Published in final edited form as:

J Mol Med (Berl). 2006 July ; 84(7): 561–572. doi:10.1007/s00109-005-0037-x.

The contribution of individual and pairwise combinations of SNPs in the *APOA1* and *APOC3* genes to interindividual HDL-C variability

C. M. Brown¹, T. J. Rea¹, S. C. Hamon², J. E. Hixson³, E. Boerwinkle³, A. G. Clark⁴, and C. F. Sing^{1,*}

¹Department of Human Genetics, University of Michigan, Ann Arbor, MI 48109, USA

²Laboratory of Statistical Genetics, Rockefeller University, New York, NY 10021, USA

³Human Genetics Center, University of Texas Health Science Center, Houston, TX 77030, USA

⁴Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA

Abstract

Apolipoproteins (apo) A-I and C-III are components of high-density lipoprotein-cholesterol (HDL-C), a quantitative trait negatively correlated with risk of cardiovascular disease (CVD). We analyzed the contribution of individual and pairwise combinations of single nucleotide polymorphisms (SNPs) in the *APOA1/APOC3* genes to HDL-C variability to evaluate (1) consistency of published single-SNP studies with our single-SNP analyses; (2) consistency of single-SNP and two-SNP phenotype-genotype relationships across race-, gender-, and geographical location-dependent contexts; and (3) the contribution of single SNPs and pairs of SNPs to variability beyond that explained by plasma apo A-I concentration. We analyzed 45 SNPs in 3,831 young African-American ($N=1,858$) and European-American ($N=1,973$) females and males ascertained by the Coronary Artery Risk Development in Young Adults (CARDIA) study. We found three SNPs that significantly impact HDL-C variability in both the literature and the CARDIA sample. Single-SNP analyses identified only one of five significant HDL-C SNP genotype relationships in the CARDIA study that was consistent across all race-, gender-, and geographical location-dependent contexts. The other four were consistent across geographical locations for a particular race-gender context. The portion of total phenotypic variance explained by single-SNP genotypes and genotypes defined by pairs of SNPs was less than 3%, an amount that is miniscule compared to the contribution explained by variability in plasma apo A-I concentration. Our findings illustrate the impact of context-dependence on SNP selection for prediction of CVD risk factor variability.

Keywords

APOA1 gene; *APOC3* gene; High-density lipoprotein-cholesterol; Cardiovascular disease

*e-mail: csing@umich.edu Tel.: +1-734-6473151 Fax: +1-734-7635277.

CHRISTINE M. BROWN received her B.S. in Zoological Anthropology from the University of Michigan, Ann Arbor, MI, and is currently working towards her Masters degree in Epidemiology in the School of Public Health. She is presently a Research Associate in the Dept. of Human Genetics at the University of Michigan, Ann Arbor, MI.

CHARLES F. SING received his Ph.D. in Statistics and Genetics from North Carolina State University in Raleigh, NC. He is currently a Professor in the Dept. of Human Genetics at the University of Michigan, Ann Arbor, MI.

Electronic Supplementary Material Supplementary material is available in the online version of this article at <http://dx.doi.org/10.1007/s00109-005-0037-x>

Introduction

Understanding the roles of environmental and genetic factors in the determination of risk of cardiovascular disease (CVD) remains a crucial research objective. Among the primary risk factors are increases in plasma lipid and lipoprotein levels such as cholesterol, triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-C) [1]. Risk of CVD is negatively correlated with plasma high-density lipoprotein-cholesterol (HDL-C) concentration. Substantial effort has been devoted to evaluating the influence of HDL-C variability on observed interindividual variability in CVD onset, progression, severity, and response to therapy. Experimental evidence supports the role of plasma HDL-C in influencing risk of CVD, including inhibiting LDL oxidation and coagulant activity, reducing levels of adhesion proteins, and transporting excess cholesterol from peripheral cells to the liver in a process called reverse cholesterol transport (RCT) [2,3].

HDL particles are a heterogeneous class of lipoproteins composed of apolipoproteins and lipids such as TG, phospholipids, cholesterol, and cholesteryl esters. Apolipoproteins (apo) A-I and C-III are integral structural and functional gene products that participate in HDL particle formation and lipid metabolic processes. Apo A-I combines with phospholipid to form pre- β HDL which acts as an acceptor for unesterified cholesterol from peripheral cells [4]. Plasma apo C-III is known to inhibit triglyceride-rich lipoprotein catabolism by lipoprotein lipase (LPL) and the HDL receptor activity of the scavenger receptor B1 [5,6]. Genes coding for apo A-I and C-III, *APOA1* and *APOC3*, are located on chromosome 11q23-q24 and have been utilized extensively as candidates in phenotype-genotype association studies of plasma lipoprotein levels and research to elucidate the genetics of CVD risk [7].

Several studies indicate that 28–48% of interindividual variability in the blood level of HDL-C in the population at large is attributable to genetic variation (reviewed in [8]). Numerous publications have reported statistically significant associations between variation in HDL-C levels and individual genetic polymorphisms, including single nucleotide polymorphisms (SNPs) located in the *APOA1/APOC3* genes. Nevertheless, these significant findings have rarely replicated across multiple studies of groups of individuals defined by race, ethnicity, or gender [9–13]. Inconsistency of gene effects has prompted speculation on the utility of genetic information for predicting variability in quantitative CVD risk factors [14].

These considerations lead to three questions that are the focus of the present study: (1) Are the results from published studies of associations of variation in blood levels of HDL-C with variation in individual *APOA1/APOC3* SNPs consistent with results from our association study of four race-gender groups? (2) Are the single-SNP and two-SNP phenotype-genotype relationships selected to explain the greatest amount of HDL-C variability consistent across contexts defined by race, gender, and geographical location? (3) Do the selected SNPs, or pairs of SNPs, contribute to explaining variability in HDL-C beyond that explained by plasma apo A-I concentration? To address these questions, we analyzed data on 45 SNPs in the *APOA1/APOC3* gene region collected by the Coronary Artery Risk Development in Young Adults (CARDIA) study.

We found three SNPs that contributed in a statistically significant manner to HDL-C variability in both the literature and at least one, but not all, of the four race-gender strata considered in our study. Of five SNPs selected to explain the greatest amount of variability in the CARDIA study, we observed consistency of four phenotype-SNP genotype relationships across field centers for a particular race-gender context. Only one of these SNPs had a consistent phenotype-genotype relationship across all race, gender, and field

center contexts. Differences in both relative genotype frequencies and genotype effects across contexts defined by race, gender, or geographical location confound interpretations of phenotype–genotype associations. Finally, we found that the contribution of genetic information to HDL-C variability beyond plasma apo A-I concentration was small relative to the contribution of plasma apo A-I. These findings have important implications for SNP selection for predicting variability in CVD risk factors.

Materials and methods

Sample

The sample used in this study consisted of individuals participating in the CARDIA study [15]. It included young, unrelated individuals 18–30 years old who were recruited between 1985 and 1986 without regard to health status from four geographical locations: residents of Birmingham, AL; selected census tracts in Chicago, IL, and Minneapolis, MN; and the Kaiser Permanente Medical Care Program (KPMCP) in Oakland, CA. This is a longitudinal study, but all phenotypic data examined here are baseline measurements taken shortly after recruitment. Genotype and phenotype data were collected from 3,831 individuals: 1,075 African–American females; 783 African–American males; 1,041 European–American females; and 932 European–American males. Venous blood was drawn after a 12-h fast. Individuals who did not fast for 12 h were excluded from analyses in this study. Plasma HDL-C concentration was determined using standard enzymatic methods [16]. Plasma apo A-I concentration was ascertained by radioimmunoassay [17].

Resequencing and genotyping

Seventy-six variants were identified in the *APOA1/APOC3* gene region by complete resequencing of this region in 24 individuals from each of three samples: African–Americans from Jackson, MS; Europeans from North Karelia, Finland; and European–Americans from Rochester, MN [18]. The *APOA1/APOC3* region encompasses approximately 10 kbp on chromosome 11q23–q24, including exons, introns, intergenic region, and ~1,000 bp upstream of the *APOA1* and *APOC3* genes. After excluding singletons (i.e., SNPs that varied in only one individual) and insertion/deletion polymorphisms, assays for 45 SNPs were successfully developed using a MassARRAY System from Sequenom, Inc (San Diego, CA). Figure 1a,b shows the locations of these SNPs in *APOA1* and *APOC3*. Genotyping of these 45 SNPs in 3,831 CARDIA study participants was afforded by polymerase chain reaction (PCR) amplification of genomic DNA across a short region that included the polymorphic site, followed by mass spectrometry detection of allele-specific mass differences of the extension product. Approximately 5% of samples had a blind duplicate, which resulted in approximately 99% agreement between the duplicates and the originals. Of the seven *APOA1* SNPs (Fig. 1a), one is located in the 5' flanking region and six are located in introns. Of the 26 *APOC3* SNPs (Fig. 1b), ten are located in the 5' flanking region, 15 are intronic, and one SNP is located in exon 3. Variation in the *APOC3* coding SNP (cSNP), at position 7761, does not result in an amino acid change. An additional 12 SNPs are located in the common 3' *APOA1/APOC3* intergenic region (SNPs 2954 to 5124, included in Fig. 1a). A detailed description of each of the 45 SNPs, including SNP nomenclature, dbSNP ID, and location, is listed in Table S1.

Statistical analyses

Plasma HDL-C levels were adjusted by fitting a race–gender–field center-specific linear regression model containing age, age², age³, and body mass index (BMI) and then adding the residuals to the race–gender-specific mean. Bartlett's test was used to evaluate homogeneity of quantitative trait variances among field centers within each race–gender

stratum. Differences in quantitative trait means among race–gender-specific field centers were evaluated using Welch’s test when field center variances were significantly different. Pearson’s chi-squared test was used to evaluate homogeneity of relative allele frequencies among field centers within each race–gender stratum and across races. Hardy–Weinberg equilibrium was evaluated in African–Americans and European–Americans using a chi-squared goodness-of-fit test.

To select the single SNP and the pair of SNPs that explained the greatest HDL-C variability in each race–gender stratum, we plotted the $-\log(p \text{ value})$ against the $-\log(p \text{ value rank})$ for each SNP or SNP pair, essentially selecting the most highly significant SNP or SNP pair (lowest p value) [19]. For single SNPs, p values from both the combinatorial partitioning method (CPM) and the one-way analysis of variance (ANOVA) were plotted [20,21]. We used CPM because it evaluates the contribution of genotype variation by considering all possible partitions of genotypes, selecting the partition that maximizes phenotypic differences between partition means, and minimizing differences between genotype means within partitions [20]. We used the one-way ANOVA to estimate the contribution of genotypes defined by pairs of SNPs to explaining HDL-C variability.

Non-additivity between pairwise SNP effects was tested using an overparameterized general linear model that compares average phenotypic values of two-SNP genotypes [22,23]. This overparameterized model was also used to test for single-SNP genotype by field center interaction within each of the race–gender strata.

We used a nested ANOVA to estimate the proportion of sample variability in HDL-C attributable to variation in plasma apo A-I concentration, and to the single SNPs and pairs of SNPs that explained the greatest amount of HDL-C variability, after adjustment for variation in center means and variation in age, BMI, and plasma apo A-I concentration within each center.

We did not assume Hardy–Weinberg equilibrium when evaluating pairwise linkage disequilibrium (LD) between SNPs. Consequently, we used the composite test of LD, i.e.

$\frac{\widehat{\Delta}_{AB}^2}{p_A p_b p_a p_b}$, where $\Delta_{AB} = 2p_{AABB} + p_{AaBB} + p_{AABb} + \frac{1}{2}p_{AaBb}$ [24]. P values for the test of $\Delta_{AB}=0$ were

obtained using the χ^2 test statistic, i.e. $\frac{\widehat{\Delta}_{AB}^2}{\text{Var}(\widehat{\Delta}_{AB})}$.

Statistical significance was defined as $p \leq 0.05$ in all analyses unless otherwise noted. All cases of statistical significance refer to the nominal significance without correction for multiple comparisons.

Results

Description of the samples

A description of each of the four race–gender strata is shown in Table 1. Age and BMI means differed significantly among field centers in European–American females only. The variance of BMI was significantly different among field centers in all race–gender strata except African–American females. Unadjusted HDL-C means and variances differed significantly across field centers in all four race–gender strata. After adjustment of HDL-C concentration for age and BMI, the estimates of interindividual variability of HDL-C remained significantly heterogeneous among field centers in African–American females, African–American males, and European–American males.

Characterization of the distribution of relative allele frequencies among race–field center strata

In addition to the observed heterogeneity in the distribution of the HDL-C phenotype, we also found significant differences in relative allele frequencies between races and among field centers within races. Relative allele frequencies for each SNP, a test of Hardy–Weinberg equilibrium within African–Americans and European–Americans, and the tests of homogeneity of relative allele frequencies within and between races are presented in Fig. 1a,b.

Of the seven SNPs in *APOA1* (Fig. 1a), genotypes of one SNP (523) in African–Americans and one SNP (1616) in European–Americans deviated significantly from Hardy–Weinberg expectations. The test of homogeneity of relative allele frequencies among field centers within races revealed that SNP 1280 differed significantly among African–American field centers, while allele frequencies at SNPs 1280 and 1616 were significantly heterogeneous among European–American field centers. Except for SNP 1616, the relative allele frequency of the common allele in European–Americans was significantly different between the races for each of the *APOA1* SNPs.

Of the 12 SNPs in the *APOA1/APOC3* 3' intergenic region (also shown in Fig. 1a), significant deviations from Hardy–Weinberg equilibrium were observed for three SNPs in African–Americans and five SNPs in European–Americans. Significant relative allele frequency heterogeneity among field centers was found at SNP 3710 in African–Americans and at SNPs 3732 and 5124 in European–Americans. The relative frequency of the common allele in European–Americans was significantly different between races for each of these 12 intergenic SNPs.

Of the 26 SNPs in the *APOC3* gene (Fig. 1b), genotypes for each of six SNPs in African–Americans and nine SNPs in European–Americans were not in Hardy–Weinberg equilibrium. Differences in the relative allele frequencies among field centers were significant for seven SNPs in African–Americans and five SNPs in European–Americans. The relative frequency of the common allele in European–Americans was significantly different between races for 23 of the 26 *APOC3* SNPs.

A comparison of single-SNP effects reported in the literature with estimates from CARDIA

To evaluate consistency between published studies and results from our analyses, we first searched the literature for studies of the association of variation in HDL-C with single-SNP variations in the *APOA1/APOC3* region. For this comparison, the published studies had to include SNPs that were also included in our study, the study participants had to be ascertained without regard to health, and the sample size had to be ≥ 99 . Table 2 summarizes the studies of five SNPs that satisfy these criteria. HDL-C levels have been significantly associated ($p \leq 0.10$) with three of these SNPs: SNP 1046 in white females [25] and SNPs 9127 and 9154 [29] in Asian–Indians.

For the five SNPs found in published reports and genotyped in the CARDIA study, the results of the test of association of HDL-C with genotype variation using the CARDIA data are presented in Table 3. The three significant associations with SNPs in published studies also contributed significantly to explaining HDL-C variability in particular CARDIA race–gender strata. SNP 1046 predicted HDL-C variability in African–American females and European–American females and males. However, in African–American females, this result is confounded by statistically significant heterogeneity of genotype effects among field centers. Additionally, SNPs 9127 and 9154 made a statistically significant contribution to explaining HDL-C variability in African–American females. In these cases, neither the test

of a genotype by field center interaction nor the test of homogeneity of the relative allele frequency among field centers was statistically significant.

Selection of single SNPs that explain the greatest HDL-C variability

Figure 2a–d presents the results of the selection strategy for identifying the SNP that explained the greatest amount of HDL-C variability in each race–gender stratum. The CPM and the ANOVA identified the same SNP as explaining the greatest amount of HDL-C variability in three of the four race–gender strata. SNPs 5124 and 9648, selected in African–American females and African–American males, respectively, did not contribute significantly to variability in the other two strata. SNP 2110 explained the greatest HDL-C variability in European–American males. This SNP also predicted significant variability in African–American females ($p=0.0160$, CPM and $p=0.0422$, ANOVA) and European–American females ($p=0.0445$, CPM only). Two SNPs (2957 and 8521) were selected in the sample of European–American females. SNP 2957, selected by the CPM analysis, was also significant in African–American females ($p=0.0121$, CPM and $p=0.0122$, ANOVA) and European–American males ($p=0.0005$, CPM and $p=0.0004$, ANOVA). SNP 8521, selected by the ANOVA, also contributed significantly to HDL-C variability in European–American males ($p=0.0020$, CPM and $p=0.0020$, ANOVA).

Phenotype–genotype relationships for SNPs that explain the greatest HDL-C variability

The analyses of consistency of phenotype–genotype relationships across field center strata for each of the five SNPs selected to explain the greatest amount of variability in each race–gender stratum are summarized in Table 4. For purposes of this study, phenotype–genotype relationships were considered to be consistent if the differences between genotypic means (milligram per deciliter) were in the same direction (either positive or negative differences) across at least three of the four field centers within a race–gender stratum. In each case, the SNP that explained the greatest amount of variability had a consistent phenotype–genotype relationship among field centers for at least one contrast of genotypes. In addition to missing genotype classes, heterogeneity of relative allele frequencies among field centers (SNPs 5124, 9648, and 8521) and a significant genotype by field center interaction (SNP 2957, contrast AA–AG) confounded the evaluation of replication of phenotype–genotype relationships in other race–gender strata. Only one of the five SNPs (2110, contrast CC–CT) had a consistent phenotype–genotype relationship across all 16 race–gender–field center strata, and there was no statistically significant evidence of heterogeneity of relative allele frequency or genotype by field center interaction effects.

Selection of pairs of SNPs that explain the greatest HDL-C variability

The results of the analyses of the associations of HDL-C variability with genotypes defined by pairs of SNPs are presented in Fig. 3a–d. Pairs of SNPs that explained the greatest amount of variability in each race–gender stratum did not explain the greatest HDL-C variability in any other stratum. Selected pairs of SNPs for the African–American female (4281–6156) and African–American male (4797–9648) samples did not explain statistically significant variation in any other race–gender stratum. However, the pair of SNPs that explained the greatest HDL-C variability in European–American females (1616–5124) also made a statistically significant contribution to explaining variability in African–American females. The SNP pair that explained the greatest HDL-C variability in European–American males (2110–5904) also made a statistically significant contribution to explaining variability in African–American females. Of the four selected pairs, only one SNP pair (1616–5124), selected in European–American females, did not involve statistically significant single-SNP genotype effects. In African–American females, each of these SNPs had a statistically significant genotype effect.

Contribution of SNPs to HDL-C variability beyond that explained by field center, age, BMI, and plasma apo A-I concentration

Table 5 presents estimates of the contribution of single SNP (s) and pair(s) of SNPs selected because they explained the greatest amount of HDL-C variability in a particular race–gender stratum (shown in Figs. 2a–d and 3a–d). Variability among field center means explained 1.16 to 4.34% of sample variability across race–gender strata. Age and BMI variability within field centers explained 8.94 to 11.16% of sample variability across race–gender strata. After adjustment for the contribution of variability among field centers and age and BMI variability within field centers, the contribution of plasma apo A-I concentration within field centers to explaining HDL-C variability ranged from 22.08 to 32.35% of sample variability across race–gender strata. After adjustment for age, BMI, and plasma apo A-I, selected SNPs explained only 0.24 to 1.38% of HDL-C variability, while the selected pairs of SNPs explained from 1.04 to 3.04% of sample variability across race–gender strata.

Discussion

Although biochemical evidence has clearly demonstrated a functional relationship between HDL particles and plasma apo A-I and apo C-III, our study further documents inconsistency of statistical associations between plasma HDL-C variation and SNP variation in the *APOA1/APOC3* region. A comparison of our results with similar single-SNP studies reported in the literature and the observation that different single SNPs explained the greatest HDL-C variability in each race–gender stratum suggest that there is little support for invariant effects of single-SNP genotypes in the *APOA1/APOC3* genes. Furthermore, none of the SNPs selected because it explained the greatest amount of variation in a particular race–gender stratum overlapped with any of the previously published SNPs, and none of these SNPs was selected in more than one race–gender stratum. Only one SNP (2110), selected because it explained the greatest amount of variation in European–American males, had a consistent phenotype–genotype relationship across all 16 race–gender–field center strata of the CARDIA study. No other statistically significant SNP included in our study had a consistent phenotype–genotype relationship across all race–gender strata that was not confounded by heterogeneity of relative allele frequencies or genotype effects among field centers in a particular race–gender stratum (data not shown). Additionally, a substantial number of SNP genotypes deviated significantly from Hardy–Weinberg expectations in both African–Americans and European–Americans. Significant departures from Hardy–Weinberg equilibrium were, in fact, observed in two of the SNPs selected to explain the greatest HDL-C variability (5124 in both races and 9648 in African–Americans). While it is possible that these deviations are attributable to genotyping error, they could also be due to hidden population stratification across field centers within racial strata caused by pooling groups of individuals with different genotype frequencies [30]. Analyses of the distribution of relative allele and genotype frequencies by Clark et al. (unpublished data, August 2005) using the CARDIA samples (including the *APOA1/APOC3* SNPs studied here) provide evidence in support of the latter possibility.

The lack of consistent significant genotype effects in our study and in published reports strongly suggests that the contribution of variation in single-SNP genotypes to phenotypic variability is not invariant across samples of individuals defined by race and gender. Thus, the growing popularity of meta-analyses, whereby data from multiple genetic association studies are combined to increase statistical power to detect small genotype effects, is largely unjustified. Meta-analyses are based on the assumption that the inconsistency of phenotype–genotype relationships is due to inadequate sample size or chance [31]. Combining subsets of individuals from different race–gender strata, each with a unique genetic and environmental background, may increase statistical power to detect an effect in the pooled

sample, but this approach also inflates the risk of false positives if the samples are heterogeneous, and inferences will not reflect the biological reality of any particular stratum.

The pattern of LD among pairs of SNPs in African-Americans and European-Americans further confounds the contributions of individual SNPs to explaining HDL-C variability. An analysis of pairwise LD among the five SNPs selected from the literature (Table 3) and the five SNPs selected to explain the greatest HDL-C variability (Table 4) reveals strong LD ($r^2 \geq 0.75$) for seven pairs (9127–9154, 1046–2110, 1046–2957, 1046–8521, 2110–2957, 2110–8521, and 2957–8521). Strong LD between SNPs 9127–9154 in the CARDIA sample of African-Americans suggests that the observed statistically significant associations of HDL-C with each of these SNPs are not independent and may be marking the same “functional” variation. Further, strong LD of SNPs 9127 and 9154 with 9312, a SNP that was not included in published studies, implies that neither 9127 nor 9154 may be the SNP responsible for trait variability. In European-Americans, we found strong LD between SNPs 2110 (selected in European-American males), 2957, and 8521 (selected in European-American females). Although selected to explain the greatest variability in particular race-gender strata, these three SNPs each had statistically significant genotype effects in both European-American females and males. Additionally, one SNP selected from published studies that was significant in CARDIA European-American females and males (SNP 1046) was also in strong LD with 2110, 2957, and 8521. In European-Americans, we also find extensive pairwise LD among SNPs deviating from Hardy-Weinberg equilibrium. These considerations further emphasize the futility in assigning functionality to a particular SNP using only statistical results from an association study.

Our study suggests that heterogeneity of both relative allele frequencies and genotype effects among field centers within race-gender strata can be a major consideration in evaluating replicability of single-SNP genotype effects. The difficulty of obtaining replicate samples of a particular context defined by unmeasured genetic and environmental factors is a major deterrent in sorting out context-dependent SNP effects from false-positive/type I errors. Although we analyzed multiple SNPs, we did not apply any corrections to control for the type I error rate, such as Bonferroni, false discovery rate, or permutation testing [32–34]. However, we took a “conservative” approach by selecting only those single SNPs which explained the greatest HDL-C variability in each race-gender stratum (e.g., the most statistically significant). We also found evidence that the contribution of a particular SNP to HDL-C variability was consistent across geographically diverse field centers in particular contexts defined by race and gender. This suggests, but does not prove, biological effects that are distinguishable from false-positive tests that arise in the study of any particular geographical location. Such replicability is essential for sorting out the context-dependent effects from type I errors that are characteristic of quantitative traits like HDL-C that have a complex genetic architecture in the population at large.

Identification of pairs of SNPs that explain the greatest amount of variability reveals the importance of non-additivity of SNP effects. Single SNPs contributed 0.24–1.38% HDL-C variability, while pairs of SNPs contributed 1.01–3.04%. It is likely that significant interactions between combinations of SNP effects that, when considered independently do not have a measurable impact on quantitative trait variability, are numerous throughout the genome. The dependency of SNP effects on the context defined by other SNPs has been a major deterrent to accurately characterizing the impact of gene variation on quantitative trait variability. Hamon et al. [35] studied the impact of non-additivity between pairs of SNPs in the *APOA1/C3/A4/A5* cluster on variability in total cholesterol (TC) levels in the CARDIA sample. They found significant non-additive SNP effects on TC levels for 14 pairs of SNPs in both African-American and European-American females. Eight of these SNP pairs also had a consistent phenotype-genotype relationship across field centers. While in Hamon et

al. [35], none of the pairs of SNPs explaining the greatest variability in this study significantly influenced TC levels, in European–American females, we do find significant evidence of non-additive effects of SNPs 1616 and 5124 on variability in HDL-C levels. It is interesting to note that in Hamon et al. [35], SNP 5124 combined non-additively with two other SNPs (*APOA1/APOC3* intergenic SNPs 3132 and 3581, respectively) to impact TC levels in females. The widespread use of statistical models of phenotype–genotype relationships that do not consider interactions between SNP effects has been more a function of perpetuated popularity or apparent ease of calculation rather than an informed approach for characterizing the associations between genetic variations and variability in traits such as HDL-C [36].

Although the contribution of the selected single SNPs and pairs of SNPs to explaining HDL-C variability was statistically significant, the amount of total variability explained was a minor fraction of total sample variability (Table 5). Compared with adjusted data, these estimates using data that were not adjusted for plasma apo A-I were essentially unchanged (0.32–1.81% for the selected single SNPs and 1.25–3.43% for the selected pairs of SNPs). Furthermore, although plasma apo A-I makes a considerable contribution to explaining HDL-C variability (22–32%), the contribution of the *APOA1/APOC3* SNPs selected to explain the greatest HDL-C variability to explaining variation in plasma apo A-I concentration was small (0.27–1.46% for single SNPs and 0.66–1.89% for pairs of SNPs). Given that 28–48% of HDL-C variability may be attributable to genetic variation in the population at large, it is obvious that many genetic loci, in addition to the *APOA1/APOC3* structural genes, influence HDL-C via their effects on plasma apo A-I concentration and that selecting one, or a few, candidate gene loci to characterize the genetic architecture of this important quantitative CVD risk factor is woefully inadequate [8,37].

The statistically significant contribution of individual SNPs to HDL-C variability should be interpreted with caution. While a statistically significant SNP genotype effect may indicate a direct functional relationship between the SNP and its associated gene product (i.e., a SNP resulting in a nonsynonymous amino acid change that alters protein function), we have provided evidence that several factors can confound the interpretation of such statistical associations. Specifically, variation in genetic and environmental background, patterns of LD among pairs of SNPs, and the contribution of SNPs beyond other (environmental) variables limit etiologic interpretation of statistical effects of a particular SNP genotype. Furthermore, because of the complexity of the phenotype–genotype relationship, we anticipate that single SNPs that have both an independent and biologically relevant impact on a quantitative trait with a multifactorial etiology such as HDL-C will be the exception.

Racial/ethnic, gender, and regional differences in (nongenetic) CVD risk factor variability and associated risk of disease are widely recognized and have been well-documented in CARDIA and other study populations across the USA [38–43]. Additionally, the presence of confounding effects of heterogeneity of the genetic substructure among racially/ethnically/geographically diverse groups has been documented by numerous studies of population-specific recombination rates, population diversity, *Fst* statistics, measures of population admixture, and the distribution of relative allele and haplotype frequencies among populations [44–48]. Despite such compelling evidence, many studies of phenotype–genotype relationships continue to pool individuals from different races, genders, or geographical locations [49]. Inconsistent phenotype–genotype findings are likely a consequence of false assumptions about homogeneity of the populations of inference and the invariance of genotype effects. As an alternative, we must restate the genetic question and ask which subset of genetic variants, in which genes, in which genetic and environmental strata of the population at large explain/predict CVD risk factor variability [50]. Our study identified race–gender-specific genotype effects that are consistent across field centers

within each race–gender stratum. These results illustrate the importance of genetic and environmental background in characterizing the contribution of SNPs to variability in quantitative CVD risk factors such as plasma HDL-C concentration.

Acknowledgments

This work was supported by NIH grants HL072810, HL072905, HL039107, GM065509, HC095095, HC048047, HC048048, HC048049, HC048050, HC045134, and HC05187. The authors would like to thank Lynn Illeck, Paul Kopec, Deborah Theodore, and Kenneth G. Weiss for their technical support and research assistance.

References

1. National Heart Lung, and Blood Institute. 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). National Institutes of Health, Bethesda, MD. *Circulation*. 2002; 106 (25):3143–3421. [PubMed: 12485966]
2. Asztalos BF. HDL Atherosclerosis Treatment Study. High-density lipoprotein metabolism and progression of atherosclerosis: insights from the HDL Atherosclerosis Treatment Study. *Curr Opin Cardiol*. 2004; 19:385–391. [PubMed: 15218401]
3. Tall AR, Small DM. Plasma high-density lipoproteins. *N Engl J Med*. 1978; 299:1232–1236. [PubMed: 213711]
4. Lund-Katz S, Liu L, Thuahnai ST, Phillips MC. High density lipoprotein structure. *Front Biosci*. 2003; 8:d1044–d1054. [PubMed: 12700101]
5. Wang CS, McConathy WJ, Kloer HU, Alaupovic P. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J Clin Invest*. 1985; 75:384–390. [PubMed: 3973011]
6. Xu S, Laccotripe M, Huang X, Rigotti A, Zannis VI, Krieger M. Apolipoproteins of HDL can directly mediate binding to the scavenger receptor SR-BI, an HDL receptor that mediates selective lipid uptake. *J Lipid Res*. 1997; 38:1289–1298. [PubMed: 9254056]
7. Groenendijk M, Cantor RM, de Bruin TW, Dallinga-Thie GM. The apoAI-CIII-AIV-AV gene cluster. *Atherosclerosis*. 2001; 157:1–11. [PubMed: 11427198]
8. Sing CF, Boerwinkle E (1987) Genetic architecture of inter-individual variability in apolipoprotein, lipoprotein and lipid phenotypes. In: Bock G, Collins GM (eds) *Molecular approaches to human polygenic disease* (Ciba Foundation Symposia 130). Wiley, Chichester, pp 99–127
9. Miller M, Zhan M. Genetic determinants of low high-density lipoprotein cholesterol. *Curr Opin Cardiol*. 2004; 19:380–384. [PubMed: 15218400]
10. Pallaud C, Gueguen R, Sass C, Grow M, Cheng S, Siest G, Visvikis S. Genetic influences on lipid metabolism trait variability within the Stanislas Cohort. *J Lipid Res*. 2001; 42:1879–1890. [PubMed: 11714857]
11. Kamboh MI, Bunker CH, Aston CE, Nestlerode CS, McAllister AE, Ukoli FA. Genetic association of five apolipoprotein polymorphisms with serum lipoprotein-lipid levels in African blacks. *Genet Epidemiol*. 1999; 16:205–222. [PubMed: 10030402]
12. Larson IA, Ordovas JM, Barnard JR, Hoffmann MM, Feussner G, Lamon-Fava S, Schaefer EJ. Effects of apolipoprotein A-I genetic variations on plasma apolipoprotein, serum lipoprotein and glucose levels. *Clin Genet*. 2002; 61:176–184. [PubMed: 12000358]
13. Waterworth DM, Talmud PJ, Bujac SR, Fisher RM, Miller GJ, Humphries SE. Contribution of apolipoprotein C-III gene variants to determination of triglyceride levels and interaction with smoking in middle-aged men. *Arterioscler Thromb Vasc Biol*. 2000; 20:2663–2669. [PubMed: 11116069]
14. Pembrey M. Genetic epidemiology: some special considerations of birth cohorts. *Paediatr Perinat Epidemiol*. 2004; 18:3–7. [PubMed: 14738540]
15. Friedman GD, Cutter GR, Donahue RP, Hughes GH, Hulley SB, Jacobs DR Jr, Liu K, Savage PJ. CARDIA: study design, recruitment, and some characteristics of the examined subjects. *J Clin Epidemiol*. 1988; 41:1105–1116. [PubMed: 3204420]

16. Warnick GR. Enzymatic methods for quantification of lipoprotein lipids. *Methods Enzymol.* 1986; 129:101–123. [PubMed: 3724535]
17. Albers JJ, Adolphson JL. Comparison of commercial kits for apoprotein A-I and apoprotein B with standardized apoprotein A-I and B radioimmunoassays performed at the Northwest Lipid Research Center. *J Lipid Res.* 1988; 29:102–108. [PubMed: 3128618]
18. Fullerton SM, Buchanon AV, Sonpar VA, Taylor SL, Smith JD, Carlson CS, Salomaa V, Stengård JH, Boerwinkle E, Clark AG, Nickerson DA, Weiss KM. The effects of scale: variation in the APOA1/C3/A4/A5 gene cluster. *Hum Genet.* 2004; 115:36–56. [PubMed: 15108119]
19. Efron B, Tibshirani R. Empirical bayes methods and false discovery rates for microarrays. *Genet Epidemiol.* 2002; 23:70–86. [PubMed: 12112249]
20. Nelson MR, Kardina SL, Ferrell RE, Sing CF. A combinatorial partitioning method to identify multilocus genotypic partitions that predict quantitative trait variation. *Genome Res.* 2001; 11:458–470. [PubMed: 11230170]
21. Rohlf FJ, Sokal RR (1994) *Biometry*. W.H. Freeman, New York
22. Hamon SC, Stengård JH, Clark AG, Salomaa V, Boerwinkle E, Sing CF. Evidence for non-additive influence of single nucleotide polymorphisms within the apolipoprotein E gene. *Ann Hum Genet.* 2004; 68:521–535. [PubMed: 15598211]
23. Searle SR (1971) *Linear models*. Wiley, New York
24. Weir BS (1996) *Genetic data analysis 2: methods for discrete population genetic data*. Sinauer, Massachusetts
25. Kamboh MI, Aston CE, Nestlerode CM, McAllister AE, Hamman RF. Haplotype analysis of two APOA1/MspI polymorphisms in relation to plasma levels of apo A-I and HDL-cholesterol. *Atherosclerosis.* 1996; 127:255–262. [PubMed: 9125316]
26. Shioji K, Mannami T, Kokubo Y, Goto Y, Nonogi H, Iwai N. An association analysis between ApoA1 polymorphisms and the high-density lipoprotein (HDL) cholesterol level and myocardial infarction (MI) in Japanese. *J Hum Genet.* 2004; 49:433–439. [PubMed: 15258834]
27. Peacock RE, Temple A, Gudnason V, Rosseneu M, Humphries SE. Variation at the lipoprotein lipase and apolipoprotein AI-CIII gene loci are associated with fasting lipid and lipoprotein traits in a population sample from Iceland: interaction between genotype, gender, and smoking status. *Genet Epidemiol.* 1997; 14:265–282. [PubMed: 9181356]
28. Dallongeville J, Meirhaeghe A, Cotel D, Fruchart JC, Amouyel P, Helbecque N. Gender related association between genetic variations of APOC-III gene and lipid and lipoprotein variables in northern France. *Atherosclerosis.* 2000; 150:149–157. [PubMed: 10781646]
29. Miller M, Rhyne J, Khatta M, Parekh H, Zeller K. Prevalence of the APOC3 promoter polymorphisms T-455C and C-482T in Asian-Indians. *Am J Cardiol.* 2001; 87:220–221. [PubMed: 11152845]
30. Hartl DL, Clark AG (1997) *Principles of population genetics*, 3rd edn. Sinauer, Massachusetts
31. Davey Smith G, Egger M. Meta-analysis. Unresolved issues and future developments. *BMJ.* 1998; 316:221–225. [PubMed: 9468698]
32. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc.* 1995; 57:289–300.
33. Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. *Genetics.* 1994; 138:963–971. [PubMed: 7851788]
34. Lynch M, Walsh B (1998) *Genetics and analysis of quantitative traits*. Sinauer Associates, Massachusetts
35. Hamon SC, Kardina SLR, Boerwinkle E, Liu K, Klos KLE, Clark AG, Sing CF. Replicate evidence for intragenic and intergenic interactions between SNP effects in the APOA1/ C3/A4/A5 gene cluster. *Hum Hered.* 2006; 61:87–96. [PubMed: 16710093]
36. Spence MA, Greenberg DA, Hodge SE, Vieland VJ. The emperor's new methods. *Am J Hum Genet.* 2003; 72:1084–1087. [PubMed: 12704569]
37. Brem RB, Kruglyak L. The landscape of genetic complexity across 5,700 gene expression traits in yeast. *Proc Natl Acad Sci U S A.* 2005; 102:1572–1577. [PubMed: 15659551]

38. American Heart Association (2005) Heart disease and stroke statistics—2005 update. American Heart Association, Dallas, TX
39. Barrett-Connor E. Sex differences in coronary heart disease. Why are women so superior? The 1995 Ancel Keys Lecture. *Circulation*. 1997; 95:252–264. [PubMed: 8994444]
40. Cooper R, Cutler J, Desvigne-Nickens P, Fortmann SP, Friedman L, Havlik R, Hogelin G, Marler J, McGovern P, Morosco G, Mosca L, Pearson T, Stamler J, Stryer D, Thom T. Trends and disparities in coronary heart disease, stroke, and other cardiovascular diseases in the United States: findings of the national conference on cardiovascular disease prevention. *Circulation*. 2000; 102:3137–3147. [PubMed: 11120707]
41. Greenlund KJ, Kiefe CI, Gidding SS, Lewis CE, Srinivasan SR, Williams OD, Berenson GS. Differences in cardiovascular disease risk factors in black and white young adults: comparisons among five communities of the CARDIA and the Bogalusa heart studies. *Coronary artery risk development in young adults*. *Ann Epidemiol*. 1998; 8:22–30. [PubMed: 9465990]
42. Kiefe CI, Williams OD, Bild DE, Lewis CE, Hilner JE, Oberman A. Regional disparities in the incidence of elevated blood pressure among young adults: the CARDIA study. *Circulation*. 1997; 96:1082–1088. [PubMed: 9286933]
43. Schoenborn CA, Adams PF, Barnes PM, Vickerie JL, Schiller JS (2004) Health behaviors of adults: United States, 1999–2001. National Center for Health Statistics. *Vital Health Stat* 10(219)
44. Helgason A, Yngvadottir B, Hrafnkelsson B, Gulcher J, Stefansson K. An Icelandic example of the impact of population structure on association studies. *Nat Genet*. 2005; 37:90–95. [PubMed: 15608637]
45. Jorgenson E, Tang H, Gadde M, Province M, Leppert M, Kardia S, Schork N, Cooper R, Rao DC, Boerwinkle E, Risch N. Ethnicity and human genetic linkage maps. *Am J Hum Genet*. 2005; 76:276–290. [PubMed: 15627237]
46. Reiner AP, Ziv E, Lind DL, Nievergelt CM, Schork NJ, Cummings SR, Phong A, Burchard EG, Harris TB, Psaty BM, Kwok PY. Population structure, admixture, and aging-related phenotypes in African-American adults: the cardiovascular health study. *Am J Hum Genet*. 2005; 76:463–477. [PubMed: 15660291]
47. Risch N, Burchard E, Ziv E, Tang H. Categorization of humans in biomedical research: genes, race and disease. *Genome Biol*. 2002; 3:1–12.
48. Sawyer SL, Mukherjee N, Pakstis AJ, Feuk L, Kidd JR, Brookes AJ, Kidd KK. Linkage disequilibrium patterns vary substantially among populations. *Eur J Hum Genet*. 2005; 13:677–686. [PubMed: 15657612]
49. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K. A comprehensive review of genetic association studies. *Genet Med*. 2002; 4:45–61. [PubMed: 11882781]
50. Sing CF, Stengård JH, Kardia SL. Genes, environment and cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 2003; 23:1190–1196. [PubMed: 12730090]

Fig. 1. Relative allele frequencies (*N*) and tests of relative allele frequency homogeneity within and between races. **a** *APOA1* (523–2110) and *APOA1/APOC3* intergenic (2954–5124) SNPs. Note that site 1787 did not vary in European–Americans. **b** *APOC3* SNPs. *Bold* denotes common allele in European–Americans. *Dashed boxes* indicate significant heterogeneity at $p \leq 0.05$, and *shaded boxes* indicate significant deviation from Hardy–Weinberg equilibrium

Fig. 2. Single-SNP CPM and ANOVA $-\log(p$ values) for each race-gender strata
a African-American females
b African-American males
c European-American females
d European-American males

Fig. 3. ANOVA $-\log(p$ values) for pairs of SNPs in *APOA1/APOC3* for each race-gender stratum

a African-American females

b African-American males

c European-American females

d European-American males Each *dot* indicates an SNP pair

Table 1

Sample descriptions by race and gender (*N*, mean, and variance; *N*=3,817)

	Birmingham			Chicago			Minneapolis			Oakland			All			Homogeneity	
	N	Mean	Var	N	Mean	Var	N	Mean	Var	N	Mean	Var	N	Mean	Var	Means	Vars
African-American females																	
Age	288	24.54	14.56	230	24.32	15.28	203	24.40	17.73	350	24.65	13.75	1071	24.50	15.02	0.760	0.219
BMI	288	25.90	38.36	230	26.13	45.48	203	25.29	44.68	350	25.92	39.56	1071	25.84	41.44	0.563	0.422
HDL-C	288	52.18	131.78	230	56.04	169.27	203	55.22	188.24	350	56.40	147.36	1071	54.96	158.20	<0.001	0.030
HDL adj	288	54.96	117.53	230	54.96	153.86	203	54.96	170.57	350	54.96	136.06	1071	54.96	141.04	NT	0.024
African-American males																	
Age	218	24.22	14.47	157	24.16	13.43	192	24.26	15.62	213	24.60	12.59	780	24.32	14.00	0.637	0.461
BMI	218	24.67	24.49	157	24.89	18.24	192	24.30	17.77	213	24.66	16.00	780	24.62	19.23	0.645	0.012
HDL-C	218	52.39	171.48	157	54.01	164.90	192	55.84	236.49	213	52.33	156.01	780	53.55	183.35	0.029	0.014
HDL adj	218	53.55	152.54	157	53.55	152.62	192	53.55	206.66	213	53.55	145.19	780	53.55	163.24	NT	0.047
European-American females																	
Age	182	24.84	11.95	223	25.15	11.69	348	26.14	9.75	283	25.69	12.81	1036	25.58	11.60	<0.001	0.100
BMI	182	22.58	23.13	223	22.65	13.71	348	23.95	22.26	283	22.83	17.09	1036	23.12	19.46	<0.001	<0.001
HDL-C	182	51.81	140.43	223	58.87	196.48	348	54.92	155.71	283	58.67	141.33	1036	56.25	164.56	<0.001	0.035
HDL adj	182	56.25	123.70	223	56.25	164.32	348	56.25	141.35	283	56.25	127.63	1036	56.25	139.04	NT	0.138
European-American males																	
Age	223	25.26	12.62	214	25.26	10.45	306	25.82	10.41	187	25.60	11.95	930	25.52	11.28	0.157	0.344
BMI	223	24.41	15.10	214	24.52	10.51	306	24.30	12.10	187	23.68	10.17	930	24.25	12.12	0.077	0.015
HDL-C	223	44.07	84.58	214	47.93	107.37	306	47.31	127.39	187	49.13	114.14	930	47.04	112.71	<0.001	0.014
HDL adj	223	47.04	74.32	214	47.04	98.54	306	47.04	115.57	187	47.04	100.93	930	47.04	98.50	NT	0.007

Bold *p* values indicate significance at ≤ 0.05

NT No test

Table 2

Association studies involving HDL-C and *APOA1/APOC3* SNPs in the literature

Race/ethnicity	Gender	Sample size	Mean age (years)	Region	P value	Reference
1046 (+83, <i>MspI</i>)						
White	Males	236	52.5	Colorado	0.280	[25]
White	Females	257	52.5	Colorado	0.010	
Black	Males	490	42.5	Nigeria	0.180	[11]
Black	Females	291	38.6	Nigeria	0.870	
Caucasian	Males	372	58.7	USA	0.940	[12]
Caucasian	Females	358	56.1	USA	0.698	
1280 (T84C, <i>HaeIII</i>)						
Japanese	Males/females	796/839	66.3/63.3	Japan	0.236	[26]
7761 (1100)						
Caucasian	Males	772	41.5	France	NS	[10]
Caucasian	Females	780	39.5	France	NS	
Icelandic	Males	101	42.2	Iceland	NS	[27]
Icelandic	Females	103	42.1	Iceland	NS	
9127 (-455)						
French	Males	499	51.2	France	NS	[28]
French	Females	508	51.4	France	NS	
Asian-Indians	Males/females	99	45.5	USA	0.080	[29]
9154 (-482)						
French	Males	498	51.2	France	NS	[28]
French	Females	507	51.4	France	NS	
Asian-Indians	Males/females	99	45.5	USA	0.020	[29]
Caucasian	Males	772	41.5	France	NS	[10]
Caucasian	Females	780	39.5	France	NS	

Study populations ascertained without regard to health status and with sample size ≥ 99 . Bold *p* values indicate statistical significance at $p \leq 0.10$. Race/ethnicity nomenclature as reported in the published study. SNP label in parentheses indicates literature nomenclature

NS Not significant where no *p* values given

Table 3
P values from analyses of previously published SNPs and HDL-C variability in CARDIA sample

Site	Test	African-American		European-American	
		Females	Males	Females	Males
1046	CPM	0.0219	0.2704	0.0266	0.0001
	G × F	0.0302	0.8747	0.4472	0.3589
	χ^2	0.3482	0.5191	0.8711	0.4293
1280	CPM	0.2521	0.1436	0.2401	0.2673
	G × F	0.8932	0.0277	0.9783	0.0330
	χ^2	0.0050	0.0213	0.2140	0.0285
7761	CPM	0.1286	0.7957	0.1317	0.9135
	G × F	0.1688	0.1244	0.5114	0.0538
	χ^2	0.6952	0.8897	0.5209	0.1335
9127	CPM	0.0448	0.0905	0.5365	0.6167
	G × F	0.2836	0.8505	0.5773	0.6063
	χ^2	0.2781	0.0006	0.0094	0.0001
9154	CPM	0.0142	0.2517	0.4681	0.8207
	G × F	0.1909	0.9098	0.9442	0.6602
	χ^2	0.0893	0.0001	0.1967	0.0003

Bold *p* values indicate significance at $p \leq 0.05$
CPM Single site Combinatorial Partitioning Method, $G \times F$ genotype by field center interaction, χ^2 chi-squared test of relative allele frequency homogeneity among field centers

Table 4
Genotypic differences in mean HDL-C (milligram per deciliter) for field centers in race-gender strata

SNP	Contrast	African-American						European-American									
		Females			Males			Females			Males						
		BIR	CHI	MIN	OAK	BIR	CHI	MIN	OAK	BIR	CHI	MIN	OAK				
5124	GG-GT	-0.06	1.54	-1.05	1.41	0.82	-0.76	0.30	-2.65	NA	-3.26	-3.26	0.99	4.44	NA	2.62	NA
	GG-TT	1.17	2.11	5.25	4.90	2.34	1.59	-1.91	-0.19	NA	-1.02	-3.14	2.30	3.19	NA	4.30	NA
	GT-TT	1.23	0.57	6.30	3.49	1.52	2.35	-2.21	2.46	-2.99	2.24	0.12	1.31	-1.25	-0.21	1.68	2.78
9648	AA-AG	NA	NA	NA	NA	-0.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	AA-GG	NA	NA	NA	NA	1.97	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	AG-GG	-1.27	2.54	0.45	2.59	2.03	9.95	1.22	6.55	NA	NA	NA	NA	NA	NA	NA	NA
2957	AA-AG	-0.62	1.67	-5.89	-0.72	-2.50	-0.23	2.67	-3.10	-0.37	-7.87	-3.27	-2.41	-0.30	-4.87	-4.03	-14.15
	AA-GG	-0.45	-4.63	-5.45	-3.50	-2.13	-0.20	0.60	-1.66	NA	NA	NA	NA	NA	NA	NA	NA
	AG-GG	0.17	-6.30	0.44	-2.78	0.37	0.03	-2.07	1.44	NA	NA	NA	NA	NA	NA	NA	NA
8521	AA-AG	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	AA-GG	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	AG-GG	NA	NA	-5.97	-2.53	NA	NA	NA	-0.69	-1.74	7.11	3.67	1.77	3.84	6.55	4.74	NA
2110	CC-CT	-0.86	1.02	-5.27	-1.88	-1.10	-0.68	-0.29	-0.96	1.08	-7.63	-3.51	-1.76	-0.73	-4.76	-5.85	-14.05
	CC-TT	1.65	-4.38	-6.68	-3.38	-3.98	3.49	2.24	-0.49	NA	NA	NA	NA	NA	NA	NA	NA
	CT-TT	2.51	-5.40	-1.41	-1.50	-2.88	4.17	2.53	0.47	NA	NA	NA	NA	NA	NA	NA	NA

Solid boxes indicate stratum in which SNP explained the greatest variability. Dashed boxes indicate significant relative allele frequency heterogeneity among centers. Shaded mean differences indicate significant genotype contrast by field center interaction.

NA Genotypic classes with less than five individuals were not included

Table 5
Contribution of selected genetic and environmental variables to sample variability of HDL-C by race and gender

Model	African-American				European-American			
	Females		Males		Females		Males	
	SNPs	R ² ×100	SNPs	R ² ×100	SNPs	R ² ×100	SNPs	R ² ×100
Total		100.00		100.00		100.00		100.00
Centers		1.91***		1.16*		4.34***		2.84***
Age and BMI/centers		8.94***		9.82***		11.16***		9.76***
Plasma apo A-I/age and BMI/centers		25.34***		32.35***		22.08***		23.13***
Best single ANOVA SNP/plasma apo A-I/ age and BMI/centers	5124	0.80**	9648	1.03**	8521	0.24*	2110	1.38***
Best pair of SNPs/plasma apo A-I/ age and BMI/centers	4281-6156	3.04***	4797-9648	1.53***	1616-5124	1.04*	2110-5904	1.74***

* Significant at $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.001$