Variations at multiple genes improve interleukin 28b genotype predictive capacity for response to therapy against hepatitis c genotype 1 or 4 infection

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\textbf{Objective}: To identify genetic factors that predict sustained virological response (SVR) to pegylated interferon (Peg-IFN)/ribavirin (RBV) in HIV/hepatitis C virus (HCV) genotype 1 or 4-coinfected patients and that enhance the predictive capacity of \textit{IL28B} genotype in this population.

\textbf{Design}: Prospective cohort study.

\textbf{Setting}: Five tertiary care centers in Spain.

\textbf{Subjects}: 205 HIV/HCV genotype 1 or 4-coinfected patients who received a complete course of Peg-IFN/RBV for 48 weeks.

\textbf{Main outcome measures}: All individuals were genotyped for 144 SNPs.

\textbf{Results}: One hundred sixty-two (79\%) patients bore HCV genotype 1. Overall SVR was achieved by 73 (36\%) individuals. SNPs at the following genes were associated with SVR: \textit{IL28B}, low-density lipoprotein receptor (\textit{LDLR}), transforming growth factor \(b\) (\textit{TGF}-\(b\)), aquaporine 2 (\textit{AQP–2}), very-low-density lipoprotein receptor, Sp110 nuclear body protein, interferon alpha/beta receptor 1, 2′–5′-oligoadenylate synthase 1 and apolipoprotein B. There was a strong synergy between SNPs at \textit{IL28B}, \textit{TGF}-\(b\) and \textit{AQP–2} genes: the number of patients reaching SVR with all three favorable genotypes versus unfavorable genotypes were 22 (78.6\%) versus 1 (7.1\%) (\(p = 2.1 \times 10^{-4}\)). HCV baseline viral load, \textit{IL28B}, \textit{TGF}-\(b\), \textit{AQP–2} and \textit{LDLR} haplotypes were independently associated with SVR.

\textbf{Conclusion}: A number of genetic factors modify the predictive capacity of \textit{IL28B} genotype. These can be used to identify HCV genotype 1 or 4 infected patients with a very high or a very low probability to respond to bitherapy with Peg-IFN/RBV. Predictive models based on these factors could be helpful to tailor direct acting antiviral-based therapy.

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\textit{AIDS} 2013, 27:000–000

Keywords: aquaporine 2, hepatitis C virus, HIV, low density lipoprotein receptor, pegylated interferon, ribavirin, transforming growth factor \(b\)

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DOI:10.1097/01.aids.0000432459.36970.a9
Introduction

In European countries, over 50% of the cases of chronic hepatitis C among HIV-infected patients are caused by hepatitis C virus (HCV) genotype 1 [1]. The rate of sustained virological response (SVR) to dual therapy with pegylated interferon (Peg-IFN) and ribavirin (RBV) are very low in this subset under real-life conditions [2]. HIV/HCV genotype 4-infected patients represent approximately 15%–20% of the HIV/HCV-coinfected population [1,2]. The response rates to Peg-IFN plus RBV observed in these individuals are somewhat higher, as compared to HIV/HCV genotype 1 infections; however, approximately two thirds do not achieve SVR [2]. Triple therapy including Peg-IFN, RBV and either telaprevir or boceprevir has recently become the standard of care against chronic hepatitis C by genotype 1 in the HIV-coinfected patient [3]. SVR rates with these regimens observed in clinical trials in treatment-naïve patients have reached up to 74% [4,5] and data obtained under real-life conditions are also promising [6,7]. However, response in all patients is not achieved. In the case of HIV/HCV genotype 4 coinfection, no alternative treatment option has been approved to date. Therefore, predictive tools to select patients with a very high or a very low probability to achieve SVR are necessary in the current clinical practice, especially for those infected with HCV genotype 4.

As it is the case for dual therapy, the standard recommended treatment duration for triple therapy in HIV/HCV-coinfected patients remains 48 weeks [3]. The finding of reliable predictors for SVR could, on the one hand, help us to identify candidates who may benefit from dual therapy and, on the other hand, allow the development of shorter treatment schedules with direct-acting antivirals (DAAs). Since tolerance to Peg-IFN plus RBV is poor especially in the setting of antiretroviral therapy, the benefit of shorter regimens is even higher in HIV-coinfected patients. Likewise, source-limited settings are in high need of predictors of SVR to dual therapy. Pharmacogenetic determinations represent cost-effective tools to predict the probability of SVR. In this context, the single nucleotide polymorphism (SNP) rs12979860 near the interleukin 28B (IL28B) gene is a potent predictor for SVR to dual therapy in HIV/HCV genotype 1 or 4-coinfected patients [2,3,9]. Likewise, it has a lower, but evident, impact on the outcome of first-generation protease inhibitor (PI)-based therapy in prior treatment-naïve patients without HIV coinfeciton [10–12]. The predictive capacity of IL28B genotype can be enhanced by its combination with viral and host factors [13–16]. In this context, the determination of genetic variations of the SNP rs14158, at the low-density lipoprotein receptor (LDLR) gene, increases the IL28B predictive performance [13], which may be caused by the HCV viral replication cycle being affected by cholesterol and fatty acid biosynthesis. However, the predictive value obtained for HIV/HCV genotype 1 or 4-infected patients even using IL28B plus rs14158 genotyping is suboptimal, as the probability of SVR in patients identified as likely responders using these parameters hardly reaches 70% [13–16].

This study aimed to identify genes other than IL28B and LDLR whose variations predict response to Peg-IFN plus RBV and which may allow us to enhance the predictive value of IL28B genotype in HIV/HCV genotype 1 or 4-infected patients.

Methods

Study population

HCV genotype 1 or 4-infected patients were selected from a cohort that was prospectively followed at the units of Infectious Diseases of five Spanish hospitals between June 2000 and December 2010. The inclusion criteria in this cohort were: i) older than 18 years, ii) prior HCV treatment naïve, iii) initiation of dual therapy with Peg-IFN plus RBV, iv) coinfection with HIV and v) stored whole blood samples available for genetic determinations. Responses were analyzed in an on-treatment approach, i.e., those who interrupted treatment due to adverse events or who voluntarily dropped out were excluded. The scheduled visits were at baseline, every 4 weeks during the first six months of treatment and every 12 weeks thereafter. In order to evaluate SVR, a visit 24 weeks after stopping therapy was conducted.

Treatment regimens and definition of response

Peg-IFN alfa–2a or alfa–2b was administered at doses of 180 μg or 1.5 μg/kg once per week, respectively, in combination with weight-adjusted RBV (1000 mg/day for <75 kg and 1200 mg/day for ≥75 kg). The scheduled treatment duration was 48 weeks for all patients. Stopping rules were applied following international guidelines in force at the moment of treatment. SVR was defined as undetectable plasma HCV RNA 24 weeks after the completion of therapy. A decline of plasma HCV RNA of less than 2 log_{10} at week 12 or lack of undetectable HCV RNA at treatment week 24 after having presented a ≥2 log_{10} decline but without reaching undetectability, was considered as non-response. Undetectable HCV RNA at the end of therapy without achieving SVR was considered as relapse. Viral breakthrough was defined as detectable plasma HCV RNA during treatment after having reached undetectability.

Selection and genotyping of SNPs and HCV RNA quantification

Genomic DNA was isolated from whole blood samples or from isolated peripheral blood mononuclear cells using the automated MagNA Pure DNA extraction method (Roche Diagnostics Corporation, Indianapolis, IN, USA).
or the Quick Pure Blood DNA extraction Kit (Macherey-Nagel, Düren, Germany). Selected SNPs included SNPs belonging to genes involved in the cholesterol metabolism and transport or in the immune response and which were identified as tag SNPs after an analysis of the genotype data obtained from Hapmap CEU population (www.hapmap.org) using Haplovew V4.1 software (https://www.broad.harvard.edu/haplovew/haplovew). Likewise, SNPs from genes which had previously been found to be associated with SVR or with liver fibrosis in HCV-infected patients were tested [17–19]. All SNPs were genotyped using a custom Golden Gate Veracode genotyping assay (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions. Those SNPs with the following characteristics were discarded: i) a minor allele frequency <0.05, ii) a Hardy-Weinberg-Equilibrium p<0.01 and iii) genotyping of less than 80%. Genotyping of IL28B and determination of plasmatic HCV-RNA was conducted as described elsewhere [8,9]. The researchers responsible for genotyping were blinded to other patient data.

Data analysis
Haplovew V4.1 Software (Haplovew V4.1 software (http://pngu.mgh.harvard.edu/~purcell/plink/gplink.shtml) was used to calculate the Hardy-Weinberg equilibrium for all SNPs. A first analysis of standard case/control allelic associations (1 degree freedom), as well as of different genetic models using PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/gplink.shtml) was performed. Those SNPs that were associated with SVR showing a p-value <0.05 were selected for further analysis. Genotypes within one SNP that showed similar rates of SVR were combined. The linkage disequilibrium (LD) values between the genetic markers studied (measured in Lewontin’s standardized disequilibrium coefficient D’), haplotype frequencies, and haplotype-based association analyses were calculated using Haplovew V4.1 for those genes with ≥2 SNPs available. Chromosome phases for LDLR haplotypes were estimated using PLINK software.

Continuous variables were compared by means of the Student’s t-test or the Mann-Whitney U-test, when applicable, and are presented as median (interquartile range, IQR). For the analysis of categorical variables, the χ² test or the Fisher’s exact test was used in 2x2 tables. SVR was the primary outcome variable. A multivariate logistic regression analysis including as covariables age, sex, as well as those factors that were associated with SVR with a p value <0.05 in the univariate analysis, was conducted. For additive genetic factor analysis, a simple unweighted genetic risk score (GRS) was calculated with those SNPs that were independently associated with SVR. In the subpopulation of IL28B genotype CC carriers, protective genotypes were added (+1 point for each favourable genotype, unfavourable genotypes were rated as 0). Likewise, in the subpopulation of patients presenting IL28B genotype non-CC, unfavourable genotypes were subtracted (-1 point for each unfavourable genotype, favourable genotypes were rated as 0). Descriptive and inferential analyses were carried out using the SPSS statistical software package release 19.0 (IBM Corporation, Somers, NY, USA).

Ethical issues
The study was designed and performed according to the Helsinki declaration and was approved by the Ethics Committee of the Hospital Universitario de Valme.

Results

Overall study population
A total of 262 patients fulfilled the inclusion criteria for this study. Of these, 57 (21.8%) individuals were excluded because they could not be assessed in an on-treatment approach. Finally, 205 patients were selected (Table 1). One hundred and sixty-two (79) and 43 (21%) bore HCV genotype 4. Sixty-seven (32.8%) presented a baseline plasma HCV RNA below 600000 IU/mL.

Response to Peg-IFN plus RBV
SVR was achieved by 73 (35.6%) individuals. Undetectable HCV RNA at week 4 of treatment was observed in 32 (16.8%) of the 190 patients in whom this information was available. Eighty-nine (43.4%) patients were non-responders and 30 (14.6%) subjects relapsed. Thirteen (6.3%) individuals experienced a viral breakthrough. Fifty-five (34%) of the patients infected with HCV genotype 1 versus 18 (41.9%) of those individuals bearing HCV genotype 4 achieved SVR (p=0.36). The rate of patients achieving SVR according to IL28B rs12979869 genotype was: 44 (55.7%) for CC, 20 (20.8%) CT and 9 (10.3%) TT.

Table 1. Baseline characteristics of the study population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tbody>
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<td>Age (years)*</td>
<td>41.6 (38.5–44.8)</td>
</tr>
<tr>
<td>Male gender, no. (%)</td>
<td>170 (82.9)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)*</td>
<td>23.4 (21.6–26.2)</td>
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<tr>
<td>Intravenous drug user, no. (%)</td>
<td>174 (84.9)</td>
</tr>
<tr>
<td>HCV genotype 1, no. (%)</td>
<td>162 (79)</td>
</tr>
<tr>
<td>IL28B rs12979860 CC, no. (%)</td>
<td>79 (38.3)</td>
</tr>
<tr>
<td>Plasma HCV viral load (log10 IU/mL)*</td>
<td>6.21 (5.52–6.72)</td>
</tr>
<tr>
<td>Advanced fibrosis, no. (%)</td>
<td>80 (47.6)</td>
</tr>
<tr>
<td>Alanine-aminotransferase(U/L)*</td>
<td>66 (44–99.5)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)*</td>
<td>168 (148–196)</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol (mg/dL)*</td>
<td>92.6 (74.9–116)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)*</td>
<td>117 (83–172)</td>
</tr>
<tr>
<td>Pegylated interferon a2a, no. (%)</td>
<td>146 (71.2)</td>
</tr>
<tr>
<td>Daily dose of ribavirin (mg/kg)*</td>
<td>14.9 (13.1–16.9)</td>
</tr>
<tr>
<td>Undetectable plasma HIV RNA, no. (%)</td>
<td>167 (81.5)</td>
</tr>
<tr>
<td>CD4 cell count (cells/μL)*</td>
<td>521 (388–730)</td>
</tr>
</tbody>
</table>

*median (IQR); †determined by liver biopsy (F≥3 according to the Scheuer Index) or a liver stiffness value ≥11 kPa, available in 168 patients.
(30%) TT, respectively (p = 8.1*10^{-6}). Individuals who achieved SVR presented a baseline median HCV RNA (IQR) of 5.7 (5.1–6.5) log_{10} IU/mL versus 6.4 (5.9–6.8) log_{10} IU/mL for those who did not (p = 4.5*10^{-6}). The median (IQR) plasmatic LDL cholesterol levels were 105 (84.6–120) mg/dL among patients with SVR versus 89 (70.5–113) mg/dL for those without SVR (p = 0.007).

**SNPs selected**

One hundred and forty-four SNPs were selected for the primary analysis (Supplement 1). Of these, 26 were excluded due to either a minor allele frequency <0.05 [20(15.9%)SNPs], a p of the Hardy-Weinberg-Equilibrium below 0.01 [1(0.7%)SNP] or a rate of genotyping below 80% [5(3.5%)SNPs]. Thus, 118 (81.9%) of the previously selected SNPs were analyzed (Supplement 1).

SNPs at the following genes were associated with SVR in the analysis conducted using PLINK software: LDLR (rs10415811, p = 0.047; rs16721261, p = 0.0067; rs1433009, p = 0.0019; rs2569540, p = 0.0044; rs95930, p = 0.046), transforming growth factor β (TGF-β; rs1800469, p = 0.0087), aquaporin 2 (AQP-2; rs2878771, p = 8*10^{-6}), very-low-density lipoprotein receptor (VLDLR; rs1454626, p = 0.015), Sp110 nuclear body protein (SP110, rs919178, p = 0.045), interferon alpha/beta receptor 1 (IFNAR1, rs1244592, p = 0.036), 2′-5′-oligoadenylate synthase 1 (OAS1; rs1131454, p = 0.047) and apolipoprotein B (APO-B; rs11126598, p = 0.0094). Genotyping was achieved in 100% (AQP-2, VLDLR, SP110, IFNAR1, OAS1 and APO-B) and 97.1% (TGF-β) of the cases, respectively. The VLDLR rs7043199 was discarded due to the low prevalence of its favourable genotype TT (2.9%), so that subsequent analysis exclusively refers to rs1454626.

**Treatment response according to newly identified SNPs**

According to the SVR rates observed for each genotype, the SNPs genotyping results were classified as favourable and unfavourable. Figure 1 presents the rates of SVR, as well as the distribution of each genotype in the study population. SVR rates according to variations of rs1131454 in the IFNAR1 gene were 18 (49%) for genotype CC and 55 (33%) for genotype AG/AA (p = 0.067).

In spite of the number of SNPs in specific genes identified as predictors for SVR in this study, haplotype analysis could only be performed for LDLR, VLDLR and TGF-β genes due to the number of SNPs analyzed in these genes (Supplement 1). In the LDLR gene, seven LD blocks were detected (Figure 2). An extended haplotype (GGAAG) in LD block number 6 defined by rs2738464, rs2738465, rs1433099, rs2738466 and rs7258950 (Figure 2) was associated with SVR (p = 0.0046). After categorization, 31 (50.8%) of the subjects with LDLR haplotype GGAAG versus 42 (29.4%) of those with the non-GGAAG haplotype showed SVR (p = 0.003). Among the HCV genotype 1-infected subjects, 22 (50%) of those presenting LDLR haplotype GGAAG versus 33 (28.2%) of those with other LDLR haplotypes reached SVR (p = 0.009). The corresponding figures for HCV genotype 4 carriers were 9 (52.9%) versus 9 (34.6%), respectively (p = 0.23). The interaction between LDLR haplotype and IL28B genotype on SVR rates are shown in Table 3. No haplotype of VLDLR or TGF-β was associated with SVR.

**Interaction of newly identified SNPs with the predictive value of IL28B rs12979860**

Forty-four (55.7%) of the patients presenting IL28B genotype CC versus 29 (23%) of those with genotype CT/TT attained SVR (p = 2*10^{-6}). The corresponding figures among the subpopulations infected with HCV genotype 1 and 4 were 34 (51.5%) versus 21 (21.9%; p = 9*10^{-4}) and 10 (76.9%) versus 8 (26.7%; p = 0.002), respectively. SVR rates according to the newly identified genotypes stratified for IL28B genotype are listed in Table 2. After categorization for IL28B, TGF-β and AQP-2 genotypes, the number of patients reaching SVR with favorable genotypes (IL28B CC, TGF-β non-AA and AQP-2 non-GG) versus unfavorable genotypes (IL28B non-CC, TGF-β AA and AQP-2 GG) were 22 out of 28 (78.6%) versus 1 out of 13 (7.7%) (p = 2.1*10^{-5}).

According to HCV genotype, the numbers of individuals with the former combinations of genotypes achieving SVR were 16 (76.2%) versus 1 (9.1%) in HCV genotype 1 (p = 3*10^{-4}) and 6 (85.7%) versus 0 in HCV genotype 4 (p = 0.023), respectively. Furthermore, an interaction between IL28B and VLDLR, SP110, IFNAR1 and APO-B, respectively, was observed (Table 2).

**Multivariate analysis**

According to the univariate analysis, SNPs in TGF-β, AQP-2, VLDLR and APO-B genes, as well as the LDLR haplotype GGAAG, were entered into a multivariate logistic regression model adjusted for age, sex, presence of advanced fibrosis, HCV baseline viral load and IL28B genotype. SP110 and OAS1 were excluded due to their considerably weak association with SVR and, mainly, because of the low prevalence of the unfavourable genotype (Figure 1). In the multivariate analysis TGF-β non-AA, AQP-2 non-GG and LDLR GGAAG were independently associated with SVR along with IL28B CC genotype and low baseline HCV RNA load (Table 3).

**Additive genetic factor analysis**

In accordance with the multivariate analysis, TGF-β, AQP-2 and LDLR haplotype were selected for this calculation. In patients presenting IL28B genotype CC, the meanGRS for patients with SVR was 1.88 versus 1.18 presented by those individuals without SVR (p = 1.3*10^{-4}). In the subpopulation of patients with IL28B non-CC genotype, mean GRS was −1 in patients presenting SVR.
versus −1.62 in those without SVR (p = 3.5 × 10⁻³). The rates of SVR according to different GRS among patients with favourable IL28B genotype were 0 out of 4 patients with GRS = 0, 38.9% (14 patients) with GRS = 1, 82.6% (19 patients) with GRS = 2 and 75% (9 patients) with GRS = 3, p = 4 × 10⁻⁴. Among patients with IL28B genotype non-CC, the rates of SVR were 55.6% (5 patients) for GRS = 0, 34.6% (18 patients) for GRS = −1, 8.9% (5 patients) for GRS = −2 and none out of 6 patients for GRS = −3, p = 0.001.

### Discussion

This study has identified genetic variations in the TGF-β and AQP-2 genes as independent predictors of SVR to Peg-IFN plus RBV in HIV/HCV genotype 1 or 4-infected patients. Additionally, a haplotype on the LDLR gene has also been found to be associated with SVR. These pharmacogenomic parameters improve the predictive capacity of IL28B genotype and may therefore play a role in the development of a tool to accurately predict response to therapy against HCV.

The IL28B genotype is a potent predictor of response to dual therapy in HIV/HCV-coinfected patients [2,8,9], which is commonly used in daily practice. However, the meaning of a favourable or unfavourable IL28B genotype in terms of the likelihood of SVR is very different depending on viral [16] and host factors, such as plasma levels of IP-10 [15] or LDLR genotype [13,14]. This study demonstrates that other genomic factors may determine the SVR rates associated with IL28B variations and that some of those factors with a greater impact

![Fig. 1. Rates of sustained virological response (SVR) according to genotypes of the SNPs newly identified and distribution of each genotype in the study. A: transforming growth factor β (TGF-β); B: aquaporine 2 (AQP-2); C: very-low-density lipoprotein receptor (VLDLR); D: Sp110 nuclear body protein (SP110); E: 2’-5’-Oligoadenylate synthase 1 (OAS1); F: apolipoprotein B (APO-B). n = Number of patients achieving SVR for each genotype. N = Number of patients bearing the specific genotype in the overall population.](image-url)
Plasma lipoproteins, including VLDLR [31] and APO-B [32], play an important role on HCV infectivity and on the outcome of therapy against HCV. Genetic variations in the VLDLR gene were observed to impact on SVR herein, although no independent association was observed in the multivariate analysis. The identification of a LDLR haplotype associated with SVR supports the findings of a previous study where an influence of a specific SNP (rs14158) in the 3'UTR of the LDLR gene on SVR was described [13]. Likewise, this study shows that using this haplotype, the predictive capacity of isolated SNPs in LDLR genes is improved.

The predictive performance of IL28B genotype can be markedly enhanced by using other genomic predictors concomitantly. This has been proven using the combination of IL28B and the rs14158 CC genotype on the LDLR gene [13]. However, almost 31% of the carriers of both IL28B and LDLR favourable genotypes do not respond to therapy, whereas 14% of those harboring both unfavourable genotypes show SVR to Peg-IFN plus RBV[13]. This points out the necessity to optimize this combination of genotypes. In this study, using three genotypes (IL28B/AQP-2/TGF-β), the probability of SVR increased to 80% for the favourable combination and it was only 7% for the triple unfavourable genotype. Unfortunately, the clinical utility of this combination is limited because the triple favourable and unfavourable genotypes are relatively uncommon; indeed, they were

**Fig. 2. Genomic position of the SNPs on the LDLR gene and linkage disequilibrium blocks analyzed.**
**Table 3. Univariate and multivariate analysis to identify factors associated with sustained virologic response (SVR).**

<table>
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<tr>
<th>Parameter</th>
<th>SVR, n (%)</th>
<th>P univariate</th>
<th>AOR (95% CI)</th>
<th>P multivariate</th>
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<td><strong>Age</strong></td>
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<tr>
<td>≤41.6 years</td>
<td>42 (41.2)</td>
<td>0.098</td>
<td>1 (0.91–1.1)</td>
<td>0.961</td>
</tr>
<tr>
<td>&gt;41.6 years</td>
<td>31 (30.1)</td>
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<td><strong>Gender</strong></td>
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<tr>
<td>Male</td>
<td>61 (35.9)</td>
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<td>0.776</td>
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<tr>
<td>Female</td>
<td>12 (34.3)</td>
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<td>0.844 (0.26–2.73)</td>
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<td><strong>Advanced fibrosis</strong></td>
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<tr>
<td>No</td>
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<td>0.093</td>
<td>2.11 (0.86–5.15)</td>
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<td>Yes</td>
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<td>≤600000 IU/mL</td>
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<td>5.87 (2.19–15.7)</td>
<td>4.3×10^{-4}</td>
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<td><strong>IL28B rs12979860</strong></td>
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<td>CC</td>
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<td>GG/AG</td>
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<td><strong>AQP-2 rs2878771</strong></td>
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</tr>
<tr>
<td><strong>APO-B rs11126598</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>51 (42.9)</td>
<td>0.011</td>
<td>1.56 (0.65–3.75)</td>
<td>0.321</td>
</tr>
<tr>
<td>AG/AA</td>
<td>22 (25.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LDLR haplotype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGAAG non GGAAG</td>
<td>31 (50.8)</td>
<td>0.003</td>
<td>5.078 (1.79–14.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>non GGAAG</td>
<td>42 (29.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SVR: sustained virological response; AOR: adjusted odds ratio; CI: confidence interval; IL28B: interleukin 28B; IU: IU; LDLR: low-density lipoprotein receptor; TGF-β; transforming growth factor β; AQP-2: aquaporine 2; VLDLR: very-low-density lipoprotein receptor; APO-B: apolipoprotein B; LDLR: low-density lipoprotein receptor. 1 available in 168 individuals.
found only in 13.7% and in 6.3% of the population analyzed herein. However, it is probable that the combination of some of the SNPs described in this study with other viral or host predictors of SVR may yield valuable predictive tools. As it can be seen with the GRS calculation, SVR rates vary considerably according to the number of risk factors both among carriers of the favourable and the unfavourable IL28B genotype. Importantly, the rates of SVR are higher for those patients bearing IL28B non-CC but no other unfavourable genotype than those patients with IL28B CC but no other favourable genotype. In the case of HCV genotype 1 infection, this information could be used to select those individuals who may greatly benefit from dual therapy. This is a critical point because PI-based therapy is unlikely to be widely available in many countries due to financial restrictions in the next few years. Likewise, HCV genotype 4-infected patients with a very high probability to respond could be motivated to undergo dual therapy. On the other hand, treatment could be deferred in those patients with a very low likelihood to respond to dual therapy, if they do not present advanced fibrosis.

The duration of dual therapy against HCV may be decided on the basis of HCV kinetics on treatment [3,33,34]. Similarly, in patients with rapid viral decline, DAA-based therapy may be also shortened without reduction of the rate of SVR [35,36]. Viral kinetics in HCV-infected patients strongly depend on the pharmacogenomic host features [37–39]. Consequently, the genomic predictors identified here may correlate with viral kinetics. If so, these predictors could be useful to identify patients who qualify for shorter double or triple treatment durations from baseline, thus avoiding very early viral load determinations. Further studies are required in order to address this topic.

This study has some limitations. First, the number of patients is relatively limited to allow classification into multiple genetic profiles. This led to categories with low numbers of cases. Because of this, these data should be reproduced with a higher number of patients and in other populations. However, the main objective of this study was to identify novel SNPs that may be used to develop a predictive model that allows calculating the individual probability of response for each patient, and, in fact, we have identified SNP potentially candidates to be entered in predictive models along with viral and other host factors. Second, these results should be analyzed in HCV-monoinfected patients, since the predictive value may be different in this population. Third, the analysis presented herein is limited to HCV genotypes 1 and 4. In genotype 3-infected individuals, a higher mortality has been observed for IL28B CC carriers [40] and these individuals would benefit from identifying alternative predictors. However, and similar to what is observed for IL28B genotype in patients with or without HIV coinfection [8,9,41,42], no association between SVR and the SNPs described herein could be detected in HCV genotype 2 or 3-infected individuals (data not shown). However, an impact of a SNP on the proprotein convertase subtilisin/kexin type 9 gene on SVR uniquely in genotype 3 infection has been described recently [39], lining out the necessity to distinguish between these genotypes. Finally, in the era of new DAAAs genomic predictors may be less important. However, an impact of IL28B genetic variations has been observed under interferon-based triple therapy [10–12] in treatment naïve patients. It also seems to play a role in interferon-free regimens [43,44], particularly with specific combinations [44]. Therefore, the value of genomic predictors is likely to remain important in the setting of DAA-based therapy. Furthermore, DAA-based therapy will not be available for all HCV-infected patients in most countries, mainly due to financial restrictions. Because of this, dual therapy may continue to be given in a significant number of patients in these settings. A combination of pharmacogenomic markers with high predictive performance may be very helpful to identify patients to be treated with dual therapy and, among them, those who may benefit from shorter courses of therapy.

In conclusion, there is a number of important genetic factors that modify the predictive capacity of IL28B genotype, as TGF-β, AQP-2 and LDLR genotype. A combination of these factors can be used to identify HIV/HCV genotype 1 or 4 infected patients with a very high or a very low probability to respond to dual therapy with Peg-IFN/RBV. Furthermore, the predictive ability of models based on these factors should be analyzed in patients on direct acting antivirals.

Financial support: This work was supported in part by the Red de Investigacion en SIDA (grant number RETIC RD06/006 / RD12/0017), the Fundación Progreso y Salud, Consejería de Salud de la Junta de Andalucía (grant number PI-0247–2010, PI-0157–2011, PI-0430–2012 PI10/01232), the Fondo de Investigaciones Sanitarias (grant number PI10/01664), the Ministerio de Sanidad y Servicios Sociales (grant number EC11–304) and the Fundación para la Investigación y la Prevención del Sida en España (grant number 121004/10). KN is the recipient of a Sara Borrell postdoctoral perfection grant from the Instituto de Salud Carlos III (grant number SCO/523/2008). JAP is the recipient of an intensification grant from the Instituto de Salud Carlos III (grant number Programa I3NSNS).

Acknowledgements

Author contribution:

K. N.: planning and conducting the study, collecting and interpreting data and drafting the manuscript.
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A. R.-J.: collecting and interpreting data.
P.B.: collecting and interpreting data.
L.M.: collecting and interpreting data.
L.M.R.: genotyping, collecting and interpreting data.
R. H.: collecting and interpreting data.
A. Camacho: collecting and interpreting data.
V. S.: planning and conducting the study, interpreting data and drafting the manuscript.
F. A. D. L.: collecting and interpreting data and drafting the manuscript.
J. M.: collecting and interpreting data and drafting the manuscript.
A. R.: planning and conducting the study, interpreting data and drafting the manuscript.
J. A. P.: planning and conducting the study, interpreting data and drafting the manuscript.

Source of Funding: D. M. has received consulting fees from Viiv Healthcare, Bristol-Myers Squibb, Abbott Pharmaceuticals, Merck Sharp & Dohme, Jansen-Cilag and Boehringer Ingelheim. V.S. has received consulting fees from Merck Sharp & Dohme and Boehringer-Ingelheim and Jansen. He has received research support from Merck Sharp & Dohme, Jansen and Gilead and Boehringer Ingelheim and received lecture fees from Gilead, Viiv Healthcare, Bristol-Myers Squibb, Merck Sharp & Dohme and Jansen. A.R. has received consulting fees from Bristol-Myers Squibb, Abbott, Gilead, Jansen Cilag, Merck Sharp & Dohme and BoehringerIngelheim, has received research support from Abbott, GlaxoSmithKline, Roche, Bristol-Myers Squibb, Abbott and BoehringerIngelheim and has received lecture fees from GlaxoSmithKline, Abbott, Bristol-Myers Squibb, Merck Sharp & Dohme, Jansen Cilag, Gilead, Boehringer Ingelheim and Schering-Plough. J. A. P. has received consulting fees from GlaxoSmithKline, Bristol-Myers Squibb, Abbott Pharmaceuticals, Gilead, Merck Sharp & Dohme, Schering-Plough, Jansen and Boehringer Ingelheim. He has received research support from GlaxoSmithKline, Roche, Bristol-Myers Squibb, Schering-Plough, Abbott Pharmaceuticals, Gilead, Merck Sharp & Dohme, Jansen and Boehringer Ingelheim, and has received lecture fees from GlaxoSmithKline, Roche, Abbott Pharmaceuticals, Bristol-

Myers Squibb, Gilead, Merck Sharp & Dohme, Jansen, Boehringer Ingelheim and Schering-Plough.

Conflicts of Interest
All other authors: none to declare.

References

Large-scale candidate gene analysis of spontaneous LDLr genotype modifies the


