# Racial/Ethnic Variation in the Association of Lipid-Related Genetic Variants With Blood Lipids in the US Adult Population

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***Background***—Genome-wide association studies (GWAS) have identified a number of single-nucleotide polymorphisms (SNPs) associated with serum lipid level in populations of European descent. The individual and the cumulative effect of these SNPs on blood lipids are largely unclear for the US population.

***Methods and Results***—Using data from the second phase (1991–1994) of the Third National Health and Nutrition Examination Survey (NHANES III), a nationally representative survey of the US population, we examined associations of 57 GWAS-identified or well-established lipid-related genetic loci with plasma concentrations of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, total cholesterol, triglycerides, total cholesterol/HDL-C ratio, and non-HDL-C. We used multivariable linear regression to examine single SNP associations and the cumulative effect of multiple SNPs (using a genetic risk score [GRS]) on blood lipid levels. Analyses were conducted in adults from each of the 3 major racial/ethnic groups in the United States: non-Hispanic whites (n=2296), non-Hispanic blacks (n=1699), and Mexican Americans (n=1713). Allele frequencies for all SNPs varied significantly by race/ethnicity, except rs3764261 in *CETP*. Individual SNPs had very small effects on lipid levels, effects that were generally consistent in direction across racial/ethnic groups. More GWAS-validated SNPs were replicated in non-Hispanic whites (<67%) than in non-Hispanic blacks (<44%) or Mexican Americans (<44%). GRSs were strongly associated with increased lipid levels in each racial/ethnic group. The combination of all SNPs into a weighted GRS explained no more than 11% of the total variance in blood lipid levels.

***Conclusions***—Our findings show that the combined association of SNPs, based on a GRS, was strongly associated with increased blood lipid measures in all major race/ethnic groups in the United States, which may help in identifying subgroups with a high risk for an unfavorable lipid profile. **(*Circ Cardiovasc Genet*. 2011;4:523-533.)**

**Key Words:** nutrition surveys ■ lipids ■ continental population groups ■ ethnology ■ cholesterol ■ genetics ■ risk



Genome-wide association studies (GWAS) have successfully identified numerous genetic loci associated with blood lipid levels, which are known risk factors for cardiovascular disease.[1–8] These studies have confirmed some risk loci identified in previous candidate gene and linkage analyses, and they have uncovered a panel of new candidate loci for lipid traits such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG). Meta-analyses of GWAS have proven fruitful as well. A recent meta-analysis of blood lipid studies reported significant associations of 95 loci with lipid traits in >100 000 individuals of European ancestry, most of which had concordant effects in non-European populations.[3] Nevertheless, studies have reported racial/ethnic variation in blood lipid levels, with a healthier lipid profile (lower TG and higher HDL-C) among African Americans than persons of European descent.[9–11] Interestingly, differences seem to exist between the genetic determinants of lipid profiles in African Americans and European Americans.[12] To date, it remains unclear whether the exact polymorphisms discovered in populations of European descent are relevant to non-European populations in the United States. The allele frequencies of common genetic variants are likely to show wide variation across multiple populations, as reported in candidate gene studies or GWAS.[13,14] This genetic variation may contribute, in part,

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to the differential distribution of diseases across racial/ethnic groups. However, other studies suggest that genetic effects for complex diseases usually are consistent across racial/ethnic groups.[3,15]

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The clinical and public health implications of these variants validated through GWAS, however, are unclear. Studies have suggested a stronger association with combinations of common variants than with individual single-nucleotide polymorphisms (SNPs),[6] although these loci explain only a small proportion of the heritable component of disease.[12,16] To date, the individual and cumulative effects of these SNPs on blood lipid levels are largely undetermined for the major racial/ethnic groups in the US population. To address these concerns, we analyzed blood lipid concentrations and their associations with 55 GWAS-identified SNPs and 2 candidate gene SNPs in *APOE* in the Third National Health and Nutrition Examination Survey (NHANES III), a large, well-designed, and population-based survey of the US population. We aimed to (1) estimate allele frequencies using this nationally representative sample of adults from each of the 3 major racial/ethnic groups in the United States, (2) evaluate the independent and combined effects of these genetic variants on serum lipid levels, and (3) examine racial/ethnic variation in the cumulative effects of these polymorphisms on lipid traits.

## Methods

### Study Population

NHANES is a cross-sectional survey designed to provide national statistics on the health and nutritional status of the civilian, noninstitutionalized US population. We included samples from the NHANES III DNA bank, which contains specimens from 7159 participants aged ≥12 years that were collected in phase 2 of NHANES III (1991–1994). The DNA bank is maintained by the Centers for Disease Control and Prevention (CDC) and has been described previously.[5] The present study included behavioral, environmental, and clinical information available in NHANES III. We restricted our analyses to participants aged ≥17 years (n=6317). Of these, we excluded persons in the "other" racial/ethnic group (n=301), pregnant women (n=122), and persons who reported taking cholesterol-lowering medications (n=186). The final sample size was 5708, which included 2296 non-Hispanic whites, 1699 non-Hispanic blacks, and 1713 Mexican Americans. We further restricted our analyses of LDL-C to participants who fasted between 8 and 24 hours (n=3405). This study was approved by the National Center for Health Statistics Ethics Review Board at the CDC.

### Laboratory Measures and Phenotype Definitions

Details of the blood collection procedures and the laboratory evaluation of LDL-C, HDL-C, TC, and TG are available online.[17] HDL-C and TG levels were measured using standard enzymatic methods. Serum LDL-C was calculated using the Friedewald equation.[18] Participants who did not fast or fasted <8 hours or who had TG levels >400 mg/dL were excluded from the analyses of LDL-C. The value of the TC/HDL-C ratio was calculated using TC levels divided by HDL-C levels. Outliers of TC/HDL-C ratio >40 also were excluded. The value of non-HDL-C was calculated by subtracting HDL-C from TC. LDL-C was examined in fasting samples only, whereas all other outcomes were examined in both fasting and nonfasting samples to increase statistical power.[19] Additionally, we conducted a subset analysis of TG among participants who fasted 8 to 24 hours (n=3386).

Phenotypic covariates included in the analyses were selected based on the Third Report of the National Cholesterol Education Program Adult Treatment Panel guidelines published in 2001.[20] The demographic and phenotypic covariates included age; sex; education completed (less than high school, high school, or college and above); alcohol intake (none, <4 drinks/week, ≥4 drinks/week); smoking status (current smoker, former smoker, never smoker); body mass index; physical activity (none, low [active <5 times/week], or high [active ≥5 times/week]); history of hypertension; history of diabetes; log of food energy (kcal); and log of percent of kilocalories from saturated fat, monounsaturated fat, and polyunsaturated fat (reported in a dietary recall from the previous 24-hour period). Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or currently taking medication to lower high blood pressure. Diabetes was defined by self-report (although women with only a history of gestational diabetes were excluded), by self-reported use of diabetes medication (diabetes pills or insulin), by fasting plasma glucose ≥7.0 mmol/L (≥126 mg/dL), or by hemoglobin A1c level ≥6.5%.[21]

### SNP Selection and Genotyping Methods

We included a total of 57 SNPs that were previously genotyped in NHANES III as follows: 55 SNPs associated with lipid levels among persons of European ancestry in GWAS,[22] and 2 *APOE* variants strongly associated with lipid levels in candidate gene association studies,[23–25] including our own.[5] These 2 *APOE* variants form the basis of the ε2, ε3, and ε4 isoforms. Online-only Data Supplement Table I lists all the variants included in the analyses. The 2 *APOE* variants were genotyped by TaqMan assay (Applied Biosystems; Foster City, CA).[26] All remaining variants were genotyped either by GoldenGate (Illumina; San Diego, CA) or by iPLEX or iPLEX Gold (Sequenom; San Diego, CA).[27] All SNPs were in Hardy-Weinberg proportions according to the National Center for Health Statistics criterion (http://www.cdc.gov/nchs/nhanes/genetics/genetic.htm).

### Construction of Genetic Risk Scores

We used the following 2 methods to construct separate genetic risk scores (GRSs) for each of the 6 lipid outcomes to examine the cumulative effect of the included SNPs on lipid levels in each racial/ethnic group: an unweighted method (calculated using a simple count of the number of risk alleles) and a weighted method (in which the effect size of each SNP factors into the calculation). More detailed methods for constructing the unweighted and weighted GRS are available in the online-only Data Supplement. Briefly, we assumed that each SNP is independently associated with risk. In cases of significantly correlated ($r^2$>0.8) SNPs in the same gene, we included only 1 variant from each gene in the GRS, which was determined by examining the allele frequency, significance of the association, and the direction of effect for each set of correlated variants. The only exception was *APOE,* for which we included both SNPs (rs429358 and rs7412) because they comprise the ε2, ε3, and ε4 isoforms of the gene.

We included in the GRS only those variants associated with each particular outcome through GWAS or candidate gene studies (eg, *APOE*), taking into account correlated variants as just described. The SNPs that were or were not included in the unweighted and weighted GRS for each lipid measure are indicated in online-only Data Supplement Tables III through VIII. Briefly, an 18-SNP GRS was constructed for HDL-C, a 19-SNP GRS for LDL-C, and a 17-SNP GRS for TG. All 9 SNPs were included in the GRS for TC. For TC/HDL-C, a 26-SNP GRS was constructed that included all polymorphisms from the GRSs for TC and HDL-C (only 1 variant was common across both outcomes). A 33-SNP GRS was constructed for non-HDL-C in a similar fashion. More details on the construction of the GRS are available in the online-only Data Supplement.

### Statistical Analyses

Analyses were performed in SAS-callable SUDAAN 10.0 (2007; Research Triangle Institute; Research Triangle Park, NC) to account

for the complex sampling design, nonresponse, and sample weights for the NHANES III genetic component. Detailed methods on calculating NHANES III sample weights have been described elsewhere.[28,29] Allele frequencies were calculated and weighted using NHANES III sample weights for each variant, stratifying by racial/ethnic group. Racial/ethnic differences in allele frequencies were tested in pooled data from all racial/ethnic groups using the Satterthwaite-adjusted F test in polytomous logistic regression.

We examined each lipid outcome using only polymorphisms that were significantly associated with increased levels of each outcome based on published data as follows: HDL-C (n=23 SNPs), LDL-C (n=23), TC (n=9), TG (n=25), TC/HDL-C ratio (n=total of 31 SNPs associated with either TC or HDL-C), and non-HDL-C (n=total of 45 SNPs associated with TC, LDL-C, or TG). TG was log-transformed in the analysis to normalize the distribution. We assumed an additive mode of inheritance and used linear regression analyses to evaluate the per-risk allele increase in lipid concentration adjusted for the covariates mentioned previously. Multivariable regression models adjusting for conventional risk factors were performed for each lipid trait by race/ethnicity. We also analyzed age- and sex-adjusted models for each lipid trait for each racial/ethnic group. To assess whether the observed concordance between effect directions (indicating that the direction of effect is concordant with prior GWAS in populations of European descent) for each lipid trait was due to chance, we tested the overall number of concordant SNPs in each racial/ethnic group through a binomial draw with a null expectation of $P=0.5$.[3] For the multivariable regression GRS analyses, we used the GRS as a continuous independent variable and categorized it into quintiles to quantify the effects and examine the linear trend. The regression models for GRS were performed separately for each racial/ethnic group. We estimated weighted $\beta$-coefficients and 95% CIs for each individual variant and for the GRS as a continuous variable. We also calculated the adjusted mean for the GRS categorized as quintiles. To test the homogeneity of effects of individual SNPs or the GRS across race/ethnicity, we included an interaction term for each variant (or the GRS) and race/ethnicity in the pooled data set. For single-variant analyses, the $P$ value from Satterthwaite-adjusted F statistics was adjusted to control for the false discovery rate (FDR)[30] for multiple testing in each racial/ethnic group separately. For the GRS analyses, the FDR-adjusted $P$ value was calculated using all 3 major racial/ethnic groups combined. Significance was determined based on a 2-sided FDR-adjusted $P<0.05$. We also estimated the genetic contribution of the included variants toward lipid concentrations by comparing nongenetic models (containing a set of conventional risk factors) with nongenetic models plus the addition of genetic variants. We report the adjusted $R^2$ (ie, the proportion of variance explained) to evaluate the proportion of the variability in models that are explained by these polymorphisms. We also report $P$ values of the trend test for statistical models with GRS categorized as quintiles.

# Results

## Characteristics of the Participants

The characteristics of the study participants are presented in Table 1 by race/ethnicity. (A full list of characteristics are included in online-only Data Supplement Table I.) All measured demographic, clinical, and dietary parameters differed by racial/ethnic group ($P<0.01$) except for total food energy intake, the intake of dietary fat subtypes, and the percentage of kilocalories from polyunsaturated fat, all of which were similar across populations ($P>0.05$). Non-Hispanic white participants had the highest mean age, were the most physically active, and had the smallest mean body mass index compared with non-Hispanic black and Mexican American participants. Non-Hispanic whites also had the lowest prevalence of diabetes and the highest measures of LDL-C, TC, TC/HDL-C ratio, and non-HDL-C compared with the other 2 populations. Among non-Hispanic black participants were the largest proportions of women and current smokers compared with non-Hispanic white and Mexican American participants. The prevalence of hypertension and diabetes were highest among non-Hispanic blacks, who showed a more-favorable lipid profile (highest levels of HDL-C and lowest levels of TG, TC/HDL-C ratio, and non-HDL-C) compared with the other 2 populations. Mexican American participants (the youngest group overall) had the highest proportion of men and never smokers; the lowest percentage with hypertension; and the lowest levels of HDL-C, LDL-C, and TC while having the highest mean TG concentration compared with non-Hispanic white and non-Hispanic black participants.

## Allele Frequency of Lipid-Related Variants

Weighted allele frequency estimates of all variants included in this study are shown in Figure 1 and online-only Data Supplement Table II. Allele frequencies for all SNPs varied significantly across racial/ethnic groups ($P=0.04$ to $<0.01$), except for rs3764261 in *CETP* ($P=0.27$).

## Single SNP Effects on Blood Lipid Concentrations

The individual associations of the studied SNPs with blood lipids within the 3 racial/ethnic groups are shown in online-only Data Supplement Tables III through VIII. Overall, significant associations were more likely to be seen among non-Hispanic white and Mexican American participants than among non-Hispanic black participants. *CETP* variants rs1800775 and rs3764261 showed associations with HDL-C consistently in fully adjusted models in all 3 racial/ethnic groups before FDR adjustment, but associations disappeared in non-Hispanic blacks after the adjustment (online-only Data Supplement Table III). The 2 candidate gene variants in *APOE* (rs429358 and rs7412) were strongly associated with LDL-C and TC across all 3 racial/ethnic groups (FDR-adjusted $P\leq0.04$, except for the borderline significance [FDR-adjusted $P=0.05$] of rs429358 in non-Hispanic blacks) (online-only Data Supplement Tables IV and V, respectively) in the fully adjusted or age- and sex-adjusted models. A small number of other variants were significantly associated with LDL-C in at least 2 racial/ethnic groups, including SNPs in *CELSR2* and *LDLR* (online-only Data Supplement Table IV). For log(TG), there were no variants significant across all 3 racial/ethnic groups in fasting or nonfasting samples (online-only Data Supplement Table VI). *APOE* (rs7412) was the only variant associated with TC/HDL-C ratio across all 3 racial/ethnic groups (FDR-adjusted $P<0.05$) (online-only Data Supplement Table VII). *APOE* rs7412, *CELSR2/PSRC1/SORT1* rs12740374, and *LDLR* rs6511720 were strongly associated with non-HDL-C in each racial/ethnic group in fully adjusted or age- and sex-adjusted models (online-only Data Supplement Table VIII). We found no evidence of differences in genetic effects across racial/ethnic groups (SNP×race/ethnicity interaction) for any lipid trait, with the exception of *APOB* rs693 with TC in the age- and sex-adjusted model (FDR-adjusted $P<0.01$) (online-only Data Supplement Table V).

Online-only Data Supplement Table IX summarizes the directions of the effects from single SNP associations by race/ethnicity. The majority of $\beta$-coefficients were in the

**Table 1. Characteristics of Participants by Race/Ethnicity: NHANES III DNA Bank (1991–1994)**

| Characteristic | n | Non-Hispanic White (n=2296) | Non-Hispanic Black (n=1699) | Mexican American (n=1713) | *P** |
|---|---|---|---|---|---|
| Mean age, y | 5708 | 44.9 (43.0–46.8) | 40.3 (38.8–41.8) | 36.2 (34.9–37.5) | <0.01 |
| Sex | 5708 | | | | |
| Male | | 49.0 (47.2–50.9) | 46.3 (43.4–49.1) | 53.2 (51.6–54.7) | <0.01 |
| Female | | 51.0 (49.1–52.8) | 53.7 (50.9–56.6) | 46.8 (45.3–48.4) | |
| Education | 5679 | | | | |
| <High school | | 18.4 (16.1–21.1) | 33.2 (28.6–38.2) | 56.9 (51.2–62.5) | <0.01 |
| High school | | 34.5 (31.0–38.2) | 38.2 (34.4–42.0) | 25.9 (22.8–29.2) | |
| College and above | | 47.0 (41.8–52.3) | 28.6 (24.0–33.7) | 17.2 (14.0–21.0) | |
| Physical activity | 5708 | | | | |
| None | | 16.8 (13.9–20.2) | 29.7 (26.3–33.4) | 32.7 (28.8–36.8) | <0.01 |
| Low (active <5 times/wk) | | 36.0 (32.9–39.2) | 30.8 (27.5–34.3) | 30.2 (28.2–32.4) | |
| High (active ≥5 times/wk) | | 47.2 (43.4–51.0) | 39.5 (35.8–43.2) | 37.1 (33.8–40.5) | |
| Alcohol intake | 5519 | | | | |
| None | | 45.5 (41.7–49.4) | 54.7 (51.0–58.3) | 50.1 (47.8–52.3) | <0.01 |
| <4 drinks/wk | | 28.6 (26.0–31.4) | 21.9 (19.8–24.1) | 25.8 (22.4–29.5) | |
| ≥4 drinks/wk | | 25.9 (22.8–29.3) | 23.5 (21.1–26.1) | 24.1 (21.1–27.5) | |
| Smoking status | 5708 | | | | |
| Current smoker | | 26.2 (22.6–30.3) | 30.3 (28.5–32.1) | 21.2 (18.7–23.9) | <0.01 |
| Former smoker | | 26.7 (23.9–29.6) | 14.0 (11.8–16.4) | 20.3 (18.2–22.5) | |
| Never smoker | | 47.1 (43.5–50.7) | 55.7 (52.5–58.9) | 58.5 (55.4–61.6) | |
| Mean BMI, kg/m$^2$ | 5700 | 26.4 (26.0–26.7) | 27.9 (27.4–28.4) | 27.5 (27.3–27.8) | <0.01 |
| Mean food energy, kcal | 5502 | 2278.2 (2193.9–2362.4) | 2183.4 (2106.5–2260.4) | 2316.0 (2262.8–2369.2) | 0.08 |
| Mean % kilocalories from saturated fat | 5502 | 11.1 (10.7–11.4) | 10.9 (10.6–11.2) | 10.2 (9.9–10.5) | 0.01 |
| Mean % kilocalories from monounsaturated fat | 5502 | 12.7 (12.4–13.1) | 13.0 (12.6–13.4) | 11.7 (11.3–12.1) | 0.01 |
| Mean kilocalories from polyunsaturated fat | 5502 | 7.1 (6.8–7.3) | 7.2 (6.9–7.4) | 7.5 (7.3–7.8) | 0.10 |
| Hypertension | 5699 | | | | |
| Yes | | 21.6 (18.6–25.0) | 28.3 (25.2–31.6) | 13.5 (11.2–16.2) | <0.01 |
| No | | 78.4 (75.0–81.4) | 71.7 (68.4–74.8) | 86.5 (83.8–88.8) | |
| Type 2 diabetes | 5708 | | | | |
| Yes | | 6.2 (5.2–7.4) | 11.4 (9.5–13.6) | 8.4 (7.0–10.1) | <0.01 |
| No | | 93.8 (92.6–94.8) | 88.6 (86.4–90.5) | 91.6 (89.9–93.0) | |
| Mean HDL-C, mg/dL | 5659 | 49.6 (48.4–50.8) | 54.1 (53.1–55.2) | 47.0 (46.0–48.1) | <0.01 |
| Mean LDL-C, mg/dL | 3405 | 125.2 (122.8–127.6) | 121.7 (119.2–124.1) | 118.9 (115.7–122.0) | <0.01 |
| Mean TC, mg/dL | 5685 | 201.3 (198.9–203.7) | 196.1 (194.1–198.1) | 195.2 (192.0–198.3) | <0.01 |
| Mean TG, mg/dL | 5685 | 142.9 (137.8–148.0) | 113.4 (110.0–116.9) | 157.6 (150.2–165.0) | <0.01 |
| Mean TC/HDL-C ratio | 5654 | 4.5 (4.3–4.6) | 3.9 (3.9–4.0) | 4.4 (4.3–4.5) | <0.01 |
| Mean non-HDL-C, mg/dL | 5655 | 151.5 (148.9–154.2) | 141.8 (140.0–143.6) | 147.7 (144.8–150.5) | <0.01 |

Data are presented as weighted % (95% CI). NHANES genetic sample weights were applied. BMI, indicates body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NHANES III, Third National Health and Nutrition Examination Survey; TC, total cholesterol; TG, triglycerides.

*$P$ values were calculated using $\chi^2$ statistics based on log odds ratios for categorical variables and Satterthwaite-adjusted F statistics for continuous variables.



expected direction (indicating that the direction of effect is concordant with prior GWAS performed in populations of European descent). In general, we saw the expected direction of association across all 3 racial/ethnic groups, although this varied tremendously by outcome. For example, among the 3 groups, we observed that 21, 16, and 20 of the 23 LDL-C SNPs had a concordant direction of the effect in fully adjusted models. However, for log(TG), the direction of effect of all examined SNPs were concordant in non-Hispanic whites, whereas only one half were concordant among non-Hispanic blacks. The likelihood is small that the directional consistency for each lipid trait is due to chance (maximal $P$ values, 0.053, 0.421, and <0.003 among non-Hispanic whites, non-Hispanic blacks, and Mexican Americans, respectively, in fully adjusted models) (online-only Data Supplement Table IX).

## Cumulative Effects of Genetic Variants on Blood Lipids

We further explored the cumulative association between blood lipids and the included SNPs by examining the GRS on

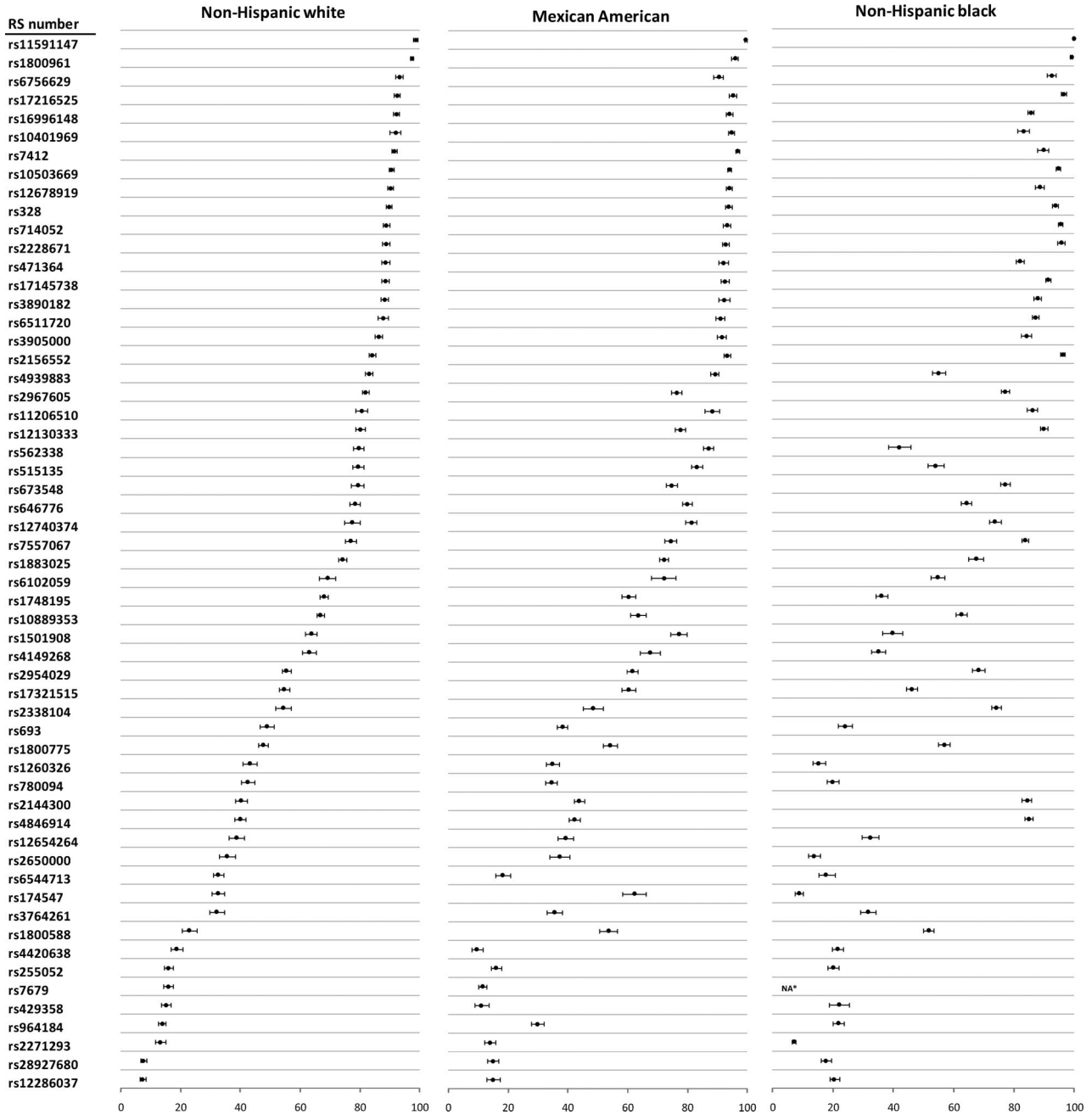


**Figure 1.** Weighted allele frequency of lipid-related variants in US adults by race/ethnicity—Third National Health and Nutrition Examination Survey DNA bank (1991–1994).



a continuous scale and categorically (divided into quintiles). Table 2 presents the adjusted $\beta$-coefficients and 95% CIs for the weighted GRS modeled as a continuous variable for each lipid trait by race/ethnicity. We observed that the weighted GRS was strongly associated (FDR-adjusted $P \leq 0.01$) with increased levels of all blood lipid traits in all racial/ethnic groups in both fully adjusted and age- and sex-adjusted models. There was no evidence of a differential effect of the GRS on any of the 6 lipid traits across race/ethnicity (observed by the interaction of GRS and race/ethnicity) in fully adjusted models except for LDL-C ($P=0.04$), whereas the race/ethnic difference was significant for 4 of the traits in age- and sex-adjusted models. When we examined the non-genetic and genetic contribution to the total variation in lipid concentrations, the data indicated that the included conventional risk factors explain 15% to ≈30% of the total variation in fully adjusted models and in age- and sex-adjusted models, depending on the outcome. Adding the continuous GRS to the conventional risk factors explains an additional 1% to 11% of the variation.

When the GRS was categorized into quintiles, the associations with the lipid outcomes were consistent with the patterns observed for the GRS modeled as a continuous variable (Figure 2**A** through 2**F**, online-only Data Supplement Tables X through XVI). A strongly significant trend across quintiles (FDR-adjusted $P$ for trend $\leq 0.01$) was seen

**Table 2. Multivariable Associations Between Genetic Risk Score and Lipid Traits by Race/Ethnicity: NHANES III DNA Bank (1991–1994)**

| Race/Ethnicity | No. Observations | GRS Median* (Range) | Estimate of $\beta$ Coefficient (95% CI)† | FDR-Adjusted *P*‡ | $R^2$ | | GRS* Race/ Ethnicity *P* |
|---|---|---|---|---|---|---|---|
| | | | | | Without GRS§ | With GRS‖ | |
| Fully adjusted model¶ | | | | | | | |
| HDL-C | | | | | | | |
| Non-Hispanic white | 1812 | 15 (3–32) | 0.53 (0.42–0.64) | <0.01 | 0.295 | 0.318 | 0.48 |
| Non-Hispanic black | 1351 | 19 (11–31) | 0.91 (0.60–1.22) | <0.01 | 0.153 | 0.187 | |
| Mexican American | 1228 | 20 (5–31) | 0.70 (0.54–0.85) | <0.01 | 0.195 | 0.245 | |
| LDL-C | | | | | | | |
| Non-Hispanic white | 928 | 27 (12–37) | 3.04 (2.47–3.61) | <0.01 | 0.150 | 0.249 | 0.04 |
| Non-Hispanic black | 718 | 28 (6–36) | 2.56 (1.94–3.18) | <0.01 | 0.163 | 0.268 | |
| Mexican American | 668 | 26 (16–36) | 2.53 (1.85–3.21) | <0.01 | 0.162 | 0.215 | |
| TC | | | | | | | |
| Non-Hispanic white | 1788 | 12 (5–18) | 4.53 (3.23–5.83) | <0.01 | 0.189 | 0.239 | 0.39 |
| Non-Hispanic black | 1343 | 17 (3–18) | 3.47 (2.57–4.38) | <0.01 | 0.164 | 0.214 | |
| Mexican American | 1240 | 13 (5–18) | 4.31 (3.00–5.61) | <0.01 | 0.174 | 0.211 | |
| Log(TG) | | | | | | | |
| Non-Hispanic white | 1681 | 16 (6–27) | 0.03 (0.03–0.04) | <0.01 | 0.258 | 0.294 | 0.33 |
| Non-Hispanic black | 1262 | 16 (0–34) | 0.01 (0.01–0.01) | <0.01 | 0.210 | 0.221 | |
| Mexican American | 1173 | 13 (4–28) | 0.03 (0.02–0.04) | <0.01 | 0.201 | 0.273 | |
| TC/HDL-C | | | | | | | |
| Non-Hispanic white | 1688 | 41 (6–52) | 0.04 (0.02–0.05) | <0.01 | 0.266 | 0.299 | 0.30 |
| Non-Hispanic black | 1269 | 40 (11–52) | 0.04 (0.03–0.05) | <0.01 | 0.210 | 0.245 | |
| Mexican American | 1147 | 31 (19–44) | 0.10 (0.08–0.12) | <0.01 | 0.229 | 0.302 | |
| Non-HDL-C | | | | | | | |
| Non-Hispanic white | 1541 | 43 (23–56) | 2.33 (1.74–2.92) | <0.01 | 0.218 | 0.295 | 0.60 |
| Non-Hispanic black | 1136 | 47 (8–61) | 1.31 (1.01–1.62) | <0.01 | 0.196 | 0.275 | |
| Mexican American | 1073 | 43 (28–61) | 2.02 (1.65–2.39) | <0.01 | 0.217 | 0.282 | |
| Age-, and sex-adjusted model# | | | | | | | |
| HDL-C | | | | | | | |
| Non-Hispanic white | 1936 | 13 (0–32) | 0.45 (0.30–0.59) | <0.01 | 0.128 | 0.148 | 0.04 |
| Non-Hispanic black | 1476 | 21 (12–32) | 0.83 (0.45–1.21) | <0.01 | 0.018 | 0.046 | |
| Mexican American | 1323 | 18 (4–30) | 0.70 (0.52–0.88) | <0.01 | 0.044 | 0.101 | |
| LDL-C | | | | | | | |
| Non-Hispanic white | 982 | 26 (11–37) | 3.00 (2.42–3.58) | <0.01 | 0.089 | 0.177 | 0.25 |
| Non-Hispanic black | 779 | 28 (8–38) | 2.52 (1.97–3.08) | <0.01 | 0.081 | 0.187 | |
| Mexican American | 718 | 26 (16–38) | 2.37 (1.68–3.07) | <0.01 | 0.122 | 0.165 | |
| TC | | | | | | | |
| Non-Hispanic white | 1912 | 12 (4–18) | 4.72 (3.59–5.85) | <0.01 | 0.138 | 0.193 | 0.12 |
| Non-Hispanic black | 1471 | 16 (3–18) | 3.55 (2.66–4.44) | <0.01 | 0.112 | 0.156 | |
| Mexican American | 1339 | 13 (6–18) | 4.49 (3.11–5.86) | <0.01 | 0.143 | 0.177 | |
| Log(TG) | | | | | | | |
| Non-Hispanic white | 1800 | 16 (6–27) | 0.03 (0.02–0.04) | <0.01 | 0.097 | 0.128 | 0.01 |
| Non-Hispanic black | 1381 | 27 (0–34) | 0.01 (0.00–0.01) | <0.01 | 0.075 | 0.085 | |
| Mexican American | 1269 | 14 (5–29) | 0.03 (0.02–0.04) | <0.01 | 0.086 | 0.139 | |
| TC/HDL-C | | | | | | | |
| Non-Hispanic white | 1804 | 38 (0–52) | 0.03 (0.02–0.04) | <0.01 | 0.095 | 0.125 | 0.01 |
| Non-Hispanic black | 1392 | 35 (7–52) | 0.04 (0.04–0.05) | <0.01 | 0.035 | 0.077 | |
| Mexican American | 1237 | 33 (19–46) | 0.09 (0.07–0.11) | <0.01 | 0.101 | 0.178 | |
| Non-HDL-C | | | | | | | |
| Non-Hispanic white | 1651 | 43 (20–58) | 2.17 (1.63–2.70) | <0.01 | 0.119 | 0.208 | 0.01 |
| Non-Hispanic black | 1242 | 43 (3–61) | 1.21 (0.93–1.50) | <0.01 | 0.088 | 0.164 | |
| Mexican American | 1158 | 43 (28–63) | 2.06 (1.67–2.44) | <0.01 | 0.152 | 0.216 | |

FDR indicates false discovery rate; GRS, genetic risk score; log(TG), log-transformed triglycerides. Other abbreviations as in Table 1.

*The number of risk alleles present. The variants included in the GRS for each lipid trait are available in online-only Data Supplement Tables III through VIII.

†Mean and 95% CIs were adjusted using the NHANES genetic sample weights.

‡*P* was calculated to test the difference in lipid levels across the weighted GRS in each racial/ethnic group using Satterthwaite-adjusted F statistics and adjusting for multiple comparisons using the FDR.

§Indicates that the model included conventional risk factors without the GRS.

‖Indicates that the model included the GRS in addition to conventional risk factors.

¶Adjusted for age, sex, education, smoking, physical activity, alcohol consumption, hypertension, diabetes, BMI, total food energy, percent of kilocalories from saturated fat, percent of kilocalories from monounsaturated fat, and percent of kilocalories from polyunsaturated fat.

#Adjusted for age and sex only.

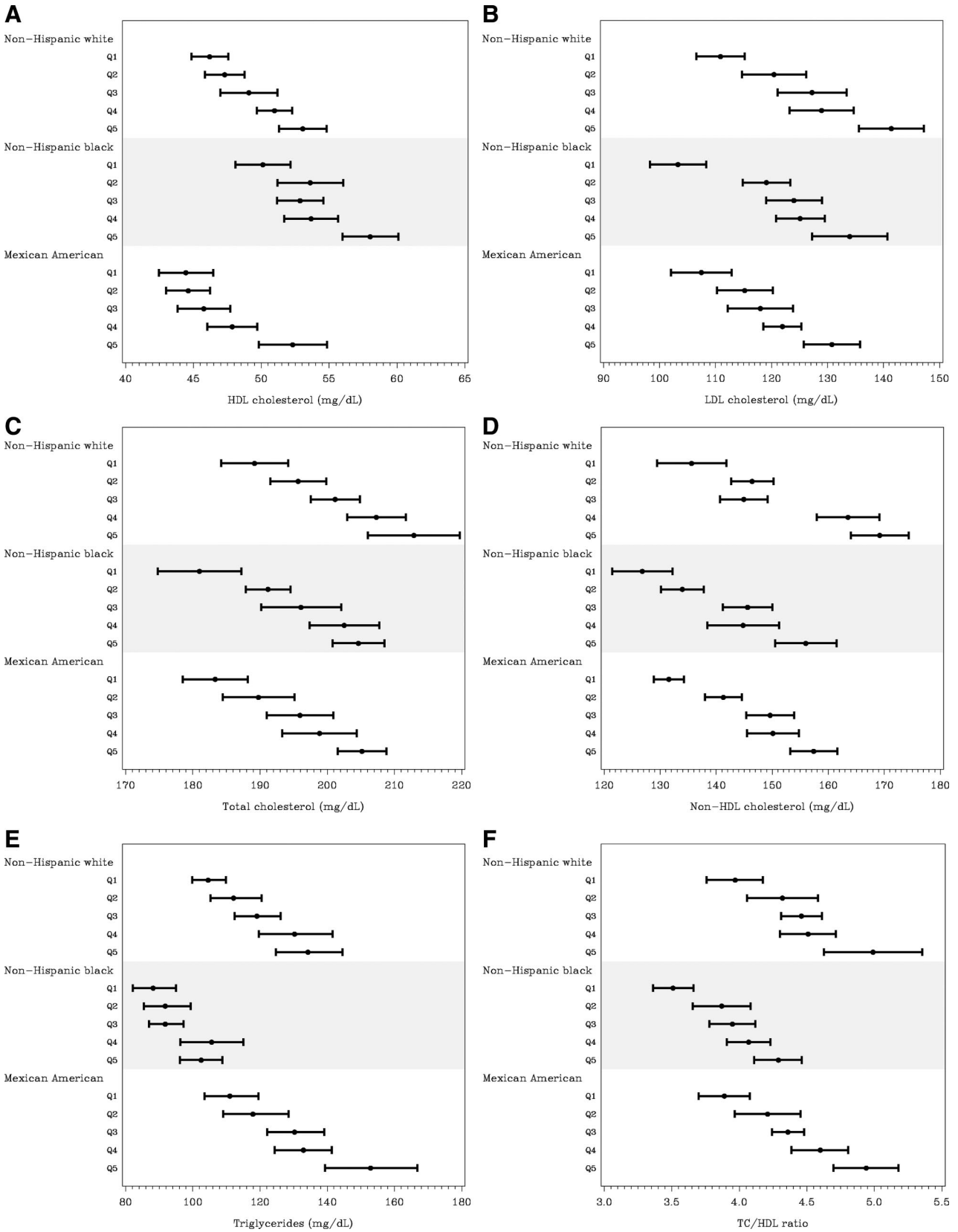


**Figure 2.** Adjusted means and 95% CIs for weighted genetic risk score in quintiles with lipid traits by race/ethnicity. **A**, HDL cholesterol. **B**, LDL cholesterol. **C**, TC. **D**, TC/HDL ratio. **E**, Triglycerides. **F**, Non-HDL cholesterol. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; Q, quintile; TC, total cholesterol.

for all lipid traits in all racial/ethnic groups. These associations were seen in both weighted and unweighted analyses of the GRS (data not shown). Results for TG were consistent when using nonfasting and fasting samples (online-only Data Supplement Table XVI). We did not find evidence of differences in the GRS across racial/ethnic groups in fully adjusted or age- and sex-adjusted models when the GRS was categorized into quintiles (online-only Data Supplement Tables X through XVI).

## Discussion

Using a population-based and nationally representative survey in the United States, we have estimated the allele frequencies and genetic associations of lipid-associated polymorphisms that have been robustly validated through GWAS and meta-analyses. Consistent with findings from the International HapMap Project,[13] the present data show that there is a wide variation in risk allele frequencies across multiple racial/ethnic populations. This variation may affect the number of individuals at increased risk for a lipid profile that is itself a risk factor for cardiovascular disease. Although we did not find evidence of differences in genetic effects across racial/ethnic groups, the proportion of the associations replicated in non-Hispanic white participants (upwards of 67%) was higher than in non-Hispanic black (<44%) and Mexican American (<44%) participants. Because these loci were discovered in white populations of European ancestry, we suspected that the marginal associations of the studied loci would more likely be seen among Mexican Americans than among non-Hispanic blacks because of differences in population admixture. A prior study reported that the average admixture estimates for Mexican Americans is ≈55% to 60% European ancestry,[31] whereas African Americans typically have ≈20% European ancestry (although this can vary widely).[32–34] Therefore, failure to replicate a genetic association should not be disregarded because the failure may provide important clues about genetic architecture.[35] Indeed, our findings may inform subsequent comprehensive fine mapping and functional studies on lipid metabolism[36] in addition to the analysis of metabolic networks across these loci in multiple racial/ethnic groups. Limited statistical power and chance may partly explain the lack of consistent findings across the 3 race/ethnicities examined in the present study. In addition, differences in lipid profile, allele frequencies, and linkage disequilibrium structure among race/ethnic groups may have impaired our ability to observe true associations, if they exist (examples of linkage disequilibrium plots are available in the online-only Data Supplement).

The present study also investigated the cumulative effects of the included variants. We constructed GRSs using the $\beta$-coefficients from our own data because effect sizes of the included SNPs are only available from studies on European populations and because the $\beta$-coefficients from GWAS have been reported using different measures. We observed that the GRS was strongly associated with increased blood lipid outcomes in all racial/ethnic groups. Further analysis may be needed to confirm that the GRS estimates we used are appropriate. To avoid collinearity, we chose to construct the GRS using only variants from each gene that were not highly correlated. However, we evaluated the robustness of the GRS to the inclusion of different SNPs (ie, exchanging highly correlated SNPs for ones that were not previously included in the GRS). Our results are indeed robust to a different choice of SNPs to create the GRS in the models (data not shown).

As concluded in prior studies,[16] the present data show that the included SNPs account for only a small fraction of the variability in blood lipid levels, indicating that a considerable number of additional common genetic variants (probably with modest effects) and rare alleles (probably with large effects) need to be identified.[16,37,38] Further research is needed regarding whether (1) each SNP included in the GRS contributed to the genetic association, (2) the clinical utility of the significant risks are attributable to a GRS, and (3) analyses of interactions (eg, gene-gene and gene-environment interactions as well as pathway-disease relationships) identify other contributors to the unexplained variance.[39]

We observed a directional consistency across the 3 racial/ethnic groups for many of the included SNPs, which may indicate that despite the importance of environmental risk exposures, common genetic variants reflect a shared biological impact on the risk of common diseases across racial/ethnic groups.[15] One strength of the present study is that we included a larger panel of validated lipid-related variants than did most of the previous studies. There is good evidence that some of these loci may directly contribute to clinical phenotypes, as a recent meta-analysis of lipid GWAS reported associations in 18 genes previously implicated in Mendelian lipid disorders.[3] We included variants in 14 of these 18 genes. Interestingly, the biological or clinical relevance of several GWAS loci have been investigated. For example, *HMGCR* has been targeted for the treatment of hyperlipidemia (statins),[40] *PLTP* has been shown to influence lipid metabolism,[41] and *CELSR2/PSRC1/SORT1* is associated with LDL-C and may alter the risk for myocardial infarction (regulated by a noncoding SNP).[42] Therefore, additional functional validation of these GWAS-confirmed SNPs may yield insights into the biological mechanisms of lipid traits.

The present study has several limitations. First, validation of the included loci in non-European populations has been limited.[43] It remains uncertain whether these variants, which have been identified in white European populations, are useful in persons of African descent because of differences in allele frequencies, risk factor prevalence, and the underlying linkage disequilibrium structure of these loci.[44] Therefore, the individual and cumulative effects estimated for these polymorphisms may not be good representations of the true effects of these loci for non-Hispanic blacks and Mexican Americans. However, a recent study on fine mapping confirmed 5 regions associated with lipid traits in European-derived populations that are relevant in African Americans.[45] In addition, Teslovich and colleagues[3] demonstrated that most of the lipid-related loci identified in their large meta-analysis contribute to the genetic architecture of lipid traits across global populations. Second, understanding the precise effect sizes for these variants and their combined effects will require large studies and extensive replication in diverse populations[46] in addition to carefully synthesizing all the accumulated evidence.[47] Because we were missing genotype

data for ≈9% of specimens for several SNPs, we had limited power (power calculations available in the online-only Data Supplement) to detect associations with some variants given their small effect sizes. Our study also was underpowered to test for gene-gene or gene-environment interactions.[48,49] Third, we were not able to assess population structure in our analyses, which grouped participants on the basis of self-reported race/ethnicity. Genetic associations of the variants evaluated in this study may be confounded by subtle population stratification and admixture. Because ancestry-informative markers are not yet available in NHANES III, we were not able to incorporate genetic ancestry information into our analysis. Fourth, in the construction of the GRS, we assumed that each SNP is independently associated with risk and weighted its own $\beta$-coefficient to account for the potentially different magnitudes of effect. We assumed additive per-allele effects in the analyses, but it is likely that not all SNPs operate by the same genetic model. We do not have a clear understanding of the underlying biology or genetics (including gene-gene and variant-variant interactions) for many of these SNPs, which may limit the validity of the GRS if nonbiologically relevant SNPs have been included. Finally, we did not limit our entire study to fasting samples because of concerns about study power (fewer than one half of the NHANES III participants provided fasting samples), and we did not consider variables that may have a profound effect on lipids, such as insulin resistance.

The present study also has notable strengths. First, we included a large, updated set of GWAS-confirmed lipid-related SNPs. In addition, we included lipid measures and other phenotype variables from a nationally representative sample of the US population, including the 3 major racial/ethnic groups in the country. Because of the design of NHANES, our estimates are generalizable to the entire US population.

In summary, we report the independent and combined effects of 57 confirmed lipid-associated variants among adults of European and non-European ancestry in a nationally representative and population-based survey of the United States. Our data show that allele frequencies of these lipid-associated polymorphisms vary significantly by race/ethnicity. Nevertheless, the patterns of influence of these SNPs on lipid levels were generally consistent across racial/ethnic groups. The included SNPs had very small individual effects on lipid levels, and only a small proportion of the total variance in lipids can be explained by the combination of all the SNPs. Our data also show that the GRS was significantly associated with increased lipid measures in all racial/ethnic groups, which may help in identifying subgroups with a high risk for an unfavorable lipid profile. In addition, as new lipid-related genes and variants continue to be identified, our ability to understand the genetic basis for these complex traits will continue to improve.



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## CLINICAL PERSPECTIVE

The present study describes the independent and combined effects of 57 genetic variants associated with lipid levels among adults of European and non-European ancestry in a unique nationally representative survey of the United States. There is good evidence that some of these loci, which were all identified through genome-wide association studies, may directly contribute to variation in clinical phenotypes, although the clinical and public health implications of many of these variants remain unclear. We report that although allele frequencies varied significantly by race/ethnicity, the patterns of genetic association with lipid levels were generally consistent across racial/ethnic groups, indicating that these common polymorphisms may reflect a shared biological influence on lipid levels. The data also show that the included single-nucleotide polymorphisms account for only a small fraction of the interindividual variability in blood lipid levels, suggesting that a considerable number of additional common variants (probably with modest effects) and rare alleles (probably with large effects) remain to be identified. Nevertheless, we observed that the genetic risk score, which assessed the combined effect of multiple single-nucleotide polymorphisms, was significantly associated with increased lipid measures in all racial/ethnic groups.