Impact of genetic factors on dyslipidemia in HIV-infected patients starting antiretroviral therapy

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Objective: The impact of host genetic factors on the incidence of dyslipidemia in antiretroviral-naive HIV patients starting antiretroviral therapy (ART) is not clear. We assessed the role of single nucleotide polymorphisms (SNPs) identified from previous genome-wide association studies adjusting for the contribution of nongenetic factors.

Methods: We assessed 192 SNPs in an HIV cohort who started ART (1997–2008) including a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor (NNRTI). Patients had fasting plasma lipids, total cholesterol (T-Chol), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides measured prior to their ART initiation and after 1 year. A logistic regression model was constructed and multiple test was corrected using 10% false discovery rate (FDR). Haplotypes and gene interactions were analysed.

Results: A total of 727 individuals were successfully genotyped ($n = 381$_PI-group; $n = 346$_NNRTI-group). Age and hepatitis C virus (HCV) coinfection were associated with increases and decreases in T-Chol and LDL-C ($P < 0.01$), respectively. Protease inhibitor containing ART showed an unfavourable association with T-Chol ($P < 0.01$) and triglycerides ($P = 7.4E{-}4$) and NNRTI-containing ART was favourably associated with HDL-C ($P < 0.01$). Moreover, SNPs in apolipoprotein B (*APOB*) were associated with an increase of LDL-C [rs10495712 ($P = 3.18E{-}4$); rs754524 ($P = 1.26E{-}3$)]. Six SNPs in three genes showed an association with a favourable effect on HDL-C levels when ART included NNRTI: *ABCA1* (rs4149313, $P = 2.97E{-}4$), *LIPC* (rs1800588, $P = 2.13E{-}3$; rs473224, $P = 3.06E{-}4$; rs261336, $P = 2.23E{-}3$) and *CETP* (rs173539, $P = 2.96E{-}3$; rs3764261, $P = 1.52E{-}3$). After 10% FDR correction for multiple testing, one and six SNPs displayed significant associations with LDL-C and HDL-C, respectively.

Conclusion: In HIV-infected patients staring ART, one SNP in *APOB* was associated with an increase of LDL-C. SNPs in *ABCA1/LIPC/CETP* were favourably associated with HDL-C when ART included NNRTI. However, an unfavourable effect on T-Chol and triglyceride levels was observed when ART included protease inhibitor. The risk of hypercholesterolaemia increased with age and decreased with HCV coinfection. These findings might help to individualize the selection of ART.

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Introduction

Treatment of HIV infection may be associated with dyslipidemia [1,2]. Not only specific drug factors may account but also concomitant conditions may play an additional role. The extent of host genetics on the risk of developing antiretroviral therapy (ART)-associated dyslipidemia is poorly known.

In HIV-infected patients, abnormalities of lipid metabolism were described before the use of ART [3,4]. An early decrease in high-density lipoprotein cholesterol (HDL-C) and a later decrease in low-density lipoprotein cholesterol (LDL-C), followed by an increase in triglyceride levels were observed with the progression of HIV infection [5]. Some studies reported the consistent finding that patients with advanced HIV infection or AIDS had higher levels of plasma triglyceride and lower levels of plasma HDL-C [4,5]. The decrease of HDL-C likely increases the risk of atherosclerosis more than the increase of LDL-C does. The effect is amplified because of the fact that HDL-C does not function optimally in the setting of infection or inflammation [6]. Certainly, numerous studies [7–10] have reported dyslipidemia and an increased risk of cardiovascular disease (CVD) in HIV-infected persons receiving ART. The DAD study (Data collection on Adverse events of anti-HIV Drugs) reported an increase of 32% in the relative risk for CVD over the 5 following years since the initiation of ART, risk that appeared to be greater with the exposure of protease inhibitor relative to NNRTI [7–10].

The severity of dyslipidemia can vary according to the ART regimen used. However, not all patients exposed to the same ART regimen develop dyslipidemia, despite having had the same ART exposure, similar demographic characteristics and being immunologically and virologically comparable. This discrepancy may be due, at least in part, to host genetic factors. The risk of dyslipidemia has been described in patients carrying unfavourable genotypes for genes encoding apolipoproteins such as apolipoprotein E, apolipoprotein C3 and apolipoprotein A. In fact, this was more plausible in those patients exposed to a ritonavir (RTV) containing treatment [11,12]. Furthermore, a genome-wide association study (GWAS) in normal population identified other polymorphisms associated with dyslipidemia in HIV-infected individuals, suggesting that genetic information should be taken into account in addition to the dyslipidemic effects of ART [13]. The main objective of this study was to assess the impact of 192 single nucleotide polymorphisms (SNPs), previously identified in six GWAS done in general population (Table_S1_supplementary, http://links.lww.com/QAD/A291), on plasma lipid levels in a cohort of 750 antiretroviral-HIV patients starting ART including a protease inhibitor or a nonnucleoside reverse transcriptase inhibitor (NNRTI). A second aim was to assess relevant nongenetic factors contributing to dyslipidemia when adjusting for genetic factors.

Materials and methods

Study design, patients and genetic variants

We studied an HIV-infected cohort prospectively followed-up at Hospital Clínic of Barcelona between 1 January 1997 and 31 December 2008.

The study population consisted of 750 naive HIV-infected patients (age between 18 and 75 years; 80% men) randomly selected who fulfill inclusion criteria of at least 1 year of follow-up without ART changes and no AIDS criteria. This project was approved by the ethics committees of our hospital. As part of the routine follow-up, these participants had measurements of serum levels of total cholesterol (T-Chol), LDL-C, HDL-C and triglyceride at baseline and after 1 month and each 3–6 months after ART initiation. All starting and stopping dates of each antiretroviral regimen and all cardiovascular medication were recorded in the database. No lipid-lowering agents or drugs that could have an influence on lipid levels were administered during the first year of treatment. Figure 1 provides the flow chart leading to the 750 DNA samples that were genotyped. In this study, 192 genetic variants (SNPs) in 87 genes from the lipid metabolism pathway were assessed. Participants gave written informed consent for genetic testing. All tested SNPs had significant genome-wide associations with serum lipid levels in previously published GWAS performed in the general population [13–18] with minor allele frequencies (MAFs) greater than 0.01 (Table_S1, http://links.lww.com/QAD/A291). Of all these variants, 26% were previously associated with T-Chol, 15% with LDL-C, 36% with HDL-C and 23% with triglyceride levels. DNA was isolated following the manufacturer's instructions with the QIAamp DNA Blood Mini Kit (QIAcube-QIAGEN; Qiagen Sciences Inc., Germantown, Maryland, USA). Genotyping was performed using the Illumina Golden Gate platform (Universitat Autònoma Barcelona, Spain). Duplicates from 10% of the samples were included either within or between plates as a genotyping quality control. The resulting genotypes were filtered prior to statistical analysis [14]: individuals with genotyping call rate less than 80% ($n = 23$ samples) and SNPs with genotyping call rate less than 95% ($n = 11$ SNPs) were excluded. Ultimately, 727 patients and 181 SNPs in 83 genes were included in this study (Fig. 1). Because the clinical relevance of prolonged dyslipidemia is well documented by genetic causes of dyslipidemia [15,16], we included all lipid measurements available for each participant and calculated the observed proportions of participants with sustained dyslipidemia during the study period. The influence of ART and lipid-lowering agents, administered after the first year of ART initiation, on lipid levels was assumed to be rapid and reversible [17].

Dyslipidemia, as in previous reports [12,18], was defined according to the cut-off level defined by the National Cholesterol Education Program Third Adult Treatment

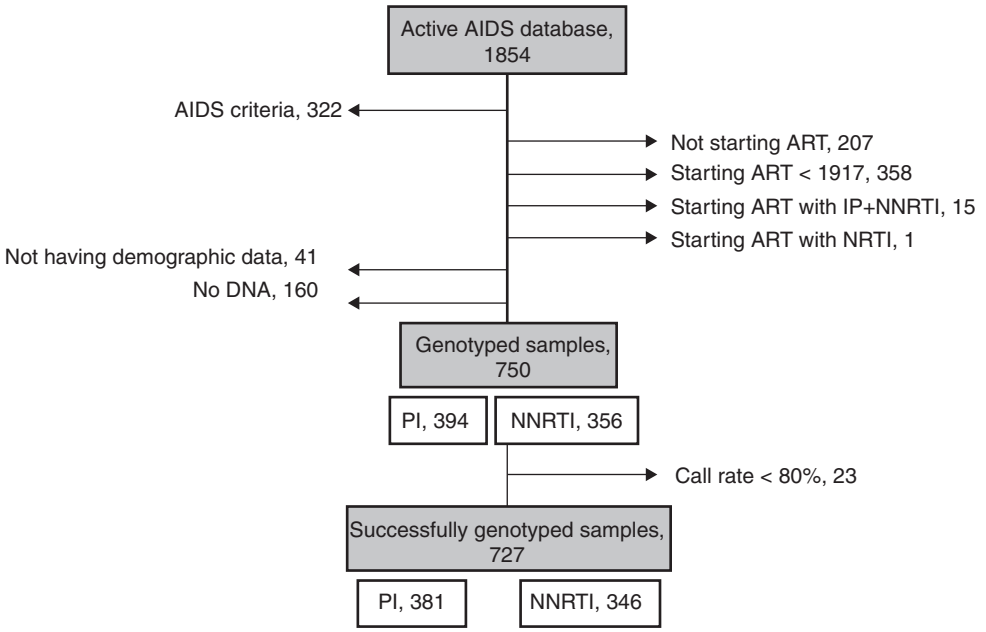

Fig. 1. Selection and exclusion criteria for samples included in the association study. NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PIs, protease inhibitors.

Program (NCEP-ATPIII). Briefly, the cut-off lipid values considered were T-Chol greater than 6.2 mmol/l (240 mg/dl); LDL-C greater than 3.4 mmol/l (130 mg/dl); HDL-C less than 1.03 mmol/l (40 mg/dl); triglyceride greater than 2.26 mmol/l (200 mg/dl). Those individuals with an increase of T-Chol/LDL-C/triglyceride or a decrease of HDL-C levels with respect to the cut-off level during the first year of follow-up once ART started were considered cases.

Statistical analysis

Data were analysed longitudinally by modelling the individual effects of the different covariates on plasma lipid fraction (T-Chol, LDL-C, HDL-C and triglyceride) levels. Time-dependent covariates considered included age, $CD4^+$ T-cells and HIV viral load. Fixed covariates included sex, ethnicity, hepatitis C virus (HCV) coinfection, first-line ART regimen either with a protease inhibitor or a nonnucleoside reverse transcriptase (NNRTI) and the genetic factors assessed. Medians were used to show central tendencies and interquartile ranges were calculated as measures of variability and statistical dispersion.

The study population was initially checked for Hardy–Weinberg equilibrium ($P > 0.001$) at all the tested SNPs and separately evaluated for different lipid fractions. PLINK1.07 was used for all association and statistical analyses [19]. Study participants were treated as cases whenever an abnormal lipid value existed at 1 year of ART exposure. Stratified analysis controlling for first-line ART was performed using the Cochran–Mantel–Haenszel (CMH) test. Then, candidate SNPs were evaluated in a multivariate analysis on the basis of a logistic regression adjusted for all genetic and nongenetic variables for each lipid fraction. For the additive effects of SNPs, the direction of the regression represented the favourable or unfavourable effect of each extra minor allele. The power/effect of each variable was assessed using the odds ratio (OR) and the variance components calculated in the multivariate analysis. For multiple testing corrections, we used the Q-value R package [20] to apply a false discovery rate (FDR) of 10%, which corresponded to a significance threshold of a P value equal to 0.000318 for the LDL-C fraction and a P value equal to 0.002969 for HDL-C. For T-Chol, triglyceride and the sum of all lipid fractions, no significant thresholds could be calculated at 10% FDR.

Significant SNPs that overcame the 10% FDR correction were assessed in a Breslow–Day test of homogeneity of odd ratios, in order to check whether different antiretrovirals from the same family had different gene ORs. This was performed in both drug families: ritonavir-boosted protease inhibitor (PI/r) versus non-boosted protease inhibitor in the protease inhibitor group and efavirenz (EFV) versus nevirapine (NVP) in the NNRTI group. Statistically significant SNPs ($P \leq 0.05$) for the Breslow–Day test were separately reassessed in a chi-square based basic association test in order to designate them as favourable or unfavourable with an OR less than 1 or an OR more than 1, respectively.

Multiple-marker analysis

In order to check nonrandom association of alleles at two or more loci, linkage disequilibrium blocks were obtained from primary genotype data using Haploview [21,22]. The haplotype-based association study was restricted to genes including more than one associated

genetic variant in the single-marker analysis. All the genotyped variants within these genes were considered. The best two-marker haplotype from all possible pairwise combinations was identified. Likewise, additional markers (up to three) were added in a stepwise manner to the initial two-SNP haplotype. Significance was estimated using 1000 permutations with the UNPHASED 3.2 software [23]. As the expectation-maximization algorithm does not accurately estimate low haplotype frequencies [24], haplotypes with frequencies less than 0.05 were excluded.

Interaction analysis

Interaction analysis between SNPs from different genes was performed by comparing it to different regression models with a likelihood ratio test using the statistical package SPSS v15.0 (SPSS Inc., Chicago, Illinois, USA). In the first model, we used the abnormal serum lipid status as a dependent variable and the two risk alleles as predictive variables (affected status = a + $bSNP_1$ + $cSNP_2$). In the second model, we included the interaction between SNPs as an independent variable (Affected status = a + $bSNP_1$ + $cSNP_2$ + $dSNP_1$*SNP_2). For each gene, the inheritance model providing the best association results in the single-marker analysis was used. To test possible interactions between haplotypes from different genes, haplotype frequencies were estimated using PHASE software and then we followed the procedure described above.

Results

Characteristics of the participants and lipid levels

Three hundred and eighty-one patients (52.4%) had a protease inhibitor and 346 (47.6%) an NNRTI prescribed in their first-line treatment.

Successful genotyping was accomplished for 727 out of 750 participants (96.9%). Their characteristics are summarized in Table 1. All of them were Spanish and whites. MAF values at all SNPs were similar to the ones previously described for ethnically equal populations (Table_S1, http://links.lww.com/QAD/A291) and all the tested SNPs were in Hardy–Weinberg equilibrium. During the study period, a median [interquartile range = upper quartile (Q3) − lower quartile (Q1)] of 8.5 (12), 5 (6), 7 (9) and 10 (13) measurements of T-Chol, LDL-C, HDL-C and triglyceride were determined in the protease inhibitor group and 9 (11), 6 (4.5), 8 (8) and 10 (13.75) in the NNRTI group.

Effect of antiretroviral regimen on plasma lipid levels

Before exposure to ART, all lipid fraction values were below the respective NCEP-ATPIII cut-off level. All lipid values except HDL-C increased once the ART treatment was initiated. At 1 year of starting treatment, 168 (44%), 193(50.6%), 283 (74.2%) and 258 (67.7%)

Table 1. Characteristics of the study participants.

Characteristic	Study participants ($n = 727$)	
	PI ($n = 381$)	NNRTI ($n = 346$)
Baseline age (years)[a]	35 (9)	36 (10)
Men/women, *n* men (%)	300 (78.8)	287 (83)
Ethnicity, whites, *n* (%)	381 (100)	346 (100)
Presumed mode of HIV transmission, *n* (%)		
MSM	156 (40.9)	197 (57.1)
Heterosexual	111 (29.1)	82 (23.7)
Bisexual	4 (1)	8 (2.2)
IDU	92 (24)	30 (8.7)
MSM and IDUs	4 (1)	8 (2.2)
Transfusion	3 (0.6)	1 (0.2)
Haemophilic	0	2 (0.6)
Unknown	12 (3.3)	18 (5.2)
Coinfection with HCV, *n* (%)	118 (30.5)	52 (15)
Follow-up period		
$CD4^+$ T-cell count (cells/μl)[a]	309 (294)	294 (221)
HIV viral load (log)[a]	4.65 (5.27)	4.8 (6.26)
Participants treated with PI, *n* (%)		
RTV-boosted PI	72 (18.9)	–
RTV-nonboosted PI	309 (81.1)	–
Participants treated with NNRTI, *n* (%)		
EFV	–	227 (65.8)
NVP	–	118 (34)
EFV and NVP	–	1 (0.2)
T-Chol determinations per participant[a]	8.5 (12)	9 (11)
LDL-C determinations per participant[a]	5 (6)	6 (4.5)
HDL-C determinations per participant[a]	7 (9)	8 (8)
TG determinations per participant[a]	10 (13)	10 (13.75)

EFV, efavirenz; HCV, hepatitis C virus; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; RTV, ritonavir; T-Chol, total-cholesterol; TG, triglycerides.
[a]Median [interquartile range = upper quartile (Q3) − lower quartile (Q1)].

Table 2. Evolution of lipid fraction values during the study follow-up period.

	PI ($n = 381$)	NNRTI ($n = 346$)
Basal plasma T-Chol level (mg/dl)[a]	141 (31)	147.5 (52.75)
Basal plasma LDL-C level (mg/dl)[a]	90 (47.5)	101 (44)
Basal pasma HDL-C level (mg/dl)[a]	35 (13)	36 (14)
Basal plasma TG level (mg/dl)[a]	132 (121)	111 (74.5)
Abnormal plasma T-Chol level (mg/dl)[a]	278.5 (51.25)	265 (42)
Abnormal plasma LDL-C level (mg/dl)[a]	160 (46)	158 (39.75)
Abnormal plasma HDL-C level (mg/dl)[a]	30 (9)	31 (8)
Abnormal plasma TG level (mg/dl)[a]	340.5 (261.75)	317 (197.75)
Individuals with abnormal T-Chol, *n* (%)	168 (44)	114 (32.9)
Individuals with abnormal LDL-C, *n* (%)	193 (50.6)	181 (52.2)
Individuals with abnormal HDL-C, *n* (%)	283 (74.2)	251 (72.5)
Individuals with abnormal TG, *n* (%)	258 (67.7)	168 (48.5)

Basal plasma lipid values (mg/dl) were measured after HIV diagnosis and before ART exposure. Abnormal lipid values (mg/dl) represent abnormality within the first year of ART exposure or until drug family was changed. HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; NNRTI, nonnucleoside reverse-transcriptase inhibitor; PI, protease inhibitor; TG, triglyceride.
[a]Median [interquartile range = upper quartile (Q3) − lower quartile (Q1)].

patients from the protease inhibitor group showed abnormal values for T-Chol, LDL-C, HDL-C and triglyceride, respectively. In the NNRTI group, 151 (32.9%), 240 (52.2%), 333 (72.5%) and 223 (48.5%) patients showed abnormal values for T-Chol, LDL-C, HDL-C and triglyceride, respectively (Table 2). The increase of triglyceride levels after exposure to ART was higher in the protease inhibitor than in the NNRTI group. Nearly 67.7% of the patients presented an abnormal triglyceride level at 1 year of exposure to a protease inhibitor versus 48.5% of the patients exposed to an NNRTI. The percentage of individuals with abnormal lipid fraction levels was not significantly different between the protease inhibitor and the NNRTI groups ($P = 0.26$) (Table 2).

Contribution of single nucleotide polymorphisms to individual abnormal plasma lipid levels

A total of 51 SNPs were significantly associated ($P < 0.05$) with a lipid fraction in the univariate analysis stratified by ART (Table_S2, http://links.lww.com/QAD/A291). From all these variants, 40 SNPs remained significant ($P < 0.05$) in the logistic regression model-based multivariate analysis (Table_S3, http://links.lww.com/QAD/A291). In the final multi-SNP model adjusted for all genetic and nongenetic covariates, only 15 variants with a significance of P value less than 0.01 were taken into account (Table 3). Ultimately, one and six SNPs displayed a significant association with LDL-C and HDL-C respectively, after 10% FDR correction for multiple testing.

Table 3. Logistic regression based multivariate analysis of the contribution of single nucleotide polymorphisms to dyslipidemia.

					CMH (ART)				Logistic regression			
	CHR	SNP	Nearest gene	MAF	*P*	OR	L95	U95	$P < 0.01$	OR	L95	U95
Total cholesterol	9	rs471364	*TTC39B*	0.13	0.00278	0.545	0.365	0.814	0.002927	0.515	0.332	0.797
LDL-cholesterol	2	rs10495712	*APOB*	0.22	0.00049	1.708	1.262	2.312	0.0003181[a]	2.013	1.376	2.947
	2	rs6754295	*APOB*	0.25	0.01284	0.688	0.511	0.925	0.009661	0.620	0.432	0.891
	2	rs754524	*APOB*	0.22	0.00128	1.656	1.217	2.255	0.001263	1.864	1.277	2.722
	7	rs2240466	*BAZ1B*	0.06	0.04356	1.695	1.011	2.842	0.009008	2.352	1.238	4.468
HDL-cholesterol	9	rs4149313	*ABCA1*	0.19	0.0004	0.550	0.393	0.768	0.000297[a]	0.516	0.361	0.739
	15	rs1800588	*LIPC*	0.26	0.00465	0.645	0.476	0.875	0.002138[a]	0.608	0.443	0.836
	15	rs473224	*LIPC*	0.14	0.00051	0.522	0.361	0.756	0.0003067[a]	0.483	0.326	0.717
	15	rs261336	*LIPC*	0.18	0.00301	0.601	0.428	0.843	0.002239[a]	0.572	0.400	0.818
	16	rs173539	*CETP*	0.29	0.00363	0.638	0.471	0.865	0.002969[a]	0.608	0.438	0.844
	16	rs3764261	*CETP*	0.29	0.00238	0.626	0.462	0.848	0.001521[a]	0.588	0.424	0.817
Triglycerides	2	rs1260326	*GCKR*	0.43	0.00725	1.367	1.088	1.718	0.008063	1.361	1.083	1.709
	2	rs780094	*GCKR*	0.44	0.00299	1.412	1.124	1.774	0.003286	1.412	1.122	1.777
	9	rs4149313	*ABCA1*	0.19	0.00843	0.684	0.516	0.907	0.00877	0.683	0.514	0.908
	11	rs174570	*FADS2*	0.17	0.00509	1.570	1.143	2.157	0.005261	1.570	1.144	2.155

Data show significant ($P < 0.01$) SNPs associated with the lipid fraction and their significance level in the univariate analysis stratified by first-line antiretroviral treatment [CMH (ART)]. CHR, chromosome; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MAF, minor allele frequency; OR, odds ratio; PI, protease inhibitor; SNP, single nucleotide polymorphism.
[a]Significant associations after 10% FDR correction for multiple testing.

T-cholesterol

A total of nine SNPs (rs515135, rs541041, rs312985, rs506585, rs1260326, rs780094, rs904743, rs471364, rs1883025) in four genes (*APOB, GCKR, TTC39B* and *ABCA1*) maintained association ($P < 0.05$) with T-Chol levels in the logistic regression analysis (Table_S3, http://links.lww.com/QAD/A291).

In the final multi-SNP model, one SNP, rs471364 (*TTC39B*; $P = 0.002927$; OR, 0.515), contributed significantly to a decrease of plasma T-Chol levels (Fig. 2, Table 3, Table_S3, http://links.lww.com/QAD/A291).

Low-density lipoprotein-cholesterol

Eleven SNPs (rs11206510, rs10198175, rs10495712, rs6754295, rs7557067, rs673548, rs754524, rs754523, rs2240466, rs714052, rs2967605) in four genes (*PCSK9, APOB, BAZ1B* and *RAB11B*) maintained association ($P < 0.05$) with LDL-C levels in the logistic regression analysis (Table_S3, http://links.lww.com/QAD/A291).

In the final multi-SNP model, three SNPs showed an unfavourable effect whereas one SNP showed a favourable effect. The SNPs that contributed significantly to an increase of plasma LDL-C levels were rs10495712 (*APOB*; $P = 0.000318$; OR, 2.013), rs754524 (*APOB*; $P = 0.001263$; OR, 1.864) and rs2240466 (*BAZ1B*; $P = 0.009008$; OR, 2.352). However, one SNP, rs6754295 (*APOB*; $P = 0.009661$; OR, 0.62) contributed favourably to plasma LDL-C levels (Fig. 2, Table 3; Table_S3, http://links.lww.com/QAD/A291). Despite the high degree of linkage disequilibrium ($r^2 > 0.9$) between the studied SNPs in *APOB*, haplotype analysis did not reveal any significant association. Only SNP rs10495712 in *APOB* with an unfavourable effect (OR, 2.013) on LDL-C remained significant after 10% FDR correction.

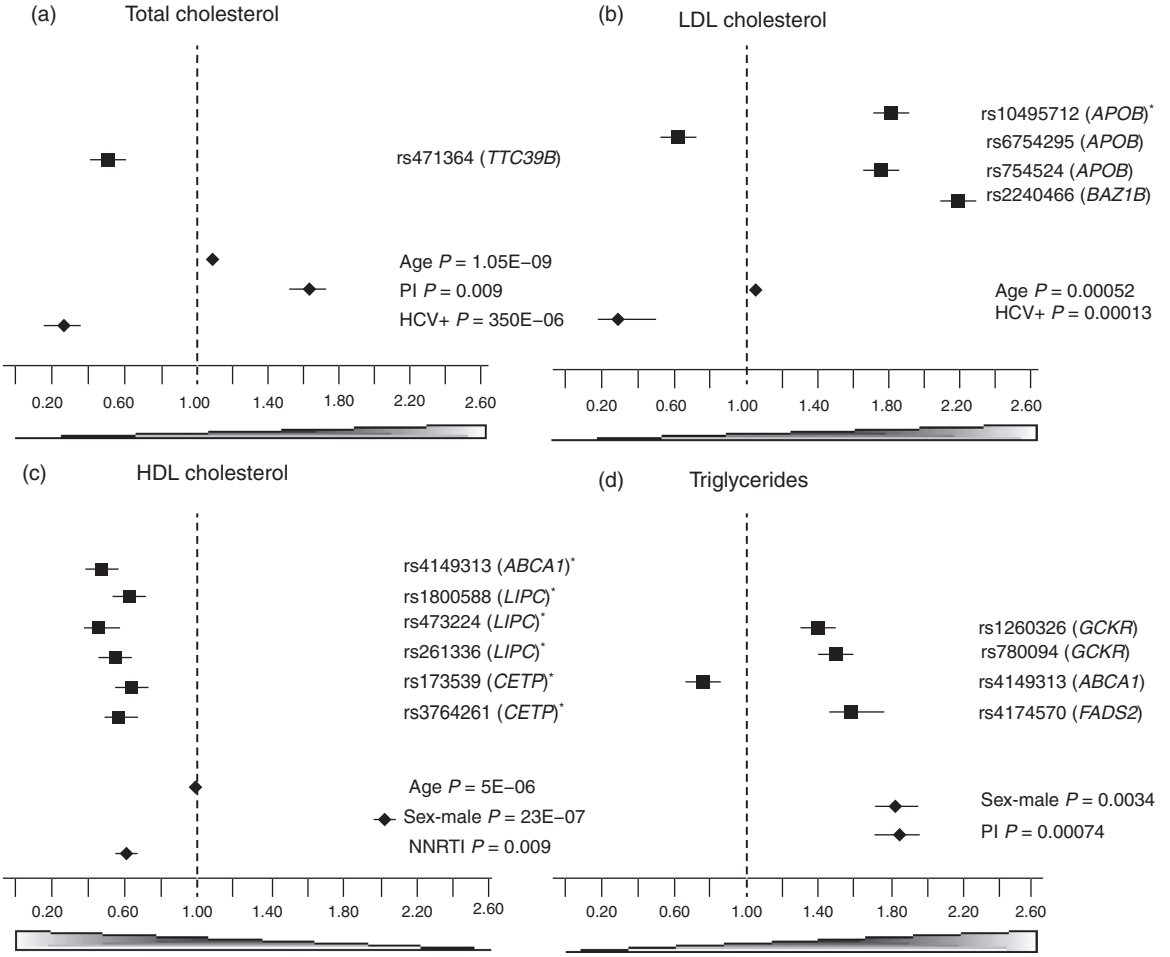

Fig. 2. Impact of genetic and nongenetic variables on dyslipidemia in the final multivariate analyses. Impact ($P < 0.01$) for (a) Total cholesterol, (b) LDL-cholesterol, (c) HDL-cholesterol and (d) Triglycerides. Results are represented as the estimated effect (OR) of the covariables and 95% confidence interval. Genetic variants are shown as black squares (■) and nongenetic variants as black rhombus (◆). HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor. *Significant genetic associations after 10% FDR correction for multiple testing.

High-density lipoprotein-cholesterol

A total of 13 SNPs (rs10487506, rs4149313, rs7903146, rs1800588, rs473224, rs261336, rs247615, rs12448528, rs173539, rs3764261, rs711752, rs708272 and rs7205804) in six genes (*LEP, ABCA1, TCF7L2, LIPC, HERPUD1* and *CETP*) showed association ($P < 0.05$) with HDL-C in the logistic regression analysis (Table_S3, http://links.lww.com/QAD/A291).

In the multi-SNP model, six SNPs in three genes showed a favourable effect on HDL-C. SNPs rs4149313 (*ABCA1*; $P = 0.000297$; OR, 0.516), rs1800588 (*LIPC*; $P = 0.002138$; OR, 0.608), rs473224 (*LIPC*; $P = 0.000307$; OR, 0.483), rs261336 (*LIPC*; $P = 0.002239$; OR, 0.572), rs173539 (*CETP*; $P = 0.002969$; OR, 0.608), rs3764261 (*CETP*; $P = 0.001521$; OR, 0.588) contributed significantly to an increase in plasma HDL-C levels (Fig. 2, Table 3, Table_S3, http://links.lww.com/QAD/A291). The results remained significant after applying a 10% FDR correction. High degrees of linkage disequilibrium ($r^2 > 0.9$) were found between the three SNPs in *LIPC* and also between the two SNPs in *CETP*. Haplotype analysis showed a protective haplotype (rs173539A/rs3764261A) in *CETP* (Table 4) associated with a favourable effect on HDL-C levels [$P = 0.000167$; OR, 0.638 (0.239–0.982)]. No associated haplotype was identified in *LIPC*. Interaction analysis was negative for all the possible gene pairs.

Triglycerides

Eleven SNPs (rs1260326, rs780094, rs1919127, rs6544713, rs5908, rs264, rs4149313, rs1800682, rs10838852, rs174547 and rs174570) in nine genes (*GCKR, C2orf16, ABCG8, HMGCR, LPL, ABCA1, FAS, OR4X1, FADS1* and *FADS2*) displayed association ($P < 0{,}05$) with triglyceride levels in the logistic regression analysis (Table _S3, http://links.lww.com/QAD/A291).

Table 4. Haplotypes associated with high-density lipoprotein-cholesterol and triglyceride levels.

	CETP	GCKR
SNPs[a]	rs173539 rs3764261	rs1260326 rs780094
Haplotype	A-A	A-A
Associated lipid fraction	HDL-C	TG
Frequency of cases	0.23	0.48
Frequency of controls	0.33	0.40
P	0.000167	0.002276
OR (L95-U95)	0.638 (0.239–0.982)	1.391 (1.281–1.888)
Effect on lipid fraction	Protective	risk

All genes with more than one SNP significantly associated with a lipid fraction ($P < 0.01$) were subjected to haplotype analysis. Combinations of SNPs in *CETP* and *GCKR* associated with HDL-C and TG levels, respectively, showed higher significance levels ($P < 0.005$) than the single-marker analysis. HDL-C, high-density lipoprotein-cholesterol; SNP, single nucleotide polymorphism; TG, triglyceride.

[a]For all markers, A is the minor allele.

In the final multi-SNP model, three SNPs showed an unfavourable effect, whereas one SNP showed a favourable effect. The SNPs that contributed significantly to increase plasma triglyceride levels were rs1260326 (*GCKR*; $P = 0.008063$; OR, 1.361), rs780094 (*GCKR*; $P = 0.003286$; OR, 1.412) and rs174570 (*FADS2*; $P = 0.005261$; OR, 1.57). Conversely, rs4149313 (*ABCA1*; $P = 0.00877$; OR, 0.683) contributed significantly to decrease plasma triglyceride levels (Fig. 2, Table 3, Table_S3, http://links.lww.com/QAD/A291). The two SNPs in *GCKR* were having high linkage disequilibrium ($r^2 > 0.9$). Haplotype analysis identified a risk haplotype in this gene (rs1260326A/rs780094A) that was associated with an unfavourable effect on triglyceride levels [$P = 0.002276$; OR, 1.391 (1.281–1.888)] (Table 4).

Contribution of nongenetic variables to plasma lipid levels (multivariate analysis)

Age was associated with plasma T-Chol [$P = 1.05E{-}09$; OR, 1.07 (1.05–1.09)], LDL-C levels [$P = 0.00052$; OR, 1.04 (1.02–1.07)] and HDL-C [$P = 5E{-}06$; OR, 0.95 (0.93–0.97)]. HCV coinfection showed a favourable association with T-Chol ($P = 3.5E{-}06$; OR, 0.31 (0.19–0.5)] and LDL-C [$P = 0.00013$; OR, 0.33 (0.2–0.58)]. However, a protease inhibitor containing ART regimen was unfavourably associated with plasma T-Chol [$P = 0.009$; OR, 1.6 (1.42–1.91)] and triglyceride [$P = 0.00074$; OR, 1.8 (1.39–1.99)] levels. First-line treatment with an NNRTI containing ART regimen [$P = 8.9E{-}03$; OR, 0.42 (0.19–0.5)] was favourably associated with plasma HDL-C levels. On the contrary, sex (male) showed an unfavourable association with both HDL-C [$P = 2.3E{-}07$; OR, 3.73 (1.16–1.44)] and triglyceride [$P = 0.0034$; OR, 1.81 (1.37–1.91)] (Fig. 2, Table_S3, http://links.lww.com/QAD/A291).

Subanalyses based on antiretroviral therapy drugs

Contribution of single nucleotide polymorphisms to plasma lipid levels depending on ritonavir-boosted protease inhibitor versus nonboosted protease inhibitor

From the global study, 72 individuals (18.9%) from the protease inhibitor group were treated with a PI/r and the other 309 (81.1%) were treated with a nonboosted protease inhibitor (Table 1). None of the SNPs showing a significant association ($P < 0.01$) in the global logistic regression model for T-Chol and triglycerides showed a significant association with an effect on these fractions depending on a nonboosted protease inhibitor versus PI/r in the Breslow–Daytest (data not shown).

Contribution of single nucleotide polymorphisms to plasma lipid levels depending on efavirenz versus nevirapine

From the global study, 227 individuals (65.8%) from the NNRTI group were treated with EFV and the other 118 (34%) were treated with NVP (Table 1). Three SNPs in

LIPC [rs1800588; $P=0.0004198$; OR, 0.39 (0.2337–0.6685)], rs473224 [$P=1.94E{-}05$; OR, 0.27 (0.1485–0.5107)], rs261336 [$P=3.35E{-}05$; OR, 0.31 (0.1789–0.5528)], which were associated with an increase of HDL-C in the global logistic regression, showed significant association with an increase of this fraction in those patients who started an EFV-containing NNRTI treatment. None of these SNPs were significant when a logistic regression analysis was performed for this subanalysis (data not shown).

Discussion

In this longitudinal study of naive HIV individuals starting ART including one protease inhibitor or one NNRTI, SNPs that have consistently been associated with dyslipidemia in the general population GWAS [25–28] were validated. Moreover, previously published association studies in HIV population related to dyslipidemia have also been considered [11–13]. In this study, nongenetic factors have been taken into account in order to explain the interindividual variation of lipid levels once ART treatment with a protease inhibitor or an NNRTI has begun. We demonstrate that nongenetic covariates such as age, sex and HCV-coinfection are also playing an important role in these lipid variations. The additive logistic regression model showed the unfavourable effect of protease inhibitors on T-Chol and triglyceride levels. On the contrary, NNRTIs present a favourable effect on HDL-C levels. However, neither protease inhibitors nor NNRTIs apparently present a direct effect on LDL-C level variations. As in the general population, significant nongenetic traits such as sex (male) and age also influence lipid fraction levels [29]. Surprisingly, a favourable association was found in our study between the presence of HCV coinfection and a decrease of T-Chol and LDL-C fractions. This could be explained by the ability of both HIV and HCV viruses, as many other infectious agents do, to impair cholesterol metabolism to satisfy their own cholesterol requirements at different stages of their life cycle [30]. Furthermore, we assessed the influence of candidate genetic variables included in the logistic regression models performed for each lipid fraction. The only genetic variation found to be associated with T-Chol fraction is a polymorphism in the tetratricopeptide repeat domain 39B gene (*TTC39B*), which has previously been described in a general population GWAS [27] to be associated with HDL-C levels. This variation regulates the *TTC39B* expression. *TTC39B* seems, in general, to function in protein–protein interactions, but it actually has no known function in humans. This variant did not overcome10% FDR correction for multiple testing.

Regarding LDL-C levels, three polymorphisms in *APOB* (rs10495712, rs6754295, rs754524) and one in *BAZ1B* (rs2240466) reached significance in the final multi-SNP model. Only rs10495712 in *APOB*, with previously described association with cholesterol and triglyceride fractions in GWAS in the general population, presented a significant association after 10% FDR. Our results show the first evidence of association between the presence of this polymorphism and the increase of LDL-C levels in HIV patients with antiretroviral treatment.

The strongest association with triglyceride levels was found between a polymorphism in *GCKR* (rs780094), encoding the glucokinase regulatory protein, previously reported in a general population GWAS [25], and an increase of this fraction. This is the first evidence of association in treated HIV population between this SNP and an unfavourable effect on triglyceride levels in the presence of a protease inhibitor containing first-line antiretroviral regimen. This association should be validated in other HIV cohorts. There were SNPs in *GCKR* (rs1260326), in the fatty acid desaturase 2, *FADS2* (rs174570), and in the ATP-binding cassette A, *ABCA1,*(rs4149313) that showed an association with triglyceride levels in the logistic regression model. An unfavourable effect was observed with an increase of triglyceride levels in the case of SNPs in *GCKR* and *FADS2*. In contrast, the effect was favourable in the case of rs4149313 in *ABCA1* with a decrease of triglyceride levels. However, the association pattern of rs4149313 is contradictory with the association found in a previously reported multiple regression analysis [13], wherein the effect was described in the opposite direction as an unfavourable variant. However, we should consider that in our analysis, these SNPs did not reach significance after 10% FDR correction for multiple testing.

Finally, the logistic regression analysis for HDL-C fractions showed a significant association with six polymorphisms in the *ABCA1*, *LIPC* and *CETP* genes after 10% FDR correction. All these SNPs were associated with a favourable effect on the increase of HDL-C in the presence of an NNRTI-containing first-line treatment. Association between rs4149313 in *ABCA1* and an increase of HDL-C levels confirmed previously reported results in HIV population [12,13]. Moreover, associations of rs173539 and rs3764261 in *CETP* showed the same effects as the ones previously published in general population GWAS [26,27] but are newly described in HIV population under ART. We describe a newly identified protective haplotype [A-A] between these two SNPs ($P=0.000167$) in *CETP*. A previously reported association between rs1800775 in *CETP* and an increase of HDL-C levels in HIV population [12] was confirmed in our study only in the univariate analysis stratified by ART, and the significance was lost in the multivariate analysis. No gene interactions were found between any of the associated genes observed for each lipid fraction. Although SNPs in *LIPC* showed a favourable association

with HDL-C when patients start an EFV-containing first-line ART regimen, these candidate polymorphisms lost association strength in the final logistic regression model performed for the NNRTI family subanalysis (EFV versus NVP). There was also a lack of association between candidate genes and protease inhibitor drug families (IP/r versus nonboosted protease inhibitor) regarding lipid variations.

In summary, our results show statistical evidence of specific polymorphisms, as well as nongenetic variables, being associated with an effect on lipid fraction levels depending on a protease inhibitor or an NNRTI containing first-line ART regimen. These variants should be validated in other cohorts and they could be used as biomarkers to identify patients at risk for dyslipidemia and to guide the rational selection of ART.

Study limitations

The strengths of this study in the field of HIV are its longitudinal design and the SNP selection based on previously reported GWA studies performed in the general population. Thus, our results allow validation of previously published results in genetic-dyslipidemia association studies in HIV population [12,13,28]. This is the first study of this kind in an HIV population with a first-line treatment, wherein complete lipid measurements of T-Chol, LDL-C, HDL-C and triglyceride have been considered. New models are required to confirm the variants implicated in ART-related dyslipidemia in HIV populations. In addition, functional studies are necessary to validate those already confirmed associations.

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Conceived and designed the experiments: M.A., E.M., J.M.G. Performed the experiments: L.E., T.E. Analysed the data: M.A., L.E., B.C. Wrote the article: M.A., L.E., B.C., E.M.

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Conflicts of interest

J.M.G. and E.M. have been a consultant on advisory boards, have participated in speakers' bureaus, have received research grants or have conducted clinical trials with Roche, Boehringer-Ingelheim, Abbott, BMS, GSK, Gilead, Janssen, Merck and Pfizer. M.A., L.E., B.C. and T.E. report no conflicts of interest relevant to this article. No other potential conflict of interest relevant to this article was reported.

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