ORIGINAL ARTICLE

HSD17B13 and other liver fat-modulating genes predict development of hepatocellular carcinoma among HCV-positive cirrhotics with and without viral clearance after DAA treatment

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Abstract

Background Genetic predisposition to accumulate liver fat (expressed by a polygenic risk score, GRS, based on the number of at-risk alleles of *PNPLA3*, *TM6SF2*, *MBOAT7* and *GCKR*) may influence the probability of developing hepatocellular carcinoma (HCC) after hepatitis C treatment. Whether this holds true taking into account carriage of the *HSD17B13:TA* splice variant, also affecting lipogenesis, and achievement of viral clearance (SVR), is unknown.

Methods *PNPLA3*, *TM6SF2*, *MBOAT7*, *GCKR* and *HSD17B13* variants were determined in a cohort of 328 cirrhotic patients free of HCC before starting treatment with direct acting antivirals (DAA).

Results SVR in the study cohort was 96%. At the end of follow-up, $N = 21$ patients had been diagnosed an HCC; none of the genes included in the GRS was individually associated with HCC development. However, in a Cox proportional hazards model, a GRS > 0.457 predicted HCC independently of sex, diabetes, albumin, INR and FIB4. The fit of the model improved adding treatment outcome and carriage of the *HSD17B13*:TA splice variant, with sex, GRS > 0.457, *HSD17B13:TA* splice variant and failure to achieve an SVR (hazard ratio = 6.75, 4.24, 0.24 and 7.7, respectively) being independent predictors of HCC.

Conclusion Our findings confirm that genes modulating liver fat and lipogenesis are important risk factors for HCC development among cirrhotics C treated with DAA.

Keywords Gene polymorphisms · Hepatitis C virus · Hepatocarcinogenesis · Prognosis · Therapy

Introduction

The introduction of direct acting antivirals (DAA) in the treatment of hepatitis C made possible to achieve sustained virological response (SVR) rates well above 90% of treated patients, with an excellent safety profile [1]. SVR is associated with a reduced risk of hepatic decompensation, as well as reduced overall and liver-related mortality [2]. The impact of DAA on the risk of developing hepatocellular carcinoma (HCC) has been the focus of a hot debate [3],

fueled by observations of HCC development or recurrence early after having completed DAA treatment [4, 5]. A likely explanation for these findings is that, due to DAA effectiveness and tolerability, far more aged patients with advanced cirrhosis were proposed treatment than in the interferon era. Larger studies have clarified that HCC development after DAA is driven by severity of liver disease and treatment failure [6]; indeed, following treatment, the incidence of HCC is the highest in patients with cirrhosis and treatment failure, while HCC risk decreased through patient's categories with cirrhosis and SVR, and no cirrhosis with and without SVR [7]. Being cirrhotic patients cleared of HCV still at risk for HCC [8], the current HCC international guidelines recommend long-life surveillance [9].

In this context, there is an ongoing search for HCC predictors, which might contribute to risk stratification by better tailoring follow-up strategies. A promising field of investigation is the genetic contribution to HCC risk [10].

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Several single nucleotide polymorphisms, many of which influencing fat accumulation in the liver, modulate liver fibrosis progression both in fatty liver disease and hepatitis C [11, 12] as well as the risk of HCC development [13–15]. A genetic risk score estimating the inherited predisposition to accumulate liver fat (GRS) has been associated with the severity of liver fibrosis in patients with nonalcoholic fatty liver disease (NAFLD), suggesting that hepatic fat accumulation has a causal role in determining CLD progression [16]. GRS combines four major risk variants: the patatin-like phospholipase domain containing 3 (*PNPLA3*) rs738409, the membrane bound O-acyltransferase domain containing 7 (*MBOAT7*) rs641738, the transmembrane 6 superfamily member 2 (*TM6SF2*) rs5842926 and the Glucokinase Regulator (*GCKR*) rs1260326. In a further study, the impact of GRS on HCC arising in a large cohort of NAFLD patients was refined by adjusting for the carriage of a 17β-hydroxysteroid dehydrogenase type 13 (*HSD17B13*) splice variant [17], known to modulate the risk of fibrosis and HCC among patients with fatty liver [18].

A recent study demonstrated an association between GRS and development of de novo HCC in patients with HCV-related cirrhosis treated with DAA, independently of liver disease severity [19]. However, these interesting findings have not been confirmed to date. Importantly, adjustment for loss-of-function *HSD17B13* variants and achievement of SVR had not been performed, although, as mentioned above, they may represent relevant confounders to taken into consideration. We conducted the present study to fill these gaps.

Methods

Study population

The present is a retrospective cohort study, based on a consecutive series of 1050 patients with chronic HCV infection who attended the liver clinic of an academic hospital in northern Italy between February 1, 2015 and August 31, 2020 to receive DAA treatment. Data were retrieved interrogating an electronic REDCap database containing the baseline demographic and anthropometric (age, gender, body mass index,), clinical (diagnosis of diabetes, FIB-4 score, liver stiffness, diagnosis of esophageal varices, treatment outcome) and laboratory (HCV genotype and HCV-RNA circulating levels, albumin, bilirubin, INR, platelets count) data. Cirrhosis was diagnosed based on either the results of transient elastography (TE) with an established liver stiffness cutoff ≥ 12 kPa or—in the absence of valid TE results—liver biopsy and/or evidence of portal hypertension.

Figure 1 presents the flow chart of the study, which has been conducted in strict accordance with the principles of the Declaration of Helsinki; all patients gave an informed
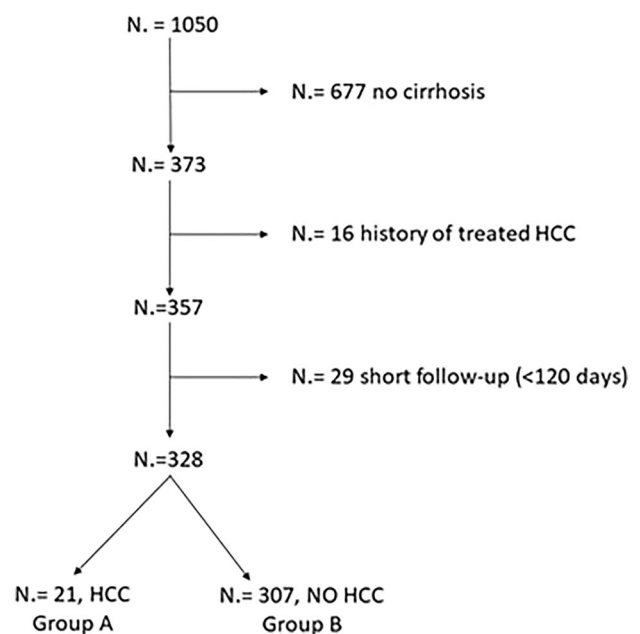

Fig. 1 Flow chart of the study. *HCC* hepatocellular carcinoma

consent to their participation to the study, including genetic analyses. The study has been approved by the local ethics committee (CE 215/19), as emended on April 21, 2021 to expand the set of genes studied.

Anti-HCV treatment

All patients were treated with direct antiviral agent(s), according to the Italian National Health System guidelines. Sustained virological response (SVR) was defined as undetectable HCV RNA 12 or 24 weeks (FU12 or FU24) after the end of treatment; relapse was defined as detectable HCV RNA at FU12 visit, with undetectable HCV RNA at the end of treatment. Patients who did not present to either the FU12 or the FU24 visit were considered dropouts.

HCC surveillance and diagnosis

Within three months before starting DAA treatment, all patients had undergone an abdominal ultrasound examination; after the end of DAA treatment, all were encouraged to continue surveillance for HCC with an interval of 6 months. Diagnosis of HCC were established based on dynamic imaging hallmarks or liver biopsy, according to the current guidelines [9].

Genetic studies

Genomic DNA was extracted from whole blood or buffy coat, using a commercial kit (Invitrogen, Carlsbad, CA,

USA), according to the manufacturer's instructions. Genotypes of *PNPLA3* (rs738409) and *HSD17B13* (rs72613567) were determined as previously reported [13]. TaqMan® SNP Genotyping Assays were used to assess the genotypes of the rs58542926 C > T (*TM6SF2* E167K), rs641738 C > T (*MBOAT7* locus) and rs1260326 C > T (*GCKR* P446L) (Life Technologies, Thermo Fisher Scientific; Carlsbad, California, US). The GRS was calculated by multiplying the number of at-risk alleles in each gene to a coefficient, based on the 1H magnetic resonance spectroscopy data quantifying hepatic triglyceride concentration in the Dallas Heart Study participants [20], as previously described [16].

Statistical analysis

Statistical analysis was conducted using the Stata statistical software package, version 15.1 (StataCorp LP, College Station, TX, USA). Continuous variables are presented as medians (interquartile ranges) and categorical variables as frequencies (percentage of the total). For the continuous variables, the differences between the groups were analyzed with the Mann–Whitney test, while in the case of categorical variables, the association was explored with Fisher's exact test or Pearson's chi square test, as appropriate.

The time-to-event analysis was based on the length of time elapsed between the day DAA treatment ended and the day the diagnostic exam for HCC was performed; the censor date was established based on last follow-up visit or telephone call. Differences in the probability of developing HCC were estimated by the log-rank test and presented graphically with a Kaplan–Meier plot. The association between de novo HCC and a set of predictor variables was evaluated building several nested Cox proportional hazards models, whose relative fitness was estimated by the likelihood ratio test. For all the tests used, the value chosen to indicate the statistical significance threshold was 0.05 (two tailed).

Results

Study population and outcome of antiviral treatment

The main features of the study population, including risk allele frequencies for each of the genes of interest (whose genotype frequencies did not depart significantly from those expected according to the Hardy–Weinberg equilibrium), are shown in Table 1.

Briefly, the typical profile in our study population was that of an elderly male with an HCV genotype 1b infection and a diagnosis of cirrhosis established with TE.

Following DAA treatment, $N = 315/328$ patients achieved an SVR; 1/328 patients did not present to neither the FU12 nor to the FU24 visits, while 12/328 patients had a viral relapse. Thus, the intention-to-treat and per protocol SVR were 96.0% and 96.3%, respectively.

HCC development

The follow-up lasted up to 55 months (median, 7.5 months) after the end of treatment. At the end of the study, $N = 21$ patients had been diagnosed an HCC (Group A). Along a time at risk of 136,813 days, the incidence rate of de novo HCC was 0.00015, while the time to event 25th, 50th and 75th percentiles were 1519 days, 1614 days and not reached, respectively. The comparison of the main characteristics of patients who developed de novo HCC vs. all other patients is presented in Table 1. Briefly, patients in group A were more commonly males and had a more advanced liver disease, as demonstrated by a higher liver stiffness, lower albumin levels and higher prevalence of esophageal varices.

Table 2 shows allele frequencies for the four genes included in the GRS and for *HSD17B13*, as well as measures of centrality and distribution for the GRS. Regarding the latter, it can be appreciated that a more inclusive cutoff (comparing the highest quartile vs. the three lower quartiles) greatly improves the discriminating ability for the score.

In Group A patients, there were 4 relapses and 1 dropout; thus, the intention-to-treat SVR for Group A was 76% vs. 97% in all remaining patients (Group B; 8 relapses) ($p = 0.001$).

De novo HCC and genetic risk factors

None of the four genes of interest included in the genetic risk score was associated with the probability of developing de novo HCC, either when taken in isolation or when included as predictor variables in a Cox proportional hazards model (data not shown). Moreover, building with our data an exact replica of the Cox proportional hazards model proposed by Degasperi et al. [19], which also included sex, diabetes, albumin levels, INR and FIB4, the categorical variable GRS ($\leq 0.597, > 0.597$) had no independent prognostic value (Supplementary Table S1).

At that cutoff value, representing the 88th percentile in our population, the sensitivity of the GRS for a diagnosis of de novo HCC was 24%, the specificity 87%, the likelihood ratio for the test positive 1.87 and the likelihood ratio for the test negative 0.87; choosing a more inclusive cutoff, i.e., a cutoff value representing the 75th percentile (> 0.457), the sensitivity of the GRS for a diagnosis of de novo HCC was 52%, the specificity 79%, the likelihood ratio for the test positive 2.51 and the likelihood ratio for the test negative 0.60.

Figure 2 shows the Kaplan–Meier estimates in the study population, grouped according to the two categories of the GRS deriving from using this latter cutoff (0.457).

Table 1 Main characteristics of the study population (presented as a whole, as well as divided in the following two groups: patients who developed de novo HCC, group A, vs. all others, Group B)

Parameter	Total $N=328$	Group A $N=21$	Group B $N=307$	p value
Age, years	67 [55–75]	67 [58–72]	67 [54–75]	0.863
Male sex, N	185 (56)	18 (86)	167 (54)	0.005
Caucasian race, N	318 (97)	21 (100)	297 (97)	1.000
Body Mass Index, kg/m^2	26.5 [23.3–30.0]	29.3 [24.5–31.5]	26.5 [23.3–29.7]	0.399
Diabetes, N	74 (23)	4 (19)	70 (23)	1.000
HCV genotype, N				
HCV-1a	48 (15)	3 (14)	45 (15)	
HCV-1b	141 (43)	11 (52)	130 (42)	
HCV-2	79 (24)	4 (19)	75 (24)	
HCV-3	42 (13)	2 (10)	40 (13)	
HCV-4	16 (5)	1 (5)	15 (5)	
Other, indeterminate	2 (<1)	0 (0)	2 (<1)	0.961
Circulating HCV RNA, IU/mL ($\times 10^3$)	772 [201–1910]	712 [255–1400]	780 [200–1937]	0.966
FIB-4	4.6 [2.9–7.7]	6.8 [3.4–8.7]	4.5 [2.9–7.4]	0.181
Liver stiffness, kPa	20 [15–27][a]	29 [22–39]	19 [14–26]	0.005
Esophageal varices, N	85 (34)[b]	11/18 (61)	74/235 (31)	0.017
Albumin, g/L	39 [35–42]	36 [35–39]	39 [35–42]	0.012
Bilirubin, mg/dL	0.9 [0.7–1.3]	1.1 [0.9–1.7]	0.9 [0.7–1.2]	0.009
INR	1.12 (1.05–1.21)[c]	1.17 [1.11–1.24]	1.12 [1.05–1.24]	0.277
Platelet count, $\times 10^9$/L	123 [85–162]	92 [61–158]	123 [86–162]	0.154

Continuous variables are presented as medians [interquartile range], categorical variables as frequencies (%). p values refer to comparison between Group A vs. Group B

[a]Data missing for 38 patients

[b]Data missing for 75 patients

[c]Use of antagonists of vitamin K by 8 patients

Table 2 Gene frequencies in the study population (as a whole, as well as divided in the following two groups: patients who developed de novo HCC, group A, vs. all others, Group B)

Parameter	Total $N=328$	Group A $N=21$	Group B $N=307$	p value
PNPLA3, CG/GG	176 (54)	14 (67)	162 (53)	0.262
G allele frequency	0.32			
MBOAT7, CT/TT	225 (69)	14 (67)	211 (69)	0.812
T allele frequency	0.45			
TM6SF2, CT/TT	43 (13)	4 (19)	39 (13)	0.498
T allele frequency	0.07			
GCKR, CT/TT	243 (74)	16 (76)	227 (74)	1.000
T allele frequency	0.48			
Genetic risk score (GRS)	0.330 [0.128–0.457]	0.458 [0.266–0.531]	0.330 [0.128–0.401]	0.068
GRS > 0.597 (85th percentile), N	38 (12)	3 (14)	35 (11)	0.722
GRS > 0.457 (75th percentile), N	75 (23)	11 (52)	64 (21)	0.002
HSD17B13, T/TA or TA/TA	140 (43)	5 (24)	135 (44)	0.108
TA allele frequency	0.24			

p values refer to comparison between Group A vs. Group B

Table 3 shows the summary results of the Cox model, built using the same variables as above, but with GRS
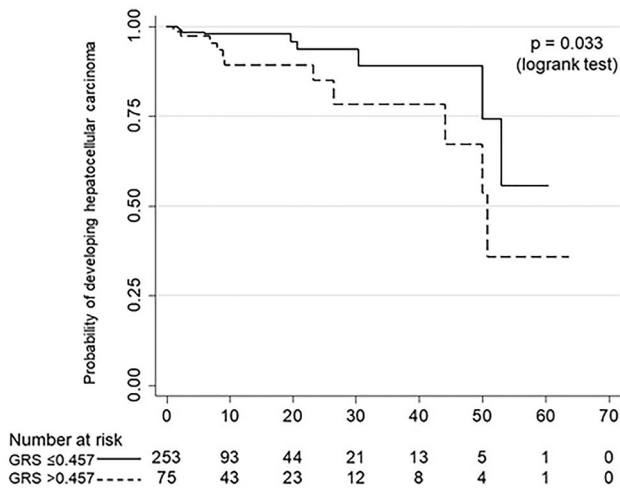
Fig. 2 Kaplan–Meier estimates of the probabilities of developing de novo hepatocellular carcinoma based on the Genetic Risk Score, using a cutoff value of 0.457 (limit of the highest quartile)

Table 3 Summary of the results of a Cox proportional hazards model, built adopting a cutoff value for the GRS corresponding to the highest quartile

Variable	Hazard ratio (HR)	95% CI of the HR	p value
Sex (female, male)	4.69	1.34–16.4	0.016
Diabetes (no, yes)	1.10	0.36–3.39	0.867
GRS > 0.457 (no, yes)	2.89	1.19–7.07	0.020
Albumin	0.32	0.11–0.89	0.029
INR	0.18	0.00–8.74	0.388
FIB4	1.04	0.96–1.14	0.322

No. of observations: 328; No. of failures: 21; time at risk (days): 136,813. Likelihood ratio chi square (6 degrees of freedom): 18.1

categorized adopting the 0.457 cutoff (model A). It can be appreciated that GRS emerged as an independent predictor of de novo HCC.

De novo HCC in relationship to possession of the HSD17B13 splice variant

A further Cox proportional hazards model was built adding to model A a further categorical variable related to *HSD17B13* genetic variants (model B). Table 4 presents the summary results of such model. Based on the likelihood ratio test, restricting the set of predictor variables to those included in model A significantly reduced its fit in comparison to model B (likelihood ratio chi square = 11.8, $p < 0.001$).

Table 4 Summary of the results of a Cox proportional hazards model, adding to the model A a categorical variable based on HSD17B13 genetic variability

Variable	Hazard ratio (HR)	95% C.I. of the HR	p value
Sex (female, male)	5.70	1.57–20.7	0.008
Diabetes (no, yes)	2.11	0.60–7.44	0.612
GRS > 0.457 (no, yes)	3.82	1.51–9.65	0.005
Albumin	0.29	0.11–0.79	0.015
INR	0.27	0.00–12.6	0.504
FIB4	1.04	0.95–1.13	0.432
HSD17B13, T/TA or TA/TA (no, yes)	0.26	0.08–0.82	0.021

No. of observations: 328, No. of failures: 21; time at risk (days): 136,813. Likelihood ratio chi-square (6 degrees of freedom): 24.4

De novo HCC in relation to treatment outcome

A final Cox proportional hazards model was built adding to model B a further categorical variable related to treatment outcome (SVR = 0, lack of SVR = 1; model C). Table 5 presents the summary results of such model. Based on the likelihood ratio test, restricting the set of predictor variables to those included in model B significantly reduced its fit in comparison to model C (likelihood ratio chi square = 9.13, p 0.003). Not unexpectedly, restricting the set of predictor variables to those included in model A significantly reduced its fit in comparison to model C (likelihood ratio chi square = 15.39, $p < 0.001$).

Discussion

The present study confirms that a polygenic risk score predicting the amount of hepatic fat can be deployed to risk stratify for de novo HCC among HCV-infected cirrhotics treated with DAA, thus confirming what observed in a recently published paper on the same topic [19]. Importantly, the contribution of the GRS to the residual risk of HCC is strengthened by adjusting for two potential confounders, treatment outcome (i.e., achievement of an SVR) and carriage of *HSD17B13* variants. The interpretation of these findings and their possible clinical implications will now be discussed at the light of current literature, starting with a summary on what is known regarding viral hepatocarcinogenesis.

The development of HCC in association with HCV infection is thought to derive from both direct and indirect mechanisms. In favor of the former are studies on transgenic animals, where HCC develops following the expression of HCV proteins with little or no inflammation in the liver parenchyma [21]. As for the latter, persistence of HCV

Table 5 Summary of the results of a Cox proportional hazards model, adding to the pre-DAA model a categorical variable related to treatment outcome (intention-to-treat)

Variable	Hazard ratio (HR)	95% CI of the HR	p value
Sex (female, male)	6.75	1.62–28.2	0.009
Diabetes (no, yes)	1.91	0.56–6.55	0.301
GRS > 0.457 (no, yes)	4.24	1.59–11.33	0.004
Albumin	0.36	0.12–1.12	0.077
INR	0.11	0.00–5.89	0.278
FIB4	1.04	0.97–1.12	0.297
HSD17B13, T/TA or TA/TA (no, yes)	0.24	0.07–0.75	0.015
Sustained viral response (yes, no)	7.70	2.38–25.0	0.001

No. of observations: 315; No. of failures: 21; time at risk (days): 132,963. Likelihood ratio chi-square (6 degrees of freedom): 25.7

induced chronic necroinflammation and subsequent generation of reactive-oxygen species is believed to facilitate chromosomal mutations and eventually malignant transformation of proliferating hepatocytes [22]. Beyond the virus, several other host and environmental factors are known contributors to hepatocarcinogenesis, including older age, male sex, alcohol abuse, severity of liver disease and ongoing liver damage [23, 24]. One further relevant risk factor for HCC is type 2 diabetes mellitus (DM). At variance with Degasperi et al. [19], our data did not show a significant association between DM and HCC in DAA treated patients. It is believed that DM favors the development of liver cancer, likely through signaling in the insulin/insulin growth factor axis [25]. However, the hepatocarcinogenetic role of DM in the setting of HCV infection is somewhat more controversial and possibly lower in comparison to other factors. Indeed, one of the earliest studies showing an association between DM and HCC development did not include cirrhotics [26]. A likely confounder is the temporal order between DM, cirrhosis and HCC as far as only incident (not prevalent) HCCs are considered. This was the case in a study conducted on residents of an area with a high prevalence of hepatitis virus infection, where DM was found to increase the risk of developing HCC only among those who were HCV negative or had a high level of total cholesterol. On the same line, several studies (reviewed by Li et al. [27]) have suggested that DM duration and type of pharmacologic treatment modulate the risk of developing HCC. Another major modifier of the risk of HCC in HCV-infected patients is alcohol consumption, not specifically addressed either in our study or in the study by Degasperi et al. In brief, possible explanations to interpret the discrepancy about DM as a risk factor between the two studies include differences in DM duration before HCC, DM pharmacologic treatment and extent of alcohol consumption.

Since the IFN era, it has been acknowledged that the risk of HCC after having achieved SVR primarily persisted in patients with advanced fibrosis or cirrhosis [28]. In an extensive retrospective cohort study including 22,500 HCV patients who received DAAs, Kanwal et al. have confirmed that the absolute risk of HCC remained high in DAA-treated HCV patients with established cirrhosis and in those who did not obtain SVR [29]. The residual risk of facing HCC onset after HCV eradication has been demonstrated to correlate with liver disease severity [30]. In a large prospective multicentric study of 2249 consecutive cirrhotic HCV patients treated with DAAs low albumin levels and platelet count, two proxies for advanced liver disease, together with failure to achieve an SVR, were independently associated with an increased risk of developing HCC during a mean follow-up of 14 months [6]. The highest predicted cumulative incidence of HCC was in patients who did not achieve SVR with albumin serum levels < 3.5 g/dL and platelet count below 120×10^9/L [6]. Thus, the established concept is that the more severe the liver disease the higher the risk of HCC and this is true whatever the parameter reflecting severity of liver disease is assessed (low albumin levels for impaired liver function, low platelet count for portal hypertension or stiffer liver for more advanced inflammatory damage and/or fibrosis). Here, while aiming at validating the independent association between a risk score based on the *PNPLA3*, *TM6SF2*, *MBOAT7* and *GCKR* and the development of de novo HCC in DAA treated cirrhotics, we outline the link between polygenic control of liver fat content and viral hepatocarcinogenesis. The improvement obtained by including into the Cox model the variable *HSD17B13* goes on the same line: indeed, carriers of the loss-of-function variant of this latter gene have less hepatic inflammation and fibrosis despite a high fat content [18]. It could be speculated that the contribution of genetic predisposition to liver fat accumulation on the one hand and the genetically determined way excess liver fat is handled, on the other, may lead to an acceleration of the natural history of hepatitis C. This process may start far earlier than at a cirrhotic stage.

Beyond the thought-provoking, proof-of-concept nature of this association between liver fat and liver cancer, one may wonder if measuring the genetically defined risk of excess hepatic fat will ever be of any practical use to stratify

HCV patients for the risk of developing HCC. Arguably, many clinicians caring for these patients would agree that the risk of developing HCC among those who have reached a cirrhosis stage prevents omitting surveillance for HCC based on a favorable GRS profile. It does not add to being confident on this regard the fact that in our series the GRS threshold identifying patients at risk for de novo HCC was 0.457, significantly lower than the previously suggested 0.597 value [19]; one possible explanation for this discrepancy between the two studies, similar for many other aspects, is that the sensitivity needed for this factor to emerge changes with the length of follow-up, being lower with the shorter observation time that we had. Without a shared threshold to categorize high vs. low-risk patients, the practical advantages of measuring the GRS of these patients are doubtful. On the other hand, the rationale for a hepatocarcinogenetic effect of a high GRS is strong and is strengthened by the observed protective effect of carrying a loss-of-function *HSD17B13* variant. The genes included in the GRS are weighted based on how strongly they modulate the hepatic fat content [16]. Fat accumulation creates a harmful environment that predisposes to enhanced oxidative stress and mitochondrial dysfunction, which in turn could be responsible for the malignant transformation of hepatocytes [31]. Thus, at least in theory, patients with high GRS may be the right target for future HCC chemoprevention strategies. It should be pointed out, however, that neither our study nor the seminal paper from Degasperi et al. [19] by design may claim that, once reached the cirrhosis stage, the hepatic fat content in this population of cirrhotics bears a relationship with the GRS. In fact, proliferating and newly regenerated liver cells are to such extent resistant to fat accumulation that, in end-stage cirrhosis due to alcoholic fatty liver disease, no steatosis remains visible [32]. Among cirrhotics, the negative effects that ensue from an unfavorable GRS profile may no longer been related to the fat content per se, but rather to multiple inflammatory mechanisms mediating transition to HCC [33]; in this scenario, anti-inflammatory drugs and/or oxygen scavengers would possibly be more valuable for HCC chemoprevention. Clearly, further studies are needed to clarify this issue.

A novel finding of our study is the demonstration that the role of GRS in predicting HCC development among DAA-treated cirrhotics is independent from viral clearance and carriage of the loss-of-function *HSD17B13* variant. The exact function of this gene variant remains incompletely understood. Proteins belonging to the HSD17B family are involved mainly in sex hormone metabolism; their expression differs between tissues [34]. The protein product of *HSD17B13* is mainly expressed in the liver, where is limited to hepatocytes in which it is targeted from the endoplasmic reticulum to lipid droplets (LDs) [35], localizing around them. *HSD17B13* overexpression is related with higher LDs and triglycerides accumulation in the liver: in fact, the increased expression of *HSD17B13* is linked to higher sterol regulatory-element binding protein 1 (SREBP-1) maturation, involved in cellular lipid homeostasis and with higher fatty acid synthase protein expression [36, 37]. Moreover, *HSD17B13* is a direct target gene of SRBP-1 and participates in de novo lipogenesis in the liver. Specifically, SRBP-1 increases HSD17B13 expression and, in turn, HSD17B13 promotes SREBP-1 maturation with positive feedback resulting in an increased fatty acid synthesis [38]. The protein has also a retinol dehydrogenase activity [39], which is interesting considering the hypothetical role of retinoids in HCC chemoprevention [40].

Carriage of the protein-truncating variant of *HSD17B13* is associated with lower levels of transaminases and with a lower risk of alcoholic liver disease and NAFLD in adults, as well as with lower hepatic steatosis in obese children [18, 41]. These protective effects may extend to HCC development both among HCV infected [13, 42] and non-HCV infected patients with chronic liver disease [43, 44], as well as in the general population [45]. Importantly, it has been shown that patients who carry the minor *HSD17B13* allele have reduced expression of the protein product in hepatocytes together with a dysregulation of genes involved in immune response, resulting in less severe histological inflammation [46]. Nonresolving inflammation is considered paramount to the development of HCC, through multiple mechanisms [47]. It must be pointed out, however, that *HSD17B13* deficiency in knock-out mice does not reproduce the protective role of *HSD17B13* loss-of-function mutants observed in humans [48]: the reasons for these interspecies differences are unknown and may include the failure of animal models to fully capture the steatohepatitis phenotype, compensation by other enzyme(s) and differences in substrate and/or functions between humans and other species.

Whatever is the mechanism by which GRS modulates the risk of developing HCC (increased fat hepatic content vs. establishment of a proinflammatory state), it does not require the presence of an ongoing HCV infection, though it may act synergistically with it. Earlier data from our group led us to suggest that, since patients with active HCC have a lower response to DAA [49], lack of achieving SVR may be considered a clue for the presence of hidden HCC foci [50]. Here we show that adding treatment outcome to other predicting variables improves the goodness-of-fit of a nested time-to-event model of HCC development including GRS, giving further emphasis to the role played by an unfavorable GRS profile in this peculiar setting.

We must acknowledge that the present study has several important limitations, starting with its retrospective nature, that may have generated a selection bias, though we took all the precautions to include all HCV cirrhotics consecutively treated with DAAs at our center. The comparatively small

sample size and short duration of follow-up are of concern; however, we think that under such experimental conditions it would have been easier to refute than to confirm a role for the GRS. Importantly, we could not agree with the cutoff proposed by Degasperi et al. to define the residual risk of de novo HCC among DAA treated cirrhotics: clearly, this issue needs refinement before considering any implication of the GRS in clinical practice. Likely, this would require pooling our cohort with (an)other cohort(s) to provide a real external validation. In such a future study it would be important to include populations with greater genetic variability compared to the seminal study than the one we provided here, as the remarkable similarity between the measures of centrality and distribution of the GRS in the two studies demonstrates. We also believe that, in future studies, quantifying the hepatic fat and/or the degree of residual liver inflammation after DAA treatment might prove of help for better understanding the implications of possessing an at-risk GRS profile in this setting.

In conclusion, we confirm that the GRS is an independent predictor of de novo HCC in DAA treated cirrhotics with hepatitis C. Further studies to better elucidate the underlying mechanisms and the clinical implications of this association should be devised.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12328-021-01578-1.

Author contributions Conceptualization, MP; Data handling, GM and RM; Statistical analysis, MP; Clinical management of patients, MEB and CR; Laboratory methods, MNB and VRM; Writing—original draft, MEB and MB; Writing—review and editing, RM and MP.

Declarations

Conflict of interest Mario Pirisi has received speaker honoraria from Gilead, Abbvie, Bayer and Eisai. All other authors declare no conflicts of interest.

Institutional review board statement The study was conducted in strict accordance with the Principles of the Declaration of Helsinki and was approved by the local ethical committee (Comitato Etico Interaziendale di Novara, filed as CE 215/19.).

Informed consent Informed consent was obtained from all subjects involved in the study.

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