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The Association of Genetic Variants with Hepatic Steatosis in Patients with Genotype 1 Chronic Hepatitis C Infection

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Abstract

Background—Single-nucleotide polymorphisms (SNPs) in the *IL28B* and *PNPLA3* gene regions have been associated with hepatic steatosis in genotype 1 (G1) chronic HCV infection but their clinical impacts remain to be determined.

Aim—We sought to validate these associations and to explore their impact on treatment response to peginterferon and ribavirin therapy.

Methods—A total of 972 G1 HCV-infected Caucasian patients were genotyped for the SNPs rs12979860 (*IL28B*) and rs2896019 (*PNPLA3*). Multivariable analysis tested *IL28B* and *PNPLA3* for association with the presence of any steatosis (>0 %); clinically significant steatosis (>5 %); steatosis severity (grade 0–3/4); and the interacting associations of the SNPs and hepatic steatosis to sustained viral response (SVR).

Results—*IL28B* and *PNPLA3* polymorphisms were associated with the presence of any steatosis (rs12979860, $p = 1.87 \times 10^{-7}$; rs2896019, $p = 7.56 \times 10^{-4}$); clinically significant steatosis (rs12979860, $p = 1.82 \times 10^{-3}$; rs2896019, $p = 1.27 \times 10^{-4}$); and steatosis severity (rs12979860, $p = 2.05 \times 10^{-8}$; rs2896019, $p = 2.62 \times 10^{-6}$). Obesity, hypertriglyceridemia, hyperglycemia, liver fibrosis, and liver inflammation were all independently associated with worse steatosis. Hepatic steatosis was associated with lower SVR, and this effect was attenuated by *IL28B*. *PNPLA3* had no independent association with SVR.

Conclusions—*IL28B* and *PNPLA3* are associated with hepatic steatosis prevalence and severity in Caucasians with G1 HCV, suggesting differing potential genetic risk pathways to steatosis. *IL28B* attenuates the association between steatosis and SVR. Remediable metabolic risk factors remain important, independently of these polymorphisms, and remain key therapeutic goals to achieve better outcomes for patients with HCV-associated hepatic steatosis.

Keywords

Polymorphism, single-nucleotide, SNP; *IL28B* protein, human; *PNPLA3* protein, human; Adiponutrin, human; Fatty liver; Abdominal obesity metabolic syndrome

Introduction

Hepatic steatosis is frequently observed in the setting of chronic hepatitis C virus (HCV) infection and has been associated with poor treatment response (SVR, sustained viral response) and more advanced hepatic fibrosis [1–3]. In genotype 3 HCV infection, viral factors are implicated in the development of steatosis, while for genotype 1 (G1) HCV, host metabolic risk factors such as obesity and insulin resistance afford increased risk [4, 5].

The increasing burden of chronic HCV infection, in combination with an epidemic of obesity in the West, suggests that hepatic steatosis will have an increasing contribution to liver disease [6–10]. Better understanding of the interactions between host genetic and metabolic risk factors for HCV-related steatosis is thus important.

Recently, an association between *IL28B* polymorphism and hepatic steatosis in Caucasian patients with mixed HCV genotype infection (324 patients with G1 HCV) was recognized [11]. The relatively small proportion of G1 HCV patients limited exploration of the interrelationships between these SNPs, hepatic steatosis, and treatment outcomes. Our group also reported the association with *IL28B* and hepatic steatosis in a combined clinical and trial cohort, but again only a limited number of patients had SVR data ($n = 164$) [12]. A non-synonymous polymorphism in the patatin-like phospholipase domain-containing 3 gene (*PNPLA3* rs738409, C > G encoding I148 M,) has been associated with hepatic fat content in non-alcoholic fatty liver disease (NAFLD) [13, 14] and histological features of NAFLD severity [15], though its functional mechanism remains unclear. European candidate gene studies have confirmed the association with *PNPLA3* and hepatic steatosis in chronic HCV infection, further correlating the risk allele with hepatic fibrosis, though with conflicting results regarding the association between *PNPLA3* and SVR [11, 16, 17]. We sought to validate the associations of *IL28B* and *PNPLA3* on G1 HCV-associated hepatic steatosis, and to assess their impact on SVR, in a large, well-characterized clinical trial cohort.

Methods

Patients

The IDEAL trial was a multi-center, randomized control trial that demonstrated similar efficacy and adverse events of differing regimens of peginterferon-alfa and ribavirin (PEG/RBV) in 3,070 North American patients with chronic G1 HCV (ClinicalTrials.gov number, NCT00081770) [18]. A pharmacogenomics sub-cohort from the trial consented to DNA testing and has been previously analyzed for genome-wide associations with treatment response [19] and ribavirin-induced hemolytic anemia [20]. To explore and validate previous European Caucasian genetic associations studies, the analysis was limited to Caucasians ($n = 972$). Clinical characteristics of the study population are presented in Table 1.

Steatosis was blindly evaluated by a single expert histopathologist (ZG) and graded on the percentage of hepatocytes with steatosis [grade 0 (0 % hepatocytes); 1 (>0–5 %); 2 (>5–32 %); 3 (>32–66 % hepatocytes affected); or 4 (>66 % hepatocytes affected)]. For inclusion in this study, patients required a minimum of six portal tracts in the liver biopsy. The mean biopsy length was 21 mm, with 59 % having liver biopsies more than 15 mm in length. The

mean body mass index (BMI) was 27.8 kg/m², with over a quarter of patients classified as obese (BMI > 30 kg/m²). In keeping with what one might expect clinically in a G1 HCV cohort, hepatic steatosis was typically not severe (15 % > grade 1).

As per the IDEAL trial protocol, a history of alcohol consumption of more than 80 g/day within 2 years of enrollment, or histological evidence of significant steato-hepatitis were exclusion criteria [18]. Patients on statin therapy at any time during the study period were excluded from the genetic analysis to avoid potential confounding ($n = 46$).

Genetic Analysis

Patients were genotyped using the Illumina Human610- quad BeadChip. Ethnicity was genetically inferred, and principal components analysis was conducted using a modified Eigenstrat method to correct for population sub-stratification [21]. Full genotyping procedures and quality-control measures undertaken have been previously described in the original study from which this dataset was extracted [19]. Here we validate genetic associations of *IL28B* (rs12979860) and *PNPLA3* (using proxy SNP rs2896019) with HCV-associated hepatic steatosis in Caucasian patients, using conventional statistical significance criteria ($p < 0.05$).

rs12979860 variant, upstream from the *IL28B* gene, is a biallelic variant where CC genotype is associated with better treatment response and genotypes with the T allele (CT and TT variants/genotypes) associated with relatively poorer treatment response. Unfortunately, the *PNPLA3* variant rs738409 is not genotyped directly on the Illumina Human610- quad BeadChip. Using open access software (SNAP 2.2, Broad Institute, Harvard University, MA, USA) [22], we searched the 1,000 Genomes Reference database [23] for an alternative, proxy SNP present on the GWAS chip that was correlated (in linkage disequilibrium, LD) with the *PNPLA3* variant rs738409. The SNP in strongest LD with rs738409 identified on the chip was rs2896019 ($R^2 = 0.688$, $D' = 0.94$).

Genome analysis was conducted with PLINK software and additional SVR analyses was conducted with STATA (StataCorp, College Station, TX, USA) and SAS 9.2 (SAS Institute, Cary, NC, USA) software [24].

Phenotype Definition of Hepatic Steatosis

Hepatic steatosis was modeled in three ways. Firstly, to assess whether genetic variants were protective for steatosis, we assessed the total absence of hepatic steatosis (binary variable in logistic regression model, 0 % or greater than 0 % hepatocytes with steatosis). Next, we assessed “clinically significant” steatosis using conventionally accepted definitions (binary variable/logistic model, 0–5 % vs. greater than 5 % steatosis). Finally, we assessed severity or grade of hepatic steatosis [ordinal logistic regression model, with grades 3 and 4 combined to satisfy proportional hazards assumptions (Supplementary Table 1)].

Covariates adjusted for in the models included age, gender, body mass index (kg/m²), baseline HCV viral load (log₁₀ IU/ml), fibrosis (binary variable as METAVIR stage F0–2 vs. F3–4), inflammation (METAVIR grade A0–1 vs. A2–3), alanine transaminase (ALT) values, baseline fasting blood glucose levels and genetically defined ancestry sub-population sets (or Eigenstrat vectors). Plasma HCV RNA concentrations were measured using the COBAS Taqman assay with a lower limit of quantitation of 27 IU/ml (Roche Diagnostics, Indianapolis, IN, USA).

Results

IL28B and PNPLA3 Variants Are Associated with Genotype-1 HCV-Induced Hepatic Steatosis Prevalence and Steatosis Severity

Genotype frequencies for *IL28B* rs12979860 were: TT: 117 (12.1 %); TC: 490 (50.8 %); CC: 357 (37.0 %). Patients with the T allele had significantly more steatosis across all grades of steatosis (Fig. 1). The presence of any steatosis (>0 %) was significantly associated with the poor response T allele (OR: 1.88, $p = 1.87 \times 10^{-7}$, Table 1). Clinically significant hepatic steatosis (>5 % steatosis) ($p = 1.82 \times 10^{-3}$) and hepatic steatosis severity were also both associated with the T allele (OR 1.80, (95 % CI 1.47–2.21), $p = 2.05 \times 10^{-8}$, Table 2).

Genotype frequency for the *PNPLA3* proxy SNP rs2896019 (where the G allele is associated with steatosis risk) were: GG: 26 (2.7 %), GT: 264 (27.2 %) and TT: 682 (70.2 %). Univariate analysis demonstrated patients with the G allele had proportionally more steatosis for each grade, and proportionally less were free of steatosis (grade 0, 33.1 vs. 42.7 % G allele vs. TT genotype, respectively, Fig. 2). On Logistic regression multivariable analysis (MVA), *PNPLA3* was independently associated with the presence of any steatosis (OR (G allele): 1.62, (95 % CI 1.22–2.14), $p = 7.56 \times 10^{-4}$, Table 3); the presence of clinically significant hepatic steatosis ($p = 1.27 \times 10^{-4}$); and more severe steatosis (OR 1.78 (95 % CI 1.40–2.27) $p = 2.62 \times 10^{-6}$, Table 3).

Finally, we sought to assess how the additional genetic information improved the model's explanation of variability in steatosis severity (as measured by pseudo R^2). Inclusion of either or both *PNPLA3* and *IL28B* SNPs provided very modest improvement in model performance as indicated by relative change in the pseudo R^2 (Table 4).

Host Metabolic Risk Factors Are the Major Determinants of Hepatic Steatosis

BMI had the most statistically significant association with steatosis severity (*IL28B* model: OR 1.11, (95 % CI 1.07–1.14), $p = 4.78 \times 10^{-11}$; *PNPLA3* model: OR 1.11, (95 % CI 1.07, 1.15), 1.41×10^{-11} , Tables 2, 3). Other clinical variables had significant association with the respective steatosis severity models, with differing effect sizes, including age, histological evidence of advanced hepatic fibrosis and inflammation, elevated ALT, elevated baseline fasting glucose and baseline triglycerides.

We explored whether these genetic associations may be working indirectly through other steatosis risk factors, or interacting with each other. For its association with steatosis severity, *IL28B* showed interaction with alanine aminotransferase (ALT) ($p = 0.003$); triglyceride (TG) levels ($p < 0.0001$); fasting glucose levels ($p = 0.045$) and fibrosis ($p < 0.0001$). However, these factors differed minimally when we compared univariate analysis between patients with the good response to poor response variants, with minor differences only in ALT levels (2.6 vs. 2.0 times the upper limit of normal, respectively, $p < 0.0001$) and fasting blood glucose levels, (mean glucose level 5.14 vs. 5.31 mmol/l, respectively, $p = 0.0066$). We observed no interaction between the effect of *PNPLA3* and *IL28B* for steatosis. On sub-analysis limited to patients with the *PNPLA3* risk allele (rs2896019 TG or GG), the p value for association between rs12979860 and steatosis is 0.00013345 based on logistic regression, while for patients without the risk genotype (rs2896019 TT genotype), for the association between rs12979860 and steatosis the p value was 0.00103807.

PNPLA3's effect on steatosis was not influenced by fibrosis, BMI, ALT, TG or glucose levels, in its association with steatosis severity (interaction p values all non-significant), thus offering little clue to pathogenic pathways linking *PNPLA3* to steatosis. The *PNPLA3* effect

on steatosis was influenced by age ($p = 0.017$), but age distributions did not differ when compared between genotypes.

PNPLA3 variant's association with hepatic fibrosis was dependent on the genetic model used (rs2896019 association with $>F2$ fibrosis, recessive model GG vs. non-GG: $p = 0.695$; dominant model GG and TG vs. TT: $p = 0.040$; and, additive model GG vs. GT vs. TT: $p = 0.089$).

***IL28B* Attenuates the Impact of Hepatic Steatosis on SVR but *PNPLA3* Has No Significant Association with SVR Directly or Indirectly Through Steatosis**

The absence of clinically significant steatosis ($< 5\%$ hepatocytes) was associated with improved SVR in a multivariable model that did not include *IL28B* or *PNPLA3* genotype (OR 2.20 (95 % CI 1.39–3.47) $p = 0.001$, Supplementary Table 2). After the inclusion of *IL28B* as a covariate into the model, the effect and significance of hepatic steatosis on treatment response was attenuated, however, steatosis remained an independently significant predictor (OR 1.73 (95 % CI 1.05–2.83) $p = 0.031$, Supplementary Table 3).

No significant independent association between *PNPLA3* and SVR was observed in a multivariable analysis ($p = 0.294$) and the inclusion of *PNPLA3* in the model did not alter the strength or significance of the association between steatosis and SVR (Supplementary Table 4). We ran this same model for the presence of any steatosis ($>0\%$) and there was similarly no significant relationship. Thus, on MVA we were unable to identify a direct association between *PNPLA3* and SVR, nor find any evidence of *PNPLA3* indirectly altering the effect of steatosis on SVR.

Finally, we assessed whether *PNPLA3* was significant when analysis was limited to those patients with advanced fibrosis (METAVIR F3). In this limited subgroup (limited further by available response data, $n = 85$), we found no association between *PNPLA3* and SVR ($p = 0.288$). Interestingly, for these patients with advanced fibrosis, the only significant predictors of SVR were total cholesterol (OR 1.82; 95 % CI: 0.67–4.75; $p = 0.033$) and fasting blood sugar (OR 2.40 (per unit decrease mmol/l); 95 % CI: 1.09–5.29; $p = 0.029$), but not HCV RNA burden ($p = 0.408$).

Discussion

We have shown that the prevalence and severity of hepatic steatosis in Caucasian patients with G1 HCV is associated with *IL28B* and *PNPLA3* genotypes. Our findings are consistent with previous candidate-gene association studies [11, 12, 16, 17]. Our results also suggest that even allowing for these significant genetic associations, metabolic and clinical risk factors continue to be significant for HCV-induced hepatic steatosis. We have shown that hepatic steatosis remains independently associated with SVR even after consideration of host genetic variants, though importantly, the association between steatosis and SVR is attenuated by *IL28B*. We found no association between the *PNPLA3*-proxy variant and SVR or fibrosis.

For clinicians, these results may help to place in context the clinical impacts of host genetic variation on hepatic steatosis. This data supports an important yet relatively modest role for host genetic variation explaining the variability of HCV-associated steatosis. Our study reiterates the clinical link between less steatosis and improved treatment responses, and may support attempts to motivate patients with lifestyle modification such as weight loss [25, 26]. Persistent fatty liver disease can remain a problem for patients with G1 HCV even with successful viral clearance [2, 3]. Thus, despite higher SVR rates anticipated in the era of HCV direct acting antiviral agents, hepatic steatosis may remain a clinical problem even for

patients cured of HCV. For patients, this may mean a “transition” from one primary liver disease (HCV) to another (NAFLD), further underscoring the importance for clinicians to seize opportunities to optimize metabolic risk factors. Host *IL28B/PNPLA3* genotype may help to identify those patients at increased risk of steatosis, and consequently those who may benefit most from lifestyle intervention.

It is important to note the differences in this analysis compared to previously reported candidate-gene associations with hepatic steatosis. A major caveat to our results was the use of a proxy-SNP for the *PNPLA3* variant rs738409. Despite this, we demonstrated significant associations between *PNPLA3* and all steatosis phenotypes, but not for SVR nor convincingly so for fibrosis. While acknowledging the possibility of false-negative associations for fibrosis and SVR due to the use of a proxy SNP, our results show some consistency with others' findings. Trepo et al. [17] demonstrated an association between *PNPLA3* and hepatic steatosis and fibrosis in multivariate models with dependence on the genetic model and no significant relationship was found between *PNPLA3* and SVR. In contrast, we found a significant relationship with the primary phenotype of steatosis in our proxy SNP irrespective of the model used, suggesting the SNP was a valid candidate to study for association. Trepo et al. found no association between *IL28B* (rs12979860) and hepatic steatosis in contrast to our highly significant association across a variety of phenotypes, further suggesting that cohort differences may be important in differing genetic association results rather than sub-optimal linkage disequilibrium of the candidate SNP.

Our study had the advantages of a large sample size, with uniform, protocol-based assessment. In particular, liver biopsy assessment was by a single, expert histopathologist, thus reducing inter-observer variability. Our cohort had proportionally less patients with advanced hepatic fibrosis than these European studies (11.6 % of this cohort with >F2 METAVIR stage compared to 26–40 %), [11, 16, 17] and thus potentially underpowered to find associations with fibrosis, or in subgroup analysis of SVR in patients with advanced fibrosis. *PNPLA3* has been associated with alcohol-related liver injury and cirrhosis, and alcohol is an important co-factor in liver injury and fibrosis in chronic HCV infection [27–29]. *PNPLA3* clinical associations with steatosis and fibrosis in chronic HCV are modulated, though not dependent on alcohol use [30, 31]. Significant alcohol intake was an exclusion criterion for this clinical trial cohort study, while for instance in the Swiss study a third of patients reported consuming more than 20 g of alcohol per day [11]. Importantly, we were not able to quantify alcohol exposure except as less than 80 g per day, yet doses less than this may contribute significantly to steatosis, and were not captured by our study. While we were able to consider for fasting glucose, the absence of insulin resistance data was a major limitation, and necessary to qualify the analyses of both steatosis and SVR. Finally, over a quarter of our cohort were obese (BMI>30 mg/kg²) with a mean BMI of 27.8 mg/kg², higher than comparative European cohort studies where median BMI varied from 23.7 to 25.9 mg/kg² [11, 16, 17]. Thus, a range of cohort factors including rates of hepatic fibrosis, contributing alcohol use, and obesity may provide differing environmental contributions to steatosis, and thus influence the relative contribution of host genetics. While reflecting the heterogeneity of genetic and environmental contributions to steatosis and fibrosis, it also underscores the importance of validating host genetic-clinical associations in variety of clinical contexts and populations.

The functional underpinning of the *IL28B* and *PNPLA3* associations with HCV-induced steatosis is not clear. A weak correlation has previously been found between interferon-stimulated gene expression and hepatic steatosis [32], and higher levels of ISG expression are correlated with the unfavorable *IL28B* genotypes [33–35]. This association may therefore represent a non-specific association between intrahepatic inflammation/ISG induction, and this might be reflected by the significant interaction between ALT and *IL28B*

for steatosis. *PNPLA3* mRNA expression has been found to be decreased in patients with advanced fibrosis or cirrhosis from alcohol and non-alcoholic fatty liver disease but intriguingly, *PNPLA3* gene expression did not differ by *PNPLA3* genotype, nor does *PNPLA3* genotype differentiate the expression patterns of other candidate-gene pathways involved in steatosis, adipogenesis, or fibrogenesis. While it is possible to speculate on inflammatory pathways as a possible link between *IL28B* and hepatic steatosis, hypotheses on potential pathogenic links between *PNPLA3* and steatosis are less clear. Further exploration of these functional pathways and genotype-based differential gene expression patterns may help understand their pathogenesis in HCV-associated hepatic steatosis.

Our observations help to clarify the role of genetic variants in G1 HCV-associated hepatic steatosis and reflect its complex, multifactorial nature. We confirm significant genetic associations between *IL28B* and *PNPLA3* and hepatic steatosis. We show that beneficial *IL28B* genotype attenuates the negative effect of hepatic steatosis with SVR, but found no equivalent relationship for *PNPLA3*. We found no significant interaction between these SNPs in their effect on steatosis in contrast to recent reports, where *IL28B* association with steatosis was apparent only in patients without the G risk allele for *PNPLA3* (rs738409) [36]. In these multivariable models, remediable risk factors such as BMI and fasting plasma glucose all remain independently significant, identifying important therapeutic opportunities to help patients modify risks. For patients with G1 HCV, improving hepatic steatosis remains an important clinical goal, irrespective of host genotype. At the population level, development of effective, lifestyle interventions will be critical to improve hepatic steatosis, achieve better HCV treatment responses and importantly, better liver health after HCV cure.

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This study is conducted for ideal investigators.

Abbreviations

HCV	Hepatitis C virus
SNP	Single-nucleotide polymorphism
IL28B	Interleukin 28B
PNPLA3	Patatin-like phospholipase domain-containing 3
G1	Genotype 1
MVA	Multivariable analysis
SVR	Sustained viral response
NAFLD	Non-alcoholic fatty liver disease

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Fig. 1. Proportion of patients by IL28B genotype (rs12979860, CC vs. CT/TT) by steatosis grade. Patients with the T allele (CT/TT genotypes) have proportionally more severe steatosis than patients with CC genotype (one-sided Wilcoxon rank-sum p value = 4.821×10^{-8})

Fig. 2.

Proportion of patients by PNPLA3 genotype (rs2896019, TT vs. GT/GG) by steatosis grade patients with the G allele (GT/GG genotypes) have proportionally more severe steatosis than patients with TT genotype (one-sided Wilcoxon rank-sum p value = 8.282×10^{-5})

Table 1

Clinical characteristics of the study population

Cohort characteristic	
<i>n</i>	972
Gender (M/F)	601/371
Age ($\pm$ SD)	47.1 ($\pm$ 7.6 years)
BMI (kg/m^2) mean, ($\pm$ SD)	27.8 ($\pm$ 4.5)
BMI > 30 kg/m^2 <i>n</i> , (%)	264 (27.2 %)
Steatosis grade 0 <i>n</i> , (%)	387 (39.8 %)
Steatosis grade 1 <i>n</i> , (%)	441 (45.4 %)
Steatosis grade 2 <i>n</i> , (%)	123 (12.7 %)
Steatosis grade 3/4 <i>n</i> , (%)	21 (2.1 %)
Hepatic fibrosis (METAVIR F3–4) <i>n</i> , (%)	113 (11.6%)
Activity grade (% with grade 2–3)	812 (83.5 %)
Viral load ($\log_{10}$ IU), median (25–75th percentile)	6.47 (6.03–6.81)
Fasting glucose (mmol/l), median (25–75th percentile)	5.10 (4.80–5.60)
Baseline triglycerides (mg/dl), median (25–75th percentile)	103.6 (77.06–148.0)
Baseline total cholesterol (mg/dl), median (25–75th percentile)	177.1 (155.8–199.2)
ALT ($\times$ ULN), median (25–75th percentile)	1.65 (1.15–2.63)
Liver biopsy length (mm) (mean)	20.9
Number of samples with a biopsy length >15 mm (%)	576 (59.3 %)
SVR (%)	433/780 (55.5 %)

SD standard deviation, *BMI* body mass index, *IU* international units, *ALT* alanine aminotransferase, *ULN* upper limit of normal, *SVR* sustained viral response

Table 2

Multivariable regression model results of *IL28B* (rs12979860, T allele) association with presence of steatosis and steatosis severity

Independent variables	Presence of steatosis ($\geq 0\%$)		Steatosis severity (grade 0–3/4)	
	Odds ratio	CI	p value	
rs12979860 [*]	1.88	(1.48, 2.39)	1.87×10^{-7}	1.80
Age	1.03	(1.01, 1.05)	1.45×10^{-3}	1.04
Gender (M/F) [§]	0.94	(0.69, 1.28)	0.70	0.91
BMI (kg/m ²)	1.11	(1.07, 1.15)	1.04×10^{-8}	1.10
Viral load (log ₁₀ IU)	0.92	(0.75, 1.13)	0.41	0.88
Advanced fibrosis [‡]	2.02	(1.16, 3.52)	1.29×10^{-2}	2.38
Activity grade [‡]	2.06	(1.38, 3.07)	3.77×10^{-4}	2.15
Baseline fasting glucose (mmol/l)	1.37	(1.11, 1.69)	3.51×10^{-3}	1.20
Baseline triglycerides (mg/dl)	1.00	(1.00, 1.01)	2.03×10^{-3}	1.00
Baseline total cholesterol (mg/dl)	1.00	(0.99, 1.00)	0.13	1.00
ALT ($\times$ ULN)	1.23	(1.11, 1.37)	1.06×10^{-4}	1.17

Model also adjusted for population sub stratification with Eigenstrat analysis (covariates not shown)

SD standard deviation, *BMI*/body mass index, *IU*/international units, *ALT*/alanine aminotransferase, *ULN*/upper limit of normal

^{*} Results correspond to the poor response T allele

[§] Female gender compared to male

[‡] Fibrosis stage F3–4 compared to F0–2

[‡] Inflammatory activity grade 2–3 compared to 0–1

Table 3

Multivariable regression model results of *PNPLA3* proxy SNP (rs2896019, G allele) association with presence of steatosis and steatosis severity

Independent variables	Presence of steatosis (>0 %)		Steatosis severity (grade 0–3/4)	
	Odds ratio	CI	p value	p value
<i>PNPLA3</i> rs2896019 G [*]	1.62	(1.22, 2.14)	7.56×10^{-4}	2.62×10^{-6}
Age	1.04	(1.02, 1.06)	5.85×10^{-4}	2.11×10^{-6}
Gender (M/F) [§]	0.98	(0.72, 1.33)	0.90	0.65
BMI (kg/m ²)	1.11	(1.07, 1.15)	3.03×10^{-9}	1.41×10^{-11}
Viral load (log ₁₀)	0.87	(0.71, 1.07)	0.18	6.69×10^{-2}
Advanced fibrosis [‡]	1.90	(1.10, 3.28)	0.02	3.66×10^{-5}
Activity grade [‡]	1.69	(1.15, 2.49)	7.72×10^{-3}	1.52×10^{-3}
Fasting plasma glucose	1.42	(1.15, 1.75)	1.05×10^{-3}	3.02×10^{-3}
Baseline triglycerides (mg/dl)	1.00	(1.00, 1.01)	1.87×10^{-3}	5.43×10^{-7}
Baseline total cholesterol (mg/dl)	0.99	(0.99, 1.00)	1.43×10^{-2}	1.69×10^{-2}
ALT (×ULN)	1.18	(1.07, 1.30)	1.04×10^{-3}	6.02×10^{-4}

Model also adjusted for population sub stratification with Eigenstrat analysis (covariates not shown)

SD standard deviation, *BMI*/body mass index, *IU*/international units, *ALT*/alanine aminotransferase, *ULN*/upper limit of normal

^{*} Results correspond to the risk G allele

[§] Male is used as base level

[‡] Fibrosis stage F0–2 is used as base level

[‡] Inflammatory activity grade 0–1 is used as base level

Table 4

Change in hepatic steatosis severity model performance by inclusion of rs12979860 and/or rs2896019 (for model covariates see Tables 2, 3)

	No SNP	rs12979860	rs2896019	Both
Pseudo R ² (%)	10.1	11.7	11.2	12.7