A genome-wide linkage scan identifies multiple quantitative trait loci for HDL-cholesterol levels in families with premature CAD and MI

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Abstract Plasma HDL cholesterol levels (HDL-C) are an independent predictor of coronary artery disease (CAD). We have completed a genome-wide linkage scan for HDL-C in a US cohort consisting of 388 multiplex families with premature CAD (GeneQuest). The heritability of HDL-C in GeneQuest was 0.37 with gender and age as covariates ($P = 5.1 \times 10^{-4}$). Two major quantitative trait loci (QTL) for log-transformed HDL-C adjusted for age and gender were identified onto chromosomes 7p22 and 15q25 with maximum multipoint logarithm of odds (LOD) scores of 3.76 and 6.69, respectively. Fine mapping decreased the 7p22 LOD score to a nonsignificant level of 3.09 and split the 15q25 QTL into two loci, one minor QTL on 15q22 (LOD = 2.73) that spanned the *LIPC* gene, and the other at 15q25 (LOD = 5.63). A family-based quantitative transmission disequilibrium test (QTDT) revealed significant association between variant rs1800588 in *LIPC* and HDL-C in the GeneQuest population ($P = 0.0067$), which may account for the minor QTL on 15q22. The 15q25 QTL is the most significant locus identified for HDL-C to date, and these results provide a framework for the ultimate identification of the underlying HDL-C variant and gene on chromosomes 15q25, which will provide insights into novel regulatory mechanisms of HDL-C metabolism.—Yang, R. L. Li, S. B. Seidelmann, G-Q. Shen, S. Sharma, S. Rao, K. G. Abdullah, K. G. MacKinlay, R. C. Elston, Q. Chen, E. J. Topol, Q. K. Wang. **A genome-wide linkage scan identifies multiple quantitative trait loci for HDL-cholesterol levels in families with premature CAD and MI. *J. Lipid Res.* 2010.** 51: **1442–1451.**

Supplementary key words genetics • linkage • single nucleotide polymorphism • high density lipoprotein cholesterol • coronary artery disease • myocardial infarction • genome-wide scan • quantitative trait locus • *LIPC* gene

Coronary artery disease (CAD) and its principal clinical complication of acute myocardial infarction (MI) represent the most important causes of death and disability in the developed world. According to the American Heart Association's 2009 statistical update, nearly 16.8 million Americans are affected with CAD, and 7.9 million have had a myocardial infarction. On average, an American will suffer from a coronary event every 26 s, and about every minute somebody will die from one (1). The lifetime risk of developing CAD after age 40 is 49% for men and 32% for women (1).

A decreased concentration of plasma HDL-cholesterol (HDL-C) is a major risk factor for CAD, and epidemiological evidence from several longitudinal studies, including the Framingham Heart Study, indicate that HDL-C is an

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Abbreviations: CAD, coronary artery disease; GWAS, genome-wide association study; HDL-C, high density lipoprotein cholesterol; LOD, logarithm of odds; MI, myocardial infarction; MCMC, Bayesian Markov Chain Monte Carlo; QTDT, quantitative transmission disequilibrium test; QTL, quantitative trait locus; SNP, single nucleotide polymorphisms.

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TABLE 1. Summary of QTLs identified for HDL-C by genome-wide linkage analysis

QTL for HDL-C[a]	Number of Families	LOD Score	Study
2q21.3	330 families (Framingham Heart Study)	3.5	Arya et al. (44)
4q21.21	13 French-Canadian families	4.6	Yu et al. (29)
5p13.3	101 Caucasian families (NHLBI Study)	3.6	Peacock et al. (31)
6q23.1 (HDL3-C)	330 families (Framingham Heart Study)	4.0	Yang et al. (45)
7p15-22	388 Caucasian families (GeneQuest)	3.76	Present study
7q31.32	295 African-American diabetic sib pairs	4.3	Adeyemo et al. (46)
8q23.1-24.22	25 Finnish families	4.7	Soro et al. (33)
8q23.1-24.22	10 Mexican-American families (San Antonio Heart Study)	4.9	Almasy et al. (30)
9p21.3	27 Mexican-American families	3.4	Arya et al. (47)
11q23.3	105 families from Utah	3.5	Kort et al. (48)
12q14.1	292 pedigrees (Quebec Family Study)	4.1	Bosse et al. (49)
15q22.31[b]	10 Mexican-American families (San Antonio Heart Study)	3.3	Almasy et al. (30)
15q21-26	388 Caucasian families (GeneQuest)	6.69	Present study
16q22.3-23.1	10 Mexican-American (San Antonio Heart Study)	4.3	Mahaney et al. (50)
16q22.3-23.1	48 Dutch and Finnish families	3.4	Pajukanta et al. (51)

Abbreviations: HDL-C, high density lipoprotein cholesterol; LOD, logarithm of odds; NHLBI, National Heart, Lung, and Blood Institute; QTL, quantitative trait locus.
[a] Chromosome location.
[b] Unesterified HDL2a-C.

independent predictor of atherosclerosis in both men and women (2, 3). Families that have early onset, heritable CAD more frequently have low HDL-C than the general population. In men with CAD, low HDL-C is the most common lipid abnormality observed, affecting half the patients (4). Due to its anti-atherogenic properties, even small variations in HDL cholesterol levels are physiologically important. For each 1 mg/dl increase in HDL-C levels, there is a decrement of 2–3% in CAD risk (5). In addition to its prevalence in CAD patients, reduced HDL-C levels are a cornerstone of the metabolic syndrome because low HDL-C is associated with insulin resistance and abdominal obesity in humans (6). With the problem of obesity continuing to escalate in the United States, metabolic syndrome poses a major public health threat affecting 22% of the adult population (7). Because low HDL-C is prevalent in patients with both metabolic syndrome and CAD, the challenge of elucidating the causes of variation in HDL-C levels and discovering new drug treatments for the condition will continue to be critical.

Plasma HDL-C levels have a strong genetic component; approximately 50% of variation in human populations is due to genetic factors (8, 9). While differences in plasma HDL-C have long been recognized to be controlled by genetic factors, our current understanding of the genetics of variation in HDL-C levels is largely based on studies of extreme monogenic HDL-C conditions. Although variation in genes caused by rare mutations may make some contribution to HDL-C levels (10, 11), the majority of genetic variation in genes that control HDL-C levels in the general population has yet to be identified (12). Identification of the genes and genetic variants that control HDL-C concentrations are critical for preventative cardiology in reducing the public health burden of CAD and the metabolic syndrome.

Genome-wide linkage and association scans provide comprehensive and unbiased approaches to identify HDL genes and may lead to the elucidation of unrecognized genetic pathways in HDL metabolism. Genome-wide association is more powerful than genome-wide linkage analysis to detect common alleles at a locus, but it is less powerful if the extreme phenotypes of interest are due to the segregation of many relatively rare alleles at that locus. Furthermore, whereas allelic associations can be due to spurious causes, especially heterogeneity/population stratification, linkage analysis is not subject to such type 1 errors. To date, multiple quantitative trait loci (QTLs) have been identified that show strong evidence of linkage for HDL-C levels (summarized in **Table 1**). Recent genome-wide association studies (GWAS) also identified multiple loci represented by various single nucleotide polymorphisms (SNP) associated with HDL-C (13–18). The previous work demonstrates the complex inheritance of genetic factors that influence this major CAD risk factor.

In the present study, we describe a whole genome linkage scan to identify chromosomal regions influencing HDL-C levels in families with premature CAD and MI. Five candidate QTLs were localized to chromosomes 3p25, 7p22, 13q12, 13q32, and 15q25 with maximum multipoint logarithm of odds (LOD) scores of 4.10, 4.21, 4.66, 3.95,

TABLE 2. Clinical and demographic features of the study population

Feature	Value
Number of pedigrees	388
Number of family members (N)	714
Gender (M/F)	480/234
Age (years)	49.6 ± 7.8[a]
Ethnicity	Caucasian
Smoking (%)	81.5
BMI (kg/m^2)	29.4 ± 5.7[a]
Hypertension	333/714 (46.7%)
CAD	694/714 (97.2%)
MI	384/714 (53.8%)
Non-CAD	20/714 (2.8%)
Diabetes (NIDDM)	78/714 (10.9%)
Diabetes (IDDM)	31/714 (4.3%)
Total cholesterol (mg/dl)	220.2 ± 55.3[a]
HDL cholesterol (mg/dl)	39.2 ± 11.4[a]
LDL cholesterol (mg/dl)	133.8 ± 43.4[a]

Abbreviations: BMI, body mass index; CAD, coronary artery disease; IDDM, insulin-dependent diabetes mellitus; MI, myocardial infarction; NIDDM, noninsulin-dependent diabetes mellitus.
[a] Data are mean ± SD.

and 7.57, respectively. After log-transformation and adjustment of age and gender, 15q25 and 7p22 QTLs remained significant with maximum multipoint LOD scores of 6.69 and 3.76, respectively. Further fine mapping resulted in the drop of the LOD score of 7p22 QTL to 3.09 and of 15q25 to 5.63. Interestingly, the promoter SNP of *LIPC* rs1800588 showed significant association with HDL in the family-based quantitative transmission disequilibrium test (QTDT) analysis. To the best of our knowledge, the 15q25 QTL is the most significant locus identified for HDL-C to date. Our study provides a framework for the ultimate cloning and identification of genes that regulate plasma HDL-C levels.

MATERIAL AND METHODS

Clinical data

The study population consists of 714 Caucasian individuals from 388 families with familial premature CAD and MI as described previously (19). Patients were recruited by cardiologists and data coordinators at the Cleveland Clinic Foundation over an approximately five-year period. Institutional review boards approved protocols, and informed consent was obtained from every study participant. For recruitment, each proband in a family was required to have a living sibling meeting the same criteria. Participants answered a health questionnaire, had anthropomorphic measures taken, and had fasted blood drawn for measurement of serum markers and DNA extraction. HDL-C was measured by standard laboratory procedures.

Genotyping

DNA was extracted from whole blood using Puregene Kits (Gentra). Genome-wide genotyping of microsatellite markers was performed by the National Heart, Lung, and Blood Institute (NHLBI) Mammalian Genotyping Services directed by Dr. James L. Weber at Center for Medical Genetics, Marshfield Clinic, using Screening Set 11 with 408 markers that span the human genome at approximately every 10 cM (http://research.marshfieldclinic.org/genetics/geneticResearch/screeningSets.asp). For fine mapping, additional markers were selected from

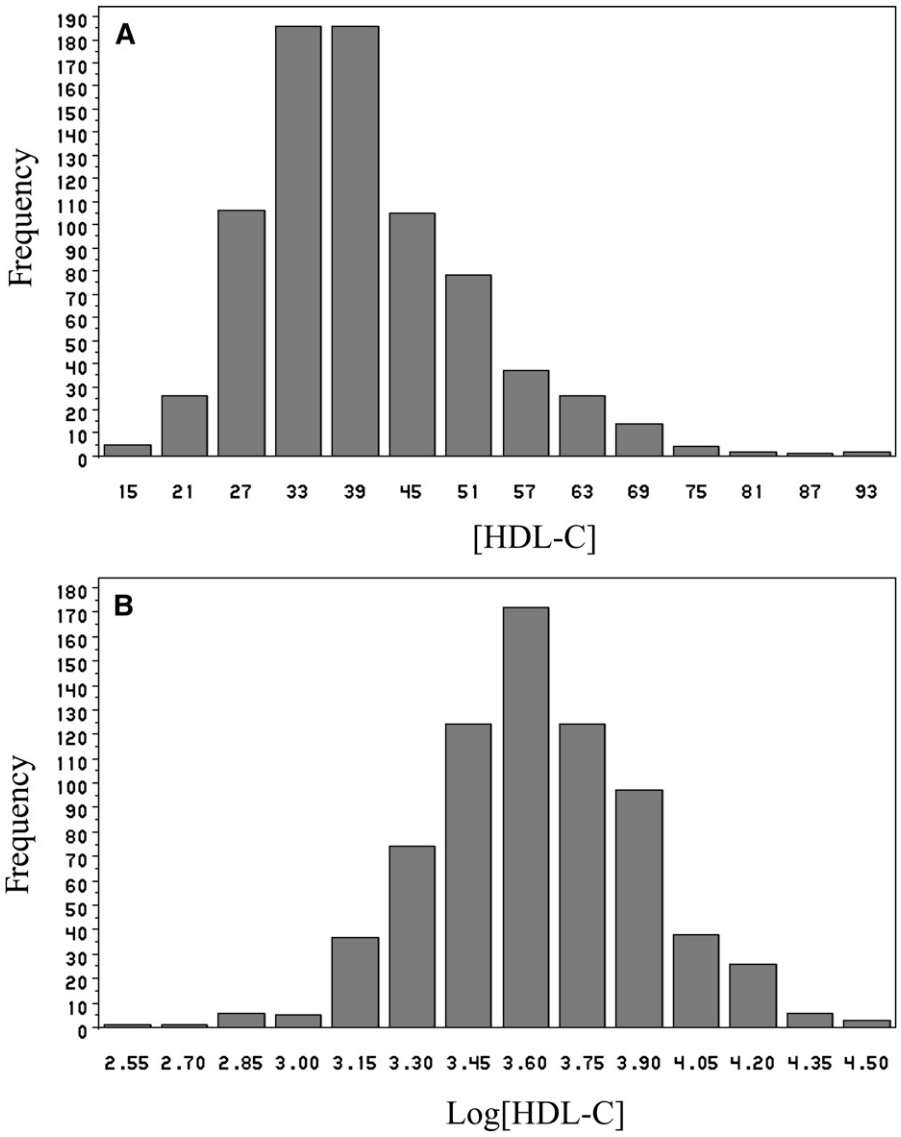

Fig. 1. Distribution of the HDL-C concentrations [HDL-C] (A) and log transformed HDL-C concentrations (B) in the study population. HDL-C, high density lipoprotein cholesterol.

the Marshfield database, synthesized, tagged with 6′-FAM (Sigma), and genotyped using an ABI 3100 genetic analyzer (Applied Biosystems) as previously described (20). The quality of genotyping for markers used for fine mapping was high.

For genotyping SNPs, the TaqMan PreDesigned SNP Genotyping Assays were performed on an ABI PRISM 7900HT Sequence Detection System as previously described (21, 22).

Genetic statistical analyses

Before linkage scanning, obvious pedigree errors, data errors, genotyping errors, and locus-order errors that commonly occur with a large-scale linkage analysis were corrected. Allele frequencies for all markers genotyped for the cohort were estimated by maximum likelihood methods using the S.A.G.E. program FREQ (23). Pedigree relationships were checked using RELTEST, which uses a Markov process model of allele sharing along the chromosome and classifies pairs of pedigree members according to their true relationship by use of genome-scan data (24). Twenty-seven of 428 pedigrees with uncorrectable errors were excluded from further linkage analysis. The S.A.G.E. program MARKERINFO was used to detect any Mendelian inheritance inconsistencies. Three families with inconsistent Mendelian inheritance were eliminated from the study. Three pairs of monozygotic twins were identified in GeneQuest and excluded from further statistical analysis. Only 388 Caucasian families with HDL-C data were analyzed for linkage.

A genome-wide linkage analysis was performed using the program GENEHUNTER (GENEHUNTER 2.1 package, Whitehead Institute, Cambridge, MA) using the sibs quantitative trait mapping function, maximum likelihood QTL variance estimation. All sib pairs were used for analysis. Positions of markers were from Center for Medical Genetics (see http://research.marshfieldclinic.org/genetics/ for Marshfield Genetic Database marker information). Age and sex were determined to be important covariates for HDL-C levels and were modeled in the linkage analysis using general linear regression with SAS Version 9.00. To evaluate the significance of the linkage results, we followed the criteria proposed by Lander and Kruglyak (25) specifically for sib pair linkage analysis in humans. LOD scores for suggestive, significant, and highly significant evidence of linkage are 2.2, 3.6, and 5.4, respectively.

A family-based association study of SNPs with HDL-C was carried out using quantitative transmission disequilibrium tests (QTDT2.5.1, http://www.sph.umich.edu/csg/abecasis/QTDT/) (26).

RESULTS

We have completed a genome-wide linkage analysis to identify QTLs for plasma HDL-C levels in a well-characterized US cohort consisting of multiplex families (GeneQuest). HDL-C values were available for 67% of the GeneQuest study population. A total of 714 Caucasian persons in 388 families with HDL-C data available were analyzed. The clinical and demographic features of the study population are shown in **Table 2**. The mean value of HDL-C in the population was low (39.2 mg/dl). As the HDL-C levels did not present with a normal distribution (**Fig. 1A**), the values were also log-transformed prior to analysis. The transformed HDL-C values are shown in Fig. 1B. Age and sex were determined to be significantly correlated with HDL-C values ($P = 0.0024$ and $P < 0.0001$, respectively). Therefore, HDL-C values were also adjusted for age and gender using general linear regression analysis.

A residual heritability estimate of HDL-C in the study population was calculated as 0.37 with gender and age as covariates ($P = 5.1 \times 10^{-4}$; SOLAR, http://solar.sfbrgenetics.org/). Genome-wide genotyping was carried out with 408 polymorphic markers that span the entire human genome at approximately every 10 cM. We performed both single-

TABLE 3. Summary of chromosomal regions linked to plasma HDL-C levels in a Caucasian premature CAD and MI population (GeneQuest)

				Uncorrected [HDL-C]		[HDL-C][a]		Log[HDL-C][a]	
Chromosome and Marker	Location	Map Position (cM)	Position (Mb)	Single-Point LOD	Multipoint LOD	Single-Point LOD	Multipoint LOD	Single-Point LOD	Multipoint LOD
Chr 3p									
MFD433	3p26	11.0	3.6	5.26	3.89	3.76	3.16	1.54	1.71
GATA131D09	3p26	19.3	5.5	2.95	3.47	2.42	3.57	0.62	1.49
D3S4545	3p25	26.0	10.8	2.60	3.11	3.40	4.10	1.76	2.27
Chr 7p									
D7S2477	7p22	0	2.6	1.81	3.10	1.99	3.37	0.05	2.73
D7S3056	7p22	7.0	4.5	1.26	4.58	1.28	4.21	1.83	3.76
D7S3047	7p21	17.0	8.5	4.26	3.51	4.12	2.55	3.62	2.07
D7S1802	7p21	33.0	20.2	4.24	4.46	4.41	3.21	2.26	1.74
D7S1808	7p15	42.0	27.9	3.69	1.79	3.02	1.76	1.19	1.56
Chr 13q									
ATA5A09N	13q12	20.0	28.7	6.42	4.79	6.17	4.66	4.24	3.10
D13S1493	13q13	26.0	32.0	2.98	2.87	2.84	2.36	1.43	1.38
Chr 13q31-32									
D13S317	13q31	64.0	81.6	2.14	3.27	2.11	3.95	1.96	1.57
D13S793	13q32	76.0	96.6	2.93	3.60	2.75	3.47	1.45	2.63
Chr 15q									
D15S643	15q21	52.0	55.3	2.87	2.10	3.12	1.72	2.42	2.20
D15S1507	15q22	60.0	60.9	4.40	2.94	4.37	4.75	3.19	3.09
D15S818	15q24	72.0	70.7	2.23	4.37	2.83	4.91	2.06	4.23
D15S655	15q25	83.0	84.2	6.28	5.91	6.76	7.57	5.79	6.69
D15S652	15q26	90.0	88.7	4.77	5.03	5.72	6.72	4.44	6.18

Abbreviations: CAD, coronary artery disease; HDL-C, high density lipoprotein cholesterol; LOD, logarithm of odds; MI, myocardial infarction.

[a] Adjusted for age and gender.

point and multipoint linkage analyses using the GeneHunter sibs quantitative trait mapping function and maximum likelihood QTL variance estimation, the results of which are shown in **Table 3** and **Figs. 2–5**.

Of the loci identified for HDL-C adjusted for age and gender, the 15q25 region displayed the strongest evidence for linkage to HDL-C. Model-free multipoint linkage analysis revealed high significance at 86 cM in a region between markers *D15S655* (83 cM) and *D15S652* (90cM) as shown in Fig. 3. Additionally, single-point linkage analysis confirmed that high significance was reached at both markers: *D15S655* (LOD 6.76) and *D15S652* (LOD 5.72) (Table 3). Therefore, for the chromosome 15 locus, the maximum multipoint and single-point LOD scores were 7.57 and 6.76, respectively.

Four additional loci were detected with significant evidence of multipoint linkage: 3p25, 7p22, 13q12, and 13q31-32. The second strongest locus for HDL-C was at 13q12 (20 cM) with maximum multipoint evidence of linkage equal to a LOD of 4.66 (Fig. 3). This locus is in close proximity to marker *ATA5A09N* (20 cM), and single-point analysis confirmed significant evidence of linkage at *ATA5A09N* with a LOD of 6.17 (Table 3). Maximum multipoint linkage for chromosome 3p25 (LOD = 4.10) was reached at 23 cM between markers *GATA131D09* (19.3 cM) and *D3S4545* (26 cM) (Table 3 and Fig. 3). The chromosome 7p locus followed with maximum multipoint evidence of linkage equal to a LOD of 4.21 at 6.1 cM (Fig. 3). Single-point analysis yielded a significant result at the closest marker *D7S3056* (LOD = 4.11; 7.0 cM) (Table 3). Finally, multipoint analysis for the 13q31-32 locus reached an LOD score of 3.95 at 68.8 cM between markers *D13S317* (64 cM) and *D13S793* (76.0 cM) (Fig. 2).

For HDL-C levels without adjustment of age and gender, we also obtained evidence of linkage for the five putative QTLs on chromosome 15q25, 3p25, 7p22, 13q12, and 13q31-32. The maximum multipoint/single-point LOD scores were 5.91/6.28, 3.47/2.95, 4.58/4.24, 4.79/6.42, and 3.60/2.93, respectively (Table 3). For HDL-C after log-transformation and adjustment for age and gender, the maximum multipoint/single-point LOD scores were 6.69/5.79, 2.27/1.76, 3.76/3.63, 3.10/4.24, and 2.63/1.9, respectively (Figs. 4 and 5; Table 3).

For the two strongest linkage loci on 7p22 and 15q25, fine mapping was carried out with additional microsatellite markers and di-allelic SNP markers. For the 7p22 QTL, we genotyped the GeneQuest families with *D7S1532* and two candidate SNPs, rs10499320 and rs10486788, which in the Framingham Heart Study showed potential association with HDL-C (13). Linkage analysis for HDL-C after log-transformation and adjustment for age and gender showed a major, complete linkage peak (half

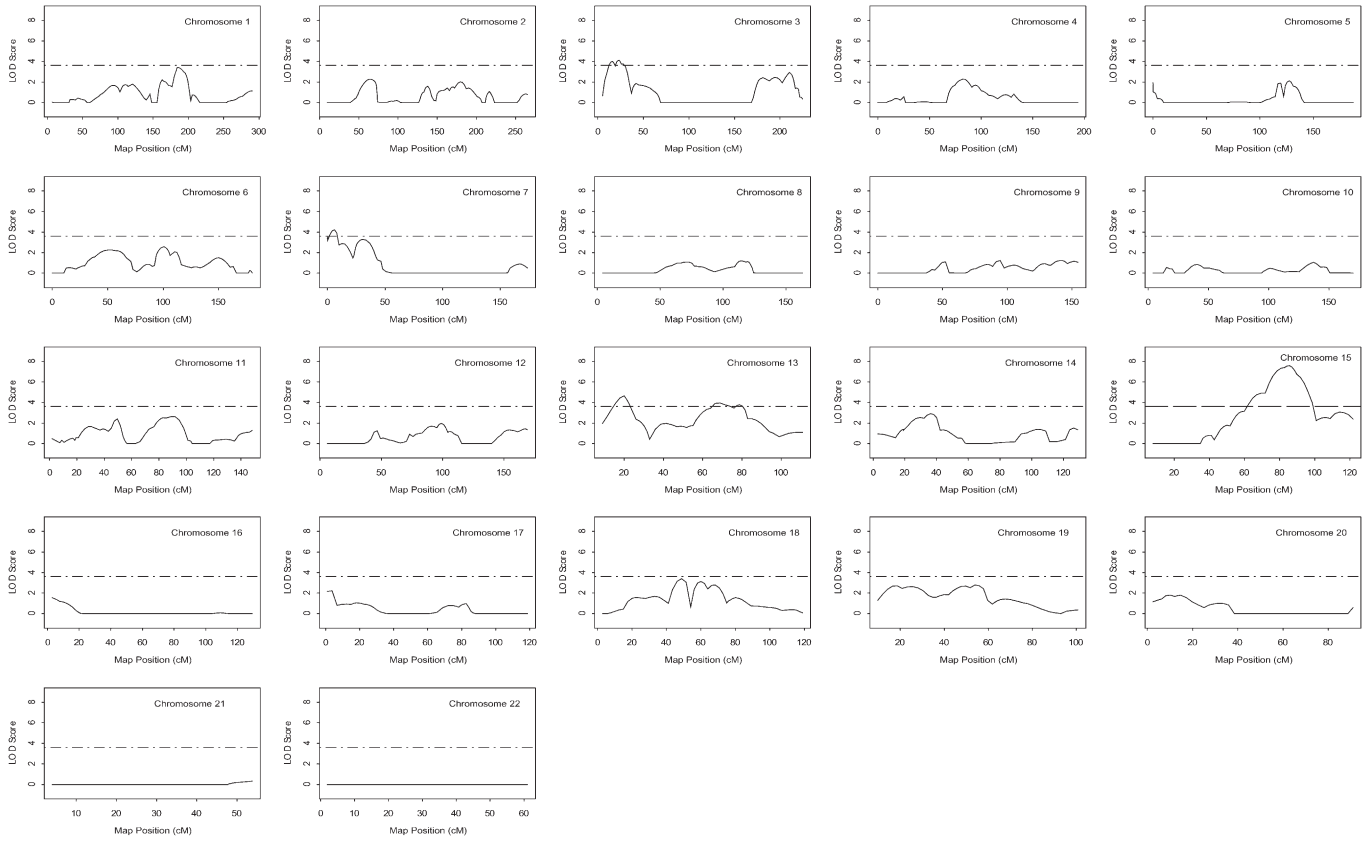

Fig. 2. Likelihood plots for QTLs for HDL-C adjusted for age and gender. The Y-axis of each plot is the LOD score; the X-axis is the marker map position. Solid lines represent the multipoint linkage analysis; horizontal dashed lines indicate the significance threshold which is equal to a LOD score value of 3.6. Significant linkages to chromosomes 3p25, 7p22, 13q12, 13q32, and 15q25 were detected with multipoint allele sharing LOD scores of 4.10, 4.21, 4.66, 3.95, and 7.57, respectively. HDL-C, high density lipoprotein cholesterol; LOD, logarithm of odds; QTL, quantitative trait locus.

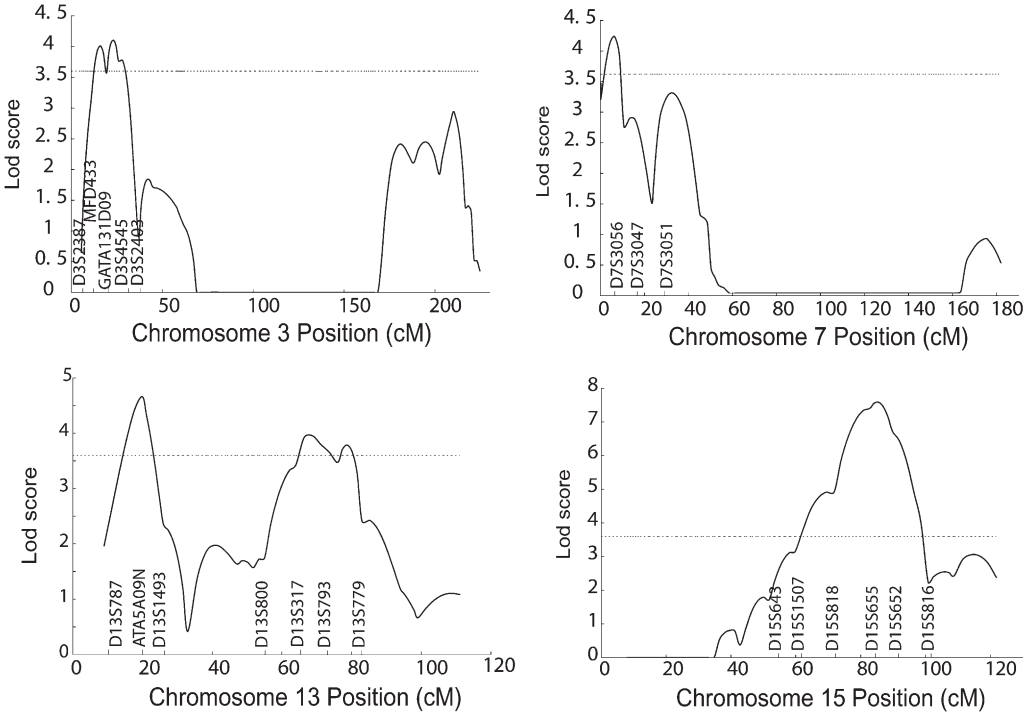

Fig. 3. Detailed likelihood plots for significant QTLs for HDL-C on chromosomes 3p25, 7p21, 13q12, 13q32, and 15q25. The Y-axis of each plot is the LOD score; the X-axis is the marker map position. Solid lines represent the multipoint linkage analysis; horizontal dashed lines indicate the significance threshold which is equal to a LOD score value of 3.6. HDL-C, high density lipoprotein cholesterol; LOD, logarithm of odds; QTL, quantitative trait locus.

peak before fine mapping). However, the maximum LOD score of 7p22 dropped to 3.09, which did not exceed the significance threshold of a LOD score of 3.6 (**Fig. 6** and **Table 4**). The size of the one-LOD drop interval was not changed by fine mapping. A QTDT did not identify any association between rs10499320 and rs10486788 and HDL-C ($P > 0.05$) (**Table 5**). Haplotypes formed by these two SNPs were predicted by PHASE software (http://stat.washington.edu/stephens/software.html), and none of the haplotypes showed any association with HDL-C levels (data not shown).

For fine mapping of the 15q25 QTL, we studied *D15S983* and two SNPs, rs1491579 and rs1638634, adjacent to marker *D15S655* with the highest LOD score. The fine mapping study splits the QTL into two linkage peaks, one with a maximum multipoint LOD score of 2.73 at chromosome 15q22 covering the *LIPC* gene (encoding hepatic lipase) and the other with a maximum multipoint LOD score of 5.63 remaining at chromosome 15q25 (Fig. 6 and Table 4). The fine mapping sharpened the major linkage peak by narrowing the one-LOD drop interval from 14.8 cM to 9.1 cM. A QTDT did not identify any association between rs1491579 and rs1638634 and HDL-C ($P > 0.05$) (Table 5). None of the haplotypes formed by these two SNPs showed any association with HDL-C levels (data not shown).

Recent genome-wide SNP association studies and earlier candidate gene analysis revealed association of SNPs in the *LIPC* gene with HDL-C levels (14, 27). Because *LIPC* is located within the small QTL for HDL-C on 15q22 (Fig. 6), we assessed its association with HDL-C in the GeneQuest families using a family-based QTDT, focusing on the promoter and exonic SNPs. For the *LIPC* promoter, we selected SNP rs1800588 because this promoter SNP has been reported to be associated with plasma HDL-C levels (14). Furthermore, two exonic SNPs, rs690 and rs6083, were selected among tagging SNPs for LIPC identified by the Tagger program and Haploview 4.1 using the threshold minor allele frequency of 0.3 and R^2 of 0.6. As shown in **Table 6,** significant association with HDL-C levels was identified for SNP rs1800588 ($P = 0.0067$), but not with rs690 or rs6083.

DISCUSSION

The present study reports evidence from genome-wide linkage analysis that multiple QTLs influence HDL-C levels in a cohort of premature CAD and MI families (GeneQuest). In particular, we identified one locus on chromosome 15q25 with highly significant evidence of linkage to date, displaying a LOD score that is greater than the cutoff LOD score of 5.40 for highly significant evidence of linkage at 86 cM. Four regions were also detected with evidence of linkage to HDL-C levels on chromosomes 3p25, 7p22, 13q12, and 13q32.

Supporting the current findings, several independent studies provide evidence for the candidate QTLs on chromosomes 15q25, 13q12, 3p25, and 13q32. Linkage for HDL-C was observed on chromosome 15 in Turkish families with dyslipidemia (LOD = 3.05 at 15q23 at 66.5 Mb) (28); French Canadian families (LOD = 1.6 at 15q25.1 at 78.1 Mb) (29); and for unesterified HDL_{2b}-C in Mexican-

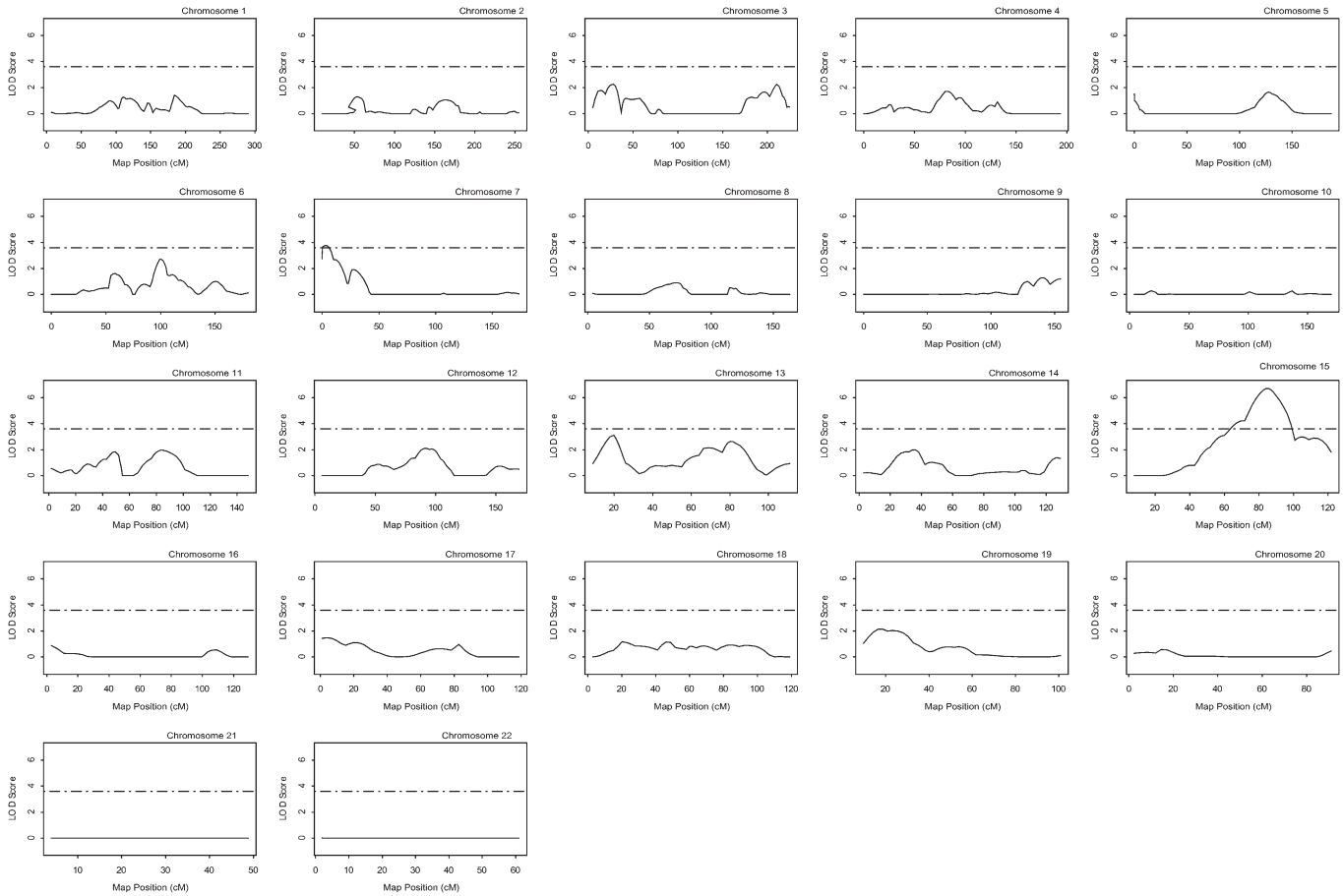

Fig. 4. Likelihood plots for QTLs for log-transformed HDL-C adjusted for age and gender. Horizontal dashed lines indicate the significance threshold which is equal to a LOD score value of 3.6. HDL-C, high density lipoprotein cholesterol; LOD, logarithm of odds; QTL, quantitative trait locus.

American families (LOD = 2.54 at 15q25.3 at 83.2 Mb) (30). Our data, together with these results, suggests that the HDL-C gene at this locus may play a role in HDL-C metabolism in several ethnic populations. On chromosome 13, suggestive evidence of linkage was detected in families from the NHLBI family heart study (LOD = 2.36 at 13q13.2 at 32.9 Mb) (31) and families with type 2 diabetes (LOD = 2.01 at 13q13-14 at 32.2-41.0 Mb) (32). For chromosome 3, suggestive evidence for an HDL-C locus was identified in 25 Finnish families (LOD = 2.1 at 3p26.3-24.3 at 0-17 Mb) (33). The chromosome 13q32 locus was previously reported using a Bayesian Markov Chain–Monte Carlo (MCMC) approach to map HDL-C QTLs in FCHL families (Intensity Ratio = 13 at 13p32 at 96.6-100.3 Mb) (34). The chromosome 13q32 region has also been identified for linkage to total cholesterol, LDL cholesterol, and various lipid-related traits in two studies involving families with familial hypercholesterolemia (35, 36). In total, these independent reports provide some support that the genetic regions identified in the current study harbor genetic variants that regulate HDL-C levels.

Our genome-wide linkage scan and heritability analysis of a US Caucasian population clearly indicate that HDL-C and CAD/MI are complex traits with mixed contributions from multiple genetic and environmental factors. Various studies using transgenic and gene-targeted mice have revealed >100 genes influencing the development of atherosclerotic lesions (37, 38), and a larger number of genes influencing HDL metabolism is expected to be identified. It is interesting to note that a recent study (39) using bivariate linkage analysis of coronary artery calcification (CAC), a measure of atherosclerosis determined by electron beam–computed tomography, provided evidence of two regions with pleiotropic effects on CAC and HDL-C on chromosomes 4p16 (MLS = 3.03, $P = 0.00084$) and 9p12 (MLS = 3.21, $P = 0.00056$), which may suggest an underlying genomic mechanism for pleiotropism. In future studies we can explore gene-gene interactions for HDL-C (including related plasma parameters) and clinical cardiovascular phenotypes as both data are available, and significant genetic loci were identified for both phenotypes.

A recent genome-wide SNP association study involving 1,087 Framingham Heart Study offspring cohort participants identified two SNPs (rs10499320, rs10486788) within the 1-LOD- and 2-LOD-drop interval of our 7p15-22 candidate HDL-C QTL that showed positive association with HDL-C levels ($P = 8.5 \times 10^{-4}$, 8.8×10^{-4}, respectively) (13). Our QTDT did not detect any association between HDL-C and SNP rs10499320 or between HDL-C and SNP rs10486788 in the GeneQuest families.

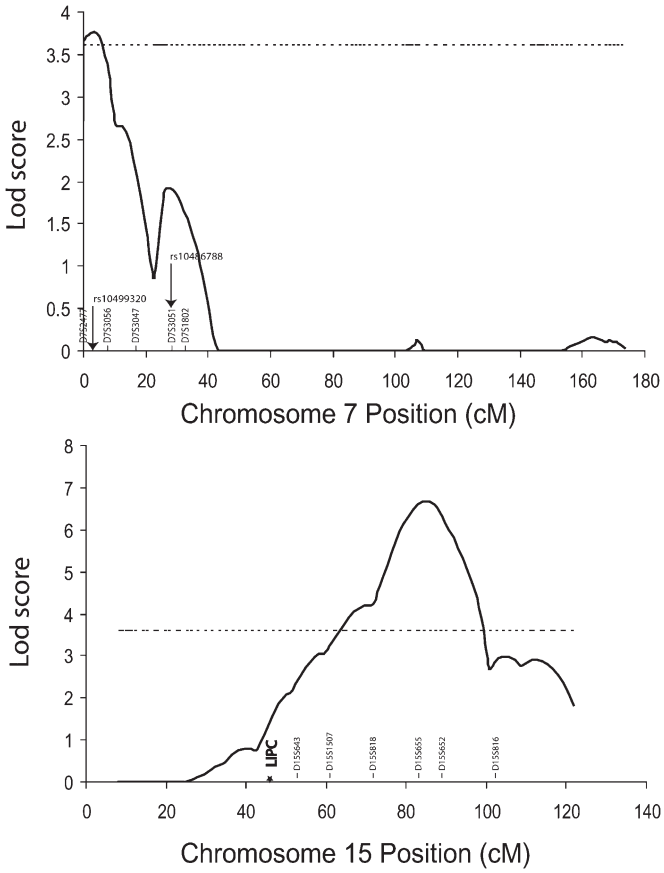

Fig. 5. Detailed likelihood plots for significant QTLs for log transformed HDL-C on chromosomes 7p21 and 15q25 adjusted for age and gender. Horizontal dashed lines indicate the significance threshold which is equal to a LOD score value of 3.6. HDL-C, high density lipoprotein cholesterol; LOD, logarithm of odds; QTL, quantitative trait locus.

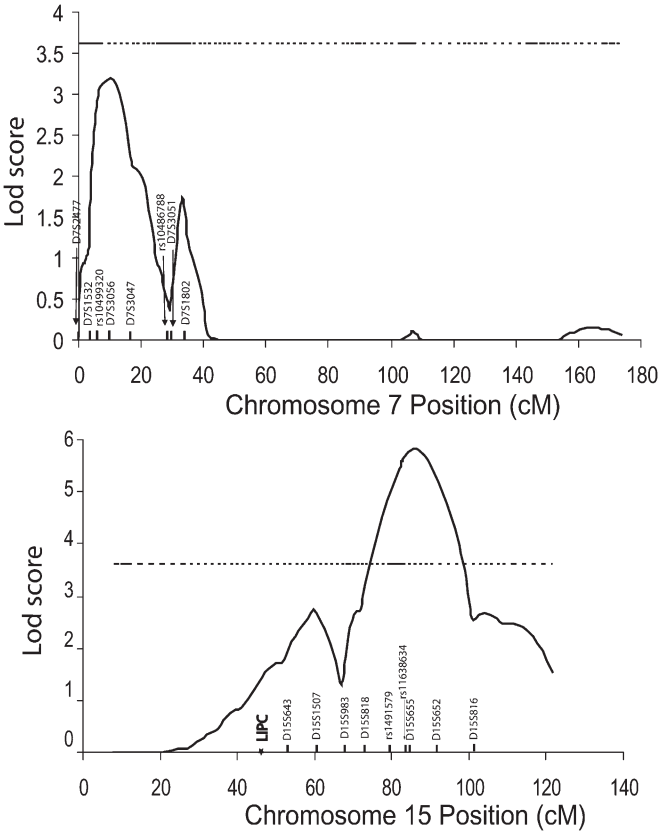

Fig. 6. Fine mapping of the 7p22 and 15q25 QTLs for log transformed HDL-C adjusted for age and gender. Horizontal dashed lines indicate the significance threshold which is equal to a LOD score value of 3.6. HDL-C, high density lipoprotein cholesterol; LOD, logarithm of odds; QTL, quantitative trait locus.

For chromosome 15q25 QTL for HDL-C, fine mapping studies split the QTL into two separate QTLs, one major QTL on 15q25 with a maximum LOD score of 5.63 and the other minor QTL on 15q22 that showed a maximum LOD score of 2.73 and covered the *LPIC* gene. Two SNPs, including rs1491579 and rs11638634 close to marker D15S655 showing the maximum LOD score, were analyzed for association with HDL-C, but no significant association was detected. For the minor QTL at 15q22, we analyzed the *LIPC* gene for its association with HDL-C using a family-based QTDT in the GeneQuest families. Interestingly, a *LIPC* promoter SNP, rs1800588, showed significant association with HDL-C, but two exonic SNPs, rs690 and rs6083, were not associated with HDL-C in the GeneQuest population (Table 6). The rs1800588 association may account for the minor QTL on 15q22 identified by fine mapping. These results are identical to those generated by a population-

TABLE 4. Fine mapping of the 7p22 and 15q25 QTLs for log[HDL-C] after adjustment for age and gender

Chromosome and Marker	Location	Map Position (cM)	Position (Mb)	Single-Point LOD	Multipoint LOD
Chr 7p					
D7S1532	7p22	3.13	2.4	0.19	1.04
rs10499320	7p22	4.15	3.1	1.07	2.15
D7S3056	7p22	7.0	4.5	1.60	3.09
D7S3047	7p21	17.0	8.5	3.37	2.12
Chr 15q					
D15S643	15q21	51.8	55.3	1.98	1.76
D15S1507	15q22	59.8	60.9	2.54	2.73
D15S983	15q23	66.9	66.4	0.79	1.30
D15S818	15q24	71.8	70.7	2.35	2.75
rs1491579	15q25	78.9	82.0	2.67	4.83
rs11638634	15q25	82.8	85.7	2.80	5.47
D15S655	15q25	82.8	85.8	4.48	5.63
D15S652	15q26	89.8	88.7	3.42	5.51

Abbreviations: HDL-C, high density lipoprotein cholesterol; LOD, logarithm of odds; QTL, quantitative trait locus.

TABLE 5. Assessment of two SNPs at chromosome 7p22 for association with HDL-C using a QTDT in GeneQuest

Chromosome 7p22		QTDT Analysis	
SNP	Gene/Position	*F*	*P*
rs10499320	N/A	0.42	0.6578
rs10486788	N/A	0.73	0.4801

Abbreviations: HDL-C, high density lipoprotein cholesterol; QTDT, quantitative transmission disequilibrium test; SNP, single nucleotide polymorphisms.

based association study reported in a Turkish population (27). To the best of our knowledge, this is the first family-based QTDT study to demonstrate the association between *LIPC* and HDL-C. On the other hand, the specific gene responsible for the major 15q25 HDL-C QTL remains to be identified.

Due to its highly significant linkage to HDL-C, the chromosome 15 locus provides the most promise for gene identification. Several interesting candidate genes reside within this genetic interval. The most promising candidate genes for HDL-C at this locus include START domain containing 5 (*STARD5*), *PL1N1, ADANTSL3,* and endonuclease VIII-like 1 (*NEIL1*). STARD5 is a member of the steroidogenic acute regulatory lipid transfer (START) domain superfamily of proteins involved in several pathways of intracellular trafficking and metabolism of cholesterol (40). PLIN1 is a cAMP-dependent protein kinase substrate in adipocytes that plays a role in lipolysis and has been shown to be associated with metabolic variables in Caucasian women (41). ADAMTSL3 belongs to the ADAMTS metalloprotease family (42). NEIL1 knockout mice developed metabolic syndrome with severe obesity, dyslipidemia, and fatty liver disease (43). It would be interesting to determine whether SNPs in these genes may account for the major 15q25 HDL-C QTL.

One limitation of the current study is that empirical *P* values for each QTL based on trait or marker resimulation data could not be estimated because such a program was not implemented in the GeneHunter package. The other limitation is that the HDL-C QTLs identified in this study were mostly derived from patients and families with CAD and MI (97.2% of the GeneQuest population) (Table 2), which may be enriched for low HDL-C levels. These QTLs may be different from those modulating HDL-C in general healthy populations.

TABLE 6. Assessment of SNPs at chromosome 15q22-25 for association with HDL-C using a QTDT in GeneQuest

Chromosome 15q22-25		QTDT Analysis	
SNP	Gene/Position	*F*	*P*
rs1800588	*LIPC*/promoter	5.05	0.0067
rs690	*LIPC*/exon 4	1.72	0.1791
rs6083	*LIPC*/exon 5	0.21	0.8128
rs1491579	*SH3GL3*/intron 4	1.56	0.2117
rs11638634	N/A	0.58	0.5576

Abbreviations: HDL-C, high density lipoprotein cholesterol; QTDT, quantitative transmission disequilibrium test; SNP, single nucleotide polymorphisms.

CONCLUSION

The genome-wide linkage analysis for HDL-C QTLs described here provides highly significant evidence for the presence of a locus on chromosome 15q25 controlling HDL-C values. The 15q25 QTL is the most significant locus identified for HDL-C and represents a novel candidate genetic region that influences plasma HDL-C levels. These results will lay the foundation for the successful identification of genetic variants that influence HDL-C at this QTL.

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