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# Effects of *IL28B* rs12979860 CC Genotype on Metabolic Profile and Sustained Virologic Response in Patients With Genotype 1 Chronic Hepatitis C

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## **Background & Aims**

Patients with genotype 1 chronic hepatitis C (G1 CHC) frequently develop steatosis and insulin resistance (IR), caused by metabolic and viral factors. These accelerate the progression of liver disease and reduce the response to therapy. A sustained virologic response (SVR) to therapy in patients with G1 CHC is associated strongly with polymorphisms near the *interleukin-28B* (*IL28B*) gene, but the interaction between *IL28B* genotype and IR, and their combined effects on SVR, have not been defined. We tested the association between the IL28B rs12979860 single-nucleotide polymorphism and metabolic features, including IR, and evaluated their effects on SVR.

### Methods

We performed genotype analysis of *IL28B* rs12979860 for 434 white G1 CHC patients who underwent consecutive biopsy analysis at 3 tertiary centers. Metabolic profile analyses included assessments of lipid levels and IR by the homeostasis model assessment.

### Results

Patients with the CC polymorphism in *IL28B* had higher levels of total and low-density lipoprotein cholesterol, lower levels of triglycerides, and a lower prevalence of IR and moderate-severe steatosis (P < .05) than patients without this genotype. By multiple logistic regression analysis, body mass index (odds ratio [OR], 1.223; P < .001), level of triglycerides (OR, 1.007; P = .006), the CC polymorphism in *IL28B* (OR, 0.378; P = .001), and levels of HCV RNA greater than 850,000 IU/mL (OR, 1.803; P = .01) were associated with IR. The CC polymorphism in *IL28B* (OR, 8.350; P < .001) and IR (OR, 0.432; P = .005), but not steatosis (OR, 0.582; P = 0.25), was associated with an SVR.

## Conclusions

In white patients with G1 CHC, the IL28B rs12979860 CC genotype is associated with reduced IR. IL28B rs12979860 genotype and IR by the homeostasis model assessment strongly affect the outcome of antiviral therapy.

#### Abbreviations used in this paper

- AUC, area under the receiver operating characteristic curve;
- BMI, body mass index;
- CHC, chronic hepatitis C;
- G, genotype;
- IL28B, interleukin 28B;
- HCV, hepatitis C virus;
- HDL, high-density lipoprotein;
- HOMA, homeostasis model assessment;
- IR, insulin resistance;
- LDL, low-density lipoprotein;
- OR, odds ratio;

- PEG-IFN/RBV, pegylated interferon/ribavirin;
- SNP, single nucleotide polymorphism;
- SVR, sustained virologic response

Hepatitis C virus (HCV) infection is characterized by a multifaceted interaction with host metabolic pathways. On the one hand, HCV is able to interrupt glucose homeostasis through direct and indirect mechanisms (up-regulation of tumor necrosis factor- $\alpha$ , down-regulation of suppressor of cytokine signaling and protein phosphatase 2A), leading to hepatic and extrahepatic insulin resistance (IR).<sup>1, 2 and 3</sup> On the other hand, the virus life cycle in all its phases (viral circulation in blood, liver cell uptake and export, and replication) relies on intracellular and extracellular lipids.<sup>4</sup> In addition, steatosis is observed in approximately 40% of livers from patients with chronic hepatitis C (CHC) after exclusion of common risk factors that are associated with steatosis.<sup>5</sup> Unlikely genotype 3 (G3) infection, in which the severity of hepatic steatosis correlates with the extent of viral replication and is reduced by successful therapy,<sup>6</sup> in genotype 1 (G1), steatosis correlates with metabolic variables, such as body mass index (BMI),<sup>7</sup> and tends to persist even after achieving a sustained virologic response (SVR).<sup>8</sup> Interestingly, greater degrees of virusinduced steatosis do not translate to high levels of IR, and IR by the homeostasis model assessment (HOMA-IR) scores are higher in patients with G1 than G3. Clinically, in G1 CHC this translates to accelerated liver disease progression (including the development of hepatocellular carcinoma),<sup>9 and 10</sup> a reduced response to antiviral therapy,<sup>11</sup> and, in susceptible individuals, an increased risk for developing type 2 diabetes.<sup>12</sup>

Single-nucleotide polymorphism (SNPs) around the gene encoding *interleukin-28B* (*IL28B*) are an important host factor for the achievement of an SVR after therapy with pegylated interferon and ribavirin. In particular, *IL28B* rs12979860 CC genotype has the strongest association with treatment-induced SVR. <sup>13 and 14</sup> However, contrasting data exist about the link between *IL28B* polymorphisms and both steatosis <sup>15, 16 and 17</sup> and IR. <sup>17 and 18</sup>

We studied a large multicenter cohort of consecutive patients with biopsy-proven G1 CHC to assess the putative association between *IL28B* polymorphisms and metabolic features, namely steatosis and IR, and their impact on the response to therapy. We show that the favorable *IL28B* genotype is associated with an improved metabolic profile. Further, IR rather than steatosis has a synergistic action with *IL28B* SNPs in the outcome of antiviral therapy.

## **Patients and Methods**

This cohort study comprised 434 patients with CHC consecutively enrolled in 3 centers (Gastro-hepatology Division of the University Hospital Torino, Italy: n = 76; Westmead Millenium Institute, Sydney, Australia: n = 84; and the Gastrointestinal and Liver Unit of the University Hospital Palermo, Italy: n = 274) and fulfilling the following inclusion criteria: (1) diagnosis of CHC G1 infection based on hepatitis C serology and viral RNA, (2) histologic diagnosis on liver biopsy, and (3) alcohol consumption less than 20 g/d in the past 12 months. A total of 341 patients had a standard course of treatment with pegylated interferon/ribavirin (PEG-IFN/RBV) with a known virologic response 6 months after treatment. All responders and most non-SVR patients received combination treatment for 12 months, whereas some non-SVR patients had 12 weeks of combination therapy but did not achieve at least a 2-log decrease in viral load. Patients were excluded from the study if they were co-infected with either the hepatitis B virus or human immunodeficiency virus, or if they were not of northern European descent. All participants who met the eligibility criteria were recruited after they provided written informed consent. Blood samples were obtained at the time of liver biopsy and stored at -80°C for further analysis.

## Anthropometric and Laboratory Evaluation

Clinical and anthropometric data were collected at the time of liver biopsy. BMI was calculated on the basis of weight in kilograms and height in meters. Subjects were classified as normal weight (BMI, 18.5–24.9 kg/m<sup>2</sup>), overweight (BMI, 25–29.9 kg/m<sup>2</sup>), and obese (BMI > 30 kg/m<sup>2</sup>). The diagnosis of type 2 diabetes was based on the revised criteria of the American Diabetes Association of a fasting blood glucose level of 126 mg/dL or higher on at least 2 occasions. In patients with a previous diagnosis of type 2 diabetes, current therapy with oral hypoglycemic agents was documented.

Serum levels of total cholesterol, triglycerides, and liver function tests were determined by routine liver failure tests on the day of liver biopsy.

Plasma glucose levels were measured by the glucose oxidase method (Beckman Instruments, Fullerton, CA; interassay coefficients of variability, 4%). Quantitative measurement of insulin in serum was performed using an immunoradiometric assay kit (Radim, Pomezia, Italy; interassay coefficients of variability, 13%). Insulin determination in the 3 centers was standardized.

HOMA-IR was determined by using the following equation: insulin resistance (HOMA-IR) = fasting insulin ( $\mu$ U/mL) × fasting glucose (mmol/L)/22.5.<sup>19</sup> HOMA-IR values greater than 3 indicated IR, as previously published.<sup>20</sup> All patients were tested at the time of biopsy for HCV RNA (limit of detection, 12 IU/mL). Genotyping was performed using the INNO-LiPA, HCV II (Bayer Diagnostics, Tarrytown, NY) kit. HCV RNA levels were quantified at baseline and viral load was classified as low (<850,000 IU/mL) or high (≥850,000 IU/mL).

## Genotyping

In 274 subjects, genotyping for *IL28B* rs12979860 was performed using the TaqMan SNP genotyping allelic discrimination method (Applied Biosystems, Foster City, CA). Custom genotyping assays were performed by submitting the SNP sequences to Kbioscience (Rotterdam, NY). Genotyping was performed with SDS software v.1.3.0 (ABI Prism 7500, Foster City, CA) and conducted in a blinded fashion relative to patient characteristics. In the remaining patients, genotyping was performed with the restriction fragment length polymorphism technique: amplified DNAs were digested with Bsh1236I restriction enzyme and DNA fragments were analyzed by agarose gel electrophoresis.

## Histopathology

Liver biopsy was performed for all patients. Liver histology was used to determine the extent of steatosis, hepatic necroinflammation, and fibrosis. Steatosis was graded as absent (<5%), mild (5%–29%), or moderate/severe (>30%). Liver fibrosis was classified as absent/mild, moderate (advanced fibrosis but short of cirrhosis), or severe (cirrhosis), whereas necroinflammatory activity was classified as absent/mild and moderate/severe (based on the degree of piecemeal necrosis and lobular necrosis) according to the Metavir scoring system.<sup>21</sup>

## **Statistical Analysis**

Continuous variables were summarized as mean ± standard deviation, and categoric variables were summarized as frequency and percentage. The *t* test and the chi-square test were used where appropriate. Multiple regression models were used to assess the factors associated with HOMA-IR, steatosis, and SVR. In the first model, the dependent variable was HOMA-IR, which was coded as 0 if it was less than 3, and coded as 1 if it was 3 or greater; in the second model, the dependent variable was steatosis, which was coded as 0 if steatosis was absent/mild, and was coded as 1 if steatosis was moderate/severe; in the third model the dependent variable was the outcome, which was coded as 0 if SVR was not obtained, and was coded as 1 if SVR was obtained. Input variables included in the models were age, sex, BMI, triglycerides, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, blood

glucose, insulin, HOMA score, diabetes, steatosis, *IL28B* rs12979860 genotype, lobular inflammation, and fibrosis. The effect of *IL28B* rs12979860 was evaluated comparing the CC vs CT/TT genotypes. Variables associated with the dependent variable on simple logistic regression (probability threshold, P < .10) were included in multiple logistic regression. To avoid the effect of co-linearity, IR, HOMA score, blood glucose level, and insulin level, as well as total cholesterol, HDL cholesterol, and LDL cholesterol, were not included in the same multiple logistic regression. Receiver operating characteristic curves were applied to determine the area under the receiver operating characteristic curve (AUC) of the individual variables associated with SVR, and the capacity of the entire model to discriminate between patients with SVR and those without SVR. Regression analyses were performed using Proc Logistic, Proc *t* test, and Proc freq in SAS (SAS Institute, Inc, Cary, NC).<sup>22</sup>

### **Patient Features and Histology**

The baseline features of the 434 patients are shown in Supplementary Table 1. Despite the majority of patients being in the lean to overweight range, IR was observed in almost half of them, but only a minority had type 2 diabetes. The mean values for total cholesterol, HDL cholesterol, and triglyceride levels were within the normal range. One patient in 5 had severe fibrosis, and 1 patient in 3 had moderate to severe necroinflammation. About half of the patients had histologic evidence of steatosis, although the grading was moderate to severe in only 14.2%. A minority (15.9%) of patients had the *IL28B* rs12979860 TT polymorphism, compared with 52.5% and 31.6% with TC and CC variants, respectively. Interestingly, patients carrying the *IL28B* rs12979860 CC genotype had a different metabolic profile compared with those with TT/TC genotypes. Specifically, *IL28B* rs12979860 CC patients had a lower BMI, lower HOMA-IR and prevalence of IR, lower serum triglyceride levels but a higher total and LDL cholesterol level, and a lower prevalence of moderate-severe steatosis compared with patients with the TT/TC genotype (*P* < .001 for all). As reported previously, those with the *IL28B* CC genotype had a higher prevalence of viral load greater than 850,000 IU (*P* < .001 for all) (Table 1).

Variable	IL28B rs12979860 TT/TC n = 297 (68.4)	IL28B rs12979860 CC n = 137 (31.6)	P value
Age, y	51.7 ± 11.5	51.3 ± 13.0	.77
Male sex, %	48.8	40.1	.10
BMI, kg/m <sup>2</sup>	26.7 ± 4.9	25.6 ± 3.8	.02
Cholesterol level, mg/dL	169.2 ± 35.6	179.5 ± 37.9	.007
HDL cholesterol level, mg/dL	52.7 ± 19.8	49.6 ± 16.0	.11
LDL cholesterol level, mg/dL	106.5 ± 32.9	118.7 ± 34.7	.001
Triglyceride level, mg/dL	115.4 ± 80.3	99.3 ± 44.1	.03
Blood glucose level, mg/dL	95.3 ± 32.5	91.1 ± 35.6	.22
Insulin level, μU/mL	14.3 ± 8.5	11.1 ± 6.1	<.001
НОМА	3.42 ± 2.55	2.42 ± 1.43	<.001
IR–HOMA >3, %	48.6	26.4	<.001
Type 2 diabetes, %	10.1	5.1	.08
HCV RNA level >850,000 IU, %	48.6	67.2	<.001
Histology at biopsy			
Steatosis grade, %			
Absent-mild/moderate-severe	83.3/16.7	91.3/8.7	.03
Necroinflammatory activity, %			
Absent-mild/moderate-severe	66.2/33.8	63.1/36.9	.77
Fibrosis, %			
Absent-mild/moderate/severe	55.4/27.0/17.6	60/25.4/14.6	.6

## Table 1. Characteristics of 434 Patients With G1 CHC, According to IL8B rs12979860 CC Genotype Variable UL28B rs12979860 TT/TC n = 297 (68.4) IL28B rs12979860 CC n = 137 (31.6) P val

NOTE. Data are shown as mean ± standard deviation or as a percentage.

### **Factors Associated With Insulin Resistance**

By simple logistic regression, older age, higher BMI, lower HDL and higher LDL cholesterol levels, higher triglyceride levels, absence of *IL28B* rs12979860 CC, HCV RNA level greater than 850,000 IU, moderate-severe steatosis, moderate-severe necroinflammatory activity, and fibrosis were associated with IR (P < .10) (Table 2).

	HOMA-IR ≤3 n =	HOMA-IR >3 n =	Simple logistic regression		Multiple logistic regression	
Variable	253	181	OR (95% CI)	<i>P</i> value	OR (95% CI)	P value
Age, y	50.1 ± 12.4	53.0 ± 10.9	1.021 (1.004– 1.038)	.01	1.019 (0.997– 1.041)	.09
Male sex, %	51	55.8	1.212 (0.818– 1.795)	.33	_	
BMI, kg/m <sup>2</sup>	24.8 ± 3.5	28.5 ± 5.1	1.250 (1.175– 1.329)	<.001	1.223 (1.147– 1.303)	<.001
Cholesterol level, mg/dL	174.7 ± 37.9	169.8 ± 33.6	0.996 (0.991– 1.002)	.18	-	
HDL cholesterol level, mg/dL	53.7 ± 19.2	48.8 ± 17.8	0.986 (0.975– 0.997)	.01	0.988 (0.974– 1.002)	.09
LDL cholesterol level, mg/dL	113.4 ± 33.1	105.5 ± 35.1	0.993 (0.987– 0.999)	.02	-	
Triglyceride level, mg/dL	97.4 ± 51.5	130.4 ± 91.0	1.008 (1.004– 1.012)	<.001	1.007 (1.002– 1.012)	.006
IL28B rs12979860, % CC/TT-TC	39.4/60.6	19.8/80.2	0.379 (0.240– 0.597)	<.001	0.378 (0.218– 0.654)	.001
HCV RNA level >850,000 IU, %	49.4	59.3	1.492 (0.997– 2.232)	.05	1.803 (1.103– 2.947)	.01

Table 2. Factors Associated With IR in 434 Patients With G1 CHC

NOTE. Data are shown as mean ± standard deviation or as a percentage.

In a model excluding histologic variables, multiple logistic regression showed that IR was associated with BMI (odds ratio [OR], 1.223; 95% confidence interval [CI], 1.147–1.303; P < .001), triglycerides (OR, 1.007; 95% CI, 1.002–1.012; P = .006), *IL28B* rs12979860 CC (OR, 0.378; 95% CI, 0.218–0.654; P = .001), and HCV RNA level greater than 850,000 IU (OR, 1.803; 95% CI, 1.103–2.947; P = .01) (Table 2). After Bonferroni correction, only BMI (P < .001) and *IL28B* rs12979860 CC (P = .003) remained significantly associated with IR. When adding histologic variables, similar results were obtained, except that now fibrosis also was associated with IR (OR, 1.582; 95% CI, 1.119–2.238; P = .009). Supplementary Figure 1A shows the association between IR and *IL28B* rs12979860 genotype.

## Factors Associated With Moderate-Severe Steatosis

Older age, higher BMI, higher HOMA-IR, higher prevalence of IR, absence of *IL28B* rs12979860 CC, and moderate-severe necroinflammatory activity were associated with moderate-severe steatosis by simple logistic regression (P < .10) (Table 3).

	Steatosis <30% n =	Steatosis ≥30% n = 62	Simple logistic regression		Multiple logistic regression	
Variable	372		OR (95% CI)	<i>P</i> value	OR (95% CI)	P value
Age, y	51.0 ± 12.0	54.7 ± 11.0	1.028 (1.003– 1.054)	.03	1.022 (0.993– 1.050)	.13
Male sex, %	53.7	57.6	1.084 (0.623– 1.888)	.57	_	
BMI, kg/m <sup>2</sup>	26.0 ± 4.5	28.4 ± 4.5	1.098 (1.041– 1.159)	<.001	1.059 (0.997– 1.125)	.06
Cholesterol level, mg/dL	172.0 ± 36.2	171.8 ± 37.3	1.000 (0.992– 1.008)	.98	_	
HDL cholesterol level, mg/dL	51.2 ± 18.5	53.6 ± 21.3	1.008 (0.994– 1.024)	.39	_	
LDL cholesterol level, mg/dL	111.2 ± 34.0	104.2 ± 32.4	0.994 (0.985– 1.003)	.16	_	
Triglyceride level, mg/dL	110.3 ± 73.2	111.7 ± 64.5	1.000 (0.996– 1.004)	.89	_	
Blood glucose level, mg/dL	92.6 ± 33.4	102.4 ± 36.8	1.007 (1.001– 1.013)	.04	_	
Insulin level, μU/mL	12.5 ± 7.0	17.9 ± 11.2	1.070 (1.036– 1.106)	<.001	_	
НОМА	2.86 ± 1.90	4.55 ± 3.27	1.295 (1.157– 1.449)	<.001	_	
IR–HOMA >3, %	37.7	65.4	3.123 (1.718– 5.677)	<.001	2.124 (1.102– 4.092)	.02
Type 2 diabetes, %	7.8	13.5	1.838 (0.794– 4.253)	.15	_	
IL28B rs12979860, % CC/TT-TC	67.4/32.6	81.3/18.7	0.474 (0.237– 0.947)	.03	0.444 (0.199– 0.990)	.04
HCV RNA level >850,000 IU, <i>%</i>	54.8	54.3	1.067 (0.606– 1.877)	.94	_	

Table 3. Simple and Multiple Logistic Regression of Factors Associated With Steatosis of 30% or More

NOTE. Data are shown as mean ± standard deviation or as a percentage.

After exclusion of histologic variables, multiple logistic regression confirmed only HOMA-IR (OR, 2.124; 95% CI, 1.102–4.092; P = .02) and *IL28B* rs12979860 CC (OR, 0.444; 95% CI, 0.199–0.990; P = .04) as predictors of moderate-severe steatosis (Table 3). No variables remained significantly associated with moderate-severe steatosis after Bonferroni correction. When introducing other histologic variables, necroinflammatory activity as expected was associated with moderate-severe steatosis (OR, 2.983; 95% CI, 1.582–5.628; P = .001). Supplementary Figure 1*B* shows the association between steatosis grade and *IL28B* rs12979860 genotype.

#### Factors Associated With Sustained Virologic Response

SVR was achieved in 43.4% of the 341 patients who underwent antiviral treatment. A comparison of the variables associated with SVR is reported in Table 4. A multiple logistic regression was performed that included histologic features. Surprisingly, steatosis was not associated with SVR (OR, 0.582; 95% CI, 0.229– 1.476; P = .25), but the analysis confirmed other well-known predictors, such as age (OR, 0.972; 95% CI, 0.947–0.997; P = .02), serum LDL level (OR, 1.014; 95% CI, 1.005–1.022; P = .003), IR (OR, 0.432; 95% CI, 0.242–0.773; P = .005), *IL28B* rs12979860 CC (OR, 8.350; 95% CI, 4.235–16.463; P < .001), and HCV RNA level greater than 850,000 IU (OR, 0.273; 95% CI, 0.145–0.514; P < .001) (Table 4). After Bonferroni correction, LDL (P = .009), IR (P = .01), *IL28B* rs12979860 CC (P < .001), and HCV RNA greater than 850,000

IU (P < .001) remained significantly associated with SVR. The AUC of *IL28B* for the prediction of SVR was 0.67, whereas that of HOMA greater than 3 was 0.62. When combining these 2 variables, their AUC for SVR prediction was 0.73. Finally, the discriminant ability of the rule generated by the entire model was high (AUC, 0.81) (Figure 1).

Simple logistic regression Multiple logistic regression No SVR n = Variable SVR n = 148 193 Р Р OR (95% CI) OR (95% CI) value value 0.967 (0.948-52.7 ± 10.7 <.001 0.972 (0.947-0.997) .02 48.3 ± 12.2 Age, y 0.986) 0.873 (0.549-Male sex, % 45.1 49.3 .43 1.295) 0.970 (0.927-BMI,  $kg/m^2$  $26.6 \pm 4.5$  $25.9 \pm 5.1$ .19 1.015) 1.009 (1.002-Cholesterol, mg/dL  $167.5 \pm 34.5$ 177.9 ± 35.3 .007 1.015) 0.995 (0.983-HDL cholesterol, mg/dL 51.5 ± 18.7 49.8 ± 17.8 .40 1.007) 1.014 (1.007-LDL cholesterol, mg/dL 105.7 ± 31.4 120.4 ± 33.2 <.001 1.014 (1.005-1.022) .003 1.021) 0.998 (0.994-Triglycerides, mq/dL 117.1 ± 85.2 105.0 ± 59.6 .14 1.001) 0.987 (0.978-Blood glucose, mg/dL 96.9 ± 36.4 86.6 ± 27.2 .004 0.996) 0.928 (0.895-Insulin, μU/mL  $14.4 \pm 7.6$  $11.1 \pm 5.8$ <.001 0.962) 0.734 (0.633-HOMA <.001 3.48 ± 2.52  $2.43 \pm 1.52$ \_ 0.850) 0.324 (0.202-IR-HOMA >3, % 24.7 <.001 0.432 (0.242-0.773) .005 50.3 0.520) 0.258 (0.096-.004 3.3 Type 2 diabetes, % 11.9 0.697) 5.403 (3.295-8.350 (4.235-IL28B rs12979860, % CC/TT-TC 17.1/82.9 52.7/47.3 <.001 <.001 16.463) 8.859) 0.479 (0.310-HCV RNA >850,000 IU, % 60.1 41.9 .001 0.273 (0.145-0.514) <.001 0.740) Histology at biopsy Steatosis grade Absent-mild/moderate-0.385 (0.187-74.4/25.6 53.7/46.3 .01 0.582 (0.229-1.476) .25 severe 0.793) Necroinflammatory activity Absent-mild/moderate-0.590 (0.309-64.7/35.3 75.2/24.8 .12 1.012) severe Fibrosis 47.8/32.6/19.6 60.9/26.8/12.3 Absent-.05 0.965 (0.658-1.417) .85 mild/moderate/severe

**Table 4.** Simple and Multiple Logistic Regression of Factors Associated With SVR in 341 Patients With G1CHC

NOTE. Data are shown as mean ± standard deviation or as a percentage.



Evaluation of the rule predicting the SVR by receiver operating curve and AUC (0.81).

Of particular interest and clinical utility in this difficult-to-treat cohort was that the combination of IR and *IL28B* genotype progressively increased the likelihood of achieving an SVR from being least likely in patients carrying both negative predictors (IR and rs12979860 TT/TC; SVR, 20.7%), to intermediate in those with only one predictor (IR or rs12979860 CC; SVR, 38.7% or 51.7%, respectively), to the highest likelihood in patients with no IR and rs12979860 CC (SVR, 78.4%) (Figure 2).





Likelihood of SVR according to different patterns of baseline predictors.

#### Discussion

The principal findings of this study in a large cohort of white patients with G1 CHC and a low prevalence of obesity and type 2 diabetes is that the *IL28B* rs12979860 CC genotype is associated with both reduced steatosis and IR, despite higher serum levels of total and LDL cholesterol and viral load. As expected, steatosis and IR were associated negatively with the *IL28B* rs12979860 CC genotype after correction for BMI and lipid profile. These results suggest that the *IL28B* genotype is associated intimately with IR and steatosis, above and beyond an association with LDL status, as reported previously.<sup>23</sup> From a clinical perspective, the data additionally suggest that the determination of both HOMA-IR and *IL28B* genotype together can inform the therapeutic decision making in the context of combination therapy with PEG-IFN plus RBV, which still is widely used.

*IL28B* genotype is a well-established, host-related pretreatment predictor of SVR after PEG-IFN and RBV therapy in patients with CHC. <sup>13 and 14</sup> In our study, as expected, we confirmed the strong association of *IL28B* CC genotype with SVR, as well as its previously observed relationship with higher serum LDL <sup>23</sup> levels and viral load. <sup>13 and 14</sup>

Considering the presence of contrasting data on the association between *IL28B* genotype and both steatosis <sup>15, 16 and 17</sup> and IR <sup>17 and 18</sup> in G1 CHC, we sought to clarify these issues in a large cohort of well-characterized patients. Specifically, we showed that *IL28B* CC genotype is linked to a lesser degree of steatosis, in addition to known metabolic risk factors. This finding was not observed in other smaller reports, <sup>16 and 17</sup> although it is in agreement with what was observed by Tillmann et al<sup>15</sup> in 2 cohorts of G1 CHC. However, the latter study was performed in populations with a higher BMI, and correction for IR was not performed.<sup>15</sup> Another relevant finding of our study was that we showed an association between *IL28B* CC genotype and a lower prevalence of IR, after correction for known metabolic and virologic risk factors. This was not observed in a cohort of 202 patients with mixed infection (G1 and G4), who were also younger and had a lower prevalence of IR compared with our study population.

Our cross-sectional study was not designed to assess the pathogenic mechanisms linking the *IL28B* genotype to IR, but a few hypotheses may be entertained. One possibility is that lower IR (and steatosis) and higher levels of circulating lipids are linked to reduced intrinsic interferon-stimulated gene expression in the liver of *IL28B* CC patients as reported previously, <sup>24</sup> leading to less interference with insulin signaling on the one hand, and on the other hand to a more efficient export of lipids from hepatocytes. Indeed, experimental studies have shown that IFN-γ up-regulates suppressor of cytokine signaling 3 expression, which can impair insulin signaling and favor IR development.<sup>25</sup> As for lipid metabolism, high interferon expression is known to suppress lipoprotein lipase activity, leading to reduced conversion of very low density lipoprotein to LDL, and inducing fat accumulation in the liver.<sup>26</sup> Accordingly, in patients with HCV infection, exogenous interferon decreases LDL cholesterol and increases triglyceride levels in very low density lipoprotein by suppressing lipoprotein lipase.<sup>27, 28 and 29</sup> Alternatively, it could be speculated that interactions between *IL28B* genotype and hepatic necroinflammation or innate immunity may contribute to the phenotype. However, we did not find a significant association between *IL28B* genotype and necroinflammatory activity as reported recently, <sup>30, 31, 32 and 33</sup> perhaps as a consequence of differences in demographic, metabolic, virologic, and histologic characteristics of the studied populations.

A second important outcome of the present study was that when assessing the relative and combined effects of all variables on SVR, both *IL28B* rs12979860 CC genotype and the absence of IR, but not steatosis, predicted a better outcome. Ogawa et al <sup>34</sup> recently observed similar results in an older Asian population, in which another *IL28B* SNP was evaluated. In that study, the prevalence of steatosis was lower (36%), and was evaluated in a smaller subgroup (186 of 328). <sup>34</sup> Our results suggest that steatosis influences SVR only indirectly, perhaps through both metabolic (IR) and genetic pathways (*IL28B*). Second, from a clinical perspective, we suggest that the assessment of IR and *IL28B* genotype pretherapy can help guide decision

making with regard to the timing and choice of antiviral therapy, particularly in the context of the advent of direct-acting antiviral agents. Thus, the likelihood of SVR is low in patients with both unfavorable predictors (IR and *IL28B* rs12979860 TT/TC; 21% SVR), but is increased between 2- and 4-fold when patients had only one or none (~80% SVR) of the adverse markers. In an era in which scarcity of economic resources is a concern, our data could allow the identification of a subgroup of patients (ie, *IL28B* CC and no IR) at high likelihood of SVR, for whom dual therapy with PEG-IFN/RBV is equivalent to triple therapy, but with reduced costs and side effects. Our results are similar to those reported in other studies by combining different SVR predictors, for example, vitamin D and *IL28B* genotype, <sup>35 and 36</sup> and fibrosis and *IL28B* genotype, <sup>14</sup> underlying the need to identify, after external validation, easy-to-treat G1 CHC patients suitable for PEG-IFN/RBV therapy. Future studies need to evaluate if the reported interplay between IR and *IL28B* also will exert a role during triple therapy. In this context, preliminary results have suggested that HCV protease inhibitors improve insulin sensitivity by decreasing viral load, <sup>37 and 38</sup> and that IR does not affect SVR likelihood.<sup>38</sup> However, these data were obtained in a cohort of mostly normal weight young patients without data on the *IL28B* SNPs, and therefore needs validation.

The main limitation of this study was its cross-sectional nature, making it impossible to dissect the relationship between *IL28B* genotype, metabolic alterations, and their interplay with the likelihood of SVR in G1 CHC patients. A further methodologic issue is the potentially limited external validity of the results for different populations and settings. Thus, replicating our results in external cohorts is important. Lack of data on serum levels of adipocytokines, and on other polymorphisms that conceivably could confound the data, also may have affected the results. In conclusion, we found that in a large cohort of G1 CHC patients, the *IL28B* rs12979860 CC genotype, which is associated with a higher SVR rate, is also characterized by a better metabolic profile, including lower IR and hepatic steatosis. These results clarify controversial recently reported data and point to a complex interaction between *IL28B* genotype and metabolic pathways in the host. Although first-generation, direct-acting antiviral agents have been approved in many countries, <sup>39 and 40</sup> combination treatment with PEG-IFN/RBV is still widely used and our results have clinical utility with regard to the timing and choice of therapy.

#### Supplementary material

#### Supplementary Table 1.

Baseline Demographic, Laboratory, Metabolic, and Histologic Characteristics of 434 Patients With G1 CHC

Variable	G1 CHC (N = 434)
Age, y	51.5 ± 12.0
Male sex, %	53.9
BMI, kg/m <sup>2</sup>	26.3 ± 4.6
BMI, %	
<25	38.7
25–29.9	45.7
≥30	15.6
Cholesterol, mg/dL	172.5 ± 36.5
HDL cholesterol, <i>mg/dL</i>	51.7 ± 18.7
LDL cholesterol, <i>mg/dL</i>	110.4 ± 33.9
Triglycerides, mg/dL	110.3 ± 71.2
Blood glucose, mg/dL	93.9 ± 33.5
Insulin, μU/mL	13.3 ± 8.0
HOMA-IR	3.10 ± 2.31
IR–HOMA >3 <i>, %</i>	41.6
Type 2 diabetes, %	8.5

Variable	G1 CHC (N = 434)
IL28B rs12979860, % CC/TT-TC	31.6/68.4
HCV RNA >850,000 IU, %	54.7
Histology at biopsy	
Steatosis grade, %	
0 (<5%)	53.7
1 (5%–29%)	32.1
2 (≥30%)	14.2
Necroinflammatory activity, %	
Absent/mild	65.2
Moderate/severe	34.8
Fibrosis, %	
Absent/mild	56.9
Moderate	26.5
Severe	16.6

NOTE. Data are shown as mean ± standard deviation or as a percentage.



Metabolic alterations and *IL28B* genotype. Prevalence of (*top*) insulin resistance and (*bottom*) moderate-severe steatosis according to *IL28B* rs12979860 genotype

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