Variants for HDL-C, LDL-C, and Triglycerides Identified from Admixture Mapping and Fine-Mapping Analysis in African American Families

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**Background**—Admixture mapping of lipids was followed-up by family-based association analysis to identify variants for cardiovascular disease in African Americans.

**Methods and Results**—The present study conducted admixture mapping analysis for total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides. The analysis was performed in 1905 unrelated African American subjects from the National Heart, Lung and Blood Institute’s Family Blood Pressure Program (FBPP). Regions showing admixture evidence were followed-up with family-based association analysis in 3556 African American subjects from the FBPP. The admixture mapping and family-based association analyses were adjusted for age, age², sex, body mass index, and genome-wide mean ancestry to minimize the confounding caused by population stratification. Regions that were suggestive of local ancestry association evidence were found on chromosomes 7 (low-density lipoprotein cholesterol), 8 (high-density lipoprotein cholesterol), 14 (triglycerides), and 19 (total cholesterol and triglycerides). In the fine-mapping analysis, 52,939 single-nucleotide polymorphisms (SNPs) were tested and 11 SNPs (8 independent SNPs) showed nominal significant association with high-density lipoprotein cholesterol (2 SNPs), low-density lipoprotein cholesterol (4 SNPs), and triglycerides (5 SNPs). The family data were used in the fine-mapping to identify SNPs that showed novel associations with lipids and regions, including genes with known associations for cardiovascular disease.

**Conclusions**—This study identified regions on chromosomes 7, 8, 14, and 19 and 11 SNPs from the fine-mapping analysis that were associated with high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides for further studies of cardiovascular disease in African Americans. (*Circ Cardiovasc Genet. 2015;8:106-113. DOI: 10.1161/CIRCGENETICS.114.000481*)

**Key Words:** admixture mapping ■ African Americans ■ association studies ■ genetics ■ lipids

Inadequate control of lipids is a key indicator of poor cardiovascular health, which is a substantial public health issue worldwide. Approximately 2.6 million deaths worldwide annually are caused by poorly controlled cholesterol.1 Variability in lipids is affected by genetic and environmental factors, and the heritability of key lipids has been estimated to range between 58% and 69% for high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides, based on studies from the Framingham Heart Study Offspring cohort.2

Several genome-wide association studies (GWAS) have successfully identified genetic variants that are associated with lipid traits.2–10 Recently, 2 association analyses using GWAS data suggested that several single-nucleotide polymorphisms (SNPs) associated with lipids in European ancestry populations may have effects in the same direction across different populations, including those of African and Hispanic ancestry.5,11 The empirical results, combined with those authors’ reports of allelic heterogeneity and
population-specific variants for lipids, emphasize the importance of conducting genetic studies in diverse study populations to identify variants.

Admixture mapping is a tool that may be used to discover these variants by using the disparities in disease prevalence between ancestral populations of admixed populations to identify genomic regions and genetic variants that are associated with a phenotype. In these analyses, the frequency of a risk allele is expected to vary between ancestral populations of an admixed study population. It is assumed that, in a recently admixed population, affected subjects have increased ancestry for the population that has a greater frequency of the risk allele at the causal variant, compared with controls. Ancestry at the causal variant is also expected to be greater than the average genome-wide ancestry. This increased ancestry has been previously reported in hypertension cases. In African Americans, some genomic regions have been identified previously for lipids using admixture mapping, including 8q11-8q21 for HDL-C.

In the current study, admixture mapping analyses were conducted to identify genomic regions associated with lipid traits: total cholesterol (TC), HDL-C, LDL-C, and triglycerides. The regions showing association evidence with local ancestry were followed up by fine-mapping association analysis using SNPs in families.

Methods

Sample

The study samples were obtained from the National Heart, Lung and Blood Institute (NHBLI)’s family-based blood pressure program (FBPP). The FBPP consists of 4 networks: GenNet, Genetic Epidemiology Network of Atherosclerosis (GENOA), Hypertension Genetic Epidemiology Network (HyperGEN), and SAPPHIRE. Each network is a multicenter study that focuses on the genetic causes of hypertension and associated traits in African Americans, Asians and Asian Americans, European Americans, and Mexican Americans. The FBPP study design was set up for linkage analysis, and the 4 networks in the FBPP ascertainment families through probands with increased blood pressure or hypertension.

The current study was conducted in African Americans who were recruited from 3 FBPP networks: HyperGEN in Birmingham, Alabama, and Forsyth County, North Carolina; the GENOA in Jackson, Mississippi; and the GenNet study in Maywood, Illinois. Further details of the FBPP networks are available elsewhere. The current study was approved by the University Hospitals Case Medical Center institutional review committee.

The admixture mapping analysis was conducted in 1905 unrelated subjects that were selected from the overall FBPP data as described previously. Briefly, the normotensive control was selected first from the families, if available. If multiple controls were available, the oldest control was chosen. In the cases, the youngest hypertensive case in the family was selected. Subjects with age <19 years or age >80 years or average global African ancestry <2.5% were removed from the data set.

For the fine-mapping study, chromosomal regions that showed local ancestry association evidence for the phenotypes were selected for follow-up analysis. There were 3556 subjects with complete phenotype and genotype information in 1459 pedigrees from the FBPP in the fine-mapping analysis.

Genotyping

The FBPP networks obtained blood samples from the study participants, and DNA was extracted by standard methods. 2593 evenly spanned ancestry-informative markers (AIMs) were identified across the genome that maximized the difference in allele frequency for the ancestral populations, HapMap CEU (Utah residents with Northern and Western European ancestry from the CEPH collection) and HapMap YRI (Yoruba in Ibadan, Nigeria). These SNPs were selected from those that were available on the Illumina Human 1 M, Illumina 650K, and Affymetrix 6.0 arrays. These AIMs were genotyped for the 1905 unrelated subjects using the Illumina iSelect Custom Bead Chip at the University of California San Francisco. After dropping SNPs with call rate <0.95 and Illumina GenTrain score <0.7, a final data set of 2507 AIMs in 1905 unrelated subjects was used in the admixture mapping analysis.

Two of the 4 FBPP Networks had GWAS data. The subjects from the GENOA network were genotyped using the Affymetrix Array 6.0 and Illumina 1 M array, and SNPs were imputed to obtain a complete data set (imputation R² >0.5). The subjects from the HyperGEN network were genotyped using the Affymetrix Array 6.0 and the Affymetrix Array 5.0, and imputation was performed for a complete data set (imputation R² >0.3). Quality control measures were performed separately for the GENOA and HyperGEN samples. SNPs with call rates <95% were excluded. There were an additional 872 African American subjects from the HyperGEN and GENOA networks who were not genotyped with conventional GWAS platforms; these individuals were genotyped using the Affymetrix Axiom Genome-Wide 1 array. SNPs were called using the Affymetrix Genotyping Console by analyzing CEL files of intensity calculations from the Affymetrix Axiom Genome-Wide 1 arrays (www.affymetrix.com). SNPs with call rates <95% were excluded. The 3 data sets were combined in the fine-mapping analysis, and the total sample size was 3556 subjects with complete information.

The combined genotyping data were examined for Mendelian errors and departures from Hardy–Weinberg Equilibrium (P<0.001), and 30 SNPs on chromosome 19 were removed. This analysis also helped to reduce the effect of using different genotyping arrays. After all of the quality control measures, 52,939 SNPs were analyzed in 5 regions identified from the admixture mapping analysis.

Statistical Analyses

Two software programs, ADMIXPROGRAM and SABER, were used for estimating an individual’s local ancestry. Both programs use maximum likelihood methods to estimate locus-specific ancestry, using SNPs as AIMS. ADMIXPROGRAM uses a Hidden Markov Model to estimate the ancestry at each locus, where conditional on a hidden state, the genotype depends on its ancestral allele frequency, assuming that AIMS are in linkage equilibrium in the ancestral populations. It also uses a continuous gene-flow model to model the chromosome crossovers between ancestral populations, and this model is applicable to admixed populations with 2 ancestral populations, such as African Americans. SABER uses a Markov–Hidden Markov Model for local ancestry estimation, and this model uses densely spaced AIMS, such as those from a GWAS, to account for pair-wise linkage disequilibrium in the ancestral populations. In addition, SABER models the local ancestry probability with a transition matrix using an intermixing model that accounts for more than 2 ancestral populations with separate admixing times, such as Hispanic populations. The performance of the software was compared and the admixture mapping results were similar. As the population of interest had 2 ancestral populations, the estimates from ADMIXPROGRAM were used. The regions showing local ancestry association evidence with the estimates from ADMIXPROGRAM were followed up with fine-mapping association analysis.

Linear regression analysis was performed similarly to previous admixture mapping studies. Specifically, let be the trait value of individual . Let be the African ancestry at the th AIM and be the average African Ancestry of individual . Let be estimated by ADMIXPROGRAM and was calculated as the mean of for all AIMS. The linear regression analysis was performed as

and tested the null hypothesis , which was used to assess statistical significance in admixture mapping. To test the null hypothesis , a P value <0.001 was considered as suggestive evidence of association in admixture mapping analysis. This P value was chosen to compromise between the power of admixture mapping and of follow-up association analysis, that is, to retain an adequate number of genomic regions in admixture mapping analysis, although reducing
the number of SNP tests performed in the association analysis. A region of admixture mapping was defined as comprising the markers that were within the 1 unit drop of $-\log_{10}(P)$ value from the peak signal on either side. Average African ancestry was included in the regression model to adjust for population stratification.

In the fine-mapping analysis, family-based association analysis using the ASSOC program in S.A.G.E. software v. 6.3.0 was performed. ASSOC was selected for this analysis because it can easily incorporate covariates, and it is more powerful than the transmission disequilibrium test. ASSOC is a linear regression model incorporating familial correlation among members within a pedigree through a mixed model, which is similar to GWAF. Briefly, ASSOC applies a linear mixed model: $y = \beta_0 + \beta_1 g + \varepsilon$, where $y$ is the residual after adjusting for covariates, $g \sim MVN(0,V)$, and $V$ is a variance–covariance matrix. For members between families, the correlation is 0. For members within the same family, the correlation is twice of the kinship coefficient. The maximum likelihood method is used to estimate the parameters. Residuals were calculated for each trait after adjusting for the covariates (age, age$^2$, sex, body mass index, and 10 principal components [PCs]). For each trait, we did not observe substantial departure from a normal distribution. The SNP-specific heritability was calculated by the formula:

$$\frac{4\beta^2 p(1-p)}{\sigma^2}$$

where $\beta$ is the estimated effect size, $p$ is the allele frequency of the effect allele, and $\sigma^2$ is the total trait variance.

For the family samples, the first 10 PCs were estimated to account for population stratification as described elsewhere. Briefly, the PCs for all founders were calculated and the PCs of the nonfounders were projected according to their genotype data. PC analysis was conducted using FamCC v.1.0 software. The PCs of founders and nonfounders were similar to the PCs that were calculated from founders only.

The analyses in this study were conducted with PLINK software, SAS 9.2 (SAS Institute Inc., Cary, North Carolina, USA), Stata 9.2 (StataCorp. 2005. Stata Statistical Software: Release 9. College Station, TX: StataCorp LP). Additional information was obtained from the database of single-nucleotide polymorphisms, the University of California Santa Cruz Genome Browser database, and the SNP Annotation and Proxy Search tool.

Results

Admixture Mapping

Admixture mapping analyses were conducted genome-wide for TC, HDL-C, LDL-C, and triglycerides in a sample of 1905 unrelated African American subjects in the FBPP. The descriptive statistics were presented in Table 1. The admixture mapping analyses for TC, HDL-C, LDL-C, and triglycerides were presented in Figures IA—ID in the Data Supplement.

In the admixture mapping analysis, chromosomal regions suggesting evidence of local ancestry association were those with $P$ value <0.001 ($-\log_{10}(P)$ value $\geq 3$). The results for each phenotype were reported with the associated peak, corresponding SNP, $-\log_{10}(P)$ value of the SNP, and region of admixture mapping (Table 2).

For TC, local ancestry association evidence was suggested on chromosome 19 (4.9–40.1 Mega base pairs [Mb]). The maximum $-\log_{10}(P)$ value in this region was at SNP rs901792 (19p13, $-\log_{10}(P)$ value $= 3.06$) in TRIC-A (TMEM38A). For HDL-C, suggested ancestry association evidence was found on chromosome 8 (16.8–27.4 Mb), and the peak was at SNP rs13438843 (8p21, $-\log_{10}(P)$ value $= 3.05$). For LDL-C, ancestry association evidence was suggested on chromosome 7 (16.8–23.9 Mb), and the peak SNP in this region was at rs7793253 (7p15, $-\log_{10}(P)$ value $= 3.21$). For triglycerides, ancestry association evidence was suggested on chromosome 19 (1.6–8.1 Mb) with the peak at rs8110664 (19p13, $-\log_{10}(P)$ value $= 3.96$). These regions on chromosomes 7, 8, 14, and 19 were selected for the fine-mapping association analysis.

Fine-Mapping Analysis

For each trait, only the chromosomal regions showing local ancestry association evidence in the admixture mapping analysis were studied in the family-based association analysis. The association models were adjusted for age, age$^2$, sex, body mass index, and the first 10 PCs. A summary of the significant fine-mapping results were reported in Table 3.

Overall, 52939 SNPs were tested in the fine-mapping analysis, and the number of SNPs tested for each phenotype was TC (17258 SNPs), HDL-C (10995 SNPs), LDL-C (6964 SNPs), and triglycerides (15328 SNPs on chromosome 14 and 2394 SNPs on chromosome 19). Quantile–quantile plots (Figures 1A—1E) presented the observed and expected $-\log_{10}(P)$ value from the fine-mapping analyses. These plots showed substantial deviation from the diagonal line after accounting for population stratification for HDL-C, LDL-C, and triglycerides (chromosome 14), suggesting that there were true variants associated with these traits in these regions. Genomic inflation factors ($\lambda$) were calculated for each plot as described previously and noted below the quantile–quantile plots. Genomic inflation factors $\geq 1.00$ usually indicated that population stratification was not well-controlled. However, as these regions suggested association evidence in the admixture mapping, the observed inflated genomic factors were reasonable and can be attributed to the linkage disequilibrium among the SNPs and true associated variants in these regions.

For HDL-C, 2 SNPs were significant after testing 10995 SNPs. The most significant SNP, rs10096633 ($P = 4.17 \times 10^{-7}$; 8p21) was not in any known genes, but it was near LPL (lipoprotein lipase). The second SNP rs13702 (P=3.44x10^{-8}; 8p21) was located within LPL. These results were consistent with the well-established association between LPL and HDL-C.

Table 1. Summary Statistics

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>Min, Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean African Ancestry</td>
<td>1905</td>
<td>83.7%</td>
<td>85.5%</td>
<td>33.5%, 98.8%</td>
</tr>
<tr>
<td>Age, y</td>
<td>1905</td>
<td>48.8</td>
<td>49.0</td>
<td>19.0, 80.0</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>1905</td>
<td>31.1</td>
<td>29.9</td>
<td>13.8, 70.7</td>
</tr>
<tr>
<td>Sex</td>
<td>1905</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>682</td>
<td>(35.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1223</td>
<td>(64.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>1530</td>
<td>197.7</td>
<td>194.0</td>
<td>80.0, 418.0</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>1529</td>
<td>54.6</td>
<td>52.0</td>
<td>22.0, 171.0</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>1479</td>
<td>119.2</td>
<td>116.8</td>
<td>25.6, 290.4</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>1530</td>
<td>122.2</td>
<td>105.0</td>
<td>23.0, 3170.0</td>
</tr>
</tbody>
</table>

- BMI indicates body mass index; HDL-C, high-density lipoprotein cholesterol; and LDL-C, low-density lipoprotein cholesterol.
For LDL-C, 4 SNPs were significant after testing 6964 SNPs. The most significant SNP was rs12534314 (P = 9.29 × 10^-6; 7p15), which was in high LD (D^2 = 1.00, r^2 = 1.00) with rs12531660 (P = 2.13 × 10^-5; 7p15). SNPs rs12534314 and rs6966083 (P = 1.02 × 10^-10; 7p21) were not located within genes, but SNP rs10486301 (P = 9.37 × 10^-6; 7p21) was located within HDAC9.

On chromosome 14, there were 15328 SNPs tested for triglycerides in the fine-mapping analysis, and 5 of these SNPs were significant. Three of the 5 SNPs were in high LD (D^2 = 1.00, r^2 = 1.00), resulting in 3 independent SNPs for triglycerides. The most significant SNP was rs10483943 (14q31) (P = 3.35 × 10^-7), which was not in any known gene. SNPs rs757645 (P = 1.92 × 10^-6) and rs759512 (P = 7.74 × 10^-7) were in close proximity to rs10483943. In addition, SNPs rs10130530 within TTC7B (14q24) in close proximity to rs10483943. In addition, SNPs rs69473414 (14q24) in NRXN3 and rs11620666 (14q32) in TTC7B were significantly associated with triglycerides (P = 2.13 × 10^-6 and P = 3.35 × 10^-5, respectively).

Graphs were created to summarize the results from the admixture mapping, family-based fine-mapping analysis, and large GWAS in the literature (Figures 2A—2E). The SNP results from the fine-mapping analysis and the SNP results from literature were overlaid on the admixture mapping results from the present study.

Table 2: Regions Suggested by Admixture Mapping

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Chromosome and Region, Mb*</th>
<th>Peak SNP in the Region</th>
<th>−log_(10)(P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>Chromosome 19: 4.9–40.1</td>
<td>rs901792</td>
<td>3.06</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Chromosome 8: 16.8–27.4</td>
<td>rs13438843</td>
<td>3.05</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Chromosome 7: 16.8–23.4</td>
<td>rs7793253</td>
<td>3.21</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>Chromosome 14: 78.3–95.6</td>
<td>rs3825663</td>
<td>3.26</td>
</tr>
<tr>
<td></td>
<td>Chromosome 19: 1.6–8.1</td>
<td>rs8110664</td>
<td>3.96</td>
</tr>
</tbody>
</table>

HDLC indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Mb, Mega base pairs; and SNP, single-nucleotide polymorphism.

*Region is the 1 unit drop of −log_(10)(P value) of the peak SNP in the region.

An in silico replication of the lipid results was performed using data from the NHLBI’s Candidate Gene Association Resource (CARe) Consortium. The CARe Consortium is a multisite, multiethnic study comprised of 9 cohort studies, including 5 African American cohorts, that examines genetic variation in lipids, blood pressure, and many other traits. Admixture mapping analysis of lipid traits was conducted using data from the NHLBI’s Candidate Gene Association Resource (CARe) Consortium. The CARe Consortium is a multisite, multiethnic study comprised of 9 cohort studies, including 5 African American cohorts, that examines genetic variation in lipids, blood pressure, and many other traits.34,35 In Lipid analysis, the residuals of both HDL-C and LDL-C were standardized, resulting in smaller estimated genetic effect sizes than the current study. However, the different transformations did not affect replication result. For HDL-C (N = 7813), SNPs rs10096633 (P = 1.48 × 10^-6) and rs13702 (P = 2.49 × 10^-5) were significantly associated with the phenotype (Table 3). These results had the same direction of effect as the FBPP results, and the P values were more significant in the CARe results.

For LDL-C (N = 7565), only rs6966083 (P = 0.01181) was significantly associated with the trait, whereas rs12534314 (P = 0.9183) and rs10486301 (P = 0.8083) were not. Only the result for rs10486301 was in the same direction for both FBPP and CARe. In addition, the P values were more significant for LDL-C in the FBPP results.

Discussion
Admixture mapping analysis of lipid traits was conducted for unrelated African Americans in the FBPP, and the

Table 3: Significant Fine-Mapping Results

<table>
<thead>
<tr>
<th>Phenotype (Chr)</th>
<th>No. of SNPs Tested</th>
<th>Bonferroni-Corrected Significance Level</th>
<th>Significant SNPs [call rate]</th>
<th>Beta</th>
<th>SE</th>
<th>Wald P Value</th>
<th>Effect Allele Frequency in CEU/YRI</th>
<th>SNP-Specific Heritability</th>
<th>CARE Replication Beta (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL (8)</td>
<td>10995</td>
<td>4.55 × 10^-4</td>
<td>rs10096633 [99.97%]</td>
<td>2.10</td>
<td>0.41</td>
<td>4.17 × 10^-2</td>
<td>T 0.142/0.493</td>
<td>0.017</td>
<td>0.1008 (1.48 × 10^-9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs13702 [99.92%]</td>
<td>-1.89</td>
<td>0.41</td>
<td>3.44 × 10^-4</td>
<td>T 0.712/0.418</td>
<td>0.014</td>
<td>-0.0981 (2.49 × 10^-9)</td>
</tr>
<tr>
<td>LDL (7)</td>
<td>6964</td>
<td>7.18 × 10^-4</td>
<td>rs12534314* [99.92%]</td>
<td>-8.50</td>
<td>1.92</td>
<td>9.29 × 10^-4</td>
<td>T 0.185/0.003</td>
<td>0.013</td>
<td>0.0031 (0.9183)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs6966083 [100%]</td>
<td>-18.27</td>
<td>4.14</td>
<td>1.02 × 10^-5</td>
<td>T 0.509/0.194</td>
<td>0.010</td>
<td>0.183 (0.01181)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs12531660† [99.92%]</td>
<td>-8.82</td>
<td>1.93</td>
<td>2.13 × 10^-5</td>
<td>G 0.172/0.025</td>
<td>0.014</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs10486301 [100%]</td>
<td>-8.34</td>
<td>2.14</td>
<td>9.37 × 10^-5</td>
<td>C 0.231/0.008</td>
<td>0.018</td>
<td>-0.0092 (0.8083)</td>
</tr>
<tr>
<td>Triglycerides (14)</td>
<td>15328</td>
<td>3.26 × 10^-4</td>
<td>rs10483943† [100%]</td>
<td>36.95</td>
<td>7.24</td>
<td>3.35 × 10^-2</td>
<td>G 0.185/0.008</td>
<td>0.012</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs759512† [100%]</td>
<td>35.97</td>
<td>7.28</td>
<td>7.74 × 10^-3</td>
<td>T 0.194/0.004</td>
<td>0.011</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>rs757645† [100%]</td>
<td>33.05</td>
<td>6.94</td>
<td>1.92 × 10^-4</td>
<td>A 0.169/0.017</td>
<td>0.009</td>
<td>NA</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>rs10130530 [100%]</td>
<td>33.28</td>
<td>11.24</td>
<td>2.13 × 10^-4</td>
<td>A 0.023/0.008</td>
<td>0.024</td>
<td>NA</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>rs11620666 [100%]</td>
<td>23.85</td>
<td>5.11</td>
<td>3.08 × 10^-4</td>
<td>T 0.418/0.008</td>
<td>0.005</td>
<td>NA</td>
</tr>
</tbody>
</table>

CEU indicates Utah residents with Northern and Western European ancestry from the CEPH collection from the International HapMap Project; Chr, chromosome number; HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NA, not available; SNP, single-nucleotide polymorphism; and YRI, Yoruba in Ibadan, Nigeria from the International HapMap Project.

*In linkage disequilibrium (D = 1.00, r^2 = 1.00) on chromosome 7.
†In linkage disequilibrium (D = 1.00, r^2 = 1.00) on chromosome 14.
chromosomes that contained regions of admixture evidence were followed-up with fine-mapping association analysis using family data. This procedure has been suggested to identify SNP associations, which may be missed by traditional GWAS.\textsuperscript{20,26} The admixture mapping analysis capitalized on the recent admixture of African American population to narrow down regions of interest for a trait, and the family-based analysis allowed for fine-mapping of the regions.

Five regions in the admixture mapping analysis that showed local ancestry association evidence were reported for association with lipids. In the fine-mapping analysis, 11 SNPs (8 independent SNPs) were associated with lipid traits. Findings

Figure 1. Quantile–quantile plots of fine-mapping analysis in families. 52,939 SNPs were tested, and the number of SNPs tested for each phenotype was total cholesterol (17,258 SNPs), HDL-C (10,995 SNPs), LDL-C (6,964 SNPs), and triglycerides (15,328 SNPs on chromosome 14 and 2,394 SNPs on chromosome 19). Significant results were reported for HDL-C, LDL-C, and triglycerides (chromosome 14).

A, Total cholesterol (Greek \(\text{lda}=0.97\)); B, HDL-C (Greek \(\text{lda}=1.02\)); C, LDL-C (Greek \(\text{lda}=1.10\)); D, triglycerides on chromosome 14 (Greek \(\text{lda}=1.00\)); E, triglycerides on chromosome 19.

Figure 2. Combined analyses plots. The SNP results from the fine-mapping analysis and from the literature were overlaid on the admixture mapping results from the present study. Results from the present study were represented by circles, and results from the literature were represented by triangles. Additional significant results were reported by Teslovich et al for LDL-C at rs12670798 (21.6 Mb) and rs2072183 (44.5 Mb) on chromosome 7 and for total cholesterol at rs6511720 (11.1 Mb) and rs4420638 (60.1 Mb) on chromosome 19 without \(P\) values. A, Total cholesterol; B, HDL-C; C, LDL-C; D, triglycerides on chromosome 14; E, triglycerides on chromosome 19. Mb, Mega base pairs; and SNP, single-nucleotide polymorphism.
for 4 SNPs were consistent with previously reported results and the other 4 were novel associations.

Significant admixture associations were reported for TC and triglycerides on chromosome 19, but significant fine-mapping results were not found in these regions. For TC, the reported admixture region included 4 SNPs that were previously reported for TC, HDL-C, and LDL-C in a large GWAS of blood lipids in populations of European ancestry populations.6 For HDL-C, the most significant SNP from the fine-mapping, rs10096633, was near LPL and the second SNP rs13702 was located within LPL. This gene has a key role in the hydrolysis of the triglyceride component of several lipoproteins and lipid metabolism.36–38 The relationship between LPL and HDL-C has been well-established, and several studies have identified common variants in LPL that are associated with reduced HDL-C (Ser447stop, Asn291Ser, and D9N).39–41 Recent studies have also identified LPL variants in diverse populations and have reported associations between rare LPL variants and reduced response to fenofibric acid treatment for low HDL-C.5,42,43

For LDL-C, the admixture region on chromosome 7 included rs12670798 (DNAH1), which was previously reported for association with LDL-C and TC.6 The most significant SNP in the fine-mapping analysis, SNP rs12534314, was for LDL-C and was located near STEAP1B. Although not directly associated with a lipid phenotype, the region around STEAP1B was previously reported for association with all-cause death on dialysis in African Americans with type 2 diabetes mellitus.44 SNP rs10486301 in HDAC9 was reported for association with stroke and obesity phenotypes.45,46

For triglycerides, the admixture analysis peak on chromosome 14 was located at rs3825663 in TDP1, which was reported for association with carotid plaque in a Dominican family study.47 In the fine-mapping, SNP rs10130530 in NRXN3 was previously reported for association with obesity, body mass index, and waist circumference in GWAS.48–50 SNP rs11620666 in TTC7B was reported in a GWAS of European-ancestry individuals for cytomegalovirus antibody response, and cytomegalovirus was found to be associated with atherosclerosis and acute coronary events.51–53 The admixture regions for triglycerides included SNPs that were not previously reported for association in the lipids GWAS.6

Finally, the results from the admixture mapping analysis, the fine-mapping association analysis, and GWAS were evaluated concurrently (Figures 2A—2E).3,9,43,54–57 As expected, the fine-mapping SNPs for HDL-C, LDL-C, and triglycerides (chromosome 14) were consistent with the admixture mapping results with regards to the location of the peak SNPs. Yet, it seemed that the precision and magnitude of the SNP results benefitted from the testing of dense SNPs in the family-based, fine-mapping analysis. For example, for LDL-C, the locations of the most significant SNPs were consistent with the peaks from the admixture analysis; however, the magnitude of the P values indicate that the strength of evidence showing departure from the null hypothesis (of no association) was stronger in the fine-mapping analysis than in the admixture mapping analysis. Consequently, the admixture mapping results were narrowed down to the significant SNPs from the fine-mapping analysis. Further, these graphs demonstrated consistencies between the studies, despite being performed in different populations, which was also reported in recent studies.5,21 The graphs also showed that the current study’s use of African American subjects revealed novel findings that were not found in studies of other populations.

By conducting a 2-stage analysis, this study reduced the number of SNPs tested in the family-based association study by focusing on variants in candidate regions. This method of analysis has the potential to uncover the variants missed by traditional studies, such as GWAS.20,26

In summary, the current study reports 5 chromosomal regions that showed association of local ancestry evidence with cardiovascular phenotypes. In the follow-up fine-mapping analysis in families, 8 independent SNPs were associated with lipid traits. These regions and SNPs may aid in identifying potential therapeutic targets for cardiovascular disease traits. Furthermore, these regions are candidates for future sequencing studies and further lipid studies, especially in African American populations. The present study also suggested the benefits of multistage analysis methods for genetic studies, particularly in recently admixed populations that were not well-studied with traditional association studies.

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Disclosures

None.

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Lipids Variants Identified Using Admixture Mapping

Shetty et al

We reported several admixture regions and single-nucleotide polymorphisms that were associated with high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides in African Americans. Although the clinical effects of these variants have not yet been identified, they may provide biological insight to lipids. Because admixture mapping will detect trait associated variants with substantial allele frequency in ancestral populations, the identified variants may partially explain the disparity in disease prevalence between African Americans and European Americans. In addition, these variants can be informative in identifying true causal variants for lipids and cardiovascular disease through sequencing and functional studies. Finally, the genetic variants may help identify novel drug targets and patients at risk of cardiovascular diseases.
Fine-Mapping Analysis in African American Families

Variants for HDL-C, LDL-C, and Triglycerides Identified from Admixture Mapping and Fine-Mapping Analysis in African American Families


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SUPPLEMENTAL MATERIAL

Appendix:

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Tracy RP
Vasan RS
Waters KM
Wilks R
Wilson JG
Fabsitz RR
Gabriel SB
Kathiresan S
Boerwinkle E
Supplemental Figures 1: Admixture Mapping

The ADMIXPROGRAM results are the dashed lines and the SABER results are the dotted lines.

A: Total Cholesterol
B: HDL-C
C: LDL-C
D: Triglycerides