Supplemental Material can be found at:
http://www.jlr.org/content/suppl/2006/06/23/M600078-JLR200.DC1.html

Quantitative trait loci influencing low density lipoprotein particle size in African Americans

Iftikhar J. Kullo,[1],* Keyue Ding,* Eric Boerwinkle,† Stephen T. Turner,§ and Mariza de Andrade**

Divisions of Cardiovascular Diseases,* Nephrology and Hypertension,§ and Biostatistics,** Mayo Clinic and Foundation, Rochester, MN 55905; and Human Genetics Center and Institute of Molecular Medicine,† University of Texas-Houston Health Science Center, Houston, TX 77030

Abstract **Genomic regions that influence LDL particle size in African Americans are not known. We performed family-based linkage analyses to identify genomic regions that influence LDL particle size and also exert pleiotropic effects on two closely related lipid traits, high density lipoprotein cholesterol (HDL-C) and triglycerides, in African Americans. Subjects (n = 1,318, 63.0 ± 9.5 years, 70% women, 79% hypertensive) were ascertained through sibships with two or more individuals diagnosed with essential hypertension before age 60. LDL particle size was measured by polyacrylamide gel electrophoresis, and triglyceride levels were log-transformed to reduce skewness. Genotypes were measured at 366 microsatellite marker loci distributed across the 22 autosomes. Univariate and bivariate linkage analyses were performed using a variance components approach. LDL particle size was highly heritable ($h^2 = 0.78$) and significantly ($P < 0.0001$) genetically correlated with HDL-C ($\rho_G = 0.32$) and log triglycerides ($\rho_G = -0.43$). Significant evidence of linkage for LDL particle size was present on chromosome 19 [85.3 centimorgan (cM), log of the odds (LOD) = 3.07, $P = 0.0001$], and suggestive evidence of linkage was present on chromosome 12 (90.8 cM, LOD = 2.02, $P = 0.0011$). Bivariate linkage analyses revealed tentative evidence for a region with pleiotropic effects on LDL particle size and HDL-C on chromosome 4 (52.9 cM, LOD = 2.06, $P = 0.0069$). These genomic regions may contain genes that influence interindividual variation in LDL particle size and potentially coronary heart disease susceptibility in African Americans.**—Kullo, I. J., K. Ding, E. Boerwinkle, S. T. Turner, and M. de Andrade. **Quantitative trait loci influencing low density lipoprotein particle size in African Americans.** ***J. Lipid Res.*** **2006.** 47: **1457–1462.**

Supplementary key words bivariate • genetic linkage • high density lipoprotein cholesterol • triglycerides

Low-density lipoprotein particles vary in size, and smaller LDL particles are considered to be more atherogenic than larger particles (1–4). Together with decreased high density lipoprotein cholesterol (HDL-C) and increased triglyceride levels, small LDL particle size constitutes the syndrome of atherogenic dyslipidemia, which is associated with a significant increase in the risk for coronary heart disease (5, 6). Several studies have investigated the genetic basis of LDL particle size [reviewed by Bosse, Perusse, and Vohl (7)]. Most of the studies have been limited to non-Hispanic whites, and genomic regions that influence interindividual variation in LDL particle size in African Americans are unknown. African Americans have lower levels of triglycerides and higher levels of HDL-C than do non-Hispanic whites (8), and Haffner et al. (9) reported that LDL particle size may also vary with ethnicity. Thus, the genetic determinants of LDL particle size in African Americans cannot be extrapolated from studies in other ethnic groups and will need further delineation.

Because of the well-recognized correlation between LDL particle size, HDL-C, and triglycerides, it is likely that genes with pleiotropic effects on these lipid traits exist. We (10) and others (11) have demonstrated that shared genetic effects (pleiotropy) account for a significant proportion of the phenotypic correlations between LDL particle size, HDL-C, and triglycerides. Therefore, in addition to univariate linkage analyses to identify genetic regions that influence LDL particle size, we performed bivariate linkage analyses in African American sibships to identify additional regions that may exert pleiotropic effects on pairwise combinations of LDL particle size, HDL-C, and triglycerides. Such studies have the potential to yield new insights into the genetic basis of the interindividual variation of LDL particle size and the susceptibility to coronary heart disease in African Americans (12).

METHODS

Sample

Subjects included African Americans from Jackson, Mississippi, participating in the Genetic Epidemiology Network of Arteriopathy (GENOA) study, a multicenter, community-based study of hypertensive sibships that aims to identify genes

Manuscript received 14 February 2006 and in revised form 13 April 2006.
Published, JLR Papers in Press, April 19, 2006.
DOI 10.1194/jlr.M600078-JLR200

---

[1] To whom correspondence should be addressed.
e-mail: kullo.iftikhar@mayo.edu

influencing blood pressure levels and the development of target organ damage as a result of hypertension (13). Recruitment into the initial phase of the GENOA study (September 1995 to June 2001) has been described previously (14). Between December 2000 and October 2004, 1,350 of the original GENOA-Jackson participants returned for a second study visit to undergo measurement of risk factors and traits, including LDL particle size and assessment of target organ damage resulting from hypertension. Genotypic (microsatellite markers) and phenotypic (LDL particle size) data were available for 1,318 Jackson participants in phase II. The study was approved by the Institutional Review Board of the University of Mississippi (Jackson). Written informed consent was obtained from each participant.

Height was measured by stadiometer, weight by electronic balance, and body mass index (BMI) was calculated as kg/m^2. Resting systolic and diastolic blood pressure levels were measured in the right arm with a random-zero sphygmomanometer (Hawksley and Sons, West Sussex, UK). Participants were considered diabetic if they reported using insulin or oral hypoglycemic agents or if they reported a physician diagnosis of diabetes but were not currently taking a pharmacological agent to control their high glucose levels. Information about the use of lipid-lowering medications was obtained from participants at the time of the study.

Measurement of lipid variables

Blood samples were obtained by venipuncture after an overnight fast. Standard enzymatic methods were used to measure total cholesterol, HDL-C, and triglycerides (15). LDL particle size was measured by polyacrylamide gel electrophoresis as described previously (16, 17). The mean LDL particle size was then determined by averaging weighted diameters for each fraction. Two controls (mean particle diameters of 264 and 254 Å) were analyzed with every 10 study samples. Interassay coefficients of variation were 0.77% (SD = 2.0 Å) and 1.46% (SD = 3.7 Å), respectively.

Genotyping

DNA was extracted from 10 ml of EDTA-anticoagulated blood drawn from all study participants according to standard procedures. Microsatellite markers (CHLC/Weber screening set 9.0, n = 366) were genotyped using standard PCR-based methods by the Mammalian Genotyping Center of the Marshfield Medical Research Foundation. Marker order and genetic map distances were those provided by the Marshfield Medical Research Foundation (research.marshfieldclinic.org/genetics/). Inconsistencies of the genotypes with pedigree structure were identified by the Lange and Goradia algorithm (18) as implemented in the PedCheck software (19). Instances that could not be resolved as genotyping errors were considered as missing data.

Statistical analyses

Before genetic linkage analyses, we assessed whether the assumption of normality in the distribution of each lipid trait was violated (20). Triglyceride levels were positively skewed and were therefore log-transformed. LDL particle size, HDL-C, and log triglycerides were adjusted for age, sex, BMI, diabetes, history of smoking, and statin use in the genetic analyses. Genetic and environmental correlations between adjusted lipid traits were estimated by variance decomposition using maximum likelihood methods (21), and the phenotypic correlations between traits were calculated based on genetic and environmental correlations (22). The heritability (h^2) of each trait was calculated as the proportion of the total phenotypic variance attributable to additive genetic effects.

Univariate and bivariate linkage analyses were performed using the S-Plus library Multic, which employs a multivariate variance components approach that is an extension of the univariate approach described by Amos (23) and de Andrade et al. (24). Multipoint identity-by-descent sharing among pairs of relatives was calculated using the SIMWALK2 software program (25). This program estimates multipoint identity-by-descent probabilities using an alternative Markov chain Monte Carlo algorithm to efficiently accommodate large sibships and provides estimates in agreement with deterministic methods.

To test for genetic linkage, a likelihood ratio test (LRT) was used in which the LRT is defined as $-2\times$[(log likelihood under the null hypothesis) $-$ (log likelihood under the alternative hypothesis)]. Under the null hypothesis, the linked gene parameter(s) are restricted to equal zero ($\sigma_{g.ij}$ are zero for all i,j). For the bivariate linkage analysis, the distribution of the bivariate test that the linked-gene components and covariance are zero is a mixture of $\frac{1}{4}\chi^2_0$, $\frac{1}{2}\chi^2_1$, and $\frac{1}{4}\chi^2_3$, as described by Self and Liang (26).

All log of the odds (LOD) scores for the multipoint linkage analyses were calculated from the LRT values as LRT/(2 × *ln*[10]). For the univariate linkage analyses of LDL particle size, HDL-C, and log triglycerides, we considered multipoint LOD scores of $\geqslant$3.00 as statistically significant evidence of linkage, scores of $\geqslant$2.00 as suggestive evidence of linkage, and scores of $\geqslant$1.30 as tentative evidence of linkage (27). These multipoint LOD score thresholds correspond to genome-wide *P* values of $\leqslant$0.0001, $\leqslant$0.001, and $\leqslant$0.007, respectively (27). Bivariate linkage analyses were performed for each pairwise combination of the traits. To achieve levels of statistical significance comparable to the univariate linkage thresholds, we considered bivariate LOD scores of $\geqslant$4.00 as statistically significant evidence of linkage, scores of $\geqslant$2.87 as suggestive evidence of linkage, and scores of $\geqslant$2.06 as tentative evidence of linkage. These higher bivariate thresholds were calculated using asymptotic values of a mixture of $\frac{1}{4}\chi^2_0$, $\frac{1}{2}\chi^2_1$, and $\frac{1}{4}\chi^2_3$ distribution. We considered a region to have possible pleiotropic effects if the bivariate LOD score was at least 2.06 and the *P* value was less than either of the univariate maxima.

RESULTS

The study sample of 1,318 subjects belonged to 674 sibships and included 1,133 sibpairs; there were 312 sibships of size 1, 201 sibships of size 2, and 161 sibships of size 3 or greater. Seventy percent of the subjects were women, 71% were hypertensive, and 18.3% were on lipid-lowering medications (**Table 1**).

TABLE 1. Descriptive characteristics of the subjects (n = 1,318)

Characteristic	Mean ± SD or (%)	Range
Age (years)	63.0 ± 9.5	26.4–91.6
Women (%)	927 (70.3)	
BMI (kg/m^{-2})	31.5 ± 6.6	16.4–60.3
Hypertension	1040 (79.0)	
History of smoking (%)	534 (40.5)	
LDL particle size (Å)	268.8 ± 4.9	251–285
HDL-C (mg/dl)	57.4 ± 18.0	21.7–175.7
Triglycerides (mg/dl)	119.0 ± 69.1	28.5–813.5
Log triglycerides	4.66 ± 0.46	3.35–6.70
Statin use	241 (18.3)	

BMI, body mass index; HDL-C, high density lipoprotein cholesterol. Values shown are means ± SD for quantitative traits or percentages for categorical traits.

TABLE 2. Heritabilities (on the diagonal), phenotypic correlations (above the diagonal), and genetic correlations (below the diagonal) of the three lipid traits

Trait	LDL Particle Size	HDL-C	Log Triglycerides
LDL particle size	**0.78**	0.23	−0.46
HDL-C	0.32	**0.65**	−0.39
Log triglycerides	−0.43	−0.57	**0.40**

Traits were adjusted for age, sex, body mass index, diabetes, smoking status, and statin use. The estimates of heritability and genetic correlations were significant ($P < 0.0001$).

Heritability and genetic correlations

LRT results indicated significant heritability ($P < 0.001$) for each of the three lipid traits: LDL particle size, HDL-C, and triglycerides (**Table 2**). After adjustment for age, sex, BMI, diabetes, history of smoking, and statin use, more than three-quarters of the residual variance in LDL particle size, more than half of the residual variance in HDL-C, and more than one-third of the residual variance in log triglycerides was attributable to additive genetic effects (Table 2).

Genetic correlations between the three lipid traits were significantly different from zero ($P < 0.0001$) based on LRT results (Table 2). The genetic correlations between LDL particle size and log triglycerides ($\rho_G = -0.43$) and between HDL-C and log triglycerides ($\rho_G = -0.57$) were negative, whereas the genetic correlation between LDL particle size and HDL-C was positive ($\rho_G = 0.32$).

Linkage analyses

In univariate linkage analyses, there was suggestive evidence of linkage for LDL particle size near D12S1064 on chromosome 12q21 [90.8 centimorgan (cM), LOD = 2.02, $P = 0.0011$] (**Table 3**, **Fig. 1A**). Significant evidence of linkage for LDL particle size was observed near D19S589 on chromosome 19q13 (85.3 cM, LOD = 3.07, $P = 0.0001$) (Table 3, Fig. 1B). Univariate linkage analyses of HDL-C demonstrated tentative evidence of linkage on chromosome 7q35 (159.1 cM, LOD = 1.32, $P = 0.0068$) and 10q21 (81.5 cM, LOD = 1.48, $P = 0.0046$). For log triglycerides, there was tentative evidence of linkage on chromosome 8q21 (104.3 cM, LOD = 1.64, $P = 0.0030$) (Table 3).

Bivariate linkage analyses identified a region near D4S2632 on chromosome 4p15 that may have pleiotropic effects on LDL particle size and HDL-C (52.9 cM, bivariate LOD = 2.06, $P = 0.0069$) (Table 3, **Fig. 2A, B**). There was suggestive evidence of pleiotropic effects on the pairwise combination of HDL-C and log triglycerides on two chromosomes: chromosome 4q13 (75.9 cM, bivariate LOD = 3.17, $P = 0.0006$) (Fig. 2A, B) and chromosome 16 (91.0 cM, bivariate LOD = 2.29, $P = 0.0042$) (Fig. 2C, D).

DISCUSSION

This study is the first to report heritability and linkage analyses for LDL particle size in African Americans. Numerous analyses have been performed to assess the heritability of LDL particle size in either twin (28, 29) or family studies (30–33). These studies, comprising mostly non-Hispanic whites or Hispanics, found that 30–60% of the variance in LDL particle size was attributable to genetic factors. We found LDL particle size to be significantly heritable in African Americans: 78% of the variance in LDL particle size could be attributed to additive genetic effects.

Although linkage scans for genetic loci affecting LDL particle size have been performed in several previous studies (**Table 4**), none of these included African Americans. In this study of African American sibships ascertained on the basis of hypertension, we found significant evidence of linkage (LOD = 3.07) of chromosome 19q13 (LOD-1 interval: 73.3–96.7 cM) (Fig. 1B). Allayee et al. (34) found tentative evidence for linkage for peak LDL particle size in this region of chromosome 19q13 (78.1 cM, LOD = 1.6) in Dutch Caucasian families. These results

TABLE 3. Maximum multipoint LOD scores (and positions in cM) and P values for the univariate and bivariate linkage analyses LDL particle size, HDL-C, and log triglycerides

	Univariate Linkage Analyses			Bivariate Linkage Analyses		
Chromosome	LDL Size	HDL-C	Log Triglycerides	LDL Size and HDL-C	LDL Size and Log Triglycerides	HDL-C and Log Triglycerides
4	0.52 (176.10)	0.68 (65.93)	0.25 (167.55)	**2.06 (52.94)**	0.72 (206.98)	**3.17 (75.90)**
	0.0608	0.0383	0.1399	**0.0069**	0.1237	**0.0006**
7	0.97 (64.52)	**1.32 (159.06)**	1.16 (121.54)	1.18 (155.10)	1.33 (122.39)	1.55 (120.70)
	0.0171	**0.0068**	0.0104	0.0455	0.0334	0.0206
8	0.059 (94.08)	1.12 (158.37)	**1.64 (104.26)**	0.85 (161.86)	1.33 (102.56)	1.87 (109.34)
	0.3015	0.0116	**0.003**	0.091	0.0326	0.0105
10	0.48 (28.31)	**1.48 (81.53)**	0.16 (59.03)	1.26 (13.82)	0.48 (120.97)	1.61 (82.30)
	0.0696	**0.0046**	0.1936	0.0388	0.2064	0.0182
12	**2.02 (90.80)**	0.62 (48.70)	0.64 (19.68)	2.07 (90.80)	2.09 (89.11)	1.10 (51.22)
	0.0011	0.0457	0.0434	0.0068	0.0064	0.0541
16	0.06 (100.39)	1.15 (93.56)	0.15 (10.36)	0.82 (95.28)	0.09 (10.36)	**2.29 (91.02)**
	0.2974	0.0107	0.2011	0.0986	0.4883	**0.0042**
19	**3.07 (85.26)**	0.06 (23.96)	0.21 (9.84)	2.97 (85.26)	3.06 (84.46)	0.29 (9.84)
	0.0001	0.2942	0.1639	0.001	0.0008	0.3026

cM, centimorgan; LOD, log of the odds. Variables were adjusted for age, sex, BMI, diabetes, smoking, and statin use. Positions are distances from p terminus in cM. The following LOD scores are highlighted in boldface: ⩾1.3 in univariate linkage analyses, and ⩾2.06 in bivariate linkage analyses with the P value lower than for either of the univariate maxima. Data from chromosomes without any LOD scores of ⩾1.3 in univariate or ⩾2.06 in bivariate linkage analyses are not shown.

Supplemental Material can be found at:
http://www.jlr.org/content/suppl/2006/06/23/M600078-JLR200.DC1.html

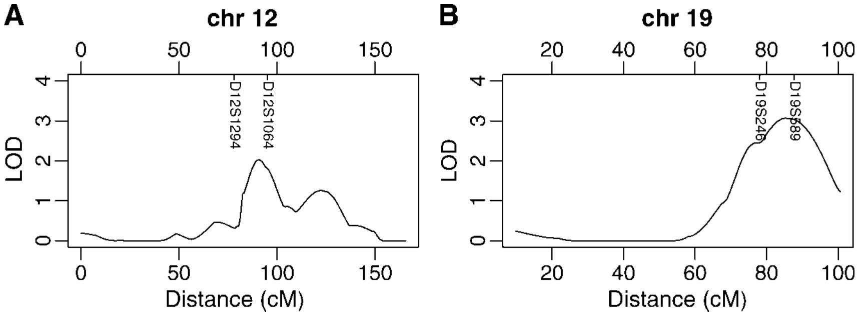

Fig. 1. Results of univariate multipoint variance component linkage analyses for LDL particle size on the chromosome 12 (A) and chromosome 19 (B). Markers adjacent to the log of the odds-1 (LOD-1) interval are shown along the top of each plot. cM, centimorgan.

suggest the presence of a quantitative trait locus on chromosome 19q13 that influences LDL particle size. The linkage peak for LDL particle size on chromosome 12q21 (LOD = 2.02) (Fig. 1A) appears to be novel and has not been reported previously. Univariate linkage analyses for HDL-C and log triglycerides yielded only weak linkage signals (LOD < 2.0).

Bivariate linkage analyses may provide greater statistical power to identify genetic loci with effects too small to be detected in single-trait analyses (35). Using such an approach, we previously found a locus with pleiotropic effects on LDL particle size and HDL-C on chromosome 1 (LOD = 4.48, 4 cM) in non-Hispanic whites participating in the GENOA study (17). In the present study, bivariate analysis of pairwise combinations of the three lipid traits revealed three genomic loci that may have pleiotropic effects on these traits (Fig. 2B, D). In the loci identified, the univariate LOD scores were not suggestive of linkage to any of the individual traits, but the bivariate analyses yielded a higher LOD score with a lower *P* value than for any of the univariate maxima. Given that common metabolic pathways influence these traits, a gene with pleiotropic effects on the traits, rather than tightly linked genes, is likely to be responsible for the linkage signal of the bivariate phenotype.

An overlap of the bivariate LDL particle size-HDL-C and HDL-C-log triglyceride linkage signals was noted in the chromosome 4q13 region, raising the possibility that the region may have a pleiotropic effect on all three traits. We performed trivariate linkage analyses to assess whether a quantitative trait locus in the region influenced the three lipid traits. A significant increase in the LOD score was not noted, suggesting the presence of two separate loci in-

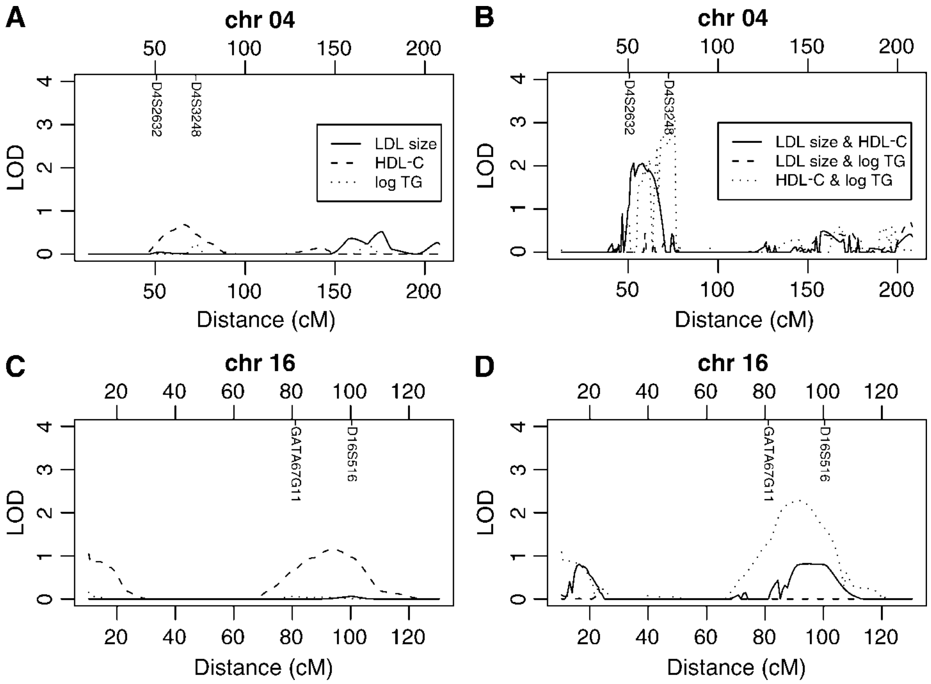

Fig. 2. Results of univariate (A, C) and bivariate (B, D) multipoint variance component linkage analyses for the three lipid traits on chromosome 4 (A, B) and chromosome 16 (C, D). Markers adjacent to the LOD-1 interval are shown along the top of each plot. HDL-C, high density lipoprotein cholesterol; TG, triglycerides.

TABLE 4. Linkage studies for LDL particle size

Reference	Study Sample	Phenotype	Location of Linkage Signal	Peak LOD Score
Rotter et al. (37)	Caucasian coronary heart disease families	LDL peak particle size	19p13	na[a]
			16q21	
			11q23	
			6q25	
Allayee et al. (41)	Dutch Caucasian familial combined hyperlipidemia families	LDL peak particle size	6q25	na
			16q21	
			11q23	
Rainwater et al. (32)	Mexican-American families	Cholesterol content of LDL size subfractions	3q28	4.11
			4q25	4.11
Bosse et al. (42)	French-Canadian families	LDL peak particle size	17q21	6.76
			2q33	2.27
			4p15	2.11
Badzioch et al. (43)	White pedigrees with familial combined hyperlipidemia	LDL peak particle size	9p	3.70
			16q23	3.00
			11q22	3.71
			14q24-31	2.37
Kullo et al. (17)	Non-Hispanic white sibships ascertained on the basis of hypertension	Average LDL particle size	2p	1.92

[a] LOD scores not available.

fluencing the LDL particle size-HDL-C and the HDL-C-log triglyceride traits (analyses not shown).

We attempted to identify potential positional candidate genes for the lipid traits in the LOD-1 regions of the linkage signals (i.e., LOD ⩾ 1.3 in univariate and LOD ⩾ 2.06 in bivariate linkage analyses). Of the genes suggested by previous linkage or association studies to influence LDL particle size, an apolipoprotein gene cluster (*APOE/C1/C4/C2*) was noted to be adjacent to the linkage peak on chromosome 19q13. Two important genes involved in lipid metabolism, *CETP* (cholesteryl ester transfer protein; chromosome 16q21) and *LCAT* (lecithin-cholesterol acyltransferase; chromosome 16q22), were present under the HDL-C-log triglyceride bivariate signal on chromosome 16q22. We did not find any obvious candidate genes under the remaining linkage signals.

We also performed a functional network analysis to identify the interaction of known candidate genes for LDL particle size with genes under the linkage signals, using the Ingenuity® Pathway Analysis tool (www.ingenuity.com) (see supplementary figure). Ingenuity® Pathway Analysis is a knowledge-based discovery tool and the largest curated database of previously published findings on mammalian biology (36). Three genes, *CLEC11A* (C-type lectin domain family 11, member A), *KIT* (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog), and *KITLG* (KIT ligand), under the linkage signals on chromosome 19q13, chromosome 4p15, and chromosome 12q21, respectively, interact with the LDL receptor gene (*LDLR*) shown previously to be a positional candidate gene for LDL particle size (37). In this network, *CLEC11A* increases the phosphorylation of *KIT* (38), there is a protein-protein interaction between *KIT* and *KITLG* (39), and in turn, *KITLG* can increase the expression of *LDLR* (40). These interactions, by affecting *LDLR*, may influence LDL particle size, although this needs confirmation in additional studies.

In conclusion, our findings indicate the presence of a quantitative trait locus on chromosome 19 that influences LDL particle size in African American sibships ascertained based on hypertension. In addition, there is suggestive evidence for a locus on chromosome 12 that influences this trait and a locus on chromosome 4 that may have pleiotropic effects on LDL particle size and HDL-C. Linkage analysis of LDL particle size and correlated lipid traits is a step toward identifying genetic variants that influence coronary heart disease susceptibility in African Americans.

This work was supported by Grants RR-17720 and HL-75794 to I.J.K., grant HL-54457, and the General Clinical Research Center Grant M01 RR-00585 from the National Institutes of Health.

REFERENCES

1. Freedman, D. S., J. D. Otvos, E. J. Jeyarajah, J. J. Barboriak, A. J. Anderson, and J. A. Walker. 1998. Relation of lipoprotein subclasses as measured by proton nuclear magnetic resonance spectroscopy to coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1046–1053.
2. Kuller, L., A. Arnold, R. Tracy, J. Otvos, G. Burke, B. Psaty, D. Siscovick, D. S. Freedman, and R. Kronmal. 2002. Nuclear magnetic resonance spectroscopy of lipoproteins and risk of coronary heart disease in the Cardiovascular Health Study. *Arterioscler. Thromb. Vasc. Biol.* **22:** 1175–1180.
3. Blake, G. J., J. D. Otvos, N. Rifai, and P. M. Ridker. 2002. Low-density lipoprotein particle concentration and size as determined by nuclear magnetic resonance spectroscopy as predictors of cardiovascular disease in women. *Circulation.* **106:** 1930–1937.
4. Carmena, R., P. Duriez, and J. C. Fruchart. 2004. Atherogenic lipoprotein particles in atherosclerosis. *Circulation.* **109:** III2–III7.
5. Austin, M. A., J. D. Brunzell, W. L. Fitch, and R. M. Krauss. 1990. Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. *Arteriosclerosis.* **10:** 520–530.
6. Reaven, G. M., Y. D. Chen, J. Jeppesen, P. Maheux, and R. M. Krauss. 1993. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. *J. Clin. Invest.* **92:** 141–146.
7. Bosse, Y., L. Perusse, and M. C. Vohl. 2004. Genetics of LDL particle heterogeneity: from genetic epidemiology to DNA-based variations. *J. Lipid Res.* **45:** 1008–1026.
8. Jain, T., R. Peshock, D. K. McGuire, D. Willett, Z. Yu, G. L. Vega, R. Guerra, H. H. Hobbs, and S. M. Grundy. 2004. African Americans and Caucasians have a similar prevalence of coronary calcium in the Dallas Heart Study. *J. Am. Coll. Cardiol.* **44:** 1011–1017.

9. Haffner, S. M., R. D'Agostino, Jr., D. Goff, B. Howard, A. Festa, M. F. Saad, and L. Mykkanen. 1999. LDL size in African Americans, Hispanics, and non-Hispanic whites: the Insulin Resistance Atherosclerosis Study. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2234–2240.
10. Kullo, I. J., M. de Andrade, E. Boerwinkle, J. P. McConnell, S. L. Kardia, and S. T. Turner. 2005. Pleiotropic genetic effects contribute to the correlation between HDL cholesterol, triglycerides, and LDL particle size in hypertensive sibships. *Am. J. Hypertens.* **18:** 99–103.
11. Edwards, K. L., M. C. Mahaney, A. G. Motulsky, and M. A. Austin. 1999. Pleiotropic genetic effects on LDL size, plasma triglyceride, and HDL cholesterol in families. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2456–2464.
12. Pajukanta, P., H. E. Lilja, J. S. Sinsheimer, R. M. Cantor, A. J. Lusis, M. Gentile, X. J. Duan, A. Soro-Paavonen, J. Naukkarinen, J. Saarela, et al. 2004. Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). *Nat. Genet.* **36:** 371–376.
13. The FBPP Investigators. 2002. Multi-center genetic study of hypertension: the Family Blood Pressure Program (FBPP). *Hypertension.* **39:** 3–9.
14. O'Meara, J. G., S. L. Kardia, J. J. Armon, C. A. Brown, E. Boerwinkle, and S. T. Turner. 2004. Ethnic and sex differences in the prevalence, treatment, and control of dyslipidemia among hypertensive adults in the GENOA study. *Arch. Intern. Med.* **164:** 1313–1318.
15. Kottke, B. A., P. P. Moll, V. V. Michels, and W. H. Weidman. 1991. Levels of lipids, lipoproteins, and apolipoproteins in a defined population. *Mayo Clin. Proc.* **66:** 1198–1208.
16. Hoefner, D. M., S. D. Hodel, J. F. O'Brien, E. L. Branum, D. Sun, I. Meissner, and J. P. McConnell. 2001. Development of a rapid, quantitative method for LDL subfractionation with use of the Quantimetrix Lipoprint LDL System. *Clin. Chem.* **47:** 266–274.
17. Kullo, I. J., S. T. Turner, E. Boerwinkle, S. L. Kardia, and M. de Andrade. 2005. A novel quantitative trait locus on chromosome 1 with pleiotropic effects on HDL-cholesterol and LDL particle size in hypertensive sibships. *Am. J. Hypertens.* **18:** 1084–1090.
18. Lange, K., and T. M. Goradia. 1987. An algorithm for automatic genotype elimination. *Am. J. Hum. Genet.* **40:** 250–256.
19. O'Connell, J. R., and D. E. Weeks. 1998. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am. J. Hum. Genet.* **63:** 259–266.
20. de Andrade, M., B. Fridley, E. Boerwinkle, and S. T. Turner. 2003. Diagnostic tools in linkage analysis for quantitative traits. *Genet. Epidemiol.* **24:** 302–308.
21. Lange, K., and M. Boehnke. 1983. Extensions to pedigree analysis. IV. Covariance components models for multivariate traits. *Am. J. Med. Genet.* **14:** 513–524.
22. Jaquish, C. E., M. C. Mahaney, J. Blangero, S. M. Haffner, M. P. Stern, and J. W. MacCluer. 1996. Genetic correlations between lipoprotein phenotypes and indicators of sex hormone levels in Mexican Americans. *Atherosclerosis.* **122:** 117–125.
23. Amos, C. I. 1994. Robust variance-components approach for assessing genetic linkage in pedigrees. *Am. J. Hum. Genet.* **54:** 535–543.
24. de Andrade, M., T. J. Thiel, L. Yu, and C. I. Amos. 1997. Assessing linkage on chromosome 5 using components of variance approach: univariate versus multivariate. *Genet. Epidemiol.* **14:** 773–778.
25. Sobel, E., H. Sengul, and D. E. Weeks. 2001. Multipoint estimation of identity-by-descent probabilities at arbitrary positions among marker loci on general pedigrees. *Hum. Hered.* **52:** 121–131.
26. Self, S., and K-Y. Liang. 1987. Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under non-standard conditions. *J. Am. Stat. Assoc.* **82:** 605–610.
27. Morton, N. E. 1998. Significance levels in complex inheritance. *Am. J. Hum. Genet.* **62:** 690–697.
28. Lamon-Fava, S., D. Jimenez, J. C. Christian, R. R. Fabsitz, T. Reed, D. Carmelli, W. P. Castelli, J. M. Ordovas, P. W. Wilson, and E. J. Schaefer. 1991. The NHLBI Twin Study: heritability of apolipoprotein A-I, B, and low density lipoprotein subclasses and concordance for lipoprotein(a). *Atherosclerosis.* **91:** 97–106.
29. Austin, M. A., B. Newman, J. V. Selby, K. Edwards, E. J. Mayer, and R. M. Krauss. 1993. Genetics of LDL subclass phenotypes in women twins. Concordance, heritability, and commingling analysis. *Arterioscler. Thromb.* **13:** 687–695.
30. Bosse, Y., M. C. Vohl, J. P. Despres, B. Lamarche, T. Rice, D. C. Rao, C. Bouchard, and L. Perusse. 2003. Heritability of LDL peak particle diameter in the Quebec Family Study. *Genet. Epidemiol.* **25:** 375–381.
31. Barzilai, N., G. Atzmon, C. Schechter, E. J. Schaefer, A. L. Cupples, R. Lipton, S. Cheng, and A. R. Shuldiner. 2003. Unique lipoprotein phenotype and genotype associated with exceptional longevity. *J. Am. Med. Assoc.* **290:** 2030–2040.
32. Rainwater, D. L., L. Almasy, J. Blangero, S. A. Cole, J. L. VandeBerg, J. W. MacCluer, and J. E. Hixson. 1999. A genome search identifies major quantitative trait loci on human chromosomes 3 and 4 that influence cholesterol concentrations in small LDL particles. *Arterioscler. Thromb. Vasc. Biol.* **19:** 777–783.
33. Austin, M. A., K. L. Edwards, S. A. Monks, K. M. Koprowicz, J. D. Brunzell, A. G. Motulsky, M. C. Mahaney, and J. E. Hixson. 2003. Genome-wide scan for quantitative trait loci influencing LDL size and plasma triglyceride in familial hypertriglyceridemia. *J. Lipid Res.* **44:** 2161–2168.
34. Allayee, H., K. M. Dominguez, B. E. Aouizerat, R. M. Krauss, J. I. Rotter, J. Lu, R. M. Cantor, T. W. de Bruin, and A. J. Lusis. 2000. Contribution of the hepatic lipase gene to the atherogenic lipoprotein phenotype in familial combined hyperlipidemia. *J. Lipid Res.* **41:** 245–252.
35. Turner, S. T., S. L. Kardia, E. Boerwinkle, and M. de Andrade. 2004. Multivariate linkage analysis of blood pressure and body mass index. *Genet. Epidemiol.* **27:** 64–73.
36. Calvano, S. E., W. Xiao, D. R. Richards, R. M. Felciano, H. V. Baker, R. J. Cho, R. O. Chen, B. H. Brownstein, J. P. Cobb, S. K. Tschoeke, et al. 2005. A network-based analysis of systemic inflammation in humans. *Nature.* **437:** 1032–1037.
37. Rotter, J. I., X. Bu, R. M. Cantor, C. H. Warden, J. Brown, R. J. Gray, P. J. Blanche, R. M. Krauss, and A. J. Lusis. 1996. Multilocus genetic determinants of LDL particle size in coronary artery disease families. *Am. J. Hum. Genet.* **58:** 585–594.
38. Tatton, L., G. M. Morley, R. Chopra, and A. Khwaja. 2003. The Src-selective kinase inhibitor PP1 also inhibits Kit and Bcr-Abl tyrosine kinases. *J. Biol. Chem.* **278:** 4847–4853.
39. Heinrich, M. C., D. C. Dooley, and W. W. Keeble. 1995. Transforming growth factor beta 1 inhibits expression of the gene products for steel factor and its receptor (c-kit). *Blood.* **85:** 1769–1780.
40. Kolbus, A., M. Blazquez-Domingo, S. Carotta, W. Bakker, S. Luedemann, M. von Lindern, P. Steinlein, and H. Beug. 2003. Cooperative signaling between cytokine receptors and the glucocorticoid receptor in the expansion of erythroid progenitors: molecular analysis by expression profiling. *Blood.* **102:** 3136–3146.
41. Allayee, H., B. E. Aouizerat, R. M. Cantor, G. M. Dallinga-Thie, R. M. Krauss, C. D. Lanning, J. I. Rotter, A. J. Lusis, and T. W. de Bruin. 1998. Families with familial combined hyperlipidemia and families enriched for coronary artery disease share genetic determinants for the atherogenic lipoprotein phenotype. *Am. J. Hum. Genet.* **63:** 577–585.
42. Bosse, Y., L. Perusse, J. P. Despres, B. Lamarche, Y. C. Chagnon, T. Rice, D. C. Rao, C. Bouchard, and M. C. Vohl. 2003. Evidence for a major quantitative trait locus on chromosome 17q21 affecting low-density lipoprotein peak particle diameter. *Circulation.* **107:** 2361–2368.
43. Badzioch, M. D., R. P. Igo, Jr., F. Gagnon, J. D. Brunzell, R. M. Krauss, A. G. Motulsky, E. M. Wijsman, and G. P. Jarvik. 2004. Low-density lipoprotein particle size loci in familial combined hyperlipidemia: evidence for multiple loci from a genome scan. *Arterioscler. Thromb. Vasc. Biol.* **24:** 1942–1950.