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Type of manuscript: Article

Title: *IL28RA* polymorphism (rs10903035) is associated with insulin resistance in HIV/HCV-coinfected patients

Running head: *IL28RA* polymorphism and insulin resistance

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Abstract

Hepatitis C virus (HCV) infection is associated with insulin resistance (IR), although mechanisms leading to IR in these patients are not completely understood. The aim of this study was to evaluate the association of *interleukin 28B* (*IL28B)* and interleukin 28 receptor alpha (*IL28RA*) polymorphisms with IR among human immunodeficiency virus (HIV)/HCV coinfected patients. We carried out a cross-sectional study on 203 patients. *IL28B* (rs8099917) and *IL28RA (*rs10903035) polymorphisms were genotyped by GoldenGate® assay. IR was defined as homeostatic model assessment (HOMA) values ≥3.00. Univariate and multivariate Generalized Linear Models were used to compare HOMA values and the percentage of patients with IR according to *IL28B* and *IL28RA* genotypes. In total, 32% (n=65/203) of the patients had IR. *IL28B* rs8099917 TT was not significantly associated with HOMA values and IR. In contrast, rs10903035 AA was significantly associated with high HOMA values taking into account all patients (p=0.024), as well as the subgroups of patients with significant fibrosis (p=0.047) and infected with HCV genotype 3 (p=0.024). Additionally, rs10903035 AA was significantly associated with IR (HOMA ≥3.00) in all patients (adjusted odds ratio (aOR)=2.02; p=0.034), in patients with significant fibrosis (aOR=2.86; p=0.039), and HCV genotype 3 patients (aOR=4.89; p=0.031). **In conclusions,** *IL28RA* polymorphism (rs10903035) seems to be implicated in the glucose homeostasis because AA genotype increases the likelihood of IR, but this association was different depending on hepatic fibrosis and HCV genotype.

Key words: AIDS; hepatitis C; insulin resistance; liver fibrosis; SNPs

INTRODUCTION

Chronic hepatitis C (CHC) has become a major cause of morbidity and mortality in human immunodeficiency virus (HIV)/hepatitis C virus (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). In addition, HCV infection is associated with metabolic abnormalities. CHC alters lipid metabolism leading to steatosis, interacts with glucose metabolism promoting insulin resistance (IR) and diabetes and is associated with an increased risk of cardiovascular diseases (4-6). On the other hand, the use of combined antiretroviral therapy (cART) induces also significant metabolic disturbances such as dyslipidemia and insulin resistance (IR) (7), facilitating the emergence of comorbidities such as metabolic syndrome, type 2 diabetes, and cardiovascular disease (8, 9).

In CHC, IR is directly associated with steatosis and fibrosis progression, irrespective of the HCV genotype (10). Moreover, CHC patients with IR achieve lower rates of sustained virologic response (SVR) to combination therapy with pegylated interferon and ribavirin (10). However, the mechanisms by which CHC leads to IR are not fully understood. HCV through both direct and indirect pathways, affects the insulin signaling pathways, promoting IR at a cellular level by proteins such as suppressors of cytokine signaling (SOCS)-3 (10). This protein is encoded by *SOCS-3* gene, which is an interferon-stimulated gene (ISGs) induced during the immune response against viral infections. SOCS-3 negatively regulates Janus tyrosine kinase-signal transducers and activators of transcription (Jak-STAT) signaling, which enables the cell to rapidly initiate a transcriptional response to external stimulation. IFN and other molecules (such as interleukins, growth factors), through a cascade of tyrosine phosphorylation events, activate STAT transcription factors, which are mainly involved in immunity and hematopoiesis and induce the expression of genes involved in the antiviral response (11, 12). As negative regulator of Jak-STAT pathway, SOCS-3 makes the cell less responsive to IFN signaling (13, 14).

The *interleukin 28B* (*IL28B)* gene encodes for a type III interferon (IFN) cytokine that has potent antiviral activity in liver via innate immunity pathway (14-16), which induces HCV antiviral activity through ISGs (17). It is known that polymorphisms around the *IL28B* gene are strongly associated with HCV treatment outcomes in both HCV monoinfected and HIV/HCV coinfected patients (18). Patients carrying the so-called favorable *IL28B* genotypes (associated with better HCV treatment response) seem to have reduced hepatic expression levels of IL28B and ISGs at baseline, but are induced more strongly after administration of IFN-α treatment (13). Besides, accelerated liver disease also seems to be probably influenced by *IL28B* polymorphisms (19-25). On the other hand, IFN-λ3 induces its cellular activity through a heterodimer receptor (IFN-λ receptor) composed of interleukin 28 receptor alpha (*IL28RA*) and interleukin 10 receptor beta (*IL10RB*), which influence IFN type III signaling (26, 27). Polymorphisms in *IL28RA* have been scarcely studied so far but also seem to influence on response to HCV treatment in HIV/HCV coinfected patients (28).

Type 2 diabetes mellitus is a complex disease in which genetic and environmental factors interact to produce alterations in insulin action, leading to hyperglycemia. (29-31). Although results are still controversial (32, 33), it has been recently reported that IR seems to be more common in carriers of unfavorable *IL28B* genotype than in favorable *IL28B* genotype among HCV monoinfected patients (34, 35), which could partly explain the poor outcome of peginterferon/ribavirin therapy in HCV monoinfected patients. However, there is no data about HIV/HCV coinfected patients. For this reason, the aim of our study was to evaluate the association of *IL28B* and *IL28RA* polymorphisms with IR among non-diabetic 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. The study was conducted in accordance with the Declaration of Helsinki and it was approved by the Research Ethic Committee of the Instituto de Salud Carlos III (ISCIII).

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 cART or no need for cART. Patients with diabetes, active opportunistic infections or active drug addiction were excluded.

From our cohort of 361 HIV/HCV coinfected patients with liver biopsy data, 223 of them had DNA sample available for *IL28B* and *IL28RA* genotyping and only 203 patients had HOMA data available. All patients were European whites.

Clinical and laboratory data

The following information was obtained from medical records when liver biopsy was performed: age, gender, risk category, weight, height, 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 biochemical liver panel tests. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

Biochemistry panel was determined using a fully automated clinical chemistry analyzer (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): fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5 (36). IR was considered to be altered when the HOMA score was ≥3.0 (35).

Liver biopsies were performed on an outpatient basis following the recommendations of the Patient Care Committee of the American Gastroenterological Association (37). Liver fibrosis was estimated according to Metavir score (10). 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. We have considered that hepatic steatosis was clinically significant when fatty hepatocytes exceed 10% of parenchyma hepatic.

Genotyping

Genomic DNA was extracted from peripheral blood with Qiagen columns (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). One single nucleotide polymorphism (SNP) near the *IL28B* gene (rs8099917) and one SNP within the 3'UTR at *IL28RA* gene (rs10903035) were genotyped by the Spanish National Genotyping Center (CeGen; http://www.cegen.org/). Genotyping was performed by using GoldenGate® assay with VeraCode® Technology (Illumina Inc. San Diego, CA, USA).

Statistics

All statistical tests were performed with the 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 $p<0.05$.

For the genetic association study, univariate and multivariate Generalized Linear Models (GLM) were used to compare the HOMA values and the percentage patients with HOMA ≥3.0 according

to *IL28B* and *IL28RA* polymorphisms. Several genetic models were developed in order to choose the inheritance model that best fitted the data. Besides, the goodness of fit of GLM tests were evaluated by Akaike information criterion (AIC) value and Bayesian information criterion (BIC) (38). On the one hand, a GLM with a gamma distribution (log-link) was used to investigate the association between *IL28B* and *IL28RA* polymorphisms and HOMA values. This test gives the differences between groups and the arithmetic mean ratio (AMR) in HOMA values between groups. On the other hand, a GLM with binomial distribution (logit-link) was used to investigate the association of *IL28B* and *IL28RA* polymorphisms with IR (HOMA ≥3.0). This test gives the differences between groups and the odds ratio (OR) for IR. GLM tests were adjusted by the most important clinical and epidemiological characteristics. In each adjusted regression analysis, we included a SNP and the most significant clinical and epidemiological covariables (*backward* criterion with a p-value for exit of 0.20). The covariables used were gender, age, BMI, nadir CD4+ T-cells, undetectable HIV viral load (<50 copies/mL), time with cART, cART with protease inhibitor, HCV genotype (GT1/4 vs. GT2/3), HCV viral load ≥500,000 IU/ml, and significant fibrosis (F≥2).

RESULTS

Characteristics of patients

Our study included 203 non-diabetic HIV/HCV-coinfected patients, whose characteristics at the time of liver biopsy are shown in **Table 1**.

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

	All patients
No. HIV-1 patients	203
Gender (male)	149 (73.4%)
Age (years)	39.8 (37.5; 44.0)
Body mass index (BMI, kg/m2)	22.4 (20.9; 24.9)
Injection drug users	177 (87.2%)
CDC category C	55 (27.1%)
cART	
3 NRTI-based	18 (8.9%)
NRTI /NNRTI-based	102 (50.2%)
NRTI /IP-based	43 (21.2%)
NRTI/NNRTI/IP-based	3 (1.5%)
Time on cART (years)	4.42 (2.39; 6.39)
Metavir fibrosis stage	
No fibrosis (F0)	22 (10.8%)
Portal fibrosis (F1)	83 (40.9%)
Periportal fibrosis (F2)	55 (27.1%)
Advanced fibrosis (F≥3)	43 (21.2%)
HIV markers	
Nadir CD4+ T-cells	195 (83; 315)
CD4+ T-cells/uL	472 (336.0; 676.7)
HIV-RNA < 50 cp/mL	149 (73.4%)
HCV markers	
HCV genotype (n= 199)	
1	113 (55.7%)
2	4 (2%)
3	48 (23.6%)
4	34 (16.7%)
HCV RNA >500,000 UI/mL	149 (73.4%)
Biochemical parameters	
HOMA	2.09 (1.26; 3.64)
ALP (UI/L)	102 (75.7; 163.0)
AST (UI/L)	54 (36.0; 80.0)
GGT (UI/L)	96.5 (47.2; 212.0)
ALT (UI/L)	72 (45.0; 112.0)
Cholesterol	162 (125; 192)
Triglycerides	122 (112; 155)
HDL	36 (34; 52)
LDL	88 (65; 99)

Values are expressed as absolute number (percentage) and median (percentile 25; percentile 75).

Abbreviations: ALT: alanine aminotransferase. ALP: alkaline phosphatase. AST: aspartate aminotransferase. GGT: gamma glutamyl transpeptidase. 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; LDL: low density lipoprotein; HDL: high density lipoprotein.

Patients with null or mild fibrosis (F0/F1) had lower HOMA values than patients with significant fibrosis (F≥2) (median: 1.75 (Interquartile range (IQR): 1.86) and 2.54 (IQR: 2.98), respectively; p< 0.001). In total, 32% (n= 65/203) of the patients had a HOMA ≥3.0 and were considered to be insulin resistant. The percentage of IR was lower in patients with F0/F1 than in patients with significant fibrosis (F≥2) [22.9% (n= 24/105) and 41.8% (n= 41/98], respectively; p= 0.004). HOMA and IR were similar among patients stratified by HCV genotype, BMI ≥25 Kg/m^2, or steatosis (data not shown).

In order to investigate those clinical and epidemiological characteristics independently associated with HOMA values and IR, GLM with gamma and binomial distribution, respectively, was performed simultaneously including the following variables: TT rs8099917, AA rs10903035 , gender, age, BMI, nadir CD4+ T-cells, undetectable HIV viral load (<50 copies/mL), time with cART, cART with protease inhibitor, HCV genotype (GT1/4 vs. GT2/3), HCV viral load ≥500,000 IU/ml, and significant fibrosis (F≥2). We observed that AA genotype of rs10903035 (p= 0.024), nadir CD4+ T-cells (0.008), cART with protease inhibitor (p= 0.017) and significant fibrosis (F≥2) (p=0.001) remained independently associated with HOMA values. However, only AA genotype of rs10903035 (p=0.047) and significant fibrosis (F≥2) (p=0.05) were independently associated with IR (HOMA≥3.0).

Association of IL28B and IL28RA genotypes with insulin resistance

IL28B and IL28RA SNPs were in Hardy-Weinberg equilibrium (p>0.05), and fulfilled the minimum allele frequency (MAF) >0.05 for all samples and displayed less than 5% of missing values. The allelic and genotypic frequencies for rs8099917 (*IL28B*) were T (82.6%, major allele), G (17.4%, minor allele), TT (n=140; 69%), GT (n=55, 27.1%) and GG (n=8; 3.9%) and for rs10903035 (*IL28RA*) were A (68.3%, major allele), G (31.7%, minor allele), AA (n=95; 46.8%), AG (n=87; 42.9%) and GG (n=21; 10.3%). The statistical analysis was carried out according to a dominant genetic model, which was the model that best fit to our data.

Figure 1 shows HOMA values according to *IL28B* and *IL28RA* polymorphisms. In regards to *IL28B* polymorphisms, patients with favorable genotype of rs8099917 (TT) tended to have lower HOMA values than patients carrying rs8099917 GT/GG genotype, but this difference was not significant (p=0.201; **Figure 1A**). There were also no significant differences in the percentage of patients with IR (HOMA ≥3.00) according to rs8099917 genotype (p=0.215; **Figure 1B**). On the other hand, patients with rs10903035 AA genotype (*IL28RA*) had higher HOMA values than patients carrying rs10903035 AG/GG genotypes (p=0.010; **Figure 1C)**. Forty percent of the rs10903035 AA patients and 25% of the rs10903035 AG/GG patients had IR (HOMA ≥3.00) (p= 0.023) (**Figure 1D**).

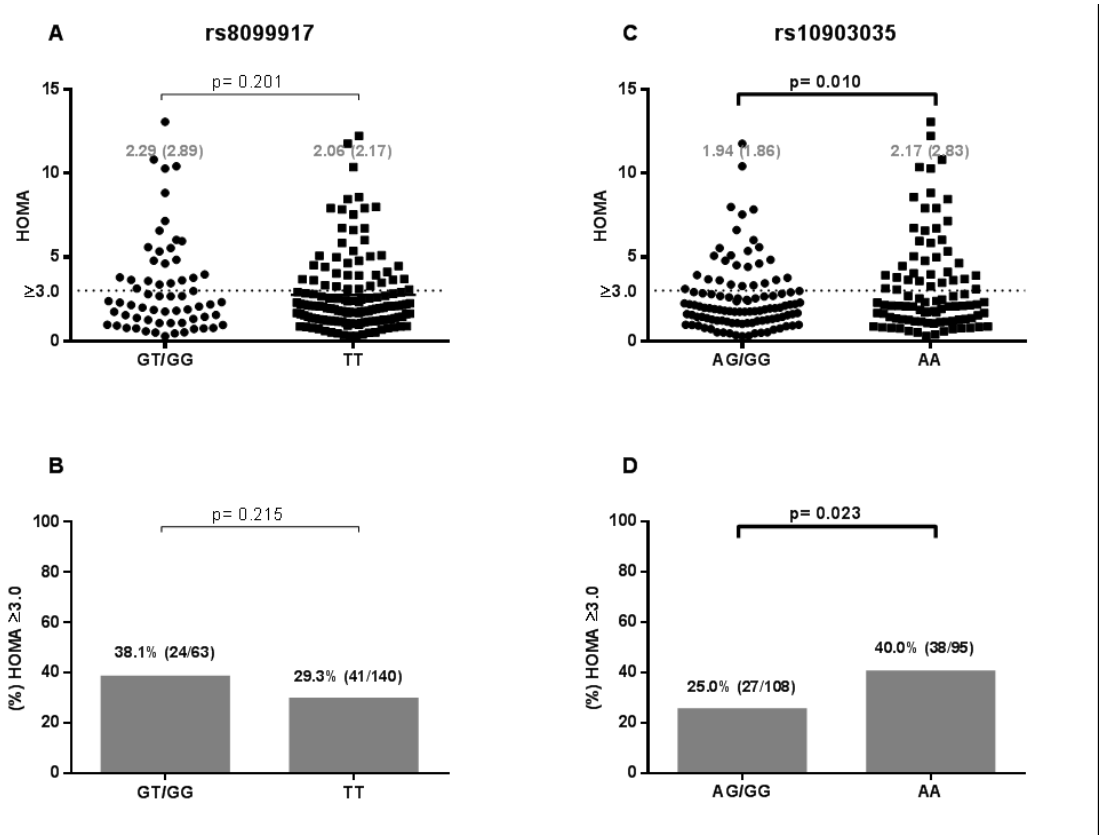

Figure 1. Distribution of HOMA values and HIV/HCV coinfected patients with insulin resistance (HOMA ≥3.00) according to *IL28B* and *IL28RA* polymorphisms. P-values were calculated by Generalized Linear Model (GLM) tests with a gamma distribution (HOMA values) and binomial distribution (HOMA ≥3.0). Data in grey indicate median (interquartile range).

When patients were stratified by fibrosis, no significant differences in HOMA values according to *IL28B* and *IL28RA* genotypes were found in patients with F0/F1 (**Figure 2**). In patients with F≥2, those with rs8099917 TT tended to have lower HOMA values than those carrying the G allele (p=0.090; **Figure 2A**); but this difference was not significant. However, 35.1% of the rs8099917 TT patients and 62.5% of the rs8099917 GT/GG patients with F≥2 had IR (p= 0.021; **Figure 2B**). On the other hand, rs10903035 AA patients with F≥2 had higher HOMA values than those carrying the G allele (p= 0.025; **Figure 2C**). In this setting, 53.2% of the rs10903035 AA patients had IR, while only 31.4% of the rs10903035 AG/GG patients had IR (p= 0.030; **Figure 2D**).

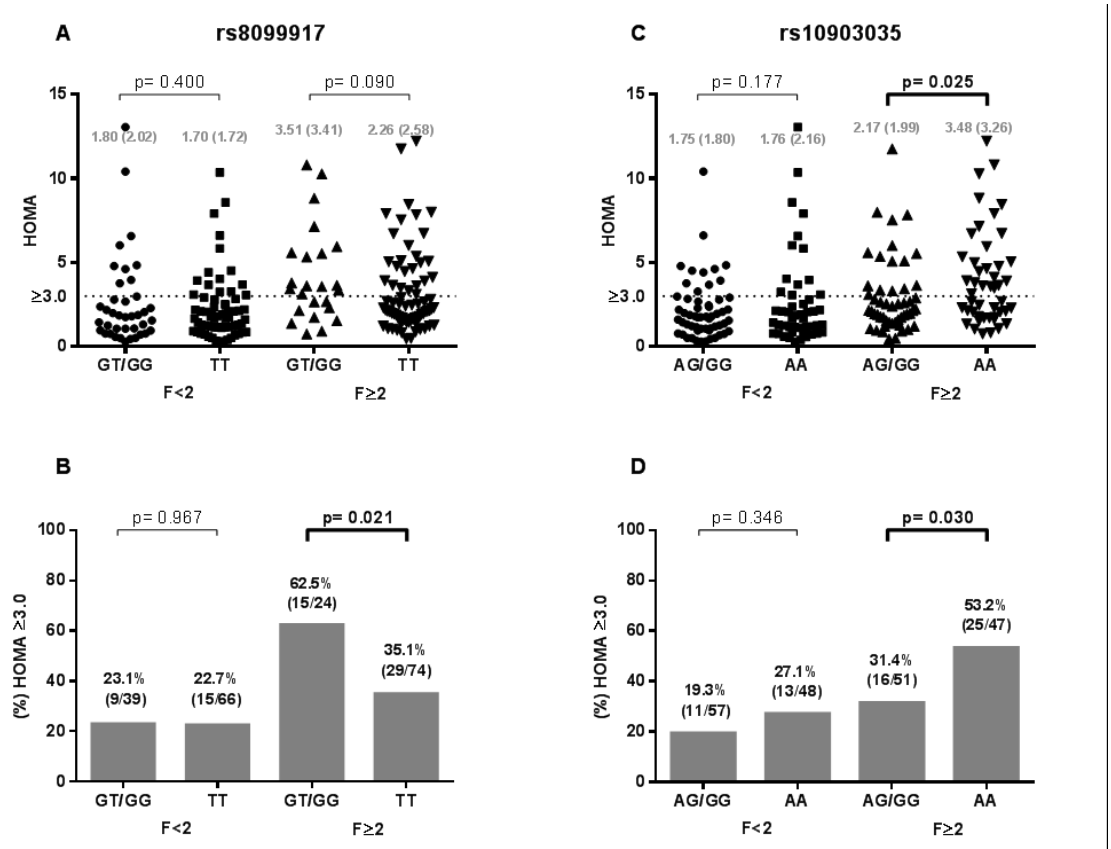

Figure 2. Distribution of HOMA values and HIV/HCV coinfected patients with insulin resistance (HOMA ≥3.00) according to *IL28B* and *IL28RA* polymorphisms and stratified by significant fibrosis. P-values were calculated by Generalized Linear Model (GLM) tests with a gamma distribution (HOMA values) and binomial distribution (HOMA ≥3.0). Data in grey indicate median (interquartile range).

When patients were stratified by HCV genotype, patients infected with GT1/4 showed no significant association of *IL28B* or *IL28RA* genotypes with HOMA values or IR (**Figure 3**). In regards to GT3 patients, rs8099917 TT carriers tended to have lower HOMA values and percentage of IR than those carrying the G allele, but differences were not significant (p= 0.131 (**Figure 3A**) and p= 0.053 (**Figure 3B**), respectively). In contrast, rs10903035 AA patients with GT3 had higher HOMA values than those carrying the G allele (p= 0.015; **Figure 3C**). In this setting, 45.5% of the rs10903035 AA patients had IR and only 15.4% of the rs10903035 AG/GG patients had IR (p= 0.022; **Figure 3D**).

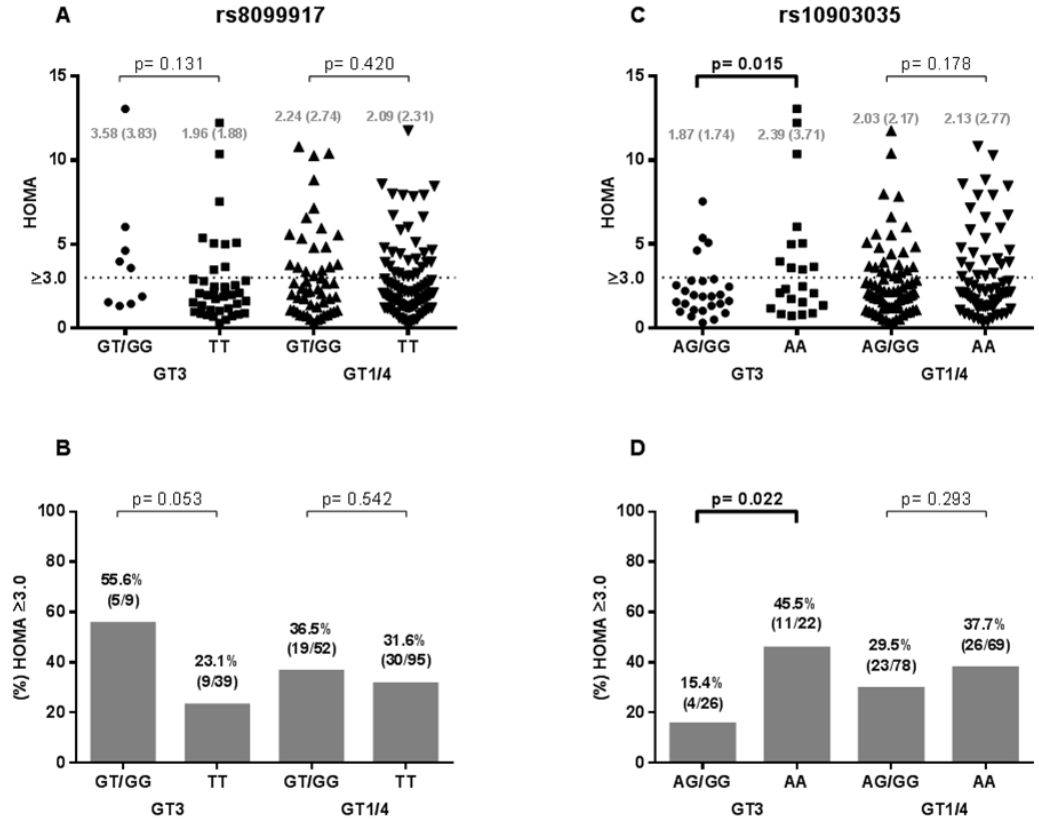

Figure 3. Distribution of HOMA values and HIV/HCV coinfected patients with insulin resistance (HOMA ≥3.00) according to *IL28B* and *IL28RA* polymorphisms and stratified by HCV genotypes. P-values were calculated by Generalized Linear Model (GLM) tests with a gamma distribution (HOMA values) and binomial distribution (HOMA ≥3.0). Data in grey indicate median (interquartile range).

Figure 4 shows the influence of *IL28B* and *IL28RA* polymorphisms on HOMA values and IR via adjusted GLM tests. *IL28B* rs8099917 TT was not significantly associated with HOMA values and IR. In contrast, rs10903035 AA was significantly associated with high HOMA values taking into account all patients (p= 0.024), as well as patients subgroups with F≥2 (p= 0.047) and infected with GT3 (p= 0.024) (**Figure 4A**). Additionally, rs10903035 AA was significantly associated with IR in all patients (p= 0.034), in patients with F≥2 (p= 0.039), and GT3 patients (p= 0.031) (**Figure 4B**).

We did not find any significant association of *IL28B* and *IL28RA* polymorphisms with BMI ≥25 Kg/m^2 and steatosis (data not shown).

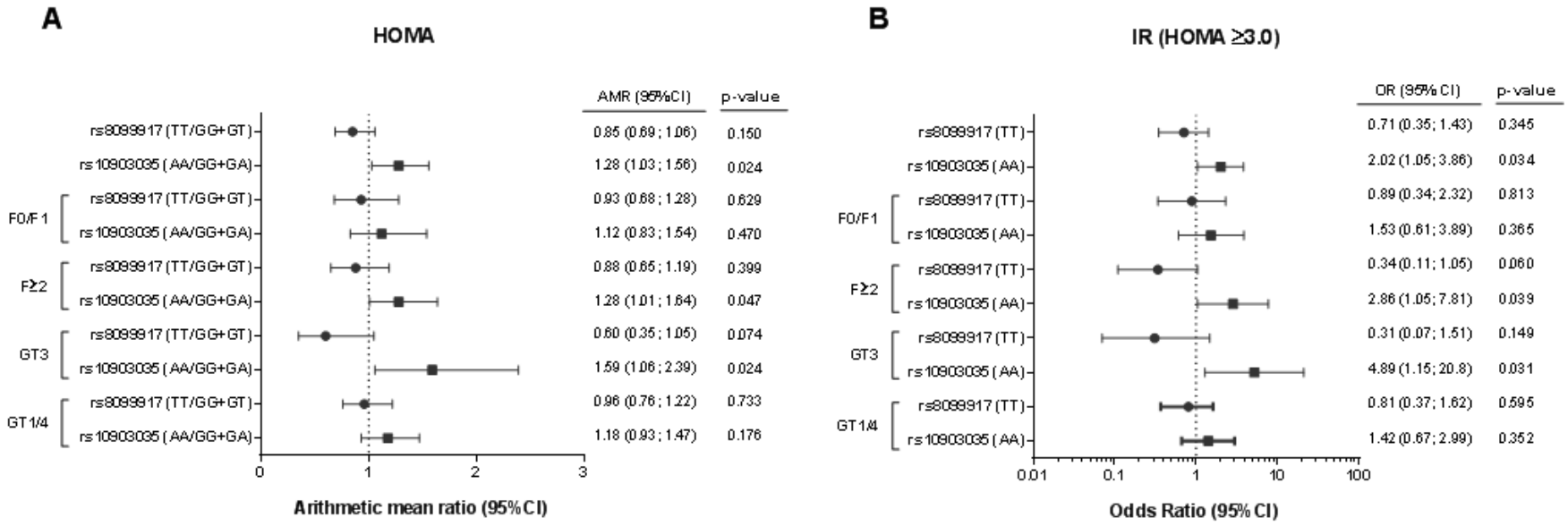

Figure 4. Influence of *IL28B* and *IL28RA* polymorphisms on HOMA values and insulin resistance (HOMA ≥3.0) in HIV/HCV coinfected patients. Data were calculated by Generalized Linear Model (GLM) tests with a gamma distribution (HOMA values) and binomial distribution (HOMA ≥3.0). GLM were adjusted by the most relevant epidemiological and clinical variables selected by *backward* criteria. In bold, p-values with statistical significance. Abbreviations: AMR, arithmetic mean ratio; OR, odds ratio; 95% CI, 95% of confidence interval, p, significant value.

DISCUSSION

In our cohort, we previously found an association of the polymorphism rs8099917 (*IL28B*) with SVR (39) and with fibrosis progression, steatosis, and elevated ALT (20). Moreover, unfavorable genotypes of *IL28B* (rs8099917) and *IL28RA* (rs10903035) polymorphisms were also associated with early failure to HCV antiviral therapy (28). In this current study, we found that rs8099917 genotype did not have any association with HOMA values; whilst rs10903035 AA carriers had significantly higher HOMA values and frequency of IR than AG/GG carriers. To our knowledge, this is the first report that has shown the association between *IL28RA* polymorphisms and IR among non-diabetic HCV/HIV coinfected patients.

Many studies have described a high prevalence of IR in patients with hepatitis C (40), although mechanisms leading to IR in these patients are not completely understood. There is growing evidence that DNA polymorphisms may contribute to differences in complex disease traits between individuals. In this setting, several studies have investigated the relationship between *IL28B* polymorphisms and IR in HCV monoinfected patients, with controversial results. Ciesla et al (33) and Del Campo et al (32) reported that rs12979860, a SNP located upstream from the *IL28B* gene, was not associated with IR in European patients. In contrast, Stättermayer et al (35) and Petta et al (34) have recently described that IR is less common in carriers of the favorable rs12979860 genotype. In our study, as rs12979860 had been previously analyzed in Caucasian population, we studied other *IL28B* polymorphism (rs8099917), which has been less studied. To our knowledge, in contrast to rs12979860, the association between rs8099917 and insulin resistance has only been studied in Asian population so far. In respect to the *IL28B* polymorphism analyzed in our study (rs8099917), Ogawa et al (41) recently reported no significant difference in *IL28B* genotype distribution according to HOMA values in HCV monoinfected patients. As frequencies of certain allelic variants and mutations vary among different ethnicities, we considered important to study the association between rs8099917 and IR in European population. Finally, we obtained results consistent with Ogawa et al., showing a lack of association between rs8099917 and HOMA values in European HCV/HIV coinfected patients. Therefore, rs8099917 does not seem to be implicated in the regulation of the glucose homeostasis both in HCV monoinfected and in HCV/HIV coinfected patients.

To date, few studies have investigated the role of *IL28RA* polymorphisms in HCV infection (28, 42). To the best of our knowledge, this study is the first evidence of association between *IL28RA* polymorphism and IR in HCV/HIV coinfected patients. However, the role of rs10903035 seems to be controversial. On the one hand, in our study, those patients carrying rs10903035 AA genotype had high HOMA values and a higher likelihood of IR than patients carrying AG/GG genotypes. On the other hand, in a recent published article, rs10903035 AA genotype was associated with high rate of early virological response (EVR) among HIV/HCV coinfected patients, although no significant association was found with sustained virological response (SVR) (28). This apparent paradox might indicate that there are different mechanisms by which rs10903035 could influence either the CHC evolution leading to IR and the response to HCV therapy. As rs10903035 is located in a regulatory region (3'UTR) within the *IL28RA* gene, this polymorphism could be exerting an effect on *IL28RA* expression and therefore, on the activation of the IFN signaling pathway. Signaling through the IFN-λ receptor (IL28RA/IL10RB) complex results predominantly in the activation of Jak-STAT (43), which have antiviral effect and could lead to a better response to HCV therapy (13); but IFN-λ through its receptor (IL28RA/IL10RB) can also up-regulate SOCS-3 expression (44), which is involved in the insulin signaling promoting IR (10) and besides, regulates negatively the Jak-STAT signaling (11). These effects could be responsible for the association of rs10903035 with EVR and the lack of association between this SNP and SVR (28).

The mechanism of action of rs10903035 polymorphism is unknown. One possibility is that rs10903035 polymorphism might affect microRNA binding to mRNA of *IL28RA*. By analyzing the *IL28RA* sequence via PATROCLES database (http://www.patrocles.org/) (45), we found that the A allele of rs10903035 generates two putative target sites (GGAGTGA and AGTGATT) for two microRNAs (hsa-miR-483-3p and hsa-miR-34b respectively). In contrast, the G allele generates a different putative target site for a different microRNA (GTGGTTT-hsa-miR-497-3p) and disrupts the target sites mentioned above. MicroRNAs are small RNA molecules that post-transcriptionally regulate the expression of an extensive number of protein-coding genes by silencing expression via mRNA degradation or by preventing mRNA translation (46). Therefore, rs10903035 could have a major role in regulating the *IL28RA* expression, and consequently altering the course of HCV infection, including the susceptibility to IR development. Functional studies would be needed in order to clarify the mechanisms involved in the susceptibility to IR in patients carrying AA genotype of rs10903035.

Insulin resistance and hepatic fibrosis are closely related in HIV/HCV co-infected patients (47, 48). Since approximately 50% of portal insulin is cleared by the liver during first-past transit (49), liver fibrosis could lead to impaired hepatic clearance of insulin and, consequently, it could affect HOMA values. When we evaluated the influence of *IL28B* and *IL28RA* polymorphisms on IR regarding hepatic fibrosis, none of *IL28B* and *IL28RA* polymorphisms were associated with HOMA values and IR in patients with no or mild fibrosis (F0/F1). However, in patients with F≥2, the rs10903035 AA genotype was associated with a higher likelihood of elevated HOMA values and IR than the unfavorable genotype (TG/GG). Thus, rs10903035 AA genotype could play an important role on alterations in insulin metabolism secondary to significant fibrosis.

Moreover, we also evaluated the influence of *IL28B* and *IL28RA* polymorphisms on IR, considering HCV genotype. Significant association between rs10903035 and IR was only found in patients with GT3. It is known that patients with GT3 show a clear trend towards faster fibrosis progression compared with patients with other HCV genotypes (50). In our study, the majority of patients with GT3 had a high rate of significant fibrosis versus patients with other genotypes (62.5% vs. 43.7%, respectively). Therefore, the association found between rs10903035 and IR in patients with GT3 could indirectly be a reflection of the association found in patients with significant fibrosis due to the high percentage of significant fibrosis among GT3 patients. These considerations should be taken into account for a correct interpretation of the data.

This study has several limitations that must be taken into account for the correct interpretation of the data. Our study has a low number of patients especially in some groups such as patients with HCV genotype 3. Since p-value is depending on the sample size, it may be possible that we did not find any significant adjusted p-value in some comparisons due to a size-limited population. 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 one of them with modest individual effects (51). Thus, only big effects would be detected in small populations. Moreover, 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. On the other hand, this study was carried out entirely in Europeans; therefore, as 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.

In summary, *IL28RA* polymorphism (rs10903035) seems to be implicated in the glucose homeostasis because AA genotype increases the likelihood of IR, but this association was

different depending on hepatic fibrosis and HCV genotype. These results could help to predict the risk of developing IR in non-diabetic HCV/HIV coinfected patients.

ACKNOWLEDGEMENTS, CONFLICTS OF INTEREST, AND FUNDING

Acknowledgements

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

Funding/Support

This work has been supported by grants given by Fondo de Investigacion de Sanidad en España (FIS) [Spanish Health Founds for Research] [grant numbers PI08/0738, PI11/00245; PI08/0928, and PI11/01556], Red Española de Investigación en SIDA (RIS) [AIDS Research Network] [grant numbers RD12/0017/0024 and RD12/0017/0004] and "Fundación para la Investigación y la Prevención del Sida en España" (FIPSE) [grant number 361020/10].

AFR, MGF, MGA, DPT and MAJS are supported by "Instituto de Salud Carlos III" [grant numbers PI11/00245, CM09/00031, CD12/00442, CM12/00043, and CM10/00105, respectively].

Competing interests

The authors have no conflicts of interest or funding to disclose.

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