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Title: Association of adiponectin (*ADIPOQ*) rs2241766 polymorphism and dyslipidemia in HIV/HCV coinfected patients

Short title: *ADIPOQ* polymorphism and dyslipidemia

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ABSTRACT

Background: The adiponectin (*ADIPOQ)* rs2241766 polymorphism is related to metabolic abnormalities. The aim of this study was to evaluate the association of the *ADIPOQ* rs2241766 polymorphism with serum dyslipidemia and insulin resistance (IR) in human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfected patients.

Methods: We carried out a cross-sectional study on 262 patients. *ADIPOQ* rs2241766 polymorphisms were genotyped by GoldenGate® assay. Generalized Linear Models (GLM) were used to compare continuous outcome variables (total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), non-HDL-C, and homeostatic model assessment (HOMA)); and categorical outcome variables (TC≥200 mg/dL, TG≥170 mg/dL, LDL-C≥100 mg/dL, HDL-C≤35 mg/dL, non-HDL-C≥120 mg/dL, and HOMA≥3.8) according to *ADIPOQ* genotype under a dominant inheritance model.

Results: Patients with the rs2241766 GG/GT genotype had significantly lower serum TC levels (p=0.038) and percentages of TC≥200 mg/dL (p=0.022) than rs2241766 TT carriers. When adjusted GLM was performed, rs2241766 GG/GT was associated with low serum TC levels (Arithmetic mean ratio (AMR)=0.92 ((95%CI=0.85; 0.99) p=0.024) and low likelihood of TC≥200 mg/dL (Odds ratio (OR)=0.32 ((95%CI=0.11; 0.88) p=0.027). When stratifying by steatosis, no significant values were found for patients without steatosis. However, for patients with steatosis, rs2241766 GG/GT genotypes were related to low TC serum values of TC (AMR= 0.89; p= 0.027), LDL-C (AMR= 0.85; p= 0.039), and non-HDL-C (AMR= 0.86; p= 0.015). No significant associations were found between rs2241766 and HOMA values.

Conclusions: The presence of the *ADIPOQ* rs2241766 G allele (GG/GT genotype) was associated with a protective effect against dyslipidemia, primarily in HIV/HCV coinfected patients with steatosis.

Key words: Adiponectin; chronic hepatitis C; AIDS; SNPs; metabolic disturbance; dyslipidemia.

BACKGROUND

In human immunodeficiency virus (HIV) infected patients, combined antiretroviral therapy (cART) has decreased the rates of morbidity and mortality . However, cART has been linked to metabolic disturbances, such as insulin resistance (IR) and dyslipidemia (reductions in total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)) [1], which facilitate the possible emergence of other previously hidden comorbidities such as metabolic syndrome, type 2 diabetes mellitus (T2DM) and cardiovascular disease [2, 3]. Moreover, hepatitis C virus (HCV) infection has been associated with steatosis and metabolic abnormalities such as IR, T2DM, and dyslipidemia in HCV monoinfected patients [4]. These metabolic disturbances have also been associated with an increased risk of cardiovascular disease [4-6].

Chronic hepatitis C (CHC) has become a major cause of morbidity and mortality in HIV/HCV coinfected patients [7, 8]. In fact, HIV/HCV coinfection in the cART era has been associated with a significantly increased risk of cardiovascular disease among HIV-infected patients [9]. Moreover, the presence of dyslipidemia in these patients is not well established because HCV coinfection appears to reduce the lipid build-up associated with cART during HIV infection [10]. In any case, HIV/HCV coinfection appears to exacerbate metabolic disturbance and induce prothrombotic changes in patients [10].

Adipose tissue has been recognized as an active secreting organ that releases a variety of proteins, collectively named adipokines, which may be directly involved in metabolic fluxes to the amount of stored energy [11]. The most abundant adipokine is adiponectin (ADIPOQ), which is a protein that plays a critical role in lipid metabolism and glucose homeostasis [12, 13]. The activity of adiponectin in glucose and lipid metabolism confers protection against obesity and metabolic syndrome traits [14]. In CHC, ADIPOQ exhibits a hepatoprotective and antifibrogenic effect in cases of liver injury, as well as protecting against liver steatosis [15, 16]. Thus, ADIPOQ attenuates inflammatory activity [15], improves insulin sensitivity [17], counteracts ectopic fat deposition and visceral obesity [18], and has a protective effect against hepatocellular carcinoma [19].

The adiponectin gene (ADIPOQ) is located on chromosome 3q27, spanning a 16 kb region, and has more than 160 genetic variants [20]. Several single nucleotide polymorphisms (SNPs) of ADIPOQ have been associated with metabolic disturbance and cardiovascular disease [21-23]. The rs2241766 (+45T>G) mutation is one of the most studied, but contradictory results have been published about the association of the ADIPOQ rs2241766 polymorphism with dyslipidemia and IR in the general population [22, 23].

The aim of our study was to evaluate the association of the ADIPOQ rs2241766 polymorphism with the lipid profiles [TC, HDL-C, LDL-C, non-HDL-C, and triglycerides (TG)] and IR in white European HIV/HCV coinfected patients.

PATIENTS AND METHODS

Study design

We carried out a cross-sectional study on 262 non-diabetic HIV/HCV coinfected patients at Hospital General Universitario Gregorio Marañón (Madrid, Spain) between September 2000 and July 2009. The study was approved by the Institutional Review Board and the Research Ethic Committee of the Instituto de Salud Carlos III (ISCIII). This study was conducted in accordance with the Declaration of Helsinki and patients gave their written consent. Study reporting conforms to the STROBE statement along with references to STROBE and the broader EQUATOR guidelines [24].

All subjects included in our study were HCV treatment-naive patients who were potential candidates for HCV therapy and, in most cases, underwent a liver biopsy. The Inclusion criteria for the study were: 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 successful cART or no need for cART. Patients with active opportunistic infections, active drug and/or alcohol addiction, and other concomitant diseases or conditions, such as nephropathies, autoimmune diseases, hemochromatosis, primary biliary cirrhosis, Wilson's disease, a1-antitrypsin deficiency and neoplasia, were excluded.

Of the 495 HIV/HCV coinfected patients who met the criteria described above, 293 had a DNA sample available for genotyping. Additionally, 13 patients were excluded due to genotyping problems, and 18 had to be excluded because of no homeostatic model assessment (HOMA) or lipid data. The final study sample consisted of 262 HIV/HCV coinfected patients, 212 of which had a liver biopsy performed. All patients were European whites.

Clinical and laboratory data

The following information was obtained from medical records when HCV therapy was started and/or liver biopsy was performed: age, gender, HIV transmission category, weight, height, nadir CD4+ T cell count, antiretroviral therapy, HCV genotype, CD4+ T-cell count, plasma HIV viral load (HIV-RNA), plasma HCV viral load (HCV-RNA), 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.

The biochemistry panel was measured using an autoanalyzer Hitachi 912 (Boehringer Mannheim, Germany), while patients were fasting. We collected data of TC, HDL-C, and TG. The LDL-C was calculated by Friedewald estimation (LDL-C = TC - HDL-C - (TG/5)) [25], and non-HDL-C was calculated as TC minus HDL-C [26].

The degree of IR was estimated for each patient using HOMA described by Matthews et al [27]: fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5.

Liver biopsy

Liver biopsies were performed on an outpatient basis following the recommendations of the Patient Care Committee of the American Gastroenterological Association [28]. Liver fibrosis was estimated according to METAVIR score [29]. 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. 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.

Genotyping of *ADIPOQ* polymorphisms

Genomic DNA was extracted from peripheral blood with Qiagen columns (QIAamp DNA Blood Midi/Maxi; Qiagen, Hilden, Germany). A SNP within the *ADIPOQ* gene (rs2241766 (+45T>G)) was genotyped at the Spanish National Genotyping Center (CeGen). Genotyping was performed by using GoldenGate® assay with VeraCode® Technology (Illumina Inc. San Diego, CA, USA).

Outcome variables

Lipid disturbance: a) serum concentration of TC, LDL-C, HDL-C, non-HDL-C, and TG; b) thresholds of lipid profile: TC ≥200 mg/dL, LDL-C ≥100 mg/dL, HDL-C <35 mg/dL, non-HDL-C ≥120 mg/dL,and TG ≥170 mg/dL [26, 30].

Insulin resistance: a) HOMA continuous values; b) HOMA ≥3.8 [29].

Statistics

All statistical tests were performed with the Statistical Package for the Social Sciences (SPSS) 19.0 software (IBM Corp., Chicago, USA). Graphics were generated by GraphPad PRISM 6.01 (GraphPad Software INC, La Jolla, USA). All p-values were two-tailed and statistical significance was defined as $p<0.05$. Continuous variables were expressed as median (interquartile range) and categorical variables as percentage (absolute frequency).

For the description of the study population, p-values were estimated with a nonparametric test: Mann-Whitney U test was used for continuous variables and Chi-square for categorical variables. Hardy-Weinberg equilibrium (HWE) was assessed by a Chi-square test, considering equilibrium when $p>0.05$.

For the genetic association study, univariate and multivariate Generalized Linear Models (GLM) were used to compare the outcome variables (lipid profile and IR) according to ADIPOQ polymorphism. We analyzed the data according to several models, including dominant, recessive and additive. The analysis was carried out according to a dominant genetic model for G allele (GG/GT versus TT), which was the model that best fit our data according to the statistical power to detect significant associations.

On the one hand, a GLM with a gamma distribution (log-link) was used to investigate the association between *ADIPOQ* polymorphisms and continuous outcome variables. This test gives the differences between groups and the arithmetic mean ratio (AMR) in continuous outcome variables between groups. On the other hand, a GLM with binomial distribution (logit-link) was used to investigate the association of ADIPOQ polymorphisms with categorical outcome variables. This test gives the differences between groups and the odds ratio (OR) for categorical outcome variables. 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 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 on cART, cART with protease inhibitor, specific antiretroviral drugs (saquinavir, efavirenz, ritonavir, etc.), HCV genotype (GT1/4 vs. GT2/3), and HCV viral load ≥500,000 IU/ml. The goodness of fit of GLM tests against other statistical tests were evaluated by Akaike information criterion (AIC) value and Bayesian information criterion (BIC) [31].

RESULTS

Characteristics of patients

Table 1 shows the main characteristics of the 262 HIV/HCV coinfected patients participating in the study. No significant differences were found when stratifying by rs2241766 genotypes (GG/GT versus TT).

Table 1. Epidemiological and demographic characteristics of HIV/HCV coinfected patients. Categorical variables are expressed in absolute numbers (percentage of patients). Continuous variables are expressed in median (interquartile range). P-values were estimated with a nonparametric Mann-Whitney U test for continuous variables and Chi-square for categorical variables.

Characteristics	All Patients	rs2241766 GG/GT	rs2241766 TT	p-value
N (%)	262 (100%)	81 (30.9%)	181 (69.1%)	
Male, n (%)	197 (75.2%)	61 (75.3%)	136 (75.1%)	0.976
Age, years	40.8 (IQR=6.9)	40.9 (IQR=6.6)	40.9 (IQR=7)	0.213
BMI, kg/m2	22.5 (IQR=3.7)	22.4 (IQR=3.6)	22.5 (IQR=3.9)	0.936
HIV acquired by IVDU, n (%)	224 (85.5%)	73 (90.1%)	151 (83.4%)	0.565
Prior AIDS, n (%)	76 (29%)	23 (28.4%)	53 (29.3%)	0.884
Time on cART, years	4.8 (IQR=5)	4.4 (IQR=5.5)	5.0 (IQR=4.9)	0.760
Current cART protocols, n (%)				
Any NRTIs + any PI	64 (24.2%)	20 (24.7%)	44 (24.3%)	0.947
Any NRTIs + PI + NNRTI	2 (0.8%)	0 (0%)	2 (1.1%)	0.342
Any NRTIs + any NNRTI	135 (51.1%)	36 (44.4%)	99 (54.7%)	0.125
Only NRTIs	19 (7.3%)	5 (6.2%)	14 (7.7%)	0.652
Specific antiretroviral drugs, n (%)				
Thymidine analogues + ddI	156 (59.5%)	42 (51.9%)	114 (63%)	0.090
Efavirenz	79 (30.2%)	19 (23.5%)	60 (33.1%)	0.114
Saquinavir	3 (1.1%)	2 (2.5%)	1 (0.6%)	0.178
Fosamprenavir	6 (2.3%)	2 (2.5%)	4 (2.2%)	0.897
Ritonavir	16 (6.1%)	6 (7.4%)	10 (5.5%)	0.556
HIV markers				
Nadir CD4+, cells/μL	206 (IQR=222.8)	210 (IQR=226.5)	203 (IQR=229)	0.909
Nadir CD4+ <200 cells/μL, n (%)	127 (48.5%)	39 (48.1%)	88 (48.6%)	0.944
CD4+ T, cells/μL	462 (IQR=332.3)	483 (IQR=300)	456 (IQR=350)	0.540
CD4+ ≥ 500 cells/μL, n (%)	113 (43.3%)	38 (46.9%)	75 (41.7%)	0.429

HIV-RNA < 50 copies/mL, n (%)	200 (76.6%)	56 (69.1%)	144 (80%)	0.055
HCV markers, n (%)				
HCV-GT 1	143 (56.5%)	42 (55.3%)	101 (57.1%)	0.791
HCV-GT 2	5 (2%)	1 (1.3%)	4 (2.2%)	0.625
HCV-GT 3	65 (25.6%)	17 (22.4%)	48 (27%)	0.442
HCV-GT 4	41 (16.1%)	16 (21.1%)	25 (14%)	0.165
HCV-RNA ≥ 500.000 IU/ml	187 (74.8%)	57 (76%)	130 (74.3%)	0.775
Metavir, n (%)				
Liver biopsy patients	210 (80.2%)	66 (81.5%)	144 (79.6%)	0.620
Significant fibrosis (F≥2)	103 (49%)	28 (42.4%)	75 (52.1%)	0.194
Moderate or severe activity (A≥2)	108 (52.2%)	29 (43.9%)	79 (56%)	0.105
Steatosis	116 (57.4%)	36 (58.1%)	80 (57.1%)	0.903

Abbreviations: AIDS, acquired immunodeficiency syndrome; BMI, body mass index; cART, combined antiretroviral therapy; ddl, didanosine; GT, Genotype; HCV, Hepatitis C virus; HCV-RNA, HCV plasma viral load; HDL-C, High-density lipoprotein; HIV, human immunodeficiency virus; HIV-RNA, HIV plasma viral load; HOMA, homeostatic model assessment method; IQR, interquartile range; IVDU, intravenous drug users; LDL-C, low-density lipoprotein; NNRTI, no nucleoside analog reverse-transcriptase inhibitors; NRTI, nucleoside analog reverse-transcriptase inhibitors; PI, protease inhibitors.

Frequencies of the *ADIPOQ* polymorphism

Allele frequencies for the *ADIPOQ* rs2241766 polymorphism were 0.17 (G allele) and 0.83 (T allele). Genotype frequencies for the *ADIPOQ* rs2241766 polymorphism were 0.03 (GG), 0.28 (GT), and 0.69 (TT). These frequencies in our dataset were in accordance with data listed on the NCBI SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=2241766). The rs2241766 SNP had fulfilled the minimum allele frequency (MAF) >0.05 for all samples and displayed less than 5% of missing values. Furthermore, rs2241766 was in HWE (p= 0.940).

Lipid profiles and the *ADIPOQ* polymorphism

Patients with rs2241766 GG/GT genotype had significantly lower serum TC levels (p= 0.038; **Figure 1A**) and lower percentages of TC values ≥200 mg/dL (p= 0.022; **Figure 1B**) than patients with rs2241766 TT. When adjusted GLM was performed, rs2241766 GG/GT genotype was associated with low serum TC levels (AMR= 0.92; p= 0.024; **Figure 2A**) and low likelihood of TC values ≥200 mg/dL (OR= 0.32; p= 0.027; **Figure 2B**), supporting the protective effect of the G allele. However, although rs2241766 GG/GT carriers seem to have reduced values of LDL-C, HDL-C, non-HDL-C, and triglycerides, no significant differences were observed (**Figure 1 & Figure 2**).
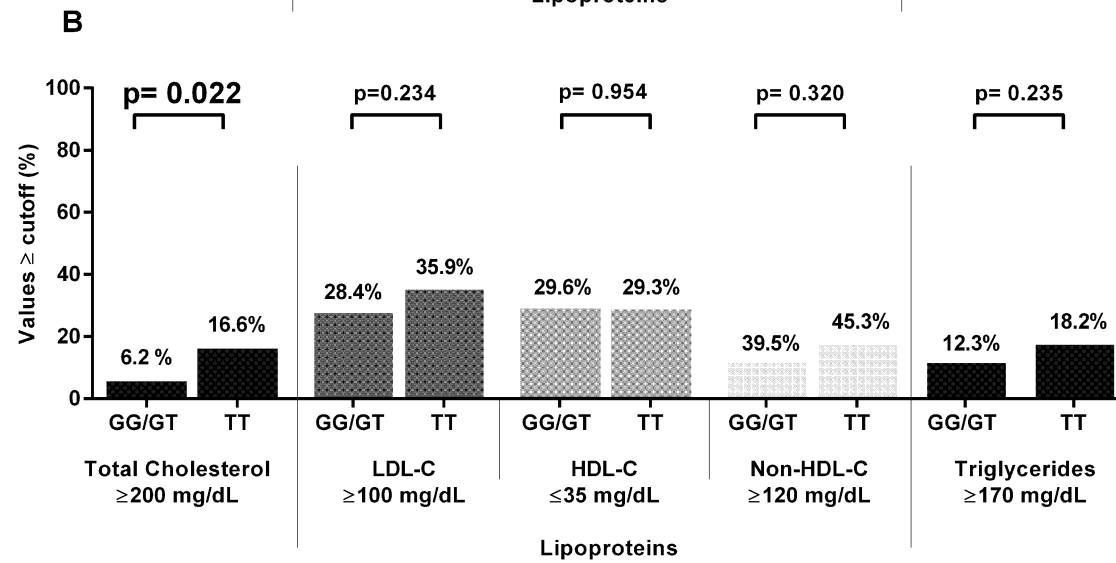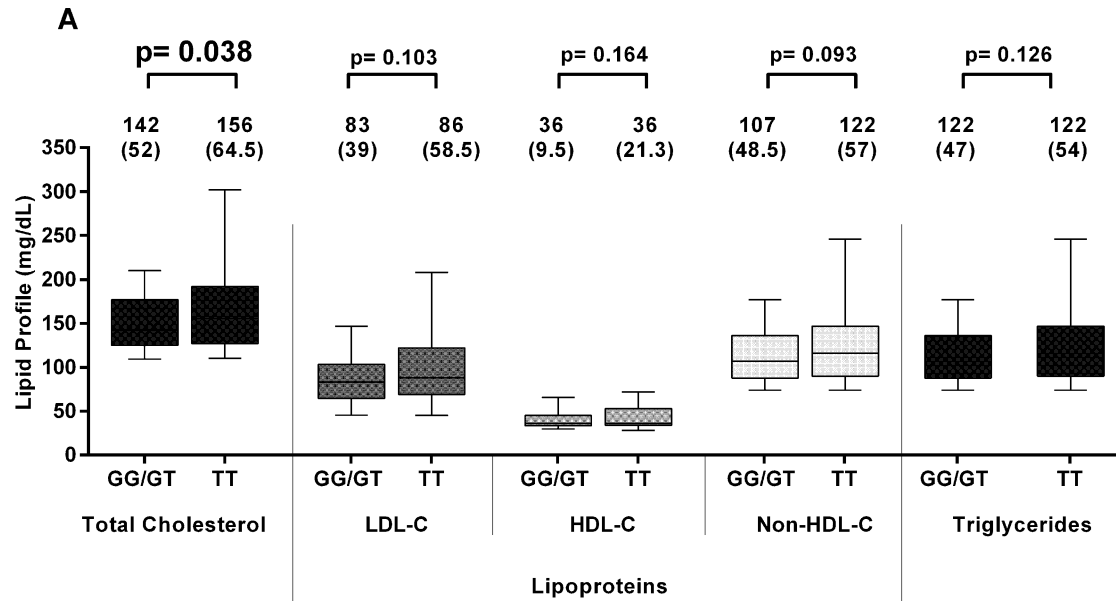
A

p= 0.038   p= 0.103   p= 0.164   p= 0.093   p= 0.126

| 142 (52) | 156 (64.5) | 83 (39) | 86 (58.5) | 36 (9.5) | 36 (21.3) | 107 (48.5) | 122 (57) | 122 (47) | 122 (54) |

B

p= 0.022   p=0.234   p=0.954   p=0.320   p= 0.235

Total Cholesterol: GG/GT 6.2 %, TT 16.6%
LDL-C: GG/GT 28.4%, TT 35.9%
HDL-C: GG/GT 29.6%, TT 29.3%
Non-HDL-C: GG/GT 39.5%, TT 45.3%
Triglycerides: GG/GT 12.3%, TT 18.2%

Total Cholesterol ≥200 mg/dL
LDL-C ≥100 mg/dL
HDL-C ≤35 mg/dL
Non-HDL-C ≥120 mg/dL
Triglycerides ≥170 mg/dL

Lipoproteins

10

Figure 1. Distribution of lipid profile values according to *ADIPOQ* rs2241766 polymorphism in HIV/HCV coinfected patients. The median (interquartile range) and p-values (unadjusted GLM with gamma distribution) are shown for continuous variables (**A**). The percentage of patients and p-values (unadjusted GLM with binomial distribution) are shown for thresholds (**B**).
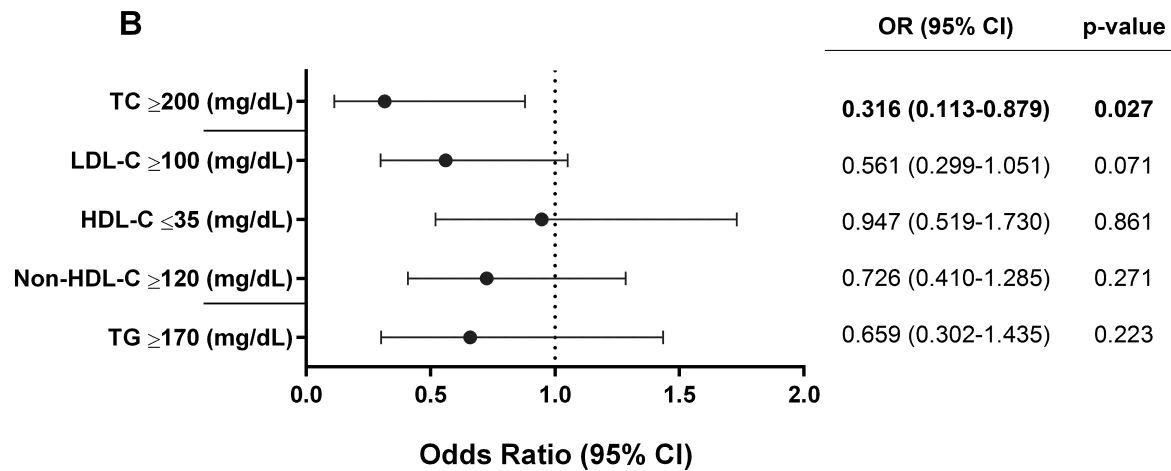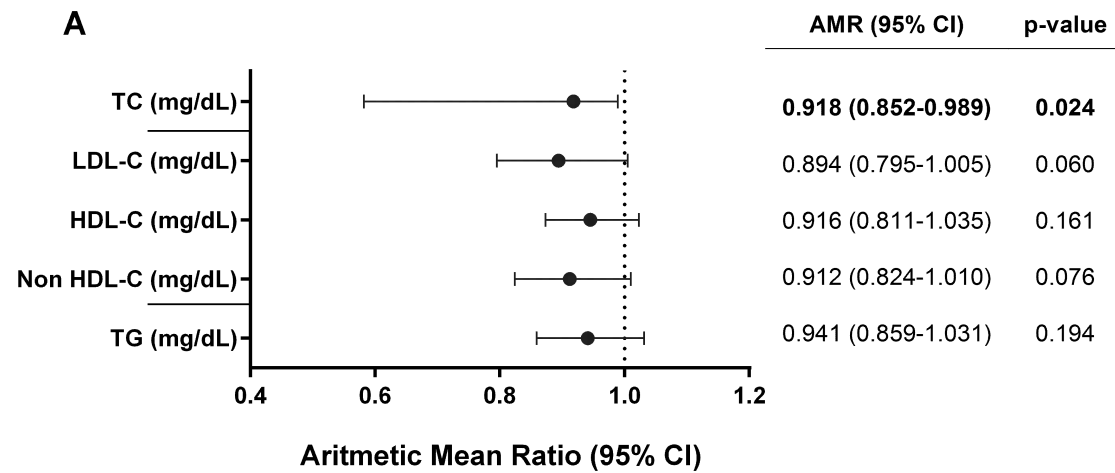
Figure 2. Association of *ADIPOQ* rs2241766 polymorphisms with serum lipid values (**A**) and dyslipidemia thresholds (**B**) in HIV/HCV coinfected patients. Multivariate GLM were adjusted by several epidemiological and clinical factors [age, gender, BMI, nadir CD4+, HIV plasma viral load, HCV plasma viral load, time on cART and specific antiretroviral drugs].

Additionally, we analyzed the association between the *ADIPOQ* rs2241766 polymorphism and serum lipid values stratified by steatosis (**Figure 3**). No significant values were found for patients without steatosis (**Figure 3A**). However, for patients with steatosis (**Figure 3B**), rs2241766 GG/GT carriers had lower serum values of TC (p= 0.039), LDL-C (p= 0.036), and non-HDL-C (p= 0.021) than rs2241766 TT carriers. When adjusted GLM was performed, the rs2241766 GG/GT genotype was related with low serum values of TC (AMR= 0.89; p= 0.027), LDL-C (AMR= 0.85; p= 0.039), and non-HDL-C (AMR= 0.86; p= 0.015) (**Figure 4A**).
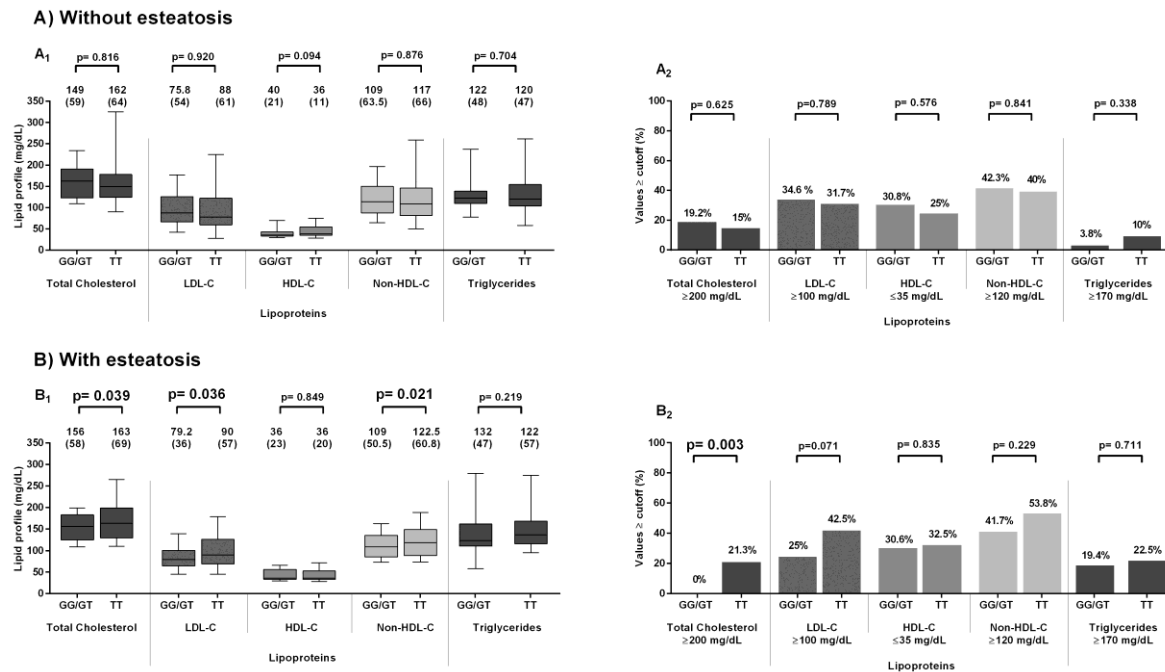

Figure 3. Distribution of lipid profile values according to *ADIPOQ* rs2241766 polymorphism in HIV/HCV coinfected patients stratified by steatosis. The median (interquartile range) and p-values (unadjusted GLM with gamma distribution) are shown for continuous variables (A_1, B_1). The percentage of patients and p-values (unadjusted GLM with binomial distribution) are shown for thresholds (A_2, B_2).

A

	AMR (95% CI)	p-value
TC	**0.893 (0.807-0.987)**	**0.027**
LDL-C	**0.846 (0.721-1.992)**	**0.039**
HDL-C	0.996 (0.888-1.118)	0.950
Non HDL-C	**0.856 (0.755-0.970)**	**0.015**
TG	0.892 (0.785-1.014)	0.081

Aritmetic Mean Ratio (95% CI)

B

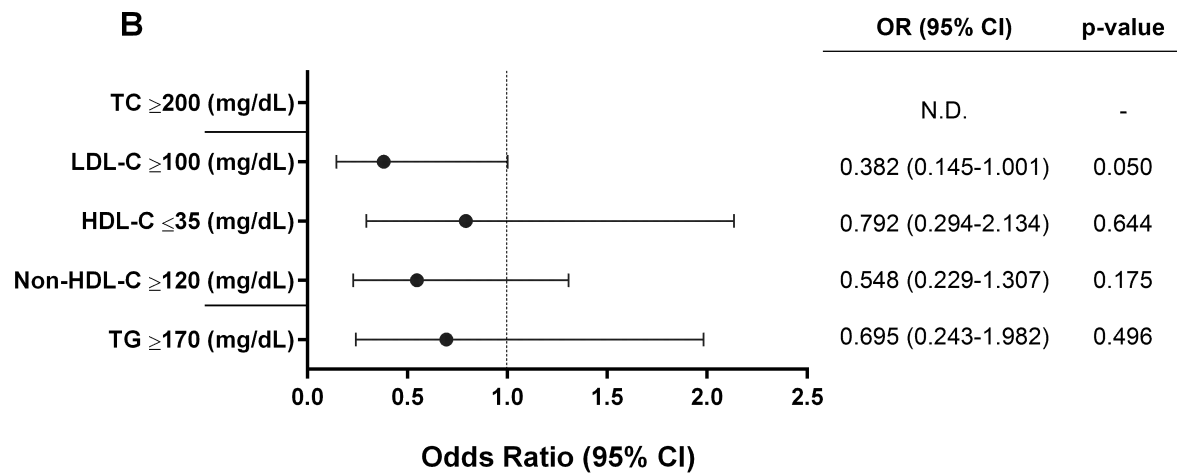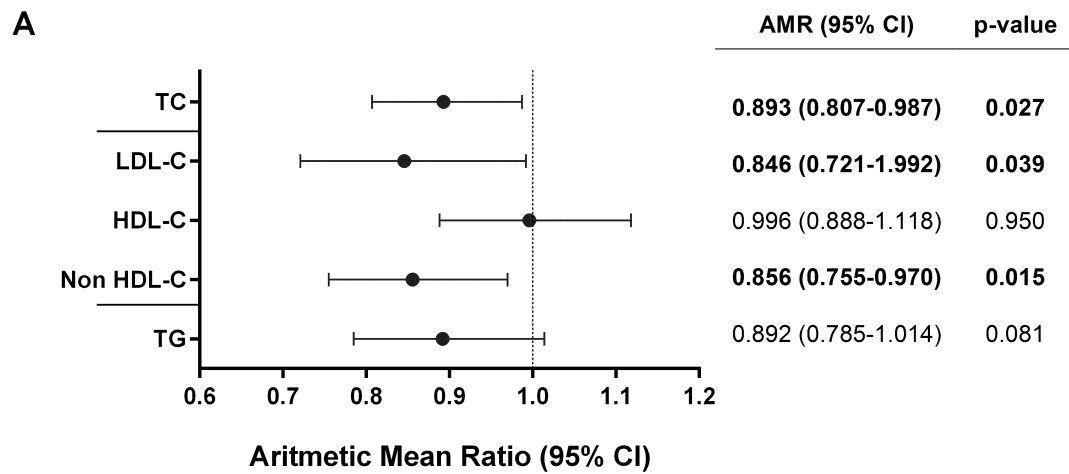|	OR (95% CI)	p-value
TC ≥200 (mg/dL)	N.D.	-
LDL-C ≥100 (mg/dL)	0.382 (0.145-1.001)	0.050
HDL-C ≤35 (mg/dL)	0.792 (0.294-2.134)	0.644
Non-HDL-C ≥120 (mg/dL)	0.548 (0.229-1.307)	0.175
TG ≥170 (mg/dL)	0.695 (0.243-1.982)	0.496

Odds Ratio (95% CI)

Figure 4. Association of *ADIPOQ* rs2241766 polymorphisms with serum lipid values (**A**) and dyslipidemia thresholds (**B**) by multivariate GLM tests in HIV/HCV coinfected patients with steatosis. These tests were adjusted by several epidemiological and

clinical factors [age, gender, BMI, nadir CD4+, HIV plasma viral load, HCV plasma viral load, time on cART and specific antiretroviral drugs].

Insulin resistance and the *ADIPOQ* polymorphism

When analyzing the association of the *ADIPOQ* rs2241766 polymorphism with HOMA values and the percentage of patients with HOMA ≥3.8, no significant differences were found for all patients, nor for patients stratified by significant liver fibrosis (**Supplemental Figure 1**). Additionally, when adjusted GLM was performed, no significant association was found (**Supplemental Figure 2**).

DISCUSSION

To the best of our knowledge, there is no data about the relationship of the *ADIPOQ* rs2241766 polymorphism with metabolic disturbances in HIV/HCV coinfected patients. In this study, we found that the rs2241766 GG/GT genotype was significantly associated with lower cholesterol levels (TC, LDL-C, and non-HDL-C) mainly in patients with liver steatosis.

Adiponectin is a potent modulator of lipid metabolism and an indicator of metabolic disorders [11]. Thus, serum adiponectin levels have been found to be inversely associated with components of metabolic syndrome, such as obesity, IR and T2DM [11]. The serum adiponectin concentration has a strong genetic component, with heritability estimated at 88% [32], but the mechanism of how the rs2241766 (+45T>G) *ADIPOQ* polymorphism is related to metabolic disturbance remains to be determined. The *ADIPOQ* rs2241766 polymorphism is a synonymous mutation (GGT→GGG, Gly→Gly), and the way in which this variation influences metabolic phenotypes remains to be elucidated. The G allele at the rs2241766 polymorphism has been associated with higher serum adiponectin concentrations and higher adiponectin mRNA expression in adipose tissue [33, 34]. However, associations between the *ADIPOQ* rs2241766 polymorphism and adiponectin concentration in population studies have not always been confirmed [35-37]. In our study, we did not find any association between *ADIPOQ* genotypes and values of serum adiponectin (*data not shown*). This lack of significance might be due to the limited number of patients used in the analysis (only 92 samples were available), or also due to the possible distortive effect of direct and indirect factors related to HIV and HCV infection [38].

Dyslipidemia and IR are two complex metabolic disturbances in which genetic and environmental factors interact to produce homeostatic abnormalities [39-42]. The *ADIPOQ* rs2241766 polymorphism has been extensively studied among HIV and HCV seronegative subjects in relation to the development of T2DM, blood lipids and blood pressure, but the influence of the *ADIPOQ* rs2241766 polymorphism on metabolism disturbance is not clear [22, 23]. Thus, the rs2241766 G allele has been associated with obesity, adverse lipid profiles, T2DM, hyperglycemia and IR [43-49], while other studies proposed a similar association for the rs2241766 T allele [50, 51] or a lack of associations between rs2241766 and alterations in the T2DM status, obesity, dyslipidemia or hypertension [36, 52]. In our study, the rs2241766 GG/GT genotype had a protective effect against dyslipidemia, with lower cholesterol levels mainly in HIV/HCV coinfected patients with steatosis. Additionally, no significant association between rs2241766 and HOMA values were found in our study.

Our findings are not consistent with a recent meta-analysis, which shows that the G allele of the *ADIPOQ* rs2241766 polymorphism is associated with a higher risk of cardiovascular disease [21]. However, other recent meta-analysis has shown that the presence of +45T>G (rs2241766) does not appear to influence the development of type 2 diabetes [22]. These discrepancies could be due to the characteristics of the study population, since HIV/HCV coinfected patients had an intrinsic metabolic deregulation due to the HIV and HCV coinfection [38]. In addition, cART is often associated with severe metabolic disorders, such as IR and hyperlipidemia [38]. Liver steatosis in CHC has been associated with metabolic disturbance such as IR and the metabolic syndrome [4]; reductions in TC as well as HDL-C and LDL-C are observed in HCV-infected patients

compared to matched controls [4]. Thus, these observations highlight that HIV/HCV coinfected patients form a very unique population, where perhaps our findings need not coincide with those of the general population.

Some aspects must be taken into account for a correct interpretation of our data: a) this is a cross-sectional study with a relatively low sample size, however our population provides adequate statistical power to detect the significant associations that are shown in this study (**Supplemental Table 1**); b) it is uncertain whether our results would be observed in HCV mono-infected patients; c) the patients selected for our study were patients who met a set of criteria for starting HCV treatment (for example 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; d) this study was carried out entirely in white 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; e) the rs2241766 polymorphism is located in a linkage disequilibrium (LD) block spanning from the intron 1 to exon 3 of the adiponectin gene [20], and it is possible that other genetic variants in the LD with the rs2241766 polymorphism are causal variants for the associations.

In conclusion, the presence of the rs2241766 G allele (GG and GT genotypes) was associated with a protective effect against dyslipidemia, mainly in HIV/HCV coinfected patients with steatosis. Thus, our results suggest that rs2241766 may play a significant role in metabolic disorders in HIV/HCV coinfected patients. Further studies in larger populations will be required to determine whether the *ADIPOQ* polymorphisms play a pivotal role in energy metabolism of HIV/HCV coinfected patients.

AUTHORS' CONTRIBUTIONS:

JB and SR participated in the study concept and design. DPT and SR performed all statistical analysis, interpretation of the data and wrote the manuscript. JB, CD, AC, PC and TA participated in patient selection, collection of samples and acquisition of data. DPT, PGC, MAJS, AFR, and MGA 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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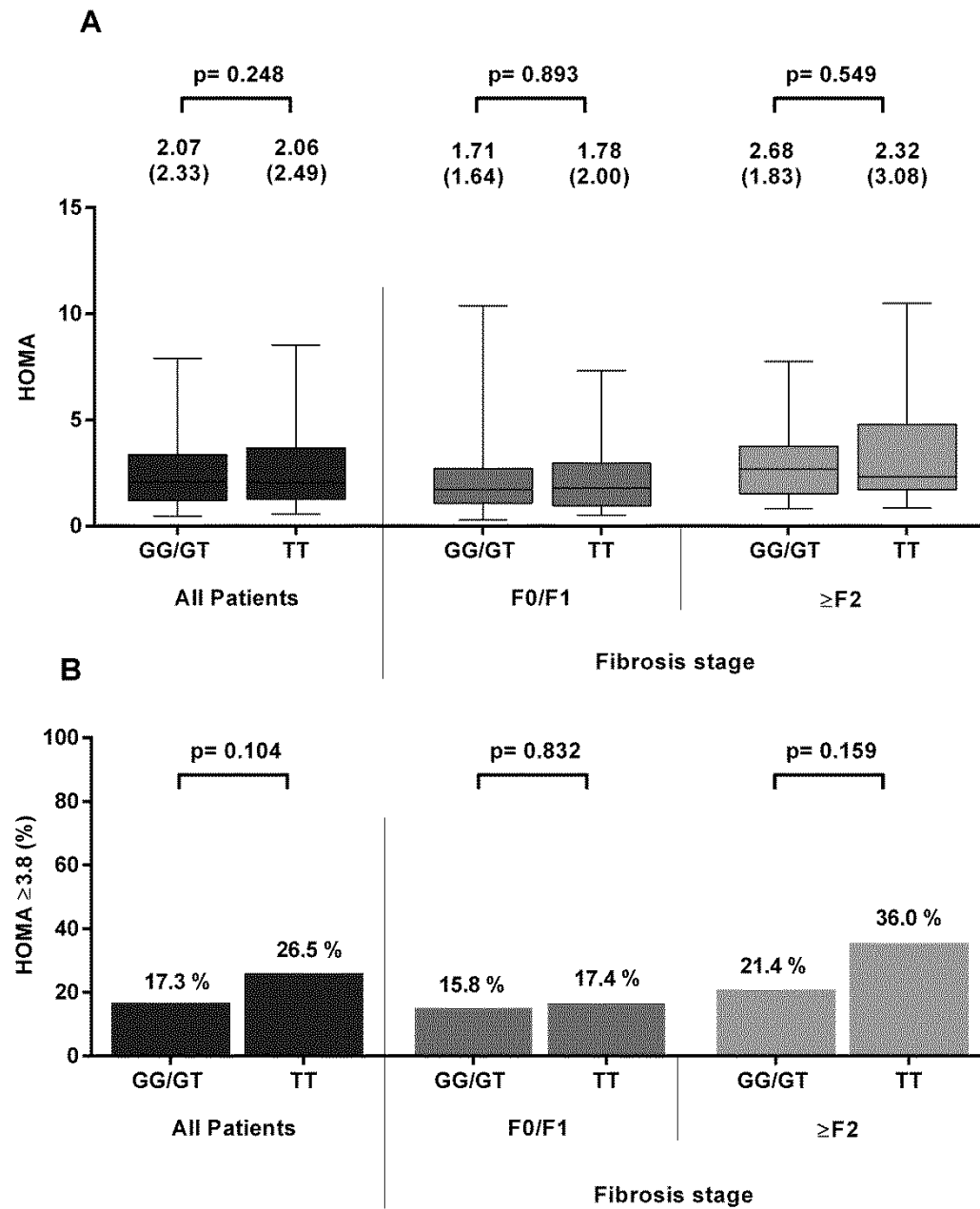
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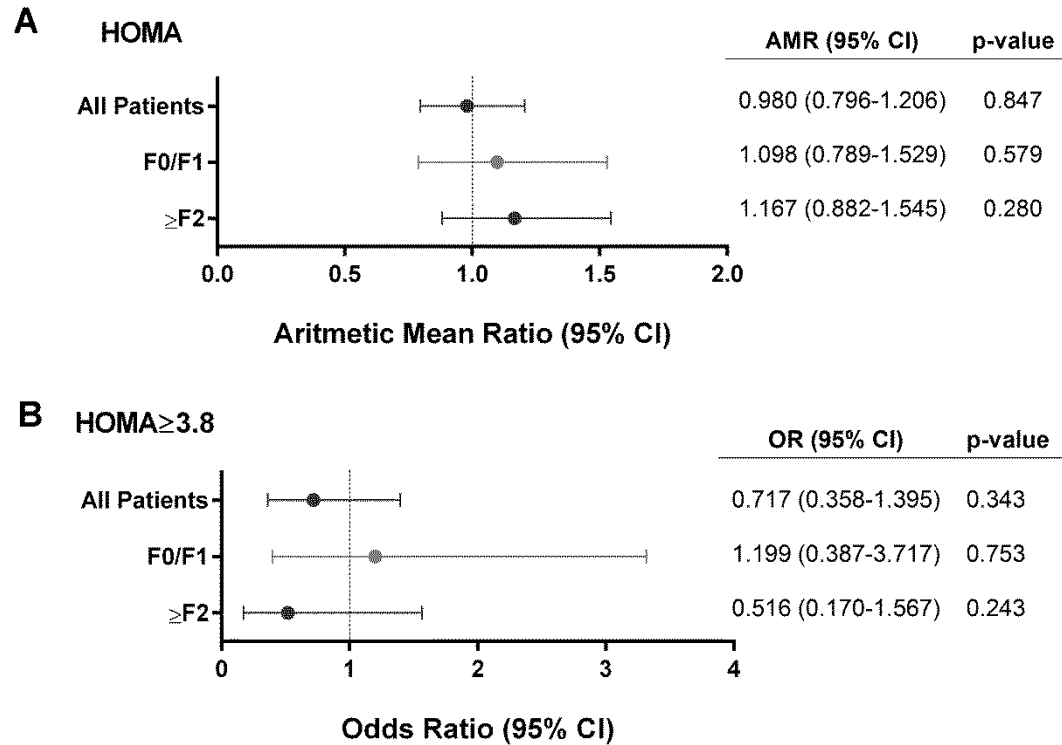
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SUPPLEMENTAL DATA

Supplemental Figure 1. Distribution of HOMA values and insulin resistance according to *ADIPOQ* rs2241766 polymorphism and stratified by liver fibrosis in HIV/HCV coinfected patients. The median (interquartile range) and p-values (unadjusted GLM test with gamma distribution) are shown for continuous values (**A**). The percentage of patients and p-values (unadjusted GLM with binomial distribution) are shown for HOMA ≥3.8 (**B**).

A

p= 0.248   p= 0.893   p= 0.549

2.07   2.06   1.71   1.78   2.68   2.32
(2.33)   (2.49)   (1.64)   (2.00)   (1.83)   (3.08)

HOMA

15

10

5

0

GG/GT   TT   GG/GT   TT   GG/GT   TT

All Patients   F0/F1   ≥F2

Fibrosis stage

B

p= 0.104   p= 0.832   p= 0.159

HOMA ≥3.8 (%)

100

80

60

40

20

0

17.3 %   26.5 %   15.8 %   17.4 %   21.4 %   36.0 %

GG/GT   TT   GG/GT   TT   GG/GT   TT

All Patients   F0/F1   ≥F2

Fibrosis stage

24

Supplemental Figure 2. Association of *ADIPOQ* rs2241766 polymorphism with HOMA (**A**) and insulin resistance (HOMA≥3.8) (**B**) by Generalized Linear Model (GLM) tests in HIV/HCV coinfected patients. These tests were adjusted by several epidemiological and clinical factors [age, gender, BMI, nadir CD4+, HIV plasma viral load, HCV plasma viral load, time on cART and specific antiretroviral drugs].

A HOMA

	AMR (95% CI)	p-value
All Patients	0.980 (0.796-1.206)	0.847
F0/F1	1.098 (0.789-1.529)	0.579
≥F2	1.167 (0.882-1.545)	0.280

Aritmetic Mean Ratio (95% CI)

B HOMA≥3.8

	OR (95% CI)	p-value
All Patients	0.717 (0.358-1.395)	0.343
F0/F1	1.199 (0.387-3.717)	0.753
≥F2	0.516 (0.170-1.567)	0.243

Odds Ratio (95% CI)

Supplemental Table 1. Estimation of statistical power performed *a posteriori* for two independent groups (http://www.imim.cat/ofertadeserveis/software-public/granmo/), in those cases where the statistical significance was found. The simulation was performed accepting an alpha risk of 0.05 in a two-sided test with 81 subjects in the GG/GT group and 181 in the TT group.

	p-value	Statistical power
Non stratified		
Total Cholesterol	0.038	69%
Total Cholesterol ≥200 mg/dL	0.022	71%
Liver steatosis		
Total Cholesterol	0.039	67%
Total Cholesterol ≥200 mg/dL	0.003	99%
LDL-C	0.036	77%
Non-HDL-C	0.021	78%