Contribution of 20 single nucleotide polymorphisms of 13 genes to dyslipidemia associated with antiretroviral therapy

Mireia Arnedo[a], Patrick Taffé[b], Roland Sahli[a], Hansjakob Furrer[c], Bernard Hirschel[d], Luigia Elzi[e], Rainer Weber[f], Pietro Vernazza[g], Enos Bernasconi[h], Roger Darioli[i], Sven Bergmann[j], Jacques S. Beckmann[j,k], Amalio Telenti[a,l], Philip E. Tarr[l] and the Swiss HIV Cohort Study

Background **HIV-1 infected individuals have an increased cardiovascular risk which is partially mediated by dyslipidemia. Single nucleotide polymorphisms in multiple genes involved in lipid transport and metabolism are presumed to modulate the risk of dyslipidemia in response to antiretroviral therapy.**

Methods **The contribution to dyslipidemia of 20 selected single nucleotide polymorphisms of 13 genes reported in the literature to be associated with plasma lipid levels (*ABCA1*, *ADRB2*, *APOA5*, *APOC3*, *APOE*, *CETP*, *LIPC*, *LIPG*, *LPL*, *MDR1*, *MTP*, *SCARB1*, and *TNF*) was assessed by longitudinally modeling more than 4400 plasma lipid determinations in 438 antiretroviral therapy-treated participants during a median period of 4.8 years. An exploratory genetic score was tested that takes into account the cumulative contribution of multiple gene variants to plasma lipids.**

Results **Variants of *ABCA1*, *APOA5*, *APOC3*, *APOE*, and *CETP* contributed to plasma triglyceride levels, particularly in the setting of ritonavir-containing antiretroviral therapy. Variants of *APOA5* and *CETP* contributed to high-density lipoprotein-cholesterol levels. Variants of *CETP* and *LIPG* contributed to non-high-density lipoprotein-cholesterol levels, a finding not reported previously. Sustained hypertriglyceridemia and low high-density lipoprotein-cholesterol during the study period was significantly associated with the genetic score.**

Conclusions **Single nucleotide polymorphisms of *ABCA1*, *APOA5*, *APOC3*, *APOE*, and *CETP* contribute to plasma triglyceride and high-density lipoprotein-cholesterol levels during antiretroviral therapy exposure. Genetic profiling may contribute to the identification of patients at risk for antiretroviral therapy-related dyslipidemia.**
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[a]Institute of Microbiology, University of Lausanne, Switzerland, [b]Swiss HIV Cohort Study Data Center, Lausanne, Infectious Diseases Service, University Hospitals of [c]Bern, [d]Geneva, [e]Basel, [f]Zurich, [g]Kantonsspital, St Gallen, [h]Ospedale Civico, Lugano, [i]Lipidology, University Medical Polyclinic, [j]Department of Medical Genetics, University of Lausanne, [k]Medical Genetics Service and [l]Infectious Diseases Service, University Hospital, Lausanne, Switzerland

Correspondence to Philip E. Tarr, MD, Infectious Diseases Service, University Hospital, CHUV BH 07-865, 1011 Lausanne, Switzerland
Tel: +41 21 314 3020; fax: +41 21 314 1008; e-mail: philiptarr@yahoo.com or Amalio Telenti, MD, PhD, Institute of Microbiology, University of Lausanne, 1011 Lausanne, Switzerland
Tel: +41 21 314 0550; e-mail: amalio.telenti@chuv.ch

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Introduction

Dyslipidemia has emerged as a major long-term concern in individuals with HIV infection who are treated with combination antiretroviral therapy (ART) [1–3]. Data from the large, prospective data collection on adverse events of HIV drugs (D.A.D.) study suggests an increased cardiovascular risk, particularly in patients treated with protease inhibitors (PIs), that is at least partially mediated by dyslipidemia [4,5]. ART-associated dyslipidemia may include hypertriglyceridemia, decreased high-density lipoprotein cholesterol (HDL-C) levels, and increased non-HDL-C (NHC) and low-density lipoprotein cholesterol levels [6–10]. Each of these lipid abnormalities is considered a cardiovascular risk factor by the National Cholesterol Education Program, Third Adult Treatment Program (NCEP-ATPIII) [11], whose guidelines are also recommended for the treatment of dyslipidemia in HIV-infected patients [2].

The type and severity of dyslipidemia may vary according to the ART regimen in use. Hypertriglyceridemia most frequently occurs during treatment with ritonavir (RTV) [6,7], whereas increased low-density lipoprotein cholesterol or NHC is a feature of several ART agents, including stavudine (d4T), PIs and non-nucleoside reverse transcriptase inhibitors (NNRTI), particularly efavirenz [6,8,9]. NNRTIs, particularly nevirapine, may be associated with increased HDL-C levels [6,12]. Dyslipidemia does not, however, develop in all patients, despite similar ART exposure and comparable demographic, immunologic, and virologic characteristics. This discrepancy may be related to host genetic factors. We have reported an

increased risk of dyslipidemia in HIV-infected carriers of unfavorable apolipoprotein *E* (*APOE*) and *APOC3* genotypes, which was most pronounced in the setting of RTV-containing ART [13]. A significant number of patients with abnormal lipid levels had favorable *APOE* and *APOC3* genotypes, however, suggesting that additional genes modify the lipid response to ART. The aim of this study was to identify associations of plasma lipid levels during ART with additional genetic variants. To increase the validity of our results, the assessed gene variants were selected on the basis of the existing knowledge, that is, a previously published link to dyslipidemia. A second, exploratory aim was to evaluate appropriate methods to take into account the cumulative contribution of the different genetic variants to lipid levels, as a background for future studies of genetic testing before initiation of ART.

Patients and methods

Patients, antiretroviral therapy

Study participants were followed in the Swiss HIV Cohort Study (SHCS) (*www.shcs.ch*). The SHCS Genetics Project was approved by the ethics committees of all participating centers. Patients were included if they gave written, informed consent for genetic testing and were treated with ART during the study period which was from 1 January 1999 to 22 October 2005. The multiplicative, independent contribution of different antiretroviral drugs to plasma lipid levels was evaluated using regression modeling (see Statistical analysis). ART agents were distributed into groups according to published reports of their lipid effects [6–10,12–16] and the results of the regression analysis (Table 1). At each lipid determination, the specific ART medication and ART group in use was recorded. Lipid values were re-assigned to a new ART group or to 'no ART', respectively, if ART changes or interruptions were made. Thus, each participant could contribute lipid values to more than one ART group.

Table 1 Groups of ART used in this study

Plasma lipid analyzed	ART group 1	ART group 2	ART group 3
Triglycerides	No ART	Single protease inhibitor-containing ART (except ritonavir)	Ritonavir-containing ART (except ATV/r)
	Nucleoside reverse transcriptase inhibitors (NRTI) only	ATV/r	
	Nevirapine-containing ART without a PI	Efavirenz	
HDL-cholesterol	No ART NRTI only	PI	NNRTI
Non-HDL cholesterol	No ART	PI except ATV/r	NA
	NRTI only ATV/r	NNRTI	

ATV/r, ritonavir-boosted atazanavir; ART, antiretroviral therapy; NA, not applicable; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitor.

Lipids

All determinations of total cholesterol, HDL-C, and triglycerides (TG), made during the study period were included. NHC was calculated as NHC = total cholesterol – HDL-C. The fasting state was recorded for all blood draws. Sustained dyslipidemia was defined as > 2/3 of all lipid values of a participant during the study period being above/below the respective NCEP-ATPIII cutoff levels [TG: > 2.26 mmol/l (200 mg/dl); HDL-C: < 1.03 mmol/l (40 mg/dl); and NHC: > 4.9, 4.1, or 3.4 mmol/l (190, 160, and 130 mg/dl), respectively) [11]. Sustained dyslipidemia provides a clinically more relevant summary of an individual patient's plasma lipid status over time than single elevated or extreme values, as was selected in our previous report [13].

Single nucleotide polymorphisms

Candidate SNPs were identified via review of PubMed reports of SNP associations with dyslipidemia in the general population, or among HIV-infected individuals. Following recommendations for the conduction of gene association studies [17], the supporting reports are listed in detail herein (Supplementary Table 1). Genotyping methods, primers and probes are listed in Supplementary Table 2. Equivocal samples were retested and confirmed by a second observer. For quality assurance, 40 random samples per SNP were re-assayed, and two heterozygous and two homozygous samples for each of the common and rare allele were confirmed by direct sequencing. Results were entered into the central SHCS genetic database without knowledge of lipid values.

Genotype categories

The following, previously reported composite genotypes/haplotypes were used: *APOA5* [composite 64 G > C/ – 1131 T > C haplogenotype: *1/*1 (common alleles) vs. non-*1/*1 haplotypes], and *APOE* (ε3/ε3 genotype vs. non-ε3/ε3 genotypes). Formal haplotype analysis was performed for the three *APOC3* variants (– 482 C > T, – 455 T > C, and 3238 C > G) and for the *APOC3*–*APOA5* locus using *Haploview* [18] (accessible online at *www.broad.mit.edu/mpg/haploview/*). For all other genes, individual SNPs were analyzed: ABC transporter A1 (*ABCA1*) 969 G > A and 2962 A > G, β 2-adrenergic receptor (*ADRB2*) 265 A > G and 298 C > G, cholesteryl ester transfer protein (*CETP*) 279G > A and – 629 C > A, hepatic lipase (*LIPC*) – 250 G > A, endothelial lipase (*LIPG*) 584 C > T, lipoprotein lipase (*LPL*) 1595 G > C, multidrug resistance gene 1 (*MDR1*) 3435 C > T, microsomal triglyceride transfer protein (*MTP*) – 493 G > T, scavenger receptor type I (*SCARB1*) 41 C > T, and tumor necrosis factor (*TNF*) – 308 G > A.

Statistical analysis

The data were analyzed longitudinally by modeling the individual effects of the different covariables on plasma lipid levels. We improved the model used in our previous report [13], by using a multiplicative model with $\log_{10}$ transformation of lipid values, to make the distribution more symmetric and stabilize variances. A multivariate model was used in which the covariance structure arises from consideration of three residual components [19,20], that is a variance components model. Advantages of this approach include the ability to accommodate unbalanced repeated measurements made at irregular time intervals [21–23]. To check for the weak stationarity assumption, the follow-up time scale was discretized annually and the Pearson's correlations of the ordinary least square residuals between the different time periods were calculated. In addition, inspection of a graph of the residuals over time, as well as of a scatter plot matrix were used [20,21]. An informal check of the assumed covariance structure and the importance of each component can be gained by estimating nonparametrically the empirical semi-variogram [19,24].

Time-dependent covariables included in the regression model were ART regimen, age, body mass index, waist circumference, fasting state, treatment with lipid lowering agents, smoking status, CD4 + T cell count, and HIV viral load. Fixed covariables included were sex, presumed mode of HIV transmission, ethnicity, diabetes mellitus status, and the genetic factors assessed. The influence of ART and lipid lowering agents on lipid levels was assumed to be rapid and reversible [25]. For appropriate confounding adjustment, cross-sectional and longitudinal relationships between the continuous, time-dependent explanatory variables and the response variable were distinguished [21,26], and adequate functional form was assessed using fractional polynomials [27].

Model selection [28,29] was based on confounding adjustment, Student *t*-test and Akaike information criterion. We started with a full model including all candidate SNPs and used a backward elimination procedure, removing at each step the least significant SNP, and re-estimating the model until a P-value < 0.05 for genetic variants and a plausible biological dose–response relationship was obtained. To validate this selection procedure, starting from the selected model we sequentially re-introduced each SNP and compared the Akaike information criterion calculated on models with the same sample size. Adjustment for multiple testing was not performed, as the SNPs assessed had already been published and the goal of the study was to confirm previously reported associations [30,31]. We assessed gene × gene and gene × drug interactions in the model [32,33]. Goodness of fit was evaluated by comparing mean observed and predicted measurements as well as studentized conditional and marginal residuals on time plots. Different serial correlation processes, for example exponential or Gaussian correlation models, were investigated and compared by superimposition of the parametric and the nonparametric estimated variograms [19,20,24].

We validated final regression models by using an established cross-validation procedure [34]. The Pearson correlation between the observed and predicted values was calculated to estimate the fit shrinkage [34]. Time plots of the prediction errors in both the $\log_{10}$ and untransformed scales were also used to assess the predictive ability of the model. All statistical analyses were conducted using SAS version 9.1 (SAS Institute, Cary, North Carolina, USA) and STATA 9.2 (StataCorp, College Station, Texas, USA).

Results

Study participants

The characteristics of the participants are shown in Table 2. Results are based on 404, 418, and 419 patients with no missing demographic or ART data and with successful genotyping of gene variants previously linked to TG, HDL-C, and NHC, respectively. The contribution of demographic variables to lipid levels was consistent with published reports (Table 3). The allelic frequencies and the distribution of genotypes were comparable with published frequencies in ethnically similar populations (Supplementary Table 1). Each patient contributed a median of 10 lipid values (interquartile range, 9–12) during the study period (median follow-up, 4.8 years); overall, more than 4400 lipid values were analyzed. Participants with a median TG, HDL-C, and NHC level above the median for the study population contributed similar numbers of lipid values as did those with lower lipid levels (data not shown).

Contribution of antiretroviral therapy regimen to plasma lipid levels

A mean of 2.5 ART modifications were made during the study period (median, 2; IQR, 2–3). The contribution of ART to lipid levels was consistent with published reports (Tables 1 and 3). Compared with group 1, the population-averaged, observed median TG levels during the study period were higher in participants treated with ART from the second ($P = 0.01$) and third ($P < 0.001$) groups. Median TG values were similar among participants receiving different regimens *within* each ART group, including those receiving different RTV doses, d4T, or both d4T and RTV (data not shown). For analysis of HDL-C, ART regimens were divided into three groups (Table 1). Compared with group 1, observed median HDL-C levels were higher in the second ($P = 0.001$) and third ($P < 0.001$) groups. Median HDL-C values were similar for different regimens *within* each group, but

Table 2 Participant characteristics

Characteristic	Study participants ($n=438$)
Baseline age in years, median (IQR)	40 (35.3–47.2)
Men/ women, no. (% men)	342/96 (78.1)
Ethnicity, no. (%)	
Caucasian	387 (88.4)
Black	29 (6.6)
Hispanic	10 (2.3)
Asian	11 (2.5)
Unknown/other	1 (0.2)
Presumed mode of HIV transmission, no. (%)	
Men who have sex with men	184 (42)
Heterosexual	151 (34.5)
Injection drug use	86 (19.6)
Unknown/other	17 (3.9)
Follow-up period	
Duration of, median (IQR), years	4.8 (4.5–5)
Dropout or death, no. (%)	22 (5)
CD4+ T cell count, median (IQR), cells/μl	461 (319–654)
HIV RNA, no (%) with <400 copies/ml	3821 (85.6)
Treated with lipid-lowering agents, no. (%)	85 (19.4)
Diabetes mellitus, no. (%)	23 (5.2)
Plasma TG level during follow-up, median (IQR), mmol/l	1.9 (1.2-3)
No. of TG values determined during follow-up (median no. of determinations per participant)	4682 (10)
Plasma HDL-C level during follow-up, median (IQR), mmol/l	1.2 (0.9–1.5)
No. of HDL-C values determined during follow-up (median no. of determinations per participant)	4506 (10)
Plasma NHC level during follow-up, median (IQR), mmol/l	4 (3.2–5)
No. of NHC values determined during follow-up (median no. of determinations per participant)	4495 (10)

HDL-C, high-density lipoprotein-cholesterol; IQR, interquartile range; NHC, non–high-density lipoprotein-cholesterol; TG, triglycerides.

higher during nevirapine (NVP) than efavirenz (EFV) exposure (1.31 vs. 1.25 mmol/l; $P=0.02$). For NHC, ART regimens were divided into two groups (Table 1). Compared with group 1, median NHC levels were higher in the second group ($P<0.001$), but similar for different regimens *within* each group. The number of patients in each ART group is shown in Figs 1A (triglycerides), 2A (HDL-C), and 3 (NHC); note that the combined number of participants in the different ART groups is greater than the total number of study participants, because participants may contribute lipid values to several ART groups.

Contribution of single nucleotide polymorphisms to plasma lipid levels

No significant contribution to plasma TG, HDL-C, or NHC was identified in the present dataset for *ADRB2* 265 A > G and 298 C > G, *LIPC* −250 G > A, *LIPG* 584 C > T, *LPL* 1595 G > C, *MDR1* 3435 C > T, *MTP* −493 G > T, *SCARB1* 41 C > T, and *TNF* −308 G > A. All allelic frequencies and the contribution of SNPs to lipid levels are listed in Supplementary Table 1.

Triglycerides

As shown in Table 3, homozygous variant *ABCA1* 2962A > G, non-*1/*1 haplotypes of *APOA5*, and non-ε3/ε3 genotypes of *APOE* contributed to increased plasma TG levels, after adjusting for the nongenetic covariates.

Table 3 Multivariate analysis of the contribution of demographic factors, antiretroviral therapy, and SNPs to dyslipidemia

Variable	Triglycerides ($n=404$)	HDL-Cholesterol ($n=418$)	Non-HDL-Cholesterol ($n=419$)
	Mean (95% confidence interval) predicted effect of a one unit change on plasma lipid levels (mmol/l)		
Reference plasma lipid level[a] (mmol/l)	1.68 (1.41; 2.00)	1.45 (1.33; 1.59)	3.72 (3.39; 4.08)
Baseline age (change per year)	0.05 (0.03; 0.08) $P<0.001$	0.00 (−0.01; 0.01) $P=0.43$	0.07 (0.04; 0.10) $P<0.001$
Male sex	0.10 (−0.15; 0.40) $P=0.44$	−0.25 (−0.35; −0.15) $P<0.001$	0.26 (−0.27; 0.35) $P=0.87$
Fasting state	−0.06 (−0.12; −0.01) $P=0.02$	0.01 (−0.02; 0.03) $P=0.66$	−0.04 (−0.09; 0.02) $P=0.19$
Treated with lipid-lowering agents	−0.05 (−0.15; 0.06) $P=0.40$	−0.03 (−0.07; 0.02) $P=0.28$	−0.33 (−0.43; −0.22) $P<0.001$
Antiretroviral treatment			
Group 1	Reference	Reference	Reference
Group 2	0.11 (0.02; 0.21) $P=0.01$	0.07 (0.02; 0.11) $P=0.001$	0.58 (0.48; 0.68) $P<0.001$
Group 3	1.06 (0.89; 1.25) $P<0.001$	0.18 (0.13; 0.23) $P<0.001$	n.a.
SNPs			
ABCA1 2962 GA	0.11 (−0.08; 0.33) $P=0.29$	NS	NS
ABCA1 2962 GG	0.57 (0.13; 1.11) $P=0.008$	NS	NS
APOA5 non-*1/*1 haplotypes	0.32 (0.11; 0.56) $P=0.002$	−0.12 (−0.19; −0.05) $P=0.003$	NS
APOC3 1 or 2 variants	0.10 (−0.08; 0.30) $P=0.25$	NS	NS
APOC3 3-variant haplotype[b]	0.25 (−0.01; 0.55) $P=0.05$	NS	NS
APOE genotype other than ε3/ε3	0.21 (0.03; 0.41) $P=0.02$	NS	NS
CETP 279 GA	−0.10 (−0.25; 0.07) $P=0.24$	NS	NS
CETP 279 AA	−0.24 (−0.43; −0.02) $P=0.04$	NS	NS
CETP −629 CA	NS	0.11 (0.02; 0.20) $P=0.02$	0.09 (−0.13; 0.32) $P=0.46$
CETP −629 AA	NS	0.25 (0.14; 0.37) $P<0.001$	0.28 (0.00; 0.59) $P=0.04$
LIPG 584 CT	NS	NS	0.18 (−0.03; 0.40) $P=0.10$
LIPG 584 TT	NS	NS	0.42 (0.07; 0.79) $P=0.02$

Values shown are adjusted for all variables listed above. 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. The number of participants varies in each column as only participants without missing values for demographic, treatment, or genetic variables are included.

n.a., not applicable; NS, not significant (all coefficients and *P* values are listed in Supplementary Table 1).

[a]The reference for the regression model represents the lipid level for a virtual 39-year-old, Caucasian woman, heterosexual HIV acquisition, not fasting, body mass index of 23 kg/m^2, nonsmoker, not diabetic, not treated with ART or lipid-lowering agents, with a CD4+ T cell count of 500 cells/μl, undetectable HIV viremia, and common alleles at all tested polymorphic markers.

[b]*APOC3* haplotype 8 in Supplementary Table 3.

The increases in mean predicted TG levels were 0.57, 0.32, and 0.21 mmol, respectively. Homozygous variant *CETP* 279G > A was associated with a decrease in mean predicted TG levels of 0.24 mmol/l. Heterozygous carriers of each of these variants had no significant change in TG levels. The contribution of homozygous variant *ABCA1* 2962 A > G to increased TG levels was large, with evidence of a gene-dose effect, but has not been reported previously and thus should be considered exploratory. In two previous studies, significant TG elevations were seen in carriers of three *APOC3* SNPs [13,35]. Haplotype analysis confirmed the high degree of linkage disequilibrium among the three *APOC3* SNPs [36,37]. The simultaneous presence of all three SNPs on one chromosome (i.e. carriage of haplotype 8 in Supplementary Table 3) was associated with an increase in TG levels (of 0.25 mmol/l), equivalent to that captured by the quantitative *APOC3* variable used in previous reports [13,35]. Haplotypes containing only 1 or 2 variants did not significantly contribute to TG levels. *APOC3* and *APOA5* polymorphisms are located in close proximity on chromosome 11q23. *APOC3*/*APOA5* haplotypes reflected linkage dysequilibrium between *APOA5* 64G (associated with hypertriglyceridemia), and the common *APOC3* −455T, indicating that the simultaneous presence of hypertriglyceridemic variants of both genes is uncommon on the same chromosome (data not shown). This supported separate analysis of the *APOC3* and *APOA5* loci. No relevant gene × gene or gene × ART group interactions were identified.

HDL-Cholesterol

Non-*1/*1-haplotypes of *APOA5* contributed to decreased (−0.12 mmol/l) mean predicted HDL-C levels (Table 3). Heterozygous and homozygous variant *CETP* −629 C > A contributed to increased HDL-C levels, with evidence of a gene-dose effect (increase in mean predicted HDL-C levels; 0.11 and 0.25 mmol/l, respectively). A second *CETP* variant, 279 G > A, was in linkage disequilibrium with *CETP* −629 C > A, and only *CETP* −629 C > A was retained [38]. No gene × gene or gene × ART group interactions were identified.

Non-HDL-Cholesterol

Homozygous variants *CETP* −629 C > A and *LIPG* 584 C > T contributed to NHC levels (Table 3). As these effects have not been reported previously, they should be considered exploratory. No genotype × genotype or genotype × ART group interactions were identified.

Potential approaches to genetic prediction of antiretroviral therapy-related dyslipidemia

Taking into account concurrently multiple genetic variants and ART known to contribute to dyslipidemia has the potential to increase the accuracy of genetic prediction of dyslipidemia in ART-treated patients.

Triglycerides

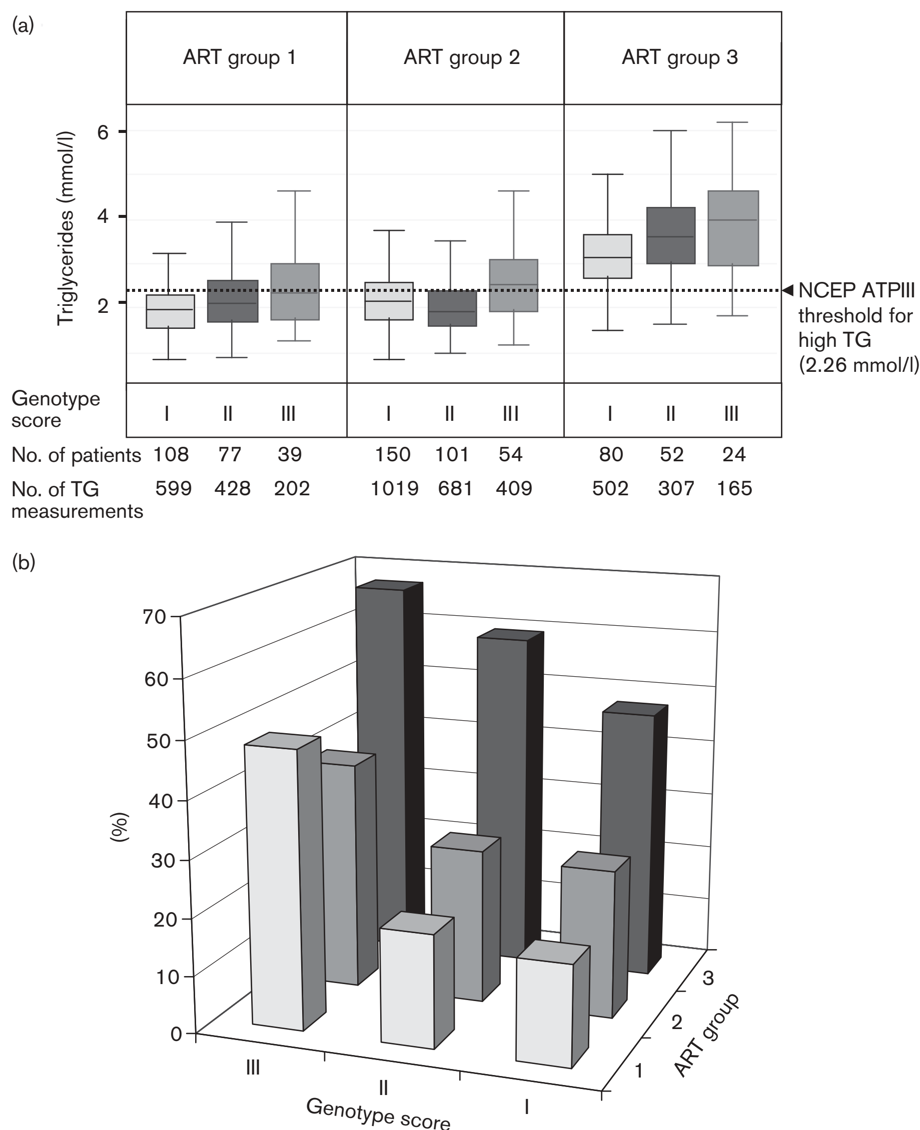
Compared with a model that contained all covariables but no genetic information, a final model that considered the composite *ABCA1*/*APOA5*/*APOC3*/*APOE*/*CETP* genotype improved the Akaike information criterion by ∼20 points. To provide a clinically meaningful, illustrative summary of the results of the multivariate analysis, we added up, for each patient, the estimated regression coefficients of the genetic variants (i.e. the rounded numeric $\log_{10}$ contribution to TG), multiplied by 100. Specifically, carriers of *ABCA1* 2962 GG, *APOA5* non-*1/*1 haplotypes, *APOC3* 3-variant haplotype, and *APOE* non-ε3/ε3 genotypes, whose contribution to TG levels in regression analysis was 0.127 $\log_{10}$, 0.076 $\log_{10}$, 0.059 $\log_{10}$, and 0.051 $\log_{10}$ mmol/l, respectively, received 13 points, eight points, six points, and five points, respectively. In carriers of *CETP* 279 GG, whose contribution to plasma TG levels in regression analysis was a −0.066 $\log_{10}$ mmol/l reduction, seven points were subtracted. For illustrative purposes, we then divided the patients into three exploratory genotype scores: genotype score I (favorable), II (intermediate), and III (unfavorable) (Supplementary Table 4).

Observed, median TG levels during the study period were 1.47 and 2.27 mmol/l ($P < 0.001$) in participants with genotype scores I and III, respectively, when treated with regimens from ART group 1; and 2.63 and 4.12 mmol/l ($P = 0.001$), respectively, during RTV exposure (ART group 3). We then evaluated the contribution of ART group and genotype score to sustained, NCEP-ATPIII-defined hypertriglyceridemia. Sustained hypertriglyceridemia was observed in 17 of 97 (17.5%) of participants with the most favorable gene-drug profile (Fig. 1). This proportion was increased in those with a single unfavorable determinant (genetic or ART); 38 of 80 (47.5%; $P < 0.001$) individuals in ART group 3 with genotype score I, and 14 of 29 (48.3%; $P = 0.001$) individuals in ART group 1 and genotype score III. The highest risk of sustained hypertriglyceridemia was seen in those with both unfavorable genes and unfavorable ART; 15 of 24 individuals (66.7%); $P < 0.001$.

HDL-Cholesterol

Compared with a model that contained all covariables but no genetic information, a final model that considered the composite *APOA5*/*CETP* genotype improved the Akaike information criterion by ∼22 points. Analogous to analysis of TG, the estimated regression coefficients of the genetic variants, multiplied by 100, were added up. Specifically, carriers of *CETP* −629 CA and *CETP* −629 AA whose contribution to HDL-C levels in multivariate analysis was 0.031 $\log_{10}$ and 0.069 $\log_{10}$ mmol/l, received three points and seven points, respectively. In carriers of *APOA5* non-*1/*1 haplotypes whose contribution to plasma HDL-C levels in regression

Fig. 1

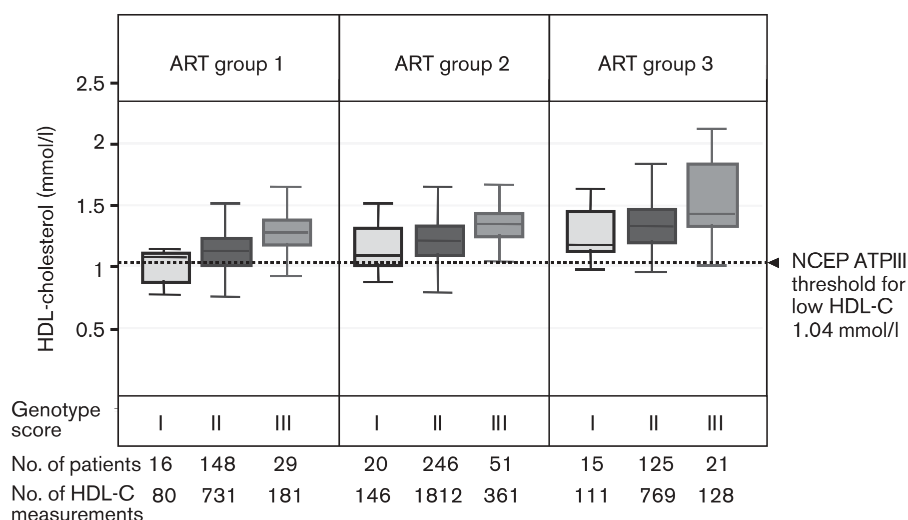


(a) Effect of the cumulative *ABCA1/APOA5/APOC3/APOE/CETP* genotype score and ART group on predicted (adjusted) median plasma triglyceride levels. Box-and-whisker plot showing the medians plus interquartile ranges (boxes) and upper and lower adjacent values (whiskers) of the plasma TG levels during the study period. Participants are stratified according to ART group and genotype score. The number of participants is greater than the total number of study participants, because participants may contribute TG values to several ART groups. (b) Observed proportion of participants with sustained hypertriglyceridemia according to genotype score and ART group. Cross-tabulation of the three genotype scores and three ART groups generates nine genotype score-by-ART group categories. ART, antiretroviral therapy; TG, triglycerides.

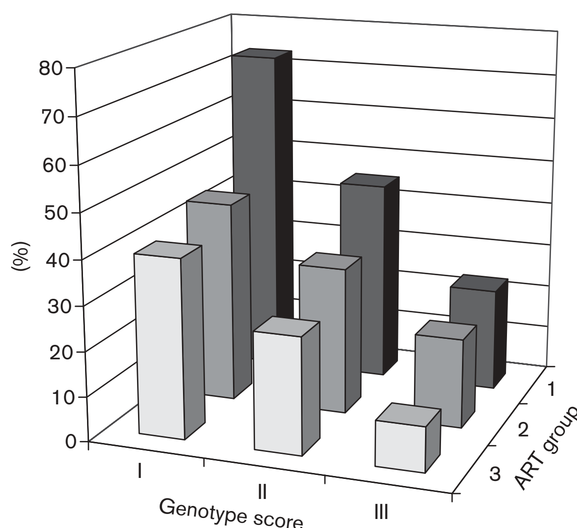
analysis was a -0.038 $\log_{10}$ mmol/l reduction in HDL-C levels, four points were subtracted. For illustrative purposes, we then divided the patients into three exploratory genotype scores: genotype score I (unfavorable), II (intermediate), and III (favorable) (Supplementary Table 5).

Observed, median HDL-C levels during the study period were 1.11 and 1.25 mmol/l ($P < 0.001$) in participants with genotype scores I and III, respectively, when treated with regimens from ART group 1; 1.15 and 1.31 mmol/l ($P < 0.001$) during PI exposure; and 1.17 and 1.50 mmol/l ($P < 0.001$) during NNRTI exposure. Sustained low HDL-C was observed in 11 of 15 (73.3%) of individuals with the most unfavorable *APOA5/CETP*-ART profile (Fig. 2). This proportion was reduced in individuals with a single favorable determinant (genetic or ART); 6 of 15 (40%; $P = 0.07$) individuals in ART group 3 with genotype scores I, and 9 of 39 (23.1%; $P = 0.001$) individuals in ART group 1 and genotype score III. The

Fig. 2



(b)



(a) Effect of *APOA5/CETP* genotype score and ART group on predicted (adjusted) median plasma HDL-cholesterol levels. Box-and-whisker plot as in Fig. 1A. (b) Observed proportion of participants with sustained low HDL-cholesterol according to genotype score and ART group. Cross-tabulation of the three genotype scores and three ART groups generates nine genotype score-by-ART group categories. ART, antiretroviral therapy; HDL, high-density lipoprotein cholesterol.

lowest risk of sustained low HDL-C was seen in those with both favorable genes and favorable ART; 2 of 20 individuals (10%); $P < 0.001$.

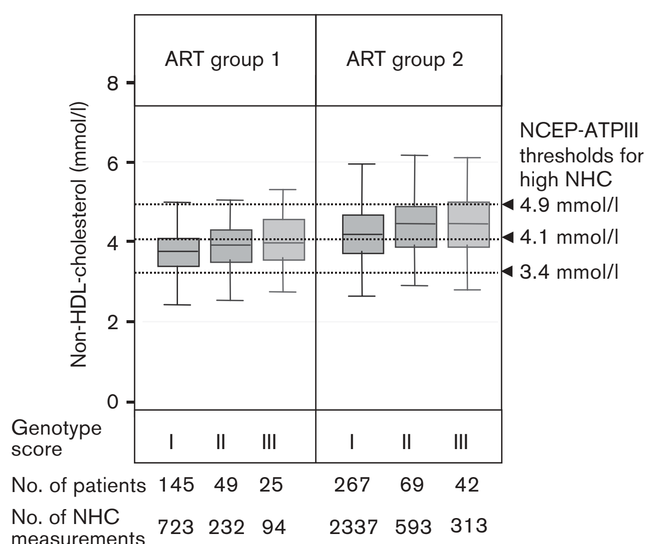
Non-high-density lipoprotein-cholesterol

Compared with a model that contained all covariables but no genetic information, a final model that considered the composite *CETP/LIPG* genotype improved the Akaike information criterion by $\sim$3 points. The proportion of participants with sustained high NHC during PI-exposure or NNRTI-exposure was not influenced by the composite *CETP/LIPG* genotype ($P = 0.71$; data not shown).

Validation of multivariate models

Goodness of fit of the TG, HDL-C, and NHC models was excellent. The different residuals behaved appropriately and the parametric estimates of the variograms were close to the nonparametric estimates. Results were similar when the analysis was restricted to participants of Caucasian ethnicity, and when further adjusted for the presence of lipoatrophy (data not shown). Definitions of sustained dyslipidemia of different stringency (i.e. $\geq 1/2$, $\geq 2/3$, and $\geq 3/4$ of an individual's lipid values above/below the NCEP-ATPIII threshold during the study period) gave similar results (data not shown). Cross-validation showed shrinkage index values of 11% for

Fig. 3



Effect of genotype score and ART group on predicted (adjusted) median plasma non-HDL-cholesterol levels. Box-and-whisker plot as in Fig. 1A. ART, antiretroviral therapy; HDL, high-density lipoprotein.

TG, 10% for HDL-C, and 15% for NHC, suggesting that the models did not overfit and may be useful for prediction in other datasets.

Discussion

Major findings

In this study, we show that the genetic background influences the risk of dyslipidemia in HIV-infected patients during ART. The genetic risk of ART-associated dyslipidemia was defined through re-evaluation of 20 SNPs of 13 genes proposed in the literature as influencing plasma lipid levels. Using regression modeling, we were able to validate SNPs of five genes (*ABCA1*, *APOA5*, *APOC3*, *APOE*, and *CETP*) as contributing to hypertriglyceridemia, and SNPs of two genes (*APOA5* and *CETP*) as contributing to low HDL-C. These data allowed the generation of genotype scores to provide a clinically meaningful, illustrative view of multigene-ART influences on lipid levels. Although variants of *LIPG* and *CETP* contributed to NHC levels, this association has not been previously reported and needs to be confirmed in other patient populations before it can be considered a true finding.

Experimental approach

Several SNPs proposed in the literature (*ADRB2*, *LIPC*, *LIPG*, *LPL*, *MDR1*, *MTP*, *SCARB1*, and *TNF*) did not contribute to plasma lipids in the present dataset, which may reflect a limited net effect of these SNPs, or the known difficulty to replicate and validate previously reported genetic associations [39,40]. In view of such concerns, particular attention was paid to preliminary guidelines [17,40] for the conduction of genetic association studies. We did not test any novel genes or SNPs but validated existing knowledge by selecting candidate genes/SNPs on the basis of their previously reported influence on lipid levels, mostly in HIV-seronegative populations, and on biological plausibility. In general, SNPs with considerable evidence in the literature supporting a true lipid association were also retained in our final models. In contrast, SNPs with contradictory reports or limited documentation in the literature, mostly from single-time point, cross-sectional studies, were not associated with plasma lipid levels in the present study. As our study design was robust, by exploiting prospectively collected data analyzed longitudinally over > 4.5 years, representing more than 4400 lipid determinations, the most likely explication for our failure to validate certain SNPs are false-positive genetic associations in the literature. Cross-sectional studies are unable to account for the variability of lipid levels and other factors over time, whereas in our study, participants served as their own controls through periods of ART interruptions and modifications. The authors of an AIDS Clinical Trial Group study who evaluated the association of *APOC3* variants with the lipid response to PI-based ART expressed concern that the single-time point, cross-sectional design of their study may have been statistically underpowered [41]. A randomized, clinical trial in ART-naive patients is the most rigorous study design to assess the lipid response to a small number of ART regimens, stratified by genetic background. A clinical trial is, however, unable to take into account interruptions and changes of ART or other factors over time, as can a cohort study, which more closely reflects the 'real life' situation of HIV clinical practice.

Exploratory genotype scores

Consistent with the current understanding of drug-related dyslipidemia as the phenotypic expression of a complex genetic trait [42,43], there is evidence that consideration of multiple genetic variants increases the validity of predictive genetic testing [44–46]. In exploratory analyses, we present a genotype score that, in addition to considering the number of favorable or unfavorable genetic variants carried by the patient, also reflects the cumulative, net contribution of these variants to plasma lipids. Using this scoring system, the proportion of patients with sustained, NCEP-ATPIII-defined dyslipidemia varies considerably as a function of ART and the genetic background. We are fully aware that the scores were derived from and applied to the same dataset, and are thus exploratory. Nonetheless, the scores illustrate in which way predictive genetic testing might in the future be used in clinical practice.

Study limitations

The multigene prediction model needs to be reproduced in an independent cohort or clinical trial. Future studies need to include a greater proportion of women and patients of non-Caucasian ethnicity, as the majority of participants were white men. In addition, the power of gene × gene and gene × drug interaction testing in our dataset was limited by the large number of possible interactions and the resulting small number of participants in some resulting strata. The prediction models and, ultimately, multiplexing genotyping systems, can be refined as we learn more about new SNPs that contribute to ART-related dyslipidemia. As an illustration of the limitation of our current prediction models, 18% of participants in this study had sustained hypertriglyceridemia and 10% had sustained low HDL-C, despite both favorable genes and favorable ART. Similarly, 33% of participants did *not* have sustained hypertriglyceridemia and 27% did *not* have low HDL-C, despite an unfavorable gene-drug profile.

Outlook

Technical improvements are now progressively lowering the cost of genotyping, which can be performed on a single occasion to identify patients at risk of dyslipidemia. If the accuracy of genetic prediction can be further improved, by consideration of additional gene variants and by the development of clinically useful multigene scoring systems, these findings may provide the background for prospective trials of the value of genotyping before ART initiation. The goal of multigene prediction is to avoid dyslipidemic ART regimens and their associated consequences, including the prescription of lipid lowering medication, polypharmacy, drug interactions, and, ultimately, increased cardiovascular risk.

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References

1 Grinspoon S, Carr A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. *N Engl J Med* 2005; **352**:48–62.
2 Dube MP, Stein JH, Aberg JA, Fichtenbaum CJ, Gerber JG, Tashima KT, *et al.* Guidelines for the evaluation and management of dyslipidemia in human immunodeficiency virus (HIV)-infected adults receiving antiretroviral therapy: recommendations of the HIV Medical Association of the Infectious Disease Society of America and the Adult AIDS Clinical Trials Group. *Clin Infect Dis* 2003; **37**:613–627.
3 Manuel O, Thiebaut R, Darioli R, Tarr PE. Treatment of dyslipidaemia in HIV-infected persons. *Expert Opin Pharmacother* 2005; **6**:1619–1645.
4 Friis-Moller N, Sabin CA, Weber R, d'Arminio MA, El-Sadr WM, Reiss P, *et al.* Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med* 2003; **349**:1993–2003.
5 Friis-Moller N, Reiss P, El-Sadr W, D'Arminio Monforte A, Thiebaut R, De Wit S, *et al.* Exposure to PI and NNRTI and risk of myocardial infarction: results from the D:A:D study. Program and Abstracts of the 12th Conference on Retroviruses and Opportunistic Infections, Denver, 5–8 February 2006 (abstract No. 144).
6 Fontas E, van LF, Sabin CA, Friis-Moller N, Rickenbach M, d'Arminio MA, *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**:1056–1074.
7 Periard D, Telenti A, Sudre P, Cheseaux JJ, Halfon P, Reymond MJ, *et al.* Atherogenic dyslipidemia in HIV-infected individuals treated with protease inhibitors. The Swiss HIV Cohort Study. *Circulation* 1999; **100**:700–705.
8 Doser N, Sudre P, Telenti A, Wietlisbach V, Nicod P, Darioli R, *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**:389–390.
9 Martinez E, Arnaiz JA, Podzamczer D, Dalmau D, Ribera E, Domingo P, *et al.* Substitution of nevirapine, efavirenz, or abacavir for protease inhibitors in patients with human immunodeficiency virus infection. *N Engl J Med* 2003; **349**:1036–1046.
10 Young J, Weber R, Rickenbach M, Furrer H, Bernasconi E, Hirschel B, *et al.* Lipid profiles for antiretroviral-naive patients starting PI- and NNRTI-based therapy in the Swiss HIV cohort study. *Antivir Ther* 2005; **10**:585–591.
11 Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP). Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA* 2001; **285**:2486–2497.
12 van Leth F, Phanuphak P, Stroes E, Gazzard B, Cahn P, Raffi F, *et al.* Nevirapine and efavirenz elicit different changes in lipid profiles in antiretroviral-therapy-naive patients infected with HIV-1. *PLoS Med* 2004; **1**:e19.
13 Tarr PE, Taffe P, Bleiber G, Furrer H, Rotger M, Martinez R, *et al.* Modeling the influence of APOC3, APOE, and TNF polymorphisms on the risk of antiretroviral therapy-associated lipid disorders. *J Infect Dis* 2005; **191**:1419–1426.
14 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**:2093–2099.
15 Johnson M, Grinsztejn B, Rodriguez C, Coco J, DeJesus E, Lazzarin A, *et al.* Atazanavir plus ritonavir or saquinavir, and lopinavir/ritonavir in patients experiencing multiple virological failures. *AIDS* 2005; **19**:153–162.
16 Fellay J, Boubaker K, Ledergerber B, Bernasconi E, Furrer H, Battegay M, *et al.* Prevalence of adverse events associated with potent antiretroviral treatment: Swiss HIV Cohort Study. *Lancet* 2001; **358**:1322–1327.
17 Freimer NB, Sabatti C. Guidelines for association studies in Human Molecular Genetics. *Hum Mol Genet* 2005; **14**:2481–2483.
18 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**:263–265.
19 Diggle PJ. An approach to the analysis of repeated measurements. *Biometrics* 1988; **44**:959–971.

20 Diggle PJ, Heagerty P, Liang KY, Zeger S. *Analysis of longitudinal data*. 2nd edition. Oxford: Oxford University Press; 2002.
21 Laird NM, Donnelly C, Ware JH. Longitudinal studies with continuous responses. *Stat Meth Med Res* 1992; **1**:225–247.
22 Jones RH, Boadi-Boateng F. Unequally spaced longitudinal data with AR(1) serial correlation. *Biometrics* 1991; **47**:161–175.
23 Chi EM, Reinsel G. Model for longitudinal data with random effects and AR(1) errors. *J Am Stat Societ* 1989; **84**:452–459.
24 Diggle PJ, Verbyla AP. Nonparametric estimation of covariance structure in longitudinal data. *Biometrics* 1998; **54**:401–415.
25 Purnell JQ, Zambon A, Knopp RH, Pizzuti DJ, Achari R, Leonard JM, *et al*. Effect of ritonavir on lipids and post-heparin lipase activities in normal subjects. *AIDS* 2000; **14**:51–57.
26 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.
27 Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. *Int J Epidemiol* 1999; **28**:964–974.
28 Zucchini W. An introduction to model selection. *J Math Psychol* 2000; **44**:41–61.
29 Murtaugh PA. Methods of variable selection in regression modelling. *Commun Statist: Simul Comput* 1998; **27**:711–734.
30 Saville DJ. Multiple comparison procedures: the practical solution. *Am Statist* 1990; **44**:174–180.
31 Roback PJ, Askins RA. Judicious use of multiple hypothesis tests. *Conserv Biol* 2003; **19**:261–267.
32 Chunrong AI, Norton EC. Interaction terms in logit and probit models. *Econ Lett* 2003; **80**:123–129.
33 Starr JR, McKnight B. Assessing interaction in case-control studies: type I errors when using both additive and multiplicative scales. *Epidemiology* 2004; **15**:422–427.
34 Timm NH, Wiesner AJ. Model selection and prediction in multiple linear regression. Unpublished report ftp://ftp.pitt.edu/users/t/i/timm/Publications/Linpapr.PDF. 2006.
35 Fauvel J, Bonnet E, Ruidavets JB, Ferrieres J, Toffoletti A, Massip P, *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**:2397–2406.
36 Pennacchio LA, Olivier M, Hubacek JA, Krauss RM, Rubin EM, Cohen JC. Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. *Hum Mol Genet* 2002; **11**:3031–3038.
37 Talmud PJ, Hawe E, Martin S, Olivier M, Miller GJ, Rubin EM, *et al*. Relative contribution of variation within the APOC3/A4/A5 gene cluster in determining plasma triglycerides. *Hum Mol Genet* 2002; **11**:3039–3046.
38 Dachet C, Poirier O, Cambien F, Chapman J, Rouis M. New functional promoter polymorphism, CETP/-629, in cholesteryl ester transfer protein (CETP) gene related to CETP mass and high density lipoprotein cholesterol levels: role of Sp1/Sp3 in transcriptional regulation. *Arterioscler Thromb Vasc Biol* 2000; **20**:507–515.
39 Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. *Nat Genet* 2001; **29**:306–309.
40 Colhoun HM, McKeigue PM, Davey SG. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003; **361**:865–872.
41 Foulkes AS, Wohl DA, Frank I, Puleo E, Restine S, Wolfe ML, *et al*. Associations among race/ethnicity, ApoC-III genotypes, and lipids in HIV-1-infected individuals on antiretroviral therapy. *PLoS Med* 2006; **3**:e52.
42 Han Z, Heath SC, Shmulewitz D, Li W, Auerbach SB, Blundell ML, *et al*. Candidate genes involved in cardiovascular risk factors by a family-based association study on the island of Kosrae, Federated States of Micronesia. *Am J Med Genet* 2002; **110**:234–242.
43 Rodondi N, Darioli R, Ramelet AA, Hohl D, Lenain V, Perdrix J, *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**:582–589.
44 Pharoah PD, Antoniou A, Bobrow M, Zimmern RL, Easton DF, Ponder BA. Polygenic susceptibility to breast cancer and implications for prevention. *Nat Genet* 2002; **31**:33–36.
45 Janssens AC, Pardo MC, Steyerberg EW, van Duijn CM. Revisiting the clinical validity of multiplex genetic testing in complex diseases. *Am J Hum Genet* 2004; **74**:585–588.
46 Weedon MN, McCarthy MI, Hitman G, Walker M, Groves CJ, Zeggini E, *et al*. Combining information from common type 2 diabetes risk polymorphisms improves disease prediction. *PLoS Med* 2006; **3**:e374.