Genome-wide scan for quantitative trait loci influencing LDL size and plasma triglyceride in familial hypertriglyceridemia

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Abstract **Small, dense LDLs and hypertriglyceridemia, two highly correlated and genetically influenced risk factors, are known to predict for risk of coronary heart disease. The objective of this study was to perform a whole-genome scan for linkage to LDL size and triglyceride (TG) levels in 26 kindreds with familial hypertriglyceridemia (FHTG). LDL size was estimated using gradient gel electrophoresis, and genotyping was performed for 355 autosomal markers with an average heterozygosity of 76% and an average spacing of 10.2 centimorgans (cMs). Using variance components linkage analysis, one possible linkage was found for LDL size [logarithm of odds (LOD) = 2.1] on chromosome 6, peak at 140 cM distal to marker F13A1 (closest marker D6S2436). With adjustment for TG and/or HDL cholesterol, the LOD scores were reduced, but remained in exactly the same location. For TG, LOD scores of 2.56 and 2.44 were observed at two locations on chromosome 15, with peaks at 29 and 61 cM distal to marker D15S822 (closest markers D15S643 and D15S211, respectively). These peaks were retained with adjustment for LDL size and/or HDL cholesterol. These findings, if confirmed, suggest that LDL particle size and plasma TG levels could be caused by two different genetic loci in FHTG.**—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. **Genome-wide scan for quantitative trait loci influencing LDL size and plasma triglyceride in familial hypertriglyceridemia.** ***J. Lipid Res.*** **2003.** 44: **2161–2168.**

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The causal relationship between LDL cholesterol and risk of coronary heart disease (CHD) is definitively established (1). But growing evidence reveals that other lipoproteins, including small, dense LDL particles, as well as increased plasma triglycerides (TGs) and lower HDL cholesterol levels, all characteristics of the metabolic syndrome (2), are also convincingly associated with atherosclerosis risk. Although these risk factors are all intercorrelated (3, 4), each of them is also an independent risk factor. For example, a meta-analysis of three prospective studies in middle-aged men (4–6) showed a 60% increased risk for CHD for every 10 Å decrease in LDL size (7). Adjustment for TG and HDL cholesterol reduced this to a 30% increased risk, but the odds ratio remained statistically significant, demonstrating that small LDL is an independent risk factor. Other studies have found even higher risk associated with CHD among young women (8), but lower risks among older populations (9, 10).

Elevated plasma TG is now also recognized as an important independent risk factor for CHD (11–14). In familial hypertriglyceridemia (FHTG), baseline TG levels predicted increased risk of cardiovascular disease mortality among first-degree relatives of probands during 20 years of follow-up, independent of baseline cholesterol levels and other covariates (15). Even more convincing data demonstrate that increasing HDL cholesterol levels can reduce risk of CHD among men with low HDL cholesterol levels (16, 17).

Numerous studies have demonstrated both genetic and environmental influences on LDL size, plasma TG, and HDL cholesterol (18, 19). For example, segregation analyses (20, 21) have consistently demonstrated single major gene effects on LDL size. These and other studies have

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also shown that LDL size is influenced by sex, age, central obesity (22), and in women, menopausal status and hormone use (23–25). Several candidate gene studies have investigated the effects of apolipoprotein structural genes (26, 27), receptors (28, 29), and enzymes involved in lipoprotein metabolism (30–33) on LDL size, with varying results.

Using a quantitative genetic analysis approach, we have previously demonstrated pleiotropic genetic effects on LDL particle size, TG, and HDL cholesterol levels in the familial forms of hypertriglyceridemia (34). These findings illustrated that the well-established phenotypic correlations between these variables (4) reflect strong, underlying genetic correlations in these types of families. For example, the phenotype correlation between LDL size and TG (ρ_p) was −0.66, and the genetic correlation was even higher ($\rho_g = -0.87$, $P < 0.001$). However, the results also demonstrated the presence of substantial nonshared effects on these risk factors: 55% of the variance in LDL size was attributable to effects not shared with TG, while 52% of the variance in TG was due to effects not shared with LDL size. Taken together, these results demonstrate the complexity of characterizing genetic influences on these important risk factors.

The present study uses a whole-genome scan approach to identify chromosomal locations influencing LDL size, TG, and HDL cholesterol. Each lipoprotein risk factor was adjusted for the other two risk factors in order to isolate the nonshared genetic effects, and the analysis focuses on FHTG families to minimize genetic heterogeneity. Thus, the purpose of this study was to perform a genome-wide scan in FHTG families to genetically map chromosomal regions influencing LDL particle size, TG, and HDL cholesterol.

METHODS

Study sample

Data for this study were obtained as part of an historical cohort family study, the Genetic Epidemiology of Hypertriglyceridemia Study, based on hypertriglyceridemic families originally ascertained as part of two studies conducted at the University of Washington (35, 36) in the early 1970s (baseline), and followed up between 1994 and 1997 (15). Details of recruitment procedures have been published (34). Briefly, a self-administered medical history questionnaire and a fasting blood sample were requested from all living relatives who could be contacted. Eligible family members were age 18 or over, were not pregnant, and were not too ill to participate. For the present analysis, 26 extended FHTG kindreds with informative genotype data, lipoprotein data, and medical history information were used, all but one of which was Caucasian, using the family classifications determined at baseline (35, 36).

Baseline study participants provided written, informed consent at the time they were enrolled in the early 1970s. The University of Washington Institutional Review Board approved the methods used to recontact living family members who had participated at baseline and to obtain blood samples and medical history data from living family members. All study information was kept confidential and was not shared with other family members.

Laboratory measurements

LDL subclasses were characterized using 2–14% polyacrylamide gels produced at the University of Washington and by applying electrophoresis procedures originally described by Krauss and Burke (37). The gels were produced using the method described by Austin et al. (38) and Rainwater and colleagues (39). The estimated diameter for the major peak from the gel scan was designated "LDL peak particle diameter" (LDL size) and was used as a continuous variable in the linkage analyses. Plasma TG measurements and other lipid and lipoprotein analyses were performed at the Core Laboratory, Northwest Lipid Research Laboratories in Seattle, one of the five reference laboratories of the National Reference System for Cholesterol, coordinated by the Centers for Disease Control (40–42). Assays of apolipoprotein A-I (apoA-I) and apoB were performed nephelometrically (42, 43).

Genetic markers and genotyping

Lymphocyte DNA samples for each study participant were used to genotype microsatellite markers distributed throughout the genome, including 355 autosomal markers with an average heterozygosity of 76% and an average spacing of 10.2 centimorgans (cMs). Sex chromosome markers were also genotyped for the purpose of checking for correct sex. Automated multiplex genotyping used PCR with fluorescently labeled primer pairs from the Cooperative Human Linkage Center (MapPairs version 8, Research Genetics, Inc.), followed by electrophoresis on an ABI DNA Sequencer (Model 377).

A total of 143 FHTG family members were genotyped. All genotypes were checked for Mendelian consistency using the GENCHECK and INFER programs in the PEDSYS package (44), and discrepancies were checked by retyping samples. For three family members, patterns of non-Mendelian inheritance were observed for 200 or more markers. These individuals were excluded from the linkage analysis. Among the remaining individuals, the genotyping error rate was less than 0.26%. These 140 subjects were used in the linkage analysis, except for the markers with discrepancies, which were not included. Thus, the linkage analysis reported here is based on 140 relatives in 26 FHTG families (**Table 1**).

Building the genetic marker map

We estimated marker locus-specific identity-by-descent (IBD) probabilities for the pedigrees using a pairwise maximum likelihood-based procedure (45). To permit multipoint analysis for quantitative trait locus (QTL) mapping, we employed an extension of the method of Fulker, Cherny, and Cardon (46) to estimate IBD probabilities at 1 cM intervals along each chromosome, using a constrained linear function of observed IBD probabilities of markers at known locations within the region. This multipoint procedure, which yields substantially greater power to localize QTLs than two-point, locus-specific methods, enabled better localization of the QTL. For the current data set, a loga-

TABLE 1. Characteristics of FHTG kindreds

Number of Kindreds	26
Size range of kindreds	2–15
Number of family members	140
Age (mean ± SD)	48.5 ± 16.2
Sex (% female)	57.9
Oral contraceptive use (%)[a]	10.0
Postmenopausal (%)[a]	44.9
Postmenopausal hormone use (%)[a]	23.8

FHTG, familial hypertriglyceridemia.
[a] Based on women only. Menopausal status missing for three women.

rithm of odds (LOD) score evaluation was performed every cM along each chromosome. Relying on published orders for human marker loci (e.g., http://research.marshfieldclinic.org/genetics/Map_Markers/maps), determination of distances between markers was facilitated by the expert system program MultiMap (47–49), which implements routines of the computer program CRIMAP (47) for computation of two-point and multipoint likelihoods.

Linkage analysis using variance components methods

Multipoint variance components analysis was used to test for linkage between genetic locations and LDL particle size using procedures contained in the Sequential Oligogenic Linkage Analysis Routines (SOLAR) package (45). In this approach, the expected genetic covariances between relatives are expressed as a function of the IBD relationship at a genomic location that is assumed to be closely linked to a QTL influencing the risk factor. For a given kindred, the covariance matrix for a completely additive model is given in Equation 1 by

$$\Pi\sigma_q^2 + 2\Phi\sigma_g^2 + I\sigma_e^2 \qquad \textit{(Eq. 1)}$$

where Π is the matrix of IBD probabilities at the location of interest, Φ is the matrix of kinship coefficients, and I is the identity matrix. The components of variance correspond to the additive genetic variation for the QTL linked to the location being tested (σ_q^2), the residual additive genetic effects (σ_g^2), and residual variation (σ_e^2). Assuming multivariate normality, a QTL linked to the location of interest can be detected by evaluating whether $\sigma_q^2 = 0$.

Analyses for LDL particle size, TG, and HDL cholesterol were repeated without adjustment for covariates, using stepwise selection for covariates and adjusting for all covariates. The results of these three analyses were similar, and thus only the findings based on the "full models," including all covariates, are reported here. These covariates were sex and age, and in women, oral contraceptive use, menopausal status, and hormone replacement, based on medical history questionnaire data. For the latter three variables, women who reported "uncertain" for these items were coded as "no." Menopausal status was missing for three women, and they were excluded from the linkage analysis. Although the statistical significance of these covariates varied in different models, they were all included in the models, because previous studies have consistently demonstrated their effects on LDL particle size (3, 24, 50).

In addition to the full models with the above covariates, the genome scan linkage analysis for LDL size was repeated with adjustments for TG, for HDL cholesterol, and for both TG and HDL cholesterol. Similarly, the genome scan linkage analysis for TG was repeated with adjustment for LDL size, for HDL cholesterol, and for both LDL size and HDL cholesterol. Finally, the genome scan linkage analysis for HDL cholesterol was repeated with adjustments for TG, for LDL size, and for both TG and LDL size. A one-LOD score support interval was calculated for the maximum LOD score result for each lipoprotein variable (51).

Oligogenic linkage analysis, in which multiple QTL effects are jointly estimated, was also performed when a single LOD score peak was identified in the analysis. Specifically, when only one linkage peak was observed, the multipoint linkage analysis was repeated, fixing the location of the QTL with the highest LOD score and performing a conditional genome screen (45).

RESULTS

Characteristics of FHTG families

The characteristics of the FHTG family members included in the genome-wide scan are presented in **Tables** 1 and **2**.

TABLE 2. Lipid, lipoprotein, and apolipoprotein characteristics of FHTG family members

	Mean ± SD	Minimum, Maximum
LDL particle size (Å)	264.4 ± 9.4	246, 287
TG (mg/dl)	175.8 ± 158.8	29, 1,250
HDL cholesterol (mg/dl)	46.8 ± 16.2	15, 117
Total cholesterol (mg/dl)	194.4 ± 36.5	95, 281
LDL cholesterol (mg/dl)	112.9 ± 32.3	49, 211
ApoA-I (mg/dl)	136.9 ± 30.0	75, 250
ApoB (mg/dl)	100.7 ± 25.9	36, 165

ApoA-I, apolipoprotein A-I; TG, triglyceride.

Family sizes ranged from two to 15 family members, with an average age of ~49 years, and slightly more than half of the family members were female. Of the women in the families, 10% used oral contraceptives, approximately half were postmenopausal, and about one-fourth were taking postmenopausal hormones. Among all the family members, the mean LDL particle size was ~265 Å, ranging from 246 Å to 287 Å. Similar to previous studies (50, 52), the distribution of LDL size members was bimodal (data not shown), with a skewness value of 0.08. Plasma TG values ranged widely, from 29 to 1,250 mg/dl. Because of the skewness of the distribution (skewness coefficient = 3.66), a natural log (*ln*) transformation was used in the analysis, reducing the skewness value to 0.28. The average HDL cholesterol level was 46.7 mg/dl, with a wide range of 15 to 177 mg/dl.

Linkage analysis for LDL size

Without any lipoprotein covariates in the model, residual heritability for LDL size was 0.26 ($P = 0.025$) in the FHTG families (**Table 3**), while the other covariates (sex, age, oral contraceptive use, menopausal status, and hormone replacement therapy) explained 21% of the variance in LDL size. For the whole-genome scan, only one chromosomal region provided possible evidence for linkage to LDL particle size on chromosome 6, ~140 cM distal from marker F13A1, with an LOD score of 2.1 (Table 3). This QTL explained all of the residual heritability at this location in the FHTG families, and the closest marker was D6S2436, located at 137.6 cM from F13A1. **Figure 1** shows the multipoint LOD score profile for chromosome 6 in the FHTG families (solid line), the one-LOD score support interval, and locations of markers. Although the peak LOD score is clearly seen, the width of the support interval is large. LOD scores at this location ranged from −0.45 to 0.84 among the 26 individual FHTG families. When this chromosome 6 location was fixed for the oligogenic linkage analysis, no conditional LOD scores >1.0 were found.

When the lipoprotein covariates (*ln* TG and/or HDL cholesterol) were included in the models, the residual heritability values were reduced, and, as expected, the proportion of variance explained by all the covariates increased (Table 3). However, the maximum LOD scores for these models remained in exactly the same location on chromosome 6, ~140 cM distal from marker F13A1. The

TABLE 3. Linkages analysis of adjusted LDL particle size in FHTG families

Lipoprotein Covariates in Model[a]	Residual Heritability[b]	Proportion of Variance Due to All Covariates (Significant Covariates)	Maximum LOD Score, Nearest Flanking Marker	Chromosome (Location[c]), 1-LOD Score Support Interval[c]
None	0.26 ($P = 0.025$)	0.21 (sex)	2.10, D6S2436	6 (140) 116–158
ln TG ($P < 0.001$)	0.12 ($P = 0.168$)	0.54 (sex)	1.82, D6S2436	6 (138) 120–155
HDL cholesterol ($P < 0.001$)	0.15 ($P = 0.121$)	0.43 (sex, hormone replacement therapy)	1.84, D6S2436	6 (138) 110–160
ln TG ($P < 0.001$) and HDL cholesterol ($P = 0.002$)	0.10 ($P = 0.213$)	0.67 (sex, hormone replacement therapy)	1.79, D6S2436	6 (138) 120–155

ln, natural log; LOD, logarithm of odds.
[a] Additional covariates: sex, age, oral contraceptive use, menopausal status, and hormone replacement therapy.
[b] Heritability remaining after accounting for the mean effects of covariates.
[c] Locations refer to approximate cM distal to F13A1 on chromosome 6; flanking markers for location 140 are D6S2436 and D6S305.

multipoint LOD score profiles were also very similar, as illustrated in Fig. 1, with the model adjusting for both *ln* TG and HDL cholesterol (dotted line).

Linkage analysis for TG

For *ln* TG, the highest LOD score in the genome scan was 2.56, ~39 cM distal to marker D15S822 on chromosome 15 (**Table 4** and **Fig. 2**, solid line). The closest marker was D15S643, located at 39.9 cM from D15S822. An apparent second peak was seen at location 61 (LOD = 2.44). The flanking marker was D15S211, located 62.1 cM from D15S822. LOD scores at the highest peak, location 39, ranged from −0.30 to 0.72 among the individual FHTG kindreds. With adjustments for LDL size, HDL cholesterol, or both, residual heritability values changed somewhat but remained statistically significant (Table 4). Similar to the results for LDL size, the LOD score peak locations remained virtually identical with these adjustments, and LOD score profiles remained similar, as shown by the model, adjusting for both LDL size and HDL cholesterol (Fig. 2, dotted line). In addition, the second peak location for each of these models remained similar: LOD = 1.37 at location 62, LOD = 1.21 at location 66, and LOD = 1.35 at location 67, with adjustments for LDL size, HDL cholesterol, and both LDL size and HDL cholesterol, respectively. The only other possible linkage was for *ln* TG, without adjustment for lipoproteins, with an LOD score of 2.11 on chromosome 5, ~113 cM from marker D5S2488. However, with adjustment for LDL size, the LOD score at this location was reduced to 0.64.

Linkage analysis for HDL cholesterol

The results for HDL cholesterol were less consistent and are presented in **Table 5**. Without adjustment for lipoproteins, the highest LOD score in the genome scan linkage analysis was 1.97 on chromosome 8. With adjustment for *ln* TG, the highest LOD score in the genome scan was 1.95, on a different chromosome, 15. With adjustment for LDL size, a similar LOD score of 1.90 was found on a third chromosome, 18. In the final model adjusting HDL cholesterol for both *ln* TG and LDL size, an LOD score value of 2.02 was also found on chromosome 18, ~42 cM from the marker GATA178F11. The closest flanking marker was D18S877, located at 42.1 cM from GATA178F11.

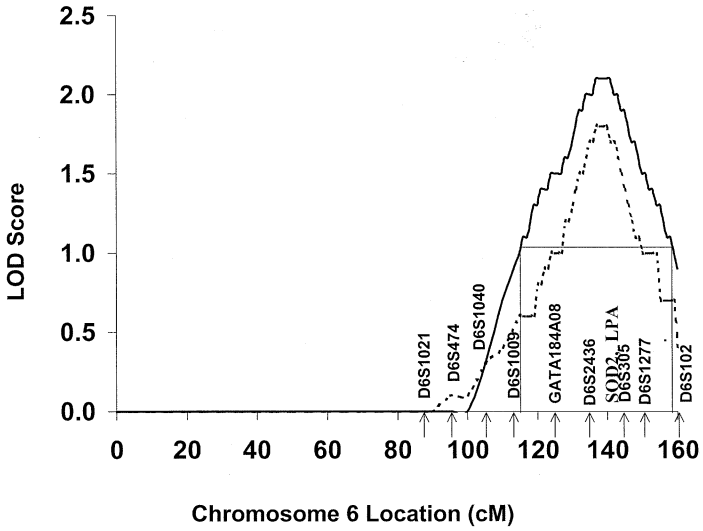

Fig. 1. Chromosome 6 multipoint logarithm of odds (LOD) score profile for LDL particle size in familial hypertriglyceridemia (FHTG) families adjusted for sex and age, and in women, oral contraceptive use, menopausal status, and hormone replacement therapy (solid line, full model). The one-LOD score support interval is shown, and locations of markers are indicated by arrows. The approximate locations of the superoxide dismutase gene (SOD2) and lipoprotein(a) (LPA) gene are also shown. Dotted line, LOD score profile for LDL particle size in FHTG families with additional adjustment for natural log (*ln*) triglyceride (TG) and HDL cholesterol.

TABLE 4. Linkage analysis of adjusted *ln* TG in FHTG families

Lipoprotein Covariates in Model[a]	Residual Heritability[b]	Proportion of Variance Due to All Covariates (Significant Covariates)	Maximum LOD Score, Nearest Flanking Marker	Chromosome (Location), 1-LOD Score Support Interval[c]
None	0.49 ($P = 0.002$)	0.13 (age)	2.56, D15S643	15 (39), 30–46
			2.44, D15S211	15 (61), 48–71
LDL particle size ($P < 0.001$)	0.34 ($P = 0.036$)	0.50 (age, oral contraceptive use)	1.67, D15S643	15 (40), 25–80
			1.37, D15S211	15 (62), 20–75
HDL cholesterol ($P < 0.001$)	0.53 ($P = 0.008$)	0.39 (age, oral contraceptive use, hormone replacement therapy)	1.90, D15S643	15 (36), 23–57
			1.21, D15S655	15 (66), 15–85
LDL particle size ($P < 0.001$) and HDL cholesterol ($P < 0.001$)	0.42 ($P = 0.027$)	0.55 (age, oral contraceptive use)	2.13, D15S643	15 (37), 23–44
			1.35, D15S655	15 (67), 15–80

[a] Additional covariates: sex, age, oral contraceptive use, menopausal status, and hormone replacement therapy.
[b] Heritability remaining after accounting for the mean effects of covariates.
[c] Locations refer to approximate cM distal to D15S822 on chromosome 15; flanking markers for location 39 are D15S659 and D15S643.

DISCUSSION

In this genome-wide scan of kindreds with FHTG, the highest LOD score for LDL size was 2.10 on chromosome 6, ~140 cM distal to marker F13A1, and the linkage peak remained in the same location after adjustment for *ln* TG, HDL cholesterol, or both (Table 3). However, the residual heritability of LDL size was reduced in these adjusted analyses, perhaps due to the strong genetic correlations of LDL size with *ln* TG ($\rho_g = -0.87$, $P < 0.001$) and HDL cholesterol ($\rho_g = 0.65$, $P < 0.001$) in these families (34). For TG, two apparent peaks were noted on chromosome 15, with LOD scores of 2.56 and 2.44, ~39 cM and 61 cM, respectively, distal to marker D15S822. However, it is also possible that this is one broad linkage peak, as suggested by the overlapping one-LOD support intervals in adjusted analyses (Table 4). These linkage peaks remained at the same locations with adjustment of *ln* TG for LDL size, HDL cholesterol, or both. Similar to the findings for LDL size, these results suggest the presence of a genetic locus influencing TG in FHTG families that does not involve pleiotropic effects with LDL size or HDL cholesterol. Two previous genome-wide scan studies have reported linkage of TG to this region of chromosome 15, one in Mexican-American families (LOD = 3.88 near D15S165) (53) and the other in white sibling pairs from the HyperGEN study (LOD = 1.9) (54). Taken together, these results support the presence of specific chromosomal locations independently influencing LDL size and TG, despite the strong genetic correlations between these two risk factors (34).

The region of chromosome 6 showing linkage to LDL particle size in the FHTG families includes the superoxide dismutase gene (SOD2). This candidate gene is of interest because of the findings that small, dense LDL particles are more susceptible to oxidation than are large LDL particles (55, 56). Two previous studies have investigated link-

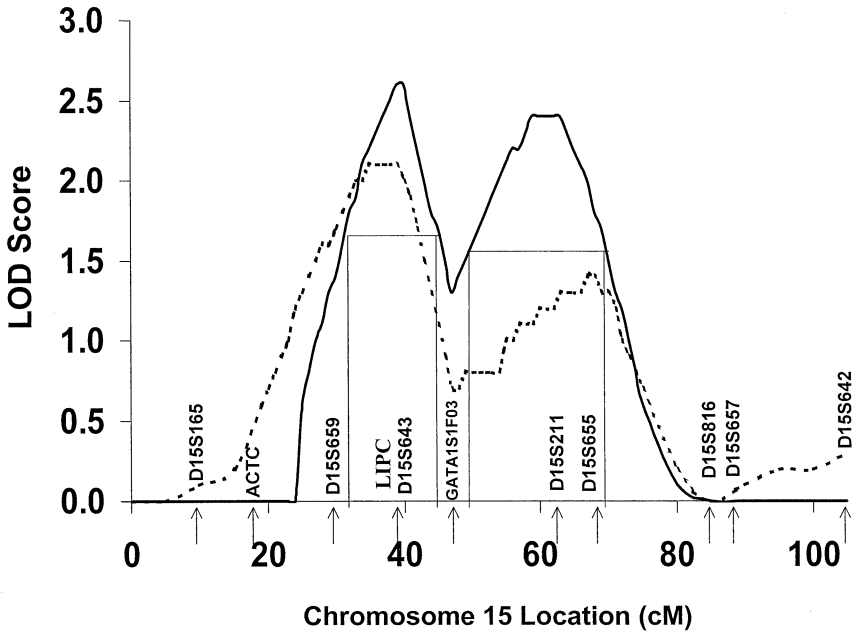

Fig. 2. Chromosome 15 multipoint LOD score profile for *ln* TG in FHTG families, adjusted for sex and age, and in women, oral contraceptive use, menopausal status, and hormone replacement therapy (solid line, full model). The one-LOD score support intervals are shown, and locations of markers are indicated by arrows. The approximate location of the hepatic lipase (LIPC) gene is also shown. Dotted line, LOD score profile for *ln* TG with additional adjustment for LDL size and HDL cholesterol.

TABLE 5. Linkage analysis of adjusted HDL cholesterol in FHTG families

Lipoprotein Covariates in Model[a]	Residual Heritability[b]	Proportion of Variance Due to All Covariates (Significant Covariates)	Maximum LOD Score, Nearest Flanking Marker	Chromosome (Location), 1-LOD Score Support Interval[c]
None	0.52 ($P = 0.004$)	0.29 (sex, age, hormone replacement therapy)	1.97, D8S1179	8 (123) 97–137
ln TG ($P < 0.001$)	0.59 ($P = 0.013$)	0.49 (oral contraceptive use, hormone replacement therapy)	1.95, D15S657	15 (92) 80–106
LDL particle size ($P < 0.001$)	0.39 ($P = 0.045$)	0.49 (hormone replacement therapy)	1.90, D18S877	18 (40) 20–72
ln TG ($P < 0.001$) and LDL particle size ($P = 0.003$)	0.50 ($P = 0.029$)	0.54 (oral contraceptive use, hormone replacement therapy)	2.02, D18S877	18 (42) 25–70

[a] Additional covariates: sex, age, oral contraceptive use, menopausal status, and hormone replacement therapy.
[b] Heritability remaining after accounting for the mean effects of covariates.
[c] Locations refer to approximate cM distal to D8S264 on chromosome 8, D15S822 on chromosome 15, and GATA178F11 on chromosome 18. Flanking markers for location 40 on chromosome 18 are D18S542 and D18S877.

age of this locus to LDL size variation. Among sib pairs from coronary artery disease families, evidence for linkage of LDL size to this gene was reported (57). However, among families of hyperlipidemic probands, and using LDL subclass phenotype classifications, strong evidence against linkage was reported (29). Using a different definition of LDL heterogeneity, results from the San Antonio Family Heart Study show an LOD score of 2.92 for cholesterol concentration in small LDL particles in the same region of chromosome 6 as reported here (33).

The lipoprotein(a) gene is also located within the support interval of linkage on chromosome 6 among the FHTG families. Although no linkage or association studies of LDL particle size and the lipoprotein(a) gene have been performed, a recent analysis demonstrated that LDL peak density correlated with lipoprotein(a) density ($r = 0.71$, $P < 0.001$), suggesting a metabolic interrelationship between LDL particles and lipoprotein(a) particles (58).

With the exception of these two candidate genes, no linkage signals were detected in the sample of FHTG families to any genes previously reported as candidates for LDL particular size. These include the LDL receptor gene (28), the apoE and apoB genes (26, 27), the lipoprotein lipase gene (31), and the cholesterol ester transfer protein gene (32). Although the relatively small sample size in this study may have limited statistical power to detect the linkages, the results suggest that these candidate genes may not influence LDL size in this familial form of hypertriglyceridemia.

The regions of linkage to *ln* TG on chromosome 15 include only one known candidate gene, hepatic lipase (LIPC). This gene includes 9 exons spanning ~35 kb (59), and hepatic lipase enzyme plays an important role in lipoprotein metabolism. After secretion by the liver, hepatic lipase remains on the surface of hepatic endothelial cells and hepatocytes, bound to proteoglycans, where it acts on chylomicron remnants, IDLs, and HDLs. Genetic variants in the LIPC gene, especially promoter polymorphisms, have been most consistently associated with variation in HDL cholesterol levels and HDL subfractions, including in samples of Caucasians, Japanese, African-American men, and normolipidemic men with CHD (60–64). Furthermore, the common −514C > T promoter variant has been reported to determine clinical response to intensive lipid-lowering treatment (30), and another promoter polymorphism (−480C > T) has recently been associated with coronary calcification in type 1 diabetes (65). The only report of linkage between LIPC and TG was a sib pair analysis in FCHL families ($P < 0.026$), in which linkage was also observed to LDL size ($P < 0.019$) and HDL cholesterol ($P < 0.003$) (66). In another sample of FCHL families, an LIPC polymorphism was associated with both dyslipidemia and insulin resistance (67). Additional genomic studies will be needed to determine whether the chromosome 15 linkage reported here is attributable to the LIPC gene.

Although the results for HDL cholesterol in the FHTG families were not as consistent as the findings for LDL size and TG, data from the San Antonio Family Heart Study have also reported linkage to regions of chromosomes 8 and 15 (68) similar to the results reported here for the model with no lipoprotein covariates and the model adjusting HDL cholesterol for *ln* TG (Table 5), respectively. In the San Antonio study, the proposed chromosome 8 locus was specific to unesterified HDL cholesterol levels, while the chromosome 15 linkage influenced a variety of HDL-related phenotypes. Both of these chromosomal regions are homologous to mouse QTL regions for HDL cholesterol as well (69). To our knowledge, suggestive linkage of HDL cholesterol to chromosome 18, as found here in models that adjust for LDL size, has not been previously reported.

The only other locus associated with FHTG is the ileal bile acid transporter gene, SLC10A2, on chromosome 13q33. A frameshift mutation in exon 4 of this gene results in a nonfunctional, truncated protein and has been characterized in one FHTG patient (70). In addition, no evidence was found in this study for linkage of LDL size to chromosome 17q, as was recently reported in the Quebec Family Study (71).

In summary, this whole-genome scan in 26 FHTG kindreds revealed possible linkage for LDL particle size on

chromosome 6 (LOD = 2.1) and for TG on chromosome 15 (LOD = 2.6), independent of other lipoprotein risk factors. Identifying and characterizing the genes responsible for characteristic phenotypes of these familial lipid disorders will increase our understanding of genetic susceptibility to atherosclerosis.

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