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

Philip E. Tarr,[1] Patrick Taffé,[3] Gabriela Bleiber,[2] Hansjakob Furrer,[5] Margalida Rotger,[2] Raquel Martinez,[2] Bernard Hirschel,[8] Manuel Battegay,[9] Rainer Weber,[6] Pietro Vernazza,[7] Enos Bernasconi,[10] Roger Darioli,[4] Martin Rickenbach,[3] Bruno Ledergerber,[6] Amalio Telenti,[1,2] and the Swiss HIV Cohort Study[a]

[1]Infectious Diseases Service, University Hospital, [2]Institute of Microbiology, University of Lausanne, [3]Swiss HIV Cohort Study Data Center, and [4]Lipidology, University Medical Policlinic, Lausanne, and [5]Infectious Diseases, University Hospital, Bern, [6]Infectious Diseases, University Hospital, Zurich, [7]Infectious Diseases, Kantonsspital, St. Gallen, [8]Infectious Diseases, University Hospital, Geneva, [9]Infectious Diseases, University Hospital, Basel, and [10]Ospedale Civico, Lugano, Switzerland

(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* −482C→T, −455T→C, and 3238C→G; ε2 and ε4 alleles of *APOE*; and *TNF* −238G→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* −238G→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 ε4* and *ε2* 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

Received 26 July 2004; accepted 8 November 2004; electronically published 22 March 2005.

Reprints or correspondence: Dr. Philip E. Tarr, Infectious Diseases Service, University Hospital, CHUV BH 07-865, 1011 Lausanne, Switzerland (philip.tarr@chuv.ch).

Financial support: Swiss HIV Cohort Study (SHCS; study project 394). The SHCS is supported by the Swiss National Science Foundation (project no. 3347-069366/1).

[a] Members of the Swiss HIV Cohort Study are listed after the text.

The Journal of Infectious Diseases 2005;191:1419–26

0022-1899/2005/19109-0007$15.00

in the 3′ untranslated region (the *Sst*I 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 Centers 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 *Fok*I [13, 16] or *Hha*I [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* ε3/ε3 versus ε2-containing *APOE* genotypes (i.e., ε2/ε2, ε2/ε3, and ε2/ε4) versus *APOE* ε3/ε4 and *APOE* ε4/ε4, as well as (2) *APOE* ε3/ε3 versus all alleles other than ε3/ε3. For the analysis of plasma cholesterol levels [18], we assessed *APOE* ε3/ε3 versus ε2-containing *APOE* genotypes (i.e., ε2/ε2, ε2/ε3, and ε2/ε4) versus *APOE* ε3/ε4 and *APOE* ε4/ε4. 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 lipid-lowering agents, smoking status, CD4 T cell count, HIV load, and the genetic factors tested. The influence of ART and of lipid-lowering 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.*

Table 2. Patient characteristics.

Characteristic	Study participants (n = 329)	SHCS participants (n = 6724)
Baseline age, median (IQR), years	39.8 (35.5–47.3)	38.2 (33.3–44)
Men/women, no. (% men)	261/68 (79.3)	4619/2105 (68.7)
Ethnicity, no. (%)		
White	289 (87.8)	5290 (78.7)
Black	23 (7)	690 (10.3)
Hispanic	9 (2.7)	116 (1.7)
Asian	6 (1.8)	186 (2.8)
Unknown/other	2 (0.6)	442 (6.6)
CD4 T cell count, median (IQR), cells/μL		
Baseline	407 (256–602)	399 (237–592)
During follow-up period	443 (298–629)	426 (275–614)
Presumed mode of HIV transmission, no. (%)		
Homosexual sex	139 (42.2)	2232 (33.2)
Heterosexual sex	113 (34.3)	2476 (36.8)
Intravenous drug use	61 (18.5)	1773 (26.3)
Unknown	16 (4.9)	243 (3.6)
Viral load, no (%) with <400 RNA copies/mL		
Baseline	246 (80.3)	3276 (51.8)
During follow-up period	2182 (84)	22,575 (64.5)
Follow-up period		
Duration, median (IQR), years	3.4 (3.2–3.6)	2.8 (1.2–3.4)
Dropouts during, no. (%)	7 (2.1)	1099 (16.3)
Deaths during, no. (%)	2 (0.6)	380 (5.6)
Treated with lipid-lowering agents, no. (%)	48 (14.6)	580 (8.6)
Diabetes mellitus, no. (%)	15 (4.6)	166 (2.5)
Plasma HDL cholesterol level during follow-up period, median (IQR), mmol/L	1.2 (0.9–1.4)	1.2 (0.9–1.4)
Plasma non-HDL cholesterol level during follow-up period, median (IQR), mmol/L	4.1 (3.2–5)	3.8 (3–4.7)
Plasma triglyceride level during follow-up period, median (IQR), mmol/L	2 (1.3–3.2)	1.7 (1.1–2.8)
Assessments at which lipoatrophy was present during follow-up period, no. (%)	519 (26.8)	5254 (20.5)

NOTE. HDL, high-density lipoprotein; IQR, interquartile range; SHCS, Swiss HIV Cohort Study.

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 regrouping of subcategories into strata was performed using the global χ^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.

	Effect on plasma level					
	HDL cholesterol		Non-HDL cholesterol		Triglycerides	
Variable	Mean (95% CI) change, mmol/L	*P*	Mean (95% CI) change, mmol/L	*P*	Mean (95% CI) change, mmol/L	*P*
Baseline age (change per year)	−0.01 (−0.02 to −0.002)	.025	0.07 (0.04 to −0.10)	<.001	0.04 (−0.01 to 0.08)	.101
Male sex	−0.18 (−0.3 to −0.06)	.004	−0.09 (−0.45 to 0.26)	.610	−0.36 (−0.85 to 0.13)	.153
Fasting state	−0.01 (−0.03 to 0.02)	.500	0.05 (−0.03 to 0.14)	.219	−0.18 (−0.37 to 0.02)	.073
ART						
PI sparing	0.11 (0.03–0.17)	.006	−0.27 (−0.49 to −0.04)	.019	0.23 (−0.29 to 0.75)	.381
Single PI	0.06 (−0.01 to 0.13)	.080	0.29 (0.07–0.52)	.010	0.20 (−0.31 to 0.71)	.448
RTV containing	−0.01 (−0.08 to 0.07)	.823	0.65 (0.41–0.89)	<.001	1.71 (1.16–2.25)	<.001
Treated with lipid-lowering agents	−0.03 (−0.98 to 0.03)	.298	−0.29 (−0.50 to −0.09)	.005	0.60 (0.20–0.99)	.003
APOE genotype						
ε2/ε2, ε3/ε2, and ε4/ε2	−0.04 (−0.16 to 0.07)	.467	0.02 (−0.30 to 0.33)	.917	NA	…
ε3/ε4 and ε4/ε4	−0.06 (−0.15 to 0.04)	.236	0.27 (0.02–0.53)	.038	NA	…
All genotypes other than ε3/ε3	NA	…	NA	…	0.73 (0.41–1.04)	.001
APOC3 genotype						
1 or 2 variant alleles	0.26 (−0.05 to 0.11)	.523	−0.19 (−0.40 to 0.03)	.088	0.03 (−0.28 to 0.35)	.828
All 3 variant alleles	0.03 (−0.07 to 0.14)	.534	−0.27 (−0.55 to 0.002)	.052	0.57 (0.16–0.98)	.006

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, 0.78–2.35) 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/m^2, had a waist circumference of 85 cm, had a CD4 T cell count of 500 cells/μL, had a viral load of 1.7 $\log_{10}$ 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).

Interaction	Plasma level		
	HDL cholesterol	Non-HDL cholesterol	Triglycerides
ART**APOE*	.799	.087	.474
ART**APOC3*	.202	.100	.233
*APOE***APOC3*	.738	.197	.025[a]
ART**APOE***APOC3*	.584	.173	.003[b]

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

[a] The only genotype category that contributed significantly was *APOE* genotypes other than ε3/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 ε3/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, and ε4/ε2 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

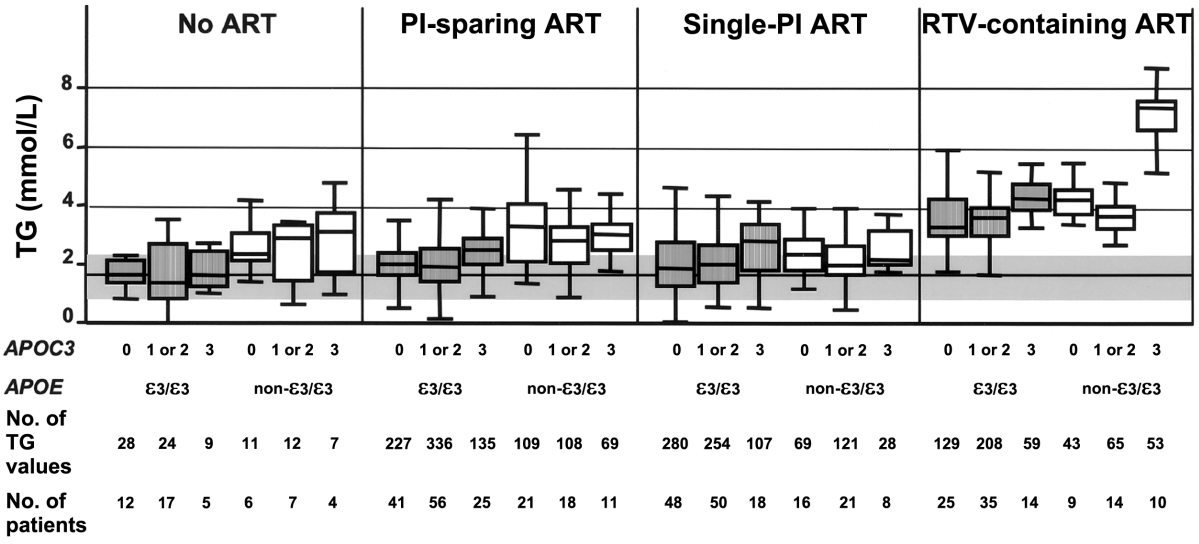

Figure 1. Effect of genotype–antiretroviral therapy (ART) interactions on adjusted plasma triglyceride (TG) levels. The medians plus interquartile ranges *(boxes)* and upper and lower adjacent values *(whiskers)* show the adjusted distribution of plasma TG levels. The median *(black horizontal line)* and estimated range *(gray horizontal stripe)* of plasma TG levels for patients receiving no ART regimen who have common *APOE* or *APOC3* alleles is shown. Patients are grouped according to the number of variant alleles of *APOC3* they have (none, 1 or 2 variant alleles, or all 3 variant alleles), and patients who have the common *APOE* ε3/ε3 genotype *(gray boxes)* are compared with patients who have *APOE* non-ε3/ε3 genotypes *(white boxes)*. PI, protease inhibitor; RTV, ritonavir.

Table 5. Adjusted contribution of demographic factors, antiretroviral therapy (ART), and genotypes to lipoatrophy in study participants.

Variable	Odds ratio (95% CI)	*P*
Baseline age, increase per year	1.042 (1.013–1.071)	.004
Female sex	0.508 (0.235–1.096)	.084
Cumulative exposure to ART, per month		
No ddI or d4T or PI	1.000 (0.988–1.011)	.999
ddI and/or d4T but no PI	1.035 (1.018–1.053)	<.001
PI but no ddI or d4T	1.009 (0.997–1.021)	.153
ddI and/or d4T and PI	1.021 (1.009–1.033	<.001
BMI at baseline, increase per point	0.719 (0.55–0.941)	.016
Waist circumference, increase per cm	1.148 (1.046–1.26)	.004
APOE genotype		
ε2/ε2, ε3/ε2, or ε4/ε2	1.464 (0.73–2.939)	.283
ε3/ε4 or ε4/ε4	1.152 (0.621–2.138)	.653
APOC3 genotype		
1 or 2 variant alleles	1.044 (0.623–1.751)	.870
All 3 variant alleles	1.870 (1.013–3.453)	.046
TNF −238G→A	1.261 (0.494–3.215)	.625

NOTE. Multivariate analysis, adjusted for time (increase in age), diabetes mellitus status, ethnicity, smoking status, body mass index (BMI), waist circumference, and viral load. CI, confidence interval; d4T, stavudine; ddI, didanosine; PI, protease inhibitor.

regards plasma TG levels, *APOE* genotypes other than ε3/ε3 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 ε3/ε3 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-ε3/ε3 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-ε3/ε3 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 ε3/ε4 and ε4/ε4 *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 ε3/ε3 (12.2% of determinations) and to patients who had variant alleles of *APOC3* (10.8% of determinations) than to patients who had the *APOE* ε3/ε3 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 cumulative exposure to ddI/d4T, irrespective of the associated use of a PI ($P < .001$). Exposure to a PI in the absence of ddI and/or d4T was not associated with lipoatrophy ($P = .153$). 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.

SWISS HIV COHORT STUDY

The members of the Swiss HIV Cohort Study are M. Battegay, E. Bernasconi, J. Böni, H. Bucher, P. Bürgisser, S. Cattacin, M. Cavassini, R. Dubs, M. Egger, L. Elzi, P. Erb, K. Fantelli, M. Fischer, M. Flepp, A. Fontana, P. Francioli (president), H. Furrer (chairman of the Clinical and Laboratory Committee), M. Gorgievski, H. Günthard, B. Hirschel, L. Kaiser, C. Kind, T. Klimkait, U. Lauper, B. Ledergerber, M. Opravil, F. Paccaud, G. Pantaleo, L. Perrin, J.-C. Piffaretti, M. Rickenbach (head of the Data Center), C. Rudin (chairman of the Mother and Child Substudy), P. Schmid, J. Schüpbach, R. Speck, A. Telenti, A. Trkola, P. Vernazza (chairman of the Scientific Board), R. Weber, and S. Yerly.

References

1. Friis-Moller N, Sabin CA, Weber R, et al. Combination antiretroviral therapy and the risk of myocardial infarction. N Engl J Med **2003**; 349:1993–2003.
2. Bernasconi E, Boubaker K, Junghans C, et al. Abnormalities of body fat distribution in HIV-infected persons treated with antiretroviral drugs: the Swiss HIV Cohort Study. J Acquir Immune Defic Syndr **2002**; 31:50–5.
3. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor–associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. Lancet **1999**; 353:2093–9.
4. Fontas E, van Leth F, Sabin CA, et al. Lipid profiles in HIV-infected

patients receiving combination antiretroviral therapy: are different antiretroviral drugs associated with different lipid profiles? J Infect Dis **2004**; 189:1056–74.
5. Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet **2000**; 1:507–37.
6. Li WW, Dammerman MM, Smith JD, Metzger S, Breslow JL, Leff T. Common genetic variation in the promoter of the human apo CIII gene abolishes regulation by insulin and may contribute to hypertriglyceridemia. J Clin Invest **1995**; 96:2601–5.
7. Dammerman M, Sandkuijl LA, Halaas JL, Chung W, Breslow JL. An apolipoprotein CIII haplotype protective against hypertriglyceridemia is specified by promoter and 3′ untranslated region polymorphisms. Proc Natl Acad Sci USA **1993**; 90:4562–6.
8. Nolan D, Moore C, Castley A, et al. Tumour necrosis factor–α gene −238G/A promoter polymorphism associated with a more rapid onset of lipodystrophy. AIDS **2003**; 17:121–3.
9. Maher B, Alfirevic A, Vilar FJ, Wilkins EG, Park BK, Pirmohamed M. TNF-α promoter region gene polymorphisms in HIV-positive patients with lipodystrophy. AIDS **2002**; 16:2013–8.
10. Bastard JP, Caron M, Vidal H, et al. Association between altered expression of adipogenic factor SREBP1 in lipoatrophic adipose tissue from HIV-1–infected patients and abnormal adipocyte differentiation and insulin resistance. Lancet **2002**; 359:1026–31.
11. Lister RK, Youle M, Nair DR, Winder AF, Rustin MH. Latent dysbetalipoproteinaemia precipitated by HIV-protease inhibitors. Lancet **1999**; 353:1678.
12. Grunfeld C, Doerrler W, Pang M, Jensen P, Weisgraber KH, Feingold KR. Abnormalities of apolipoprotein E in the acquired immunodeficiency syndrome. J Clin Endocrinol Metab **1997**; 82:3734–40.
13. Fauvel J, Bonnet E, Ruidavets JB, et al. An interaction between apo C-III variants and protease inhibitors contributes to high triglyceride/low HDL levels in treated HIV patients. AIDS **2001**; 15:2397–406.
14. Behrens G, Schmidt HH, Stoll M, Schmidt RE. ApoE genotype and protease-inhibitor–associated hyperlipidaemia. Lancet **1999**; 354:76.
15. Bonnet E, Ruidavets JB, Tuech J, et al. Apoprotein c-III and E-containing lipoparticles are markedly increased in HIV-infected patients treated with protease inhibitors: association with the development of lipodystrophy. J Clin Endocrinol Metab **2001**; 86:296–302.
16. Groenendijk M, Cantor RM, Blom NH, Rotter JI, de Bruin TW, Dallinga-Thie GM. Association of plasma lipids and apolipoproteins with the insulin response element in the apoC-III promoter region in familial combined hyperlipidemia. J Lipid Res **1999**; 40:1036–44.
17. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hha*I. J Lipid Res **1990**; 31: 545–8.
18. Rodondi N, Darioli R, Ramelet AA, et al. High risk for hyperlipidemia and the metabolic syndrome after an episode of hypertriglyceridemia during 13-*cis* retinoic acid therapy for acne: a pharmacogenetic study. Ann Intern Med **2002**; 136:582–9.
19. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics **1986**; 42:121–30.
20. Diggle PJ. Analysis of longitudinal data. Oxford: Oxford University Press, **2002**.
21. Purnell JQ, Zambon A, Knopp RH, et al. Effect of ritonavir on lipids and post-heparin lipase activities in normal subjects. AIDS **2000**; 14: 51–7.
22. Lichtenstein KA, Ward DJ, Moorman AC, et al. Clinical assessment of HIV-associated lipodystrophy in an ambulatory population. AIDS **2001**; 15:1389–98.
23. Periard D, Telenti A, Sudre P, et al. Atherogenic dyslipidemia in HIV-infected individuals treated with protease inhibitors. The Swiss HIV Cohort Study. Circulation **1999**; 100:700–5.
24. Ware JH, Dockery DW, Louis TA, Xu XP, Ferris BG Jr, Speizer FE. Longitudinal and cross-sectional estimates of pulmonary function decline in never-smoking adults. Am J Epidemiol **1990**; 132:685–700.
25. Ware JH. Linear models for the analysis of longitudinal studies. Am Stat **1985**; 39:95–101.
26. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol **1999**; 28:964–74.
27. Royston P, Altman DG. Regression using fractional polynomials of continuous covariates. Appl Stat **1994**; 43:429–67.
28. Battino M, Giunta S, Galeazzi L, et al. Coenzyme Q10, antioxidant status and ApoE isoforms. Biofactors **2003**; 18:299–305.
29. Doser N, Sudre P, Telenti A, et al. Persistent dyslipidemia in HIV-infected individuals switched from a protease inhibitor–containing to an efavirenz-containing regimen. J Acquir Immune Defic Syndr **2001**; 26:389–90.