The genetic architecture of lipoprotein subclasses in Gullah-speaking African American families enriched for type 2 diabetes: The Sea Islands Genetic African American Registry (Project SuGAR)

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Abstract We sought to partition the genetic and environmental influences on lipoprotein subclasses and identify genomic regions that may harbor genetic variants that influence serum lipoprotein levels in a sample of Gullah-speaking African-Americans. We genotyped 5,974 SNPs in 979 subjects from 418 pedigrees and used the variance component approach to compute heritability estimates, genetic and environmental correlations, and linkage analyses for selected lipoprotein subclasses. The highest heritability estimate was observed for large VLDL particle concentration (0.56 ± 0.14). Mean LDL particle size and small LDL particle concentration (−0.94) had the strongest genetic correlation estimate. The highest logarithm of odds (LOD) score detected (3.0) was on chromosome 6p24 for small LDL particle concentration. The strongest signal, obtained with the reduced sample of diabetic individuals only, was observed on chromosome 20p13 for small LDL particle concentration. The highest bivariate linkage signal (LOD 2.4) was observed on chromosome 6p24 for mean LDL particle size and small LDL particle concentration.jlr Our results suggest a significant genetic contribution to multiple lipoprotein subclasses studied in this sample and that novel loci on chromosomes 6, 10, 16, and 20 may harbor genes contributing to small, atherogenic LDL particle concentration and large, triglyceride-rich VLDL particle concentration.—Divers, J., M. M. Sale, L. Lu, W-M. Chen, K. H. Lok, I. J. Spruill, J. K. Fernandes, C. D. Langefeld, and W. T. Garvey. **The genetic architecture of lipoprotein subclasses in Gullah-speaking African American families enriched for type 2 diabetes: The Sea Islands Genetic African American Registry (Project SuGAR). *J. Lipid Res.* 2010.** 51: **586–597.**

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Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among individuals with diabetes (1–3). For example, the risk of coronary heart disease is 2–4 times higher in diabetic patients compared with nondiabetic individuals (4–8). Dyslipidemia is thought to be a potential common link between these two conditions (9, 10). The principal lipoprotein classes, VLDL, LDL, and HDL, have received considerable attention as cardiovascular disease risk factors in the epidemiology literature. Several genes have also been reported to be associated with these lipoprotein phenotypes (11–13). In fact, genetic correlation and genes with possible pleiotropic effect on LDL,

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Abbreviations: BMI, body mass index; CVD, cardiovascular disease; GENNID, Genetics of NonInsulin Dependent Diabetes; GWAS, genome-wide association studies; IDL, intermediate density lipoprotein; LOD, logarithm of odds; T2DM, type 2 diabetes mellitus; Project SuGAR, Sea Islands Genetic African American Registry; SNP, single nucleotide polymorphism; TG, triglyceride; UAB, University of Alabama at Birmingham.

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triglycerides (TG), and HDL are described in the literature (14, 15), and it is reasonable to expect this type of pleiotropic effect may be observed with the lipoprotein subclasses.

When assessed by the conventional lipid panel, dyslipidemia in type 2 diabetes mellitus (T2DM) and metabolic syndrome are characterized by high TG and low HDL-C, while total cholesterol and calculated LDL-C are not consistently affected (15–17). However, there is heterogeneity in particle size and density within the major lipoprotein subclasses, which is not measured by the conventional lipid panel, and these alterations can confer cardiovascular disease risk. For example, in the general population, increased levels of small dense LDL (18–21) and an excess of the small over large HDL particles (22, 23) have been shown to be associated with increased risk for atherosclerosis. The lipoprotein subclasses can be quantified in plasma using NMR. NMR generates unique spectra for different lipoprotein subclasses based on a bulk lipid signal that reflects particle size, with the amplitude being proportional to the lipoprotein subclass particle concentration (23–26).

We used NMR to demonstrate that insulin resistance affects subclasses for all 3 principal lipoprotein classes (VLDL, LDL, and HDL) (27, 28). First, in insulin-resistant individuals, the NMR lipoprotein subclass profile indicated that large VLDL particles, produced primarily by the liver, are markedly increased without consistent changes in medium or small VLDL. This is important because large VLDL particles may confer more cardiovascular disease risk (29, 30). Second, the NMR data demonstrated a shift of LDL particles from large, buoyant particles to small, dense particles, with the net result of little or no change in overall LDL-cholesterol. This would also result in an observed shift to smaller mean LDL particle size. This latter change could also contribute to CVD, because increments in small LDL particle concentration have been shown to represent increased CVD risk, independent of LDL-cholesterol levels (21, 31). Another important aspect demonstrated only in the NMR data was that the total number of LDL particles was increased by insulin resistance, which has also been shown to confer increased risk of cardiovascular disease (21). Third, the NMR data showed that any decrease in HDL-cholesterol was entirely explained by specific loss of the cardio-protective large HDL subclass, while noncardio-protective intermediate and small HDL particles may even have increased. Thus, the NMR lipoprotein subclass profile provides direct measurements relevant to cardiovascular risk that are either not provided or are obscured in the conventional lipid profile. However, little is known about the genetic determinants of lipoprotein subclass concentration and particle size. The dearth of information is even more pronounced in minority populations.

We describe the genetic architecture of particle size and concentrations of VLDL, LDL, and HDL subclasses in the context of the Sea Islands Genetic African American Registry (Project SuGAR). This project recruited African-Americans living in coastal communities and on the Sea Islands of South Carolina and northeast coastal Georgia. These individuals are believed to be direct descendants of slaves who were forcibly deported from the "rice or windward coast" of West Africa and transported to these areas because their rice-growing expertise was critical for the culture of this cash crop in colonial America (32). Studies of mitochondrial and Y-chromosomal markers have determined that the genetic distance between the Gullah and Sierra Leonese tribes is shorter than other African-American populations (33–36). Our intention was to capitalize upon the relative ancestral homogeneity, common diet, and increased prevalence and familial clustering of diabetes to explore the genetic architecture of these traits in the African-American Gullah population using a high resolution SNP linkage panel. Traditional lipid panel measures were included to calculate genetic and environmental correlations between conventional lipid measures and lipoprotein classes and to determine whether underlying genetic contributors to these traits differed.

RESEARCH DESIGN AND METHODS

Subjects

This study was conducted under Institutional Review Board approval from the Medical University of South Carolina, the University of Alabama at Birmingham (UAB), and Wake Forest University School of Medicine, and adhered to the tenets of the Declaration of Helsinki. Project SuGAR enlisted medical clinics, churches, and established organizations on the Sea Islands to aid in identifying patients with T2DM who belonged to families with multiple affected members (37). Inclusion criteria included self-described African-American race, at least one T2DM-affected sibling pair, no more than one of the parents affected with T2DM, and at least one parent still living. Probands and their parents were all born and raised in the South Carolina low country. Project SuGAR assessed medical, anthropometrical, and metabolic information on all consenting affected and nonaffected family members. The data were collected based on a multi-page questionnaire, detailed family history and medical history, standardized blood pressures, physical examination, body dimensions and estimation of percent body fat, and laboratory testing. Weights were determined using electronic calibrated scales (Detecto, Cleveland, OH) at 8–10AM after voiding and before breakfast. Heights were measured with a portable Harpenden statiometer. Body mass index (BMI) (kg/m^2) was calculated. Standard arm, waist, hip, and thigh circumferences were recorded using a tension-controlled tape measure (Novel Products, Rockton, IL). Laboratory testing included complete blood count, electrolytes, creatinine/BUN, liver function tests, hemoglobin A_{1C}, fasting lipid panel (cholesterol, triglycerides, HDL), circulating islet cell antibodies (if diabetic), fasting glucose, and urine albumin-creatinine ratio. All participating nondiabetic family members were evaluated with an oral glucose tolerance test or by fasting glucose. Criteria established by the Expert Committee of the American Diabetes Association (38) were used to define subjects as diabetic, impaired fasting glucose, impaired glucose tolerance, and normal glucose tolerance. Clinical records and medical history were reviewed to exclude individuals with probable type 1 diabetes on the basis of time to insulin dependence and/or islet cell antibodies. When the entire sample was used in the analysis, covariate adjustments were made for diabetes status, age, gender, BMI, and lipid and hypertension medication.

There was no need to adjust for diabetes status in the diabetic-only analysis.

Lipoprotein subclasses were measured by LipoScience, Inc.. (Raleigh, NC) using NMR spectroscopy (39). All measurements were made prior to 2004 and the availability of the NMR LipoProfile-II. The NMR spectrum of each plasma sample was modeled as the sum of the signals from 16 discrete subpopulations of lipoprotein particles: chylomicrons, six VLDL (V1–V6), intermediate density lipoprotein (IDL), three LDL (L1–L3), and five HDL (H1–H5). Our analyses focused on a subset of grouped subclasses with higher measurement precision and stronger cardiovascular disease outcome associations: large VLDL (V5+V6); large, cardioprotective HDL (H4+H5), and small, dense atherogenic LDL (L1).

The current genome scan involved a total of 967 individuals, including 791 T2DM-affected subjects and 176 unaffected relatives who were recruited from 418 families. We included all phenotyped individuals in the ascertained families that were informative for linkage in the full sample analysis and all diabetic individuals who were informative for linkage in the diabetic-only analysis.

Genotyping

DNA was extracted from 20–40 ml of venous blood using a standardized DNA isolation kit (Gentra Systems, Minneapolis, MN). The Project SuGAR registry includes 70 sib-pairs plus available parents, totaling 162 participants who were part of the Genetics of NonInsulin Dependent Diabetes (GENNID) study. For the GENNID subjects, blood was sent to the central laboratory for lymphocyte transformation and DNA extraction was performed by Coriell Cell Repositories.

A genome-wide linkage scan was completed by the Center for Inherited Disease Research using Illumina's Human Linkage Panel IVb. The genetic map position for SNPs on this panel is based on the National Center for Biotechnology Information's build 35. A total of 5,974 SNPs were successfully genotyped, with a mean spacing of 0.65 cM (518 kb). The missing data rate was 0.26% (17,434 missing genotypes/6,626,408 total genotypes), and the Mendelian consistency rate, after correction or removal of likely misspecified relationships as determined using the genetic data (see below), was 99.9% (535 events/6,292,704 study genotypes). The blind duplicate reproducibility rate was 99.9% (7 events/321,713 paired genotypes). Thirteen SNPs were removed from analyses because they violated Hardy-Weinberg assumptions ($P < 0.0001$).

Quality control checks

Each pedigree was examined for consistency of familial relationships using the Pedigree Relationship Statistical Test (40). When the self-reported familial relationships were strongly inconsistent with the genotypic data for that pedigree, then the pedigree was modified when the identity-by-descent statistics suggested a very clear alternative, or the entire genotypic data was converted to missing for those individuals whose familial relationship could not be resolved. A total of 58 pedigrees (~14%) exhibited probable misspecified familial relationships and were modified as above. Sibling relationship in 45 pedigrees had to be changed from full to half sib after reviewing the PREST (41) output. There were six pedigrees with duplicated samples; we retained the most complete observation from each pair. There were seven pedigrees containing unrelated individuals; these individuals were removed prior to the analysis. After modifying all family relationships that appeared to be inconsistent with the genome scan data, there were a total of 1,196 parent-child, 320 grandparent-child, 36 great grandparent-grandchild, 281 full sibling, 79 half-sibling, 136 avuncular, 34 first cousin, and 60 more distant relationship pairs. The number of generations observed varied between two and four, with only 19 pedigrees containing individuals from four different generations. The smallest pedigree contained three people; we observed 11 such pedigrees. The largest pedigree contained 15 people, and the average pedigree size was about 5.6 people. Each marker was examined for Mendelian inconsistencies using PedCheck (42), and sporadic problem genotypes were converted to missing. Map distances were based on the Rutgers's genetic map (43). These distances were computed using the Kosambi map function. Where two SNPs displayed LD values of $r^2 > 0.3$, we removed one SNP of the pair; 230 SNPs were removed for this reason.

Statistical analysis

We used the variance component approach as implemented in the program SOLAR (44) to compute estimates of heritability genetic and environment correlations and to complete the multipoint linkage analyses. Multipoint estimates of allele sharing IBD probability required by SOLAR were computed using MERLIN (45). The pedigree sizes analyzed in this study were within the specifications of the algorithms employed in MERLIN. Statistical analyses were conducted both on the entire sample and on the smaller, but more homogenous, sample of diabetic individuals only. All analyses were adjusted for sex, age, and BMI. We also adjusted for diabetes status when the statistical analysis was computed on the entire sample. Box-Cox transformations were applied as needed to transform lipoprotein variables whose residual distribution deviated significantly from the normality assumption or exhibited heterogeneity of variance.

We also ran bivariate linkage analysis in an effort to identify genes that may have a pleiotropic and/or coincidence effect on a pair of traits. Bivariate linkage analysis exploits the additional information contained in the correlation pattern between the two quantitative traits. It has shown increased power over univariate linkage analysis to detect linkage when the phenotypic variables are correlated (46, 47). We caution that one should not interpret bivariate LOD scores similarly as univariate LOD scores because they follow a different distribution under the null hypothesis. LOD scores were obtained at 1 cM intervals for both the univariate and bivariate linkage analyses.

Multiple testing is always a concern whenever a considerable number of tests are conducted. As will be seen in the "Results" section, a number of these traits are highly correlated, which argues against a Bonferroni type adjustment because it will likely be too conservative. Consequently, following the recommendations of Lander and Kruglyak (48), we decided to display the highest LOD scores observed on each chromosome and focus on genomic regions where a LOD score of 2.2 or higher was observed. We did so because we think that it is important to provide as much detail as possible regarding potentially interesting genomic regions. It is unlikely that more linkage studies will be done in this minority population, and providing details, even if they do not reach genome-wide significance while adjusting for multiple phenotypes, is important. With these results, we are simply suggesting that the identified regions can potentially be of interest, particularly in subsequent genome-wide association studies. Examination of the correlation of LOD scores and genetic variation predisposing to complex genetic traits suggest that regions with intermediate LOD scores are potentially of value.

We also provide the LOD-1 interval for these regions. The LOD-1 interval can be seen as the support interval for which the LOD score equals the observed maximum LOD score − 1 (49). It is in a sense a crude estimate of the 95% confidence interval around the marker with the highest LOD score in the genomic region under consideration.

RESULTS

Population characteristics

The demographic and lipid characteristics for the Project SuGAR participants are summarized in **Table 1**. The participants were mostly females (~77%), about 80% of the family members had T2DM, and 11% of them were taking lipid-controlling medication.

Heritability of lipid related phenotypes

Table 2 displays the heritability estimates and their standard error. These estimates were computed after adjusting for gender, age, diabetes status, BMI, and whether the participants were on lipid-controlling medication, and range from 0.33 to 0.56. The highest heritability estimates were observed for large VLDL particle concentration (0.56 ± 0.14) and small LDL particle concentration (0.54 ± 0.15), while large HDL particle concentration (0.33+-0.16) and VLDL mean particle size(0.29+-14) had the lowest heritability estimates.

The initial bivariate analyses indicate that significant genetic and environmental correlation exists between almost all pairs of traits considered (**Tables 3** and **4**). A star next to a value in Tables 3 and 4 indicates that the *P*-value corresponding to the estimated correlation value is between 0.01 and 0.05; 2 stars imply that a *P*-value is <0.01. These two tables support the hypothesis that shared genetic and environmental factors account for a significant portion of the total variance for most of these traits. In general, a significant nonzero squared genetic correlation between two traits provides a measure of the extent to which they are both affected by the same underlying genetic variants (50). Estimates of genetic and environmental correlations considering the entire sample are shown in Table 3. Table 4 depicts these estimates when the analysis was restricted to only diabetic individuals. The genetic correlation estimates are shown below the main diagonal in both tables, whereas the environmental correlation estimates are shown above the diagonal. The highest genetic correlation estimates are observed between the LDL average particle size and the small LDL particle concentration (−0.94) and between the average VLDL particle size and large VLDL particle concentration (−0.88). These genetic correlations were observed in the diabetic-only analysis. The second strongest genetic correlations in the combined sample are observed between the cardioprotective large HDL particle concentration and the average LDL particle size (0.75) and between the average LDL particle size and triglyceride concentration (0.72). Note that some of these traits have been transformed to satisfy the normality assumption made about the distribution of each trait. If an inverse transformation is needed for one trait while no transformation is needed for another one, the sign of the relationship between the two traits will be different than what would be expected if both variables were used on their original scale. Consequently, we suggest that the reader focus more on the absolute value of the correlation estimate, which measures the strength of the correlation instead of its directionality. Subsequent tests for pleiotropy rejected the hypothesis that the genetic correlations were equal to one, a finding that suggests these genes might have a differential effect on each trait.

Single trait linkage results

We ran linkage analysis on the complete sample as well as on a reduced sample of diabetic-only individuals. Results for the cholesterol traits were adjusted for the effect of lipid and /or hypertension controlling medications.

Complete sample analysis

The maximum LOD scores observed on each chromosome are summarized in **Table 5** for single trait linkage results using the complete sample. Following the recommendations of Kruglyak and Lander (48, 50), we focus on

TABLE 1. Clinical characteristics and measurements of lipids/lipoproteins

		Subjects with T2DM		Nondiabetic Subjects	
Clinical	All Subjects	Females	Males	Females	Males
Number	N = 967	N = 614	N = 177	N = 127	N = 49
Age (years) (mean ± SD)	52.7 ± 15.5	55.0 ± 14.2	55.0 ± 15.0	45.0 ± 16.1	36.2 ± 15.6
BMI (kg/m2) (mean ± SD)	33.5 ± 7.9	34.3 ± 7.9	31.3 ± 6.53	34.1 ± 8.7	29.3 ± 7.4
HbA1c (%) (mean ± SD)	8.87 ± 2.22	8.94 ± 2.13	8.85 ± 2.41	5.79 ± 0.97	NA
% Taking lipid-lowering medications	11.89%	14.73%	10.98%	2.84%	0.57%
		Conventional Lipid Panel			
Total cholesterol (mg/dl) (mean ± SD)	196.7 ± 44.	200.8 ± 44.8	190.9 ± 44.8	191.0 ± 43.4	183.0 ± 42.0
TG (mg/dl) (mean ± SD)	124.2 ± 85.1	128.0 ± 78.8	139.7 ± 110.8	91.6 ± 63.3	107.6 ± 83.4
HDL cholesterol (mg/dl) (mean ± SD)	49.0 ± 14.0	50.2 ± 13.6	43.2 ± 13.2	52.6 ± 15.0	46.2 ± 13.3
Calculated LDL cholesterol (mg/dl) (mean ± SD)	124.2 ± 40.0	126.1 ± 40.8	123.1 ± 39.7	119.3 ± 37.7	117.1 ± 36.8
VLDL (mean ± SD)	23.1 ± 12.5	24.2 ± 12.6	24.5 ± 12.3	17.4 ± 10.6	19.5 ± 11.6
		NMR Lipoprotein Subclass Analysis			
Large VLDL particle concentration (mg/dl) (mean ± SD	22.6 ± 38.9	22.0 ± 36.8	33.9 ± 54.2	11.1 ± 17.6	14.3 ± 23.5
VLDL mean particle size (nm) (mean ± SD)	45.7 ± 7.4	45.8 ± 7.3	47.0 ± 8.6	43.2 ± 5.5	46.1 ± 8.4
Small LDL particle concentration (mg/dl) (mean ± SD)	20.1 ± 29.4	18.3 ± 28.3	31.7 ± 36.1	15.3 ± 23.4	10.8 ± 13.6
LDL mean particle size (nm) (mean ± SD)	21.0 ± 0.6	21.0 ± 0.6	20.7 ± 0.7	21.2 ± 0.6	21.1 ± 0.4
Large HDL particle concentration (mg/dl) (mean ± SD)	23.7 ± 11.5	25.0 ± 11.5	18.0 ± 10.04	25.8 ± 12.0	19.5 ± 7.0
HDL mean particle size (nm) (mean ± SD)	9.0 ± 0.4	9.0 ± 0.4	8.8 ± 0.4	9.1 ± 0.4	9.0 ± 0.4

TABLE 2. Heritability estimates for lipid related traits

Quantitative Traits	Heritability ($h^2 \pm SD$), (*P*-value)
Large VLDL particle concentration (mg/dl)	0.56 ± 0.14, (0.00008)
VLDL mean particle size (nm)	0.29 ± 0.14, (0.02)
Small LDL particle concentration (mg/dl)	0.54 ± 0.15, (0.0003)
LDL mean particle size (nm)	0.46 ± 0.15, (0.002)
Large HDL concentration (mg/dl)	0.33 ± 0.16, (0.02)
HDL mean particle size (nm)	0.41 ± 0.17, (0.007)
Triglyceride concentration (mg/dl)	0.37 ± 0.11, (0.00046)

LOD scores ⩾ 2.2. Two such LOD scores were observed. The largest, a LOD score of 3.0, was detected on chromosome 6p24 for small LDL particle concentration. The SNP corresponding to this LOD score was rs1328132. The LOD-1 interval spans about 12 cM and is determined by rs2815155 to the left and rs1891284 to the right. The second largest, a LOD score of 2.2, was identified on chromosome 10p11 for large VLDL particle concentration. This LOD score was observed with rs867992. Its accompanying LOD-1 interval spans about 6 cM starting at rs959629 and ending at rs1441027.

Six other regions of the genome yielded LOD scores that were between 1.5 and 2.2. We present these results here in an effort to provide as complete a summary as possible given that they were obtained in such a unique population. We also note that these results can potentially be useful in efforts to prioritize marker selection following other studies, including genome-wide association studies (51). Two of these LOD scores were observed for large VLDL particle concentration on chromosome 3p26 (LOD score = 1.7) and on chromosome 5p15 (LOD score = 1.7). A LOD score of 1.7 was also observed on chromosome 9p24 for LDL mean particle size. Two additional linkage signals were detected for small LDL particle concentration: the first was located on chromosome 12p24 and the second on chromosome 18q21. Finally, a LOD score of 1.5 was observed on chromosome 9p11 for TG concentration. There were no LOD scores above this threshold for large HDL particle concentration, HDL mean particle size, and VLDL mean particle size.

In summary, large VLDL particle concentration and small LDL particle concentration both display substantial evidence of linkage at various genomic regions, albeit none of them reached genome-wide statistical significance according to thresholds set in Lander and Kruglyak (48).

Diabetics-only analysis

We also completed a linkage analysis on the sample of diabetic individuals. These results are shown in **Table 6**. Some of the diabetic sample linkage signals yielded maximum LOD scores that varied in magnitude and location relative to that of the entire sample. This is to be expected with different partitions of the data. The LOD score of 3.0 observed on chromosome 6p24 for small LDL particle concentration was now reduced to 1.7. However, the LOD score of 1.3 observed on chromosome 20p13 for small LDL particle concentration strengthened to 2.7 in the

TABLE 3. Genetic and environmental correlation in the combined sample

Traits	Large VLDL Particle Concentration (mg/dl)	VLDL Mean Particle Size (nm)	Small LDL Particle Concentration (mg/dl)	LDL Mean Particle Size (nm)	Large HDL Particle Concentration (mg/dl)	HDL Mean Particle Size (nm)	Triglyceride Concentration (mg/dl)	Total Cholesterol	HDL Cholesterol	LDL Cholesterol	VLDL Cholesterol
Large VLDL particle concentration (mg/dl)	**NA**	−0.75 **	0.14 **	−0.53 **	−0.30 **	0.31 **	−0.75 **	−0.24 **	−0.16 **	−0.338 **	−0.77 **
VLDL mean particle size (nm)	−0.88 **	**NA**	−0.19 **	0.41 **	0.07	0	0.26 **	0.17 **	0.08 *	0.35 **	0.28 **
Small LDL particle concentration (mg/dl)	0.53 **	−0.12 **	**NA**	−0.94 **	−0.43 **	0.45 **	−0.30 **	0.12 **	−0.17 *	0.12 **	−0.31 **
LDL mean particle size (nm)	−0.61 **	0.25 **	−0.53 **	**NA**	0.64 **	−0.66 **	0.47 **	0.04	0.37 **	0.54 **	0.47 **
Large HDL particle concentration (mg/dl)	−0.44 **	0.23 **	−0.71 **	0.75 **	**NA**	0.72 **	0.42 **	−0.09 *	0.79 **	−0.22 **	0.45 **
HDL mean particle size (nm)	0.49 **	−0.39 **	0.60 **	−0.63 **	0.47 **	**NA**	−0.42 **	0.32 **	−0.6 **	0.37 **	−0.47 **
Triglyceridecon centration (mg/dl)	−0.63 **	0.46 **	−0.38 **	0.72 **	0.33 **	−0.53 **	**NA**	−0.28 **	0.32 **	−0.26 **	0.99 **
Total cholesterol	0.40 **	0.17 **	0.13 **	−0.15 **	0.04	−0.11 **	−0.43 **	**NA**	0.20 **	0.94 **	−0.30 **
HDL cholesterol	−0.44 **	0.12 **	−0.53 **	0.68 **	0.85 **	−0.60 **	0.23 **	0.20 **	**NA**	1 **	0.30 **
LDL cholesterol	0.38 **	0.33 **	0.17 **	0.46 **	−0.003	−0.06	−0.18 **	0.91 **	0.73 **	**NA**	−0.22 **
VLDL cholesterol	−0.60 **	0.41 **	−0.39 **	0.71 **	0.30 **	−0.44 **	0.99 **	−0.46 **	0.22 **	−0.22 **	**NA**

The numbers shown above the diagonal (in bold) represent the environment correlation whereas numbers below the diagonal represent the genetic correlations.
*, *P*value between 0.01 and 0.05.
**, *P*-value < 0.01.

TABLE 4. Genetic and environmental correlation in the diabetic only samples

Traits	Large VLDL Particle Concentration (mg/dl)	VLDL Mean Particle Size (nm)	Small LDL Particle Concentration (mg/dl)	LDL Mean Particle Size (nm)	Large HDL Particle Concentration (mg/dl)	HDL Mean Particle Size (nm)	Triglyceride Concentration (mg/dl)	Total Cholesterol	HDL Cholesterol	LDL Cholesterol	VLDL Cholesterol
Large VLDL particle concentration (mg/dl)	**NA**	−0.81 **	−0.31 **	−0.12 *	−0.15 **	0.11 *	−0.67 **	−0.53 **	−0.24 **	−0.57 **	−0.68 **
VLDL mean particle size (nm)	−0.92 **	**NA**	0.004	0.35 **	0.03	0.04	0.27 **	0.11 **	0.32 **	0.72 **	0.26 **
Small LDL particle concentration (mg/dl)	0.85 **	−0.41 **	**NA**	−0.31 **	−0.31 **	0.58 **	0.03	0.03	0.01	0.11 *	−0.002
LDL mean particle size (nm)	−0.86 **	0.52 **	−0.94 **	**NA**	0.53 **	−0.54 **	0.18 **	0.28 **	0.17 **	0.10 *	0.23 **
Large HDL particle concentration (mg/dl)	−0.64 **	0.38 **	−0.92 **	0.87 **	**NA**	−0.87 **	0.27 **	0.03	0.79 **	−0.20 **	0.28 **
HDL mean particle size (nm)	0.64 **	−0.50 **	0.47 **	−0.71 **	−0.88 **	**NA**	−0.28 **	0.25 **	−0.67 **	0.43 **	−0.32 **
TG concentration (mg/dl)	−0.67 **	0.44 **	−0.68 **	0.93 **	0.68 **	−0.70 **	**NA**	−0.23 **	0.39 **	−0.22 **	0.99 **
Total cholesterol	0.58 **	0.42 **	0.24 **	−0.32 **	−0.19 **	0.03	−0.43 **	**NA**	0.09 *	0.97 **	−0.28 **
HDL cholesterol	−0.36 **	−0.05	−0.63 **	0.73 **	0.85 **	−0.55 **	0.23 **	0.29 **	**NA**	−0.005	0.37 **
LDL cholesterol	0.50 **	−0.73 **	0.22 **	−0.19 **	−0.06	−0.07	−0.24 **	0.90 **	−0.008	**NA**	−0.18 **
VLDL cholesterol	−0.67 **	0.45 **	−0.69 **	0.92 **	0.72 **	−0.67 **	0.99 **	−0.44 **	0.22 **	−0.26 **	**NA**

The numbers shown above the diagonal (bold) represent the environment correlation, whereas numbers below the diagonal represent the genetic correlations.
*, *P*-value between 0.01 and 0.05.
**, *P*-value less than 0.01.

TABLE 5. Maximum LOD score obtained with single point linkage analysis of each trait using the combined sample

	Large HDL Particle Concentration (mg/dl)		HDL Mean Particle Size (nm)		Small LDL Particle Concentration (mg/dl)		LDL Mean Particle Size (nm)		TG Concentration (mg/dl)		Large VLDL Particle Concentration (mg/dl)		VLDL Mean Particle Size (nm)	
Chromosome	position	LOD	position	LOD	position	LOD	position	LOD	position	LOD	position	LOD	position	LOD
1	68	0.7	73	0.2	55	0.8	59	0.5	63	0.3	88	1.3	220	0.9
2	117	0.2	259	0.4	261	0.4	261	0.2	262	0.7	260	0.8	2	0.4
3	168	1.1	167	1.5	85	0.8	85	0.5	43	0.4	35	1.7	132	0.7
4	104	0.3	30	0.8	48	0.7	35	0.4	81	0.3	30	0.6	3	1.1
5	174	0.6	174	0.1	31	0.6	126	0.5	125	0.8	64	1.7	67	0.5
6	38	0.6	39	0.8	23	**3.0**	18	0.2	139	0.8	89	0.5	83	0.6
7	3.9	0.3	126	1.2	43	1.3	39	0.7	39	0.3	167	1.2	180	0.7
8	16	0.4	17	0.8	7.9	0.5	7.9	1.1	24	0.4	106	1.3	125	1.3
9	35	0.3	67	0.9	35	1.2	47	1.7	44	1.5	60	1.0	69	0.2
10	104	0.7	108	0.8	146	1.3	107	0.3	97	0.6	59	**2.2**	21	1.0
11	81	0.3	83	0.4	154	0.4	154	0.2	120	0.0	45	0.5	87	0.5
12	154	0.1	67	0.0	127	1.6	59	0.9	19	1.2	32	1.2	36	0.9
13	55	0.5	29	0.4	55	0.0	106	0.9	91	0.3	128	0.3	26	0.1
14	30	0.2	2	0.0	107	0.2	105	0.1	26	0.3	49	0.2	26	0.5
15	114	0.1	122	0.4	120	0.7	130	0.3	37	0.2	129	0.7	47	0.5
16	101	0.0	57	0.1	130	0.7	65	0.2	4	0.4	63	0.8	82	0.8
17	120	0.1	122	0.1	69	0.3	89	0.3	116	0.4	95	0.2	97	0.2
18	31	0.8	95	0.2	42	1.7	43	0.2	43	0.1	73	1.5	71	1.1
19	64	0.7	59	0.8	51	0.28	56	0.6	49	0.5	53	0.4	49	0.3
20	63	0.5	32	0.7	8.8	1.26	24	0.7	24	0.2	23	0.6	17	0.7
21	7.6	0.1	7.6	0.0	47	0.16	45	0.5	45	0.4	45	0.1	7.6	0.0
22	0	0.0	53	0.1	42	0.21	12	0.1	44	0.5	36	0.3	34	0.2

diabetic-only sample. This LOD score was observed near rs600832 and its LOD-1 interval spans 17 cM starting at rs1342137 and ending at rs755662. The LOD score of 2.2 observed on chromosome 10p11 with the complete sample also increased to 2.4 with the reduced sample.

In summary, four LOD scores > 2.2 were observed in the analysis conducted on the diabetic-only subset compared with three in the combined sample. Three of these LOD scores were identified with the same trait, small LDL particle concentration, on chromosomes 12q24, 16p13, and 20p13. The fourth LOD score > 2.2 was observed on chromosome 10p11 for large VLDL particle concentration, which as described above has a LOD-1 interval that spans about 6 cM starting at rs959629 and ending at rs1441027.

Bivariate linkage analysis

The bivariate analyses support the univariate linkage signal identified on chromosome 6 for small LDL particle concentration. The largest bivariate LOD score (2.3) was observed at 6p24 when analyzed with LDL mean particle size, and the second largest score (2.2) was located at the same position, but it was observed with large VLDL parti-

TABLE 6. Maximum LOD score obtained with single point linkage analysis of each trait using only the diabetic individuals

	Large HDL Particle Concentration (mg/dl)		HDL Mean Particle Size(nm)		Small LDL Particle Concentration (mg/dl)		LDL Mean Particle Size(nm)		TG Concentration (mg/dl)		Large VLDL Particle Concentration (mg/dl)		VLDL Mean Particle Size(nm)	
Chromosome	Position	LOD	Position	LOD	Position	LOD	Position	LOD	Position	LOD	Position	LOD	Position	LOD
1	59	0.4	59	0.3	54	1.4	69	0.5	53	0.8	260	0.8	131	0.4
2	101	0.5	102	0.9	100	0.8	57	0.3	55	0.8	253	0.5	180	0.3
3	168	0.8	167	1.6	85	0.3	160	0.1	167	0.7	31	1.5	39	0.5
4	30	0.6	30	1.1	42	0.6	18	0.4	16	0.6	3	1.2	128	0.6
5	174	0.4	174	0.3	26	0.4	150	0.2	124	0.3	67	0.6	67	0.2
6	28	0.6	22	0.9	23	1.7	103	0.4	7.9	0.6	89	1.2	93	0.5
7	3.9	0.4	126	1.4	38	1.2	12	0.1	39	0.8	35	0.9	187	0.6
8	16	0.3	16	0.5	9.9	0.2	142	0.4	0	0.6	128	1.5	124	0.8
9	104	0.2	104	0.7	35	0.7	47	0.5	84	1.0	65	1.1	120	0.3
10	104	1.2	108	0.9	101	1.7	34	0.3	108	1.0	60	**2.4**	34	0.8
11	79	0.3	81	0.4	85	0.2	128	0.1	69	0.4	120	1.0	73	0.3
12	154	0.2	154	0.1	155	**2.2**	39	0.6	19	0.8	32	1.4	24	0.5
13	55	0.2	29	0.3	55	0.3	95	0.6	91	0.4	26	0.2	110	0.4
14	28	0.2	2	0.0	28	0.1	16	0.1	66	1.0	26	0.1	26	0.3
15	112	0.3	120	0.3	97	0.5	65	0.0	38	0.8	17	1.3	47	0.4
16	55	0.1	8.9	0.6	6	**2.3**	83	0.1	127	0.6	87	0.7	86	0.7
17	30	0.1	59	0.3	66	0.8	87	0.1	125	0.9	101	0.2	97	0.4
18	93	0.6	93	0.6	42	1.3	12	0.1	99	0.2	73	1.8	72	0.8
19	63	0.7	63	1.0	2	0.4	73	0.4	51	0.4	106	0.2	49	0.3
20	31	0.8	35	1.6	8.8	**2.7**	5.9	0.4	30	1.1	16	2.0	16	0.8
21	7.6	0.2	7.6	0.0	5.7	0.1	35	0.0	5.7	0.2	5.7	0.3	35	0.1
22	0	0.0	0	0.1	75	0.0	9.9	0.2	34	0.3	30	0.1	30	0.1

cle concentration. LOD scores between 1.8 and 1.9 were also observed with large VLDL particle concentration and TG at 4p16 (LOD = 1.9), 1 cM on chromosome 14p13 (LOD = 1.8), and at 10p12 (LOD = 1.81).

The highest linkage signal (LOD = 2.02) observed after the sample was reduced to diabetics only was on chromosome 20p13 for the bivariate effect of VLDL mean particle size and small LDL particle concentration**Table 7**. This signal was identified with rs600832; its LOD-1 interval, which spans 17 cM, starts with rs1342137 and ends with rs755662. Suggestive evidence for bivariate linkage can be found on chromosome 8p23 (LOD = 1.8) and on chromosome 11q24 (LOD = 1.7) for TG and large HDL particle concentration. Bivariate linkage signals $\geqslant$ 2.0 are summarized in **Table 8**. We note that the choice of 2.0 is arbitrary, as Kruglyak and Lander's (48,50) recommendation will not apply for the bivariate linkage analysis, because the distribution of the LOD scores under the null hypothesis is different than that of the univariate LOD score.

DISCUSSION

African-Americans from the Sea Islands of South Carolina remain a relatively homogenous population with limited European ancestral genetic admixture and have managed to conserve a relatively substantial portion of their African heritage and culture. Project SuGAR was designed to recruit families with T2DM for genetic studies. Sale et al. identified (52) genomic regions showing evidence of linkage with T2DM in this population. The principal aim of our analysis was to evaluate the relative influence of genes and environment on mean size of VLDL, LDL, and HDL lipoprotein classes, as well as particle concentrations for lipoprotein subclasses known to have significant effects on various cardiovascular outcomes (52, 53).

All the lipid traits considered in this analysis display evidence of familial aggregation. Large VLDL particle concentration and small dense LDL particle concentration have the two strongest heritability estimates, 0.56 and 0.54, respectively. However, even the lowest heritability values were close to 0.30, suggesting a substantial genetic contribution to small particle size and concentration for all lipid traits. Although these lipoprotein traits have not been extensively studied, the heritability estimate of the total LDL particle concentration is in line with previously published estimates (15, 54). As expected, the genetic correlation between LDL particle size and particle concentration is high. Previous reports have found an overall correlation of -0.80 between these traits (55), but partitioning between genetic (-0.94) and environmental (-0.53) correlations suggests a stronger genetic contribution, possibly through a gene with a pleiotropic effect on both traits. The strong correlation between VLDL particle size and large VLDL particle concentration (-0.88) is similarly predictable. The genetic correlation between small LDL particle concentration and TG level is estimated at 0.72. The correlation between these two traits has been observed even in nonobese, normolipidemic individuals (15, 56) and underscores the fact that the dyslipidemia associated with insulin-resistant states is routinely characterized by high TG and an increase in small dense LDL particles.

The largest LOD score observed was equal to 3.0, which is considered the threshold that must be reached before any linkage finding can be considered statistically significant. However, we think that LOD scores $\geqslant$ 2.2, as suggested by many before us, are suggestive of genomic regions that may harbor genes that influence these traits.

Single locus linkage analyses reveal various genomic regions that might host possible genes that affect these traits. The strongest signals are observed on chromosome 6p24 for small LDL particle concentration, and on chromosome 10p11 for large VLDL particle concentration (Table 5). We note that these two traits also had the highest heritability estimates (Table 2). One promising candidate under the 6p small LDL particle concentration signal is the gene for elongation of very long chain fatty acids 2, an elongase of PUFA (57). There do not appear to be any obvious known candidates under the chromosome 10 signal for large VLDL particle concentration. We also found suggestive evidence of linkage in other regions for these two traits and others (Table 5). Interestingly, the 9p LOD-1 intervals for the modest LDL mean particle size and TG concentration signals overlap with a linkage signal for TG in African-Americans (58) and encompass a region previously associated with CAD and T2DM (59). There do not appear to be any other overlaps with prior linkage studies in African-derived populations of standard lipid panel traits (60–64) or lipoprotein subclasses determined using polyacrylamide gel electrophoresis (29). However, evidence of linkage in this region was previously found in four large multi-generational pedigrees of European descent (65).

The only other study to date of heritability and linkage of lipid subclasses has been conducted in a German population (66). This study found a similar range of heritability estimates but showed no apparent overlap of linkage with our results. However, we did observe a LOD score of 1.6

TABLE 7. Summary of LOD scores greater than 2.2 for the single locus analysis

	Combined Sample			Diabetics Only		
Trait	Position	LOD	SNP	Position	LOD	SNP
Small LDL particle concentration (mg/dl)	6p24	3.0	rs1328132			
				12q24	2.2	rs6489226
				16p13	2.3	rs757601
				20p13	2.7	rs600832
Large VLDL particle concentration (mg/dl)	10p11	2.2	rs867992	10p11	2.4	rs727345

TABLE 8. Summary of observed bivariate LOD scores by analysis

Bivariate Combination	Position	LOD	SNP	Sample
TG × large HDL particle concentration	8q22	1.8	rs714046	Diabetics only
	11q23	1.7	rs665035	Diabetics only
	8q22	1.9	rs2453628	Combined sample
	10p14	1.8	rs2439903	Combined sample
	14p11	1.8	rs944398	Combined sample
Small LDL particle concentration × HDL mean particle size	1p33	1.8	rs1015099	Diabetics only
	20p13	1.9	rs6047134	Diabetics only
Small LDL particle concentration × LDL mean particle size	6p24	2.3	rs1328132	Combined sample
Small LDL particle concentration × large VLDL particle concentration	6p24	2.2	rs1328132	Combined sample
Small LDL particle concentration × VLDL mean particle size	20p13	2.0	rs600832	Diabetics only
TG × HDL mean particle size	20q11	1.9	rs803880	Diabetics only
Small LDL particle concentration × large HDL particle concentration	20p13	1.8	rs600832	Diabetics only
TG × small LDL particle concentration	16p13	1.8	rs757601	Diabetics only
	20p13	2.0	rs6038727	Diabetics only
Small LDL particle concentration × large HDL particle concentration	20p13	1.8	rs600832	Diabetics only
TG × LDL mean particle size	20q11	1.7	rs2225471	Diabetics only

with small LDL particle concentration on 12q24, which is near the location where they estimated a LOD score of 2.9 with HDL particle size. However, their strongest result was a LOD score of 3.3 observed on chromosome 18 at 33 cM with HDL particle concentration. The maximum LOD score that we detected on this chromosome with this trait in both analyses (combined and diabetic only) is 0.8. However, we observed LOD scores of 1.7 at 42 cM with small LDL particle concentration in the combined sample and 1.8 at 73 cM with large VLDL particle concentration in the diabetic only sample on the same chromosome.

There have been a number of recent genome-wide association studies (GWAS) for lipid and lipoprotein traits that comprise the standard clinical lipid panel of LDL cholesterol, HDL cholesterol, and/or TG (67–71). These studies have confirmed prior genomic regions of association and identified at least 6 novel loci (72). There does not appear to be any overlap between associated regions and the corresponding lipoprotein subclass linkage signals from our study in Gullah-speaking African-Americans. There are a number of possible explanations. The cohorts investigated in reported lipid GWAS have been predominantly European, and European GWAS signals for other traits, such as T2DM (73), have failed to translate to African-American populations. Additionally, the lipid profile in African-Americans differs significantly from European-Americans (74), suggesting the possibility of differential genetic contributors. Lastly, the reported GWAS have examined conventional lipid panel measures, which may not reflect lipid subfractions measured using NMR (37). In fact, insulin resistance is known to affect small dense LDL particle concentration and mean LDL size independent of LDL cholesterol levels (11). This is consistent with our observation of linkage for small LDL particle concentration at a locus 6p23 that has not yielded evidence for linkage or association in previous studies.

One limitation of this study is that families were ascertained on the basis of T2DM, although there is no reason to suspect heritabilities will be substantially different in nondiabetic families. This study has several strengths, including the use of fasting lipid subclass measures and the relatively low rates of lipid-lowering medication use (11%). For example, the percentage of participants on lipid-lowering therapy in the study of Kaess et al. (66) was 62.8% in index cases and 39.9% in affected sibs.

Several studies have shown that mean LDL particle size and concentration are more reliable predictors of cardiovascular outcomes than overall LDL cholesterol (66, 75–78). Recent evidence also suggests that overproduction of large VLDL particles, associated with higher levels of small dense LDL and lower levels of HDL cholesterol, precede a diagnosis of T2DM (79). To our knowledge, this is only the second study to explore the heritability and linkage of lipoprotein subclasses measured using NMR spectroscopy. However, it is the first to conduct these investigations in an African-American population, to use fasting lipid values, to use genotype data from the more informative SNP linkage panel, and to report partitioning of correlations between genetic and environmental contributions. Also, while Kaess et al. (66) found significant evidence for linkage to HDL particle size and concentration, our results have revealed novel suggestive loci for small LDL and large VLDL particle concentrations. Our findings suggest that there is a significant genetic contribution to the majority of the lipoprotein subclasses studied and that loci on chromosomes 6 and 10 may harbor genes contributing to atherogenic, small dense LDL particles and TG-rich large VLDL particles, respectively.

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