-risk patients out of screening. Net benefit in screened patients is calculated across a range of HCC risk thresholds (defined as the minimum probability of disease at which biannual screening would be warranted), as the proportion of patients with true positive results minus the proportion with false-positives multiplied by the odds at the threshold probability (HCC risk/1 – HCC risk). Positive status is defined at each risk threshold when predicted probability from the models exceeds the threshold probability. Decision curves thus display the difference (net benefit) between surveillance benefits and surveillance harms. DCA can be used to calculate the net benefit from implementing a decision rule using a range of HCC risk models, each with different levels of discrimination, in comparison to two extreme clinical strategies of screening no patients and screening all (equivalent to current recommendations). Risk models with higher discriminative power will provide higher net benefit, as evidenced by the highest plotted decision curves.

Unadjusted analyses were conducted on complete cases without missing information, while imputation using the random forests with the R package *missForest* was performed in all multivariate Fine-Gray regression analyses. Statistical analyses were performed using Stata 16.0 (StataCorp, College Station,

TX) and R v4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). *p* values <0.05 were considered to be statistically significant.

Results

Selection, baseline characteristics of patients and genotyping results

A total of 2,321 patients with compensated cirrhosis who underwent HCC surveillance and were included in either of the two cohorts were considered (see flowchart, Fig. S1). Among them, 1,176 were excluded, mostly because of HBV or HIV infection or persistent HCV viral infection during follow-up or missing data for SNPs. As in all previously published analyses conducted in the CirVir and other cohorts,[37] end of treatment was defined as time 0 for patients with SVR during follow-up evaluation because patients with undetectable HCV RNA at that time were considered to have SVR. The remaining 1,145 patients had either alcohol-related cirrhosis and/or cured HCV infection and were included in all subsequent analyses. Their

Table 1. Baseline characteristics and genotyping results.

	Available data, n	HCV-cured n = 659	Alcohol n = 486	Total N = 1,145	*p* value
Ancestry	1,142				<0.001
European		593 (90.0)	469 (97.1)	1,062 (93.0)	
Sub-Saharan African		52 (7.9)	13 (2.7)	65 (5.7)	
East Asian		14 (2.1)	1 (0.2)	15 (1.3)	
Age (years)	1,145	57 [51 – 65]	58 [52 – 64]	58 [51 – 65]	0.634
Male sex	1,145	421 (63.9)	333 (68.5)	754 (65.9)	0.102
BMI (kg/m^2)	906	25.7 [22.9 – 29.3]	27.5 [24.1 – 30.9]	26.5 [23.5 – 30.1]	<0.001
BMI (kg/m^2)	906				<0.001
<25		179 (41.2)	154 (32.7)	333 (36.75)	
[25; 30]		169 (38.8)	164 (34.8)	333 (36.75)	
≥30		87 (20.0)	153 (32.5)	240 (26.5)	
Diabetes	1,144	130 (19.7)	110 (22.7)	240 (21.0)	0.225
Past excessive alcohol consumption	1,109	191 (30.7)	486 (100)	677 (61.1)	<0.001
Platelet counts (10^3/mm^3)	1,056	150.0 [99.0–197.0]	142.0 [102.0–192.0]	147.0 [1010.5–195.5]	0.291
AST (IU/L)	1,054	31.0 [25.0–42.0]	35.0 [27.0–50.0]	32.0 [25.0–47.0]	<0.001
ASTx normal (n = 40)	1,054	0.78 [0.63–1.05]	0.88 [0.68–1.25]	0.80 [0.63–1.18]	<0.001
ALT (IU/L)	1,058	29.0 [21.0–43.0]	26.0 [20.0–40.0]	27.5 [21.0–42.0]	0.007
ALTx normal (n = 40)	1,058	0.73 [0.53–1.08]	0.65 [0.50–1.00]	0.69 [0.53–1.05]	0.007
GGT (IU/L)	998	48.0 [29.0–86.0]	114.0 [54.0–228.0]	66.0 [38.0–151.0]	<0.001
GGTx normal (n = 45)	998	1.07 [0.64–1.91]	2.53 [1.20–5.07]	1.47 [0.84–3.36]	<0.001
PT (%)	939	89.0 [79.0–100.0]	78.0 [67.0–91.0]	84.0 [73.0–96.0]	<0.001
Serum albumin (g/L)	902	42.7 [39.6–46.0]	40.0 [36.9–43.0]	41.5 [38.0–44.3]	<0.001
Total bilirubin (μmol/L)	975	10.0 [7.0–15.0]	14.0 [9.7–20.5]	12.0 [8.0–18.0]	<0.001
AFP (ng/ml)	824	3.6 [2.4–5.6]	3.8 [2.5–5.7]	3.7 [2.4–5.6]	0.628
aMAP score	840	57.2 [52.4–62.0]	60.2 [54.2–64.7]	58.8 [53.3–63.5]	<0.001
PNPLA3 (rs738409)	1,145				0.001
C:C		349 (53.0)	217 (44.7)	566 (49.4)	
C:G		255 (38.7)	197 (40.5)	452 (39.5)	
G:G		55 (8.3)	72 (14.8)	127 (11.1)	
TM6SF2 (rs58542926)	1,145				0.381
C:C		567 (86.0)	405 (83.3)	972 (84.9)	
C:T		89 (13.5)	79 (16.3)	168 (14.7)	
T:T		3 (0.5)	2 (0.4)	5 (0.4)	
TM6SF2 (rs187429064)	1,145				0.163
A:A		647 (98.2)	471 (96.9)	1,118 (97.6)	
A:G		12 (1.8)	15 (3.1)	27 (2.4)	
HSD17B13 (rs72613567)	1,145				0.108
-:-		408 (61.9)	318 (65.4)	726 (63.4)	
A:-		218 (33.1)	155 (31.9)	373 (32.6)	
A:A		33 (5.0)	13 (2.7)	46 (4.0)	
APOE (rs429358)	1,145				<0.001
C:C		11 (1.7)	6 (1.2)	17 (1.5)	
C:T		102 (15.5)	120 (24.7)	222 (19.4)	
T:T		546 (82.8)	360 (74.1)	906 (79.1)	
MBOAT7 (rs641738)	1,145				0.122
C:C		197 (29.9)	124 (25.5)	321 (28.0)	
C:T		336 (51.0)	249 (51.2)	585 (51.1)	
T:T		126 (19.1)	113 (23.3)	239 (20.9)	
WNT3A-WNT9A (rs708113)	1,145				0.491
A:A		251 (38.1)	188 (38.7)	439 (38.3)	
A:T		322 (48.9)	224 (46.1)	546 (47.7)	
T:T		86 (13.0)	74 (15.2)	160 (14.0)	

Comparisons were made by *t* tests or Mann–Whitney rank-sum tests for continuous variables and the chi-squared test or Fisher's exact test for categorical variables (level of significance *p* <0.05).

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; PT, prothrombin time.

baseline characteristics and genotyping results are displayed in Table 1.

HCC occurrence, competing event incidence and impact of genetic variants on outcomes

After a median follow-up time of 43.7 (95% CI 41.6–46.7) months, 86 (7.4%) patients developed HCC, with a corresponding 5-year incidence of 8.8% (95% CI 6.9–10.9). During the same timeframe, 142 (12.4%) patients died (causes of death: HCC-related in 19 [16.2%]; liver-related in 34 [29.1%]; extrahepatic cause in 64 [54.7%]; missing data in 25). The 5-year non-HCC mortality incidence was 11.4% (95% CI 9.2-13.8). Table 2 shows the HCC SHR for each SNP in the CirVir and CIRRAL cohorts and then on the whole population under study.

Patients with at least one G-*PNPLA3* allele (n = 579) had a higher HCC incidence (n = 53 [9.2%] with a 5-year HCC incidence of 11.3% [95% CI 8.4–14.7]) than CC-*PNPLA3* homozygotes (n = 33/566 [5.8%] with a 5-year HCC incidence of 6.2% [95% CI 4.0–9.0]) (SHR = 1.64; 95% CI 1.06–2.53, $p = 0.025$). *PNPLA3* (rs738409) did not influence non-HCC liver-related mortality (SHR = 1.44; 95% CI 0.73–2.87; $p = 0.29$).

Patients with at least one T-*TM6SF2* rs58542926 allele (n = 173) had a similar HCC incidence (n = 17 [9.8%] with a 5-year HCC incidence of 11.2% [95% CI 6.4–17.5]) as CC-*TM6SF2* rs58542926 homozygotes (n = 69/972 [7.1%] with a 5-year HCC incidence of 8.3% [95% CI 6.3–10.7]) (SHR = 1.42; 95% CI 0.83–2.42; $p = 0.201$). *TM6SF2* rs58542926 did not influence non-HCC liver-related mortality (SHR = 0.18; 95% CI 0.02–1.30; $p = 0.09$).

Patients with at least one G-*TM6SF2* rs187429064 allele (n = 27) had a similar HCC incidence (n = 2 [7.4%] with a 5-year HCC incidence of 3.7% [95% CI 0.3–15.9]) as AA-*TM6SF2* rs187429064 homozygotes (n = 84/1118 [7.5%] with a 5-year HCC incidence of 8.9% [95% CI 7.0–11.1]) (SHR = 1.02; 95% CI 0.26–4.00; $p = 0.979$). *TM6SF2* rs187429064 did not influence non-HCC liver-related mortality (SHR = 1.22; 95% CI 0.17–9.02; $p = 0.85$).

Patients with at least one A-*HSD17B13* allele (n = 419) had a similar HCC incidence (n = 27 [6.4%] with a 5-year HCC incidence of 6.6% [95% CI 4.0–10.1]) as -:–*HSD17B13* homozygotes (n = 59/726 [8.1%] with a 5-year HCC incidence of 10.0% (95% CI [7.5–12.9]) (SHR = 0.76; 95% CI 0.48–1.20; $p = 0.235$). *HSD17B13* (rs72613567) did not influence non-HCC liver-related mortality (SHR = 1.34; 95% CI 0.68–2.63; $p = 0.40$).

TT-*APOE* homozygous patients (n = 906) had a similar HCC incidence (n = 71 [7.8%] with a 5-year HCC incidence of 9.6% [95% CI 7.4–12.2]) as patients with at least one C-*APOE* allele (n = 15/239 [6.3%] with a 5-year HCC incidence of 5.6% [95% CI 2.8–9.7]) (SHR = 1.23; 95% CI 0.71–2.14; $p = 0.464$). *APOE* (rs429358) did not influence non-HCC liver-related mortality (SHR = 1.15; 95% CI 0.48–2.78; $p = 0.75$).

Patients with at least one T-*MBOAT7* allele (n = 824) had a similar HCC incidence (n = 69 [8.4%] with a 5-year HCC incidence of 10.2% [95% CI 7.8–13.0]) as CC-*MBOAT7* homozygotes (n = 17/321 [5.3%] with a 5-year HCC incidence of 5.2% [95% CI 2.9–8.6]) (SHR = 1.66; 95% CI 0.98–2.83; $p = 0.06$). *MBOAT7* (rs641738) did not influence non-HCC liver-related mortality (SHR = 1.60; 95% CI 0.69–3.70; $p = 0.27$).

AA-*WNT3A-WNT9A* homozygous patients (n = 439) had a higher HCC incidence (n = 42 [9.6%], 5-year HCC incidence 10.3% [95% CI 7.2–14.1]) than patients with at least one T-WNT allele (n = 44/706 [6.2%], 5-year HCC incidence 7.8% [95% CI 5.6–10.5]) (SHR = 1.57; 95% CI 1.03–2.39; $p = 0.037$). *WNT* (rs708113) did not influence non-HCC liver-related mortality (SHR = 0.91; 95% CI 0.45–1.83; $p = 0.79$).

Construction of GRSs

The combined influence of the six SNPs modulating liver fat content (*PNPLA3*, *TM6SF2* rs58542926 and rs187429064,

Table 2. HCC subhazard ratios (Fine-Gray regression modelling, level of significance $p < 0.05$).

SNP	HCV-cured n = 659	Alcohol n = 486	Total N = 1,145
PNPLA3 (rs738409)			
C:C (Ref)	SHR = 1.64; 0.86–3.15;	SHR = 1.52; 0.85–2.73;	SHR = 1.64; 1.06–2.53;
C:G or G:G	$p = 0.134$	$p = 0.158$	$p = 0.025$
TM6SF2 (rs58542926)			
C:C (Ref)	SHR = 1.01; 0.39–2.59;	SHR = 1.66; 0.86–3.19;	SHR = 1.42; 0.83–2.42;
C:T or T:T	$p = 0.983$	$p = 0.129$	$p = 0.201$
TM6SF2 (rs187429064)			
A:A (Ref)	SHR = 3.93; 1.10–14.04;		SHR = 1.02; 0.26–4.00;
A:G	$p = 0.035$	n.a.	$p = 0.979$
HSD17B13 (rs72613567)			
-:-	SHR = 1.15; 0.58–2.25;	SHR = 1.45; 0.79–2.66;	SHR = 1.31; 0.84–2.06;
A:- or A:A (Ref)	$p = 0.691$	$p = 0.227$	$p = 0.235$
MBOAT7 (rs641738)			
C:C (Ref)	SHR = 1.43; 0.68–3.01;	SHR = 1.83; 0.85–3.94;	SHR = 1.66; 0.98–2.83;
C:T or T:T	$p = 0.345$	$p = 0.122$	$p = 0.060$
APOE (rs429358)			
C:C or C:T (Ref)	SHR = 1.54; 0.55–4.35;	SHR = 1.23; 0.63–2.39;	SHR = 1.23; 0.71–2.14;
T:T	$p = 0.414$	$p = 0.544$	$p = 0.464$
WNT3A-WNT9A (rs708113)			
A:A	SHR = 0.90; 0.46–1.77;	SHR = 2.34; 1.33–4.13;	SHR = 1.57; 1.03–2.39;
A:T or T:T (Ref)	$p = 0.770$	$p = 0.003$	$p = 0.037$

HCC, hepatocellular carcinoma; SHR, subhazard ratio.

HSD17B13, APOE, and *MBOAT7* genotypes) was first analysed in the whole population by coding as 0, 1, and 2 for non-carriers and heterozygous and homozygous carriers of the HCC risk-increasing allele of each variant, respectively. In a first step, a combined 6-SNP GRS was calculated as the unweighted sum of these HCC risk-increasing alleles (range, 0-12) for each participant. Because of low numbers in some groups, subsequent analyses were conducted as a function of three genotypic associations (Group 1: scores 0-4, n = 363; Group 2: scores 5-6, n = 622; Group 3: scores ≥7, n = 160). The 5-year HCC incidence increased progressively from Group 1 (4.8%; 95% CI 2.7–7.8), to Group 2 (9.1%; 95% CI 6.5–12.2), to Group 3 (16.8%; 95% CI 10.1–24.9), *Pglobal* = 0.011 (Fig. 1A). After exclusion of non-European patients, the 6-SNP GRS remained associated with the 5-year HCC incidence (*Pglobal* = 0.007).

In a second step, *WNT3A-WNT9A* genotypes were similarly added to the previous GRS, yielding a range from 0 to 14 for the 7-SNP score. Subsequent analyses were conducted as a function of three genotypic associations (Group 1: scores 0-6, n = 627; Group 2: score 7, n = 276; Group 3: scores ≥8, n = 242). The 5-year HCC incidence increased progressively from Group 1 (5.4%; 95% CI 3.5–7.8), to Group 2 (10.7%; 95% CI 6.6–15.9), to Group 3 (15.3%; 95% CI 10.2–21.4); *Pglobal* <0.001 (Fig. 1B). After exclusion of non-European patients, the 7-SNP GRS remained associated with the 5-year HCC incidence (*Pglobal* = 0.001).

When these scoring systems were restricted to CIRRAL (Fig. S2) or CirVir cohorts (Fig. S3), the 7-SNP GRS was the only score significantly associated with HCC in patients, while the 6-SNP score nearly reached statistical significance in both cohorts.

Neither the 6-SNP GRS nor the 7-SNP GRS affected non-HCC liver-related mortality (Fig. S4A,B, respectively).

Features associated with HCC occurrence

Table 3 displays the results from univariate analyses using Fine-Gray regression models. The model identified several parameters as HCC risk factors considering the competing risk of death. Similarly, the aMAP score was also associated with HCC occurrence. Multivariate analyses were subsequently performed to assess the added prognostic value of the 6- or 7-SNP GRSs to either an internally derived routine basis model or the aMAP score. Table 4 shows the results of the Fine-Gray multivariate analyses. Both 6- and 7-SNP GRSs were independently associated with a higher HCC risk regardless of the applied clinical scoring system. When applied to the CIRRAL and CirVir cohorts, the 7-SNP GRS was the only GRS selected by the multivariate models to be associated with HCC, an effect which was restricted to the CIRRAL cohort regardless of routine or aMAP score (see Tables S1 and S2).

Table S3 shows the clinical characteristics of patients as a function of the 7-SNP GRS. Overall, patients with the highest scores were older, had higher liver test alterations, and more pronounced signs of severe liver disease. These patients also had the highest aMAP scores. Rates of BCLC 0/A HCC were 82.1%, 45.0%, and 66.7% for the lowest, intermediate and highest scores, respectively.

HCC risk stratification model performance and decision curve analyses

Fig. 2 shows the discriminative performances of internal and aMAP scores alone or following the incorporation of the 6-SNP or 7-SNP GRSs. The internally derived routine model yielded a C-index of 0.769 for 5-year HCC risk prediction. When incorporating the genetic features into the model, the C-index increased to 0.782 after adding the 6-SNP GRS and to 0.786 after adding the 7-SNP GRS.

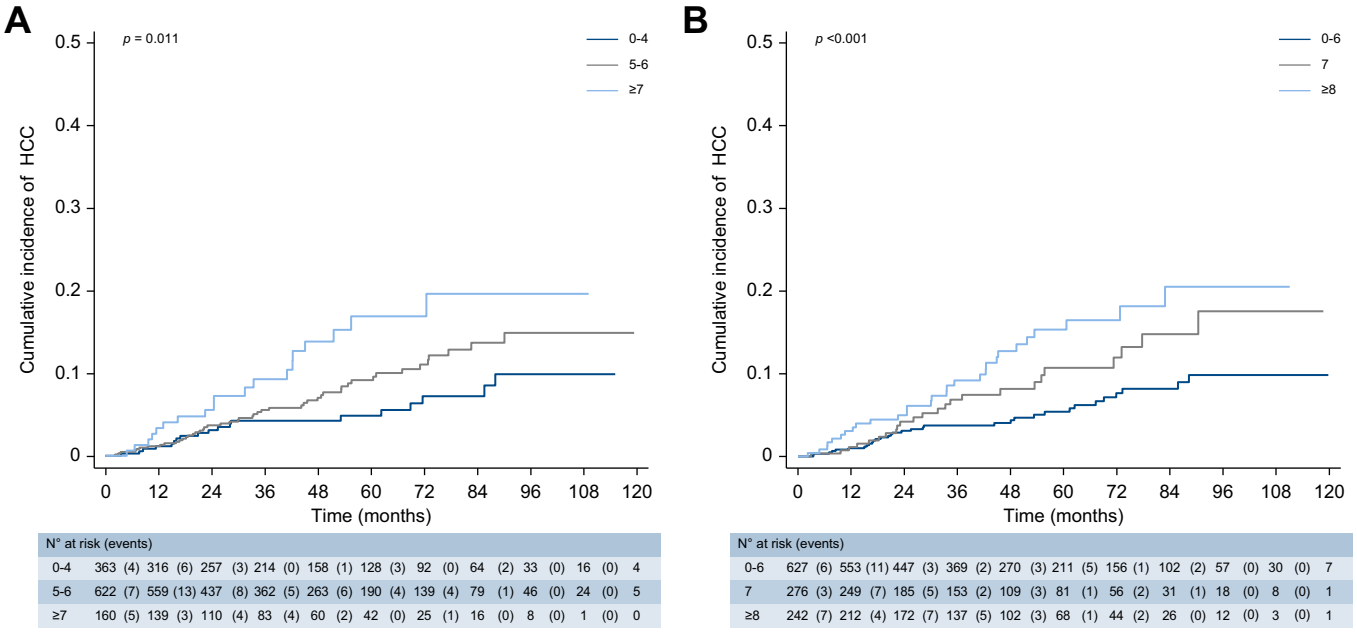


Fig. 1. HCC incidence as a function of genetic risk scores. (A) Six-SNP genetic risk score (*PNPLA3, TM6SF2* rs58542926 and rs187429064, *HSD17B3, APOE, MBOAT7*). (B) Seven-SNP genetic risk score (*PNPLA3, TM6SF2* rs58542926 and rs187429064, *HSD17B13, APOE, MBOAT7, WNT3A-WNT9A*). Unadjusted comparisons of incidence curves were performed using the Gray test (level of significance $p < 0.05$). HCC, hepatocellular carcinoma; SNP, single nucleotide polymorphism.

Table 3. Features associated with HCC occurrence in univariate analysis (Fine-Gray regression modelling, level of significance p <0.05).

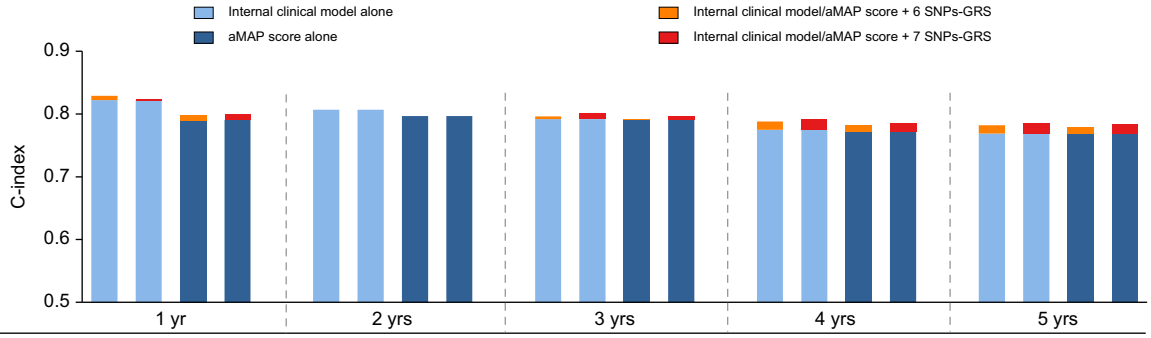
	No HCC, n = 1,059	HCC, n = 86	SHR [95% CI]	p value
Age (years)	58 [51– 64]	61 [55–70]	1.04 [1.02–1.07]	0.001
Male sex	684 (64.6)	70 (81.4)	2.38 [1.39–4.09]	0.002
BMI (kg/m^2)	26.3 [23.5–30.1]	27.8 [24.6–31.9]	1.05 [1.01–1.090]	0.025
BMI (kg/m^2)				0.173
<25	313 (37.7)	20 (26.7)	Ref	
[25; 30]	304 (36.6)	29 (38.7)	1.37 [0.78–2.41]	0.276
≥30	214 (25.7)	26 (34.6)	1.75 [0.97–3.14]	0.061
Diabetes	208 (19.6)	32 (37.7)	2.29 [1.48–3.54]	<0.001
Past excessive alcohol consumption	614 (60.0)	63 (74.1)	1.57 [0.97–2.55]	0.068
Platelet counts (10^3/mm^3)	149 [103–197]	105.5 [79.5–151.5]	0.991 [0.986–0.995]	<0.001
AST (IU/L)	32 [25–46]	36 [29–53]	1.004 [1.001–1.008]	0.026
ASTx normal (n = 40)	0.80 [0.63–1.15]	0.90 [0.73–1.33]	1.19 [1.02–1.39]	0.026
ALT (IU/L)	27 [20–41.5]	29[22–42]	1.004 [0.999–1.009]	0.098
ALTx normal (n = 40)	0.68 [0.50–1.04]	0.73 [0.55–1.05]	1.17 [0.97–1.41]	0.098
GGT (IU/L)	63.0 [36.0–140.0]	131.5 [64.0–256.0]	1.000 [0.999–1.000]	0.083
GGTx normal (n = 45)	1.40 [0.80–3.11]	2.92 [1.42–5.69]	1.01 [0.99–1.01]	0.083
PT (%)	85 [73–96]	76 [70–84]	0.98 [0.97–0.99]	<0.001
Serum albumin (g/L)	41.8 [38.0–44.5]	39.7 [36.4–42.1]	0.92 [0.88–0.95]	<0.001
Total bilirubin (μmol/L)	11.0 [8.0–17.0]	15.0 [9.1–20.0]	1.03 [1.01–1.05]	<0.001
AFP (ng/ml)	3.7 [2.4–5.5]	4.0 [2.8–6.3]	1.000 [0.999–1.002]	0.626
aMAP score	58.1 [52.8–62.9]	64.0 [60.6–68.1]	1.14 [1.09–1.19]	<0.001
Cirrhosis aetiology				
HCV-cured	622 (58.7)	37 (43.0)	Ref	
Alcohol	437 (41.3)	49 (57.0)	1.50 [0.98–2.29]	0.062
6-SNP GRS				0.014
0-4	344 (32.5)	19 (22.1)	Ref	
5-6	574 (54.2)	48 (55.8)	1.54 [0.91–2.62]	0.110
7-11	141 (13.3)	19 (22.1)	2.58 [1.36–4.88]	0.004
7-SNP GRS				0.001
0-6	594 (56.1)	33 (38.4)	Ref	
7	252 (23.8)	24 (27.9)	1.73 [1.03–2.92]	0.040
8-13	213 (15.3)	29 (33.7)	2.50 [1.52–4.11]	0.004

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; PT, prothrombin time.

Table 4. Multivariate analyses using an internally derived routine basis model or the aMAP score (Fine-Gray regression modelling, level of significance p <0.05).

	Routine basis model alone		Routine basis model + 6-SNP GRS		Routine basis model + 7-SNP GRS	
	aSHR [95% CI]	p value	aSHR [95% CI]	p value	aSHR [95% CI]	p value
Age (years)	1.04 [1.02–1.07]	0.001	1.04 [1.02–1.07]	0.001	1.04 [1.02–1.07]	0.001
Male sex	2.70 [1.57–4.60]	<0.001	2.60 [1.52–4.44]	<0.001	2.64 [1.55–4.51]	<0.001
Diabetes	1.63 [1.03–2.57]	0.037	1.69 [1.07–2.66]	0.025	1.69 [1.04–2.61]	0.032
Platelet count (10^3/mm^3)	0.992 [0.987–0.996]	<0.001	0.992 [0.987–0.997]	0.001	0.992 [0.988–0.997]	0.001
GGTx normal (n = 45)	1.010 [1.002–1.019]	0.012	1.010 [1.002–1.019]	0.014	1.012 [1.004–1.020]	0.005
Serum albumin (g/L)	0.95 [0.91–0.99]	0.018	0.95 [0.91–0.99]	0.033	0.95 [0.91–0.99]	0.040
6-SNP GRS				0.042		
0–4			Ref			
5–6			1.38 [0.79–2.41]	0.256		
7–11			2.26 [1.18–4.31]	0.014		
7-SNP GRS						0.012
0–6					Ref	
7					1.66 [0.96–2.88]	0.070
8–13					2.12 [1.28–3.52]	0.004
	aMAP score alone		**aMAP score + 6-SNP GRS**		**aMAP score + 7-SNP GRS**	
	aSHR [95% CI]	**p value**	**aSHR [95% CI]**	**p value**	**aSHR [95% CI]**	**p value**
aMAP score	1.14 [1.10–1.18]	<0.001	1.14 [1.10–1.18]	<0.001	1.13 [1.09–1.18]	<0.001
6-SNP GRS				0.048		
0–4			Ref			
5–6			1.42 [0.83–2.43]	0.200		
7–11			2.21 [1.17–4.17]	0.014		
7-SNP GRS						0.014
0–6					Ref	
7					1.0 [1.00–2.90]	0.049
8–13					2.06 [1.25–3.39]	0.005

aSHR, adjusted subhazard ratio; GRS, genetic risk score; SNP, single nucleotide polymorphism.



C-index assessed at	1 yr	2 yrs	3 yrs	4 yrs	5 yrs
6 SNPs-GRS (3 classes)	0.588	0.548	0.570	0.606	0.623
7 SNPs-GRS (3 classes)	0.620	0.556	0.607	0.627	0.639
Internal clinical model alone	0.822	0.807	0.792	0.775	0.769
Clinical model + 6 SNPs-GRS	0.829	0.801	0.796	0.788	0.782
Clinical model + 7 SNPs-GRS	0.824	0.803	0.801	0.791	0.786
aMAP score alone	0.790	0.796	0.791	0.772	0.768
aMAP score + 6 SNPs-GRS	0.798	0.788	0.792	0.782	0.779
aMAP score + 7 SNPs-GRS	0.799	0.788	0.796	0.785	0.783

Fig. 2. Performance of HCC prediction models according to the Wolber's C-index for prognostic models with competing risks. GRS, genetic risk score; HCC, hepatocellular carcinoma; SNP, single nucleotide polymorphism.

Similarly, the aMAP model yielded a C-index of 0.768 for 5-year HCC risk prediction. When incorporating the genetic features into the model, the C-index increased to 0.779 after adding the 6-SNP GRS and to 0.783 after adding the 7-SNP GRS. Similar trends were observed when restricting the analyses to the CIRRAL and CirVir cohorts (see Tables S4 and S5).

Fig. 3 shows the comparison of cumulative 5-year incidence of HCC for high/low-risk patients, defined by clinical model *vs.* clinical model + the 7-SNP GRS. High/low risk was defined according to illustrative percentile cut-off points. Fig. S5 shows a similar analysis using the aMAP score instead. Overall, the addition of the 7-SNP GRS to the internal clinical model or the aMAP score marginally improved the discrimination of high/low-risk patients.

Decision curves were finally plotted to test the clinical utility of the 7-SNP GRS alone or as a refinement of the internal routine model or the aMAP score for 3- or 5-year HCC risk prediction (Fig. 4), in comparison to two reference clinical strategies of screening all patients or none. Regardless of the approach, all predicted models demonstrated superior net clinical benefit to reference screening strategies of all or no patients, as evidenced by their overall greater net benefit values. Among predictive models, the 7-SNP GRS alone showed the weakest net benefit compared with the two routine models. When the latter were applied, their clinical utility was modestly improved by the addition of the 7-SNP GRS.

Similar trends were observed when restricting the analyses to the CIRRAL and CirVir cohorts (see Figs. S6 and S7).

Discussion

This study, based on the analysis of large prospective cohorts of patients included in HCC surveillance programmes with extensive bioclinical characterisation, long follow-up and prospective analysis of events based on patients' medical files allows us to draw several conclusions. First, a GRS reflecting lipid metabolism has independent predictive value for HCC development. Second, the addition of the recently identified locus, involved in the Wnt-β-catenin pathway, increased the performance of this GRS. Third, the incorporation of this genetic information into clinical models modestly improves HCC risk stratification.

Hepatic fat content has been shown to be influenced by genetic variants,[13] the latter being associated with the presence of HCC in European populations.[10–12] However, these case-control approaches included heterogeneous populations comprising healthy individuals or patients with mild forms of liver disease who are not the target for HCC surveillance, thus introducing several interpretation biases. In contrast, the assessment of these SNPs in the present longitudinal cohorts provides robust arguments suggesting a direct link with hepatocarcinogenesis. The study of patients with ALD and cured HCV showed that *PNPLA3* and *MBOAT7* (to a lesser extent) exerted the highest oncogenic effect when both cohorts were combined (Table 2), reflecting the selective influence of lipid metabolism on the oncogenic process in patients in whom the pro-carcinogenic effect of viral replication has been suppressed; indeed, the association of liver fat-modulating SNPs with HCV-related HCC is debated.[38] However, this inconclusive observation holds true in patients with active HCV replication: recent longitudinal studies conducted in patients who achieved SVR have indeed suggested that this genetic background may impact HCC occurrence.[39] This observation reinforces the hypothesis that alcohol-related and/or metabolic and/or cured HCV-related cirrhosis can be viewed as a "universal" phenotype in this context, particularly given the high prevalence of excessive alcohol consumption (more than 30%) or features of metabolic syndrome (nearly 60%) in HCV-cured patients (see Table 1). Consequently, based on the rigorous clinical definition of included patients (biopsy-proven cirrhosis, exclusion of

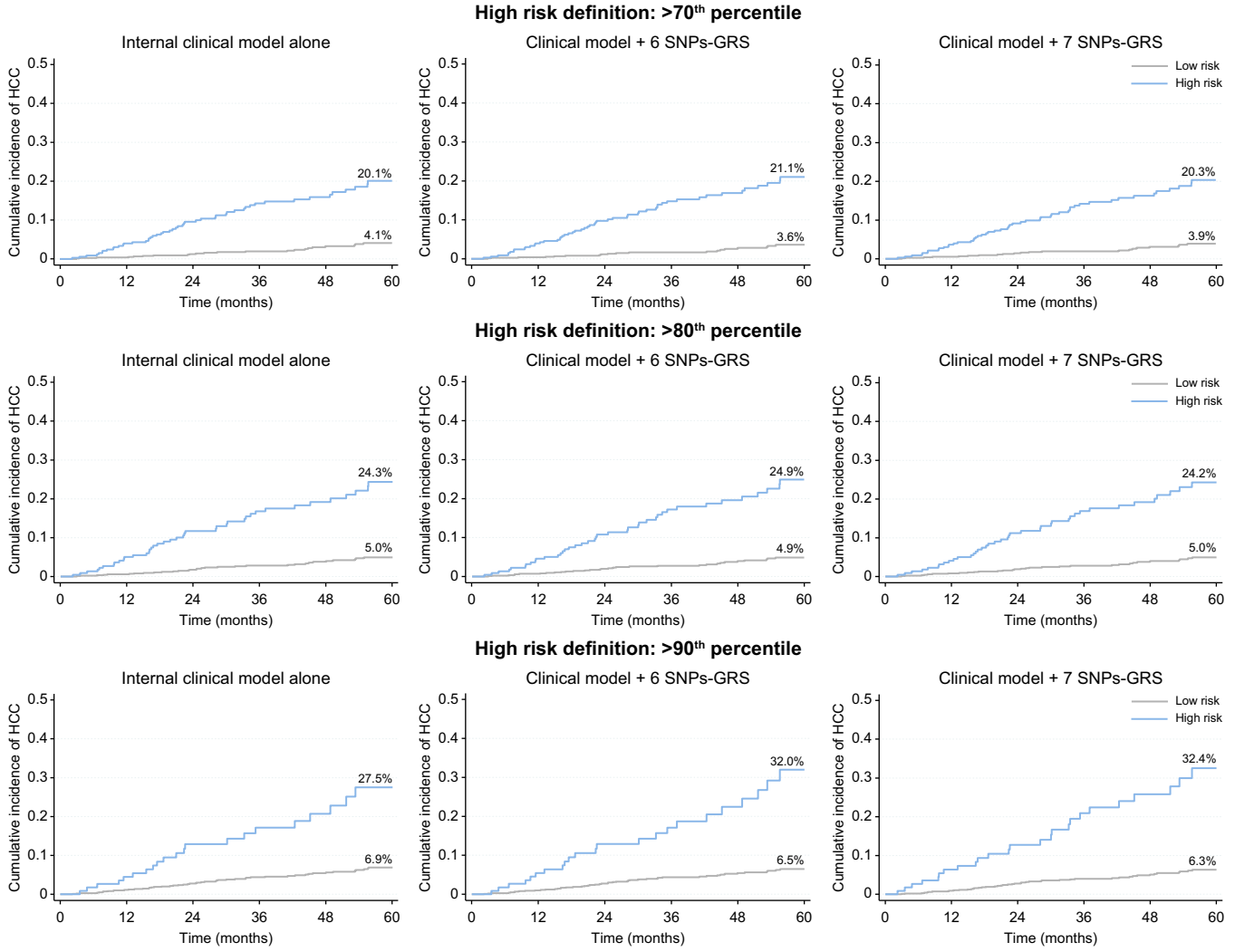


Fig. 3. Comparison of cumulative incidence of HCC at 5 years for high/low-risk patients, defined by clinical model *vs*. clinical model combined with GRS. High/low risk was defined according to illustrative percentile cut-off points. As an example, the 70th percentile definition means that individuals whose score was in the 80th percentile or greater (*i.e.* in the top 20%) were categorised as high risk, and the remainder were categorised as low risk. GRS, genetic risk score; HCC, hepatocellular carcinoma; SNP, single nucleotide polymorphism.

patients with active viral replication, extensive clinical description, protocolised monitoring), the 6-SNP GRS fairly stratified this population into different HCC risk classes (Fig. 1A). Moreover, all outcomes were considered during a long follow-up, enabling HCC development to be considered within a competing risks framework; in this context, the 6-SNP GRS did not predict non-HCC mortality despite an association with more pronounced liver function impairment (see Tables S3 and Fig. S4A). Finally, this GRS was an independent factor associated with HCC occurrence (Table 4). Taken together, these observations provide the strongest clinical arguments to date for the direct impact of this genetic heterogeneity on liver cancer development.

Following the recent identification of a new HCC susceptibility locus affecting the Wnt-β-catenin pathway,[14] we sought to investigate its additional impact on HCC occurrence. Indeed, this GWAS highlighted an additional genetic variation in the *WNT3A-WNT9A* locus that modifies HCC risk in patients with ALD. The rs708113[T] allele was associated with lower rates of HCC in these patients, an effect that seemed independent from liver fibrosis status and suggested a more direct effect on liver carcinogenesis compared with SNPs modulating hepatic fat content. Translational experiments suggested the promotion of a liver inflammatory environment by the rs708113[T] allele, which may prevent the activation of oncogenic β-catenin, thus decreasing the oncogenic process. The present report confirms this specific association by externally validating the impact of *WNT3A-WNT9A* rs708113 on HCC occurrence in the CIRRAL cohort. When considering the whole population, the addition of *WNT3A-WNT9A* rs708113 to the six aforementioned variants improved HCC risk stratification through higher SHRs (Fig. 1B and Table 4).

The extent to which GRS may impact clinical practice deserves to be proven. For that matter, one must not only consider genetic factors, but also simple routine parameters already known to accurately stratify patients who are eligible for HCC surveillance into various risk classes. Several clinical scoring systems have been developed,[40] and the widespread

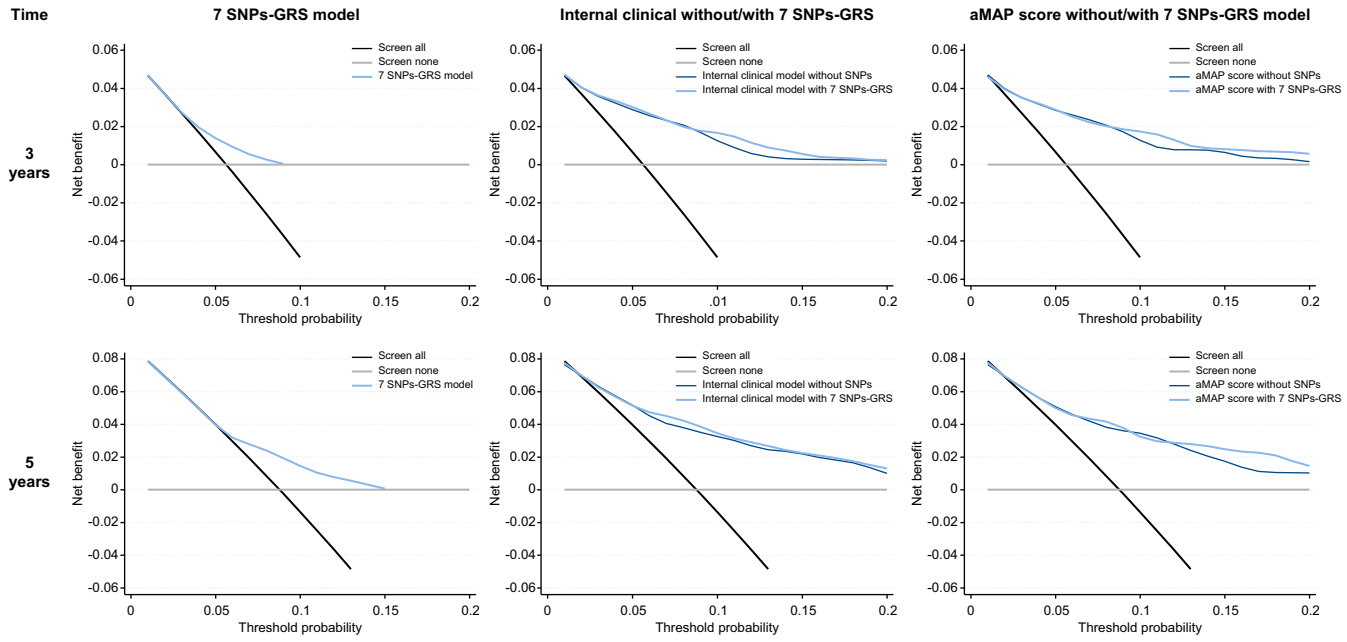


Fig. 4. Decision curve analyses. Decision curves display the net benefit between surveillance benefits and surveillance harms. Net benefit in screened patients is calculated across a range of HCC risk thresholds (defined as the minimum probability of disease at which biannual screening would be warranted), as the proportion of patients with true positive results minus the proportion with false-positives multiplied by the odds at the threshold probability (HCC risk/1 – HCC risk). The net benefits of the risk prediction models were compared with those from two different reference strategies of screening all patients or none. Risk models with higher discriminative power will provide higher net benefit, as evidenced by the highest plotted decision curves. GRS, genetic risk score; HCC, hepatocellular carcinoma; SNP, single nucleotide polymorphism.

use of antivirals has led to the construction of simple scores that can be applied to patients without viral replication regardless of the cause of liver disease.[20] In this context, we constructed an internal model using results of the multivariate model and also applied the previously developed aMAP score as an external model.[21] Both models performed well in this population, with a similar 5-year C-Index of 0.769. When enriched by 6- or 7-SNP GRSs, both models performed better, but this improvement was modest (Figs 2 and 3). This observation was further strengthened by DCAs, which confirmed the modest improvement of both internal and external clinical scoring systems incorporating the 7-SNP GRS (Fig. 4). Our results are in line with a recent report conducted in patients with HCV-cured cirrhosis, albeit HCC occurrence was not the specific outcome studied.[41] A similar analysis was performed in nearly 200,000 UK biobank participants, which evaluated the enrichment of several liver prognostic scoring systems by several SNPs;[42] although the outcome was also a mixed endpoint encompassing "liver-related complications", this large-scale study showed that the most performant scores were similarly only marginally improved by the addition of genetic variants. Nevertheless, other analyses conducted in the very same UK biobank yielded opposite conclusions:[43] this fact once again highlights the pivotal role of prospective cohorts of patients with pre-defined outcomes and events accurately recorded in clinical centres for delineating the basis of future precision medicine.

The main limitation of our study is underlined by potential underpowered analyses when stratified by liver disease as suggested by the mild predictive value of *PNPLA3* and *MBOAT7* genotypes in the CIRRAL and CirVir cohorts independently and a stronger one when both populations were combined (see Table 2). The same observation was made for the different GRSs: when the cohorts were considered separately, the 7-SNPs GRS was the only informative genetic score, an effect which was restricted to the CIRRAL cohort (see Figs. S2 and S3). After association of the two cohorts, the 6-SNP GRS combining only liver fat-modulating SNPs was clearly associated with HCC occurrence. Similar observations were made when multivariate analyses were performed. Indeed, the 6-SNP GRS combining only liver fat-modulating SNPs was (not surprisingly) not associated with HCC in either the CirVir or CIRRAL cohorts (see Tables S1 and S2), while it was highlighted as an independent risk factor whether considering the internal or external aMAP clinical scoring system (see Table 4). This fact is partially the consequence of the cautious selection of patients from both cohorts (see flowchart Fig. S1). In addition, while the prospective design of these longitudinal cohorts limits the ability to follow-up large numbers of patients using standardised surveillance protocols recorded in clinical centres in the long term, such a rigorous approach means that our conclusions can be interpreted with confidence. This clinical approach is in sharp contrast with the aforementioned registry studies,[10,42] which in turn suffer from limited clinical information and outcomes. These statistical issues provide further justification to combine patients with cirrhosis and HCV eradication with other causes of non-viral liver diseases, as highlighted by the development of universal scoring systems such as the aMAP score. While limited longitudinal single-centre studies comprising biobanks are emerging,[39] prospective and protocolised multicentric efforts similar to the CirVir and CIRRAL cohorts are currently ongoing

in other countries and will ultimately enable the refinement of our observations when made available. In this setting, the extent to which our findings are generalisable to non-European populations warrants further investigation. Ultimately, ongoing international efforts to gather large-scale longitudinal cohorts of patients recruited in European countries will provide further insight on both HCC genetic predisposition and risk stratification.

In conclusion, patients with cirrhosis included in HCC surveillance programmes can be stratified by genetic scores, using variants affecting lipid turnover and the Wnt-β-catenin pathway, into various HCC risk classes. This genetic information modestly improves the performance of clinical scores for HCC risk allocation. The continuous enrichment with yet to be identified or validated circulating biological components (genetic or not) might ultimately pave the way for personalisation of HCC surveillance using more effective tools in a cost-effective manner, if they are proven to substantially improve the performance of routine scoring systems. In the meantime, ongoing randomised clinical trials aimed at gathering clinical evidence for the benefits of HCC risk-based screening interventions will rely solely on clinical scoring systems.

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Abbreviations

ACLD, advanced chronic liver disease; ALD, alcoholic liver disease; GRS, genetic risk score; GWAS, genome-wide association studies; HCC, hepatocellular carcinoma; MRI, magnetic resonance imaging; SVR, sustained virological response; US, ultrasound; SNP, single nucleotide polymorphism.

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Conflicts of interest

Pr Nahon has received honoraria from and/or consults for AstraZeneca, Abbvie, Bayer, Bristol-Myers Squibb, Eisai, Gilead, Ipsen, MSD and Roche. He received research grants from AstraZeneca, AbbVie, Bristol-Myers Squibb and Eisai. Pr Trépo received research support from Gilead and a speaker fee from Abbvie. Pr Ganne-Carrié consults for and/or has received personal fees from Abbvie, Bayer, Gilead, Ipsen, and Shionogi outside the submitted work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Drs Nahon and Audureau had full access to all data in the study and take responsibility for data integrity and the accuracy of data analysis.

Study concept and design: Nahon, Audureau. *Acquisition of data:* Nahon, Layese, Ganne-Carrié, Chaffaut, Zucman-Rossi, Bamba-Funck, Sutton. *Analysis and interpretation of data:* Nahon, Layese, Bamba-Funck, Sutton, Audureau. *Drafting of the manuscript:* Nahon, Layese, Audureau, Bamba-Funck, Sutton. *Critical revision of the manuscript for important intellectual content:* Ganne-Carrié, Ziol, Trépo, Zucman-Rossi.*Statistical analysis:* Layese, Audureau. *Obtained* funding*:* Pr Jean-Claude Trinchet, Pr Nathalie Ganne-Carrié, Pr Pierre Nahon. *Administrative, technical and material* support*:* Nahon, Cagnot, Layese, Audureau, Bamba-Funck, Sutton. *Study supervision:* Nahon, Audureau, Sutton.

Data availability statement

Data are available on reasonable request to the corresponding author.

Role of the funding sources

The funding sponsors had no role in the design or conduct of the study, the collection, management, analysis or interpretation of the data, or the preparation, review or approval of the manuscript.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2022.11.003.

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