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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 (OR 11.37; 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- λ 3 are strongly associated with the response to treatment of chronic hepatitis C (15-21). In particular, the

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

11 We sought to extend our understanding of the impact of these recently reported genetic
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13 polymorphisms on treatment outcome by investigating the independent association of candidate
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15 genetic (including the *rs8099917*, *rs12979860* and *rs12980275* markers), host (including IP-10
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17 levels and HOMA-IR score) and viral factors on virological response in a cohort of treatment-naïve,
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19 non-diabetic Caucasian patients with chronic hepatitis C.
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22 23 **PATIENTS AND METHODS**

24 25 *Patient population*

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27 Patients were recruited in an observational prospective cohort study, the Italian Hepatitis C Cohort
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29 Study (ITAHECS), designed to evaluate factors that influence response to antiviral therapy,
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31 including host metabolic factors. Overall, 500 consecutive, treatment-naïve patients with chronic
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33 hepatitis C were enrolled and treated between June 2006 and June 2007 at seven tertiary referral
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35 Italian liver units. Eligibility criteria for therapy included Caucasian ancestry, age between 18 and
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37 65 years, detectable HCV RNA in serum by polymerase chain reaction (PCR) (see below) and
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39 alanine aminotransferase (ALT) above the upper limit of normal within 6 months of treatment
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41 initiation. Patients were not considered for therapy if pregnant or breast-feeding, had
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43 decompensated cirrhosis or any other contraindication to therapy with the combination of pegylated
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45 IFN- α and ribavirin, were positive for hepatitis B surface antigen or anti-HIV antibodies, or
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47 reported a high daily alcohol intake (>40 g/day). All patients were asked to completely abstain
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49 from alcohol during antiviral treatment. Therapy was based on the combination of pegylated IFN-
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51 α_{2a} (180 μ g/week) or pegylated IFN- α_{2b} (1.5 μ g/kg/week) plus ribavirin (800-1200 mg/day) for 48
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1 weeks in HCV genotype 1 or 4 patients, and for 24 weeks in HCV genotype 2 or 3 patients. A rapid
2 virological response (RVR) was defined as undetectable HCV RNA in serum at week 4 of therapy.
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4 virological response (RVR) was defined as undetectable HCV RNA in serum at week 4 of therapy.
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6 Early virological response (EVR) was defined as serum HCV RNA negativity or any $>2 \log_{10}$
7
8 decline in HCV RNA levels at week 12 of therapy compared with baseline. Patients with sustained
9
10 virological response (SVR) were those with undetectable HCV RNA in serum 24 weeks after
11
12 stopping therapy. Treatment failures included patients who had a $< 2 \log_{10}$ drop in viral load at week
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14 12 as compared to baseline, those who were still detectable at week 24 (i.e. non-responders, according to
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16 international guidelines [3]), and those who had undetectable HCV RNA at the end of therapy but
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18 detectable HCV RNA at 24 weeks after cessation of therapy (i.e. relapsers). The study was
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20 approved by all local ethical committees and all patients gave informed consent at enrollment, in
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22 accordance with the Helsinki declaration.

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

40 *Anthropometric and laboratory evaluations*

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

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2 immunoassay (Insulina Immulite 2000, Medical System SpA, Genova, Italy; inter-assay coefficient
3 of variation of 4.9%) using an autoanalyzer. The HOMA-IR score was calculated as reported (24).

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6 HOMA-IR scores were considered as continuous or dichotomous variable (i.e. $<$ or ≥ 2 , because this
7 threshold has been reported in the literature as capable of discriminating SVR vs. non-SVR) (7, 8).

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10 HCV genotyping was performed by INNO-LiPA HCV II assay (Innogenetics, Zwijndrecht,
11 Belgium). Serum HCV RNA was quantified at baseline and at week 12 of therapy by reverse
12 transcription-PCR using Cobas Amplicor HCV Monitor Test, v 2.0 (Roche, Basel, Switzerland).

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14
15 Qualitative HCV RNA assessment was performed at weeks 4, 12, 24, 48 during treatment and 24
16 weeks after stopping therapy using Cobas Amplicor HCV, v2.0 (Roche, Basel, Switzerland; limit of
17 detection: 50 IU/mL). High baseline viral load was defined as HCV RNA levels >400.000 IU/ml.

22 23 *Liver biopsy*

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25 Liver biopsy specimens were coded and scored by a single pathologist (MG at the University of
26 Padua, Italy) who was blinded to clinical and biologic data, using the METAVIR score (25).

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29 Advanced fibrosis was defined as the presence of F3 or F4. Steatosis was recorded according to the
30 criteria proposed by Brunt et al. (26) and classified as absent ($<5\%$), mild (5-33%),
31 moderate ($>33-66\%$) and severe ($>66\%$).

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36 37 *IL28B genotyping*

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

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

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8 *IP-10 quantification*

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

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15 *Statistical analysis*

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17 Continuous variables were summarized as mean \pm standard deviation (SD) and categorical variables
18
19 as frequency and percentage. Individual characteristics between groups were compared using the
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21 analysis of variance, Student t-test or the Mann-Whitney *U* test for continuous variables, and
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23 contingency tables and the χ^2 -test or the Fisher's exact test for categorical data. Two-sided *P*
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25 values < 0.05 were considered statistically significant. However, because of the multiple
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27 comparisons between subjects with and without RVR or SVR, we considered of interest and
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29 commented only the results of statistical tests with a p-value < 0.01 , to reduce the risk of false
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31 positive results due to chance only. Multivariate logistic regression analysis was used to identify
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33 factors significantly associated with RVR or SVR (dependent variable coded as 0 = absent or 1 =
34
35 present). Variables significantly associated with the dependent variable on univariate analysis were
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37 included in the multivariate logistic regression model at the first step, and removed if not
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39 significantly associated with the dependent variable at the 0.1 p-value. Subsequently, a multivariate
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41 logistic regression analysis with backward selection was used to identify factors independently
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43 associated with RVR or SVR at a 0.05 p-value. The predictive value of RVR toward SVR was
44
45 evaluated fitting a separate logistic regression model with SVR as the response variable and RVR as
46
47 the only predictor. Sensitivity, specificity, positive predictive values and likelihood ratios of a
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49 combination of variables associated with RVR or SVR in HCV genotype 1 patients were estimated.

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

18 *Patients characteristics*

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21 Two-hundred and eighty patients were included in this analysis. Their baseline demographic,
22 biochemical and virological characteristics are reported in Table 1. There were 166 (59%) males
23 and the mean age was 46 ± 11 years. Mean BMI was 24.9 ± 3.8 , 130 (46%) patients had BMI >25
24 and 90 (32 %) had central obesity. The mean HOMA-IR score was 2.9 ± 3.0 . HCV infection was
25 due to genotype 1 in 121 (43%) cases, to genotype 2 in 104 (37%) and to genotype 3 in 55 (20%).
26
27 HCV RNA >400,000 IU/mL was found in 189 (67%) patients. A pre-treatment liver biopsy was
28 available in 177 patients. Liver histology showed moderate/severe (Metavir scores A2-A3) activity
29 in 78 (44%) and advanced fibrosis (F3-F4) in 29 (16%) patients. Steatosis was moderate/severe in
30 25 (15%) cases.
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40 The frequency of the favourable *rs8099917* TT genotype tended to be higher in HCV genotype 3
41 compared to HCV genotype 1 or 2 patients, although the statistical significance was borderline
42 (p=0.049) (Table 2). There was a non-significant trend in the same direction for the favourable
43 *rs12979860* CC (p=0.19) and *rs12980275* AA genotypes (p=0.21).
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48 [Quantification of IP-10 was performed in 226 out of 280 patients: their baseline features are shown](#)
49 [in Supplemental Table 1.](#) HCV genotype 1 patients had higher baseline IP-10 levels compared to
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1 patients infected with genotype 2 or 3 ($p=0.01$) (Table 2). In HCV genotype 1 patients, significantly
2 higher baseline IP-10 levels were observed in carriers of one or two of the risk G at *rs12980275* as
3 compared to homozygous carriers of the favorable AA at *rs12980275* ($p = 0.01$) (Table 3); there
4 was a non-significant trend in the same direction in carriers of one or two of the risk T at
5 *rs12979860* ($p=0.05$) or of the risk G at *rs8099917* ($p=0.07$). No significant association was noted
6 between basal HCV RNA and *IL28B* SNP variants in HCV genotype 1 patients (Table 3). In HCV
7 genotype 2 or 3 patients, the *IL28B* allele distribution showed no correlation with baseline IP-10
8 levels or HCV RNA (data not shown).

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10 [The baseline characteristics of patients infected with HCV genotype 4 \(n=15\) and those with](#)
11 [diabetes \(n=28\), excluded from further analyses, are shown in Supplemental Table 2.](#)
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18 *Predictors of RVR*

19 A RVR was observed in 172/280 (61%) patients, i.e. 33 (27%) with HCV genotype 1, 95 (91%)
20 with genotype 2, and 44 (80%) with genotype 3.
21

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

45 By multivariate analysis of HCV genotype 1 patients, independent predictors of RVR were the
46 baseline HCV RNA <400,000 IU/ml (OR= 11.37; 95% CI 3.03-42.6), *rs12980275* type AA (vs.
47 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
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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

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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.

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

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6 To assess the performance of *IL28B* type compared with baseline HCV RNA and IP-10 levels for
7
8 predicting RVR in our cohort of genotype 1 patients, we calculated sensitivity, specificity, positive
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10 predictive value (PPV), negative predictive value (NPV) and positive and negative likelihood ratios
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12 (Table 6). The *rs12980275* marker and HCV RNA had similar NPV, PPV, specificity and
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14 sensitivity. However, the combination of *rs12980275* marker and HCV RNA levels had rather high
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16 PPV (87%) and high specificity (98%) though low sensitivity (39%). The addition of baseline IP-10
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18 levels to the model show similar specificity, higher sensitivity but lower PPV (76 %).

19
20 In HCV genotype 1 patients, the *rs12980275* AA genotype, HCV RNA levels < 400,000 IU/ml and
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22 the combination of *rs12980275* AA genotype and HCV RNA levels < 400,000 IU/ml were present
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24 in 35%, 30% and 12% of patients, respectively.

25
26 Among the 88 HCV genotype 1 patients without RVR, the PPV for SVR were 68%, 67% and 64%
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28 for *rs12980275* marker, age ≤ 40 years and combining *rs12980275* marker and age, respectively
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30 (Table 7). The combination of the two variables had a sensitivity of 61% and a specificity of 80%.

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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.

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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](#)).

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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

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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

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[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

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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*

Deleted: Indeed, IP-10 levels in these two categories of patients were largely overlapping (data not shown), and they could be added to the predictive model as continuous variable after log-transformation. Clinically meaningful cutoff levels of IP-10 should probably be independently validated by large scale prospective studies, or be reassessed by meta-analyses of individual patients' data.

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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

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

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Deleted: and metabolic syndrome. Since our series included very few patients with the metabolic syndrome, we may hypothesize that when IR is increased due to HCV, this may not significantly affect response, and that the HOMA-IR score may predict poor response to therapy only when it is harbinger of the metabolic syndrome. We may also speculate that this association may not be causal, i.e. the HOMA-IR may be a surrogate marker of some metabolic event unrelated to insulin signaling.

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

23 In conclusion, genetic variation in *IL28B* and pre-therapy levels of IP-10 and HCV RNA may be
 24 useful as first-line tools to identify the majority of HCV genotype 1 patients achieving RVR with
 25 the currently available dual therapy. This may be 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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For Peer Review

REFERENCES

1. **Shepard CW**, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005;**5**:558-567.
2. **Seeff LB**. Natural history of chronic hepatitis C. *Hepatology* 2002;**36** (Suppl 1):S35-46.
3. **Ghany MG**, Strader DB, Thomas DL, *et al*. Diagnosis, management, and treatment of hepatitis C: An update. *Hepatology* 2009;**49**:1335-1374.
4. **Zeuzem S**. Heterogeneous virologic response rates to interferon-based therapy in patients with chronic hepatitis C: who respond less well? *Ann Intern Med* 2004;**140**:370-381.
5. **Bressler BL**, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003;**38**:639-644.
6. **Romero Gomez M**, Del Mar Vilorio M, Andrade RJ, *et al*. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005;**128**:636-641.
7. **Poynard T**, Ratziu V, McHutchison J, *et al*. Effect of treatment with peginterferon or interferon alfa-2b and ribavirin on steatosis in patients infected with hepatitis C. *Hepatology* 2003;**38**:75-85.
8. **Poustchi H**, Negro F, Hui J, *et al*. Insulin resistance and response to therapy in patients infected with chronic hepatitis C virus genotypes 2 and 3. *J Hepatol* 2008; **48**: 28-34.
9. **Durante-Mangoni E**, Zampino R, Portella G, *et al*. Correlates and prognostic value of the first-phase hepatitis C virus RNA kinetics during treatment. *Clin Infect Dis* 2009;**10**:43-49.
10. [Yu ML, Dai CY, Huang JF, et al. Rapid virologic response and treatment duration for chronic hepatitis C genotype 1 patients: a randomized trial. *Hepatology* 2008;**47**:1884-1893.](#)

Deleted: Jensen DM,

Deleted: Morgan TR, Marcellin P, *et al*. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40kd)/ribavirin therapy. *Hepatology* 2006;**43**:954-960.

Deleted: Yu ML.

Deleted: Dai CY, Huang JF, *et al.*
Rapid virologic response and treatment
duration for chronic hepatitis C genotype
1 patients: a randomized trial. *Hepatology*
2008;**47**:1884-1893.

11. [Poordad FF](#). Review article: the role of rapid virological response in determining treatment duration for chronic hepatitis C. *Aliment Pharmacol Ther* 2010;**31**:1251-1267.
12. **Askarieh G**, Alsiö A, Pugnale P, *et al.* Systemic and intrahepatic interferon-gamma-inducible protein 10 kDa predicts the first-phase decline in hepatitis C virus RNA and overall viral response to therapy in chronic hepatitis C. *Hepatology* 2010;**51**:1523-1530.
13. **Romero AI**, Lagging M, Westin J, *et al.* Interferon (IFN)-gamma inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFN-alpha 2a and ribavirin for chronic hepatitis C virus infection. *J Infect Dis* 2006;**194**:895-903.
14. **Lagging M**, Romero AI, Westin J, *et al.* IP-10 predicts viral response and therapeutic outcome in difficult-to-treat patients with HCV genotype 1 infection. *Hepatology* 2006;**44**:1617-1625.
15. **Ge D**, Fellay J, Thompson AJ, *et al.* Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;**461**:399-401.
16. **Suppiah V**, Moldovan M, Ahlenstiel G, *et al.* *IL28B* is associated with response to chronic hepatitis C interferon- α and ribavirin therapy. *Nat Genet* 2009;**41**:1100-1104.
17. **Tanaka Y**, Nishida N, Sugiyama M, *et al.* Genome-wide association of *IL28B* with response to pegylated interferon- α and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009;**41**:1105-1109.
18. **Rauch A**, Kutalik Z, Descombes P, *et al.* Genetic variation in *IL28B* is associated with chronic hepatitis C and treatment failure: A genome-wide association study. *Gastroenterology* 2010;**138**:1338-1345.
19. **Mc Carthy JJ**, Li HJ, Thompson A, *et al.* Replicated association between an *IL28B* gene variant and a sustained response to pegylated interferon and ribavirin. *Gastroenterology* 2010;**138**:2307-2314.

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20. **Thompson AJ**, Muir AJ, Sulkowski MS, *et al.* Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in hepatitis C virus-1 patients. *Gastroenterology* 2010;**139**:120-9.e18.
21. **Mangia A**, Thompson AJ, Santoro R, *et al.* *IL28B* polymorphism determines treatment response of patients with hepatitis C genotypes 2 or 3 who do not achieve a rapid virologic response. *Gastroenterology* 2010;**139**:821-7, 827.e1.
22. **Rallón NI**, Naggie S, Benito JM, *et al.* Association of a single nucleotide polymorphism near the interleukin-28B gene with response to hepatitis C therapy in HIV/hepatitis C virus-coinfected patients. *AIDS* 2010;**24**:F23-9.
23. **Fukuhara T**, Taketomi A, Motomura T, *et al.* Variants in *IL28B* in liver recipients and donors correlate with response to peg-interferon and ribavirin therapy for recurrent hepatitis C. *Gastroenterology* 2010;**139**:1577-85, 1585.e1-3.
24. **Bonora E**, Targher G, Alberiche M, *et al.* Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 2000;**23**:57-63.
25. **Bedossa P**, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. *Hepatology* 1996;**24**:289-293.
26. **Brunt EM**, Janney CG, Di Bisceglie AM, *et al.* Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;**94**:2467-74.
27. **Poordad F**, McCone J, Bacon BR, *et al.* Boceprevir (BOC) combined with peginterferon alfa-2b/ribavirin (P/R) for treatment-naïve patients with hepatitis C virus (HCV) genotype (G) 1: SPRINT-2 Final Results. *Hepatology* 2010;**52(suppl)**:107A.
28. **Bacon BR**, Gordon SC, Lawitz E, *et al.* HCV RESPOND-2 final results: high sustained virologic response among genotype 1 previous non-responders and relapsers to peginterferon/ribavirin when retreated with boceprevir plus PEGINTRON (Peginterferon alfa-2b)/Ribavirin. *Hepatology* 2010;**52(suppl)**:430A.

Deleted: <#>Guidelines Committee. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens* 2003;**21**:1011-53.¶
<#>Expert panel on detection evaluation and treatment of high blood cholesterol in adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001;**285**:2486-2497.¶

Deleted: <#>Hézode C, Forestier N, Dusheiko G, *et al.* Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009;**360**:1839-50.¶

- 1
2 29. **Chen L**, Borozan I, Feld J, *et al*. Hepatic gene expression discriminates responders and
3 nonresponders in treatment of chronic hepatitis C viral infection. *Gastroenterology*
4 2005;**128**:1437-1444.
5
6
7
8 30. **Asselah T**, Bieche I, Narguet S, *et al*. Liver gene expression signature to predict response to
9 pegylated interferon plus ribavirin combination therapy in patients with chronic hepatitis C.
10 *Gut* 2008;**57**:516-524.
11
12
13 31. **Sarasin-Filipowicz M**, Oakeley EJ, Duong FH, *et al*. Interferon signaling and treatment
14 outcome in chronic hepatitis C. *Proc Natl Acad Sci USA* 2008;**105**:7034-7039.
15
16
17 32. **Lagging M**, Askarieh G, Bochud P, *et al*. IP-10 is associated with IL28B variation and
18 predicts the first phase decline of HCV RNA and outcome of therapy in chronic hepatitis C.
19 *Hepatology* 2010;**52**(suppl):766A.
20
21
22
23 33. **Honda M**, Sakai A, Yamashita T, *et al*. Hepatic ISG expression is associated with genetic
24 variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C.
25 *Gastroenterology* 2010;**139**:499-509.
26
27
28
29 34. **Darling JM**, Aerssens J, Fanning G, *et al*. Quantitation of pretreatment serum IP-10
30 improves the predictive value of an *IL28B* gene polymorphism for hepatitis C treatment
31 response. *Hepatology* 2010;**52**(suppl): 382A.
32
33
34
35 35. **D'Souza R**, Sabin CA, Foster GR. Insulin resistance plays a significant role in liver fibrosis
36 in chronic hepatitis C and in the response to antiviral therapy. *Am J Gastroenterol*
37 2005;**100**:1509-15.
38
39
40
41 36. **Moucari R**, Ripault MP, Martinot-Peignoux M, *et al*. Insulin resistance and geographical
42 origin: major predictors of liver fibrosis and response to peginterferon and ribavirin in HCV-
43
44
45
46
47 37. **Dai CY**, Huang JF, Hsieh MY, *et al*. Insulin resistance predicts response to peginterferon-
48 alpha/ribavirin combination therapy in chronic hepatitis C patients. *J Hepatol* 2009;**50**:712-
49
50
51
52
53
54
55
56
57
58
59
60

Deleted: <#>Neville LF, Mathiak G, Bagasta O. The immunobiology of interferon-gamma inducible protein 10 kD (IP-10): a novel, pleiotropic member of the C-X-C chemokine superfamily. *Cytokine Growth Factor Rev* 1997;**8**:207-219.¶

Deleted: <#>Romero-Gómez M, Del Mar Vilorio M, Andrade RJ, *et al*. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005;**128**:636-41.¶

Deleted: <#>Poustchi H, Negro F, Hui J, *et al*. Insulin resistance and response to therapy in patients infected with chronic hepatitis C virus genotypes 2 and 3. *J Hepatol* 2008;**48**:28-34.¶

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42
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45
46
47
48
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60
38. **Mizuta T**, Kawaguchi Y, Eguchi Y, *et al.* Whole-body insulin sensitivity index is a highly specific predictive marker for virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients with genotype 1b and high viral load. *Dig Dis Sci* 2010;**55**:183-9.
39. **Khatab M**, Eslam M, Sharwae MA, *et al.* Insulin resistance predicts rapid virologic response to peginterferon/ribavirin combination therapy in hepatitis C genotype 4 patients. *Am J Gastroenterol* 2010;**105**:1970-7.
40. **Petta S**, Cammà C, Di Marco V, *et al.* Time course of insulin resistance during antiviral therapy in non-diabetic, non-cirrhotic patients with genotype 1 HCV infection. *Antivir Ther* 2009;**14**:631-9.
41. **Angelico F**, Francioso S, Del Ben M, *et al.* Clinical trial: low plasma cholesterol and oxidative stress predict rapid virological response to standard therapy with peginterferon and ribavirin in HCV patients. *Aliment Pharmacol Ther* 2009;**30**:444-51.
42. **Akuta N**, Suzuki F, Hirakawa M, *et al.* Amino acid substitutions in the hepatitis C virus core region of genotype 1b are the important predictor of severe insulin resistance in patients without cirrhosis and diabetes mellitus. *J Med Virol* 2009;**81**:1032-9.
43. **Merchante N**, de los Santos-Gil I, Merino D, *et al.* Insulin resistance is not a relevant predictor of sustained virological response to pegylated interferon plus ribavirin in HIV/HCV co-infected patients. *J Hepatol* 2009;**50**:684-92.
44. **Cacoub P**, Carrat F, Bédossa P, *et al.* Insulin resistance impairs sustained virological response rate to pegylated interferon plus ribavirin in HIV-hepatitis C virus-coinfected patients: HOMAVIC-ANRS HC02 Study. *Antivir Ther* 2009;**14**:839-45.
45. **Ryan P**, Resino S, Miralles P, *et al.* Insulin resistance impairs response to interferon plus ribavirin in patients coinfecting with HIV and hepatitis C virus. *J Acquir Immune Defic Syndr* 2010;**55**:176-81.

- 1
2 46. **Neumann AU**, Bain VG, Yoshida EM, *et al.* Early prediction of sustained virological
3 response at day 3 of treatment with albinterferon-alpha-2b in patients with genotype 2/3
4 chronic hepatitis C. *Liver Int* 2009;**29**:1350-5.
5
6
7 47. **Durante-Mangoni E**, Zampino R, Portella G, *et al.* Correlates and prognostic value of the
8 first-phase hepatitis C virus RNA kinetics during treatment. *Clin Infect Dis* 2009;**49**:498-
9 506.
10
11 48. **Bortoletto G**, Scribano L, Realdon S, *et al.* Hyperinsulinaemia reduces the 24-h virological
12 response to PEG-interferon therapy in patients with chronic hepatitis C and insulin
13 resistance. *J Viral Hepat* 2010;**17**:475-80.
14
15 49. **Nasta P**, Gatti F, Puoti M, *et al.* Insulin resistance impairs rapid virologic response in
16 HIV/hepatitis C virus coinfecting patients on peginterferon-alfa-2a. *AIDS* 2008;**22**:857-61.
17
18 50. **Grasso A**, Malfatti F, De Leo P, *et al.* Insulin resistance predicts rapid virological response
19 in non-diabetic, non-cirrhotic genotype 1 HCV patients treated with peginterferon alpha-2b
20 plus ribavirin. *J Hepatol* 2009;**51**:984-90.
21
22 51. **Lam KD**, Bacchetti P, Abbasi F, *et al.* Comparison of surrogate and direct measurement of
23 insulin resistance in chronic hepatitis C virus infection: impact of obesity and ethnicity.
24
25
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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)	<i>p</i> *
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/m ²)	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
META VIR A2/A3 (n=177)	78(44)	47 (48)	18 (35)	13 (46)	0.26
META VIR 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 ($\mu\text{U/ml}$)	13.4 \pm 12.8	15.3 \pm 15.7	11.9 \pm 10.2	12 \pm 9.4	0.11
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Data are expressed as mean \pm 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.

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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)	<i>p</i> *
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

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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
		<i>rs12980275</i> 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	<i>rs12980275</i> 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	<i>rs12980275</i> 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 -
<i>rs12980275</i> 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
<i>rs12980275</i> AA and HCV RNA < 400,000 IU/ml	39	98	87	81	17.33	0.62
<i>rs12980275</i> 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.

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2 **Table 7. Performance of *IL28B* rs12980275 AA genotype and age in predicting sustained**
3 **virological response in HCV genotype 1 patients without rapid virological response**
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8 Factors considered in the model	Sensitivity %	Specificity %	PPV%	NPV %	LR +	LR -
9 <i>rs12980275</i> AA vs non-AA	39	89	68	71	3.61	0.68
10 age ≤ 40 vs. > 40 yrs	30	90	67	68	3.33	0.76
11 <i>rs12980275</i> AA and age ≤ 40 yrs	61	80	64	77	3.03	0.49

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17 [Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LR+, likelihood](#)
18 [ratio positive; LR-, likelihood ratio negative.](#)
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2 **Statement of Interests**
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4 The Corresponding Author has no competing interests to declare.
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7
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9

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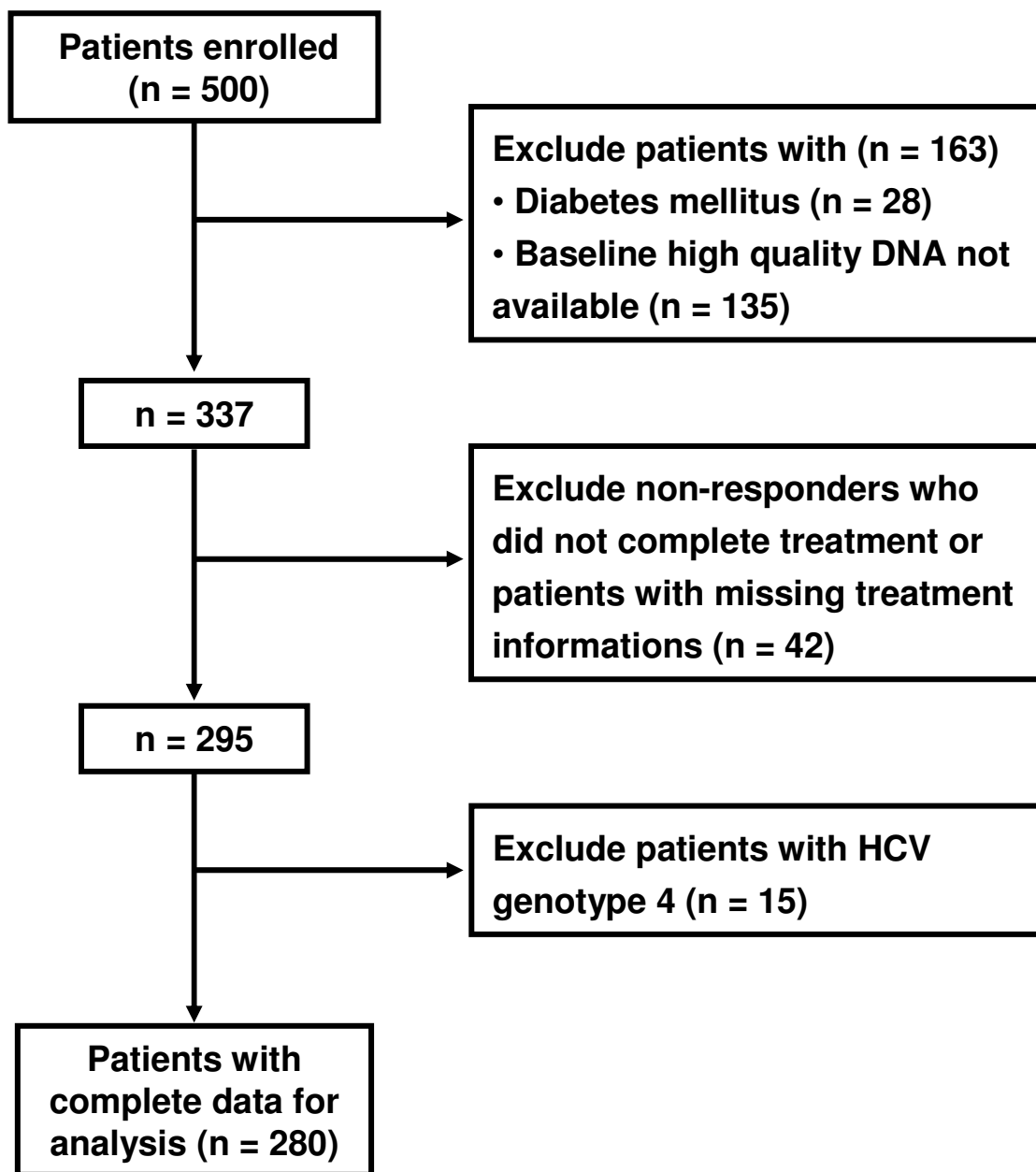
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Figure 1. Flow of the recruited patients.

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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)	<i>p</i> *
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 (log₁₀ 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 10³/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 ± 0.37	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
<i>IL28B</i> 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 (log₁₀ 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 10³/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
<i>IL28B</i> 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
<i>IL28B</i> 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)	0.000006	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 10³/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)	<i>P</i>	SVR (n = 96)	No SVR (n = 8)	<i>P</i>	SVR (n = 48)	No SVR (n = 7)	<i>P</i>
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
<i>IL28B</i> 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 10³/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

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