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Running head: *IL28RA* SNP and failure of anti-HCV therapy

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ABSTRACT

Due to the poor rate of response to hepatitis C virus (HCV) treatment in HCV/HIV coinfected patients, key factors for predicting failure would be useful to inform about initiation of HCV-treatment with pegylated-interferon alpha plus ribavirin. We performed a retrospective study on 291 patients on HCV-treatment, who had early virological response data. *IL28B* and *IL28RA* polymorphisms were performed by using the GoldenGate® assay. Unfavorable genotypes at *IL28B* (rs12980275 AG/GG and rs8099917 GT/GG) and an unfavorable allele at *IL28RA* (rs10903035 G) were associated with early treatment failure (ETF). However, only the rs12980275 AG/GG genotype and rs10903035 G allele remained independently associated with ETF in the overall population (OR= 4.15 (95%CI= 1.64-10.54) and OR= 2.00 (95%CI= 1.19-3.36), respectively) as well as in GT1/4 patients (OR= 5.07 (95%CI= 1.81-14.22) and OR= 2.03 (95%CI= 1.13-3.66), respectively). Next, a decision tree showed ETF rate increased from 37.1% to 65.5% when the unfavorable rs12980275 AG/GG and rs10903035 AG/GG genotypes and HCV-RNA≥ 500.000 IU/ml were taken into account in GT1/4 patients. In contrast, ETF rate decreased from 37.1% to 11.9% when the favorable rs12980275 AA and rs10903035 AA genotypes were detected. The percentage of patients correctly classified was 78.4% and AUROC was 0.802±0.028. Regarding GT3 patients, the presence of the GCGCA haplotype (all unfavorable alleles) was associated with ETF, while no association was observed for the *IL28B* polymorphisms. In conclusion, the *IL28RA* polymorphism was associated with ETF independently of the *IL28B* SNPs. The combination of *IL28B* and *IL28RA* polymorphisms might be a valuable tool for predicting ETF before starting HCV-treatment.

Key words: *chronic hepatitis C, HIV, IL28B, IL28RA, interferon, polymorphism.*

INTRODUCTION

Hepatitis C virus (HCV) infection is the most common cause of chronic liver disease. The consequences of HCV infection are worse in human immunodeficiency virus (HIV)/HCV coinfected patients, which is a common scenario because both viruses share the same transmission pathways (1, 2). HCV-related liver disease progression occurs faster and mortality by decompensated cirrhosis is frequent in this population (3).

The standard of care for chronic hepatitis C (CHC) in HIV/HCV coinfected patients consists of pegylated-interferon alpha (pegIFNα) plus weight-based ribavirin (RBV) (4). The rate of response is lower than in HCV monoinfected patients and sustained virological response (SVR) is achieved only in 27%-40% of HIV/HCV coinfected patients. Some of the causes explaining this poor response rate could include immunodeficiency, potential drug interactions with HAART, higher rates of serious side effects, and poor adherence to therapy (5-7). Despite the emergence of new direct-acting antivirals like Telaprevir and Boceprevir, treatment with pegIFNα/RBV is still standard, including as part of combination therapy with these newer drugs (8).

Due to the poor rate of response to HCV treatment, key factors predicting response or failure would be useful to inform about initiation and/or duration of therapy and therefore, reduce the cost and adverse effects caused by ineffective treatment. Some HCV factors such as viral genotype 1/4 and high baseline HCV viral load provide information about therapy success. Regarding host factors, advanced age, male gender, black ethnicity, presence of fibrosis and cirrhosis, and initial non-response to pegIFNα/RBV therapy have also been associated with unfavorable outcomes (9). However, there is an unexplained variability in treatment outcomes, suggesting that genetic background has an important role in this matter, as well.

It is known that polymorphisms around the *interleukin 28B* (*IL28B*) gene have been strongly associated with HCV treatment outcomes in either HCV monoinfected and HIV/HCV coinfected patients (10-13). The *IL28B* gene is located on chromosome 19 and encodes IFN-λ3, a type III interferon (IFN) cytokine, which has potent antiviral activity (14). IFN type III induces its cellular activity through a heterodimer receptor (IFN-λ receptor) composed of interleukin 28 receptor alpha (*IL28RA*) and interleukin 10 receptor beta (*IL10RB*), which influence IFN type III signaling (15, 16). Polymorphisms in the IFN-λ receptor have been scarcely studied so far but could also influence response to treatment.

A pharmacogenomic treatment approach for HIV/HCV coinfected patients could be envisaged with the incorporation of host genetic, clinical, epidemiological, and viral factors into an algorithm for predicting HCV treatment response or failure (17-19). The aim of our study was to elucidate the association and predictive value of *IL28B* and *IL28RA* polymorphisms to early treatment failure (ETF) of pegIFNα/RBV in HIV/HCV coinfected patients.

PATIENTS AND METHODS

Patients

We carried out a retrospective study in HIV/HCV coinfected patients who started HCV treatment with pegIFNα/RBV on regular follow-up at two reference HIV hospitals located in Madrid, Spain. The study population was represented by HIV/HCV–coinfected individuals who had completed a course of pegIFNα/RBV therapy and were genotyped for *IL28B* and *IL28RA* polymorphisms. Only patients who were IFN-naïve were included in this analysis. All patients were of European ancestry.

This work was conducted in accordance with the Declaration of Helsinki. All patients gave their written consent to be included in the study and the Institutional Ethics Committee approved the study.

Clinical data

The following information was obtained from medical records: age, gender, risk category, weight, height, nadir $CD4^+$ T cell count, antiretroviral therapy, and HCV genotype. In addition to this, other data were obtained during HCV therapy (complete blood counts, $CD4^+$ T-cells, plasma HIV viral load (HIV-RNA) and plasma HCV viral load (HCV-RNA)).

Liver fibrosis stage was characterized by two different protocols: a) in the Hospital General Universitario Gregorio Marañón, fibrosis score was estimated following the criteria established by the METAVIR Cooperative Study Group (20): F0, no fibrosis; F1, portal fibrosis; F2, periportal fibrosis or rare portal-portal septa; F3, fibrous septa with architectural distortion but with no obvious cirrhosis (bridging fibrosis); and F4, definite cirrhosis; and b) the Hospital Carlos III used transient elastometry (FibroScan®, Echosens). Liver stiffness values were separated into four ranges (≤7.0, 7.1 – 9.4, 9.5 – 12.4, and ≥12.5) and were considered to correspond to Metavir scores F0-F1, F2, F3, and F4, respectively (21).

DNA Genotyping

Genomic DNA was extracted from peripheral blood with Qiagen columns (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). DNA samples were sent to the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) in order to genotype four polymorphisms within or near the *IL28B* gene (rs12980275, rs8099917, rs7248668, and rs11881222) and five polymorphisms within the region of the *IL28RA* gene (rs10903032, rs10903034, rs10903035, rs11249002, and rs11249006). Genotyping was performed by using GoldenGate® assay with VeraCode® Technology (Illumina Inc. San Diego, CA, USA).

Hepatitis C therapy

HCV treatment regimens included pegIFNα 2a or 2b at standard doses (180 µg/week or 1.5 µg/kg/week, respectively) plus weight-adjusted RBV dosing (1000 mg/day for patients weighing <75 kg and 1200 mg/day for patients weighing ≥75 kg). Following international guidelines (4), patients with HCV genotypes 1 or 4 (GT1/4) received either 48 or 72 weeks of treatment, and patients with HCV genotype 3 (GT3) were treated for 24 or 48 weeks, depending on the virologic response at week 4. None of the patients in these series were infected with HCV genotype 2. Early stopping rules were applied to subjects with suboptimal virologic responses at weeks 12 and 24 (4).

HCV and HIV plasma viral load

HCV-RNA viral load was measured by polymerase chain reaction (PCR) (Cobas Amplicor HCV Monitor Test, Branchburg, NJ, USA) and real-time PCR (COBAS AmpliPrep/COBAS TaqMan HCV test); results were reported in terms of international units per milliliter (IU/mL), with a lower limit of detection of 10 IU/mL.

Outcome variables

This study was focused on investigating the association of *IL28B* and *IL28RA* polymorphisms with ETF as defined as the lack of achieving early virologic response (EVR) (7). These patients were identified in our study as null-responders.

Statistics

Statistical analysis was carried out by on-treatment (OT) analysis of observed data. Patients that prematurely interrupted their HCV treatment due to adverse events, abandonment, or loss of follow-up were discarded from the analysis. All p-values were two-tailed. Statistical significance was defined as $p<0.05$.

Categorical data were analyzed using the chi-squared test or Fisher's exact test. We performed a multivariate logistic regression analysis to investigate the association of *IL28B* and *IL28RA* polymorphisms with SVR and ETF. We selected the inheritance model that best fit the data (dominant model for *IL28B* SNPs and additive model for *IL28RA* SNPs). In order to adjust the logistic regression model, we selected the most significant covariables by a stepwise algorithm (at each step, factors are considered for removal or entry: a p-value for entry of 0.20 and exit of 0.15). The covariables analyzed by the stepwise algorithm were gender, age, body mass index (≥25 kg/m^2), nadir CD4$^+$ T-cells, undetectable HIV-RNA (<50 copies/mL), CD4+ T-cells, HAART, HCV-RNA ≥500,000 IU/ml, HCV genotype (GT1/4 vs. GT3) and significant fibrosis (F≥2).

We carried out a decision tree analysis, which is a useful tool to evaluate the influence of predictive factors on a particular outcome. Classification and regression tree (CART) was used to classify patients according to ETF using HCV genotype, HCV viral load and *IL28B* and *IL28RA* genotypes. CART is a prognostic system with a hierarchical structure, based on recursive portioning that builds a decision tree to identify subgroups at higher chance of ETF. The accuracy was evaluated by calculating the area under the receiver operating characteristic curves (AUROC). Criteria to qualify for accuracy were as follows: 0.90–1 = excellent, 0.80–0.90 = good, 0.70–0.80 = fair, and 0.60–0.70 = poor. All above-mentioned analyses were performed with the Statistical Package for the Social Sciences (SPSS) 15.0 (SPSS INC, Chicago, IL, USA).

Hardy-Weinberg equilibrium (HWE) of all single nucleotide polymorphisms (SNPs) was assessed by a $\chi2$ test, considering equilibrium when $p>0.05$ (22). In addition, pair-wise linkage disequilibrium (LD) analysis was computed to detect the inter-marker relationship using the standardized D' and r^2 values by Haploview 4.2 software (23). Haplotype-based association testing was performed by multivariate logistic regression adjusted by the covariates resulting from the stepwise algorithm, as above cited. Each haplotype was tested against all others. These analyses were performed with PLINK software (24).

RESULTS

Patients

Our cohort included 291 HIV/HCV coinfected patients on HCV treatment and for whom EVR data was available. Clinical and epidemiological characteristics of these patients are shown in **Table 1**. Two hundred and eleven out of 291 patients (72.5%) reached an EVR and 162 out of 291 patients (55.7%) reached a SVR. We did not have HCV genotype data for one patient. A total of 162 out of 290 patients (56.0%) were GT1, 94 (32.5%) were GT3, and 34 (11.8%) were GT4. When we stratified these data by HCV genotype, patients infected with GT1/4 had a rate of EVR and SVR of 62.8% and 42.7% of 196, respectively. For GT3 patients, EVR and SVR rates were 92.6% and 83.0% of 94, respectively.

Table 1. Main epidemiological and clinical characteristics of HIV/HCV coinfected patients on HCV antiviral therapy.

	Characteristic
No.	291
Male *	222 (76.3%)
Age (years) †	42 (38.5 – 45.5)
IVDU *	258 (88.7%)
HAART *	247 (84.9%)
Anthropometric values	
Height (m) †	1.70 (1.65 – 1.75)
Weight (Kgr) †	68 (60 - 76)
BMI (kg/m^2) †	23.2 (21.1 – 25.3)
BMI ≥25 kg/m ^{2} (n=280)	82 (29.3%)
HIV markers	
Nadir CD4+ T-cells/µL †	224 (119 - 329)
Nadir CD4+ <200 cells/µL *	125 (42.9%)
Baseline CD4+ T-cells/µL †	468 (307.5 – 628.5)
Baseline CD4+ ≥500 cells/µL (n=290) *	130 (44.8%)
HIV-RNA <50 copies/ml (n=289) *	224 (77.5%)
HCV markers *	
HCV-genotype (n=290)	
1	162 (56.0%)
3	94 (32.5%)
4	34 (11.8%)
HCV-RNA ≥500,000 IU/mL (n=286)	208 (72.7%)
Log_{10} HCV-RNA (IU/mL) †	6.13 (5.58 – 6.68)
Liver fibrosis (n= 254)*	
Significant fibrosis (F≥2)	159 (62.5%)
Advanced fibrosis (F≥3)	83 (32.6%)
Cirrhosis (F4)	42 (16.5%)

(*), Absolute number (percentage); (†), Median (interquartile range).

Abbreviations: BMI, body mass index; IVDU, intravenous drug users; HAART, highly active antiretroviral therapy; HCV, Hepatitis C virus; HCV-RNA, HCV plasma viral load; HIV-1, Human immunodeficiency virus type 1; HIV-RNA, HIV plasma viral load.

Linkage disequilibrium

We analyzed four *IL28B* polymorphisms (rs12980275, rs11881222, rs8099917, and rs7248668) and five *IL28RA* polymorphisms (rs10903032, rs10903034, rs10903035, rs11249002, and rs11249006). All analyzed SNPs were in HWE, fulfilled the minimum allele frequency (MAF) >0.05 for all samples and displayed less than 10% of missing values.

Strong LD was found among the following pairs of *IL28B* polymorphisms: rs12980275/rs11881222 (D´= 0.98; r^2= 0.94) and rs8099917/rs7248668 (D´= 0.98; r^2= 0.97) (**Supplemental Figure 1**). Regarding the *IL28RA* polymorphisms, strong LD was found between the following pairs: rs10903034/rs10903032 (D´= 0.99; r^2= 0.97), rs10903032/rs11249002 (D´= 1.0; r^2= 0.98), and rs10903034/rs11249002 (D´= 1.0; r^2= 0.99) (**Supplemental Figure 1**). Consequently, in order to avoid redundancy, we selected two *IL28B* SNPs (rs12980275 and rs8099917) and three *IL28RA* SNPs (rs10903034, rs10903035, and rs11249006) to analyze their association with ETF.

Genotype frequencies of IL28B and IL28RA SNPs according to HCV virological failure

Allelic and genotypic frequencies of *IL28B* and *IL28RA* polymorphisms are shown in **Supplemental Table 1**. The percentage of ETF was lower in patients carrying favorable genotypes (rs12980275 AA, rs8099917 TT and rs10903035 AA) (**Figure 1)**. When data were stratified by HCV genotype, GT1/4 patients with favorable genotypes (rs12980275 AA, rs8099917 TT, and rs10903035 AA) had a significantly lower proportion of ETF in comparison with those carrying unfavorable genotypes, whereas there were no significant differences between favorable and unfavorable *IL28B* and IL28RA genotypes for GT3 patients.

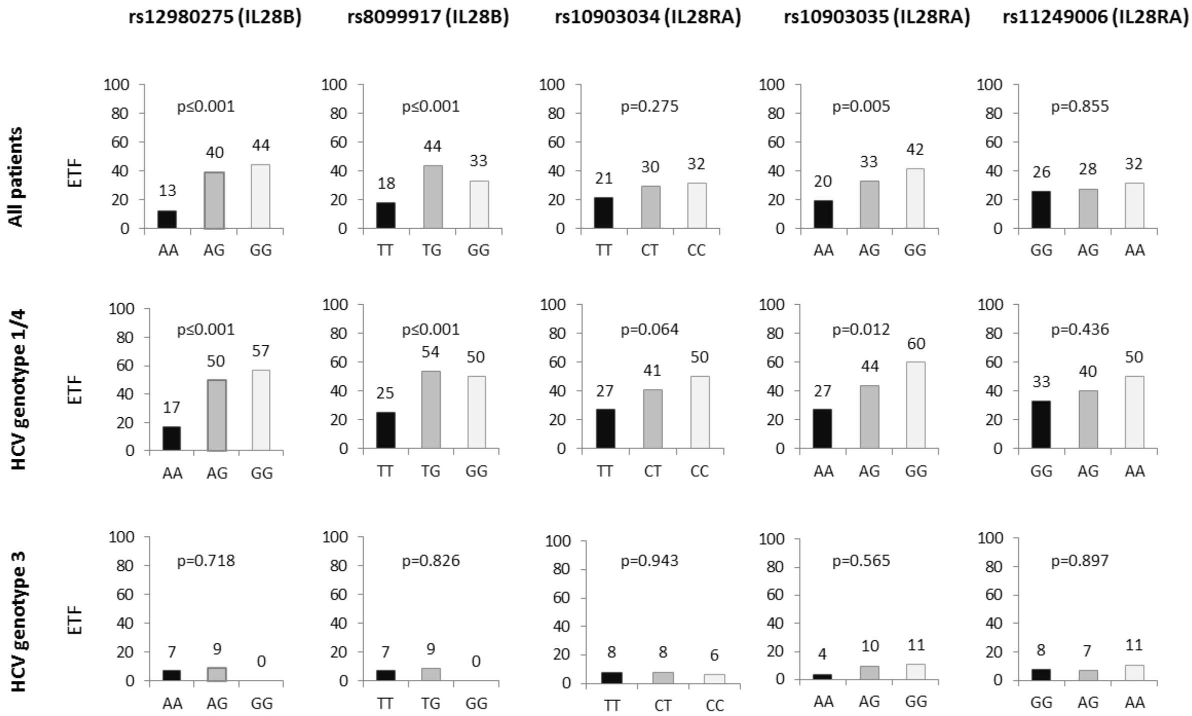

Figure 1. Percentages of HCV early treatment failure (ETF) according to HCV genotype and *IL28B* or *IL28RA* genotype among HIV/HCV coinfected patients on HCV treatment.

Multivariable logistic regression analysis

We examined the independent contribution of the *IL28B* and *IL28RA* alleles/genotypes on ETF via logistic regression analysis adjusted by the most relevant covariables (gender, HAART, HCV-RNA ≥500,000 IU/ml, F≥2 and GT1/4) identified by a stepwise algorithm (**Table 2**). Age, body mass index (≥25 kg/m^2), nadir $CD4^+$ T-cells, undetectable HIV-RNA, and CD4+ T-cells were excluded from the model due to their limited influence on the outcome variable.

Table 2. Association among favorable genotypes of *IL28B* and *IL28RA* with ETF to HCV therapy in HIV/HCV coinfected patients.

			All		Genotype 1/4		Genotype 3	
Gene	**SNPs**	**Inheritance Model***	**Adjusted OR (95%CI)**	**p-value**	**Adjusted OR (95%CI)**	**p-value**	**Adjusted OR (95%CI)**	**p-value**
IL28B	rs12980275 (AG/GG)	Dominant	5.00 (2.43-10.3)	**<0.001**	6.03 (2.71-13.43)	**<0.001**	1.98 (0.25-15.95)	0.520
	rs8099917 (TG/GG)	Dominant	2.88 (1.55-5.35)	**0.001**	3.11 (1.60-6.07)	**0.001**	2.11 (0.27-16.19)	0.474
IL28RA	rs10903034 (C)	Additive	1.54 (0.97 – 2.44)	0.068	1.65 (1.00 – 2.73)	0.051	1.04 (0.28 – 3.90)	0.951
	rs10903035 (G)	Additive	2.14 (1.30 – 3.52)	**0.003**	2.13 (1.23 – 3.68)	**0.007**	2.15 (0.58 – 8.01)	0.254
	rs11249006 (A)	Additive	1.44 (0.88 – 2.37)	0.145	1.43 (0.84 – 2.45)	0.188	2.02 (0.43 – 9.42)	0.372

*It should be taken into account that effect size is per genotype for the dominant model and per allele for the additive model.
Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; SNPs, single-nucleotide polymorphisms.
Data have been adjusted by gender, HAART, HCV-RNA ≥500,000 IU/ml, F≥2 and HCV-genotype 1/4.

Patients with unfavorable genotypes at *IL28B* (rs12980275 AG/GG and rs8099917 GT/GG) or an unfavorable allele at *IL28RA* (rs10903035 G) had increased odds of ETF (OR of 5.00, 2.88 and 2.14, respectively). Similar results were observed when this analysis was applied only to GT1/4 patients, whereas there was no significant influence of the *IL28B* and *IL28RA* alleles on ETF among GT3 patients (**Table 2**). Additionally, when we stratified these data by plasma HCV-RNA; patients with unfavorable genotypes at *IL28B* (rs12980275 AG/GG and rs8099917 GT/GG) or an unfavorable allele at *IL28RA* (rs10903035 G and rs10903034 C) had increased odds of ETF only among those with HCV-RNA ≥500,000 IU/ml (see **Supplemental Table 2**). However, we found that significant fibrosis had little influence on results when we took *IL28B* genotypes into account, while patients with rs10903035 G did have increased odds of ETF among patients with significant fibrosis but not other degrees of fibrosis (see **Supplemental Table 3**).

Moreover, when logistic regression was performed simultaneously on the three significant SNPs mentioned above, only the unfavorable rs12980275 AG/GG genotype and the unfavorable rs10903035 G allele remained significantly and independently associated with ETF in the overall population (OR= 4.15 (95%CI= 1.64-10.54) and OR= 2.00 (95%CI= 1.19-3.36), respectively), as well as in GT1/4 patients (OR= 5.07 (95%CI= 1.81-14.22) and OR= 2.03 (95%CI= 1.13-3.66), respectively).

Finally, we examined whether *IL28B* and/or *IL28RA* haplotypes were associated with ETF (**Table 3**). GT1/4 patients with all unfavorable alleles for *IL28B* polymorphisms (GGGA haplotype; comprised of rs12980275, rs11881222, rs8099917, and rs7248668, respectively) had increased odds of ETF (OR=2.78 (95%CI=1.53–5.08; p<0.001)) (**Table 3**). In contrast, patients with all unfavorable alleles for *IL28RA* polymorphisms (GCGCA haplotype; formed by rs10903032, rs10903034, rs10903035, rs11249002, and rs11249006, respectively) had increased odds of ETF for both GT1/4 patients (OR=2.28 (95%CI=1.19–4.39; p=0.011)) and GT3 patients (OR=4.67 (95%CI=1.02–21.4; p=0.043)) (**Table 3**).

Table 3. Association of *IL28B* and *IL28RA* haplotypes with HCV treatment failure in HIV/HCV coinfected patients.

Haplotype (*IL28B*; *IL28RA*)					**All**			**Genotype 1/4**		**Genotype 3**	
rs12980275	**rs11881222**	**rs8099917**	**rs7248668**		**Freq.**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
A	A	T	G		0.672	0.34 (0.20 – 0.55)	**<0.001**	0.32 (0.18 – 0.54)	**<0.001**	0.33 (0.07– 1.58)	0.161
G	G	G	A		0.194	2.28 (1.33 – 3.92)	**0.003**	2.78 (1.53 – 5.08)	**<0.001**	1.04 (0.12 – 9.12)	0.970
G	G	T	G		0.117	1.92 (1.04 – 3.53)	**0.035**	1.79 (0.94 - 3.41)	0.072	6.40 (0.60 – 68.7)	0.125
rs10903032	**rs10903034**	**rs10903035**	**rs11249002**	**rs11249006**							
A	T	A	T	G	0.584	0.66 (0.41 – 1.04)	0.071	0.61 (0.37 – 1.01)	0.052	0.96 (0.26 – 3.59)	0.951
G	C	G	C	A	0.182	2.59 (1.43 - 4.69)	**0.001**	2.28 (1.19 - 4.39)	**0.011**	4.67 (1.02 – 21.4)	**0.043**
G	C	G	C	G	0.119	1.16 (0.59 – 2.26)	0.664	1.33 (0.65 – 2.70)	0.431	NA	NA
G	C	A	C	A	0.110	0.54 (0.26 - 1.14)	0.094	0.68 (0.31 - 1.46)	0.306	NA	NA

Values expressed as odds ratio (OR) and 95% confidence interval (95% CI).
NA: it was not possible to calculate because of a low number in one of the groups.

Prediction of ETF

We constructed a decision tree (**Figure 2**) taking into account the following variables: HCV genotype, HCV viral load, and *IL28RA* and *IL28B* genotypes. The ETF rate of HCV-GT1/4 patients increased from 37.5% to 50.4%, 55.8% or 65.5% when the unfavorable genotypes of rs12980275 (AG/GG; IL28B), HCV-RNA≥500.000 IU/ml or the unfavorable genotypes of rs10903035 (AG/GG; IL28RA), respectively, were taken into account sequentially. In contrast, this rate decreased from 37.1% to 11.9% when the favorable genotypes (rs12980275 AA and rs10903035 AA) were considered sequentially. The overall percentage of patients correctly classified was 78.4% and the AUROC of this decision tree was 0.802±0.028. In order to evaluate our decision tree, a cross-validation strategy was performed by dividing data into 25 subsets. Decision trees were generated without including data for each subset (the first tree was based on all cases except the first subset of samples, the second tree was based on all cases except the second subset, etc.). The resulting overall percentage of patients correctly classified after validation was 77.3%.

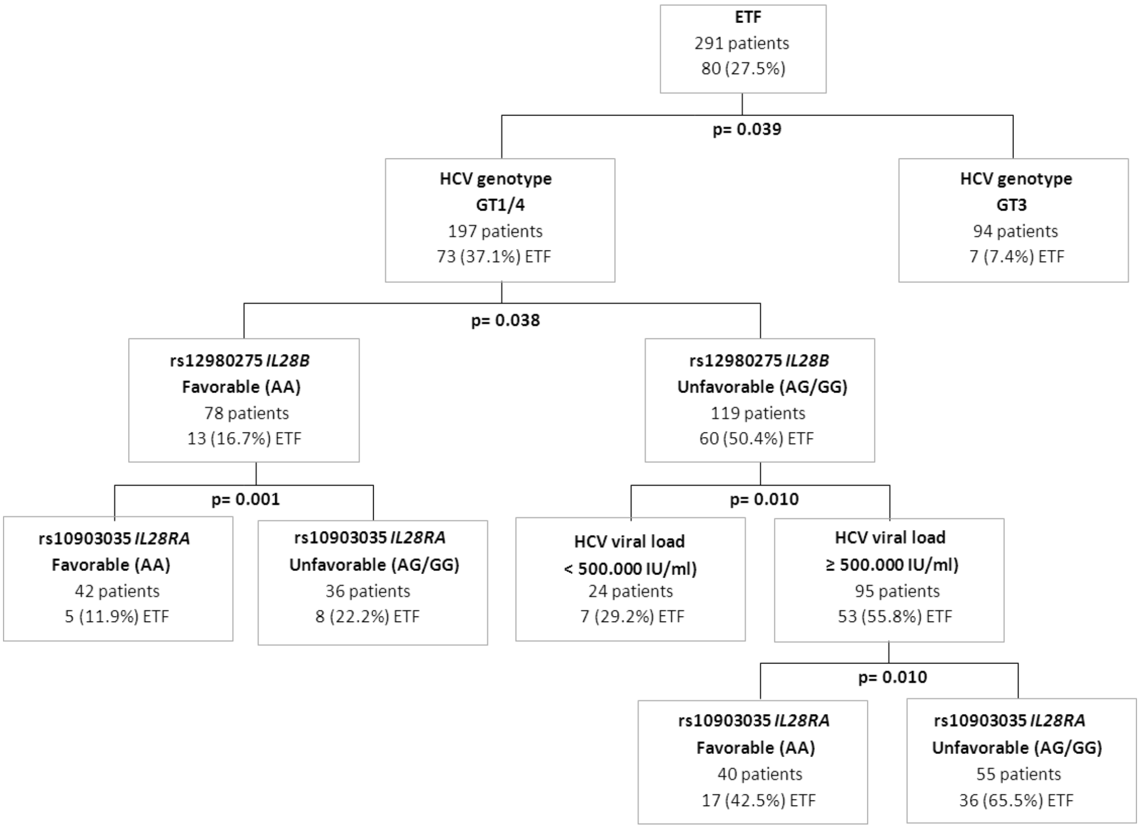

Figure 2. Representation of the decision tree for early treatment failure (ETF) among HIV/HCV coinfected patients on HCV treatment. Only significant nodes are shown.

DISCUSSION

In our study, we assessed four *IL28B* SNPs and five *IL28RA* SNPs in HIV/HCV coinfected patients under HCV treatment. As expected, *IL28B* polymorphisms were associated with SVR, but no association was found among *IL28RA* polymorphisms and SVR (data not shown). However, in respect to ETF, we found an association of an *IL28RA* polymorphism (rs10903035) and four *IL28B* polymorphisms (rs12980275, rs11881222, rs8099917, and rs7248668) with HCV therapy failure. rs10903035 is located at the 3' untranslated region (UTR) of the *IL28RA* gene, and each one of the *IL28B* SNPs studied are located at different positions around the *IL28B* gene (rs12980275 is downstream; rs11881222 is within intron 2; rs8099917 and rs7248668 are both upstream). The mechanism underlying this association is still unknown. On the one hand, those SNPs located in regulatory regions might play a key role in the modulation of gene expression and function, affecting the control of HCV replication, and therefore the response to HCV treatment. But on the other hand, these polymorphisms seem to be just tag SNPs that are in LD with the causal mutation.

To date, many articles have assessed the influence of IL28B polymorphisms on the virologic response in HIV/HCV coinfected patients, where rs12979860 has been the most studied, especially in patients with HCV genotypes 1 and 4 (17). In our study, we did not analyzed rs12979860, but we studied four other IL28B SNPs (rs12980275, rs8099917, rs7248668, and rs11881222), which were in high linkage disequilibrium with rs12979860 in European population (25, 26). In addition, they have been less studied than rs12979860 in Caucasian populations and thus, additional results including them would be of interest.

To our knowledge, this study shows the first evidence that HCV/HIV coinfected patients carrying the unfavorable allele of rs10903035 (G) have a high probability of ETF. At the same time, our study confirms the impact of the unfavorable genotypes of *IL28B* SNPs on HCV treatment failure during the first weeks of treatment (27-30). Besides, when stratifying patients by HCV genotype, similar results were obtained for HCV-GT1/4, but no association of any of the *IL28B* or *IL28RA* polymorphisms for HCV-GT3 was detected. Regarding HCV genotype 1 patients, our results indicated that *IL28RA* polymorphisms are similar but not better predictors of ETF than the *IL28B* polymorphisms. In considering another approach, we analyzed whether the use of rs10903035 together with the *IL28B* SNPs could improve the prediction of ETF. In order to check this hypothesis, a decision tree was built with HCV genotype, HCV viral load, rs12980275 and rs10903035 polymorphisms as the most important predictive factors. Our data suggested that rs10903035 (*IL28RA*) genotype provides an additional value to rs12980275 (*IL28B*) genotype for the classification of null-responder patients. In this setting, when unfavorable genotypes were considered, an increased ETF rate was detected. Thus, the inclusion of rs10903035 in the algorithm allowed us to improve the correct classification of patients who experience early failure to HCV treatment.

Although *IL28RA* polymorphisms have been previously described in several immune-driven pathologies such as multiple sclerosis and psoriasis among others (31, 32), its implication in infectious diseases has been scarcely studied. To our knowledge, only one article has described the relationship between *IL28RA* polymorphisms and HCV clearance outcomes (33). Cui et al. found in Chinese HCV monoinfected patients an association between the A allele at rs10903035 and HCV persistent infection compared to uninfected controls and the spontaneous HCV clearance group (33). Based on this study, we would expect the A allele to also be related to ETF, but we observed the opposite, that the G allele was associated to an increased likelihood of ETF. Different reasons may account for this anomaly. In the first case, the two studies were conducted on populations with different ethnic origins. Ethnicity seems to have a clear influence as observed by differential LD (the Chinese population showed a strong LD between rs10903035 and rs11249006, whereas it was not detected in our European population). Secondly, Cui et al. did not include HIV coinfected patients, and the aim of its study was different (33).

Haplotypes were also investigated to analyze whether they could improve the prediction of ETF compared to the SNPs alone. Although the IL28B GGGA haplotype (four unfavorable alleles) was the one most associated with ETF, this haplotype had similar OR values as the individual *IL28B* polymorphisms. The lack of prediction improvement provided by *IL28B* haplotypes has been previously published in reference to spontaneous HCV clearance (34). Similarly, we observed that the *IL28RA* GCGCA haplotype (all unfavorable alleles) was associated with ETF with similar OR as the individual *IL28RA* polymorphisms among HCV-GT1/4 patients. However, while no association was found between the individual *IL28RA* polymorphisms and ETF in HCV-GT3 patients, the combination of the polymorphisms did in fact show a significant association with ETF. Patients with the GCGCA haplotype showed almost five times higher odds of ETF compared to the other *IL28RA* haplotypes. To date, there does not yet exist an excellent genetic predictive marker of early failure to HCV treatment for HCV-GT3 patients (35, 36). According to our findings, the *IL28RA* haplotype might help in predicting ETF among HCV-GT3 patients, although additional studies are necessary to corroborate this finding.

Additionally, we evaluated the influence of *IL28B* and *IL28RA* polymorphisms on ETF considering HCV viral load and hepatic fibrosis. According to our results, the association between both *IL28B* and *IL28RA* polymorphisms with ETF seems be applicable only in patients with high HCV viral load (HCV-RNA ≥500.000 IU/ml). However, when we analyzed the influence of hepatic fibrosis, we found association of *IL28B* polymorphisms and ETF independently of liver fibrosis, and we only found a selective association of rs10903035 and ETF in patients with significant fibrosis (F≥2). These considerations should be taken into account in order to evaluate which patients could benefit from the predictive value of these polymorphisms.

Finally, for the correct interpretation of the data, it should be taken into account that our study had some limitations. First of all, our study design was retrospective. Secondly, our study was carried out entirely on Caucasians, therefore since the frequency of these alleles varies among ethnicities, it would be interesting to perform an independent repetition of these analyses on different ethnic groups. Finally, our study included only HIV/HCV co-infected patients, who have higher ETF rates than HCV mono-infected individuals and their HCV viral load decline is slower.

In conclusion, four *IL28B* polymorphisms (rs12980275, rs11881222, rs8099917, and rs7248668) and one *IL28RA* polymorphism (rs10903035) were individually associated with ETF in HCV genotype 1/4 patients. However, these polymorphisms did not predict treatment outcome in HCV genotype 3 patients. The *IL28RA* polymorphism was associated with ETF independently of *IL28B* SNPs. Additional use of rs10903035 improves the predictive value of *IL28B* for predicting ETF. Therefore, the combination of *IL28B* and *IL28RA* SNPs might be a valuable pretreatment tool to predict ETF among HIV/HCV coinfected patients. Additionally, the presence of a GCGCA haplotype for IL28RA polymorphisms could be an adequate predictor of ETF in patients with GT3, in which IL28B polymorphisms have shown a lack of association with ETF. Further studies on a large population are needed in order to conclusively corroborate these results.

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SUPPLEMENTAL MATERIALS

Supplemental Table 1. Allelic and genotypic frequencies of IL28B and IL28RA polymorphisms.

Gene	SNP	Allele/Genotype	All patients	Non ETF	ETF
IL28B	rs12980275 (n=290)	A	0.68	0.74	0.53
		G	0.32	0.26	0.47
		AA	0.46	0.56	0.21
		AG	0.44	0.37	0.64
		GG	0.09	0.07	0.15
	rs8099917 (n=291)	T	0.8	0.84	0.69
		G	0.2	0.16	0.31
		TT	0.63	0.71	0.41
		TG	0.34	0.27	0.55
		GG	0.03	0.03	0.04
IL28RA	rs10909034 (n=283)	T	0.59	0.61	0.54
		C	0.41	0.39	0.46
		TT	0.33	0.36	0.26
		TC	0.51	0.50	0.56
		CC	0.16	0.15	0.18
	rs10903035 (n=284)	A	0.70	0.73	0.61
		G	0.30	0.27	0.39
		AA	0.49	0.54	0.35
		AG	0.43	0.40	0.52
		GG	0.08	0.07	0.13
	rs11249006 (n=281)	G	0.71	0.72	0.70
		A	0.29	0.28	0.30
		GG	0.49	0.50	0.47
		GA	0.44	0.43	0.45
		AA	0.07	0.06	0.08

Abbreviations: ETF, early treatment failure; SNPs, single-nucleotide polymorphisms.

Supplemental Table 2. Association among favorable genotypes of *IL28B* and *IL28RA* with early HCV therapy failure according to HCV viral load in HIV/HCV coinfected patients.

			HCV-RNA <500.000 IU/ml		HCV-RNA ≥500.000 IU/ml	
Gene	**SNPs**	**Inheritance Model***	**Adjusted OR (95%CI)**	**p-value**	**Adjusted OR (95%CI)**	**p-value**
IL28B	rs12980275 (AG/GG)	Dominant	2.96 (0.75 - 11.65)	0.121	7.12 (2.85 - 17.76)	**<0.001**
	rs8099917 (TG/GG)	Dominant	2.66 (0.67 - 10.62)	0.165	3.07 (1.51 - 6.23)	**0.002**
IL28RA	rs10903034 (C)	Additive	0.82 (0.28 - 2.41)	0.721	1.87 (1.09 - 3.2)	**0.022**
	rs10903035 (G)	Additive	1.44 (0.48 - 4.31)	0.510	2.39 (1.34 - 4.24)	**0.003**
	rs11249006 (A)	Additive	0.93 (0.32 - 2.67)	0.896	1.74 (0.97 - 3.13)	0.064

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; SNPs, single-nucleotide polymorphisms.
Data have been adjusted by gender, HAART, F≥2 and HCV-genotype 1/4.

Supplemental Table 3. Association among favorable genotypes of *IL28B* and *IL28RA* with early HCV therapy failure according to significant fibrosis in HIV/HCV coinfected patients.

			F<2		F≥2	
Gene	**SNPs**	**Inheritance Model***	**Adjusted OR (95%CI)**	**p-value**	**Adjusted OR (95%CI)**	**p-value**
IL28B	rs12980275 (AG/GG)	Dominant	9.89 (1.97 - 49.63)	**0.005**	4.13 (1.8 - 9.52)	**0.001**
	rs8099917 (TG/GG)	Dominant	5.53 (1.71 - 17.9)	**0.004**	2.24 (1.06 - 4.72)	**0.034**
IL28RA	rs10903034 (C)	Additive	1.39 (0.59 - 3.28)	0.446	1.66 (0.95 - 2.92)	0.075
	rs10903035 (G)	Additive	2.31 (0.84 - 6.33)	0.105	2.13 (1.19 - 3.82)	**0.011**
	rs11249006 (A)	Additive	1.78 (0.75 - 4.18)	0.189	1.36 (0.73 - 2.53)	0.333

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; SNPs, single-nucleotide polymorphisms.
Data have been adjusted by gender, HAART, HCV-RNA ≥500,000 IU/ml, and HCV-genotype 1/4.

Supplemental Figure 1. Pairwise linkage disequilibrium (LD) patterns for four polymorphisms through the *IL28B* and *IL28RA* regions. Each diagonal represents a different SNP, with each square representing a pairwise comparison between two SNPs.