Original article

Title: Association between *IL7RA* polymorphisms and the successful therapy against HCV in HIV/HCV-coinfected patients

Running head: *IL7RA* polymorphisms and SVR

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ABSTRACT

Purpose: Interleukin-7 (IL-7) is a critical factor in maintaining or inducing of an effective antiviral CD4+ and CD8+ T cell responses. The aim of this study was to examine the association of *interleukin-7 receptor-α (IL7RA)* polymorphisms with the sustained virological response (SVR) after hepatitis C virus (HCV) therapy with pegylated-interferon-alpha plus ribavirin (pegIFNα/ribavirin) in 177 human immunodeficiency virus (HIV)/HCV-coinfected patients.

Methods: We performed a retrospective study in 177 naïve patients who started HCV treatment. The *IL7RA* rs6897932, rs987106, and rs3194051 polymorphisms were genotyped by GoldenGate® assay. The SVR was defined as undetectable HCV viral load through 24 weeks after the end of HCV treatment.

Results*:* The highest SVR rate was found in patients with rs6897932 CC (p=0.029) and rs3194051 GG (p=0.002) genotypes; and HCV GT2/3 infected patients with rs987106 AA genotype (p=0.048). Additionally, carriers of rs3194051 GG genotype had higher likelihood of achieving a SVR (adjusted odds ratio (aOR)=5.32 (95CI%=1.07;26.94); p=0.040) than patients with rs3194051 AA/AG genotype, while rs6897932 CC (aOR=0.63; p=0.205) and rs987106 AA (aOR=0.60; p=0.213) were not significant. Moreover, three major haplotypes were found: 46.6% for CTA, 32.4% for CAG and 20.7% for TAA haplotypes. Patients infected with GT2/3 and carriers of CTA haplotype had lower odds of achieving a SVR (aOR=0.08; p=0.004) and CAG haplotype (favorable alleles) had higher odds of achieving a SVR than other haplotypes (aOR=21.96; p<0.001).

Conclusions*: IL7RA* polymorphisms seem to play a significant role in the virological response to pegIFNα/ribavirin therapy in HIV/HCV-coinfected patients; in particular among patients infected with HCV-GT2/3.

Key words: HIV/AIDS; chronic hepatitis C; CD127; IL-7; HCV therapy; SNPs; SVR

INTRODUCTION

The treatment for hepatitis C virus (HCV) with pegylated interferon alpha plus ribavirin (pegIFNα/ribavirin) is still used in human immunodeficiency virus (HIV)/HCV-coinfected patients [1]; but the rate of sustained virological response (SVR) is around 20-40% for patients infected with HCV genotype 1/4 (GT1/4) and 50-60% in HCV genotype 2/3 (GT2/3) patients [2, 3]. Despite the emergence of new direct-acting antivirals (DAAs), pegIFNα/ribavirin is included as part of the combination therapy with DAAs, particularly in difficult-to-treat patients [1, 4]. Furthermore, the potential use of these DAAs in HIV/HCV-coinfected patients has numerous challenges, such as the choice of the patients to treat, possible interaction between antiretroviral drugs and the DAAs, uncertainty regarding the safety and effectiveness of the combination therapy in this population [1, 4].

Persistent viral infection depends upon effective antiviral CD4+ and CD8+ T cell responses [5]. Interleukin-7 (IL-7) is a critical factor in maintaining or inducing a cytotoxic T-lymphocyte response against viruses, due to its ability to enhance memory T-cell expansion and thus promote the Th1 response and increase specific CD8+ T-cell cytotoxicity against the virus [6]. In HCV infection, hepatocytes are stimulated by type I IFN, being able to produce IL-7 during viral hepatitis and eventually lead to viral clearance and disease resolution in the liver [7]. The early expression of the memory precursor marker CD127 (α-chain of the IL-7 receptor (IL7Rα)) on HCV-specific T cells predicts the outcome of acute HCV infection [8], but persistent HCV infection impairs HCV-specific cytotoxic T cell reactivity due to CD127 down-regulation [9].

The IL7Rα (CD127) is encoded by *IL7RA* gene, which forms a receptor complex with the common cytokine receptor gamma chain (CD132) [10]. In HIV infection, *IL7RA* single nucleotide polymorphisms (SNPs) have been related to mortality and decrease of CD4+ cell count in untreated HIV-infected subjects [11, 12], and CD4+ T-cell recovery after initiation of combination antiretroviral therapy (cART) [13]. However, there are still no data concerning the *IL7RA* polymorphisms and HCV infection.

The aim of this study was to examine the association between *IL7RA* polymorphisms and SVR after HCV therapy with pegIFNα/ribavirin in HIV/HCV-coinfected patients.

PATIENTS AND METHODS

Patients

We carried out a retrospective study in European white patients from Hospital Gregorio Marañón (Madrid, Spain) who started treatment with pegIFNα/ribavirin on regular follow-up from October 2000 to June 2010. The study was conducted in accordance with the Declaration of Helsinki and patients gave their written consent for the study. The Institutional Review Board and the Research Ethic Committee of the Instituto de Salud Carlos III approved the study.

The criteria for starting HCV antiviral treatment were: A) <u>Inclusion criteria</u>: Chronic hepatitis C (presence of detectable HCV replication for at least six months after HCV infection), negative hepatitis B surface antigen, availability of DNA sample, no clinical evidence of hepatic decompensation, detectable HCV RNA by polymerase chain reaction at baseline, CD4+ count higher than 200 cells/mm^3, and stable cART for at least 6 months before study entry or no need for cART according to treatment guidelines used in the study period [14, 15]. B) <u>Exclusion criteria</u>: Active opportunistic infections, active drug or alcohol addiction, and other concomitant diseases or conditions such as diabetes, nephropathies, autoimmune diseases, haemochromatosis, primary biliary cirrhosis, Wilson's disease, a1-antitrypsin deficiency and neoplasia.

We included only patients who had an available DNA sample for DNA genotyping. Of the 495 HIV/HCV coinfected patients who were potential candidates for HCV therapy during the study period, 328 patients were treated with pegIFNα/ribavirin. Of them, 177 patients had a DNA sample collected for SNPs genotyping.

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 [16]. The duration of HCV infection was not calculated when the date of initiation of their HCV infection could be determined with certainty.

Biochemistry panel was measured using an autoanalyzer Hitachi 912 (Boehringer Mannheim, Germany) in fasting patients. The degree of insulin resistance (IR) was estimated for each patient using the homeostatic model assessment (HOMA), according the following formula [17]: fasting plasma glucose (mmol/l) x fasting serum insulin (mU/l) / 22.5.

Liver biopsy

Liver biopsies were performed on 142 out of 177 patients basis following the recommendations of the Patient Care Committee of the American Gastroenterological Association [18] as previously we described [19]. Liver fibrosis and necroinflammatory activity were estimated according to Metavir score as follows: F0, non-fibrosis; F1, mild fibrosis; F2, significant fibrosis; F3, advanced fibrosis; and F4, definite cirrhosis. Activity grade was scored as follows: A0, non-activity; A1, mild activity; A2, moderate activity; A3, severe activity. Liver steatosis was evaluated according to the existence of hepatocytes containing visible macrovesicular fat droplets. We considered hepatic steatosis to be clinically significant when fatty hepatocytes exceeded 10% of the hepatic parenchyma.

HCV assays

HCV infection was documented in all patients by enzyme-linked immunosorbent assay (ELISA) and PCR test. HCV genotype was determined by hybridization of biotin-labeled PCR products to oligonucleotide probes bound to nitrocellulose membrane strips (INNO-LiPA HCV

II, Innogenetics, Ghent, Belgium). Plasma 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); and results were reported in terms of international units per milliliter (IU/mL), with a lower limit of detection of 10 IU/mL.

Hepatitis C therapy

Following both international and national guidelines [14, 15, 20, 21], 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). 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. A SVR was defined as an undetectable serum HCV-RNA level (<10 IU/mL) at week 24 after the end of the treatment.

Genotyping of DNA polymorphisms

Genomic DNA was extracted from peripheral blood with Qiagen kit (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). DNA samples were sent at the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) for genotyping *interleukin 28B* (*IL28B*) rs12980275 polymorphism and *IL7RA* polymorphisms (rs6897932, rs987106, and rs3194051), which are located in putative regulatory intronic region (rs987106), exon 6 (rs6897932) and exon 8 (rs3194051).

Statistical analysis

For the description of the study population, p-values were estimated by using nonparametric tests: Chi-square test for categorical variables. All SNPs were analysed for Hardy-Weinberg equilibrium (HWE) using Chi-square test, considering equilibrium when p>0.05.

The genetic analysis was carried out according to additive, recessive and dominant models, selecting the model that best fitted the outcome variable analyzed in each case (see **Supplementary data 1**). For association study, logistic regression analysis was used to investigate the relationship among *IL7RA* polymorphisms and HCV-therapy response. These analyses were adjusted by the most important clinical and epidemiological characteristics, which were selected by a "Stepwise" algorithm (a p-value for entry and exit of 0.05 and 0.10, respectively). The covariables used were gender, age, BMI, HOMA, nadir CD4+ T-cells, time on cART, HIV-RNA, HCV genotype, HCV-RNA viral load, liver fibrosis, and *IL28B* rs12980275 polymorphism. The percentages of patients that were excluded in each of multivariable analyses due to incomplete data of covariates were always less than 5%.

These analyses were performed by using the Statistical Package for the Social Sciences (SPSS) 19.0 software (IBM Corp., Chicago, USA). In addition, pair-wise linkage disequilibrium (LD) analysis was computed by Haploview 4.2 software, and haplotype-based association testing was performed using Plink software (http://pngu.mgh.harvard.edu/~purcell/plink/). All p-values were two-tailed and statistical significance was defined as p<0.05.

In silico analysis

We analysed the possible functional implication of *IL7RA* polymorphisms using the VarioWatch web tool (http://genepipe.ncgm.sinica.edu.tw/variowatch/main.do) [22]. VarioWatch retrieves information about a genomic locus from several sources like NCBI, Uniprot, HapMap and Ensembl and displays it concisely.

RESULTS

Patients

The epidemiological and clinical characteristics of 177 patients on HCV treatment stratified by HCV genotype are shown in **Table 1**: 56 patients were infected with GT2/3 and 116 with GT1/4.

Table 1. Clinical and epidemiological characteristics of HIV/HCV-coinfected patients.

Characteristics	All Patients	HCV GT2/3	HCV GT1/4
No., (%)	177 (100%)	56 (31.6%)	116 (65.5%)
Male, n (%)	133 (75.1%)	41 (73.2%)	87 (75%)
Age, years	40.6 (37.9-44.6)	41 (38.4-44.6)	40.2 (37.8-44.6)
BMI, kg/m2	22.5 (20.9-24.6)	22.5 (20.6-24.4)	22.5 (20.9-25.2)
BMI ≥25 kg/m2	41 (23.2%)	9 (16.1%)	30 (25.9%)
HOMA			
HOMA ≥3	62 (35.8%)	17 (30.9%)	44 (38.9%)
HIV acquired by IVDU, n (%)	153 (86.4%)	49 (87.5%)	99 (85.3%)
Years since HCV infection	20.4 (15.4-23.6)	19.5 (15.2-23.8)	20.6 (15.7-23.5)
Prior AIDS, n (%)	50 (28.2%)	11 (19.6%)	38 (32.8%)
cART, n (%)	146 (82.5%)	46 (82.1%)	97 (83.6%)
Time on cART, years (*)	4.8 (3.41-7.86)	4.2 (2.57-7.90)	5.1 (4.09-7.85)
Current cART protocols, n (%)			
Any NRTIs + any PI	41 (23.2%)	10 (17.9%)	31 (26.7%)
Any NRTIs + PI + NNRTI	1 (0.6%)	0 (0%)	1 (0.9%)
Any NRTIs + any NNRTI	92 (52%)	32 (57.1%)	58 (50%)
Only NRTIs	151 (85.3%)	48 (85.7%)	99 (85.3%)
HIV markers			
Nadir CD4+, cells/µL	216 (95.5-355.5)	230 (138-379.5)	203.5 (72-313.5)
Nadir CD4+ <200 cells/µL, n (%)	82 (46.6%)	24 (42.9%)	57 (49.1%)
CD4+ T, cells/µL	457 (330-674.2)	433 (374-686.2)	467 (324-675)
CD4+ ≥ 500 cells/µL, n (%)	77 (43.8%)	23 (41.1%)	52 (44.8%)
HIV-RNA copies/mL (**)	49 (49-149.5)	49 (49-49)	49 (49-237.5)
HIV-RNA < 50 copies/mL, n (%)	127 (72.2%)	42 (76.4%)	82 (70.7%)
HCV therapy			
Type of pegIFNα			
PegIFNα-2a	78 (44.1%)	24 (42.9%)	16 (13.8%)
PegIFNα-2b	69 (39.0%)	20 (35.7%)	53 (45.7%)
Unknown	30 (16.9%)	12 (21.4%)	47 (40.5%)
Ribavirin dose			
800 mg/day	38 (21.5%)	11 (19.6%)	27 (23.3%)
1000 mg/day	101 (57.1%)	35 (62.5%)	63 (54.3%)
1200 mg/day	31 (17.5%)	5 (8.9%)	24 (20.7%)
2000 mg/day	7 (4%)	5 (8.9%	2 (1.7%)
HCV markers, n (%)			
HCV-RNA ≥ 500.000 IU/ml	118 (68.6%)	36 (65.5%)	79 (69.3%)
Metavir score, n (%)			
Liver biopsy patients	142 (80.2%)	42 (75%)	97 (83.6%)
Significant fibrosis (F≥2)	91 (64.1%)	27 (64.3%)	62 (63.9%)

Moderate or severe activity (A≥2)	90 (64.7%)	25 (61%)	64 (67.4%)
Steatosis	75 (54%)	29 (65.9%)	46 (49.5%)
IL28B rs12980275			
AG/GG (%)	88 (49.7%)	40 (71.5%)	46 (39.7%)
AA (%)	89 (50.3%)	16 (28.5%)	70 (60.3%)

Values are expressed as absolute numbers (%) and median (percentile 25-percentile 75). (*), values were exclusively calculated with data of patients on ART. (**), The detection limit of the technique was 50 copies/ml.

Abbreviations: AIDS, acquired immunodeficiency syndrome; BMI, body mass index; cART, combination antiretroviral therapy; GT, genotype; HCV, hepatitis C virus; HCV-RNA, HCV plasma viral load; HIV, human immunodeficiency; HIV-RNA, HIV plasma viral load; IVDU, intravenous drug users; NNRTI, no nucleoside analogue reverse-transcriptase inhibitors; NRTI, nucleoside analogue reverse-transcriptase inhibitors; PI, protease inhibitors.

Characteristics of *IL7RA* polymorphisms

The allele frequencies for rs6897932, rs987106 and rs3194051 polymorphisms at *IL7RA* gene are shown in **Table 2**. All SNPs had a minimum allele frequency >5%, were in HWE (p>0.05), and displayed missing values <5% (only 2 out 179 samples failed for SNPs genotyping). Additionally, a strong LD among *IL7RA* SNPs was found (D´=0.999), meaning that there is no evidence for recombination between these SNPs. However, the r-square among SNPs was low (R-square<0.50), meaning that the *IL7RA* SNPs did not provide exactly the same information and the *IL7RA* SNPs cannot substitute one for another (**Figure 1**).

Table 2. Summary of the allele frequencies for *IL7RA* polymorphisms.

Gene	SNP	Position (Chr. 5)	HWE p-value	Alleles	MAF
IL7RA	rs6897932	35874575	1.000	C>T	0.207
	rs987106	35875593	0.632	A>T	0.466
	rs3194051	35876274	0.775	A>G	0.324

Abbreviations: HWE, Hardy-Weinberg Equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

IL7RA polymorphisms and HCV virological response

The number of patients who failed to complete HCV therapy were 16 (12 adverse events and 4 abandonments) and 161 patients had a full course of HCV therapy.

Table 3 shows the relationship among *IL7RA* polymorphisms and SVR by an analysis by intention-to-treat. The highest SVR rate was found in patients with rs6897932 CC (p=0.029) and rs3194051 GG (p=0.002) genotypes; and HCV GT2/3 infected patients carriers of rs987106 AA genotype (p=0.048). Additionally, carriers of rs3194051 GG genotype had higher likelihood of achieving a SVR (adjusted odds ratio (aOR)=5.32; p=0.040). We did not find any significant association for rs6897932 CC (aOR=0.63; p=0.205) and rs987106 AA (aOR=0.60; p=0.213).
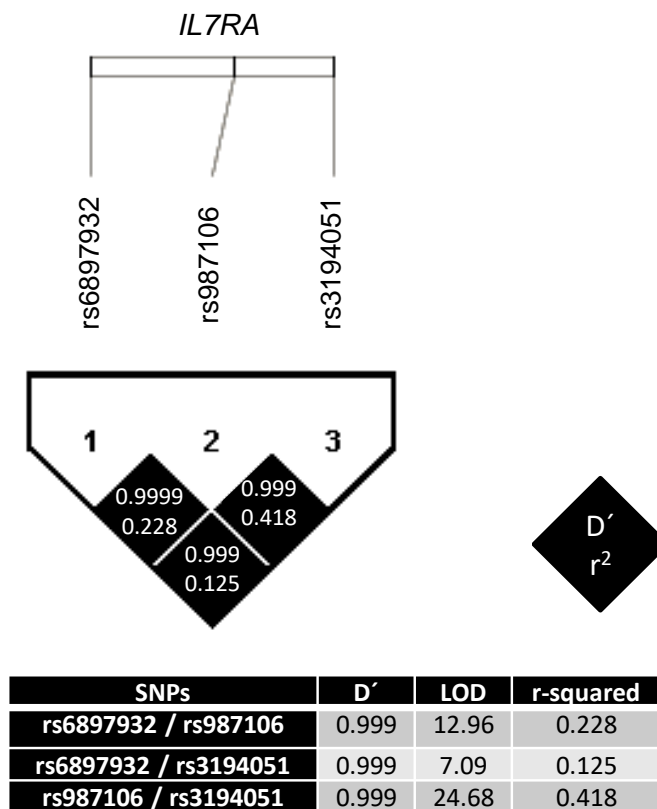
Figure 1. Pairwise linkage disequilibrium (LD) patterns for three polymorphisms through IL7RA regions. Each diagonal represents a different SNP, with each square representing a pairwise comparison between two SNPs.

Abbreviations: SNP, single nucleotide polymorphism; LOD, LOD score.

The patients with the favourable rs12980275 AA genotype had the highest SVR rates (see **Supplementary data 2**). Among patients with the unfavourable rs12980275 AG/GG, the highest SVR rates were found in carriers of rs987106 AA (p=0.043) and rs3194051 GG (p=0.007) genotypes. However, we did not find any significant association between favourable rs12980275 AA genotype and *IL7RA* polymorphisms (see **Supplementary data 2**).

IL7RA haplotypes and HCV virological response

Table 4 shows the relationship among *IL7RA* haplotypes (comprised of rs6897932, rs987106, and rs3194051) and SVR. Three major *IL7RA* haplotypes were found: 46.6% for CTA, 32.4% for CAG and 20.7% for TAA haplotypes. In the analysis by intention-to-treat, patients infected with GT2/3 and carriers of CTA haplotype had lower odds of achieving a SVR (aOR=0.08; p=0.004) and CAG haplotype (favorable alleles) had higher odds of achieving a SVR than other haplotypes (aOR=21.96; p<0.001).

Table 3. Relationships among *IL7RA* polymorphisms and sustained virologic responses to HCV treatment in HIV/HCV-coinfected patients, considering intention-to-treat approach.

IL7RA Genotypes				p-value (a)	aOR (95% CI)	p-value (b)
rs6897932 (D)	**All genotypes**	**CC**	**CT/TT**			
All patients	54.5% (96/176)	60.9% (67/110)	43.9% (29/66)	**0.029**	0.63 (0.30-1.28)	0.205
HCV GT2/3	83.9% (47/56)	85.7% (36/42)	78.6% (11/14)	0.529	0.75 (0.15-3.81)	0.736
HCV GT1/4	40% (46/115)	44.6% (29/65)	34% (17/50)	0.249	0.56 (0.24-1.01)	0.171
rs987106 (D)	**All genotypes**	**AA**	**AT/TT**			
All patients	54.8%(97/177)	64.7% (33/51)	50.8% (64/126)	0.092	0.60 (0.28-1.32)	0.213
HCV GT2/3	83.9% (47/56)	100% (16/16)	77.5% (31/40)	**0.048**	NA	NA
HCV GT1/4	40.5% (47/116)	45.5% (15/33)	38.6% (32/83)	0.495	0.96 (0.39-2.38)	0.944
rs3194051 (R)	**All genotypes**	**AA/AG**	**GG**			
All patients	54.5%(96/176)	50.6% (80/158)	88.9% (16/18)	**0.002**	5.32 (1.07-26.9)	**0.040**
HCV GT2/3	83.9% (47/56)	80.4% (37/46)	100% (10/10)	0.127	NA	NA
HCV GT1/4	40% (46/115)	38% (41/108)	71.4% (5/7)	0.080	3.94 (0.68-22.6)	0.124

Statistical significant differences are shown in bold. (a), P-values were calculated by Chi-squared test; (b), P-values were calculated by multivariate logistic regression adjusted by the most important clinical and epidemiological characteristics (see **statistical analysis** section).

Abbreviations: aOR, adjusted odds ratio; HCV-GT, hepatitis C virus genotype, D, dominant inheritance; R, recessive inheritance; NA, not available because the number of patients is equal to 0 in one of the groups analysed within the crosstab; 95%CI, 95% of confidence interval.

Table 4. Association of *IL7RA* haplotypes (comprised of rs987106, rs6897932 and rs3194051) and sustained virological response in HIV/HCV-coinfected patients on HCV therapy, considering intention-to-treat approach.

IL7RA haplotypes			All			HCV GT2/3			HCV GT1/4		
rs6897932	rs987106	rs3194051	No. (%)	aOR (95% CI)	p-value	No. (%)	aOR (95% CI)	p-value	No. (%)	aOR (95% CI)	p-value
C	T	A	79 (46.6%)	0.95 (0.57;1.58)	0.851	50 (44.6%)	0.08 (0.008;0.73)	**0.004**	26 (47.8%)	1.49 (0.80;2.78)	0.197
C	A	G	55 (32.4%)	1.45 (0.81; 2.59)	0.200	44 (39.2%)	21.96 (2.00;242)	**<0.001**	15 (28.7%)	0.81 (0.40;1.63)	0.555
T	A	A	35 (21.0%)	0.73 (0.40;1.35)	0.328	18 (16.0%)	0.60 (0.13;2.81)	0.529	13 (23.5%)	0.77 (0.38-1.59)	0.486

Statistically significant values are shown in bold. P-values were calculated by logistic regression analysis adjusted by the most important clinical and epidemiological characteristics (see **statistical analysis** section).
Abbreviations: aOR, adjusted odds ratio; 95% CI, 95% confidence interval; HCV GT, HCV genotype; Freq., frequencies.

DISCUSSION

In this study, we found that carriers of *IL7RA* rs3194051 GG genotype (homozygous for the minor allele) had the highest SVR rate to pegIFNα/ribavirin therapy. Furthermore, both rs987106 and rs6897932 polymorphisms had a significant negative association with SVR in univariate analysis but it disappeared in the adjusted regression model. Besides, the *IL7RA* haplotypes were also related to SVR in patients infected with GT2/3. To our knowledge, this is the first description of the relation between the *IL7RA* polymorphisms and SVR in HIV/HCV-coinfected patients on pegIFNα/ribavirin therapy.

Chronic infection caused by HCV is characterised by a poor HCV-specific CD8+ T-cell response, which displays an exhausted phenotype and is unable to control viral replication [9, 23], as well as the suppression of T cell function by CD4+ Treg cells is emerging as one of the most important mechanisms during chronic HCV infection [24]. In this regard, IL-7 is a cytokine essential for the differentiation and maintenance of memory T cells, preventing their functional exhaustion [5]. However, the expression of IL-7 is significantly diminished in livers from CHC patients [25] and the expression of *IL7RA* gene (CD127) on HCV-specific T cells is decreased while an exhausted phenotype is also observed [26]. Moreover, IFN-α therapy may increase the frequency of CD127+CD8+ T cells [27] and IL-7 may increase the antiviral efficacy of CD127+CD8+ T cells [28], which may inhibit HCV replication ex vivo [28]. Thus, the expression of CD127 on HCV-specific T cells predicts the favourable outcome of HCV acute infection in HCV monoinfected patients [8, 29] and SVR in HCV/HIV co-Infected Individuals undergoing IFN-α therapy [27]. These patients with SVR had higher expression of CD127 on CD4+ T cells, lower T cell exhaustion status and better HIV and HCV proliferative responses at baseline; and an increase in the frequency of CD127+CD8+ T cells in all treated patients [27]. Besides, early therapeutic intervention with pegIFNα rescued polyfunctional memory T cells expressing high levels of CD127 and Bcl-2, cells that were detectable for up to 1 year following discontinuation of therapy [30]. In this regard, *IL7RA* polymorphisms has been correlated with levels of soluble IL7RA (sCD127) in plasma and CD127 on T-cell, [13, 31, 32]. Thus, the decreased expression of membrane-bound IL7RA in T-cell and the increased of sCD127 levels (block the IL-7 circulating) could cause a decreased of the effect of IL-7 in the immune system. Regarding the above described, it would be of great value to know the level of IL-7 and sCD127 in plasma, and the frequency and level of CD127 positive T cells in the patients included in our study. However, we did not have access to biological samples to complete our data with these immunological analyses.

In our study, rs3194051 GG genotype was related to higher SVR rate than AA/AG genotypes in HIV/HCV coinfected patients on pegIFNα/ribavirin therapy. The rs3194051 GG genotype tags the *IL7RA* haplotype 4 in European populations and it has been associated with lower sCD127 levels and appears to be protective against multiple sclerosis [32]. Besides, Rajasuriar et al. also found that homozygous carriers of the G-allele at rs3194051 experienced faster time to CD4 T-cell >500 cells/mm3 compared with homozygous carriers of the A-allele. Moreover, rs6897932 and rs987106 polymorphisms have been related to levels of soluble IL7RA (sCD127) in HIV infected patients in other reports. Thus, rs6897932 CC genotype has been associated to higher levels of sCD127 in peripheral blood compared to CT/TT genotypes [13], while rs6897932 TT genotype was a predictor for faster CD4+ recovery in HIV infected patients compared to rs6897932 CC/CT genotypes [33]. Besides, rs987106 AA genotype was associated with lower sCD127 levels in HIV infected patients compared to the presence of rs987106 T allele [13]. In our study, when the haplotype analysis was performed, carriers of CTA haplotype (comprised of rs6897932 C, rs987106 T, and rs3194051 A, three alleles associated with higher sCD127 levels) had lower odds of

achieving a SVR; since carriers of CAG haplotype (comprised of one allele associated with higher sCD127 levels and two alleles related to lower sCD127 levels) had higher odds of achieving a SVR. Thus, although we do not have any data about the functional effect of these SNPs on the expression of CD127 and function of CD8 cytotoxic cells, we could speculate on the possible role of *IL7RA* polymorphisms in the immunological control of HCV during pegIFNα/ribavirin therapy, independently of *IL28B* rs12980275 polymorphism.

We have also performed an *in silico* analysis, finding that the *IL7RA* polymorphisms could generate possible targets for splicing regulation. The *IL7RA* polymorphisms are located in putative regulatory intronic region (rs987106), exon 6 (rs6897932) and exon 8 (rs3194051). The change C>T at rs6897932 polymorphism is linked to a diminished of exonic splicing enhancers (ESEs) motif, which play important roles in constitutive and alternative splicing. In fact, rs6897932 is a missense change involved in the splicing regulation that influence on ratio of IL7RA isoforms (membrane bound and soluble) [24]. Similarly, the change A>G at rs3194051 polymorphism is also a missense change linked to ESEs motif diminished, which might also influence on ratio of IL7RA isoforms. Finally, we did not find any significant possible function for rs987106 polymorphism.

We should also mention the fact that the effect of *IL7RA* polymorphisms on SVR seems be dependent on HCV genotype. However, we did not have an satisfactory explanation that may help understand why the association between *IL7RA* polymorphisms and SVR was stronger in patients infected with GT2/3 than in GT1/4 patients. Perhaps, the fact that patients with GT1/4 had a lower SVR rate might be influencing the lack of significant association.

HCV therapy with pegIFNα/ribavirin is still in use among HIV/HCV coinfected patients but this treatment has a limited effectiveness and serious side effects [20]. Currently, new IFN-free regimens are being used in GT2/3 patients and are also being developed for GT1 patients, in both cases with a very high response rate [34]. In this new context, the combination therapy with potent DAAs might obscure the influence on HCV treatment efficacy of *IL7RA* polymorphisms and other SNPs. However, the new DAAs are extremely expensive and there are serious restrictions for its administration, and in many regions in the world these drugs are inaccessible. Also, not all patients have indications to be treated with these new antivirals, such as happen with some patients coinfected with HIV and HCV. In fact, treatment with pegIFNα/ribavirin remains the only option of therapy for many patients in the world. Moreover, nowadays, the new DAAs are generally being administered in combination with pegIFNα/ribavirin [1, 4]. Thus, it is still essential the potential ongoing relevance of understanding pegIFNα/ribavirin response predictors.

For the correct interpretation of the data, it must be taken into account that the study design was retrospective and the number of patients was limited, which could limit the achievement of statistically significant values. Secondly, HCV therapy regimens were not identical for all patients. Thirdly, all selected patients met a set of criteria for starting HCV treatment and this may have introduced a selection bias. Fourthly, this study was performed on patients with European ancestry, and it would be interesting to perform these analyses on different ethnic groups. Finally, our study only included HIV/HCV-coinfected patients and it would be interesting to know the role of studied *IL7RA* SNPs in HCV monoinfected patients, but we did not have access to a cohort of HCV monoinfected patients.

In conclusion, *IL7RA* polymorphisms seem to play a significant role in the virological response to pegIFNα/ribavirin therapy in HIV/HCV-coinfected patients; in particular, the presence of homozygous for the minor allele of *IL7RA* rs3194051 polymorphism was

related to higher odds of achieving a SVR. Further analyses would be needed to determine its potential use as a predictive marker.

ABBREVIATIONS

aOR, adjusted odds ratio
BMI, Body mass index
cART, combination antiretroviral therapy
CD127 or IL7Rα, α-chain of the IL-7 receptor
DAAs, new direct-acting antivirals
GT1/4, HCV genotype 1/4
GT2/3, HCV genotype 2/3
HCV, hepatitis C virus
HIV, human immunodeficiency virus
HOMA, homeostatic model assessment
HWE, Hardy-Weinberg equilibrium
IL28B, interleukin 28B
IL-7, Interleukin-7
IL7RA, gene of IL7Rα
ITT, intention-to-treat
LD, linkage disequilibrium
NA, not available due to a low number of patients in one of the groups
pegIFNα/ribavirin, pegylated interferon alpha plus ribavirin
SNPs, single nucleotide polymorphisms

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

AUTHORS CONTRIBUTIONS

MGF and SR performed all statistical analysis, interpretation of the data and wrote the manuscript.

JB and SR participated in the study concept and design.

JB, TAE, AC, CD, and FT, participated in patient selection, collection of samples and acquisition of data.

DPT, MAJS, MGA, SV, and VB participated in sample preparation, DNA isolation and genotyping pre-procedure, and contributed with critical revision of the manuscript.

SR supervised the study.

All authors revised the manuscript from a draft by SR.

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SUPPLEMENTARY DATA

Supplementary data 1.

Association between IL7RA rs6897932 polymorphism and sustained virologic response (SVR) (n=176, crude analysis)

Model	Genotype	No SVR	SVR	OR (95% CI)	P-value	AIC	BIC
Dominant	C/C	43 (53.8%)	67 (69.8%)	1.00	**0.029**	241.7	248.1
	C/T-T/T	37 (46.2%)	29 (30.2%)	**0.50 (0.27-0.93)**			
Recessive	C/C-C/T	78 (97.5%)	91 (94.8%)	1.00	0.350	245.7	252
	T/T	2 (2.5%)	5 (5.2%)	2.14 (0.40-11.35)			
Log-additive	---	---	---	0.66 (0.39-1.12)	0.120	244.1	250.5

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; SVR, sustained virologic response; AIC, Akaike information criterion; BIC, Bayesian information criterion

Association between IL7RA rs987106 polymorphism and sustained virologic response (SVR) (n=177, crude analysis)

Model	Genotype	No SVR	SVR	OR (95% CI)	P-value	AIC	BIC
Dominant	A/A	18 (22.5%)	33 (34%)	1.00	0.092	244.9	251.2
	A/T-T/T	62 (77.5%)	64 (66%)	0.56 (0.29-1.10)			
Recessive	A/A-A/T	62 (77.5%)	78 (80.4%)	1.00	0.640	247.5	253.9
	T/T	18 (22.5%)	19 (19.6%)	0.84 (0.41-1.73)			
Log-additive	---	---	---	0.74 (0.49-1.14)	0.170	245.9	252.2

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; SVR, sustained virologic response; AIC, Akaike information criterion; BIC, Bayesian information criterion

Association between IL7RA rs3194051 polymorphism and sustained virologic response (SVR) (n=176, crude analysis)

Model	Genotype	No SVR	SVR	OR (95% CI)	P-value	AIC	BIC
Dominant	A/A	42 (52.5%)	37 (38.5%)	1.00	0.064	243.1	249.4
	A/G-G/G	38 (47.5%)	59 (61.5%)	1.76 (0.97-3.22)			
Recessive	A/A-A/G	78 (97.5%)	80 (83.3%)	1.00	**0.002**	235.6	241.9
	G/G	2 (2.5%)	16 (16.7%)	**7.80 (1.74-35.05)**			
Log-additive	---	---	---	**1.98 (1.22-3.21)**	**0.004**	238.3	244.7

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval; SVR, sustained virologic response; AIC, Akaike information criterion; BIC, Bayesian information criterion

Supplementary data 2. Percentage of co-carriages of the *IL28B* rs12980275 and *IL7RA* polymorphisms in relation to sustained virologic response (SVR) rates among HIV/HCV-coinfected patients.

	IL7RA Genotypes			p-values
rs6897932 (D)	**All genotypes**	**CC**	**CT/TT**	
rs12980275 AG/GG	37 (42.5%)	23 (46.9%)	14 (36.8%)	0.345
rs12980275 AA	59 (66.3%)	44 (72.1%)	15 (53.6)	0.085
rs987106 (D)	**All genotypes**	**AA**	**AT/TT**	
rs12980275 AG/GG	38 (43.2%)	16 (59.3%)	22 (36.1%)	**0.043**
rs12980275 AA	59 (66.3%)	17 (70.8%)	42 (64.6%)	0.582
rs3194051 (R)	**All genotypes**	**AA/AG**	**GG**	
rs12980275 AG/GG	37 (42.5%)	32 (39.0%)	5 (100%)	**0.007**
rs12980275 AA	59 (66.3%)	48 (63.2%)	11 (84.6%)	0.130

P-values were calculated by Chi-squared test. Statistical significant differences are shown in bold.
Abbreviations: HCV-GT, hepatitis C virus genotype, D, dominant inheritance; R, recessive inheritance.