Original Article

Analysis of Quantitative Lipid Traits in the Genetics of NIDDM (GENNID) Study

Alka Malhotra, Johanna K. Wolford, and the American Diabetes Association GENNID Study Group*

Coronary heart disease (CHD) is the leading cause of death among individuals with type 2 diabetes. Dyslipidemia contributes significantly to CHD in diabetic patients, in whom lipid abnormalities include hypertriglyceridemia, low HDL cholesterol, and increased levels of small, dense LDL particles. To identify genes for lipid-related traits, we performed genome-wide linkage analyses for levels of triglycerides and HDL, LDL, and total cholesterol in Caucasian, Hispanic, and African-American families from the Genetics of NIDDM (GENNID) study. Most lipid traits showed significant estimates of heritability ($P < 0.001$) with the exception of triglycerides and the triglyceride/HDL ratio in African Americans. Variance components analysis identified linkage on chromosome 3p12.1-3q13.31 for the triglyceride/HDL ratio (logarithm of odds [LOD] = 3.36) and triglyceride (LOD = 3.27) in Caucasian families. Statistically significant evidence for linkage was identified for the triglyceride/HDL ratio (LOD = 2.45) on 11p in Hispanic families in a region that showed suggestive evidence for linkage (LOD = 2.26) for triglycerides in this population. In African Americans, the strongest evidence for linkage (LOD = 2.26) was found on 19p13.2-19q13.42 for total cholesterol. Our findings provide strong support for previous reports of linkage for lipid-related traits, suggesting the presence of genes on 3p12.1-3q13.31, 11p15.4-11p11.3, and 19p13.2-19q13.42 that may influence traits underlying lipid abnormalities associated with type 2 diabetes. *Diabetes* 54:3007–3014, 2005

Cardiovascular disease is the leading cause of death among individuals with type 2 diabetes (1). Dyslipidemia plays a major role in the development of cardiovascular disease in type 2 diabetic patients, in whom lipid abnormalities are characterized by hypertriglyceridemia and reduced levels of HDL cholesterol present mainly in the form of small, dense HDL particles (2,3). Levels of LDL cholesterol are typically normal or only mildly elevated; however, an increased level of small, dense LDL particles that are highly atherogenic is frequently a component of diabetic dyslipidemia (4,5). Disregulated lipoprotein metabolism and risk for cardiovascular disease most likely precede the development of glucose intolerance and frank diabetes (6). Haffner et al. (6) showed that among individuals who were normoglycemic at baseline examination, those who later progressed to diabetes had lower HDL levels and higher levels of LDL and total cholesterol compared with individuals who remained diabetes free. In this study, differences in lipid levels persisted following adjustments for BMI and level of glycemia but not fasting insulin concentration. Similarly, in analyses using nuclear magnetic resonance spectroscopy, Garvey et al. (7) found that increasing severity of insulin resistance, in both diabetic and nondiabetic individuals, was associated with *1*) increased VLDL size and levels of large VLDL particle concentrations (where circulating triglycerides are predominantly carried); *2*) decreased HDL size due to depletion of large HDL particles and increased levels of small HDL particles; and *3*) increased levels of small, dense LDL particles and an overall increase in the total number of LDL particles. Combined, these findings suggest that insulin resistance, and possibly hyperinsulinemia, likely underlie the lipid-related changes associated with type 2 diabetes.

Diet and exercise are among the most common environmental factors affecting lipid levels (8,9); however, genetic determinants of several monogenic lipid-related disorders have been clearly established (10). For example, mutations in the LDL receptor (*LDLR*) gene underlie familial hypercholesterolemia, which is marked by excessively high LDL levels (11), and defects in the ATP binding cassette A1 (*ABCA1*) gene lead to Tangier disease, characterized by reduced HDL levels (12–14). Despite the considerable success in identifying mutations underlying monogenic lipid-related disorders, genetic determinants of lipid traits in the general population remain largely unknown. Based on twin and pedigree analyses, genetic heritability (the proportion of variance due to genetic factors) of lipid levels has been estimated to range from 0.20 to 0.87 (15,16), suggesting roles for both environmental and genetic components.

Over 90 genome scans have been performed to identify loci affecting lipid levels (for a comprehensive review, see Bossé et al. [17]). Of these, four were performed in families who were originally ascertained for type 2 diabetes (18–21). Duggirala et al. (21) identified a major susceptibility locus for plasma triglycerides on 15q in Mexican-American families ascertained for type 2 diabetes. Linkage to chromosome 19 was identified for total cholesterol, triglycerides, and LDL in Pima Indians (19), non-Hispanic Caucasians (20), and Old Order Amish (18), respectively, in a region linked with lipid traits in nondiabetic study samples. Additional regions of linkage for lipid traits in

From the Translational Genomics Research Institute, Phoenix, Arizona.

Address correspondence and reprint requests to Johanna K. Wolford, Diabetes and Obesity Research Unit, Translational Genomics Research Institute, 445 N. 5th St., Phoenix, AZ 85004. E-mail: jwolford@tgen.org.

Received for publication 28 April 2005 and accepted in revised form 8 July 2005.

*A list of the American Diabetes Association GENNID Study Group members can be found in the APPENDIX.

CAD, coronary artery disease; CHD, coronary heart disease; GENNID, Genetics of NIDDM; LOD, logarithm of odds.

TABLE 1
Characteristics of study participants

	Caucasians		Hispanics		African Americans	
	Men	Women	Men	Women	Men	Women
n	357	462	211	371	79	161
Age (years)	53.7 ± 15.9	54.8 ± 15.7	50.6 ± 15.6	50.3 ± 15.6	50.2 ± 16.5	52.1 ± 14.9
BMI (kg/m^2)	29.2 ± 6.5	30.1 ± 7.6	28.9 ± 5.0	31.8 ± 8.0	28.8 ± 6.0	34.3 ± 9.3
Triglycerides (mg/dl)	158.4 ± 105.8	140.8 ± 103.7	156.8 ± 92.7	154.6 ± 90.0	89.9 ± 51.6	96.0 ± 50.8
LDL (mg/dl)	117.3 ± 33.8	120.0 ± 35.9	119.7 ± 34.1	118.2 ± 33.9	122.9 ± 36.7	127.1 ± 35.3
HDL (mg/dl)	34.6 ± 9.0	43.9 ± 11.4	36.2 ± 9.4	40.7 ± 9.7	44.5 ± 12.5	47.6 ± 10.7
Total cholesterol (mg/dl)	183.9 ± 37.0	192.4 ± 42.0	187.6 ± 38.1	189.4 ± 39.2	188.0 ± 39.3	195.1 ± 38.4

Data are means ± SD. Descriptive statistics of lipid levels and covariates in 819 Caucasians, 582 Hispanics, and 240 African Americans are given. Lipid concentrations represent untransformed, unadjusted values.

type 2 diabetic families include 1q21-q23 (20), 11q23 (18), and 3q (19).

The goal of this study was to identify quantitative trait loci that affect the diabetes-related plasma lipid profile in ethnically diverse populations. For this purpose, we analyzed genotype data for ~400 microsatellite markers in 153 non-Hispanic Caucasian, 113 Hispanic, and 59 African-American families who participated in the Genetics of NIDDM (GENNID) study, which comprises a repository containing extensive phenotypic and genotypic information on individuals from different ethnic groups ascertained by the presence of at least two type 2 diabetic siblings (22). Heritability estimates for lipid traits were obtained in these families, and variance components linkage analyses were performed for log-transformed plasma levels of triglycerides, HDL, LDL, and total cholesterol, as well as triglyceride/HDL and LDL/HDL ratios.

RESEARCH DESIGN AND METHODS

The GENNID study was initiated in 1993 to establish a repository of phenotypically well-characterized families ascertained by the presence of at least two siblings with type 2 diabetes. Ascertainment was completed in two phases: phase I families contained at least three other first-degree relatives and a nondiabetic ethnically matched control (i.e., an unaffected spouse) in addition to two diabetic siblings; phase II families had at least two diabetic siblings and both parents or, in cases with no available parent(s), up to two additional siblings (22). The present study used phenotypic data from both phase I and II families. Clinical and anthropometric measures, as well as family/medical histories for all GENNID participants, were collected from 13 different clinical centers throughout the U.S. Diabetes was documented according to the National Diabetes Data Group criteria (i.e., either a fasting plasma glucose concentration of ≥140 mg/dl on more than one occasion or a plasma glucose concentration of ≥200 mg/dl in a 2-h sample and in at least one other sample taken during an oral glucose tolerance test) (22). Among the clinical data collected, measures for fasting plasma levels of triglycerides and HDL, LDL, and total cholesterol were also obtained (22). Plasma levels of triglycerides, HDL, and total cholesterol were directly measured. Triglycerides were measured using a free-glycerol blanking method, HDL was measured following heparin-manganese sulfate precipitation, and total cholesterol was measured enzymatically (22). Plasma LDL concentration was estimated using the Friedewald equation [LDL = total cholesterol − HDL − (triglyceride/5)] (23).

This study used phenotypic data from the GENNID database for 819 non-Hispanic Caucasians (153 families), 582 Hispanics (113 families), and 240 African Americans (59 families). Non-Hispanic Caucasian family size ranged from 2 to 18 members with 450 parent-offspring pairs, 944 sibling pairs, 124 cousin pairs, 422 uncle/aunt-niece/nephew pairs, and 41 grandparent-grandchild pairs. Hispanic family size ranged from 2 to 16 members with 314 parent-offspring pairs, 646 sib-pairs, 70 cousin pairs, 266 uncle/aunt-niece/nephew pairs, and 4 grandparent-grandchild pairs. African-American families consisted of 2–8 individuals with 88 parent-offspring pairs, 230 sibpairs, 7 cousin pairs, 39 uncle/aunt-niece/nephew pairs, and 13 grandparent-grandchild pairs.

Genotypes. Whole-genome microsatellite marker (Marshfield screening sets 6 and 8) genotyping was performed at the Marshfield Medical Research Foundation (Marshfield, WI), and additional markers were genotyped at Glaxo Wellcome (Research Triangle Park, NC) to fill gaps in one or more of the sample populations (24). In total, 418, 424, and 373 microsatellite markers were genotyped in the non-Hispanic Caucasian, Hispanic, and African-American study groups, respectively. Average marker spacing ranged from ~8 to 9 cM, and average marker heterozygosity for each sample population was ~0.75 (24).

Statistical analyses. The SIMWALK program (25) was used to identify Mendelian genotyping errors, which were then coded with missing values. In analyses of the phenotypic data, outliers with trait values ≥3 SDs were removed to avoid any bias that might be introduced into the results (n = 12 in Caucasians, 17 in Hispanics, and 14 in African Americans). Some of the lipid traits (HDL, triglycerides, and LDL/HDL and triglyceride/HDL ratios) were not normally distributed. We therefore log- or square root–transformed these lipid concentrations, and the Statistical Analysis System package (SAS Institute, Cary, NC) was used for regression analysis of the transformed lipid traits. The covariates tested were sex, duration of diabetes, BMI, waist circumference, hip circumference, waist-to-hip ratio, age, weight, and their square and cubic terms, smoking status, and alcohol intake status. Adjustment of covariates significantly related to plasma lipid levels at the 0.05 level was performed separately in individuals affected and unaffected with diabetes to remove any effects of diabetes status. The adjusted and standardized [N (0,1)] residuals for all traits were approximately normally distributed with skewness ranging from −0.39 to 0.38 and kurtosis ranging from −0.53 to 0.77. In this study population, there were 66 Caucasians, 40 Hispanics, and 7 African Americans taking lipid-lowering drugs; data analysis was performed both including and excluding these individuals. Since the most commonly used diabetes drugs were sulfonylureas, which do not exert direct effects on lipid levels, no adjustments were made for individuals on medication for type 2 diabetes.

Genetic heritability was estimated for the adjusted and standardized residuals using the Pedigree Analysis Package (26). In addition, the six lipid traits (we will refer to the transformed, adjusted, and standardized lipid levels as total cholesterol, triglycerides, LDL, HDL, and LDL/HDL and triglyceride/HDL ratios) were subjected to variance components linkage analysis as implemented in the program GENEHUNTER (27). To assess the genome-wide significance of our linkage signals observed in Caucasians, African Americans,

TABLE 2
Heritability estimates for lipid levels in Caucasians, Hispanics, and African Americans

	Heritability estimate ± SE		
Lipid level	Caucasians	Hispanics	African Americans
Total cholesterol	0.50 ± 0.08	0.38 ± 0.08	0.47 ± 0.15
HDL	0.42 ± 0.08	0.34 ± 0.09	0.38 ± 0.15
LDL	0.42 ± 0.07	0.28 ± 0.08	0.39 ± 0.14
Triglycerides	0.54 ± 0.08	0.19 ± 0.07	0.14 ± 0.13
Triglyceride/HDL ratio	0.53 ± 0.08	0.22 ± 0.07	0.20 ± 0.13
LDL/HDL ratio	0.48 ± 0.07	0.32 ± 0.08	0.44 ± 0.14

Heritability (h^2) was estimated for 153 Caucasian, 113 Hispanic, and 59 African-American families. All h^2 estimates were significant ($P < 0.001$) except for triglycerides and triglyceride/HDL ratio in African Americans ($P > 0.05$).

TABLE 3
Regions showing evidence for linkage (multipoint LOD >1.5) in Caucasians

Chromosome	Lipid level	Multipoint LOD	2-point LOD	Closest marker	1-LOD interval (cM)
2	Triglyceride	1.71	1.36	*GATA176C01*	98–113
	Triglyceride/HDL ratio	1.74	1.41	*GATA176C01*	95–113
3	Triglyceride	3.27	1.74	*D3S3045*	116–130
	Triglyceride/HDL ratio	3.36	2.43	*D3S3045*	116–130
5	Total cholesterol	1.62	1.41	*MFD154*	145–178
	LDL	1.69	1.38	*MFD154*	149–178
9	Total cholesterol	1.63	2.06	*D9S1121*	24–63
10	LDL	2.07	1.20	*GGAA23C05*	125–160
15	LDL	2.23	2.44	*D15S107*	98–118
16	LDL/HDL ratio	2.18	1.64	*D16S748*	0–49

Variance components linkage analysis was performed using data from 153 families.

and Hispanics, 1,000 replicates of the genome were simulated using the program SIMULATE (28). The family structure and marker allele frequency information of the original dataset were used to generate the replicates. Variance components linkage analysis was then performed on all the replicates, and a logarithm of odds (LOD) score threshold was estimated for a false-positive rate anywhere in the genome of 0.05. We estimated the LOD score threshold by first subjecting each replicate to variance components linkage analysis, then obtaining the maximum LOD score for each replicate and ranking these LOD scores in ascending order. The LOD score threshold was identified as the LOD score that was exceeded in <5% of replicates. In addition, empirical *P* values were estimated as the proportion of replicates equal to or exceeding the observed LOD score. Due to time and computational constraints, we performed simulations only for those traits showing the highest LOD scores (i.e., triglycerides, triglyceride/HDL ratio, and total cholesterol).

RESULTS

The characteristics of each study sample used in our analyses are shown in Table 1. Diabetes prevalence was 45% in both Caucasians and Hispanics and 54% in African Americans. In the Caucasian sample, covariates explained 2–15% and 5–29% of the phenotypic variance in diabetic and nondiabetic individuals, respectively. In the Hispanic sample, covariates explained less of the phenotypic variance than in Caucasians: 2–10% and 7–16% in diabetic and nondiabetic individuals, respectively. Covariates in African Americans explained levels of phenotypic variance comparable to those seen in the Hispanic sample (i.e., 3–10% and 8–17% for diabetic and nondiabetic individuals, respectively).

Statistically significant estimates of heritability were observed for all plasma lipid traits in Caucasians ($P < 0.0001$) and Hispanics ($P < 0.001$) as shown in Table 2. In African Americans, total cholesterol, LDL, HDL, and LDL/HDL ratio showed substantial heritability ($P < 0.001$), but triglycerides and triglyceride/HDL ratio did not (Table 2; $P > 0.05$).

Variance components linkage analyses were performed for all lipid traits in each study population. The highest LOD scores were found on chromosome 3 for both triglyceride/HDL ratio (LOD = 3.36; empirical *P* value = 0.051) and triglycerides (LOD = 3.27; empirical *P* value = 0.059) in Caucasian families. An LOD score threshold of 3.38 was obtained following simulation of 1,000 replicates, making the LOD score for both triglycerides and triglyceride/HDL ratio slightly less than the level of statistical significance. Suggestive evidence for linkage was observed on chromosomes 5, 10, and 15 for LDL, chromosome 2 for triglycerides and triglyceride/HDL ratio, chromosomes 5 and 9 for total cholesterol, and chromosome 16 for the LDL/HDL ratio (Table 3).

Statistically significant evidence for linkage was observed for triglyceride/HDL ratio (LOD = 2.45; empirical *P* value = 0.032) on chromosome 11p in Hispanic families, where suggestive evidence for triglycerides (LOD = 2.26; empirical *P* value = 0.061) was also found. Simulation results for observing a false-positive rate of 0.05 anywhere in the genome identified an LOD score threshold of 2.31 ($P < 0.001$). Suggestive evidence for linkage was observed on chromosomes 2 and 4 for total cholesterol, 4 and 15 for LDL/HDL ratio, chromosome 11 for HDL, and chromosome 14 for triglycerides and triglyceride/HDL ratio (Table 4).

In African Americans, three chromosomal regions were identified with multipoint LOD scores >1.45 (Table 5). The strongest evidence for linkage was found on chromosome 19 for total cholesterol (LOD = 2.26; empirical *P* value = 0.105). An LOD score threshold of 2.51 ($P < 0.001$) was

TABLE 4
Regions showing evidence for linkage (multipoint LOD >1.5) in Hispanics

Chromosome	Lipid level	Multipoint LOD	2-point LOD	Closest marker	1-LOD interval (cM)
2	Total cholesterol	1.61	1.11	*D2S1352*	77–107
	Total cholesterol	1.90	1.62	*D2S1353*	171–188
4	Total cholesterol	1.79	1.95	*D4S1530*	160–204
	LDL/HDL ratio	1.61	1.18	*D4S403*	0–19
	LDL/HDL ratio	2.38	2.42	*D4S2417*	160–186
11	Triglyceride/HDL ratio	2.45*	2.18	*D4S2417*	21–46
	Triglyceride	2.26	2.09	*D11S902*	21–46
14	Triglyceride	1.54	2.12	*GATA193A07*	53–83
	Triglyceride/HDL ratio	2.07	2.46	*GATA193A07*	52–83
19	LDL/HDL ratio	2.10	1.78	*D19S47*	47–75

Variance components linkage analysis was performed using data from 131 families. *Significant evidence for linkage based on a simulation study.

TABLE 5
Regions showing evidence for linkage (multipoint LOD >1.5) in African Americans

Chromosome	Lipid level	Multipoint LOD	2-point LOD	Closest marker	1-LOD interval (cM)
19	Total cholesterol	2.26	1.69	*MFD235*	23–78
	LDL	1.62	1.58	*D19S714*	0–76
5	HDL	1.59	0.80	*D5S2500*	22–76
	LDL/HDL ratio	1.66	1.05	*D5S807*	2–43

Variance components linkage analysis was performed using data from 59 families.

obtained following simulation of 1,000 replicates, making the LOD score for total cholesterol slightly less than the level of statistical significance. Suggestive evidence for linkage for LDL was also detected in the same region on chromosome 19 (LOD = 1.62). Additional regions of linkage included chromosome 5 for LDL/HDL ratio (LOD = 1.66) and HDL (LOD = 1.59), chromosome 1 for LDL (LOD = 1.45), chromosome 17 for total cholesterol (LOD = 1.35), and chromosome 18 for triglycerides (LOD = 1.04) and triglyceride/HDL ratio (LOD = 1.14).

DISCUSSION

Genetic factors most likely underlie control of lipid traits in the general population. We found substantial genetic heritability for most lipid traits in the three populations comprising this study, and the estimates shown here correspond well with previously reported values (15,16). High estimates of genetic heritability imply familial aggregation of lipids; therefore, a gene underlying the inheritance of lipid levels may be detectable through the use of linkage and association analyses.

The highest LOD scores obtained in these analyses were found for triglyceride/HDL ratio (LOD = 3.36) and triglycerides (LOD = 3.27) on 3p12.1-3q13.31 in Caucasian families. This chromosomal region overlaps with intervals that have been identified by linkage analysis in at least five previously published studies (Fig. 1). The majority of linkage findings in this region were obtained in Caucasian populations (four of five) using different ascertainment schemes as well as different lipid-related traits (i.e., quantitative and qualitative). Analysis of the triglyceride/HDL ratio showed evidence for linkage in a coronary heart disease (CHD)-ascertained Mauritian population (29) and a randomly ascertained population from the Framingham Heart study (16). In a bivariate analysis of total cholesterol and triglycerides, evidence for pleiotropic effects of a gene residing in this region was found (30). The interval identified in the present study has also been implicated in discrete trait analyses of familial hypercholesterolemia (31) and coronary artery disease (CAD) (32). Four of the above studies (including the present study), using either randomly or disease-ascertained families, showed evidence for linkage on chromosome 3 for triglycerides and/or triglyceride/HDL ratio, and a fifth study identified linkage for CAD in this region. Because triglycerides levels are generally increased in individuals with CAD and diabetic dyslipidemia, these results strongly support the presence of a common gene on chromosome 3, which affects triglycerides levels in both randomly and disease-ascertained families.

The strongest evidence for linkage (LOD = 2.45) in the Hispanic study sample was found for triglyceride/HDL ratio on chromosome 11p15.4-11p11.3. Based on a simulation study for observing a false-positive rate of 0.05 anywhere in the genome, this LOD score was statistically significant. The linked region on chromosome 11 has also been reported in several other studies, one of which included families ascertained for type 2 diabetes (Fig. 2).

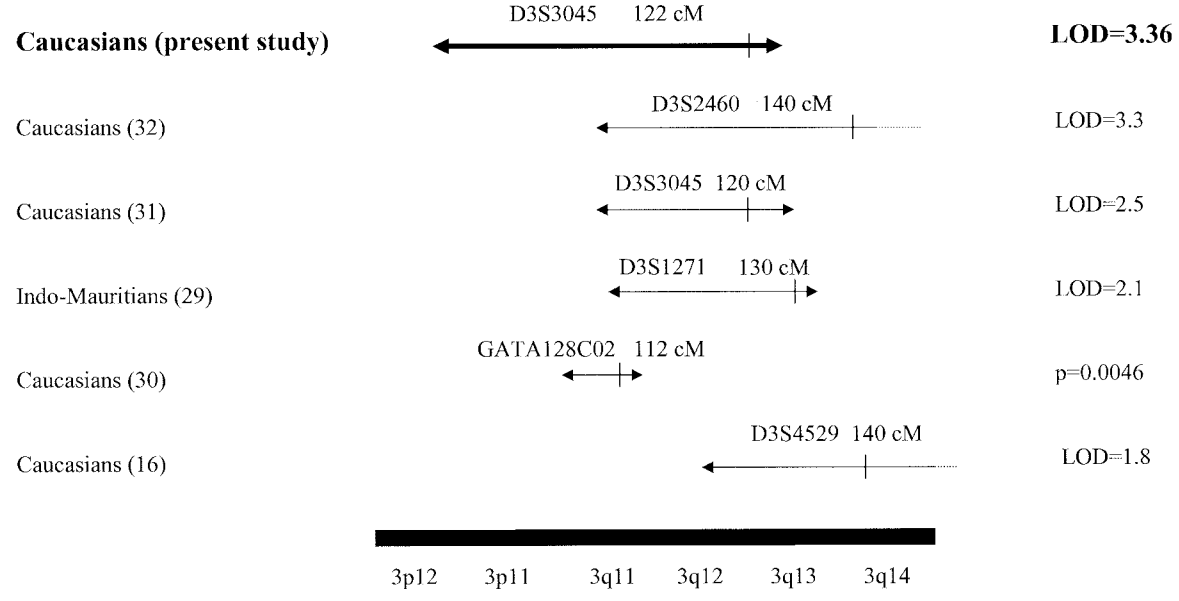

FIG. 1. A summary of studies showing evidence for linkage on chromosome 3. The ethnic background of the study population is given. Horizontal lines with arrows represent the approximate 1-LOD support interval on a cytogenetic map (bottom) for the respective studies. The closest marker and position (cM, vertical line) of the maximum LOD score or lowest *P* value (right) are indicated above the horizontal line.

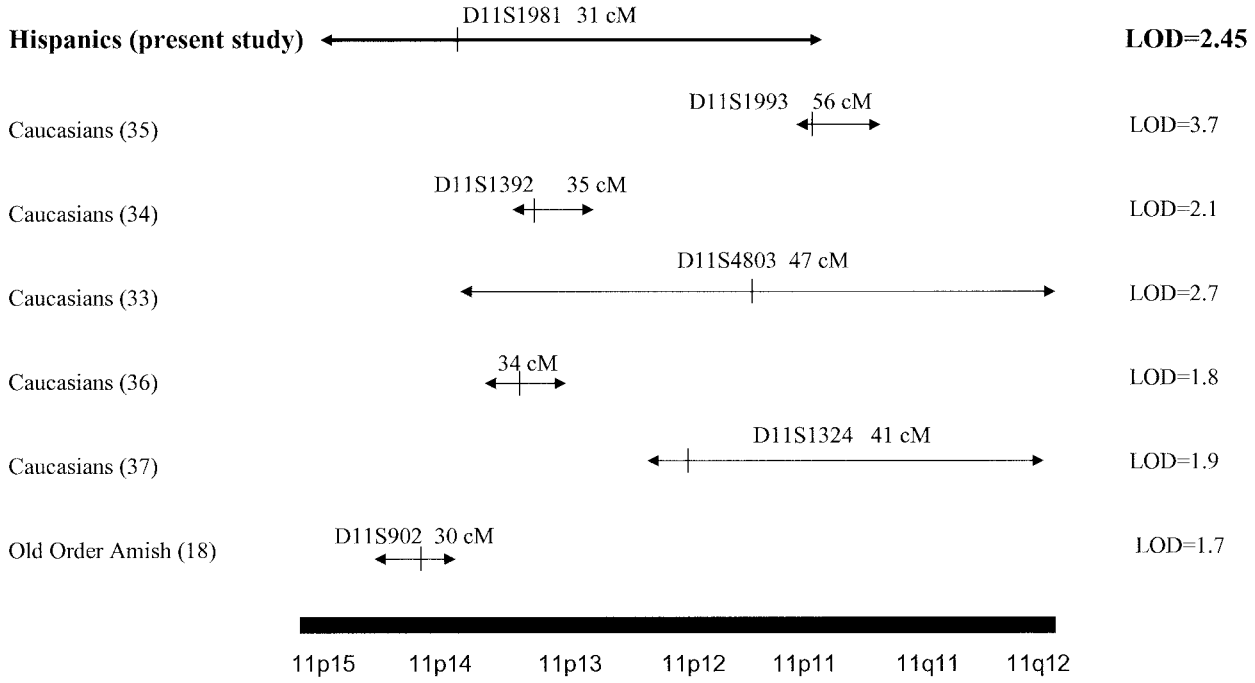

FIG. 2. A summary of studies showing evidence for linkage on chromosome 11. The ethnic background of the study population is given. Horizontal lines with arrows represent the approximate 1-LOD support interval on a cytogenetic map (bottom) for the respective studies. The closest marker and position (cM, vertical line) of the maximum LOD score (right) are indicated above the horizontal line. One study did not have closest marker information available (see ref. 36).

Evidence of linkage to this region was observed in families ascertained by the presence of familial combined hyperlipidemia (LOD = 2.7 for triglycerides and LOD = 2.04 for total cholesterol in the same region) (33) in the Quebec Family Study (LOD = 2.1 for triglycerides) using both randomly and obesity-ascertained families (34) and in the Old Order Amish (LOD = 1.7 for triglycerides) ascertained for type 2 diabetes (18). Linkage on chromosome 11p was also observed for other lipid traits. In a study combining data from CHD and randomly ascertained families, a linkage peak was identified for LDL (35). This region has also been identified in randomly ascertained individuals for total cholesterol (36) and familial combined hyperlipidemia (as a discrete trait) (37). Replication of previously published results supports the presence of a gene affecting lipid levels in this region on chromosome 11. In addition, the use of different ascertainment schemes, all of which are associated with abnormal lipid levels, may suggest pleiotropic effects of a common underlying gene in these various conditions.

Several potential candidates for control of lipid metabolism map to the linked regions on 3p12.1-3q13.31 and 11p15.4-11p11.3. The pregnane X receptor gene (also known as nuclear receptor subfamily 1, group I, member 2 [*NR1I2*]), which induces expression of genes involved in clearance of cholesterol metabolites, is located on 3q12 and has recently been shown to exert direct effects on the detoxification of cholesterol metabolites (38). The phosphotidylserine-specific phospholipase A1-α (*PLA1A*) gene, located on 3q13, is involved in the hydrolysis of fatty acids. Genes on chromosome 11 include the liver X receptor α (*LXRA*), which plays a key role in the regulation of cholesterol and lipid metabolism; oxysterol-binding protein-like protein 5 (*OSBPL5*), which belongs to a family of intracellular lipid receptors and may play a role in the regulation of cholesterol metabolism (39); and 7-dehydrocholesterol reductase (*DHCR7*), which catalyzes the conversion of 7-dehydrocholesterol to cholesterol during the final step of endogenous cholesterol biosynthesis and is linked to control of total cholesterol levels (40).

We observed a statistically significant correlation of 0.94 ($P < 0.0001$) between triglycerides and triglyceride/HDL ratio in both Caucasians and Hispanics. Given that we observed similar LOD scores for these traits in the same region but a lower LOD score for HDL (1.15 in Hispanics and <1 in Caucasians), we predict that the triglycerides level is responsible for most of the linkage signal on chromosomes 3 and 11 in Caucasians and Hispanics, respectively; however, it is possible that pleiotropic effects of an underlying gene are present. Because hypertriglyceridemia is typically observed in type 2 diabetic patients, these regions may harbor genes underlying susceptibility to developing CAD in families ascertained for type 2 diabetes.

In the African-American study sample, the strongest evidence for linkage was found on chromosome 19 for total cholesterol in a region that also overlapped a linkage peak for LDL in our analyses. Interestingly, this region has been reported for lipid traits in at least five independent studies, including three investigations of families originally ascertained for type 2 diabetes (Fig. 3). In these diabetic families, evidence of linkage was seen for total cholesterol in Pima Indians (19), triglycerides in non-Hispanic Caucasians (20), and LDL in the Old Order Amish (18). In addition, linkage for LDL concentrations was found in two non-Hispanic Caucasian populations, one randomly ascertained (41) and the other containing a combination of randomly and obesity-ascertained families (34). In a separate study, Rainwater et al. (42) fractionated LDL components and performed quantitative linkage analysis on the different LDL size fractions. Evidence for linkage of two size fractions, LDL-1 (26.4–29 nm) and

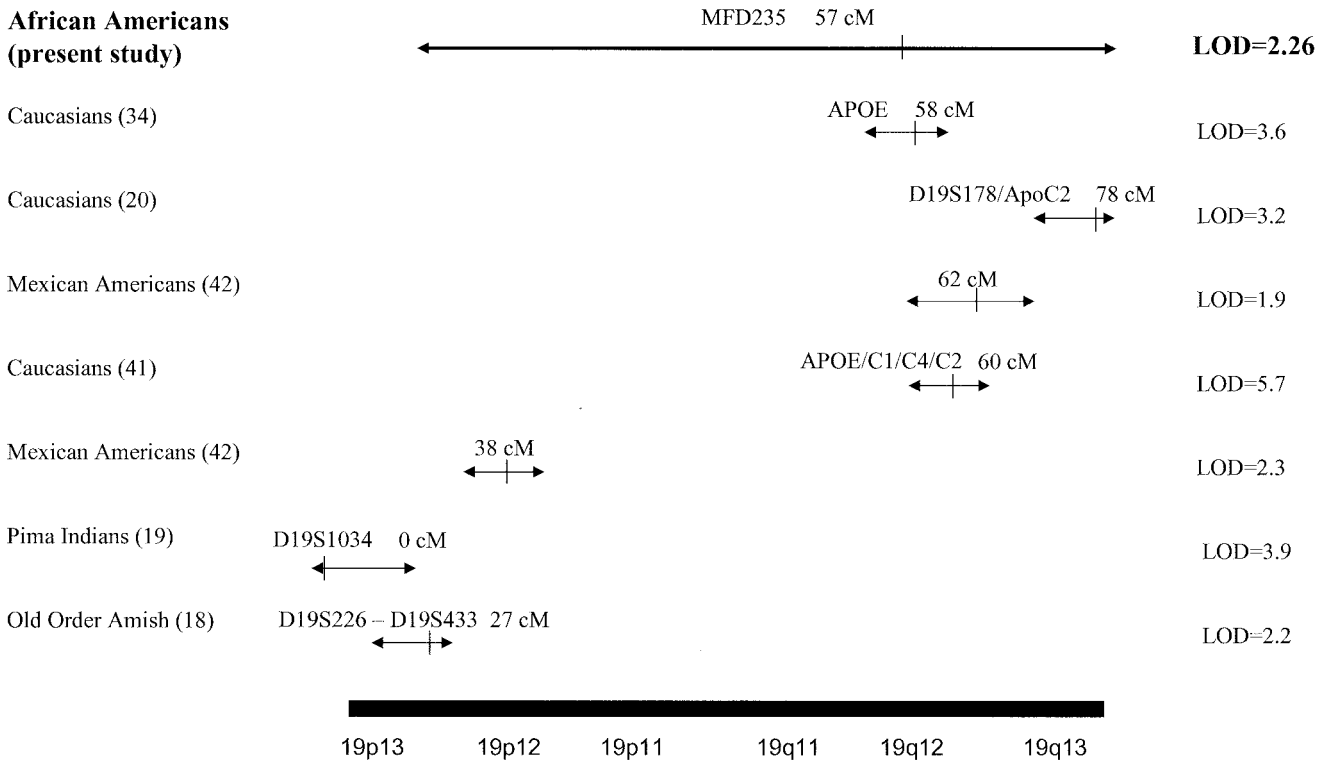

FIG. 3. A summary of studies showing evidence for linkage on chromosome 19. The ethnic background of the study population is given. Horizontal lines with arrows represent the approximate 1-LOD support interval on a cytogenetic map (bottom) for the respective studies. The closest marker and position (cM, vertical line) of the maximum LOD score or lowest *P* value (right) are indicated above the horizontal line. Two regions of linkage did not have closest marker information available (42). In addition, in the Old Order Amish study (18), the markers at the ends of the linkage interval are given.

LDL-2 (25.5–26.4 nm), were identified on 19p13.12-q12 and 19q13.12-q31.31, respectively, in Mexican Americans (42). Although the linkage signal obtained for chromosome 19 in the present study was not statistically significant, the strong support for linkage reported in other studies, particularly those using families with type 2 diabetic individuals, suggests the possibility that this region harbors genes that may impact the regulation of lipid traits in diabetic individuals.

A number of candidate genes with possible effects on lipid metabolism are located in the region of interest on chromosome 19. These include the genes encoding the LDL receptor (*LDLR*; MIM 606945), which mediates LDL uptake at the cell membrane; hormone-sensitive lipase (*LIPE*; MIM 151750), which converts cholesteryl esters to free cholesterol and hydrolyzes stored triglycerides to free fatty acids; apolipoprotein E (*APOE*; MIM 107741), which is essential for lipoprotein metabolism; and the upstream stimulatory factor 2 (*USF2*; MIM 600390), which belongs to the same nuclear hormone receptor family as *USF1* (MIM 191523), a key factor in the development of familial combined hyperlipidemia (43). In addition, several members of the cytochrome P450 family involved in cholesterol and steroid hormone biosynthesis are located in this region.

Although the present study provides strong support for the presence of loci on chromosomes 3, 11, and 19 that contribute to the control of lipid levels, a number of limitations remain. Even though many studies have shown evidence for linkage in regions overlapping the ones reported here, results from other studies do not support these findings (44,45). Disparities in linkage findings may result from different ascertainment schemes, dissimilar methods of statistical analysis, and variability in the power of a particular study sample to detect genetic linkage. Undoubtedly, failure to replicate findings among independent studies is also indicative of the complexity involved in the control of lipid levels. Furthermore, evidence for linkage of different lipid traits within the same region is suggestive of pleiotropic effects for an underlying gene(s). Although beyond the scope of the present study, multivariate analysis would be an appropriate method to address this possibility.

A second limitation of this study involves the use of lipid-lowering drugs. In the present study, we performed variance components analyses including individuals who self-reported use of lipid-lowering prescription drugs, although we recognize that the plasma lipid levels in these individuals may influence our findings. Loss of information was the primary reason for not removing these individuals from the analyses. For example, in our analyses, removal of individuals who self-reported lipid-lowering drug use (7 individuals from 6 African-American families, 40 individuals from 33 Hispanic families, and 66 individuals from 55 Caucasian families) did not eliminate the evidence for linkage but did reduce the LOD scores in the corresponding regions (2.26 to 2.07 in African Americans, 2.45 to 1.81 in Hispanics, and 3.36 to 1.69 in Caucasians; data not shown). Our results suggest that these individuals contributed to the evidence for linkage in these regions, and removing them from the variance components analyses would result in significant loss of information. Furthermore, in this study sample, removal of individuals on lipid-lowering medications may not be the most appropri-

ate way to account for drug use. First, all medication information was self-reported by the participants of the GENNID study and not confirmed by a qualified physician. Second, use of different types of drugs (i.e., statins, niacin, gemfibrozil, and resins), variable duration times of medication usage, and a lack of information on actual drug dosage make adjustments for medication use extremely complicated in the GENNID study populations. However, the fact that the results obtained in the present study identify chromosomal regions linked to quantitative lipid traits in other studies of families ascertained for type 2 diabetes suggests that our approach is tenable.

Finally, we cannot determine whether the loci identified in this study represent genes specifically linked to lipid traits under the control of hyperglycemia, hyperinsulinemia, or both. Because linkage for each trait has been found in both families ascertained for type 2 diabetes and families ascertained for other dichotomous traits such as CAD, as well as randomly ascertained samples, it may be possible that the genes underlying linkage peaks for diabetic dyslipidemia also contribute to lipid traits in the general population. That said, chromosomes 3 and 11 may harbor genes playing a role in the inheritance of lipids in both the general population and in individuals with lipid-related disorders, whereas the replication of the chromosome 19 peak in three other studies of families ascertained for type 2 diabetes suggests the presence of a gene affecting diabetic dyslipidemia in this region. However, until the specific genes within each region are identified and characterized, we cannot assign common loci to linkage signals obtained for lipid traits in differently ascertained study samples.

ACKNOWLEDGMENTS

This study was supported by the American Diabetes Association.

We thank the investigators and staff who contributed to the GENNID repository, as well as the individuals who participated in the GENNID study. We also appreciate the expert guidance and assistance of Tory Asfahani and Dr. Zhiying Wang of the American Diabetes Association.

APPENDIX

American Diabetes Association GENNID Study Group. Genetic material was collected and families characterized by Eric Boerwinkle, PhD, University of Texas Health Science Center; John Buse, MD, PhD, University of North Carolina; Ralph DeFronzo, MD, University of Texas Health Science Center; David Ehrmann, MD, University of Chicago; Steven C. Elbein, MD, University of Utah/University of Arkansas; Wilfred Fujimoto, MD, and Steven E. Kahn, MB, ChB, University of Washington; Craig L. Hanis, PhD, University of Texas Health Science Center; Richard A. Mulivor, PhD, and Jeanne C. Beck, PhD, Coriell Cell Repositories; Jill Norris, PhD, University of Colorado School of Medicine; M. Alan Permutt, MD, and Philip Behn, MD, Washington University School of Medicine; Leslie Raffel, MD, Cedars-Sinai Medical Center; and David C. Robbins, MD, Medlantic Research Institute.

REFERENCES

1. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 339:229–234, 1998
2. Schumacher MC, Maxwell TM, Wu LL, Hunt SC, Williams RR, Elbein SC: Dyslipidemias among normoglycemic members of familial NIDDM pedigrees. *Diabetes Care* 15:1285–1289, 1992
3. Laws A, Stefanick ML, Reaven GM: Insulin resistance and hypertriglyceridemia in nondiabetic relatives of patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 69:343–347, 1989
4. Krauss RM: Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care* 27:1496–1504, 2004
5. Brunzell JD, Ayyobi AF: Dyslipidemia in the metabolic syndrome and type 2 diabetes mellitus. *Am J Med* 115 (Suppl. 8A):24S–28S, 2003
6. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK: Cardiovascular risk factors in confirmed prediabetic individuals: does the clock for coronary heart disease start ticking before the onset of clinical diabetes? *JAMA* 263:2893–2898, 1990
7. Garvey WT, Kwon S, Zheng D, Shaughnessy S, Wallace P, Hutto A, Pugh K, Jenkins AJ, Klein RL, Liao Y: Effects of insulin resistance and type 2 diabetes on lipoprotein subclass particle size and concentration determined by nuclear magnetic resonance. *Diabetes* 52:453–462, 2003
8. Stamler J: Diet and coronary heart disease. *Biometrics* 38 (Suppl.):95–118, 1982
9. MacAuley D: Exercise, cardiovascular disease and lipids. *Br J Clin Pract* 47:323–327, 1993
10. Genest J: Lipoprotein disorders and cardiovascular risk. *J Inherit Metab Dis* 26:267–287, 2003
11. Davignon J, Genest J Jr: Genetics of lipoprotein disorders. *Endocrinol Metab Clin North Am* 27:521–550, 1998
12. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denefle P, Assmann G: Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat Genet* 22:352–355, 1999
13. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, Loubser O, Ouelette BF, Fichter K, Ashbourne-Excoffon KJ, Sensen CW, Scherer S, Mott S, Denis M, Martindale D, Frohlich J, Morgan K, Koop B, Pimstone S, Kastelein JJ, Genest J Jr, Hayden MR: Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat Genet* 22:336–345, 1999
14. Bodzioch M, Orso E, Klucken J, Langmann T, Bottcher A, Diederich W, Drobnik W, Barlage S, Buchler C, Porsch-Ozcurumez M, Kaminski WE, Hahmann HW, Oette K, Rothe G, Aslanidis C, Lackner KJ, Schmitz G: The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat Genet* 22:347–351, 1999
15. Beekman M, Heijmans BT, Martin NG, Pedersen NL, Whitfield JB, DeFaire U, van Baal GC, Snieder H, Vogler GP, Slagboom PE, Boomsma DI: Heritabilities of apolipoprotein and lipid levels in three countries. *Twin Res* 5:87–97, 2002
16. Shearman AM, Ordovas JM, Cupples LA, Schaefer EJ, Harmon MD, Shao Y, Keen JD, DeStefano AL, Joost O, Wilson PW, Housman DE, Myers RH: Evidence for a gene influencing the triglyceride/HDL-C ratio on chromosome 7q32.3-qter: a genome-wide scan in the Framingham study. *Hum Mol Genet* 9:1315–1320, 2000
17. Bosse Y, Chagnon YC, Despres JP, Rice T, Rao DC, Bouchard C, Perusse L, Vohl MC: Compendium of genome-wide scans of lipid-related phenotypes: adding a new genome-wide search of apolipoprotein levels. *J Lipid Res* 45:2174–2184, 2004
18. Pollin TI, Hsueh WC, Steinle NI, Snitker S, Shuldiner AR, Mitchell BD: A genome-wide scan of serum lipid levels in the Old Order Amish. *Atherosclerosis* 173:89–96, 2004
19. Imperatore G, Knowler WC, Pettitt DJ, Kobes S, Fuller JH, Bennett PH, Hanson RL: A locus influencing total serum cholesterol on chromosome 19p: results from an autosomal genomic scan of serum lipid concentrations in Pima Indians. *Arterioscler Thromb Vasc Biol* 20:2651–2656, 2000
20. Elbein SC, Hasstedt SJ: Quantitative trait linkage analysis of lipid-related traits in familial type 2 diabetes: evidence for linkage of triglyceride levels to chromosome 19q. *Diabetes* 51:528–535, 2002
21. Duggirala R, Blangero J, Almasy L, Dyer TD, Williams KL, Leach RJ, O'Connell P, Stern MP: A major susceptibility locus influencing plasma triglyceride concentrations is located on chromosome 15q in Mexican Americans. *Am J Hum Genet* 66:1237–1245, 2000
22. Raffel LJ, Robbins DC, Norris JM, Boerwinkle E, DeFronzo RA, Elbein SC, Fujimoto W, Hanis CL, Kahn SE, Permutt MA, Chiu KC, Cruz J, Ehrmann DA, Robertson RP, Rotter JI, Buse J: The GENNID study: a resource for mapping the genes that cause NIDDM. *Diabetes Care* 19:864–872, 1996
23. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502, 1972
24. Ehm MG, Karnoub MC, Sakul H, Gottschalk K, Holt DC, Weber JL, Vaske D, Briley D, Briley L, Kopf J, McMillen P, Nguyen Q, Reisman M, Lai EH,

Joslyn G, Shepherd NS, Bell C, Wagner MJ, Burns DK: Genomewide search for type 2 diabetes susceptibility genes in four American populations. *Am J Hum Genet* 66:1871–1881, 2000
25. Weeks DE, Sobel E, O'Connell JR, Lange K: Computer programs for multilocus haplotyping of general pedigrees. *Am J Hum Genet* 56:1506–1507, 1995
26. Hasstedt S: *PAP: Pedigree Analysis Package*. 5th ed. Salt Lake City, Utah, Department of Human Genetics, University of Utah, 2002
27. Pratt SC, Daly MJ, Kruglyak L: Exact multipoint quantitative-trait linkage analysis in pedigrees by variance components. *Am J Hum Genet* 66:1153–1157, 2000
28. Terwilliger JD, Speer M, Ott J: Chromosome-based method for rapid computer simulation in human genetic linkage analysis. *Genet Epidemiol* 10:217–224, 1993
29. Francke S, Manraj M, Lacquemant C, Lecoeur C, Lepretre F, Passa P, Hebe A, Corset L, Yan SL, Lahmidi S, Jankee S, Gunness TK, Ramjuttun US, Balgobin V, Dina C, Froguel P: A genome-wide scan for coronary heart disease suggests in Indo-Mauritians a susceptibility locus on chromosome 16p13 and replicates linkage with the metabolic syndrome on 3q27. *Hum Mol Genet* 10:2751–2765, 2001
30. Zhang X, Wang K: Bivariate linkage analysis of cholesterol and triglyceride levels in the Framingham Heart Study. *BMC Genet* 4 (Suppl. 1):S62, 2003
31. Hunt SC, Hopkins PN, Bulka K, McDermott MT, Thorne TL, Wardell BB, Bowen BR, Ballinger DG, Skolnick MH, Samuels ME: Genetic localization to chromosome 1p32 of the third locus for familial hypercholesterolemia in a Utah kindred. *Arterioscler Thromb Vasc Biol* 20:1089–1093, 2000
32. Hauser ER, Crossman DC, Granger CB, Haines JL, Jones CJ, Mooser V, McAdam B, Winkelmann BR, Wiseman AH, Muhlestein JB, Bartel AG, Dennis CA, Dowdy E, Estabrooks S, Eggleston K, Francis S, Roche K, Clevenger PW, Huang L, Pedersen B, Shah S, Schmidt S, Haynes C, West S, Asper D, Booze M, Sharma S, Sundseth S, Middleton L, Roses AD, Hauser MA, Vance JM, Pericak-Vance MA, Kraus WE: A genomewide scan for early-onset coronary artery disease in 438 families: the GENECARD Study. *Am J Hum Genet* 75:436–447, 2004
33. Naoumova RP, Bonney SA, Eichenbaum-Voline S, Patel HN, Jones B, Jones EL, Amey J, Colilla S, Neuwirth CK, Allotey R, Seed M, Betteridge DJ, Galton DJ, Cox NJ, Bell GI, Scott J, Shoulders CC: Confirmed locus on chromosome 11p and candidate loci on 6q and 8p for the triglyceride and cholesterol traits of combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 23:2070–2077, 2003
34. Bosse Y, Chagnon YC, Despres JP, Rice T, Rao DC, Bouchard C, Perusse L, Vohl MC: Genome-wide linkage scan reveals multiple susceptibility loci influencing lipid and lipoprotein levels in the Quebec Family Study. *J Lipid Res* 45:419–426, 2004
35. Coon H, Eckfeldt JH, Leppert MF, Myers RH, Arnett DK, Heiss G, Province MA, Hunt SC: A genome-wide screen reveals evidence for a locus on chromosome 11 influencing variation in LDL cholesterol in the NHLBI Family Heart Study. *Hum Genet* 111:263–269, 2002
36. Klos KL, Kardia SL, Ferrell RE, Turner ST, Boerwinkle E, Sing CF: Genome-wide linkage analysis reveals evidence of multiple regions that influence variation in plasma lipid and apolipoprotein levels associated with risk of coronary heart disease. *Arterioscler Thromb Vasc Biol* 21:971–978, 2001
37. Aouizerat BE, Allayee H, Cantor RM, Davis RC, Lanning CD, Wen PZ, Dallinga-Thie GM, de Bruin TW, Rotter JI, Lusis AJ: A genome scan for familial combined hyperlipidemia reveals evidence of linkage with a locus on chromosome 11. *Am J Hum Genet* 65:397–412, 1999
38. Sonoda J, Chong LW, Downes M, Barish GD, Coulter S, Liddle C, Lee CH, Evans RM: Pregnane X receptor prevents hepatorenal toxicity from cholesterol metabolites. *Proc Natl Acad Sci U S A* 102:2198–2203, 2005
39. Laitinen S, Olkkonen VM, Ehnholm C, Ikonen E: Family of human oxysterol binding protein (OSBP) homologues: a novel member implicated in brain sterol metabolism. *J Lipid Res* 40:2204–2211, 1999
40. Wassif CA, Maslen C, Kachilele-Linjewile S, Lin D, Linck LM, Connor WE, Steiner RD, Porter FD: Mutations in the human sterol delta7-reductase gene at 11q12-13 cause Smith-Lemli-Opitz syndrome. *Am J Hum Genet* 63:55–62, 1998
41. Beekman M, Heijmans BT, Martin NG, Whitfield JB, Pedersen NL, DeFaire U, Snieder H, Lakenberg N, Suchiman HE, de Knijff P, Frants RR, van Ommen GJ, Kluft C, Vogler GP, Boomsma DI, Slagboom PE: Evidence for a QTL on chromosome 19 influencing LDL cholesterol levels in the general population. *Eur J Hum Genet* 11:845–850, 2003
42. Rainwater DL, Almasy L, Blangero J, Cole SA, VandeBerg JL, MacCluer JW, Hixson JE: 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, 1999
43. Pajukanta P, Lilja HE, Sinsheimer JS, Cantor RM, Lusis AJ, Gentile M, Duan XJ, Soro-Paavonen A, Naukkarinen J, Saarela J, Laakso M, Ehnholm C, Taskinen MR, Peltonen L: Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). *Nat Genet* 36:371–376, 2004
44. Pajukanta P, Allayee H, Krass KL, Kuraishy A, Soro A, Lilja HE, Mar R, Taskinen MR, Nuotio I, Laakso M, Rotter JI, de Bruin TW, Cantor RM, Lusis AJ, Peltonen L: Combined analysis of genome scans of dutch and finnish families reveals a susceptibility locus for high-density lipoprotein cholesterol on chromosome 16q. *Am J Hum Genet* 72:903–917, 2003
45. Knoblauch H, Muller-Myhsok B, Busjahn A, Ben Avi L, Bahring S, Baron H, Heath SC, Uhlmann R, Faulhaber HD, Shpitzen S, Aydin A, Reshef A, Rosenthal M, Eliav O, Muhl A, Lowe A, Schurr D, Harats D, Jeschke E, Friedlander Y, Schuster H, Luft FC, Leitersdorf E: A cholesterol-lowering gene maps to chromosome 13q. *Am J Hum Genet* 66:157–166, 2000