Original paper

Title: *IL15* polymorphism is associated with advanced fibrosis, inflammation-related biomarkers and virologic response in HIV/HCV coinfection

Short title: *IL15* polymorphisms and fibrosis and HCV therapy

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Character count of Title: 133

Character count of Running Head: 47

Word count of Abstract: 250

Word count of Keywords: 5

Word count of Text: 3110

Count of References: 30

Count of Tables: 4

Count of Figures: 1

LIST OF ABBREVIATIONS

CHC: chronic hepatitis C

HIV: human immunodeficiency virus

HCV: hepatitis C virus

pegIFNα/RBV: pegylated-interferon-alpha plus ribavirin

DAAs: direct-acting antivirals

IL15: interleukin 15

SNPs: single nucleotide polymorphisms

IL28B: interleukin 28B

BMI: Body mass index

HGF: hepatocyte growth factor

sICAM-1: soluble intercellular adhesion molecule type 1

sVCAM-1: soluble vascular cell adhesion molecule type 1

MAF: minor allelic frequency

LD: linkage disequilibrium

SVR: sustained virological response

aOR: adjusted odds ratio

aAMR: adjusted arithmetic mean ratio

GT: HCV genotype

UTR: untranslated region

miRNAs: MicroRNAs

COMPETING INTERESTS

The authors do not have a commercial or other association that might pose a conflict of interest.

FINANCIAL SUPPORT:

This work has been supported by Fondo de Investigación de Sanidad en España (FIS) [grant numbers PI11/01556, PI14/01094, PI11/00245, PI14CIII/00011], and Red Española de Investigación en SIDA (RIS) [grant numbers RD12/0017/0024, RD12/0017/0004 and RD12/0017/0031].

MGA, MAJS, and DPT are supported by "Instituto de Salud Carlos III" [grant numbers CD12/00442, CD13/00013, and CM12/00043, respectively].

This work has been (partially) funded by the RD12/0017 project as part of the Plan Nacional R + D + I and cofinanced by ISCIII- Subdirección General de Evaluación y el Fondo Europeo de Desarrollo Regional (FEDER).

ABSTRACT

Background & Aims: IL15 is an essential cytokine in both innate and adaptive immune response against HCV infection. The aim was to analyze whether *IL15* rs10833 is associated with liver disease severity and response to pegylated-interferon-alpha plus ribavirin (pegIFN-alpha/RBV) therapy in human immunodeficiency virus (HIV)/hepatitis C virus (HCV)-coinfected patients.

Methods: A retrospective study was performed in 315 patients who started pegIFN-alpha/RBV therapy. Liver fibrosis stage was characterized in 286 patients. *IL15* rs10833 and *IL28B* rs12980275 were genotyped by GoldenGate.The primary outcomes were: a) advanced liver fibrosis evaluated by liver biopsy (F3-F4) or transient elastography (liver stiffness values ≥9.5 Kpa); b) sustained virological response (SVR). The secondary outcome variable was the levels of serum biomarkers of inflammation.

Results: Patients with rs10833 AA genotype had increased odds of having advanced fibrosis (adjusted odds ratio (aOR)=2.30; p=0.019), particularly in males (aOR=2.24; p=0.040), patients with HCV-RNA <500,000 IU/mL (aOR=5.14; p=0.018) and patients with IL28B rs12980275 AG/GG genotypes (aOR=2.51; p=0.046). Moreover, rs10833 AA genotype was significantly associated with higher levels of HGF (adjusted arithmetic mean ratio (aAMR)=1.50; p=0.016), sICAM-1 (aAMR=1.57; p=0.025) and sVCAM-1 (aAMR=1.56; p=0.007). Finally, patients with rs10833 AA genotype had increased odds of achieving SVR (aOR=3.12; p=0.006), particularly in males (aOR=3.69; p=0.005), GT1/4 patients (aOR=3.59; p=0.006), patients with advanced fibrosis (aOR=4.64; p=0.021), HCV-RNA ≥500,000 IU/mL (aOR=3.92; p=0.007) and patients with *IL28B* rs12980275 AG/GG genotype (aOR=2.98; p=0.041).

Conclusions: The presence of *IL15* rs10833 AA genotype in HIV/HCV-coinfected patients was associated with advanced liver fibrosis, inflammation-related biomarkers and increased rates of SVR to pegIFN-alpha/RBV therapy.

KEYWORDS: IL15; SNPs; AIDS; fibrosis; HCV therapy

KEY POINTS BOX:

-The *IL15* rs10833 polymorphism was linked to CHC-related outcomes in HIV/HCV-coinfected patients

-rs10833 AA genotype was associated with advanced fibrosis (F≥3)

-rs10833 AA genotype was associated with higher serum levels of HGF, sICAM-1, and sVCAM-1

-rs10833 AA genotype was linked to increased odds of achieving SVR

Introduction

The natural history of chronic hepatitis C (CHC) is highly variable, from minimal changes to advanced fibrosis or cirrhosis [1]. Several factors may accelerate the risk of these complications such as coinfection with human immunodeficiency virus (HIV) [2], where approximately 34% of patients increase at least one Metavir fibrosis stage over 2.5 years [3].

The standard of care for hepatitis C virus (HCV) infection has consisted of pegylated-interferon-alpha plus ribavirin (pegIFNα/RBV) during many years. Nowadays, the use of new direct-acting antivirals (DAAs) with improved response rate has been approved. In HIV/HCV-coinfected patients, recommendations for using pegIFNα/RBV in combination with DAAs are being prioritized. However, DAAs still have serious restrictions for its administration and are extremely expensive and inaccessible in many world regions; so pegIFNα/RBV therapy is still in use.

Immunity has a crucial role in viral persistence and tissue damage during CHC [4]. In this setting, interleukin 15 (IL15) is essential for the activation and function of cells involved in response against HCV infection [5]. This cytokine has been implicated in promoting inflammatory response in HIV/HCV-coinfected patients; where its expression has been positively associated with peripheral T-cell immune activation and hepatic stellate cells activation, which is a risk factor for accelerated liver fibrosis [6]. Moreover, IL15 level is involved in HCV clearance, where an increased IL15 level has been observed among early and late virologic responders with IFN-related therapy [7].

Genetic factors may play an important role for HCV treatment response and disease progression in CHC [8]. Apart from the well-known single nucleotide polymorphisms (SNPs) around *interleukin 28B* (*IL28B)* gene [9], other SNPs around immune-related genes have also demonstrated to have an important role. In this setting, *IL15* SNPs have been implicated in infectious diseases [10, 11], immune diseases [12] and cancers [13]. To date, there are not data about the influence of *IL15* SNPs on CHC progression and we only found an article showing a relationship with response to pegIFNα/RBV therapy [14].

Thus, the aim of this study was to analyze whether *IL15* rs10833 polymorphism is associated with liver disease severity and response to pegIFNα/RBV therapy in HIV/HCV-coinfected patients.

Materials and methods

Patients and study design

We carried out a retrospective study in HIV/HCV-coinfected patients who started HCV treatment with pegIFNα/RBV on regular follow-up, which has been previously described [15]. A cross-sectional design was used to evaluate liver disease outcomes and a longitudinal design was used to assess the HCV treatment outcome. The study was approved by the Research Ethic Committee of the Instituto de Salud Carlos III and was conducted in accordance with the Declaration of Helsinki. All patients gave their written consent.

The study population was comprised of HIV/HCV-coinfected individuals who had completed a course of pegIFNα/RBV therapy and were genotyped for *IL-15* variants. A total of 331 patients had available DNA samples, but 16 patients were excluded due to genotyping problems. Finally, 315 patients were available for genetic association analysis (**Fig.1**).

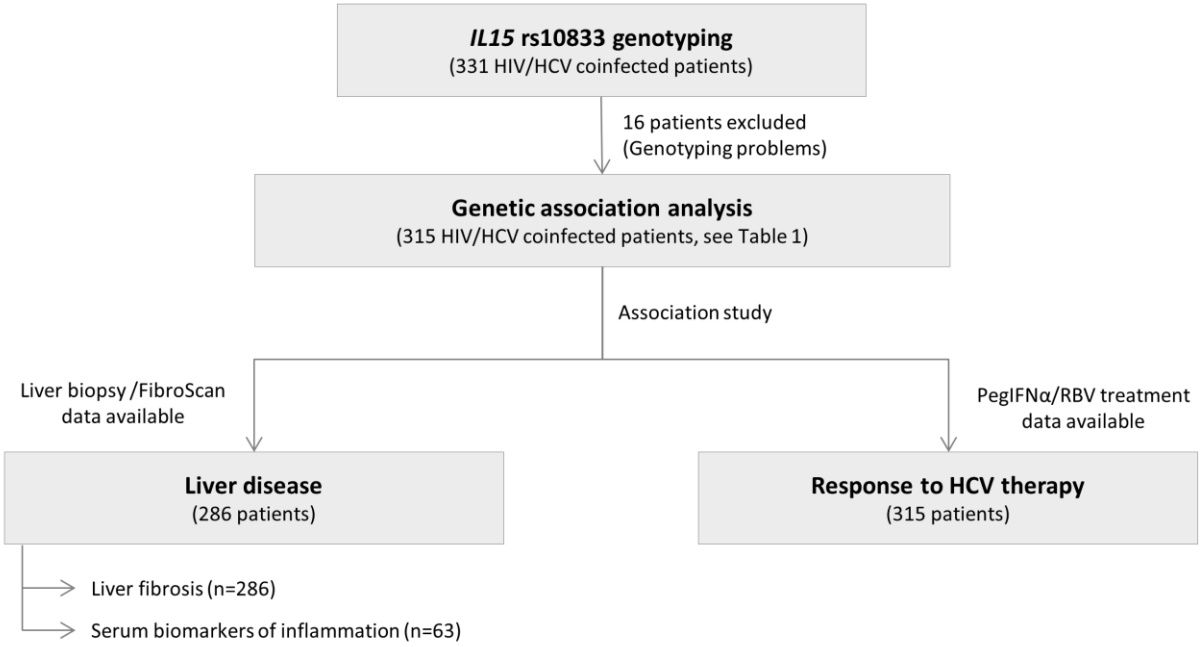

Fig.1. Flow diagram of patients selection and study design.

Epidemiological and clinical data

Clinical and epidemiological data were obtained from medical records. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. The duration of HCV infection for patients with a history of intravenous drug use (IDU) was estimated starting from the first year they shared needles and other injection paraphernalia, which are the most relevant risk practices for HCV transmission. For non-IDU patients, we only included those patients for which the initiation of their HCV infection could be determined with certainty. In this case, the initiation of HCV infection was determined only when patients reported an approximate date of transfusion prior to the introduction of HCV screening on blood donations, surgical intervention, or other HCV risk practices (sexual contact, needle piercing, etc.).

Liver fibrosis

Liver fibrosis was assessed by different methods, depending on the Hospital: a) At Hospital General Universitario "Gregorio Marañón" liver biopsy was used and fibrosis was estimated according to Metavir score as follows: F0, non-fibrosis; F1, mild fibrosis; F2, significant fibrosis; F3, advanced fibrosis; and F4, definite cirrhosis. b) At Hospital Carlos III transient elastography (FibroScan®, Echosens, Paris, France) was used and liver stiffness values ≤7.0, between 7.1 and 9.4, between 9.5 and 12.4, and ≥12.5 were considered to correspond with Metavir scores F0-F1, F2, F3, and F4, respectively.

Hepatitis C therapy

Following national guidelines [16], HCV treatment regimens included pegIFNα 2a or 2b at standard doses (180 μg/week or 1.5 μg/kg/week, respectively) plus weight-adjusted ribavirin dosing (1000 mg/day for patients weighing <75 kg and 1200 mg/day for patients weighing ≥75 kg). The virologic response to HCV treatment was measured by assessing plasma HCV-RNA at 4 weeks, 12 weeks, end-of-treatment, and 24 weeks after HCV treatment cessation. Patients

with HCV genotypes 1 or 4 received either 48 or 72 weeks of treatment, and patients with HCV genotype 2 or 3 were treated for 24 or 48 weeks, depending on the virologic response at week 4.

Multiplex ELISA

A multiplex kit (LINCOplext; LINCO Research, St Charles, MO, USA) was used to specifically evaluate several serum biomarkers using the Luminex 100™ analyser (Luminex Corporation, Austin, TX, United States): hepatocyte growth factor (HGF), soluble Fas-associated death domain protein ligand , soluble Fas-associated death domain protein , macrophage migration inhibitory factor , soluble intercellular adhesion molecule type 1 (sICAM-1), and soluble vascular cell adhesion molecule type 1 (sVCAM-1).

DNA genotyping

DNA samples were sent to the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) for DNA genotyping by using GoldenGate® assay with VeraCode® Technology (Illumina Inc. San Diego, CA, USA). The criteria for selecting *IL15* polymorphisms were: i) SNPs located in putative regulatory region; ii) minor allelic frequency (MAF) greater than 20% for CEU (Utah residents with ancestry from Northern and Western Europe) and TSI (Toscan in Italy) Hapmap population; iii) selection of tagSNPs according to linkage disequilibrium (LD)>0.8. Finally, *IL15* rs10833 polymorphism was selected.

Outcome variables

The outcome variables were: a) the advanced liver fibrosis evaluated by liver biopsy (F3-F4) or transient elastography (liver stiffness values ≥9.5 Kpa); b) the levels of serum biomarkers of inflammation; c) the sustained virological response (SVR), which was defined as no detectable HCV viral load (<10 IU/mL) at week 24 after the end of the treatment.

Statistical analysis

For the description of the study population, p-values were estimated with nonparametric tests: Mann-Whitney U test was used for continuous variable and chi-squared/Fisher's exact test for categorical variables.

The genetic association study was carried out according to a recessive genetic model (AA vs. AG/GG), which was the model that best fit to our data. For association study, logistic regression analysis was used to investigate the relationship of *IL15* rs10833 polymorphism with advanced fibrosis and SVR. Each logistic regression test was adjusted by the most significant co-variables associated with each one of the outcome variables, avoiding the over-fitting of the regression. We included the SNP (Enter algorithm) and the most relevant characteristics (Stepwise algorithm: at each step, factors are considered for removal or entry: a p-value for entry and exit of 0.15 and 0.20, respectively). The covariables used were age, gender, BMI, time of HCV infection, baseline HCV-RNA viral load (<500,000 IU/mL vs. ≥500,000 IU/mL), advanced fibrosis (F<3 vs. F≥3), *IL28B* rs12980275 polymorphism (AA vs. AG/GG), and HCV genotype (GT1/4 vs. GT2/3)). Moreover, the association between *IL15* rs10833 polymorphism and plasma biomarkers was investigated by using General Lineal Model (log-link) adjusted for the same covariates.

The statistical analyses were performed by using the IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, Chicago, Armonk, NY, USA). Besides, SNP-SNP interaction between rs12980275 and rs10833 was calculated by using the Plink software. All p-values were two-tailed. Statistical significance was defined as $p<0.05$.

Results

Patients

When patients were stratified by *IL15* rs10833 genotypes (GG/AG vs. AA), epidemiological and clinical characteristics were similar except for advanced fibrosis (p=0.036) (**Table 1**). Besides, *IL15* rs10833 AA genotype showed lower values of CD4+ cells/μL and higher percentages of CD4+<500 T-cells/μL and HIV-RNA <50 copies/ml than the *IL15* rs10833 GG/AG genotypes, but significant differences were not reached (**Table 1**).

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

		***IL15* rs10833 polymorphism**		
Characteristics	**All Patients**	**GG/AG**	**AA**	**p-value**
No.	315	270	45	
Male *	243 (77.1%)	206 (76.3%)	37 (82.2%)	0.381
Age (years) †	42 (38.7-45.9)	41.8 (38.6-45.9)	43.0 (39.2-45.0)	0.642
Anthropometric values				
Height (m) † (n=303)	1.7 (1.6-1.7)	1.7 (1.6-1.7)	1.7 (1.7-1.7)	0.955
Weight (Kgr) † (n=311)	67 (60-76)	66 (60-76)	69.0 (62.5-74.5)	0.508
BMI (kg/m^2) † (n=303)	23.1 (21.1-25.4)	23.0 (21.0-25.3)	23.9 (21.4-25.9)	0.227
BMI ≥25 kg/m ^{2} (n=303)	87 (28.7%)	74 (28.6)	13 (29.5%)	0.895
IVDU * (n=309)	277 (89.6%)	239 (90.2)	38 (86.4%)	0.441
Time of HCV infection (years)	19.6 (12.7; 23.4)	19.4 (12.7; 23.6)	19.6 (12.4; 23.3)	0.819
cART *	265 (84.1%)	223 (82.6)	42 (93.3%)	0.068
HIV markers				
Nadir CD4+ T-cells/μL †	226 (132–342)	227 (140-343)	203.0 (92-340)	0.180
Nadir CD4+ <200 cells/μL *	133 (42.2%)	111 (41.1)	22 (48.9%)	0.328
Baseline CD4+ T-cells/μL (n=312) **†**	461 (366–670)	473 (372-694)	438 (327-568)	0.100
Baseline CD4+<500 T-cells/μL (n=312)*****	171 (54.8%)	141 (52.6%)	30 (68.2%)	0.054
HIV-RNA <50 copies/ml (n=310) *****	239 (77.1%)	201 (75.3%)	38 (88.4%)	0.058
HCV markers *				
HCV genotypes (n=314)				
GT 1	180 (57.3%)	153 (56.9%)	27 (60.0%)	0.695
GT 2	1 (0.3%)	1 (0.4%)	0 (0%)	-
GT 3	99 (31.5%)	89 (33.1%)	10 (22.2%)	0.147
GT 4	34 (10.8%)	26 (9.7%)	8 (17.8%)	0.107
HCV-RNA <500,000 IU/mL (n=309)	83 (26.9%)	69 (26.1%)	14 (31.1%)	0.486

Log_{10} HCV-RNA (IU/mL) † (n=309)	6.1 (5.6-6.8)	6.1 (5.6-6.8)	6.0 (5.5-6.6)	0.416
***IL28B* polymorphism (rs12980275)**				
AA	147 (46.7%)	130 (48.1%)	17 (37.8%)	
AG	141 (44.8%)	115 (42.6%)	26 (57.8%)	0.141
GG	27 (8.6%)	25 (9.3%)	2 (4.4%)	

Values expressed as: (*), 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 serum viral load; GT, HCV genotype; HIV-1, Human immunodeficiency virus type 1; HIV-RNA, HIV plasma viral load.

IL15 polymorphism and advanced fibrosis

The rs10833 polymorphism was in Hardy-Weinberg equilibrium (p=0.368), fulfilled MAF>0.05, and displayed less than 5% of missing values.

Patients with rs10833 AA genotype had higher proportion of advanced fibrosis than patients with rs10833 GG/AG genotypes (p=0.036) (**Table 2**). When patients were stratified by gender, HCV genotype and HCV viral load, the significant differences were maintained among males and patients with HCV-RNA <500,000 IU/mL (p=0.038 and 0.017, respectively) (**Table 2**). In the multivariate analysis, patients with rs10833 AA genotype had increased odds of having advanced fibrosis (adjusted odds ratio (aOR)=2.30; p=0.019) (**Table 2; Supplemental Table 1**). After stratifying for epidemiological and clinical characteristics, rs10833 AA genotype increased the likelihood of having advanced fibrosis in males (aOR=2.24; p=0.040), patients with HCV-RNA <500,000 IU/mL (aOR=5.14; p=0.018) and patients with *IL28B* rs12980275 AG/GG genotypes (aOR=2.51; p=0.046). The SNP-SNP interaction between rs12980275 and rs10833 was not detected (p=0.735).

Serum biomarkers of inflammation

Patients with rs10833 AA genotype had higher serum levels of HGF and sVCAM-1 than patients carrying rs10833 AG/GG genotypes [p=0.038 and p=0.044; respectively] (**Table 3**). Besides, patients with rs10833 AA genotype tended to have higher levels of sICAM-1 (p=0.076) (**Table 3**). Moreover, when multivariate analyses was performed, rs10833 AA genotype was significantly associated with higher levels of HGF (adjusted arithmetic mean ratio (aAMR)=1.50; p=0.016), sICAM-1 (aAMR=1.57; p=0.025) and sVCAM-1 (aAMR=1.56; p=0.007) (**Table 3; Supplemental Table 2**).

Table 2. Association of *IL15* rs10833 polymorphism with advanced fibrosis (F≥3) in HIV/HCV coinfected patients on HCV therapy.

	Unadjusted			Adjusted	
Advanced Fibrosis (F≥3)	**GG/AG**	**AA**	**p-value [(a)]**	**aOR (95%CI)**	**p-value [(b)]**
All patients (n=286)	31.6% (78/247)	48.7% (19/39)	**0.036**	2.30 (1.15-4.61)	**0.019**
By gender					
Female (n=67)	28.8% (17/59)	37.5% (3/8)	0.614	2.18 (0.41-11.5)	0.355
Male (n=219)	32.4% (61/188)	51.6% (16/31)	**0.038**	2.24 (1.04-4.86)	**0.040**
By genotype					
GT 1/4 (n=197)	29.9% (50/167)	46.7% (14/30)	0.072	2.11 (0.95-4.67)	0.066
GT 2/3 (n=88)	34.2% (27/79)	55.6% (5/9)	0.207	3.47 (0.76; 15.8)	0.107
By HCV viral load					
HCV-RNA <500,000 IU/mL (n=77)	27.3% (18/66)	63.6% (7/11)	**0.017**	5.14 (1.32-19.8)	**0.018**
HCV-RNA ≥500,000 IU/mL (n=203)	34.3% (60/175)	42.9% (12/28)	0.379	1.69 (0.74-3.86)	0.213
By *IL28B* genotype (rs12980275)					
AA (n=131)	34.2% (40/117)	50.0% (7/14)	0.244	1.94 (0.63-5.97)	0.245
AG/GG (n=154)	29.5% (38/129)	48.0% (12/25)	0.070	2.51 (1.02-6.20)	**0.046**

Categorical variables are expressed in percentage (absolute count). Statistically significant differences are shown in bold. (a) P-values were calculated by Chi-square tests. (b) P-values were calculated by logistic regression adjusting for the most important clinical and epidemiological characteristics (see **statistical analysis** section).

Abbreviations: aOR, adjusted odds ratio; 95%CI, 95% confidence interval; HCV, Hepatitis C virus; HCV-RNA, HCV serum viral load; GT, HCV genotype; HIV-1, F3, advanced fibrosis.

IL15 polymorphism and sustained virologic response

Patients with rs10833 AA genotype had higher SVR rate than patients with rs10833 GG/AG genotypes (p=0.042) (**Table 4**). When patients were stratified according to gender, HCV genotype, advanced fibrosis and HCV viral load, a significantly higher proportion of SVR was maintained for rs10833 AA genotype among males, patients infected with GT1/4 and patients with HCV-RNA ≥500,000 IU/mL (p=0.026, p=0.002, and p=0.026; respectively). In multivariate analysis, patients with rs10833 AA genotype had increased odds of achieving SVR (aOR=3.12; p=0.006) (**Table 4; Supplemental Table 3**). After stratifying for clinical characteristics, rs10833 AA genotype increased the likelihood of achieving SVR in males (aOR=3.69; p=0.005), GT1/4 patients (aOR=3.59; p=0.006), patients with advanced fibrosis (aOR=4.64; p=0.021), HCV-RNA ≥500,000 IU/mL (aOR=3.92; p=0.007) and patients with *IL28B* rs12980275 AG/GG genotype (aOR=2.98; p=0.041). The SNP-SNP interaction between rs12980275 and rs10833 was not detected (p=0.372).

Discussion

In our study, the major findings were: (1) The minor rs10833 AA genotype was associated with advanced fibrosis, particularly in males and patients with low HCV viral load; (2) The minor rs10833 AA genotype was also associated with higher serum levels of HGF, sICAM-1, and sVCAM-1 than AG/GG carriers; (3) The minor rs10833 AA genotype was linked to increased odds of achieving SVR, particularly in males, patients with HCV genotype 1/4, advanced fibrosis and high plasma HCV-RNA.

In HIV/HCV-coinfected patients, it has been described that IL15 have a pathogenic role in the development of hepatic fibrosis by correlating with hepatic stellate cells activation [6]. In this setting, we observed higher odds of having advanced fibrosis among rs10833 AA carriers. The mechanism underlying this association is unknown and several hypotheses could be valued. On the one hand, the rs10833 polymorphism could be just a tagSNP in LD with the causal mutation. Besides, the rs10833 polymorphism is located at a regulatory region (3' untranslated region (UTR)) and thus, it could have a regulatory effect on the *IL15* gene expression. In this setting, we analyzed *in silico* whether this *IL15* SNP could be part of microRNAs (miRNAs) binding sites. The miRNAs are negative gene regulators influencing gene expression by binding at the 3'UTR level [17]. By analyzing the effect of rs10833 on putative miRNA targets via MicroSNiPer [18], we found that rs10833 A allele generates putative target sites for several miRNAs (hsa-miR-6504-3p/568/101-3p/340-5p/16-1-3p), whereas the presence of rs10833 G allele disrupts these target sites and generates others (hsa-miR-139-5p/4255, 4302/3607-3p/4495/128/3129-3p/216a-3p/3681-3p, 6499-3p). Thus, we might hypothesize that these differences in the miRNAs binding between rs10833 genotypes could be implicated in the observed association. However, further studies investigating the functional role of this SNP would be interesting.

Table 3. Association of *IL15* rs10833 polymorphism with levels of plasma biomarkers in HIV/HCV coinfected patients.

	GG/AG	AA	p-value [(a)]	aAMR (95%CI)	p-value [(b)]
HGF (pg/ml)	1399.45 (1741.73)	2549.20 (2702.74)	**0.038**	1.50 (1.08-2.10)	**0.016**
sICAM-1(ng/ml)	540.05 (688.97)	898.57 (1489.24)	0.076	1.57 (1.06-2.33)	**0.025**
sVCAM-1 (ng/ml)	818.20 (932.40)	1229.16 (1462.71)	**0.044**	1.56 (1.13-2.19)	**0.007**

Data are expressed in median (interquartile range). Statistically significant differences are shown in bold: (a) P-values were calculated by Mann-Whitney test. (b) P-values were calculated by General Lineal Model (GLM) after adjusting by the most important clinical and epidemiological characteristics (see **statistical analysis** section).

Abbreviations: aAMR, adjusted arithmetic mean ratio; 95%CI, 95% confidence interval; HGF, Hepatocyte growth factor; sICAM-1, Soluble intercellular adhesion molecule type 1; sVCAM-1, Soluble vascular cell adhesion molecule type 1.

Table 4. Association of *IL15* rs10833 polymorphism with SVR in HIV/HCV coinfected patients on HCV therapy.

	Unadjusted			Adjusted	
SVR	**GG/AG**	**AA**	**p-value [(a)]**	**aOR (95%CI)**	**p-value [(b)]**
All patients (n=315)	52.6% (142/270)	68.9% (31/45)	**0.042**	3.12 (1.37-7.09)	**0.006**
By gender					
Female (n=72)	59.4% (38/64)	62.5% (5/8)	0.865	1.58 (0.24-10.4)	0.634
Male (n=243)	50.5% (104/206)	70.3% (26/37)	**0.026**	3.69 (1.47-9.23)	**0.005**
By genotype					
GT 1/4 (n=214)	37.4% (67/179)	65.7% (23/35)	**0.002**	3.59 (1.45-8.89)	**0.006**
GT 2/3 (n=100)	82.2% (74/90)	80.0% (8/10)	0.862	1.48 (0.13; 16.1)	0.747
By advanced fibrosis					
F <3 (n=189)	56.2% (95/169)	65.0% (13/20)	0.453	2.96 (0.97-9.04)	0.056
F ≥3 (n=97)	44.9% (35/78)	63.2% (12/19)	0.153	4.64 (1.26-17.1)	**0.021**

By HCV viral load					
HCV-RNA <500,000 IU/mL (n=83)	66.7% (46/69)	71.4% (10/14)	0.729	1.28 (0.31-5.33)	0.729
HCV-RNA ≥500,000 IU/mL (n=226)	46.2% (90/195)	67.7% (21/31)	**0.026**	3.92 (1.45-10.5)	**0.007**
By *IL28B* genotype (rs12980275)					
AA (n=45)	67.7% (88/130)	88.2% (15/17)	0.082	4.31 (0.86-21.53)	0.075
AG/GG (n=269)	38.1% (53/139)	57.1% (16/28)	0.062	2.98 (1.04-8.50)	**0.041**

Categorical variables are expressed in percentage (absolute count). Statistically significant differences are shown in bold. (a) P-values were calculated by Chi-square tests. (b) P-values were calculated by logistic regression adjusting for the most important clinical and epidemiological characteristics (see **statistical analysis** section).

Abbreviations: aOR, adjusted odds ratio; 95%CI, 95% confidence interval; HCV, Hepatitis C virus; HCV-RNA, HCV serum viral load; GT, HCV genotype; HIV-1, F3, advanced fibrosis; SVR, sustained virological response.

When the effect of rs10833 polymorphism on hepatic fibrosis was evaluated under certain characteristics of patients, the association between rs10833 polymorphism and hepatic fibrosis only remained for males, patients with low HCV viral load (HCV-RNA <500,000 IU/mL) and patients with rs12980275 AG/GG genotype. The importance of gender in the effect of *IL15* polymorphisms has been reported by Pistilli et al [19], which described a significant association between *IL15* polymorphisms and metabolic parameters (BMI, insulin resistance, among others) only for male patients. Besides, the male gender is considered as a risk factor that may influence and accelerate the evolution of liver disease [20] and thus, prediction of hepatic fibrosis is highly interesting in this group. Besides, the association between rs10833 and baseline hepatic fibrosis was only observed in patients who had lower HCV viral load, as observed in the subgroup analysis. It is possible that the effect of rs10833 polymorphism on fibrosis only becomes apparent under conditions of low HCV viral load, and when HCV replication is increased, the effect of rs10833 polymorphism is diluted. However, the mechanism underlying the gender, HCV viral load and rs12980275 AG/GG genotype specific association is unknown and should be taken into account and further investigated.

Additionally, the rs10833 polymorphism was also associated to differences in serum levels of inflammatory biomarkers. Patients with rs10833 AA genotype had higher levels of HGF, sICAM-1, sVCAM-1 than AG/GG carriers. HGF is a growth factor produced in high quantities in severe stages of viral infections. Regarding CHC, HGF increases its level in concordance with the grade of liver insufficiency, being correlated with histological grading of inflammatory activity, fibrosis and cirrhosis [21]. Besides, serum HGF levels are strongly associated with obesity, insulin resistance, and metabolic syndrome [22], which are known risk factors of liver fibrosis [23]. Additionally, sVCAM-1 and sICAM1 are markers of endothelial dysfunction and inflammation, which are released from endothelial cells in response to several inflammatory cytokines (IL-1, IL-4, TNF-α, IFN-γ) [24]. During HCV infection, a process described as "chronic immune activation" or "hyperactivation" occurs accompanied by a higher expression of inflammatory cytokines in T cells [25]. Regarding HIV/HCV-coinfection, higher levels of sVCAM-1 and sICAM1 were observed in patients with advanced fibrosis and moderate to severe activity grade [26]. Thus, the increased serum levels of sICAM-1 and sVCAM-1 may indicate a high inflammatory state, which could be local (liver) or systemic (immune system activation). Overall, the association observed between rs10833 AA genotype and HGF, sICAM-1 and sVCAM-1 could be a reflection of the relationship of these biomarkers with advanced fibrosis.

An association between several SNPs located at introns of *IL15* gene and the efficacy of Peg-IFN/RBV in Chinese HCV-monoinfected patients has been reported [14]. Thus, although they did not test rs10833, the region around *IL15* gene seems to be implicated in response to HCV treatment. Besides, authors were not able to decipher the causative SNP responsible for this association and thus, the mechanisms underlying this association are still unknown. However, as previously mentioned, both intron and 3'UTR region are considered to be regulatory sites and thus, these SNPs could have an effect on the modulation of *IL15* expression. The IL15 is a crucial cytokine for the activation and maintenance of cells involved in both innate and adaptive immune response, particularly CD8+ T cells and NK cells [5]; and thus, an altered *IL15* expression or function in these cells could influence the response to HCV treatment. On the other hand, after stratifying for several important covariates, it should be taken into account that the association between rs10833 polymorphism and SVR was only maintained for males, patients with HCV-GT1/4, advanced fibrosis, high HCV viral load and rs12980275 AG/GG genotype.

To date, many articles have assessed the influence of *IL28B* polymorphisms on SVR in CHC patients, being rs12979860, rs8099917, and rs12980275 the most studied [9]. Although

rs12979860 is more likely to be correlated with SVR in the Caucasian population, we have recently shown a strong association of rs12980275 and rs8099917 with SVR in HCV/HIV-coinfected patients [15]. In the current study, we analyzed rs12980275, which is also in high linkage disequilibrium with rs8099917 and rs12979860 in the European population [27]. In addition, rs12980275 has been less studied than rs12979860 in Caucasian populations, and therefore additional results involving rs12980275 would be of interest.

Our data indicate that *IL15* polymorphism was associated with SVR independently of *IL28B* polymorphisms (rs12980275), as it is observed in the multivariate model. This finding shows that *IL28B* and *IL15* polymorphisms might influence HCV clearance by different mechanisms. Whereas rs10833 polymorphisms could exert its effect by altering NK and CD8+ T cells function, *IL28B* polymorphisms have demonstrated to have effect on SVR independently of NK cells phenotype and function, as described by Oliviero et al [28]. Besides, *IL28B* SNPs have been linked to both SVR achievement (favorable event) as well as progression of liver injury (unfavorable event) in HIV/HCV-coinfected patients [9, 29, 30]. In this setting, although the mechanism of action is not probably the same, similar effects have been observed for *IL15* rs10833 AA genotype. These findings could reflect a more vigorous immune response against the virus in *IL15* rs10833 AA carriers, which would help to control the HCV infection and could also induce liver damage.

Finally, several aspects have to be taken into account for the correct interpretation of the results.
Firstly, this report has a retrospective design with a relatively small number of patients, which could limit the achievement of significant values between the rs10833 polymorphism and the outcome variables. Besides, note that sub-group analyses could reduce the statistical power of results due to the limited sample size after stratifying patients by certain important covariates. Thus, these results should be interpreted with caution.

Secondly, the patients selected for our study were patients who met a set of criteria for starting HCV treatment, and it is possible that this may have introduced a selection bias.

Thirdly, we had no access to an uninfected control group with fibrosis, HCV-monoinfected patients or HIV-monoinfected patients with fibrosis in order to evaluate the influence of rs10833 polymorphism on these patients. Moreover, since the study was carried out entirely in European whites, and the frequency of these alleles differs among different ethnicities, it would be necessary to perform an independent replication of this study for different ethnic groups.
Fourthly, the relationship between rs10833 AA and several inflammation-related cytokines has been studied since cytokines levels were available as consequence of a previous article, however, IL15 level could not be determined by lack of samples. Its investigation could be interesting in further studies.

In conclusion, the presence of *IL15* rs10833 AA genotype in HIV/HCV-coinfected patients was associated with advanced liver fibrosis and increased rates of SVR to pegIFNα/RBV therapy. This association was different depending on gender, HCV genotype, advanced fibrosis, and HCV viral load.

ACKNOWLEDGEMENTS AND DISCLOSURES

ACKNOWLEDGEMENTS

The authors thank the Spanish National Genotyping Center (CeGen) for providing SNP genotyping services (http://www.cegen.org).

AUTHOR'S CONTRIBUTIONS

Study design: SR.

Collection data and sample preparation , and critical revision of the manuscript: MAJS, NR, DPT, MGA, SVM, JMB, JB, TAE, VS, AC.

Statistical analysis and interpretation of data: MAJS, SR.

Writing of the report: MAJS, JB, SR

REFERENCES

[1]. Westbrook R H, Dusheiko G. Natural history of hepatitis C. J Hepatol 2014; 61(1 Suppl): S58-68.

[2]. Lopez-Dieguez M, Montes M L, Pascual-Pareja J F, Quereda C, Von Wichmann M A, Berenguer J, et al. The natural history of liver cirrhosis in HIV-hepatitis C virus-coinfected patients. AIDS 2011; 25(7): 899-904.

[3]. Konerman M A, Mehta S H, Sutcliffe C G, Vu T, Higgins Y, Torbenson M S, et al. Fibrosis progression in human immunodeficiency virus/hepatitis C virus coinfected adults: prospective analysis of 435 liver biopsy pairs. Hepatology 2014; 59(3): 767-75.

[4]. Heim M H, Thimme R. Innate and adaptive immune responses in HCV infections. J Hepatol 2014; 61(1S): S14-S25.

[5]. Zhang S, Saha B, Kodys K, Szabo G. IFN-gamma production by human natural killer cells in response to HCV-infected hepatoma cells is dependent on accessory cells. J Hepatol 2013; 59(3): 442-9.

[6]. Allison R D, Katsounas A, Koziol D E, Kleiner D E, Alter H J, Lempicki R A, et al. Association of interleukin-15-induced peripheral immune activation with hepatic stellate cell activation in persons coinfected with hepatitis C virus and HIV. J Infect Dis 2009; 200(4): 619-23.

[7]. Kishida Y, Imaizumi N, Tanimura H, Haruna Y, Kashiwamura S, Kashiwagi T. Restoration of innate and adaptive immune responses by HCV viral inhibition with an induction approach using natural interferon-beta in chronic hepatitis C. Clin Dev Immunol 2012; 2012: 582716.

[8]. Stattermayer A F, Scherzer T, Beinhardt S, Rutter K, Hofer H, Ferenci P. Review article: genetic factors that modify the outcome of viral hepatitis. Aliment Pharmacol Ther 2014; 39(10): 1059-70.

[9]. Jimenez-Sousa M A, Fernandez-Rodriguez A, Guzman-Fulgencio M, Garcia-Alvarez M, Resino S. Meta-analysis: implications of interleukin-28B polymorphisms in spontaneous and treatment-related clearance for patients with hepatitis C. BMC Med 2013; 11: 6.

[10]. Zhang X, Sun X H, Li M, Zhou Z H, Gao Y Q. A promoter polymorphism (rs3806798) of interleukin-15 gene is associated with chronic hepatitis B virus infection in the Chinese Han population. Int J Immunogenet 2014; 41(4): 298-305.

[11]. Kalani M, Rasouli M, Moravej A, Kiany S, Rahimi H R. Association of interleukin-15 single nucleotide polymorphisms with resistance to brucellosis among Iranian patients. Tissue Antigens 2011; 78(5): 352-8.

[12]. Pavkova Goldbergova M, Nemec P, Lipkova J, Jarkovsky J, Gatterova J, Ambrozkova D, et al. Relation of IL-6, IL-13 and IL-15 gene polymorphisms to the rheumatoid factors, anti-CCP and other measures of rheumatoid arthritis activity. Int J Immunogenet 2014; 41(1): 34-40.

[13]. Bondurant K L, Lundgreen A, Herrick J S, Kadlubar S, Wolff R K, Slattery M L. Interleukin genes and associations with colon and rectal cancer risk and overall survival. Int J Cancer 2013; 132(4): 905-15.

[14]. Chen P J, Hwang Y, Lin C G, Wu Y J, Wu L S. The genetic differences with whole genome linkage disequilibrium mapping between responder and non-responder in interferon-alpha and ribavirin combined therapy for chronic hepatitis C patients. Int J Immunogenet 2008; 35(2): 153-7.

[15]. Fernandez-Rodriguez A, Rallon N, Berenguer J, Jimenez-Sousa M A, Cosin J, Guzman-Fulgencio M, et al. Analysis of IL28B alleles with virologic response patterns and plasma cytokine levels in HIV/HCV-coinfected patients. AIDS 2013; 27(2): 163-73.

[16]. Panel De Expertos De Gesida Plan Nacional Sobre El Sida Y Asociación Española Para El Estudio Del Hígado. [Recommendations of Gesida/PNS/AEEH for the management and treatment of the adult patient co-infected with HIV and hepatitis A, B and C virus]. Enfermedades infecciosas y microbiologia clinica 2010; 28(1): 31 e1-31.

[17]. Landi D, Barale R, Gemignani F, Landi S. Prediction of the biological effect of polymorphisms within microRNA binding sites. Methods Mol Biol 2011; 676: 197-210.

[18]. Barenboim M, Zoltick B J, Guo Y, Weinberger D R. MicroSNiPer: a web tool for prediction of SNP effects on putative microRNA targets. Hum Mutat 2010; 31(11): 1223-32.

[19]. Pistilli E E, Devaney J M, Gordish-Dressman H, Bradbury M K, Seip R L, Thompson P D, et al. Interleukin-15 and interleukin-15R alpha SNPs and associations with muscle, bone, and predictors of the metabolic syndrome. Cytokine 2008; 43(1): 45-53.

[20]. Collazos J, Carton J A, Asensi V. Gender differences in liver fibrosis and hepatitis C virus-related parameters in patients coinfected with human immunodeficiency virus. Curr HIV Res 2011; 9(5): 339-45.

[21]. Andersen E S, Ruhwald M, Moessner B, Christensen P B, Andersen O, Eugen-Olsen J, et al. Twelve potential fibrosis markers to differentiate mild liver fibrosis from cirrhosis in patients infected with chronic hepatitis C genotype 1. Eur J Clin Microbiol Infect Dis 2011; 30(6): 761-6.

[22]. Asano Y, Iimuro Y, Son G, Hirano T, Fujimoto J. Hepatocyte growth factor promotes remodeling of murine liver fibrosis, accelerating recruitment of bone marrow-derived cells into the liver. Hepatol Res 2007; 37(12): 1080-94.

[23]. Macaluso F S, Maida M, Minissale M G, Li Vigni T, Attardo S, Orlando E, et al. Metabolic factors and chronic hepatitis C: a complex interplay. Biomed Res Int 2013; 2013: 564645.

[24]. Galkina E, Ley K. Vascular adhesion molecules in atherosclerosis. Arterioscler Thromb Vasc Biol 2007; 27(11): 2292-301.

[25]. Serra A, Nuti S, Tavarini S, Sammicheli C, Rosa D, Saletti G, et al. Coligation of the hepatitis C virus receptor CD81 with CD28 primes naive T lymphocytes to acquire type 2 effector function. J Immunol 2008; 181(1): 174-85.

[26]. De Castro I F, Micheloud D, Berenguer J, Guzman-Fulgencio M, Catalan P, Miralles P, et al. Hepatitis C virus infection is associated with endothelial dysfunction in HIV/hepatitis C virus coinfected patients. AIDS 2010; 24(13): 2059-67.

[27]. Holmes J A, Desmond P V, Thompson A J. Redefining baseline demographics: the role of genetic testing in hepatitis C virus infection. Clin Liver Dis 2011; 15(3): 497-513.

[28]. Oliviero B, Mele D, Degasperi E, Aghemo A, Cremonesi E, Rumi M G, et al. Natural killer cell dynamic profile is associated with treatment outcome in patients with chronic HCV infection. J Hepatol 2013; 59(1): 38-44.

[29]. De La Fuente C, Hinojosa C, Gilabert I, Jimenez Sousa M A, Gonzalez J M, Ortiz De Lejarazu R, et al. Interleukin 28B rs12979860 (CT/TT) genotype is associated with milder hepatic damage in the natural evolution of HCV/HIV coinfection. J Interferon Cytokine Res 2013; 33(1): 43-7.

[30]. Guzman-Fulgencio M, Berenguer J, Garcia-Alvarez M, Fernandez-Rodriguez A, Jimenez-Sousa M A, Alvarez E, et al. IL28B polymorphisms are associated with severity of liver disease in human immunodeficiency virus (HIV) patients coinfected with hepatitis C virus. J Infect 2013; 66(2): 170-8.

Supporting information

Supplemental Table 1. Association of *IL15* rs10833 polymorphism with advanced fibrosis (F≥3) in HIV/HCV coinfected patients on HCV therapy by multivariate logistic regression.

	Adjusted	
Advanced Fibrosis (F≥3)	**aOR (95%CI)**	**p-value [(b)]**
All patients		
AA rs10833	2.30 (1.15-4.61)	**0.019**
By gender		
Female		
AA rs10833	2.18 (0.41-11.5)	0.355
AA_rs12980275	2.42 (0.74-7.91)	0.143
Male		
AA rs10833	2.24 (1.04-4.87)	**0.040**
By genotype		
GT 1/4		
AA rs10833	2.11 (0.95-4.67)	0.066
GT 2/3		
AA rs10833	3.47 (0.76; 15.8)	0.107
By HCV viral load		
HCV-RNA <500,000 IU/mL		
AA rs10833	5.14 (1.32-19.8)	**0.018**
HCV-RNA ≥500,000 IU/mL		
AA rs10833	1.69 (0.74-3.86)	0.213
By *IL28B* genotype (rs12980275)		
AA rs12980275		
AA rs10833	1.94 (0.63-5.97)	0.245
AG/GG rs12980275		
AA rs10833	2.51 (1.02-6.20)	**0.046**
Sex	2.19 (0.82-5.82)	0.116

Statistically significant differences are shown in bold: P-values were calculated by logistic regression adjusting for the most important clinical and epidemiological characteristics.

Abbreviations: aOR, adjusted odds ratio; 95%CI, 95% confidence interval; HCV, Hepatitis C virus; HCV-RNA, HCV serum viral load; GT, HCV genotype; HIV-1, F3, advanced fibrosis.

Supplemental Table 2. Association of *IL15* rs10833 polymorphism with levels of plasma biomarkers in HIV/HCV coinfected patients by multivariate generalized linear model.

	aAMR (95%CI)	p-value [(b)]
HGF (pg/ml)	1.50 (1.08-2.10)	**0.016**
Age	1.04 (0.99-1.07)	0.055
Sex	0.74 (0.49-1.11)	0.143
F≥3	1.76 (1.25-2.46)	**0.001**
HCV genotype	0.55 (0.37-0.81)	**0.002**
HCV-RNA ≥500,000 IU/mL	0.97 (0.68-1.38)	0.863
BMI	1.09 (1.02-1.16)	**0.015**
sICAM-1(ng/ml)	1.57 (1.06-2.33)	**0.025**
Age	1.03 (0.99-1.06)	0.085
Sex	0.95 (0.59-1.52)	0.830
F≥3	1.42 (0.99-2.04)	0.059
HCV genotype	1.11 (0.74-1.66)	0.606
HCV-RNA ≥500,000 IU/mL	1.27 (0.85-1.89)	0.248
BMI	0.95 (0.90-1.01)	0.090
sVCAM-1 (ng/ml)	1.56 (1.13-2.19)	**0.007**
Age	0.99 (0.97-1.02)	0.948
Sex	0.81 (0.60-1.11)	0.199
F≥3	1.82 (1.41-2.36)	**<0.001**
HCV genotype	2.00 (1.49-2.68)	**<0.001**
HCV-RNA ≥500,000 IU/mL	1.30 (0.99-1.71)	0.057
BMI	0.96 (0.92-1.00)	0.061

Statistically significant differences are shown in bold: P-values were calculated by General Lineal Model (GLM) after adjusting by the most important clinical and epidemiological characteristics (see **statistical analysis** section).

Abbreviations: aAMR, adjusted arithmetic mean ratio; 95%CI, 95% confidence interval; BMI, body mass index; F≥3, advanced fibrosis; HGF, Hepatocyte growth factor; sICAM-1, Soluble intercellular adhesion molecule type 1; sVCAM-1, Soluble vascular cell adhesion molecule type 1.

Supplemental Table 3. Association of *IL15* rs10833 polymorphism with SVR in HIV/HCV coinfected patients on HCV therapy by multivariate logistic regression.

	Adjusted	
SVR	**aOR (95%CI)**	**p-value [(b)]**
All patients		
AA rs10833	3.12 (1.37-7.09)	**0.006**
HCV-RNA ≥500,000 IU/mL	0.52 (0.27-1.00)	0.050
F≥3	0.50 (0.27-0.94)	**0.032**
AA rs12980275	3.34 (1.86-6.02)	**<0.001**
HCV genotype	0.10 (0.05-0.21)	**<0.001**
By gender		
Female		
AA rs10833	1.58 (0.24-10.4)	0.634
AA rs12980275	2.99 (0.82-10.90)	0.096
HCV genotype	0.16 (0.04-0.68)	**0.013**
Male		
AA rs10833	3.69 (1.47-9.23)	**0.005**
HCV-RNA ≥500,000 IU/mL	0.54 (0.26-1.12)	0.100
F≥3	0.51 (0.25-1.02)	0.058
AA rs12980275	3.48 (1.77-6.85)	**<0.001**
HCV genotype	0.09 (0.04-0.22)	**<0.001**
By genotype		
GT 1/4		
AA rs10833	3.59 (1.45-8.89)	**0.006**
Sex	0.53 (0.24-1.20)	0.130
HCV-RNA ≥500,000 IU/mL	0.40 (0.19-0.81)	**0.011**
F≥3	0.51 (0.24-1.06)	0.071
AA rs12980275	4.44 (2.23-8.81)	**<0.001**
GT 2/3		
AA rs10833	1.48 (0.13; 16.1)	0.747
Age	1.12 (0.97-1.29)	0.111
F≥3	0.33 (0.08-1.39)	0.132
BMI	0.86 (0.71-1.04)	0.124
By advanced fibrosis		
F <3		
AA rs10833	2.96 (0.97-9.04)	0.056
HCV-RNA ≥500,000 IU/mL	0.47 (0.21-1.05)	0.065
AA rs12980275	3.20 (1.55-6.64)	**0.002**
HCV genotype	0.09 (0.03-0.25)	**<0.001**
F ≥3		

AA rs10833	4.64 (1.26-17.1)	**0.021**
AA rs12980275	4.65 (1.58-13.69)	**0.005**
HCV genotype	0.11 (0.03-0.37)	**<0.001**
Age	1.11 (0.99-1.24)	0.078
By HCV viral load		
HCV-RNA <500,000 IU/mL		
AA rs10833	1.28 (0.31-5.33)	0.729
AA rs12980275	2.55 (0.92-7.09)	0.073
HCV genotype	0.33 (0.09-1.16)	0.084
HCV-RNA ≥500,000 IU/mL		
AA rs10833	3.92 (1.45-10.5)	**0.007**
F≥3	0.49 (0.23-1.06)	0.070
AA rs12980275	3.74 (1.81-7.73)	**<0.001**
HCV genotype	0.06 (0.02-0.16)	**<0.001**
By *IL28B* genotype (rs12980275)		
AA rs12980275		
AA rs10833	4.31 (0.86-21.53)	0.075
F≥3	0.50 (0.21-1.21)	0.124
HCV genotype	0.19 (0.07-0.51)	**0.001**
AG/GG rs12980275		
AA rs10833	2.98 (1.04-8.50)	**0.041**
Age	0.92 (0.85-1.00)	0.057
HCV-RNA ≥500,000 IU/mL	0.44 (0.17-1.11)	0.083
F≥3	0.45 (0.18-1.17)	0.101
HCV genotype	0.05 (0.01-0.15)	**<0.001**

Statistically significant differences are shown in bold: P-values were calculated by logistic regression adjusting for the most important clinical and epidemiological characteristics.

Abbreviations: aOR, adjusted odds ratio; 95%CI, 95% confidence interval; BMI, body mass index; HCV, Hepatitis C virus; HCV-RNA, HCV serum viral load; GT, HCV genotype; HIV-1, F3, advanced fibrosis; SVR, sustained virological response.