rs7903146 Polymorphism at *Transcription Factor 7 Like 2* Gene Is Associated with Total Cholesterol and Lipoprotein Profile in HIV/Hepatitis C Virus-Coinfected Patients

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Abstract

Transcription factor 7 like 2 (TCF7L2) rs7903146 polymorphism has been associated with metabolic disturbance and cardiovascular disease. The aim of this study was to analyze the association between *TCF7L2* rs7903146 polymorphism and potential disturbances on the lipid profile in human immunodeficiency virus (HIV)/hepatitis C virus (HCV)-coinfected patients. We performed a cross-sectional study on 263 HIV/HCV-coinfected patients. *TCF7L2* polymorphism was genotyped by GoldenGate assay. The analysis was performed by linear and logistic regression under a dominant model of inheritance. The variables analyzed were total cholesterol (TC), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), non-HDL-C, and triglycerides. Patients harboring the rs7903146 TT/TC genotype showed a diminished concentration of TC ($p=0.003$), LDL-C ($p=0.004$), HDL-C ($p=0.012$), and non-HDL-C ($p=0.013$), a lower percentage of TC ≥ 200 mg/dl ($p=0.038$), and a higher percentage of HDL ≤ 40 mg/dl ($p=0.023$). In addition, we observed that rs7903146 was differently related to fasting serum lipid levels according to the HCV-genotype (HCV-GT). With regard to HCV-GT1 patients, the rs7903146 TT/TC genotype was associated with lower levels of HDL-C [adjusted arithmetic mean ratio (aAMR)=0.91; $p=0.049$] and an elevated percentage of patients with HDL-C ≤ 40 mg/dl [adjusted odds ratio (aOR)=3.26; $p=0.003$]. For HCV-GT3 patients, the rs7903146 TT/TC genotype was associated with lower serum values of TC (aAMR=0.81; $p=0.037$), LDL-C (aAMR=0.67; $p=0.001$), and non-HDL-C (aAMR=0.75; $p=0.002$) and a reduced percentage of TC ≥ 200 mg/dl (aOR=0.089; $p=0.037$). In conclusion, the *TCF7L2* rs7903146 TT/TC genotype was associated with lower levels of TC, LDL, and HDL in HCV-GT3 patients, and lower levels of HDL-C in HCV-GT1 patients, suggesting a role in cardiovascular disease and a potential use as a biomarker in HIV/HCV-coinfected patients.

Introduction

CHRONIC HEPATITIS C (CHC) has become a major cause of morbimortality in human immunodeficiency virus (HIV)/hepatitis C virus (HCV)-coinfected patients in high-income countries.¹ An increased risk of dyslipidemia, atherosclerosis, and cardiovascular diseases has been associated with HCV and HIV infections, and also with specific antiretroviral therapies.^{2–6} In this way, HCV infection has been related to steatosis and metabolic abnormalities such as insulin resistance, type 2 diabetes mellitus (T2DM), and dyslipidemia [reductions in total cholesterol (TC), high-density lipoprotein

cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)].^{7,8} The coinfection with HIV modifies the natural history of CHC, generating higher rates of liver steatosis, cirrhosis, and end-stage liver diseases than HCV-monoinfected patients.^{9,10} Although the published data suggest that combination antiretroviral therapy (cART) might be beneficial for HIV/HCV-coinfected patients,¹⁰ it has been linked to lipodystrophy, insulin resistance, type 2 diabetes mellitus, and lipid disturbances, including higher levels of TC, LDL-C, and triglyceride (TG), and decreased levels of HDL-C.^{11–14}

Dyslipidemia is a main risk factor for cardiovascular disease in patients with HIV infection, which is related to

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cART.^{15,16} Thus, the risk of myocardial infarction is proportional to the time of cART¹⁷ and it has also been demonstrated that it could be partly explained by the dyslipidemia caused by these drugs.¹⁸ In this way, the increased cardiovascular risk is associated particularly with prolonged use of protease inhibitors, which are usually associated with a less favorable lipid profile, in particular elevated TG and TC.¹⁵ Moreover, non-nucleoside reverse transcriptase inhibitors show, in general, the best lipid profile of all antiretrovirals because they are associated with an increase in HDL-C and a significant reduction in the TC/HDL-C ratio.¹⁵ Finally, the effects of nucleoside reverse transcriptase inhibitors on lipids are generally mild and there is a high degree of heterogeneity.¹¹

The mechanisms leading to lipid disturbances are not completely understood, but there is growing evidence that genetic background may contribute to individual differences in this pathology.^{19–21} The *transcription factor 7 like 2 (TCF7L2)* is located on chromosome 10q25.3. This gene is integrated by 18 exons that suffer alternative splicing regulation. *TCF7L2* is part of the Wnt/ β -catenin signaling pathway, which is involved in glucose homeostasis and lipid metabolism.²² Recently, the rs7903146 (C>T) single nucleotide polymorphism (SNP) located at the *TCF7L2* intron 5 region has been strongly associated with metabolic syndrome,²³ type 2 diabetes mellitus,²⁴ dyslipidemia,²⁵ and cardiovascular disease^{26,27} in the general population. However, to the best of our knowledge, the effect of *TCF7L2* polymorphism has not been studied in HIV/HCV-coinfected patients.

The aim of this study was to analyze the association between *TCF7L2* rs7903146 polymorphism and potential disturbances in the lipid profile in HIV/HCV-coinfected patients.

Materials and Methods

Study design and patients

We carried out a cross-sectional study on 263 nondiabetic HIV/HCV-coinfected patients from Hospital Gregorio Marañón (Madrid, Spain) between September 2000 and July 2009. All subjects included in our study were HCV treatment-naïve patients who were potential candidates for HCV therapy.

The inclusion criteria for the study were HIV/HCV-coinfected patients who were over 18 years and HCV treatment-naïve, detectable HCV-RNA by polymerase chain reaction, negative hepatitis B surface antigen, availability of a DNA sample, no clinical evidence of hepatic decompensation, no diabetes mellitus, 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.^{28,29} Patients with active opportunistic infections, pregnant women, active drug and/or alcohol addiction, and other concomitant diseases or conditions were excluded. Thus, 495 HIV/HCV-coinfected patients met the criteria described above; 293 of them had a DNA sample available for genotyping, but only 263 patients were included in our study (12 patients were excluded due to DNA genotyping errors and 18 due to the absence of relevant clinical data). All patients were of European descent.

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 Ethics Committee of the Instituto de Salud Carlos III (ISCIII) approved the study.

Epidemiological and clinical data

The main clinical and epidemiological data of 263 patients analyzed 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.³⁰

Biochemical data

The biochemistry panel was measured by using an auto-analyzer Hitachi 912 (Boehringer Mannheim, Germany) in fasting patients. We collected data on TC, HDL-C, and TG. LDL-C was calculated by Friedewald estimation [$\text{LDL-C} = \text{TC} - \text{HDL-C} - (\text{TG}/5)$].³¹ Non-HDL-C was calculated as TC minus HDL-C.³² The degree of insulin resistance was estimated for each patient by using the homeostatic model assessment (HOMA), according to the following formula³³: fasting plasma glucose (mmol/liter) $\times$ fasting serum insulin (mU/liter)/22.5.

Liver biopsy

Liver biopsies were performed on 211 out of 263 patients following the recommendations of the Patient Care Committee of the American Gastroenterological Association³⁴ as previously described.³⁵ Liver fibrosis and necroinflammatory activity were estimated according to the Metavir score as follows: F0, nonfibrosis; F1, mild fibrosis; F2, significant fibrosis; F3, advanced fibrosis; and F4, definite cirrhosis. Activity grade was scored as follows: A0, nonactivity; A1, mild activity; A2, moderate activity; A3, severe activity.³⁶

Genotyping of the *TCF7L2* polymorphism

Genomic DNA was extracted from peripheral blood with Qiagen columns (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). DNA samples were genotyped at the Spanish National Genotyping Center (CeGen; www.cegen.org/) for the rs7903146 (C>T) SNP at the *TCF7L2* gene using the GoldenGate assay with VeraCode Technology (Illumina Inc., San Diego, CA).

Statistical analysis

All statistical tests were performed with the Statistical Package for the Social Sciences (SPSS) 19.0 software (IBM Corp., Chicago, IL). All *p*-values were two-tailed and statistical significance was defined as *p* < 0.05.

For the description of the study population, *p*-values were estimated with nonparametric tests: the Mann-Whitney *U* test was used for continuous variables and the Chi-square test for categorical variables.

Hardy-Weinberg equilibrium (HWE) for the *TCF7L2* rs7903146 polymorphism was assessed by a Chi-square test, considering equilibrium when *p* > 0.05. The genetic association study was carried out under a dominant genetic model for the T allele (CT/TT vs. CC), which was the model that best fit our data. On the one hand, lineal regression analyses were used to investigate the association between rs7903146 and continuous outcome variables, which were log₁₀ transformed. This

TABLE 1. CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF ALL HIV/HCV-COINFECTED PATIENTS STRATIFIED BY *TCF7L2* GENOTYPE

Characteristics	All patients	<i>rs7903146</i>		p-value
		TT/TC	CC	
N	263 (100%)	152 (57.6%)	111 (42.1%)	—
Male	197 (74.9%)	117 (77%)	80 (72.1%)	0.365
Age, years	40.8 (37.9; 44.7)	41.2 (38.2; 44.7)	40.2 (37.5; 44.8)	0.280
BMI, kg/m ²	22.5 (20.9; 24.7)	22.3 (20.8; 24.5)	22.9 (21.5; 25.1)	0.142
BMI ≥ 25 kg/m ²	61 (23.3%)	33 (21.9%)	28 (25.9%)	0.527
HOMA	2.1 (1.3; 3.7)	2 (1.3; 3.8)	2.3 (1.3; 3.5)	0.215
HOMA ≥ 3	91 (34.6%)	51 (33.6%)	40 (36%)	0.676
Years since HCV infection	20.9 (14.6; 24.4)	20.7 (15.4; 25.1)	21.7 (17.5; 24.0)	0.904
Prior AIDS	77 (29.3%)	48 (31.6%)	29 (26.1%)	0.337
cART	222 (84.4%)	130 (85.5%)	92 (82.9%)	0.559
Time on cART, years	4.7 (2.9; 7.8)	4.9 (3; 8)	4.7 (2.5; 7.4)	0.235
Current cART protocols				
Any NRTIs + any PI	64 (24.3%)	37 (24.3%)	27 (24.3%)	0.997
Any NRTIs + PI + NNRTI	3 (1.1%)	2 (1.3%)	1 (0.9%)	0.754
Any NRTIs + any NNRTI	134 (51%)	82 (53.9%)	52 (46.8%)	0.255
Only NRTIs	20 (7.6%)	8 (5.3%)	12 (10.8%)	0.094
Antiretroviral drugs				
Zidovudine	72 (27.4%)	42 (27.6%)	30 (27%)	0.914
Stavudine	67 (25.5%)	38 (25.0%)	29 (26.1%)	0.836
Didanosine	43 (16.3%)	21 (13.8%)	22 (19.8%)	0.193
Tenofovir	72 (27.4%)	43 (28.3%)	29 (26.1%)	0.698
Abacavir	43 (16.3%)	25 (16.4%)	18 (16.2%)	0.960
Efavirenz	80 (30.4%)	48 (31.6%)	32 (28.8%)	0.632
Ritonavir (r)	16 (6.1%)	12 (7.9%)	4 (3.6%)	0.150
Lopinavir/r	32 (12.2%)	17 (11.2%)	15 (13.5%)	0.568
Saquinavir	3 (1.1%)	1 (0.7%)	2 (1.8%)	0.388
Fosamprenavir	6 (2.3%)	3 (2.0%)	3 (2.7%)	0.696
HIV markers				
Nadir CD4 ⁺ , cells/μl	208 (92.3; 314.8)	191.5 (93.8; 301.5)	228 (83; 349)	0.351
Nadir CD4 ⁺ < 200 cells/μl	128 (48.7%)	80 (52.6%)	48 (43.2%)	0.132
CD4 ⁺ T, cells/μl	462 (338; 667)	464.5 (323.3; 657.8)	461 (363; 684)	0.688
CD4 ⁺ ≥ 500 cells/μl	113 (43.1%)	67 (44.1%)	46 (41.8%)	0.715
HIV-RNA < 50 copies/ml	202 (77.1%)	112 (73.7%)	90 (81.8%)	0.122
HCV markers, n (%)				
HCV-GT 1	142 (55.7%)	83 (56.8%)	59 (54.6%)	0.665
HCV-GT 2	5 (2%)	4 (2.7%)	1 (0.9%)	0.299
HCV-GT 3	65 (25.5%)	38 (26%)	27 (24.8%)	0.820
HCV-GT 4	43 (16.8%)	21 (14.4%)	22 (20.2%)	0.221
HCV-RNA ≥ 500.000 IU/ml	189 (75.3%)	110 (75.9%)	79 (74.5%)	0.809
Metavir score				
Liver biopsy patients	211 (80.3%)	119 (93%)	92 (92%)	0.782
Significant fibrosis (F ≥ 2)	103 (48.8%)	52 (43.7%)	51 (55.4%)	0.092
Moderate activity (A ≥ 2)	108 (51.9%)	56 (47.5%)	52 (57.8%)	0.140

Categorical variables were expressed in absolute numbers (%). Continuous variables were expressed in median (percentile 25; percentile 75). AIDS, acquired immunodeficiency syndrome; BMI, body mass index; cART, combination antiretroviral therapy; HCV, hepatitis C virus; HCV-RNA, HCV plasma viral load; GT, genotype; HIV, human immunodeficiency virus; HIV-RNA, HIV plasma viral load; HOMA, homeostatic model assessment; IVDU, intravenous drug users; NNRTI, nonnucleoside analog reverse-transcriptase inhibitors; NRTI, nucleoside analog reverse-transcriptase inhibitors; PI, protease inhibitors.

test provides the differences between groups and the arithmetic mean ratio (AMR), which provides the increase of the value with the presence of the T allele.

On the other hand, logistic regression analyses were used to investigate the association of rs7903146 with categorical outcome variables. This test provides the association between groups through the odds ratio (OR), which indicates the likelihood of having the outcome in the CT/TT genotypes versus the CC genotype. Each regression analysis was always

adjusted by the most significant covariables associated with each one of the outcome variables, avoiding overfitting of the regression. The covariables were selected by the “Stepwise” algorithm (at each step, factors are considered for removal or entry: a *p*-value for entry and exit of 0.15 and 0.20, respectively), including gender, age, BMI, nadir CD4⁺ T cells, undetectable HIV viral load (< 50 copies/ml), time with cART, specific antiretroviral drugs used by each patient (zidovudine, stavudine, didanosine, tenofovir, abacavir, efavirenz, ritonavir,

lopinavir, saquinavir, and fosamprenavir), HCV viral load $\geq 500,000$ IU/ml, and significant fibrosis ($F \geq 2$).

We also included two polymorphisms (*ADIPOQ* rs2241766 and *SLC30A8* rs13266634), which have been related to dyslipidemia in HIV/HCV-coinfected patients, according to two recent studies^{20,21} performed by our group in the same cohort.

Results

Characteristics of patients

The study included 263 patients, whose epidemiological and demographic characteristics are shown in Table 1. There were no significant differences between rs7903146 genotypes (TT/TC versus CC).

Note that patients infected with HCV-GT3 had lower serum values of TC ($p=0.013$), LDL-C ($p=0.014$), and non-HDL-C ($p=0.026$) than patients infected with HCV-GT1 (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/aid). Furthermore, liver steatosis was found in a higher percentage in HCV-GT3 patients [70.6% (36/51)] than HCV-GT1 patients [52.7% (58/110); $p=0.032$] and HCV-GT4 patients [47.1% (16/34); $p=0.029$].

Frequencies of the TCF7L2 polymorphism

Allele frequencies for the *TCF7L2* rs7903146 polymorphism were 0.64 for the C allele and 0.36 for the T allele. Genotype frequencies for the rs7903146 polymorphism were

0.42 for the CC genotype, 0.43 for the TC genotype, and 0.15 for the TT genotype. These frequencies in our dataset were in accordance with data listed on the NCBI SNP database (www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=7903146). The rs7903146 SNP fulfilled the minimum allele frequency (MAF) >0.05 and displayed less than 5% of missing values. Furthermore, rs7903146 was in Hardy–Weinberg equilibrium ($p=0.396$).

Association of rs7903146 with lipids profile

Patients with the rs7903146 TT/TC genotype showed significantly lower TC serum levels ($p=0.003$; Fig. 1A), LDL-C ($p=0.004$; Fig. 1B), HDL-C ($p=0.012$; Fig. 1C), and non-HDL-C ($p=0.013$; Fig. 1D) than CC carriers. When we stratified the patients by HCV genotype, rs7903146 TT/TC carriers infected with HCV-GT3 had diminished values of TC ($p=0.019$; Fig. 1A), LDL-C ($p=0.013$; Fig. 1B), and non-HDL-C ($p=0.020$; Fig. 1D).

Patients with the rs7903146 TT/TC genotype had a diminished percentage of TC ≥ 200 mg/dl ($p=0.038$; Fig. 2A) and non-HDL-C ≥ 130 mg/dl ($p=0.041$; Fig. 2D) and an elevated percentage of HDL-C ≤ 40 mg/dl ($p=0.028$; Fig. 2C). When patients were stratified by HCV genotype, rs7903146 TT/TC carriers infected with HCV-GT3 had a lower percentage of TC ≥ 200 mg/dl ($p=0.001$; Fig. 2A), whereas rs7903146 TT/TC carriers infected with HCV-GT1 had a higher percentage of HDL-C ≤ 40 mg/dl ($p=0.018$; Fig. 2C).

FIG. 1. Summary of the unadjusted association between *TCF7L2* rs7903146 and the concentration of serum lipid profile among HIV/HCV coinfection (A–D). Variables are expressed in median (interquartile range). p -values were calculated by univariate linear regressions. Statistically significant differences are shown in bold. GT, genotype; HCV, hepatitis C virus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

FIG. 2. Summary of the unadjusted association between *TCF7L2* rs7903146 and the NCEP ATP-III characteristics among HIV/HCV coinfection (A–D). Variables are expressed in percentages. *p*-values were calculated by univariate logistic regressions. Statistically significant differences are shown in **bold**. GT, genotype; HCV, hepatitis C virus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

When adjusted regression was performed (Table 2), rs7903146 TT/TC carriers infected with HCV-GT3 were associated with lower values of TC [adjusted AMR (aAMR)=0.81; $p=0.003$], LDL-C (aAMR=0.67; $p=0.001$), and non-HDL-C (aAMR=0.75; $p=0.002$), and a percentage of TC ≥ 200 mg/dl [adjusted OR (aOR)=0.89; $p=0.037$]. An association between rs7903146 TT/TC genotype and diminished levels of HDL-C (aAMR=0.91; $p=0.049$) and an elevated percentage of HDL-C ≤ 40 mg/dl (aOR)=3.26; $p=0.003$) was found in HCV-GT1 patients.

Discussion

In the present study, we evaluated for the first time the impact of the *TCF7L2* rs7903146 polymorphism on the serum lipid profile in HIV/HCV-coinfected patients. The major findings were (1) the rs7903146 TT/TC genotype (T allele) was related to lower fasting serum levels of TC, LDL-C, and non-HDL-C in HIV patients coinfecting with HCV-GT3 and (2) the rs7903146 TT/TC genotype was linked to diminished levels of HDL-C and an elevated percentage of patients with HDL-C ≤ 40 mg/dl among HIV patients coinfecting with HCV-GT1.

Several reports have analyzed the association of the *TCF7L2* polymorphism with serum lipid disturbances in the non-HIV/HCV-coinfected population, providing discordant results. Thus, the presence of the rs7903146 T allele disrupted the lipid metabolism, inducing low levels of HDL-C and apolipoprotein (Apo)-A1 in healthy young men,²⁵ high levels

of TG in familial combined hyperlipidemia patients,³⁷ and an atherogenic lipoprotein profile in nonalcoholic fatty liver disease.³⁸ Moreover, the rs7903146 T allele has been linked to a greater severity of coronary artery disease, a higher death ratio by cardiovascular event, and increased cardiovascular risk in nondiabetic individuals.³⁹ In contrast, it has been described that rs7903146 T carriers show a favorable lipid profile with lower TG and higher HDL-C concentration.⁴⁰ However, other studies did not find a significant association between rs7903146 and lipid metabolism.^{41,42} These inconsistencies could be due to different study designs (transversal, retrospective, cohorts, etc.), patient characteristics (age, race, etc.), environmental exposure (mainly diet), or underlying comorbidities (polycystic ovary syndrome, metabolic syndrome, type 2 diabetes mellitus, etc.).

The cardiovascular risk and atherosclerosis associated with CHC infection have been previously reported. For instance, HCV infection has been related to carotid atherosclerosis, coronary artery stenosis, and increased carotid intima-media thickness.⁴³ According to the National Cholesterol Education Program Adult Treatment Plan Guideline III (NCEP ATP-III), the evaluation of total cholesterol, triglycerides, lipoproteins, and several risk factors is useful to assess the person's risk status for CHD.⁴⁴ Elevated LDL-C is considered as a major risk factor. For this reason, cholesterol-lowering therapy is a primary goal. Treatment of patients with CHD or equivalent risks should aim at reaching an LDL < 100 mg/dl.⁴⁴ Nevertheless, the adequate interpretation of our data in the context of

TABLE 2. SUMMARY OF ADJUSTED ASSOCIATION BETWEEN RS7903146 TT/TC GENOTYPE AND LIPID PROFILE STRATIFIED BY HEPATITIS C VIRUS GENOTYPE IN HIV/HEPATITIS C VIRUS COINFECTED PATIENTS

Concentration ^a	All patients			HCV-GT1			HCV-GT3			HCV-GT4		
	AMR (95% CI)	p-value		AMR (95% CI)	p-value		AMR (95% CI)	p-value		AMR (95% CI)	p-value	
TC, mg/dl	0.91 (0.84; 0.97)	0.006		0.93 (0.85; 1.02)	0.133		0.81 (0.70; 0.93)	0.003		0.91 (0.75; 1.01)	0.325	
LDL-C, mg/dl	0.88 (0.79; 0.98)	0.016		0.93 (0.81; 1.06)	0.263		0.67 (0.54; 0.84)	0.001		0.85 (0.66; 1.11)	0.227	
HDL-C, mg/dl	0.91 (0.84; 0.98)	0.012		0.91 (0.82; 1.00)	0.049		0.89 (0.80; 1.00)	0.052		0.89 (0.76; 1.03)	0.107	
Non-HDL-C, mg/dl	0.90 (0.82; 0.98)	0.016		0.94 (0.84; 1.05)	0.255		0.75 (0.63; 0.90)	0.002		0.91 (0.71; 1.17)	0.460	
TG, mg/dl	0.99 (0.91; 1.10)	0.925		1.00 (0.89; 1.13)	0.970		1.12 (0.96; 1.30)	0.147		0.89 (0.70; 1.11)	0.291	
ATP III classification ^b	OR (95% CI)	p-value		OR (95% CI)	p-value		OR (95% CI)	p-value		OR (95% CI)	p-value	
TC ≥ 200 mg/dl	0.49 (0.21; 1.09)	0.079		1.01 (0.34; 2.99)	0.983		0.89 (0.09–0.86)	0.037		0.47 (0.06; 3.38)	0.450	
LDL-C ≥ 100 mg/dl	0.77 (0.44; 1.37)	0.374		0.86 (0.40; 1.88)	0.707		0.77 (0.21; 2.83)	0.690		1.72 (0.39; 7.65)	0.475	
HDL-C ≤ 40 mg/dl	1.96 (1.15–3.36)	0.014		3.26 (1.49–7.16)	0.003		1.72 (0.53–5.55)	0.364		2.05 (0.41–10.12)	0.380	
Non-HDL-C ≥ 130 mg/dl	0.61 (0.35–1.07)	0.087		0.71 (0.34–1.47)	0.360		0.62 (0.17–2.21)	0.464		0.61 (0.15–2.45)	0.487	
TG ≥ 150 mg/dl	0.59 (0.33–1.04)	0.067		0.72 (0.33–1.55)	0.399		0.27 (0.06–1.23)	0.091		0.16 (0.02–1.30)	0.087	

^aMultivariate linear regression was performed to compare the serum lipid values, which were log₁₀-transformed.

^bLogistic regression was performed to compare categorical variables. Both tests were adjusted by significant epidemiological and clinical factors (age, gender, BMI, nadir CD4⁺, CD4⁺ T cells/ μ l, HIV plasma viral load, HCV plasma viral load, time on cART, specific antiretroviral drugs, and related SNPs). Statistically significant differences are shown in *bold*.

AMR, arithmetic mean ratio (how many times greater is the value in the presence of the TT/TC genotype versus CC genotype); OR, odds ratio (likelihood of having the outcome in the CT/TT genotype versus CC genotype); CI, confidence interval; GT, genotype; HCV, hepatitis C virus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

cardiovascular risk in coinfecting patients is complicated, due to interactions between HIV, HCV, and cART. For instance, (1) HIV infection is strongly related to lipid disturbances in cART-naïve patients, including reduced levels of TC, HDL-C, and LDL-C, and elevated levels of TG⁴⁵; (2) cART induces increased levels of TC, LDL-C, and TG¹³; and (3) HCV replication depends on the lipid metabolism of the host cells, which generates an increased lipid cellular content, generates alterations in membrane structures, and induces dyslipidemia, mainly represented by low circulating levels of TC, LDL-C,^{2,46} and HDL-C.⁴⁶ For these reasons, we consider that the multivariate regression with continuous values of TC and lipoproteins could be a better indicator than thresholds to evaluate the impact of TCF7L2 polymorphism on relative cardiovascular risk in HIV/HCV-coinfecting patients.

It has previously been indicated that patients with CHC have a lipid profile similar to that of patients with familiar hypobetalipoproteinemia. These diminished levels on beta-lipoproteins are more commonly present in HCV-GT3 infection than HCV-GT1.⁴⁷ These facts are in agreement with our results, which reveal a key role of the rs7903146 polymorphism on beta-lipoprotein metabolism in patients infected with HCV-GT3. We observed that the presence of a minor T allele at the rs7903146 polymorphism was associated with a favorable cardiovascular profile represented by diminished serum levels of TC, non-HDL-C, and LDL-C among HCV-GT3 patients. In contrast, the rs7903146 T allele was correlated with lower levels of HDL-C and an elevated percentage of patients with T allele showed HDL-C ≤ 40 mg/dl among the HCV-GT1 patients, which is considered an independent risk factor by NCEP ATP-III.⁴⁴

Considering that the presence of the rs7903146 T allele was related to dyslipidemia, the genotyping of the TCF7L2 rs7903146 polymorphism could help to identify individuals with lipid disturbances, opening the possibility of implementing early treatment for these patients. However, the analysis of the rs7903146 T allele for the prediction of dyslipidemia according to NCEP ATP-III thresholds revealed a low diagnostic performance [area under the receiver operating characteristic curves (AUC-ROCs) < 0.6 (data not shown)]. Thus, the utility of the rs7903146 polymorphism for the prediction of dyslipidemia is limited according to our data, even though further extensive clinical research studies will be needed to clarify the impact of this SNP on cardiovascular disease in HIV/HCV-coinfecting patients.

Many studies have described the interaction of HIV, HCV infection, and antiretroviral therapy to increase the risk of insulin resistance and T2DM,^{48,49} although the mechanisms are not completely understood. Moreover, there is growing evidence that genetic background may contribute to differences between individuals in complex diseases.^{19,50} A strong association between T2DM risk and TCF7L2 SNPs was reported by Grant *et al.*⁵¹ Next, genetic variations in TCF7L2 (particularly rs7903146) were related to T2DM⁵² and glucose metabolism.^{53,54} Later, the association between a minor T allele of rs7903146 and T2DM was replicated in multiple different populations, as shown in an independent meta-analysis by Cauchi *et al.*,⁵⁵ Luo *et al.*,⁵⁶ Tong *et al.*,⁵⁷ and Peng *et al.*²⁴ Furthermore, the T allele has been associated with an increased proinsulin/insulin ratio, impaired beta cell function, and hyperglycemia.^{54,58–60} The participants of the present study were nondiabetic HIV/HCV patients, but 34.6% showed insulin resistance (HOMA ≥ 3.0). Never-

theless, an exhaustive analysis showed that the rs7903146 polymorphism was not related to HOMA values in our HIV/HCV-coinfecting population (data not shown). This lack of association with glucose metabolism could be due to the limited number of patients used in the stratified analysis, or also to the possible distortive effect of direct and indirect factors related to both HIV and HCV infections and cART.⁴⁸ Moreover, we must not discount the fact that our patients had a relatively low BMI (median: 22.5 kg/m²).

The present study has several limitations that must be taken into account for the correct interpretation of the data: (1) this is a cross-sectional study with a limited number of patients, which could limit achieving statistical significance; (2) lipid disturbances may be caused by several interacting genetic and environmental determinants, making it difficult to find the true individual effects of each disease-associated factor; (3) our study must also be performed in monoinfected and untreated patients in order to evaluate the effect of chronic hepatitis C, HIV infection, and cART separately; (4) our study was carried out entirely in whites; therefore as allele frequency differs among ethnicities,²⁴ it would be necessary to perform an independent replication of this study in different ethnicities.

Conclusions

The TCF7L2 rs7903146 TT/TC genotype was associated with lower levels of TC, LDL, and HDL in HCV-GT3 patients and lower levels of HDL-C in HCV-GT1 patients, thus suggesting its role in cardiovascular disease and its potential use as a biomarker in HIV/HCV-coinfecting patients. Nevertheless, we consider that further analyses on a large population are needed to conclusively corroborate these results.

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Author Disclosure Statement

No competing financial interests exist.

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