Modeling the Influence of APOC3, APOE, and TNF Polymorphisms on the Risk of Antiretroviral Therapy–Associated Lipid Disorders

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(See the editorial commentary by Haas, on pages 1397–400.)

Background. Single-nucleotide polymorphisms in genes involved in lipoprotein and adipocyte metabolism may explain why dyslipidemia and lipoatrophy occur in some but not all antiretroviral therapy (ART)–treated individuals.

Methods. We evaluated the contribution of APOC3/H11002482C>T, H11002455T>C, and 3238C>G; H92552 and H92554 alleles of APOE; and TNF/H11002238G>A to dyslipidemia and lipoatrophy by longitudinally modeling >2600 lipid determinations and 2328 lipoatrophy assessments in 329 ART-treated patients during a median follow-up period of 3.4 years.

Results. In human immunodeficiency virus (HIV)–infected individuals, the effects of variant alleles of APOE on plasma cholesterol and triglyceride levels and of APOC3 on plasma triglyceride levels were comparable to those reported in the general population. However, when treated with ritonavir, individuals with unfavorable genotypes of APOC3 or APOE were at risk of extreme hypertriglyceridemia. They had median plasma triglyceride levels of 7.33 mmol/L, compared with 3.08 mmol/L in the absence of ART. The net effect of the APOE*APOC3*ritonavir interaction was an increase in plasma triglyceride levels of 2.23 mmol/L. No association between TNF/H11002238G>A and lipoatrophy was observed.

Conclusions. Variant alleles of APOE and APOC3 contribute to an unfavorable lipid profile in patients with HIV. Interactions between genotypes and ART can lead to severe hyperlipidemia. Genetic analysis may identify patients at high risk for severe ritonavir-associated hypertriglyceridemia.

Adverse metabolic effects of potent antiretroviral therapy (ART) have become a major concern. Hyperlipidemia increases the risk of cardiovascular disease [1]. Lipoatrophy may stigmatize the patient and is associated with insulin resistance and hyperlipidemia [2,3]. Both hyperlipidemia and lipoatrophy have been linked to advancing age, male sex, a CD4 T cell count <200 cells/μL, and the type of ART used [2,4]. The use of ritonavir (RTV) and RTV-boosted protease inhibitors (PIs) are the most significant predictors of hypercholesterolemia and hypertriglyceridemia [4], whereas use of stavudine (d4T), didanosine (ddI), and, in some studies, PIs are associated with lipoatrophy [2]. However, these complications do not occur in all patients, despite similar exposure to ART and comparable demographic, immunologic, and virologic characteristics. The reasons for this discrepancy may be related to host genetic factors.

Single-nucleotide polymorphisms (SNPs) in APOE (specifically the APOE e4 and e2 alleles) are linked to hyperlipidemia and cardiovascular events [5] in the general population. SNPs of APOC3 at positions −455 and −482 in the promoter region and at position 3238
in the 3′ untranslated region (the 30f allele) are associated with hypertriglyceridemia [6, 7]. A SNP at position −238 in the promoter region of TNF (previous designation, TNF-α) has been linked to the more-rapid onset of lipoatrophy in ART-treated patients [8], and the rare allele was found to be present more frequently in patients with HIV who have lipoatrophy than in those who do not have the condition [9]. The APOE genotype may be functionally associated with hyperlipidemia, as the role of APOE in the transport and clearance of lipoprotein remnants from the bloodstream would suggest [5]. Through the inhibition of the activity of lipoprotein lipase, APOC3 modulates lipolysis and hepatic clearance of plasma triglycerides (TGs) [6, 7]. TNF expression in adipose tissue has been implicated in lipoatrophy because of its modulation of lipolysis and adipocyte differentiation [10]. Most reports on the metabolic complications associated with APOE and APOC3 polymorphisms have been based on studies of the general population. In HIV-infected individuals, the interpretation of the data has been limited by the small number of patients studied [11, 12], contradictory findings regarding a link between APOE genotype and hyperlipidemia [12–14], and a focus on PI-treated men [13–15]. The present study used a longitudinal approach to model the influence of APOE and APOC3 polymorphisms on ART-associated hyperlipidemia, as well as the influence of the TNF −238 polymorphism on lipoatrophy, in the context of frequent changes of therapy and other contributing factors.

PATIENTS AND METHODS

Patients. Study participants were followed in the Swiss HIV Cohort Study (SHCS) (http://www.shcs.ch) and were treated for HIV infection at 1 of 7 Swiss medical centers. The genetics project of the SHCS was approved by the ethics committees of all participating centers. Patients were included if they had given written, informed consent for genetic testing.

Lipid determinations included the routine assessments of plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and TG levels. The level of non-HDL cholesterol (NHC) was calculated by subtracting the level of HDL cholesterol from the level of total cholesterol. Patients who had a Centro for Disease Control and Prevention stage C AIDS-defining event in the preceding 3 months or a CD4 T cell count <100 cells/μL were excluded, to limit confounding by HIV-associated wasting and opportunistic illness. Lipoatrophy was defined by the patient-reported presence of fat loss at ≥1 body site (face, neck, arms, legs, or buttocks) that was confirmed by the physician on physical examination. No lipoatrophy assessments were made by use of computed tomography scanning or dual-energy x-ray absorptiometry. The fasting state (with fasting defined as ≥8 h without caloric intake) was recorded each time that a blood sample was taken.

Allelic discrimination. Genomic DNA was extracted from frozen leukocyte pellets. APOC3 promoter −482C>T [7], APOC3 3′UTR 3238C>G [7], APOE coding region 2060T>C [5], and TNF promoter −238G>A [8, 9] were investigated by TaqMan allelic discrimination (Applied Biosystems) (table 1). The polymorphisms of APOC3 −455T>C, APOE 2060T>C, and APOE 2198C>T were assessed by PCR amplification and restriction fragment–length polymorphism (RFLP) analysis using either FokI [13, 16] or HhaI [17] digestion. All equivocal results were repeated and confirmed by a second observer. Results were entered into the central SHCS database in a blinded fashion.

For statistical analysis, all variant alleles were assessed individually, and, for the purpose of clarity, were reported using previously reported composite genotypes [13, 18]. For analysis of plasma TG levels [18], we assessed (1) the presence of APOE e3/e3 versus e2-containing APOE genotypes (i.e., e2/e2, e2/e3, and e2/e4) versus APOE e3/e4 and APOE e4/e4, as well as (2) APOE e3/e3 versus all alleles other than e3/e3. For the analysis of plasma cholesterol levels [18], we assessed APOE e3/e3 versus e2-containing APOE genotypes (i.e., e2/e2, e2/e3, and e2/e4) versus APOE e3/e4 and APOE e4/e4. For APOC3, the cumulative number of variant alleles was used, as reported elsewhere [13], and thus we assessed common alleles, 1 or 2 variant alleles, and all 3 variant alleles.

Statistical analysis. The data were analyzed longitudinally by modeling the individual effects of the different covariables on plasma lipid levels and on body fat loss. The study period was defined as the follow-up period beginning after the first study visit that included measurement of plasma lipid levels (available since 1999) until 22 March 2004, when information from the SHCS database was downloaded. The analysis used a well-established statistical method developed for longitudinal data analysis, population-averaged marginal modeling [19, 20]. A multivariate distribution was fitted to the data by use of score-like methods (generalized estimating equations) [19, 20]. The advantages of this approach include the ability to accommodate repeated measurements at irregular intervals, the straightforward interpretation of regression coefficients, and the computational efficiency for relatively large data sets. Covariables included in the regression model were ART regimen, age, sex, body mass index (BMI), waist circumference, fasting state, presumed mode of HIV transmission, ethnicity, diabetes mellitus status, treatment with lipodissolving agents, smoking status, CD4 T cell count, HIV load, and the genetic factors tested. The influence of ART and of lipodissolving agents on plasma lipid levels was assumed to be rapid and reversible [21]. For evaluation of the effects of the genetic

Table 1.

The table is available in its entirety in the online edition of the Journal of Infectious Diseases.
variants on lipoatrophy, the cumulative exposure to ART was used, as was reported elsewhere [2, 22].

Exploratory analyses confirmed the published effects of individual ART agents on plasma HDL cholesterol, NHC, and TG levels and on lipoatrophy [3, 4, 23]. These data supported the rationale for dividing the ART regimens into the following groups: (1) no ART at the time that a blood sample was taken, (2) PI-sparing ART, (3) single-PI ART, and (4) RTV-containing ART. For lipoatrophy analysis, the ART regimens were divided into the following groups [2, 22]: (1) ART that did not include ddI, d4T, or a PI; (2) ART that included ddI and/or d4T but no PI; (3) ART that included a PI but not ddI or d4T; and (4) ART that included ddI and/or d4T and a PI.

When the date of the assessment of plasma lipid levels did not coincide with that of the assessment of waist circumference and weight, the values of these latter covariables were imputed by linear interpolation at the date of the assessment of plasma lipid levels. For appropriate confounding adjustment, cross-sectional and longitudinal relationships between the continuous explanatory variables and the response were distinguished [20, 24, 25]. Adequate functional form was assessed using fractional polynomials [26, 27]. The correlation structure was assumed to be autoregressive [19], because the clustering was at the individual level (repeated measurements). However, when other (e.g., exchangeable) correlation structures were used, the results were similar. Goodness of fit was evaluated visually by comparing mean observed and predicted measurements on time plots of values in strata defined by polymorphisms and ART group [20].

For analysis of lipoatrophy, the dependent variable was binary, and the logit link was used. The correlation structure was assumed to be exchangeable, because exploratory analyses indicated that lipoatrophy was rarely reversible over the follow-up period. A robust sandwich estimate of variances-covariances was used, which produces valid SEs even if the correlation structure was misspecified [19, 20].

Two-way as well as 3-way interactions between genotypes and ART groups were first tested globally. If the global test was significant, subcategories contributing to the $P$ value were screened for, and significant and consistent strata were retained. The re-grouping of subcategories into strata was performed using the global $\chi^2$ test, to address the issue of multiple comparisons.
### Table 3. Multivariate analysis of the contribution of demographic factors, antiretroviral therapy (ART), and variant alleles of APOE and APOC3 to hyperlipidemia in 329 study participants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HDL cholesterol</th>
<th>Non-HDL cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
</tr>
<tr>
<td></td>
<td>change, mmol/L</td>
<td>change, mmol/L</td>
<td>change, mmol/L</td>
</tr>
<tr>
<td>Baseline age (change per year)</td>
<td>−0.01 (−0.02 to −0.002)</td>
<td>0.07 (0.04 to −0.10)</td>
<td>0.04 (−0.01 to 0.08)</td>
</tr>
<tr>
<td>Male sex</td>
<td>−0.18 (−0.3 to −0.06)</td>
<td>−0.09 (−0.45 to 0.26)</td>
<td>−0.36 (−0.85 to 0.13)</td>
</tr>
<tr>
<td>Fasting state</td>
<td>−0.01 (−0.03 to 0.02)</td>
<td>0.05 (−0.03 to 0.14)</td>
<td>−0.19 (−0.37 to 0.02)</td>
</tr>
<tr>
<td>ART</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI sparing</td>
<td>0.11 (0.03–0.17)</td>
<td>−0.27 (−0.49 to −0.04)</td>
<td>0.23 (−0.29 to 0.75)</td>
</tr>
<tr>
<td>Single PI</td>
<td>0.06 (0.01 to 0.13)</td>
<td>0.29 (0.07–0.52)</td>
<td>0.20 (−0.31 to 0.71)</td>
</tr>
<tr>
<td>RTV containing</td>
<td>−0.01 (−0.08 to 0.07)</td>
<td>0.65 (0.41–0.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Treated with lipid-lowering agents</td>
<td>−0.03 (−0.98 to 0.03)</td>
<td>−0.29 (−0.50 to 0.09)</td>
<td>0.60 (0.20–0.99)</td>
</tr>
<tr>
<td>APOE genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ε2/ε2, ε3/ε2, and ε4/ε4</td>
<td>−0.04 (−0.16 to 0.07)</td>
<td>0.02 (−0.30 to 0.33)</td>
<td>0.23 (0.19–0.26)</td>
</tr>
<tr>
<td>ε3/ε4 and ε4/ε4</td>
<td>−0.06 (−0.15 to 0.04)</td>
<td>0.27 (0.02–0.53)</td>
<td>0.38 (0.26–0.50)</td>
</tr>
<tr>
<td>All genotypes other than ε3/ε3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>APOC3 genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or 2 variant alleles</td>
<td>0.26 (−0.05 to 0.11)</td>
<td>0.523 (−0.19 to 0.40)</td>
<td>0.088 (−0.28 to 0.35)</td>
</tr>
<tr>
<td>All 3 variant alleles</td>
<td>0.03 (−0.07 to 0.14)</td>
<td>0.534 (−0.27 to 0.05)</td>
<td>0.052 (0.16–0.98)</td>
</tr>
</tbody>
</table>

NOTE. The reference for the regression model represents a mean plasma HDL cholesterol level of 1.25 mmol/L (95% confidence interval [CI], 1.05–1.44), a mean non–high density lipoprotein (HDL) cholesterol level of 4.87 mmol/L (95% CI, 4.33–5.42), and a mean plasma triglyceride level of 1.57 mmol/L (95% CI, 1.78–2.35) for a 43-year-old woman whose plasma lipid levels were assessed in the nonfasting state, had a body mass index (BMI) of 23 kg/m², had a waist circumference of 85 cm, had a CD4 T cell count of 500 cells/µL, had a viral load of 1.7 log₁₀ copies/mL, was white, was not diabetic, was a nonsmoker, was not being treated with ART, was not being treated with lipid-lowering agents, was transmitted HIV by heterosexual sex, and had the common APOE and APOC3 genotypes. Values are adjusted for all the variables shown, as well as for diabetes mellitus status, ethnicity, smoking status, BMI, waist circumference, and viral load. For continuous variables, the effect on the plasma lipid level of each variable represents the impact of a 1-unit increase. For categorical variables, the effect represents the impact that the indicated variable has on plasma lipid levels compared with the reference value. NA, not applicable; PI, protease inhibitor; RTV, ritonavir.

Patients who had elevated plasma HDL cholesterol, NHC, and TG levels were matched by age (±5 years), BMI (±3 units), sex, and ART group to patients who had normal values for these lipid parameters, and a single-time-point, cross-sectional analysis was performed. All statistical analyses were conducted using Stata (version 8.2; StataCorp).

**RESULTS**

**Patients.** The clinical characteristics of the study patients (n = 329) are summarized in table 2. We compared their characteristics with those of the entire population of patients enrolled in the SHCS who had ≥1 follow-up visit during 1999–2003 (n = 6724). Compared with this population of the SHCS, the study patients were more likely to have suppressed HIV viremia, the duration of their follow-up period was longer, and there were fewer deaths and dropouts. These characteristics define the selection bias of patients in the SHCS Genetic Cohort, who were chosen for the quality of their clinical documentation and their ability to sign the consent form for genetic analysis.

The allelic frequencies were as follows: APOC3 −455T→C, 0.410; APOC3 −482C→T, 0.303; APOC3 3238C→G, 0.092; APOE 2060T→C, 0.093; APOE 2198C→T, 0.055; and TNF 238G→A, 0.035. These were comparable to the published allelic frequencies in ethnically similar populations [28]. The distribution of the genotypes was as follows: APOE ε3/ε3, 238 patients (72.3%); APOE genotypes other than ε3/ε3, 91 patients (27.7%); ε2/ε2, ε2/ε3, or ε2/ε4, 35 patients [10.6%]; ε3/ε4 or ε4/ε4, 56 patients [17.0%]; common alleles of APOC3, 120 patients (36.5%); 1 or 2 variant alleles of APOC3, 150 patients (45.6%); and all 3 variant alleles of APOC3, 59 patients (17.9%).

During the follow-up period, a total of 2608 determinations of plasma cholesterol (both HDL and NHC) levels (mean, 7.4 determinations of plasma cholesterol levels/patient) and 2741 determinations of plasma TG levels (mean, 7.8 determinations of plasma TG levels/patient) were recorded (range, 3–18 total lipid determinations/patient). The mean number of determinations of plasma TG levels during the follow-up period was comparable in subjects with low and high median plasma TG levels (patients with low median plasma TG levels, 7.97 determinations/patient; patients with high median plasma TG levels, 8.25 determinations/patient); the median number of determinations was identical (7 determinations/patient). Thus, potential bias due to overrepresentation of subjects with high...
Table 4. Interactions between genotypes and antiretroviral therapy (ART).

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Plasma level</th>
<th>HDL cholesterol</th>
<th>Non-HDL cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART*APOE</td>
<td>.799</td>
<td>.087</td>
<td>.474</td>
<td></td>
</tr>
<tr>
<td>ART*APOC3</td>
<td>.202</td>
<td>.100</td>
<td>.233</td>
<td></td>
</tr>
<tr>
<td>APOE*APOC3</td>
<td>.738</td>
<td>.197</td>
<td>.025(^a)</td>
<td></td>
</tr>
<tr>
<td>ART<em>APOE</em>APOC3</td>
<td>.584</td>
<td>.173</td>
<td>.003(^b)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. All data are *P* values as determined by global x\(^2\) test. HDL, high-density lipoprotein.

\(^a\) The only genotype category that contributed significantly was APOE genotypes other than \(ε2/ε3\), in association with all 3 variant alleles of APOC3.

\(^b\) The only ART category that contributed significantly was ART containing ritonavir in patients who have APOE genotypes other than \(ε2/ε3\) in association with all 3 variant alleles of APOC3.

plasma TG levels is unlikely. The median plasma lipid levels during the follow-up period are shown in table 2. Results were unchanged when plasma lipid levels in patients who had an AIDS-defining event in the preceding 3 months or a CD4 T cell count <100 cells/μL were included.

**Effects of ART regimen on plasma lipid levels.** There were a total of 9169 determinations of the type of ART regimen in use during the study period. No ART was in use at 1078 (11.8%) time points. PI-sparing ART, single-PI ART, and RTV-containing ART were in use at 2965 (32.3%), 3242 (35.4%), and 1884 (20.5%) time points, respectively. The influence of ART regimen on plasma lipid levels is summarized in table 3. The population-averaged plasma HDL cholesterol level was higher in patients treated with PI-sparing ART (\(P = .006\)). The plasma NHC level was lower in patients treated with PI-sparing ART (\(P = .019\)) and higher in patients treated with single-PI ART (\(P = .01\)) or RTV-containing ART (\(P < .001\)). Plasma TG levels were significantly higher only in patients treated with RTV-containing ART (\(P < .001\)). Because RTV can be used at different dosages, we compared plasma TG levels in patients treated with RTV alone (600 mg of RTV twice daily) or in combination with lopinavir (100 mg of RTV twice daily) or saquinavir (400 mg of RTV twice daily). The median plasma TG levels during the follow-up period in these 3 groups of patients were comparable (3.19 vs. 3.00 vs. 2.89 mmol/L, respectively; \(P = .63\)). Thus, patients treated with RTV were analyzed as a single group. As was reported elsewhere [29], there was a trend toward higher mean plasma TG levels in patients treated with PI-sparing ART that contained efavirenz (0.47 mmol/L [95% confidence interval (CI), −0.08 to 1.02 mmol/L] increase in plasma TG levels; \(P = .09\)).

**Effects of variant alleles on plasma TG levels.** APOE genotypes other than the common \(ε3/ε3\) genotype were associated with higher mean plasma TG levels (\(P < .001\)) (table 3). The increase in plasma TG levels was 0.9 mmol/L (95% CI, 0.43–1.36 mmol/L) (\(P < .001\)) for APOE genotypes \(ε2/ε2\), \(ε3/ε2\), \(ε4/ε2\), and \(ε4/ε4\) and was 0.61 mmol/L (95% CI, 0.23–1.00 mmol/L) (\(P = .002\)) for APOE genotypes \(ε3/ε4\) and \(ε4/ε4\). Thus, as
regards plasma TG levels, APOE genotypes other than e3/e3 were analyzed as a single group. Plasma TG levels were significantly elevated in patients who had all 3 variant alleles of APOC3 (P = .006) but not in patients who had only 1 or 2 variant alleles of APOC3 (P = .83).

The most unfavorable composite genotype consisted of APOE genotypes other than e3/e3 plus all 3 variant alleles of APOC3 (table 4). The predicted net effect of the APOE*APOC3 interaction was a mean 1.24 mmol/L (95% CI, 0.45–2.03 mmol/L) increase in plasma TG levels (P = .02). This unfavorable composite genotype was associated with elevations of plasma TG levels in patients in all ART groups; however, the most striking genotype-ART interaction was observed when patients who had the unfavorable composite genotype were treated with RTV-containing ART (figure 1). Here, the predicted net effect of the APOE*APOC3*RTV interaction was a mean 2.23 mmol/L (95% CI, 1.33–3.14 mmol/L) increase in plasma TG levels (P < .001). These estimates represent the contribution of the interactions in addition to the specific effects of the covariables listed in table 3. Thus, the mean plasma TG level in patients who had the unfavorable composite genotype was 3.08 mmol/L if no ART was used and increased to 7.33 mmol/L when RTV was used in the regimen (P < .001) (figure 1).

When patients were exposed to RTV, extreme hypertriglyceridemia (defined as at least 2 determinations at which plasma TG levels were above the 95th percentile of the study population [≥7.0 mmol/L]) was recorded in 4 (15.4%) of 26 patients who had common alleles of APOE and APOC3, in 3 (20.0%) of 15 patients who had all 3 variant alleles of APOC3 and common alleles of APOE, in 1 (11.1%) of 9 patients who had APOE non-e3/e3 genotypes and common alleles of APOC3, but in 6 (60.0%) of 10 patients who had the unfavorable composite genotype (P = .026). The TNF −238G−A polymorphism had no effect on plasma TG levels (P = .973).

An additional, single-time-point, cross-sectional analysis of 75 patients who had plasma TG levels ≥2.3 mmol/L and 75 control subjects who had normal plasma TG levels and were matched to patients by age, BMI, sex, and ART group was underpowered for detection of the deleterious effects of variant alleles of APOC3 or APOE. For example, individuals who had APOE non-e3/e3 genotypes had an odds ratio of 1.41 (95% CI, 0.65–3.06) for the development of hypertriglyceridemia (P = .379).

**Effects of variant alleles on plasma NHC and HDL cholesterol levels.** The e3/e4 and e4/e4 APOE genotypes were associated with elevated plasma NHC levels (P = .038) (table 3). As expected, the APOE genotype had no influence on plasma HDL cholesterol levels. There was a trend toward lower plasma NHC levels in patients who had 1 or 2 variant alleles of APOC3 (P = .088) and in patients who had all 3 variant alleles (P = .052). No significant interaction between various genotypes and ART group were identified (table 4). No effect of the TNF −238G−A polymorphism on either plasma HDL cholesterol levels or plasma NHC levels was seen (P = .592 and P = .881, respectively).

Lipid-lowering agents were given more frequently to patients who had APOE genotypes other than e3/e3 (12.2% of determinations) and to patients who had variant alleles of APOC3 (10.8% of determinations) than to patients who had the APOE e3/e3 genotype (8.7% of determinations) (P = .007) or to patients who had the common alleles of APOC3 (7.6%) (P = .023); the models were adjusted for treatment with lipid-lowering agents.

**Lipoatrophy.** A total of 2328 assessments of lipoatrophy (mean, 7.1 assessments/patient; range, 3–9 assessments/patient) were made during the follow-up period in 325 patients. The prevalence of lipoatrophy was 25.2%. Baseline age (P = .004), BMI (P = .016), and waist circumference (P = .004) were associated with lipoatrophy (table 5). Lipoatrophy was associated with elevated plasma NHC levels in patients who had the unfavorable composite genotype (P < .001) and in patients who had all 3 variant alleles (P = .026). The odds ratio for the development of lipoatrophy was higher in patients who had 3 variant alleles of APOC3 (P = .046) but was not higher in patients who had the TNF −238G−A polymorphism (P = .625).

**DISCUSSION**

We report on the identification of a significant genotype-ART interaction that may lead to severe hyperlipidemia in HIV-
infected patients treated with ART. Known genetic variants in APOC3 and APOE contributed significantly to elevations in plasma TG levels to a degree comparable to factors such as age or the use of RTV-containing ART. However, the effect of unfavorable APOC3 alleles was observed only in patients who received ART. Variant alleles of APOC3 and APOE had no significant effect on plasma HDL cholesterol levels, whereas the effect of variant alleles of APOE on plasma NHC levels was additive to that of single-PI ART. Our results extend the findings reported by Fauvel et al. [13], who documented an association between variant alleles of APOC3 and hypertriglyceridemia in 60 men treated with PIs.

The most relevant finding of our study is the identification of a complex genotype-ART interaction that may lead to extreme hypertriglyceridemia when individuals with an unfavorable composite APOC3-APOE genotype are exposed to RTV. Such a phenotypic expression of a latent genetic predisposition to hyperlipidemia has been reported by Rodondi et al. [18]. In this pharmacogenetic study, these authors identified an elevated risk of future hyperlipidemia in APOE ε2 and ε4 carriers who developed hypertriglyceridemia during retinoic acid treatment for acne. Conversely, patients who did not have elevations in their plasma TG levels during retinoic acid therapy seemed to be protected against future hyperlipidemia.

We did not identify a genetic predisposition to lipoatrophy in patients in our data set who had the TNF-238G>A polymorphism. This is in contrast to the findings of 2 other studies [8, 9]. Nolan et al. [8] used a time-to-event model to evaluate the occurrence of lipoatrophy in 191 white men. The frequency of the rare allele (f = 0.065) was higher than that in the present study (f = 0.035). After 2 years of follow-up, the effect of the TNF polymorphism was modest in the study by Nolan et al., but the effect became more pronounced over time. It is possible that we might identify a significant effect of TNF-238G>A with an extended follow-up period. The greater diagnostic accuracy in the study by Nolan et al. [8] (by the use of radiological assessments of body composition) may further explain the differences between the results of the studies. Although there was an association between lipoatrophy and variant alleles of APOC3, the biological mechanism is unclear, and this association needs to be confirmed in other patient populations before it can be considered a true finding.

Longitudinal modeling represents a powerful approach to quantitating the individual contribution of genetic factors and effects of ART in the context of complex traits. We exploited prospectively collected data consisting of >2600 lipid determinations in 329 individuals, during a study period of ≥3 years. This allowed us to evaluate the influence of the polymorphisms in APOC3 and APOE as well as multiple intervening factors of relevance to plasma lipid levels (age, sex, ethnicity, smoking status, mode of HIV transmission, ART group, CD4 T cell count, HIV viremia, BMI, fasting state, treatment with lipid-lowering agents, and diabetes mellitus status). The study patients served as their own controls, through multiple changes in ART and periods when ART was not used. This approach was selected in response to the well-recognized dilemmas in genetic association studies, which regard, particularly, the issues of statistical power, phenotype definition, and the use of appropriate statistical tools. We also attempted a conventional matched control analysis using a single time point, which proved underpowered for identification of the genetic effects. Potential bias arising from overrepresentation of subjects with high plasma lipid levels seems unlikely, because we found that the frequency of determinations was comparable in subjects with low and high plasma lipid levels.

The relatively high frequency of variant alleles of APOE and APOC3 might suggest a potential role for genetic testing before initiation of ART. This merits formal evaluation in a randomized trial. For example, 27.7% of patients in this study were carriers of variant alleles of APOE, 17.9% were carriers of all 3 variant alleles of APOC3, and 5.8% were carriers of both. The genotyping of treatment-naive patients might be an efficient method to determine the advisability of the administration of RTV-containing ART. Given the risk of hypertriglyceridemia, it might be prudent to select an alternative ART regimen for patients who have unfavorable genotypes.

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References


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