Title page

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Title: *IL28B* polymorphisms are associated with severity of liver disease in human immunodeficiency virus (HIV) patients coinfected with hepatitis C virus

Running head: *IL28B* polymorphisms and fibrosis

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ABSTRACT

Objective: To evaluate the association of *IL28B* polymorphisms and severity of liver disease among human immunodeficiency virus (HIV)/ hepatitis C virus (HCV) coinfected patients.

Methods: We carried out a cross-sectional study on 223 patients. Liver biopsies were evaluated according to Metavir score. *IL28B* polymorphisms (rs12980275, rs8099917, rs7248668, and rs11881222) were genotyped using GoldenGate® assay.

Results*:* *IL28B* polymorphisms were in strong linkage disequilibrium, especially the couples rs12980275/rs11881222 and rs8099917/rs7248668. For all patients, the rs12980275 A allele increased the odds for significant fibrosis (F≥2) (odds ratio (OR)=1.68; p=0.018) and more rapid fibrosis progression (FPR≥0.075 fibrosis units/year) (OR=1.64; p=0.035), and decreased the odds for liver steatosis (OR=0.61; p=0.046). Furthermore, the rs8099917 T allele increased the odds for F≥2 (OR=1.93; p=0.020), FPR≥0.075 (OR=2.08; p=0.021), and elevated ALT (≥80 IU/l) (OR=1.78; p=0.048). For HCV-genotype 1 patients, rs12980275 A and rs8099917 T alleles decreased the odds for liver steatosis (OR=0.22; p<0.001 and OR=0.39; p=0.048; respectively). For HCV-genotype 3 patients, the rs12980275 A allele increased the odds for F≥2 ((OR=6.30; p=0.012), FPR≥0.075 (OR=6.40; p=0.025), and elevated ALT (OR=4.12; p=0.037); and the rs8099917 T allele also increased the odds for F≥2 (OR=7.56; p=0.027), FPR≥0.075 (OR=50.8; p=0.012), and elevated ALT (OR=5.39; p=0.043). However, we did not find significant trends in patients infected with HCV-genotype 4.

Conclusion*:* The major alleles of *IL28B* (rs12980275 A, rs11881222 A, rs8099917 T, and rs7248668 G) are associated with increased odds of liver disease severity in HIV patients infected with HCV-genotype 3. In contrast, HCV-genotype 1 patients carrying the major alleles of IL28B polymorphisms had lower odds for liver steatosis.

Key-words: AIDS; Hepatitis C; SNPs; liver biopsy; fibrosis; transaminases

INTRODUCTION

Liver disease is the leading comorbidity among human immunodeficiency virus (HIV) and hepatitis C virus (HCV) coinfected patients on combination antiretroviral therapy (cART) (1). Chronic hepatitis C (CHC) has become a major cause of morbidity and mortality in HIV/HCV-coinfected patients, because HIV infection modifies the natural history of CHC, with a faster progression of fibrosis in HIV/HCV coinfected patients than in HCV monoinfected patients (1-3).

The most common treatment for CHC in HIV/HCV-coinfected patients is still a combination of pegylated-interferon alpha plus ribavirin (pegIFNα/RBV) (4), which is successful in only 20-40% of the patients infected with HCV genotype 1 (HCV-GT1) and HCV genotype 4 (HCV-GT4), and 50-60% in HCV genotype 2 (HCV-GT2) and HCV genotype 3 (HCV-GT3) patients (5, 6). However, HCV treatment outcomes is inferior among HIV/HCV-coinfected patients in whom drug interactions and numerous side effects might contribute to impair virological responses (7, 8). Given the variability of the response to HCV therapy, it is desirable to identify patients who urgently need HCV treatment (9).

Three independent groups discovered several single nucleotide polymorphisms (SNPs) located within and close to the *interleukin 28B* (*IL28B*) gene, which contribute to spontaneous and treatment induced clearance of HCV (10-12). However, the functional link between polymorphisms at *IL28B* and HCV clearance still remains unsettled. It seems that these genetic traits are related with levels of intrahepatic *IL28B* gene expression (13), and the baseline expression of interferon stimulated genes (ISGs) is significantly higher in patients carrying the minor rs8099917G allele (14, 15).

Since HCV itself is not cytopathic, liver damage in CHC is commonly attributed to immune-mediated mechanisms (16). In addition, accelerated liver disease in HIV/HCV coinfected patients seems probably influenced by differences in the composition of infiltrating inflammatory cells, and the local release of inflammatory and profibrogenic cytokines (17). *IL28B* alleles might be related to the effects on 'visiting' cell populations rather than to the hepatocytes themselves (18). *IL28B* encodes for IFN-λ3, a type III interferon cytokine with antiviral activity against HCV in liver via innate immunity pathway (18, 19). Thus, the most antiviral properties of IFN-λ3 "in vivo" depend on the proper stimulation of the host immune system rather than through induction of the antiviral state (20). Besides, *IL28B* is able to modulate adaptive immune response, favouring the Th1 immune pathway, increasing T regulatory cells and augmenting CD8 T-cell cytotoxicity and memory responses (18). Thus, it is reasonable to assume that *IL28B* polymorphism could also influence on liver fibrosis progression in CHC.

The aim of our study was to evaluate the association of *IL28B* polymorphisms and severity of liver disease among HIV/HCV coinfected patients.

PATIENTS AND METHODS

Study design

We carried out a cross-sectional study on HIV/HCV-coinfected patients who underwent a liver biopsy at Hospital Gregorio Marañón (Madrid, Spain) between September 2000 and November 2008. All patients were Caucasians.

Liver biopsies were performed on treatment-naive patients who were potential candidates for anti-HCV therapy. Inclusion criteria for the study were: no clinical evidence of hepatic decompensation, detectable HCV RNA by polymerase chain reaction (PCR), negative hepatitis B surface antigen, CD4+ lymphocyte count higher than 200 cells/mm^3, stable antiretroviral therapy or no need for antiretroviral therapy. Patients with active opportunistic infections or active drug addiction were excluded. From our cohort of 361 HIV/HCV coinfected patients with liver biopsy data, only 223 patients had DNA sample available for *IL28B* genoyping.

The study was conducted in accordance with the Declaration of Helsinki. All patients gave their written consent for the liver biopsy and 223 for the genetic testing. The Institutional Ethics Committee approved the study.

Clinical and laboratory data

The following information was obtained from medical records when liver biopsy was performed: age, gender, risk category, weight, height, alcohol intake (consumption of more than 50 g of alcohol per day for at least 12 months was considered as high intake), Centers for Disease Control (CDC) clinical category, nadir $CD4^+$ T cell count, antiretroviral therapy, HCV genotype, $CD4^+$ T-cells, plasma HIV viral load (HIV-RNA), plasma HCV viral load (HCV-RNA), activity grade and fibrosis stage of liver biopsies, and liver panel tests.

Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. The degree of insulin resistance (IR) was estimated for each patient using the homeostatic model assessment (HOMA): fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5.

Liver biopsy and outcome variables

Liver fibrosis and necroinflammatory activity were estimated according to Metavir score. Fibrosis was scored as follows: F0, no fibrosis; F1, portal fibrosis; F2, periportal fibrosis or rare portal-portal septa; F3, fibrous septa with architectural distortion; no obvious cirrhosis (bridging fibrosis); and F4, definite cirrhosis. Activity grade was scored as follows: A0, no 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 consider that hepatic steatosis was clinically significant when fatty hepatocytes exceed 10% of parenchyma hepatic.

The duration of HCV infection in 193 out of 195 patients with a history of intravenous drug use (IDU) was estimated starting from the first year that needles were shared, which are the most important risk practices for HCV transmission (21). We were able to calculate the time of HCV infection for other three non-IDUs patients. We had the date of infection for one patient who was infected by blood transfusion and other two patients who were infected by sexual contact, in whom may be dated with certainty the HCV infection. For 27 patients (2 IDUs and 25 non-IDUs), we were unable to calculate the time of HCV infection. Then, for 196 out of 223 patients with the duration of HCV infection, the fibrosis progression rate (FPR) was calculated dividing the fibrosis stage (0 to 4) by the estimated duration of HCV infection in years (fibrosis units per year) (22).

In this study, we used five outcome variables for liver disease: a) Significant fibrosis (F≥2): Values of Metavir score for fibrosis equal to or above two. b) Moderate activity grade (A≥2): Values of Metavir score for activity grade equal to or above two. c) Rapid fibrosis progression (FPR≥0.075 fibrosis units/year): Values of FPR equal to or above the median (0.075) were used as cut-off to identify patients with more rapid development of liver fibrosis. d) Liver steatosis: Presence of hepatocytes containing visible macrovesicular fat droplets. e) Elevated alanine transaminase (ALT ≥80 IU/l) at the time of

biopsy: Values of ALT equal to or above two times upper limit of normal (≥80 IU/l) were considered elevated ALT levels.

Genotyping

Genomic DNA was extracted from peripheral blood by using Qiagen columns (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). Four *IL28B* polymorphisms ((rs12980275, rs8099917, rs7248668, and rs11881222) were genotyped by the Spanish National Genotyping Centre (CeGen; http://www.cegen.org/). Genotyping was performed by using GoldenGate® assay with VeraCode® Technology (Illumina Inc. San Diego, CA, USA).

Statistics

To evaluate the association between *IL28B* SNPs and liver disease, we used Statistical Package for the Social Sciences (SPSS) 15.0 (SPSS INC, Chicago, IL, USA). All p-values were two-tailed and statistical significance was defined as $p<0.05$.

Categorical data and proportions were analysed by using the chi-squared test or Fisher´s exact test. Mann-Whitney U test was used to compare data among independent groups. We analysed the genetic data according to the genetic model that best fit our data (additive or dominant). In the dominant model, the comparison groups are homozygous for the major allele versus allele positivity (combining heterozygotes and homozygotes for the minor allele). In the additive model, the risk conferred by an allele is increased r-fold for heterozygotes and 2r-fold for homozygotes with two copies of a specific allele. In this case, homozygous for the minor allele, heterozygous, and homozygous for the major allele were coded as 0, 1, and 2, respectively.

Multivariate logistic regression analyses were adjusted by the most important clinical and epidemiological characteristics: gender, age, alcohol intake, BMI, HOMA, nadir $CD4^+$ T-cells, AIDS, undetectable HIV-RNA (<50 copies/ml), CD4+ T-cells, cART (protease inhibitor or non-nucleoside analogue), HCV-RNA ≥500,000 IU/ml. In each multiple logistic regression analysis, we included *IL28B* SNPs ("Enter" algorithm), and the most significant covariables selected by "Stepwise" algorithm (at each step, factors are considered for removal or entry: a p-value for entry and exit of 0.30 and 0.30, respectively). Thus, each logistic regression analyses were always adjusted for the most significant covariates associated with each one of the outcome variables, avoiding the over-fitting of the regression.

RESULTS

Characteristics of the patients

Table 1 shows the clinical and epidemiological characteristics of 223 HIV/HCV coinfected patients, 125 patients were infected with HCV-GT1, 3 with HCV-GT2, 51 with HCV-GT3, and 40 with HCV-GT4. There was no significant difference in baseline characteristics when the population was stratified by the HCV genotype (**Table 1**).

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

	All patients	HCV genotype 1	HCV genotype 4	HCV genotype 3
No. HIV-1 patients	223	125	40	51
Gender (male)	165 (74%)	95 (76%)	27 (67.5%)	37 (72.5%)
Age (years)	39.7 (21.1;99.6)	39.5 (29.6;54.8)	41.0 (21.0;49.9)	40.2 (29.4;53.8)
Body mass index	22.4 (15.5;35.2)	22.4 (16.3;34.3)	22.07 (16.6;34.6)	22.3 (16.5;35.2)
Epidemiological history				
Injection drug users	195 (87.4%)	108 (85.4%)	36 (90.0%)	45 (88.2%)
Years since HCV infection	21.7 (0;44.7)	21.9 (0;44.8)	21.2 (7.82;32.6)	22.1 (2.9;30.9)
High alcohol intake	90 (40.4%)	54 (43.2%)	14 (35%)	20 (39.2%)
CDC category C	61 (27.4%)	36 (28.8%)	11 (27.5%)	13 (25.5%)
Antiretroviral therapy				
Non treated	33 (14.8%)	21 (16.8%)	4 (10%)	7 (13.7%)
PI-based	50 (22.4%)	29 (23.2%)	12 (30%)	9 (17.6%)
NNRTI-based	117 (52.5%)	64 (51.2%)	20 (50%)	29 (56.9%)
3 NRTI-based	23 (10.3%)	11 (8.8%)	4 (10%)	6 (11.8%)
Metavir fibrosis stage				
No fibrosis (F0)	26 (11.7%)	15 (12%)	7 (17.5%)	3 (5.9%)
Portal fibrosis (F1)	87 (39%)	48 (38.4%)	19 (47.5%)	17 (33.3%)
Periportal fibrosis (F2)	60 (26.9%)	31 (24.8%)	8 (20%)	19 (37.3%)
Advanced fibrosis (F≥3)	50 (22.4%)	31 (24.8%)	6 (15%)	12 (23.5%)
Metavir activity grade (n=219)				
No activity (A0)	6 (2.7%)	4 (3.2%)	1 (2.5%)	0 (0%)
Mild activity (A1)	98 (43.9%)	52 (41.6%)	19 (47.5%)	24 (47.1%)
Moderate activity (A2)	91 (40.8%)	54 (43.2%)	14 (35%)	21 (41.2%)
Severe activity (A≥3)	24 (10.7%)	14 (11.2%)	4 (10%)	5 (9.8%)
Liver steatosis (n= 174)	118 (60.8%)	61 (56.5%)	16 (47.1%)	37 (80.4%)
HIV markers				
Nadir CD4+ T-cells	192 (4;991)	208 (6;991)	142 (4;594)	227 (10;652)
CD4+ T-cells/uL	468 (15;1683)	484(135;1683)	483 (15;1224)	441 (126;1584)
HIV-RNA < 50 cp/mL	165 (74%)	93 (74.4%)	29 (72.5%)	39 (76.5%)
HCV markers				
HCV genotype (n= 219)				
1	125 (56.1%)	-	-	-
2	3 (1.3%)	-	-	-
3	51 (22.9%)	-	-	-
4	40 (17.9%)	-	-	-
HCV RNA >500,000 UI/mL	165 (74%)	97 (77.6%)	29 (72.5%)	35 (68.6%)

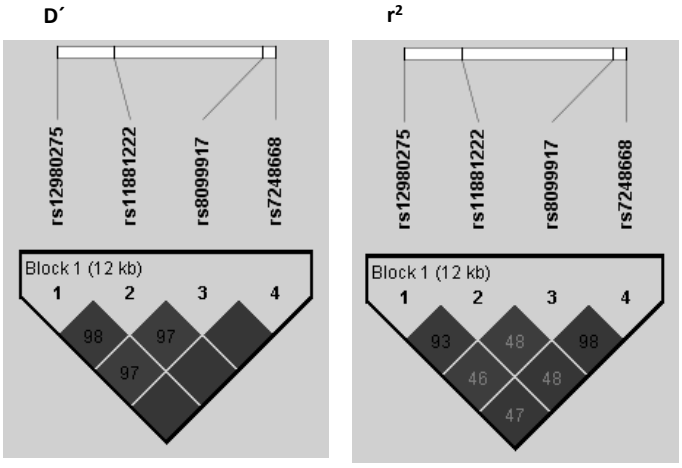
Biochemical parameters				
Glucose (mg/dL)	85 (53;170)	84.0 (53;165)	82 (60;170)	86 (53;161)
HOMA	2.10 (0.30;39.5)	2.24 (0.30;39.5)	1.78 (0.30; 38.6)	1.98 (0.31;13.07)
ALT (IU/l)	74 (12;510)	74.5 (12;491)	52 (21;510)	91 (34;231)

Values are expressed as absolute number (percentage) and m median (percentile 25; percentile 75).
Abbreviations: ALT: alanine aminotransferase. BMI: body mass index. HCV: Hepatitis C virus. HCV-RNA: HCV plasma viral load. HIV: Human immunodeficiency virus. HIV-RNA: HIV plasma viral load; HOMA: homeostatic model assessment; IVDU: intravenous drug users.

IL28B SNPs frequencies

In order to capture the maximum variability, we select four SNPs of *IL28B* located upstream (rs8099917, and rs7248668), at intron two (rs11881222) and downstream (rs12980275). All of them had a minimum allele frequency (MAF) higher than 5% and fulfilled the Hardy-Weinberg equilibrium criteria. The *IL28B* alleles, which are considered as favourable for both spontaneous and treatment-induced HCV clearance (23), showed the highest frequencies in our patients: rs12980275 (70% A), rs11881222 (71% A), rs8099917 (83% T), and rs7248668 (83% G).

Moreover, we found strong linkage disequilibrium among the four SNPs of *IL28B* (rs12980275, rs11881222, rs8099917, and rs7248668), especially between the couples rs12980275/rs11881222 and rs8099917/rs7248668 (**Figure 1**). Thus, in order to avoid redundancy, we analysed the association of only two *IL28B* SNPs (rs12980275 and rs8099917) on liver disease severity.



SNPs	D´	LOD	r^2
rs12980275/rs11881222	0.989	77.66	0.935
rs12980275/rs8099917	0.978	31.00	0.464
rs12980275/rs7248668	1.0	33.08	0.471
rs11881222/rs8099917	0.978	32.01	0.480
rs11881222/ rs7248668	1.0	34.08	0.486
rs8099917/rs7248668	1.0	65.77	0.984

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

IL28B SNPs and severity of liver disease

We explored which genetic model best fit our data and the additive model showed higher parsimony and statistical significance (*data not shown*).

The distribution of patients with liver disease according by *IL28B* polymorphisms and stratified by HCV genotypes is shown in **Figure 2**. For all patients, the percentage of significant fibrosis and more rapid fibrosis progression tended to increase with each major allele A at rs12980275 (p= 0.045 and p= 0.043; respectively). For HCV-GT1 patients, the percentage of liver steatosis tended to decrease with each major allele A at rs12980275 (p= 0.001). For HCV-GT3 patients, the percentage of significant fibrosis, more rapid fibrosis progression, and higher values of ALT tended to be greater when these patients carried the major allele A at rs12980275 (p= 0.055, p= 0.024, and p= 0.054; respectively) and the major allele T at rs8099917 (p= 0.035, p= 0.003, and p= 0.046; respectively). However, we did not find any significant results in patients infected with HCV-GT4 (**Table 2**).

Adjusted odds ratio (OR) for liver disease severity are shown in **Table 2**. For all HIV/HCV coinfected patients, the rs12980275 A allele increased the odds for significant fibrosis (odds ratio (OR) = 1.68; p= 0.018) and more rapid fibrosis progression (OR= 1.64; p= 0.035), and decreased the odds for liver steatosis (OR= 0.61; p= 0.046). Similarly, the rs8099917 T allele also increased the odds for significant fibrosis (OR= 1.93; p= 0.020) and rapid fibrosis progression (OR= 2.08; p= 0.021), while elevated ALT (OR= 1.78; p= 0.048).

Afterwards, we analysed the data by HCV genotype (**Table 2**). For HCV-GT1 patients, the rs12980275 A allele and the rs8099917 T allele decreased the odds for liver steatosis (OR= 0.22; p <0.001 and OR= 0.39; p= 0.048; respectively). For HCV-GT3 patients, the rs12980275 A allele increased the odds for significant fibrosis ((OR= 6.30; p= 0.012), more rapid fibrosis progression (OR= 6.40; p= 0.025), and elevated ALT (OR= 4.12; p= 0.037); and the rs8099917 T allele also increased the odds for significant fibrosis (OR= 7.56; p= 0.027), more rapid fibrosis progression (OR= 50.8; p= 0.012), and elevated ALT (OR= 5.39; p= 0.043). However, we did not find significant results in patients infected with HCV-GT4 (**Table 2**).

Figure 2. The distribution of HIV/HCV coinfected patients with liver disease according by *IL28B* polymorphisms and stratified by HCV genotypes. Values are expressed as percentage. P-values were calculated by linear-by-linear association chi-squared test.
Abbreviations: HCV, hepatitis C virus, HIV, HIV, human immunodeficiency virus, FPR, fibrosis progression rate; ALT, alanine aminotransferase.

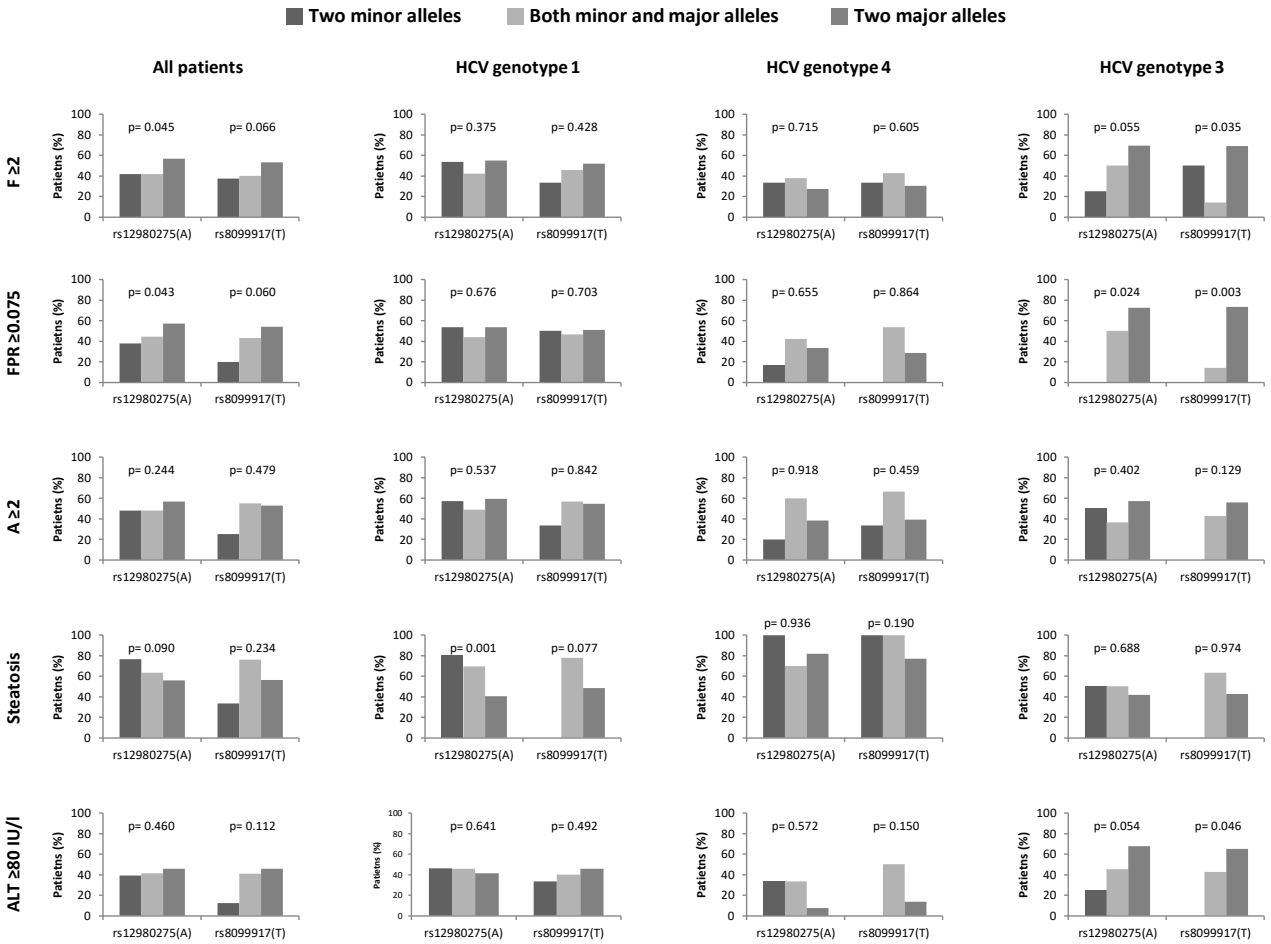


Table 2. Adjusted odds ratio (OR) of liver disease in HIV/HCV coinfected patients under an additive model of inheritance of *IL28B* polymorphisms.

		rs12980275 (major allele A)		rs8099917 (major allele T)	
		OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
All patients	**Significant fibrosis (F ≥2)**	1.68 (1.09 - 2.59)	**0.018**	1.93 (1.11 – 3.60)	**0.020**
(No.= 223)	**Rapid fibrosis progression (FPR ≥median)**	1.64 (1.03 – 2.61)	**0.035**	2.08 (1.12 – 3.88)	**0.021**
	Moderate activity grade (A≥2)	1.37 (0.88 – 2.12)	0.159	1.47 (0.85 – 2.53)	0.165
	Steatosis	0.61 (0.37 – 0.99)	**0.046**	0.78 (0.43 – 1.42)	0.430
	Elevated ALT (≥80 IU/l)	1.22 (0.78 – 1.91)	0.371	1.78 (1.01 – 3.17)	**0.048**
HCV genotype 1	**Significant fibrosis (F ≥2)**	1.21 (0.67 – 2.18)	0.516	1.27 (0.60 – 2.67)	0.525
(No.= 125)	**Rapid fibrosis progression (FPR ≥median)**	1.13 (0.61 – 2.09)	0.685	1.08 (0.48 – 2.48)	0.843
	Moderate activity grade (A≥2)	1.15 (0.63 – 2.10)	0.635	1.13 (0.52 – 2.41)	0.756
	Steatosis	0.22 (0.09 – 0.50)	**<0.001**	0.39 (0.16 – 0.99)	**0.048**
	Elevated ALT (≥80 IU/l)	0.77 (0.42 – 1.44)	0.427	1.18 (0.54 – 2.62)	0.670
HCV genotype 4	**Significant fibrosis (F ≥2)**	0.32 (0.05 – 1.98)	0.221	1.34 (0.21 – 8.31)	0.753
(No.= 40)	**Rapid fibrosis progression (FPR ≥median)**	0.34 (0.04 – 3.01)	0.333	2.38 (0.17 – 32.6)	0.514
	Moderate activity grade (A≥2)	0.35 (0.05 – 2.32)	0.279	0.16 (0.02 – 1.73)	0.133
	Steatosis	0.72 (0.21 – 2.47)	0.602	2.99 (0.53 – 16.97)	0.215
	Elevated ALT (≥80 IU/l)	0.15 (0.01 – 3.56)	0.245	0.11 (0.01 – 4.57)	0.240
HCV genotype 3	**Significant fibrosis (F ≥2)**	6.30 (1.50 – 26.4)	**0.012**	7.56 (1.29 – 45.4)	**0.027**
(No.= 43)	**Rapid fibrosis progression (FPR ≥median)**	6.40 (1.26 – 32.59)	**0.025**	50.8 (2.39 – 1080)	**0.012**
	Moderate activity grade (A≥2)	2.53 (0.71 – 7.75)	0.160	4.05 (0.81 – 20.1)	0.087
	Steatosis	1.05 (0.25 – 4.39)	0.942	NA	-
	Elevated ALT (≥80 IU/l)	4.12 (1.09 – 15.6)	**0.037**	5.39 (1.05 – 27.6)	**0.043**

Values expressed as odds ratio (OR) and 95% of confidence interval (95% CI). P-values were calculated by adjusted logistic regression. In bold, p-values with statistical significance. Abbreviations: OR, odds ratio; 95% CI, 95% of confidence interval, p, significant value; SNPs, single-nucleotide polymorphisms; HCV, hepatitis C virus, HIV, HIV, human immunodeficiency virus, FPR, fibrosis progression rate; ALT, alanine aminotransferase; NA; not applicable because there is an empty cell in the 2x2 table.

DISCUSSION

In our study, we show that the liver disease severity was more pronounced in HCV-GT3 patients carrying one or two copies of major alleles of *IL28B* (rs12980275 A, rs11881222 A, rs8099917 T, and rs7248668 G) than in patients carrying the minor alleles (rs12980275 G, rs11881222 G, rs8099917 G, and rs7248668 A). In addition, HCV-GT1 patients carrying the major alleles of IL28B polymorphisms had lower odds for liver steatosis. All logistic regression tests were adjusted by the most important factors independently associated with the progression of liver disease severity in CHC patients, such as male gender, older age, alcohol abuse, uncontrolled HIV replication, HCV genotype, obesity, insulin resistance, low CD4 counts, high serum HCV-RNA levels, or antiretroviral drug modality (24-26).

Nowadays, there are contradictory data of *IL28B* SNPs and liver disease in HCV monoinfected patients. The major rs8099917 T allele has been associated with elevated ALT, activity grade and fibrosis stage in hepatic biopsy of Japanese patients with HCV-GT1 and HCV-GT2 (27); while these associations were found in Caucasian patients with HCV-GT2/3/4 (28); or conversely, there is another study that did not find any association between rs8099917and liver disease among European patients (29). The *IL28B* SNP rs12979860 has also been associated with increased ALT levels and liver steatosis in Caucasian patients infected with HCV-GT3 (30) and HCV-GT1 (31, 32); but has not been found a clear association with liver necroinflammation and fibrosis (29, 31). Moreover, there is far less information in HIV/HCV coinfected patients. In the article of Bochud et al. (28), roughly 10% of the 1915 subjects were HIV positive in Swiss Cohort study, but no analysis was presented in the HIV positive sub-group; and Barreiro et al. (33) showed an association of rs12979860 AA genotype with increased ALT levels and cirrhosis assessed by transient elastography in Spanish HIV/HCV coinfected patients. In our study, we did not analysed rs12979860, but we studied other four *IL28B* SNPs, which have high linkage disequilibrium with rs12979860 in European population (23, 34, 35), and they have been less studied in Caucasian population.

Our data show an association between the major alleles at *IL28B* with significant fibrosis, more rapid fibrosis progression, and elevated ALT in HCV-GT3 patients, but not in those infected with HCV-GT1 or HCV-GT4. Our data partially coincide with those published by Bouchard in Caucasian patients (28), who reported an associations among rare G allele at rs8099917 —previously shown to be at risk of HCV treatment failure— with lower liver inflammation, significant fibrosis, and fibrosis progression rate in European patients with HCV-GT2/3/4. Since IL28B may induce expression of inflammatory cytokines (18), it seems reasonable that the inflammation is stronger in patients with elevated IL28B production. However, we did not find any significant association between moderate activity grade and the four *IL28B* SNPs studied. It is possible that these differences were not found because the number of patients with none or minimal activity grade (A0) was low (four with HCV-GT1 and one with HCV genotype 4). Indeed, there was an association, close to the statistical significance, between rs8099917 T allele and the moderate activity grade in HCV-GT3 patients (p=0.087). In addition, we observed that elevated ALT was more frequent among HCV-GT3 patients carrying the major alleles at *IL28B*. Although ALT levels do not always reflect the severity of liver disease, this parameter has been previously associated with liver inflammation and fibrosis progression (36).

Liver steatosis is a frequent finding in CHC and has been associated with failure of HCV treatment and advanced hepatic fibrosis (37, 38). In HCV infection with GT3, viral factors are implicated in the development of steatosis, while for HCV infection with GT1, metabolic host factors such as obesity and insulin resistance provide an increased risk of steatosis (39, 40). In our study, we only found HCV-GT1 patients carrying the minor G alleles at rs12980275 and rs8099917 had higher chance of liver steatosis, in agreement with a previous study in Japanese patients (41). This fact could have important clinical implications because persistent fatty liver disease may be a problem for HCV-GT1 patients even with successful HCV clearance (37, 38). Moreover, insulin resistance has been associated to *IL28B* SNPs in HCV monoinfected patients (42), but we did not find any association between them in our HIV/HCV coinfected patients (data not shown). This lack of association could be due to the interference of HIV infection and its antiretroviral treatments, which may cause significant metabolic alterations (43).

Moreover, a correlation between ISG expression and hepatic steatosis has been reported (44), and higher levels of ISG expression has been found in patients with unfavourable IL28B genotypes for HCV treatment (14, 15, 45). This association may therefore represent a non-specific association between intrahepatic inflammation and ISG induction, but this was not reflected by the interaction between ALT and *IL28B* SNPs for HCV-GT1 patients.

This study has several limitations, as having a cross-sectional with low number of patients. Furthermore, the patients selected for our study were patients who met a set of criteria for starting HCV treatment (eg, little alcohol abuse, high CD4 cell counts, controlled HIV replication, and good treatment adherence), and it is possible that this may have introduced a selection bias. Moreover, regarding to the statistical significance, there is a considerable controversy about adjusting the "p-value" after multiple tests on clinical-orientated studies (46, 47). It is important to have in mind that in our study there is a hypothesis supported by theory and previous reports in HCV-infected patients (27, 28, 30-32) and HIV/HCV coinfected patients (33). Therefore, we are not doing a random search of a meaningful result, and our results should not be affected by the fact of carrying out a high number of statistical tests. Moreover, since p-value is depending on the sample size, it may be possible that we did not find any significant adjusted p-value due to such a size-limited population. Thus only big effects would be detected in small populations. In addition to this, it has to be taken into account that the effect size of our study is low due to the fact that, complex human diseases are under the control of many genes that contribute each of them with modest individual effects (48).

These associations between *IL28B* polymorphisms and the outcome variables are not exactly similar. Despite these apparent discrepancies, our data show a link between major alleles of *IL28B* polymorphisms and increased odds of liver disease severity. In conclusion, the major alleles of *IL28B* polymorphisms, previously associated with both spontaneous and treatment-induced HCV clearance, are also associated with increased odds of liver disease severity in HIV patients infected with HCV-GT3. In contrast, HCV-GT1 patients carrying the major alleles of IL28B polymorphisms had lower odds for liver steatosis. These data might be useful to implement targeted therapeutic interventions in HIV/HCV coinfected patients at risk of rapid liver disease progression.

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Evaluation of hepatic biopsy: EA.
Analysis and interpretation of data: MGF, MAJS, MGA, AFR, and SR.
Drafting of the manuscript: MGF, MAJS, MGA, AFR, and SR.
Critical revision of the manuscript for important intellectual content: JB.
Statistical analysis: MGF and SR.
Administrative, technical, or material support: MAJS, MGA, and AFR.
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