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IL28B polymorphisms, IP-10 and viral load predict virological response to therapy in chronic hepatitis C

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Abbreviations: HCV, hepatitis C virus; CHC, chronic hepatitis C; IFN, interferon; SOC, standard of care; IL28B, interleukin-28B; IP-10, interferon-gamma inducible protein 10 kDa (CXCL10); HOMA-IR, homeostasis model assessment of insulin resistance; RVR, rapid virological response; SVR, sustained virological response; OR, odds ratio; CI, confidence intervals; SNP, single nucleotide polymorphism; ITAHECS, Italian Hepatitis C Cohort Study; PCR, polymerase chain reaction; ALT, alanine aminotransferase; BMI, body mass index; PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio positive; LR-, likelihood ratio negative; DAA, direct active antiviral .

Keywords: IL28B, IFN-gamma inducible protein-10 (IP-10), hepatitis C virus, antiviral therapy, chronic hepatitis

ABSTRACT

Aim: To determine the independent contribution of factors including *IL28B* polymorphisms, IFN-gamma inducible protein-10 (IP-10) levels and the homeostasis model assessment of insulin resistance (HOMA-IR) score in predicting response to therapy in chronic hepatitis C (CHC).

Methods Multivariate analysis of factors predicting rapid (RVR) and sustained (SVR) virological response in 280 consecutive, treatment-naive CHC patients treated with peginterferon alpha and ribavirin in a prospective multicenter study.

Results Independent predictors of RVR were HCV RNA < 400,000 IU/ml (OR11.37; 95% CI 3.03-42.6), *rs12980275* AA (OR 7.09; 1.97-25.56) and IP-10 (OR 0.04; 0.003-0.56) in HCV genotype 1 patients and lower baseline γ-glutamyl-transferase levels (OR = 0.02; 0.0009-0.31) in HCV genotype 3 patients. Independent predictors of SVR were *rs12980275* AA (OR 9.68; 3.44-27.18), age < 40 yrs (OR = 4.79; 1.50-15.34) and HCV RNA < 400,000 IU/ml (OR 2.74; 1.03-7.27) in HCV genotype 1 patients and *rs12980275* AA (OR = 6.26; 1.98-19.74) and age < 40 yrs (OR 5.37; 1.54-18.75) in the 88 HCV genotype 1 patients without a RVR. RVR was by itself predictive of SVR in HCV genotype 1 patients (OR 33.0; 4.06-268.32) and the only independent predictor of SVR in HCV genotype 2 (OR 9.0, 1.72-46.99) or 3 patients (OR 7.8, 1.43-42.67).

Conclusions In HCV genotype 1 patients, *IL28B* polymorphisms, HCV RNA load and IP-10 independently predict RVR. The combination of *IL28B* polymorphisms, HCV RNA level and age may yield more accurate pre-treatment prediction of SVR. HOMA-IR score is not associated with viral response.

INTRODUCTION

Hepatitis C virus (HCV) infects up to 180 million people worldwide (1) and is a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma (2). The current treatment, based on the combination of peginterferon alpha and ribavirin, leads to a sustained virological response (SVR) in ~40-50% in patients with HCV genotype 1 and in ~80% of those with genotype 2 or 3 (3). Several baseline and on-treatment variables affect the likelihood of achieving SVR (4). Older age, advanced stage of fibrosis, African-American ethnicity and HCV- related factors, including HCV genotype 1 and high viral load at baseline, predict poor response to antiviral therapy. Furthermore, metabolic factors, such as high body mass index (BMI), presence and severity of liver steatosis and increasing homeostasis model assessment of insulin resistance (HOMA-IR) score have been reported as negative predictors of response (5-9). On the other hand, early on-treatment kinetics of HCV RNA, e.g. undetectable HCV RNA at week 4, has a high positive predictive value of SVR (10, 11).

Among the baseline predictors of response, the pre-treatment activation of IFN-stimulated genes (ISG) and the host genetic polymorphisms have been the subject of recent, major studies. Regarding ISG, it has been shown that low levels of intrahepatic and systemic CXC chemokine IFN-gamma inducible protein 10 kDa (IP-10, or CXCL10), a valid surrogate marker of ISG activation, predict a more pronounced first phase decline of HCV RNA during antiviral therapy (12) and increased SVR rates (13, 14). On the other hand, several independent studies have consistently shown that single nucleotide polymorphisms (SNPs) near *IL28B*, which encodes the type III interferon IFN-λ3 are strongly associated with the response to treatment of chronic hepatitis C (15-21). In particular, the

homozygous genotypes TT at marker *rs8099917*, CC at marker *rs12979860* and AA at marker *rs12980275* are all associated with favourable treatment outcomes. These data have been confirmed in populations of different ancestry and HCV genotypes, and in various clinical scenarios (22, 23). However, very few studies are available where all these novel pre-treatment variables are analyzed together in multivariate models.

We sought to extend our understanding of the impact of these recently reported genetic polymorphisms on treatment outcome by investigating the independent association of candidate genetic (including the *rs8099917*, *rs12979860* and *rs12980275* markers), host (including IP-10 levels and HOMA-IR score) and viral factors on virological response in a cohort of treatment-naïve, non-diabetic Caucasian patients with chronic hepatitis C.

PATIENTS AND METHODS

Patient population

Patients were recruited in an observational prospective cohort study, the Italian Hepatitis C Cohort Study (ITAHECS), designed to evaluate factors that influence response to antiviral therapy, including host metabolic factors. Overall, 500 consecutive, treatment-naïve patients with chronic hepatitis C were enrolled and treated between June 2006 and June 2007 at seven tertiary referral Italian liver units. Eligibility criteria for therapy included Caucasian ancestry, age between 18 and 65 years, detectable HCV RNA in serum by polymerase chain reaction (PCR) (see below) and alanine aminotransferase (ALT) above the upper limit of normal within 6 months of treatment initiation. Patients were not considered for therapy if pregnant or breast-feeding, had decompensated cirrhosis or any other contraindication to therapy with the combination of pegylated IFN-α and ribavirin, were positive for hepatitis B surface antigen or anti-HIV antibodies, or reported a high daily alcohol intake (>40 g/day). All patients were asked to completely abstain from alcohol during antiviral treatment. Therapy was based on the combination of pegylated IFN-α_{2a} (180 μg/week) or pegylated IFN-α_{2b} (1.5 μg/kg/week) plus ribavirin (800-1200 mg/day) for 48

weeks in HCV genotype 1 or 4 patients, and for 24 weeks in HCV genotype 2 or 3 patients. A rapid virological response (RVR) was defined as undetectable HCV RNA in serum at week 4 of therapy. Early virological response (EVR) was defined as serum HCV RNA negativity or any >2 $\log_{10}$ decline in HCV RNA levels at week 12 of therapy compared with baseline. Patients with sustained virological response (SVR) were those with undetectable HCV RNA in serum 24 weeks after stopping therapy. Treatment failures included patients who had a < 2 $\log_{10}$ drop in viral load at week 12 as compared to baseline, those was still detectable at week 24 (i.e. non-responders, according to international guidelines [3]), and those who had undetectable HCV RNA at the end of therapy but detectable HCV RNA at 24 weeks after cessation of therapy (i.e. relapsers). The study was approved by all local ethical committees and all patients gave informed consent at enrollment, in accordance with the Helsinki declaration.

For the present analysis, we included all patients who achieved a SVR, independently of the effective duration of therapy and of the dose of drugs received. On the other hand, patients with a treatment failure who had not received at least 80% of the recommended dose of pegylated IFN-α and ribavirin for at least 80% of the intended duration of treatment and subjects with missing information on treatment outcome were excluded from the analysis. In addition, we excluded also patients with diabetes, those infected with HCV genotype 4, due to their small number (n = 15) and those for whom genomic DNA was not available for study (Figure 1). Given the small number of non-adherent patients (n = 42), no intent-to-treat analysis was performed.

Anthropometric and laboratory evaluations

Body mass index (BMI) was calculated as weight divided by the square of the height (kg/m^2). A BMI >25 but ≤30 Kg/m^2 was considered as overweight, while obesity was defined as a BMI >30 kg/m^2. Waist circumference was measured to the nearest 0.5 cm at the shortest point below the lower rib margin and the iliac crest. Venous blood samples were taken in the fasted state. Insulin levels were measured centrally on stored serum samples by electrochemoluminescence

Deleted: Blood pressure measurements were obtained according to the Guidelines of the International Society of Hypertension (24). The components of the metabolic syndrome were classified according to the National Cholesterol Education Program, Adult Treatment Panel III (ATP-III) (25) and subjects having three or more positive criteria were labelled as having the metabolic syndrome. ¶

immunoassay (Insulina Immulite 2000, Medical System SpA, Genova, Italy; inter-assay coefficient of variation of 4.9%) using an autoanalyzer. The HOMA-IR score was calculated as reported (24). HOMA-IR scores were considered as continuous or dichotomous variable (i.e. < or ≥2, because this threshold has been reported in the literature as capable of discriminating SVR vs. non-SVR) (7, 8). HCV genotyping was performed by INNO-LiPA HCV II assay (Innogenetics, Zwijndrecht, Belgium). Serum HCV RNA was quantified at baseline and at week 12 of therapy by reverse transcription-PCR using Cobas Amplicor HCV Monitor Test, v 2.0 (Roche, Basel, Switzerland). Qualitative HCV RNA assessment was performed at weeks 4, 12, 24, 48 during treatment and 24 weeks after stopping therapy using Cobas Amplicor HCV, v2.0 (Roche, Basel, Switzerland; limit of detection: 50 IU/mL). High baseline viral load was defined as HCV RNA levels >400.000 IU/ml.

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Liver biopsy

Liver biopsy specimens were coded and scored by a single pathologist (MG at the University of Padua, Italy) who was blinded to clinical and biologic data, using the METAVIR score (25). Advanced fibrosis was defined as the presence of F3 or F4. Steatosis was recorded according to the criteria proposed by Brunt et al. (26) and classified as absent, minimal (<5%), mild (5-33%), moderate (>33-66%) and severe (>66%).

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IL28B genotyping

Genotyping was conducted in a blinded fashion relative to baseline characteristics and treatment outcome of patients. DNA samples from patients were genotyped for the *IL28B rs8099917*, *rs12979860* and *rs12980275* polymorphisms with TaqMan SNP genotyping assays (Applied Biosystems Inc, Foster City, CA), using the ABI 7500 Fast real time thermocycler, according to manufacturers' recommended protocols. TaqMan probes and primers were designed and synthesized by Applied Biosystems Inc. Automated allele calling was performed using SDS

software from Applied Biosystems Inc. Positive and negative controls were used in each genotyping assay. Primers and probes were reported previously (18).

IP-10 quantification

Quantification of IP-10 was performed using Quantikine (R&D Systems Minneapolis, MN), a solid phase ELISA, on pretreatment samples stored at -20 °C until assayed.

Statistical analysis

Continuous variables were summarized as mean ± standard deviation (SD) and categorical variables as frequency and percentage. Individual characteristics between groups were compared using the analysis of variance, Student t-test or the Mann-Whitney *U* test for continuous variables, and contingency tables and the χ^2-test or the Fisher's exact test for categorical data. Two-sided *P* values < 0.05 were considered statistically significant. However, because of the multiple comparisons between subjects with and without RVR or SVR, we considered of interest and commented only the results of statistical tests with a p-value <0.01, to reduce the risk of false positive results due to chance only. Multivariate logistic regression analysis was used to identify factors significantly associated with RVR or SVR (dependent variable coded as 0 = absent or 1 = present). Variables significantly associated with the dependent variable on univariate analysis were included in the multivariate logistic regression model at the first step, and removed if not significantly associated with the dependent variable at the 0.1 p-value. Subsequently, a multivariate logistic regression analysis with backward selection was used to identify factors independently associated with RVR or SVR at a 0.05 p-value. The predictive value of RVR toward SVR was evaluated fitting a separate logistic regression model with SVR as the response variable and RVR as the only predictor. Sensitivity, specificity, positive predictive values and likelihood ratios of a combination of variables associated with RVR or SVR in HCV genotype 1 patients were estimated.

The effect of *IL28B* polymorphisms was evaluated comparing the TT vs. TG/GG genotypes for marker *rs8099917*, CC vs. CT/TT genotypes for marker *rs12979860* and AA vs. AG/GG genotypes for marker *rs12980275*. Patients' data were collected in a computerized central database and analyzed using the R statistical package, version 2.11.0 [R Development Core Team (2010). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org].

RESULTS

Patients characteristics

Two-hundred and eighty patients were included in this analysis. Their baseline demographic, biochemical and virological characteristics are reported in Table 1. There were 166 (59%) males and the mean age was 46 ± 11 years. Mean BMI was 24.9 ± 3.8, 130 (46%) patients had BMI >25 and 90 (32 %) had central obesity. The mean HOMA-IR score was 2.9 ± 3.0. HCV infection was due to genotype 1 in 121 (43%) cases, to genotype 2 in 104 (37%) and to genotype 3 in 55 (20%). HCV RNA >400,000 IU/mL was found in 189 (67%) patients. A pre-treatment liver biopsy was available in 177 patients. Liver histology showed moderate/severe (Metavir scores A2-A3) activity in 78 (44%) and advanced fibrosis (F3-F4) in 29 (16%) patients. Steatosis was moderate/severe in 25 (15%) cases.

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The frequency of the favourable *rs8099917* TT genotype tended to be higher in HCV genotype 3 compared to HCV genotype 1 or 2 patients, although the statistical significance was borderline (p=0.049) (Table 2). There was a non-significant trend in the same direction for the favourable *rs12979860* CC (p=0.19) and *rs12980275* AA genotypes (p=0.21).

Quantification of IP-10 was performed in 226 out of 280 patients: their baseline features are shown in Supplemental Table 1. HCV genotype 1 patients had higher baseline IP-10 levels compared to

patients infected with genotype 2 or 3 (p=0.01) (Table 2). In HCV genotype 1 patients, significantly higher baseline IP-10 levels were observed in carriers of one or two of the risk G at *rs12980275* as compared to homozygous carriers of the favorable AA at *rs12980275* (p = 0.01) (Table 3); there was a non-significant trend in the same direction in carriers of one or two of the risk T at *rs12979860* (p=0.05) or of the risk G at *rs8099917* (p=0.07). No significant association was noted between basal HCV RNA and *IL28B* SNP variants in HCV genotype 1 patients (Table 3). In HCV genotype 2 or 3 patients, the *IL28B* allele distribution showed no correlation with baseline IP-10 levels or HCV RNA (data not shown).

The baseline characteristics of patients infected with HCV genotype 4 (n=15) and those with diabetes (n=28), excluded from further analyses, are shown in Supplemental Table 2.

Predictors of RVR

A RVR was observed in 172/280 (61%) patients, i.e. 33 (27%) with HCV genotype 1, 95 (91%) with genotype 2, and 44 (80%) with genotype 3.

By univariate analysis of HCV genotype 1 patients, a significant association was found between RVR and the homozygous carriers of the genotype TT at *rs8099917* (odds ratio (OR) =3.75; 95% confidence interval, CI: 1.42-10.6), CC at *rs12979860* (OR=6.8; 95% CI 2.6-18.11) or AA at *rs12980275* (OR=8.35; 95% CI 3.13-22.88). Since the AA at rs12980275 polymorphism showed the strongest association with RVR, only this marker of *IL28B* was considered for the multivariate analysis. Other factors associated with RVR in HCV genotype 1 patients were: lower basal IP-10, lower pre-treatment HCV RNA, HCV RNA < 400,000, lower AST/ALT ratio and lower GGT (Supplemental Table 3). No predictive factors of RVR were identified in HCV genotype 2 patients. In HCV genotype 3 patients, RVR was associated only with lower GGT.

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By multivariate analysis of HCV genotype 1 patients, independent predictors of RVR were the baseline HCV RNA <400,000 IU/ml (OR= 11.37; 95% CI 3.03-42.6), *rs12980275* type AA (vs. AG/GG) (OR 7.09; 95% CI 1.97-25.56) and IP-10 (OR 0.04; 95% CI 0.003-0.56) (Table 4). No

factor predicting RVR was identified in genotype 2 infected patients. In HCV genotype 3 patients, RVR was predicted by lower baseline GGT levels (OR = 0.02 for one unit of increasing GGT; 95% CI 0.0009-0.31) (Table 4).

Predictors of SVR

SVR was achieved in 209/280 (75%) patients, i.e. 65 (53%), 96 (92%) and 48 (87%) of patients infected with HCV genotype 1, 2, or 3, respectively.

By univariate analysis of HCV genotype 1 patients, SVR was associated with the favourable genotype TT at marker *rs8099917* (OR= 4.05; 95% CI 1.78-9.3), CC at marker *rs12979860* (OR= 8.17; 95% CI 3.02-24.18) and AA at marker *rs12980275* (OR= 10.34; 95% CI 3.65-33.05). Since the AA at rs12980275 polymorphism showed the strongest association with SVR, only this marker of *IL28B* was considered for the multivariate analysis. Other factors associated with SVR in HCV genotype 1 patients were: lower basal IP-10, age < 40 years, HCV RNA < 400,000 IU/ml, lower GGT and RVR (Supplemental Table 4). In HCV genotype 2 patients no factors were associated with SVR. In HCV genotype 3 patients, SVR was associated with lower ferritin level and higher total cholesterol. *IL28B* genetic variations did not predict SVR in HCV genotype 2 or 3 patients.

By multivariate analysis of HCV genotype 1 patients, independent predictors of SVR among baseline variables, were marker AA *rs12980275* (vs. AG/GG) (OR = 9.68; 95% CI 3.44-27.18), age < 40 yrs (OR = 4.79; 95% CI 1.50-15.34) and HCV RNA <400,000 IU/ml (OR 2.74; 95% CI 1.03-7.27) (Table 5). RVR was by itself a very strong predictor of SVR in patients with HCV genotype 1, since 32 of the 33 subjects with RVR developed SVR (97%) (OR = 33.0; 95% CI 4.06-268.32). In the 88 HCV genotype 1 patients who did not achieve a RVR, predictors of SVR were AA *rs12980275* (vs. AG/GG) (OR = 6.26; 95% CI 1.98-19.74) and age <40 yrs (OR 5.37; 95% CI 1.54-18.75) (Table 5). RVR was by itself the only independent predictor of SVR in HCV genotype 2 patients (OR 9.0, 95% CI 1.72-46.99; p=0.009) and in HCV genotype 3 patients (OR 7.8, 95% CI 1.43-42.67; p=0.01).

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Performance of IL28B type and HCV RNA for predicting RVR and SVR

To assess the performance of *IL28B* type compared with baseline HCV RNA and IP-10 levels for predicting RVR in our cohort of genotype 1 patients, we calculated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and positive and negative likelihood ratios (Table 6). The *rs12980275* marker and HCV RNA had similar NPV, PPV, specificity and sensitivity. However, the combination of *rs12980275* marker and HCV RNA levels had rather high PPV (87%) and high specificity (98%) though low sensitivity (39%). The addition of baseline IP-10 levels to the model show similar specificity, higher sensitivity but lower PPV (76 %).

In HCV genotype 1 patients, the *rs12980275* AA genotype, HCV RNA levels < 400,000 IU/ml and the combination of *rs12980275* AA genotype and HCV RNA levels < 400,000 IU/ml were present in 35%, 30% and 12% of patients, respectively.

Among the 88 HCV genotype 1 patients without RVR, the PPV for SVR were 68%, 67% and 64% for *rs12980275* marker, age ≤40 years and combining *rs12980275* marker and age, respectively (Table 7). The combination of the two variables had a sensitivity of 61% and a specificity of 80%.

DISCUSSION

The present study reports on the relative impact of baseline features of chronic hepatitis C patients on the outcome of therapy with peginterferon alpha and ribavirin in the "real world", i.e. outside clinical trials. Our results confirm previous observations that *IL28B* polymorphisms are independently associated with both RVR and SVR (15-21) and that they have the strongest predictive values for SVR among HCV genotype 1 patients without RVR. Furthermore, baseline HCV RNA and IP-10 levels added to the predictiveness of *IL28B* variants, and may thus contribute to the genomic-based personalization of therapy, even at the time direct-acting antivirals (DAA) will be introduced. *Vice versa*, some metabolic variables, such as HOMA-IR score, BMI and steatosis (especially in HCV non-3 genotypes), failed to predict viral response independently of the above.

In our study, the combination of *rs12980275* marker (AA genotype) and HCV RNA levels had a very high PPV (87%) and high specificity (98%) for RVR in the relatively difficult-to-treat genotype 1 patients. Adding baseline IP-10 levels to the model had comparable specificity but higher sensitivity increasing essentially the NPV (94 vs. 81%). Since 97% of patients with RVR and genotype 1 proceeded to attain SVR (Table 6) and taking into account that the combination of *rs12980275* marker (AA genotype) and HCV RNA levels had a PPV of 87% for RVR, about 84% of patients with HCV genotype 1, *rs12980275* AA genotype and HCV RNA <400,000 IU/ml may be cured with the currently available therapy. On the other hand, the predictive value of *IL28B* genotyping was very strong also in patients failing to reach RVR. Here, 33 out of 88 (38%) HCV genotype 1 patients without RVR achieved SVR: the *rs12980275* AA genotype was able to identify two thirds of them (PPV 68%) (Table 9). Age had similar PPV, and putting together *IL28B* genotype and age did not increase the PPV any further, but had a sensitivity of 61% and a NPV of 77%. Thus, if using a 4-week lead-in strategy with the standard dual combination, failure to reach

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RVR would certainly indicate the addition of at least one direct active antiviral (DAA), with the possible exception of patients with <1 Log HCV RNA reduction, at high risk of selecting strains resistant to protease inhibitors (~40%) (27, 28).

Favorable *IL28B* variants were not associated with higher levels of baseline HCV RNA, in contrast with previous studies (15, 18). Conversely, they were associated with lower levels of IP-10, consistent with the fact that good virological responders have a counterintuitive lower level of endogenous activation of the innate, interferon associated immune response (29-31). Baseline levels of the chemokine IP-10 are an independent negative predictor of RVR, as reported previously (12-14). IP-10 levels predict especially the first-phase decline of HCV RNA during treatment (32) and are considered a valid surrogate marker of innate immune response activation. Although its negative predictive value of virological response seems counterintuitive, it is in agreement with the fact that ISG are preactivated in non-responders, who also fail to further activate genes aimed at establishing an effective antiviral state (31). Higher IP-10 levels were found in patients with the risk allele G at *rs12980275*, whereas the significance was border-line for alleles T at *rs12979860* or G at *rs8099917*, in keeping with other works (32, 33). Thus, it is clear that both IP-10 and *IL28B* genotype are independently associated with virological response and that IP-10 are not merely surrogate indicators of unfavorable *IL28B* genotypes, but that they add to their predictive value, and they indeed impair the benefit of favorable IL28B variants (34). On the other hand, we could not identify a cutoff level of IP-10 capable to discriminate responders from non-responders with sufficient accuracy.

A surprising finding of our study is the lack of predictive value of the HOMA-IR. The clinical significance of determining the HOMA-IR score before therapy is debated. Initial studies showed that chronic hepatitis C patients with HOMA-IR score <2 had significantly better chances of achieving SVR, independently of the HCV genotype (6, 8, 35, 36). The predictive value of HOMA-IR was further reported in patients of Asian (35, 37, 38) or Middle East ancestry (36, 39). However, recent works have failed to confirm these findings (40-42), and a similar controversy exists for

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chronic hepatitis C patients coinfected with HIV (43-45). Higher HOMA-IR scores and/or insulin levels are inversely correlated with the HCV RNA decay occurring during the first days (46-48) or weeks (49, 50) of therapy. In our study, we observed an association between HOMA-IR and RVR only in the genotype 3 subgroup (by univariate analysis), but no association was seen when carrying out the multivariate analysis for RVR or with SVR for any viral genotype. It is possible, however, that associations between HOMA-IR and SVR rate be confounded by several factors (e.g. adherence). In addition, it is possible that discrepancies among studies be accounted for by differences among the patients' populations, especially in terms of prevalence of central obesity.Furthermore, the accuracy of HOMA-IR score assessment has been criticized and recent work suggests that, especially in lean and/or non-obese patients, HOMA-IR may be burdened by lack of standardization (51). Thus, the future significance of assessing IR by HOMA-IR before treatment seems questionable, especially at a time when patients' stratification before therapy is better achieved by genomic-based assays.

Our study has limitations. Firstly, it was conducted exclusively on Caucasian patients. However, the vast majority of the data appeared so far in the literature have been produced among Caucasians, and limited data is available on other selected ethnical groups, such as Japanese (17), Hispanics and African-Americans (15). The overall ITAHECS patients population rarely belongs to ethnical groups other than Caucasians, since they come from the Middle East or Sub-Saharan Africa, for which no solid host gene predictive data are available. More extensive studies are warranted in this area. Second, our population contained few patients with metabolic disturbances such as central obesity: thus, data may not be generalizable to other study populations from different geographical regions and more diverse metabolic profiles.

In conclusion, genetic variation in *IL28B* and pre-therapy levels of IP-10 and HCV RNA may be useful as first-line tools to identify the majority of HCV genotype 1 patients achieving RVR with the currently available dual therapy. This may used to stratify patients, especially when novel DAAs

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will be available. It is expected that additional advances in pharmacogenomics may further improve predictive models.

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Table 1. Baseline characteristics of patients with chronic hepatitis C

	All patients (n = 280)	Genotype 1 (n = 121)	Genotype 2 (n = 104)	Genotype 3 (n = 55)	*p* *
Age (yrs)	46±11	46±11	49±11	41±8	0.03
> 40 yrs old	212 (76)	95 (79)	84 (81)	33 (60)	***0.01***
Male gender	166 (59)	78 (64)	47 (45)	41 (75)	***0.0005***
Body Mass Index (kg/m2)	24.9±3.8	25.2±4.1	24.9±3.6	24±3.1	0.07
Waist circumference >102 cm (males) or >88 cm (females)	90 (32)	37 (30)	38 (36)	8 (14)	***0.009***
HOMA-IR	2.9±3.0	3.4±3.7	2.6±2.4	3.0±2.2	0.05
HOMA-IR > 2	147 (53)	73 (61)	48 (46)	26 (47)	0.06
HCV RNA ($\log_{10}$ IU/ml	5.77±0.87	5.79±0.84	5.84±0.79	6.0±1.09	0.32
HCV RNA ≥ 400,000 IU/ml	189 (67)	85 (70)	72(69)	31 (57)	0.25
METAVIR A2/A3 (n=177)	78(44)	47 (48)	18 (35)	13 (46)	0.26
METAVIR F3/F4 (n=177)	29 (16)	21(22)	2 (4)	6 (21)	***0.006***
Moderate/severe steatosis (n=166)	25 (15)	6 (7)	8 (16)	11 (41)	***0.0001***
ALT (U/L)	93±83	92±61	75±93	129±93	***0.0001***
AST/ALT ratio	0.7±0.2	0.7±0.2	0.7±0.2	1.0±0.2	***0.0005***
γ–glutamyl-transferase (U/L)	48±45	59±50	35±38	45±38	***0.004***
Platelet count (x 10^3/μL)	218±59	215±61	225±59	213±56	0.89
Bilirubin (mg/dl)	0.77±0.3	0.8±0.3	0.75±0.3	1.0±0.3	0.36
Ferritin (ng/ml)	208±208	262±262	143±111	202±170	***0.003***
Total cholesterol (mg/dl)	173±39	170±30	190±36	144±42	***0.0001***
HDL cholesterol (mg/dl)	53±16	53±17	54±14	51±16	0.44
Triglyceride (mg/dl)	89±50	89±44	98±57	74±43	***0.0002***
Glucose (mg/dl)	88±10	90±11	87±9	87±11	***0.01***

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Insulin (μU/ml)	13.4 ±12.8	15.3±15.7	11.9±10.2	12±9.4	0.11

Data are expressed as mean ± SD or number (%)

* P values for comparison among HCV genotypes 1, 2 and 3.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model of assessment of insulin resistance.

Table 2. Frequency of *IL28B* genotypes and baseline levels of IP-10

	All patients (n = 280)	**Genotype 1** (n = 121)	**Genotype 2** (n = 104)	**Genotype 3** (n = 55)	*p* *
rs8099917					
TT	158 (56)	65 (54)	54 (52)	39 (71)	0.049
TG	107 (38)	50 (41)	41 (39)	16 (29)	
GG	15 (6)	6 (5)	9 (9)	0	
rs12979860					
CC	109 (39)	42 (35)	39 (38)	28 (51)	0.19
CT	136 (49)	60 (50)	52 (50)	24 (44)	
TT	35 (12)	19 (15)	13 (12)	3 (5)	
rs12980275					
AA	109 (39)	42 (35)	39 (38)	28 (51)	0.21
AG	136 (49)	61 (50)	51 (49)	24 (44)	
GG	35 (12)	18 (15)	14 (13)	3 (5)	
Log IP-10 (pg/mL)	2.46±0.29	2.55±0.27	2.37±0.31	2.45±0.27	***0.01***

Data are expressed as mean ± SD or number (%)

* P values for comparison among HCV genotypes 1, 2 and 3

Table 3. Baseline characteristics of HCV genotype 1 patients according to *IL28B* genetic variants

	rs8099917				rs12979860				rs12980275			
	TT (n=65)	TG (n=50)	GG (n=6)	p	CC (n=42)	CT (n=60)	TT (n=19)	p	AA (n=42)	AG (n=61)	GG (n=18)	p
HCV RNA (log_{10} IU/mL)	5.83±0.85	5.74±0.85	5.73±0.72	0.85	5.9±0.91	5.72±0.88	5.73±0.51	0.56	5.86±0.91	5.76±0.88	5.72±0.52	0.80
IP-10 (Log_{10} pg/mL)	2.49±0.29	2.62±0.23	2.56±0.20	0.07	2.48±0.26	2.56±0.26	2.68±0.25	0.05	2.45±0.27	2.57±0.25	2.69±0.26	***0.01***

Data are expressed as mean ± SD

Table 4. Multivariate logistic regression analysis of factors predictive of rapid virological response

Patients	n	Variable	OR	95% CI	*P*
HCV genotype 1	121				
		HCV RNA < 400,000 IU/mL	11.37	3.03-42.6	0.00031
		rs12980275 type (AA vs. AG/GG)	7.09	1.97-25.56	0.0027
		IP-10 ($\log_{10}$ pg/mL)	0.044	0.003-0.56	0.016
HCV genotype 3	55	γ-glutamyl-transferase (U/L)	0.017	0.0009-0.31	0.006

Table 5. Multivariate logistic regression analysis of factors predictive of sustained virological response in HCV genotype 1 patients

Patients	n	Variable	OR	95% CI	*P*
HCV genotype 1	121	*rs12980275* type (AA vs AG/GG)	9.68	3.44-27.18	0.000017
		Age < 40	4.79	1.50-15.34	0.008
		HCV RNA < 400,000 IU/ml	2.74	1.03-7.27	0.04
HCV genotype 1 without RVR	88	*rs12980275* type (AA vs AG/GG)	6.26	1.98-19.74	0.0017
		Age < 40	5.37	1.54-18.75	0.0083

Table 6. Performance of *IL28B rs12980275* genotype, baseline HCV RNA and IP-10 in predicting rapid virological response in HCV genotype 1 patients

Factors considered in the model	Sensitivity %	Specificity %	PPV%	NPV %	LR +	LR -
rs12980275 AA	70	78	55	87	3.23	0.39
HCV RNA < 400,000 IU/ml	61	82	56	85	3.33	0.48
Log IP-10*	20	97	71	76	6.7	0.82
rs12980275 AA and HCV RNA < 400,000 IU/ml	39	98	87	81	17.33	0.62
rs12980275 AA, HCV RNA < 400,000 IU/ml and Log IP-10*	52	94	76	94	8.71	0.51

*The IP-10 level was available in 92 patients only

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio positive; LR-, likelihood ratio negative.

Table 7. Performance of *IL28B rs12980275* AA genotype and age in predicting sustained virological response in HCV genotype 1 patients without rapid virological response

Factors considered in the model	Sensitivity %	Specificity %	PPV%	NPV %	LR +	LR -
rs12980275 AA vs non-AA	39	89	68	71	3.61	0.68
age ≤ 40 vs. > 40 yrs	30	90	67	68	3.33	0.76
rs12980275 AA and age ≤ 40 yrs	61	80	64	77	3.03	0.49

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood ratio positive; LR-, likelihood ratio negative.

Statement of Interests

The Corresponding Author has no competing interests to declare.

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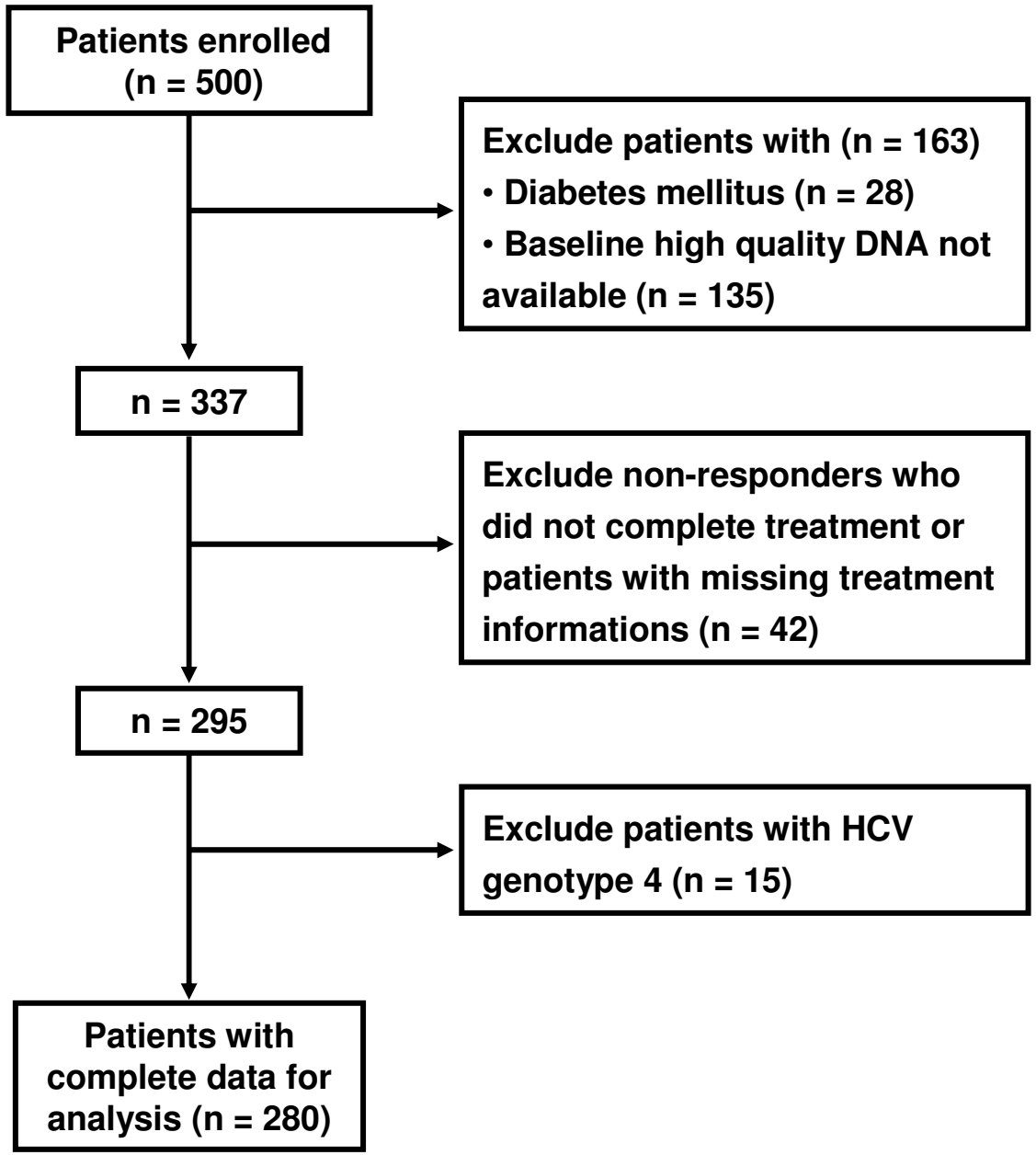
LEGEND TO FIGURE

Figure 1. Flow of the recruited patients.


Patients enrolled
(n = 500)
Exclude patients with (n = 163)
• Diabetes mellitus (n = 28)
• Baseline high quality DNA not available (n = 135)
n = 337
Exclude non-responders who did not complete treatment or patients with missing treatment informations (n = 42)
n = 295
Exclude patients with HCV genotype 4 (n = 15)
Patients with complete data for analysis (n = 280)

Supplemental Table 1. Baseline characteristics of 226 patients with chronic hepatitis C tested for IP-10

	All patients (n=226)	Genotype 1 (n = 92)	Genotype 2 (n = 87)	Genotype 3 (n = 47)	*p* *
Age (yrs)	46 ± 11	45 ± 11	48 ± 11	41 ± 8	***0.001***
> 40 yrs old	165 (72)	69 (75)	68 (78)	28 (60)	0.07
Male gender	133 (59)	59 (64)	40 (46)	34 (72)	***0.005***
Body Mass Index (kg/m2)	24.7 ± 3.8	24.9 ± 4.3	24.8 ± 3.5	23.7 ± 3.0	0.18
Waist circumference >102 cm (males) or >88 cm (females)	52 (24)	23 (26)	26 (31)	3 (7)	***0.003***
HOMA-IR	2.6 ± 2.2	2.8 ±2,0	2.5 ± 2.4	2.6 ± 2.2	0.6
HOMA-IR > 2	112 (49)	53 (58)	38 (44)	21 (44)	0.12
HCV RNA ($\log_{10}$ IU/ml	5.74 ± 0.9	5.75 ± 0.87	5.81 ± 0.81	5.62 ± 1.09	0.53
HCV RNA ≥ 400,000 IU/ml	147 (65)	61 (66)	59 (68)	27 (57)	0.46
METAVIR A2/A3 (n=142)	67 (47)	37 (51)	18 (39)	12 (52)	0.41
METAVIR F3/F4 (n=142)	21 (14)	14 (19)	2 (4)	5 (22)	0.03
Moderate/severe steatosis (n=134)	23 (17)	5 (7)	7 (16)	11 (50)	***0.0001***
ALT (U/L)	92 ± 78	95 ± 65	67 ± 69	132 ± 99	***0.0001***
AST/ALT ratio	0.7 ±0.2	0.7 ± 0.19	0.7 ± 0.2	0.6 ± 0.2	***0.0003***
γ–glutamyl-transferase (U/L)	46 ± 44	60 ± 53	31 ± 31	45 ± 40	***0.0001***
Platelet count (x 10^3/µL)	222 ± 59	225 ± 60	226 ± 60	213 ± 55	0,43
Bilirubin (mg/dl)	0.76 ± 0.39	0.78 ± 0.4	0.74 ± 0.4	0.76 ± 037	0.87
Ferritin (ng/ml)	195 ± 194	238 ± 242	137 ± 112	217 ± 179	***0.007***
Total cholesterol (mg/dl)	173 ± 41	172 ± 32	191 ± 38	145 ± 44	***0.0001***
HDL cholesterol (mg/dl)	55 ± 16	56 ± 16	55 ± 14	53 ± 17	0.58
Triglyceride (mg/dl)	88 ± 51	85 ± 39	98 ± 61	75 ± 47	***0.001***

Glucose (mg/dl)	88 ± 10	90 ± 10	87 ± 9	87 ± 12	0.02
Insulin (μU/ml)	12 ± 9	12.6 ± 8.4	11.3 ± 10.1	11.9 ± 9.5	0.66
***IL28B* polymorphism**					
rs8099917					
TT	130 (57)	49 (54)	47 (54)	34 (72)	0.12
TG	85 (38)	38 (41)	34 (39)	13 (28)	
GG	11 (5)	5 (5)	6 (7)	0 (0)	
rs12979860					
CC	92 (41)	33 (36)	34 (39)	25 (53)	0.07
CT	108 (48)	44 (48)	43 (50)	21 (45)	
TT	26 (11)	15 (16)	10 (11)	1 (2)	
rs12980275					
AA	92 (41)	33 (36)	34 (39)	25 (53)	0.2
AG	107 (47)	45 (49)	42 (48)	20 (43)	
GG	27 (12)	14 (15)	11 (13)	2 (4)	

Data are expressed as mean ± SD or number (%)
* P values for comparison among HCV genotypes 1, 2 and 3.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model of assessment of insulin resistance.

Supplemental Table 2 . Baseline characteristics of chronic hepatitis C patients with diabetes or HCV genotype 4 who were excluded from the analysis

	Patients with diabetes (n = 28)	**genotype 4** (n = 15)
Age (yrs)	58±8	47±9
> 40 yrs old	26(93)	12 (80)
Male gender	20(71)	9 (60)
Body Mass Index (kg/m2)	26±3.1	27±3.6
BMI > 25	15(54)	11 (73)
Waist circumference >102 cm (man) >88 cm (woman)	9(32)	8 (53)
HOMA-IR	NT	2.0±1.6
HOMA-IR > 2	NT	8 (53)
HCV-RNA ($\log_{10}$ IU/ml	5.7±0.4	6.0±1.043
HCV-RNA ≥ 400.000 IU/ml	18(69)	8 (53)
METAVIR A2/A3	17(74) *	7 (54) **
METAVIR F3-F4	6(26) *	2 (15) **
Moderate/severe steatosis	15(71) §	2 (15) **
ALT (U/L)	157±146	76±57
AST: ALT ratio	0.7±0.2	1.0±0.1
γ glutamyltransferase (U/L)	86±64	68±114
Platelet count (x 10^3/mmc)	184±61	221±73
Bilirubin (mg/dl)	1.1±1.4	1.0±0.1

Ferritin (ng/ml)	260±352	203±221
Total cholesterol (mg(dl)	179±43	203±48
HDL cholesterol (mg/dl)	56±17	54±14
Triglyceride (mg/dl)	81±32	159±182
Glucose (mg/dl)	152±57	88±11
Insulin (μU/ml)	27±31	11±6.0
***IL28B* polymorphism**		
rs8099917	NT	
TT		6 (40)
TG		7 (47)
GG		2 (13)
rs12979860	NT	
CC		2 (13)
CT		10 (67)
TT		3 (20)
rs12980275	NT	
AA		2 (13)
AG		10 (67)
GG		3 (20)
Log IP-10 (pg/mL)	NT	2.0±0.33

Data expressed as mean ± SD or number (%)
Liver biopsy available in * 23 out of 28 patients, § 21 out of 28 patients and ** 13 out of 15 patients
NT, not tested; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, Body Mass Index; HOMA-IR, homeostasis model of assessment of insulin resistance.

Supplemental Table 3. Baseline features of patients with chronic hepatitis C according to rapid virological response (RVR)

	Genotype 1 (n = 121)			Genotype 2 (n = 104)			Genotype 3 (n = 55)		
	RVR (n = 33)	No RVR (n = 88)	p	RVR (n = 95)	No RVR (n = 9)	p	RVR (n = 44)	No RVR (n = 11)	p
Age (yrs)	43.7 ± 11	47.8 ± 10	0.07	49.3 ± 11	48.3 ± 13	0.80	41.5 ± 7	41 ± 8	0.83
> 40 yrs old	22 (67)	73 (83)	0.08	78 (82)	6 (67)	0.37	26 (59)	7 (64)	1.0
Male gender	24 (73)	54 (61)	0.29	44 (46)	3 (33)	0.50	33 (75)	8 (73)	1.0
***IL28B* polymorphisms**									
rs8099917									
TT	25 (76)	49 (45)	***0.01***	49 (52)	5 (56)	0.88	33 (75)	6 (55)	0.26
TG	8 (24)	42(48)		38 (40)	3 (33)		11 (25)	5 (45)	
GG	0	6 (7)		8 (8)	1 (11)		0	0	
rs12979860									
CC	22 (67)	20 (23)	***0.00004***	37 (39)	2 (22)	0.38	24 (55)	4 (36)	0.31
CT	8 (24)	52 (59)		47 (49)	5 (56)		17 (39)	7 (64)	
TT	3 (9)	16 (18)		11 (12)	2 (22)		3 (6)	0	
rs12980275									
AA	23 (70)	19 (22)	***0.0000006***	37 (39)	2 (22)	0.21	23 (52)	5 (45)	0.87
AG	7 (21)	54 (61)		47 (49)	4 (44)		18 (41)	6 (55)	
GG	3 (9)	15 (17)		11 (12)	3 (33)		3 (7)	0	
Log IP-10 (pg/mL)	2.4 ± 0.28	2.6 ± 0.25	***0.002***	2.38 ± 0.31	2.3 ± 0.30	0.45	2.45 ± 0.23	2.48 ± 0.39	0.74
Body mass index (kg/m2)	25 ± 4.5	25 ± 4.0	0.67	24.8 ± 3.6	26 ± 3.3	0.31	23.8 ± 3.1	25 ± 3.1	0.19
Body mass index (kg/m2)									
BMI ≤ 25	17 (52)	44 (50)	1.0	47 (49)	4 (44)	1.0	32 (73)	6 (55)	0.29
BMI > 25	16 (48)	44 (50)		48 (51)	5 (56)		12 (27)	5 (45)	

Waist circumference >102 cm (males) or >88 cm (females)	7 (23)	30 (35)	0.27	32 (36)	6 (67)	0.08	5 (11)	3 (33)	0.12
HOMA-IR	4.0 ± 5.2	3.3 ± 3.0	0.34	2.6 ± 2.4	2.4 ± 2.4	0.80	2.3 ± 1.9	4.0 ± 2.7	0.03
HOMA-IR > 2	19 (59)	54 (61)	0.84	45 (47)	3 (33)	0.5	18 (41)	8 (73)	0.09
Log HCV RNA IU/ml	5.2 ± 0.9	6.0 ± 0.6	***0.0003***	5.8 ± 0.8	6.1 ± 0.5	0.20	5.5 ± 1.0	5.8 ± 1.1	0.45
HCV RNA ≥ 400,000 IU/ml	13 (39)	72 (82)	***0.00001***	65 (68)	7 (78)	0.72	24 (56)	7 (64)	0.74
METAVIR A2/A3 (n = 177)	8 (32)	39 (54)	0.07	17 (36)	1 (20)	0.65	10 (45)	3 (50)	1.0
METAVIR F3/F4 (n = 177)	5 (20)	16 (22)	1.0	0	2 (4)	1.0	4 (18)	2 (33)	0.58
Moderate/severe steatosis (n=166)	1 (4)	5 (8)	0.24	7 (16)	1 (25)	1.0	7 (32)	4 (80)	0.13
ALT (U/L)	106 ± 74	87 ± 55	0.12	77 ± 97	54 ± 34	0.49	120 ± 91	164 ± 96	0.17
AST/ALT ratio	0.65 ± 0.2	0.78 ± 0.2	***0.01***	0.74 ± 0.25	0.78 ± 0.18	0.69	0.61 ± 0.21	0.62 ± 0.18	0.85
γ–glutamyl-transferase (U/L)	46 ± 57	64 ± 46	***0.001***	34 ± 38	39 ± 32	0.74	36 ± 22	82 ± 62	***0.0002***
Platelet count (x 10^3/μL)	213 ± 64	216 ± 59	0.8	224 ± 58	243 ± 65	0.36	216 ± 50	200 ± 78	0.41
Bilirubin (mg/dl)	0.79 ± 0.38	0.80 ± 0.39	0.9	0.75 ± 0.35	0.83 ± 0.62	0.51	0.73 ± 0.38	0.83 ± 0.32	0.39
Ferritin (ng/ml)	245 ± 192	268 ± 283	0.71	139 ± 112	203 ± 86	0.22	179 ± 180	275 ± 105	0.14
Total cholesterol (mg/dl)	173 ± 26	169 ± 32	0.52	190 ± 37	192 ± 36	0.88	143 ± 40	145 ± 48	0.91
HDL cholesterol (mg/dl)	52 ± 12	54 ± 18	0.67	54 ± 14	55 ± 13	0.87	52 ± 16	47 ± 16	0.38
Triglyceride (mg/dl)	85 ± 34	90 ± 47	0.59	99 ± 60	87 ± 20	0.53	69 ± 42	95 ± 44	0.08
Glucose (mg/dl)	89 ± 8	91 ± 11	0.46	87 ± 9	86 ± 9	0.81	85 ± 10	90 ± 13	0.20
Insulin (μU/ml)	17 ± 22	15 ± 12	0.32	12 ± 10	10 ± 9	0.70	10 ± 8	17 ± 11	0.03

Data are expressed as mean ± SD or number (%)
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model of assessment of insulin resistance.

Supplemental Table 4. Features of patients with chronic hepatitis C according to sustained virological response (SVR)

	HCV genotype 1 (n = 121)			HCV genotype 2 (n = 104)			HCV genotype 3 (n = 55)		
	SVR (n = 65)	No SVR (n = 56)	*P*	SVR (n = 96)	No SVR (n = 8)	*p*	SVR (n = 48)	No SVR (n = 7)	*p*
Age (yrs)	44.6 ± 11	49.4 ± 10	0.02	48.9 ± 11	52.8 ± 11	0.35	40.6 ± 8	46.7 ± 6	0.06
> 40 yrs old	44 (68)	51 (91)	***0.001***	77 (80)	7 (88)	1.0	27 (56)	6 (86)	0.22
Male gender	41 (63)	37 (66)	0.8	44 (46)	3 (38)	0.73	35 (73)	6 (86)	0.66
***IL28B* polymorphisms**									
rs8099917									
TT	45 (69)	20 (36)	***0.00064***	50 (52)	4 (50)	0.87	34 (71)	45(71)	1.0
TG	18 (28)	32 (57)		37 (39)	4 (50)		14 (29)	2 (29)	
GG	2 (3)	4 (7)		9 (9)	0		0	0	
rs12979860									
CC	35 (54)	7 (13)	***0.00001***	37 (39)	2 (25)	0.38	20 (42)	3 (43)	0.79
CT	21 (32)	39 (70)		46 (48)	6 (75)		25 (52)	4 (57)	
TT	9 (14)	10 (18)		13 (14)	0		3 (6)	0	
rs12980275									
AA	36 (55)	6 (11)	***0.000001***	38 (40)	1 (13)	0.12	20 (42)	3 (43)	0.79
AG	21 (32)	40 (71)		44 (46)	7 (88)		25 (52)	4 (57)	
GG	8 (12)	10 (18)		14 (15)	0		3 (6)	0	
Log IP-10 (pg/mL)	2.47 ± 0.23	2.65 ± 0.28	***0.0009***	2.37 ± 0.31	2.33 ± 0.35	0.71	2.42 ± 0.21	2.67 ± 0.46	0.02
Body Mass Index (kg/m2)	25 ± 4.9	25 ± 3.0	0.73	25 ± 3.6	24 ± 3.8	0.39	24 ± 3.0	25 ± 3.2	0.14
Body Mass Index (kg/m2)									
BMI ≤ 25	37 (57)	24 (43)	0.14	47 (49)	4 (50)	1.0	34 (71)	4 (57)	0.66
BMI > 25	28 (43)	32 (57)		49 (51)	4 (50)		14 (29)	3 (43)	
Metabolic syndrome	2 (3)	3 (6)	0.66	8 (9)	0	1.0	3 (6)	0	1.0

HOMA-IR	3.3 ± 3.9	3.6 ± 3.4	0.74	2.6 ± 2.4	2.6 ± 2.7	0.97	2.5 ± 2.1	3.2 ± 2.7	0.49
HOMA-IR > 2	37 (58)	36 (64)	0.57	46 (48)	2 (25)	0.28	22 (46)	4 (57)	0.70
Log HCV RNA IU/ml	5.6 ± 0.8	6.0 ± 0.7	0.05	5.8 ± 0.8	6.3 ± 0.3	0.06	5.5 ± 1.1	6.1 ± 0.7	0.15
HCV-RNA ≥ 400,000 IU/ml	39 (60)	46 (82)	***0.0009***	64 (67)	8 (100)	0.06	26 (55)	5 (71)	0.69
METAVIR A2/A3 (n = 177)	21 (40)	26 (58)	0.11	17 (35)	1 (25)	1.0	10 (43)	3 (60)	0.64
METAVIR F3/F4 (n = 177)	9 (17)	12 (27)	0.33	2 (4)	0	1.0	4 (17)	2 (40)	0.29
Moderate/severe steatosis (n=166)	2 (4)	4 (9)	0.42	8 (18)	0	1.0	8 (36)	3 (60)	0.37
RVR	32 (49)	1 (2)	***0.0001***	90 (94)	5 (63)	0.02	41 (85)	3 (43)	0.02
Pegylated IFN-α2a	42 (65)	38 (68)	0.70	66 (69)	8 (100)	0.1	29 (60)	4 (57)	1.0
Pegylated IFN-α2b	23 (35)	18 (32)	0.85	30 (31)	0	0.1	19 (40)	3 (43)	1.0
Ribavirin dose									
≤ 13 mg/kg/day	19 (30)	22 (39)	0.25	47 (50)	1 (12)	0.06	18 (37)	4 (57)	0.42
> 13 mg/kg/day	45 (70)	34 (61)		47 (50)	7 (88)		30 (63)	3 (43)	
ALT (U/L)	91 ± 62	94 ± 62	0.83	78 ± 96	46 ± 31	0.36	127 ± 95	141 ± 80	0.70
AST/ALT ratio	0.72 ± 0.3	0.76 ± 0.2	0.44	0.73 ± 0.2	0.91 ± 0.3	0.04	0.61 ± 0.2	0.61 ± 0.1	0.98
γ–glutamyl-transferase (U/L)	48 ± 50	72 ± 46	***0.01***	35 ± 39	28 ± 27	0.6	42 ± 33	67 ± 64	0.1
Platelet count (x 10^3/μL)	225 ± 60	204 ± 60	0.06	226 ± 59	222 ± 60	0.88	216 ± 53	189 ± 78	0.24
Bilirubin (mg/dl)	0.74 ± 0.34	0.86 ± 0.43	0.09	0.77 ± 0.39	0.58 ± 0.2	0.19	0.73 ± 0.38	0.84 ± 0.28	0.5
Ferritin (ng/ml)	230 ± 200	297 ± 314	0.35	144 ± 112	133 ± 108	0.84	159 ± 124	434 ± 209	***0.002***
Total cholesterol (mg/dl)	170 ± 30	171 ± 31	0.94	170 ± 30	171 ± 31	0.69	150 ± 41	103 ± 21	***0.01***
HDL cholesterol (mg/dl)	52 ± 14	55 ± 20	0.34	54 ± 14	58 ± 17	0.55	52 ± 17	40 ± 6	0.08
Triglyceride (mg/dl)	85 ± 33	93 ± 54	0.35	95 ± 52	125 ± 100	0.17	77 ± 45	56 ± 17	0.24
Glucose (mg/dl)	90 ± 9	91 ± 12	0.57	87 ± 9	84 ± 10	0.39	86 ± 11	89 ± 9	0.56
Insulin (μU/ml)	15 ± 17	16 ± 13	0.77	11 ± 10	11 ± 10	0.99	11 ± 8	14 ± 12	0.48

Data are expressed as mean ± SD or number (%).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model of assessment of insulin resistance