

A genome-wide scan of serum lipid levels in the Old Order Amish

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Abstract

Elevated serum low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) and decreased high density lipoprotein cholesterol (HDL-C) levels are established risk factors for cardiovascular disease (CVD). To identify quantitative trait loci influencing lipid levels, we conducted genome-wide linkage analyses of total serum cholesterol (TSC), HDL-C, ln-transformed TG (LNTG) and LDL-C levels in 612 individuals from 28 families of the Amish Family Diabetes Study (AFDS). Subjects were genotyped for 373 microsatellite markers covering all 22 autosomes and the X chromosome at an average density of 9.7 centimorgans. All lipid traits exhibited moderate estimated heritability ($h^2 \pm$ S.E.): TSC, 0.63 ± 0.11 ; HDL-C, 0.54 ± 0.08 ; LNTG, 0.37 ± 0.08 ; LDL-C, 0.62 ± 0.10 . The highest logarithm of the odds (LOD) score observed was 2.47 ($P = 0.0003$), at 3p25 for LDL-C. LOD scores exceeding 2.0 ($P < 0.001$) were also observed at 2p23 (LOD = 2.17) and 19p13 (LOD = 2.23) for LDL-C, and at 11q23 (LOD = 2.03) for LNTG. Three additional regions exhibited LOD scores greater than 1.5, corresponding to a P -value of <0.005 . Many of the regions suggestively linked in this genome-wide scan contain genes encoding proteins with established roles in lipid metabolism, including apolipoproteins, peroxisome proliferator-activated receptor- γ and the LDL receptor. © 2003 Elsevier Ireland Ltd. All rights reserved.

Keywords: Lipids; Genome scan; Amish; Cholesterol; Triglycerides

1. Introduction

Cardiovascular disease (CVD), in particular coronary artery disease (CAD), is the leading cause of death in the United States [1]. Although prevention and improved treatment have reduced the number of deaths over the past 20 years, CVD remains a major source of disability and mortality. Elevated serum low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) levels and decreased high density lipoprotein cholesterol (HDL-C) levels are established risk factors for CAD [2]. Several monogenic lipid disorders have been described, including familial lipoprotein lipase (LPL) deficiency, apo C-11 deficiency, defective apo

B, dysbetalipoproteinemia, familial hypertriglyceridemia, chylomicronemia, familial low LDL-C, LCAT deficiency, and Tangier's disease [3,4]. However, only recently have researchers begun to search for gene variants regulating lipid levels in the general population.

Genome scans in a few populations have reported significant and/or suggestive linkages to lipid traits. Familial combined hyperlipidemia (FCHL) was linked to chromosome 1q21–23 by Pajukanta et al. with a LOD score of 5.93 [5] and replicated by Coon et al. [6]. Chromosome 11p was linked to FCHL in a set of Dutch families [7]. Total serum cholesterol (TSC) was linked to chromosome 19p in the Pima Indians [8]. LDL-C levels were linked to chromosomes 11, 5, 10, 17 and 21 in the NHLBI Family Heart Study [9]. The low HDL-C trait showed evidence for linkage to 8q, 16q and 20q in Finnish families [10]. Chromosome 16q has shown evidence of linkage in separate populations to both HDL-C levels [11] and the low HDL-C trait [12].

Studies of Mexican Americans have reported linkage of the unesterified HDL2a-C component of HDL-C to chromosomes 8 and 15 [13] and of HDL-C itself to 9p [14] as

Abbreviations: TSC, total serum cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; LNTG, ln-transformed triglyceride; LDL-C, low density lipoprotein cholesterol; LOD, logarithm of the odds

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well as a major susceptibility locus for triglyceride levels on 15q and a less significant one on chromosome 7 [15]. Triglyceride levels and the TG/HDL-C ratio were linked to chromosome 7q32.3 in the Framingham Heart Study [16]. Linkages to triglyceride levels have also been reported in Finnish families (LOD > 2.0) at chromosome 10p11.2 [17] and in the Utah Mormons at 19q [18]. A study in a large Hutterite pedigree linked triglyceride levels to chromosomes 2q14 and 9p21 [19]. The apoB concentration trait was linked to chromosome 21q21 in Finnish families [17].

We undertook a genome-wide scan for serum lipid levels in 612 individuals recruited into the Amish Family Diabetes Study (AFDS), a project devised originally to study the genetics of type 2 diabetes and related traits in the Old Order Amish (OOA) of Lancaster and surrounding counties. The OOA are a genetically homogeneous population numbering approximately 25–30,000, nearly all of whom can trace their ancestors back to a few hundred individuals who immigrated to the United States in the late 1700s [20,21]. The OOA have a strong interest in their ancestry and their genealogical relationships are well-documented. Moreover, there is considerable homogeneity in the rural lifestyle of the OOA, who eschew technological innovation. These attributes make the OOA an attractive population for genetic studies [22].

2. Subjects and methods

2.1. Subjects

Recruitment for the Amish Family Diabetes Study began in early 1995 with the goal of identifying genes influencing the risk of type 2 diabetes and related traits. The study protocol was approved by the Institutional Review Board at the University of Maryland School of Medicine, and informed consent was obtained from each study participant. With the help of liaisons from the OOA community, we identified individuals with type 2 diabetes. These probands and their first and second degree relatives and spouses aged 18 years and older were invited to participate. Between February 1995 and 1997, 727 subjects received examinations at the Amish Research Clinic in Strasburg, Pennsylvania or in their homes. Of these, 617 subjects had lipid measurements available and were genotyped for a screening panel of genetic markers (see below). We excluded from the analysis subjects taking lipid-lowering agents ($n = 5$). In the remaining group of 612 subjects, 65 had type 2 diabetes (20 prevalent cases and 45 incident cases). The recruitment strategy and study design have been described in detail previously [22,23].

2.2. Phenotypes

Blood samples were obtained from an antecubital vein after an overnight fast using a butterfly catheter at the Amish Research Clinic in Strasburg, Pennsylvania. Fasting lipid profiles (total serum cholesterol, high density

lipoprotein cholesterol, triglycerides) were performed by Quest Diagnostics (Baltimore, MD). Intra-assay CVs of duplicate samples ranged between 0.1 and 3.0%, and inter-assay CVs ranged between 0.2 and 5%. Low density lipoprotein cholesterol concentrations were estimated using Friedewald's equation ($LDL-C = TSC - HDL-C - TG/5$ for $TG < 400$ mg/dl). Height and weight were measured at the clinic visit. BMI was calculated as weight (kg) divided by height (m) squared. Details regarding the diagnostic criteria for type 2 diabetes have been described previously [22].

2.3. Genotypes

DNA was extracted from leukocytes and genotyping performed using a screening set of 373 highly polymorphic microsatellite short tandem repeat markers on the 22 autosomes and the X chromosome from the ABI Prism Linkage Mapping Set (Applied Biosystems Division/Perkin-Elmer; Foster City, CA). The mean marker heterozygosity was 0.75 (range: 0.33–0.91). The average inter-marker interval was 9.7 centimorgans (cM). The largest gap between markers was 25.4 cM, occurring on chromosome 7. The genotyping error rate based on blind replicates was 0.16% on average.

2.4. Statistical analysis

Although all subjects can be related by tracing their ancestors back multiple generations, we divided the sample into 28 discrete families for linkage analysis to reduce computational burden. These 28 families ranged in size from 3 to 69 subjects and provided a large number of relative pairs, including 418 parent-offspring pairs, 1175 sib-pairs, 1088 avuncular (aunt/uncle-niece/nephew) pairs, and 1072 first cousin pairs.

Heritability and quantitative trait multipoint linkage analyses were carried out using a variance components methodology. We partitioned variation in the lipid traits into components attributable to environmental covariates, the additive effects of genes (i.e., residual heritability), and a specific quantitative trait locus, or QTL (i.e., the linkage component). These analyses were conducted using maximum likelihood procedures as implemented in the SOLAR program [24]. The residual heritability was modeled as a function of the expected genetic covariances between relatives, and the QTL effect was modeled as a function of the identity by descent relationships at the marker locus. Allele frequencies were estimated from the data using maximum likelihood methods [24]. The hypothesis of linkage was evaluated by the likelihood ratio test, which tests whether the locus-specific effect is significantly greater than zero (i.e., $H_0: \sigma_{QTL}^2 = 0$ versus $H_A: \sigma_{QTL}^2 > 0$). In each model, we simultaneously adjusted for the effects of sex, and sex-specific age and age². Additional analyses were performed adjusting for diabetes status and/or body mass index. Because of the sex differences in HDL-C levels, a follow-up analysis of HDL-C was performed in which we

stratified by sex; that is, separate analyses were performed for men's HDL-C levels ($n = 273$) and for women's HDL-C levels ($n = 339$). Triglyceride levels were transformed by their natural logarithm in order to approximate a normal distribution. Furthermore, because of the sensitivity of variance components to violations of normality, all lipid data points whose values were greater than three standard deviations from the mean ($n = 2$ – 9 values for TSC, HDL-C, ln-transformed TG (LNTG) and LDL-C) were truncated and their values set to the value corresponding to three standard deviations from the mean (Winsorization [25]).

After initially obtaining LOD scores for linkage by the likelihood ratio test, we then evaluated the probability of obtaining false positive results by generating a large number of unlinked markers and evaluating evidence for linkage of each lipid trait to these simulated markers. We simulated a total of 10,000 unlinked markers per trait and defined the probability of obtaining a false positive result as the proportion of the 10,000 replicates for which we obtained a LOD score higher than that observed for the original linked locus. All LOD scores in this report were obtained by converting the empirical P -value obtained by simulation to its corresponding LOD score ($\text{LOD} = \chi^2/[2 \ln(10)]$).

2.5. Power estimation

We evaluated the power of our sample to detect linkage to QTLs accounting for 20, 25 and 30% of the total variation in a model trait using simulation as described previously [26]. Two hundred QTLs were simulated for each of the three effect sizes, and power to detect linkage was defined as the proportion of replicates for which we obtained LOD scores higher than selected values (e.g., 3.0 and 2.0).

3. Results

The study sample (Table 1) consisted of 273 men and 339 women who were similar in age (47.0 ± 15.2 and 47.3 ± 15.5

years, respectively) but differed with respect to BMI (men, 26.2 ± 3.6 ; women, $28.1 \pm 5.5 \text{ kg/m}^2$, $P < 0.0001$) and diabetes prevalence (men, 7.8%; women, 13.3%, $P < 0.05$), the latter likely due to differences in BMI [22]. While men and women were similar with respect to total cholesterol and triglyceride levels, women's HDL-C levels were markedly higher (53.9 ± 13.0 versus $47.2 \pm 12.1 \text{ mg/dl}$, $P < 0.0001$). Interestingly, the Amish appear to have higher total cholesterol levels but much lower triglyceride and similar HDL-C levels than their non-Amish Caucasian counterparts, as assessed through the National Health and Nutrition Examination Survey (NHANES) III [27]. Furthermore, based on food frequency data collected from volunteers recruited as spouses of participants during the AFDS, the typical Amish diet is high in fat, comprising approximately 42% (of which ~36% is saturated fat) of total caloric intake.

All four lipid traits showed substantial heritability (TSC, 0.63 ± 0.11 ; HDL-C, 0.54 ± 0.08 ; TG, 0.37 ± 0.08 ; LDL-C, 0.62 ± 0.10 ; all $P < 0.0001$). With the exception of HDL-C with LDL-C levels, which were not correlated ($r = 0.02$), all four traits tended to be moderately to highly correlated with one another, especially TSC and LDL-C, for which the correlation was 0.96.

Peak LOD scores for each trait by chromosome are given in Fig. 1, and LOD scores of 1.5 or greater are summarized in Table 2. The full genome scan can be viewed at <http://medschool.umaryland.edu/endocrinology/Amish/amlinkindex.html>. The highest LOD score (2.47; $P = 0.0004$) was for LDL-C levels occurring at 25 cM from pter near marker D3S1263 at 3p25, with a lower score at the same locus for TSC (1.67; $P = 0.003$). Other LDL-C peaks were observed between markers D2S312 and D2S220 at 2p23 (LOD = 2.17; $P = 0.0008$) and between markers D19S226 and D19S433 at 19p13 (LOD = 2.23; $P = 0.0007$). Smaller peaks for TSC were also observed at the same locations (LOD = 1.54; $P = 0.004$ for both loci). Linkage to TSC also peaked on chromosome 9q34 near marker D9S290 (LOD = 1.61; $P = 0.003$). The highest LOD score for ln-transformed triglyceride levels was 2.03

Table 1
Characteristics of the study subjects (mean \pm S.D.) and lipid trait heritabilities

Characteristic	All	Men	Women	$h^2 \pm \text{S.D.}^a$
Number	612	273	339	NA
Age (year)	47.1 ± 15.3	47.0 ± 15.2	47.3 ± 15.5	NA
BMI (kg/m^2)	27.3 ± 4.8	26.2 ± 3.6	$28.1 \pm 5.5^{**}$	0.41 ± 0.08
Diabetic ^b (%)	10.6	7.7	13.0*	NA
TSC (mg/dl)	212.3 ± 45.2	211.9 ± 46.2	212.6 ± 44.4	0.63 ± 0.11
HDL-C (mg/dl)	50.9 ± 13.0	47.2 ± 12.1	$53.9 \pm 13.0^{**}$	0.54 ± 0.08
TG ^c (mg/dl)	70.0 ± 1.7	68.5 ± 1.7	71.2 ± 1.4	0.37 ± 0.08
LDL-C (mg/dl)	145.0 ± 40.7	148.7 ± 42.0	$142.1 \pm 39.5^*$	0.62 ± 0.10

NA: not applicable.

^a Adjusted for sex-specific age and age²; All $P < 0.0001$.

^b Excluding 13 subjects with unknown diabetes status.

^c Triglyceride levels ln-transformed for analysis and back-transformed for presentation.

* $P < 0.05$, men vs. women.

** $P < 0.0001$, men vs. women.

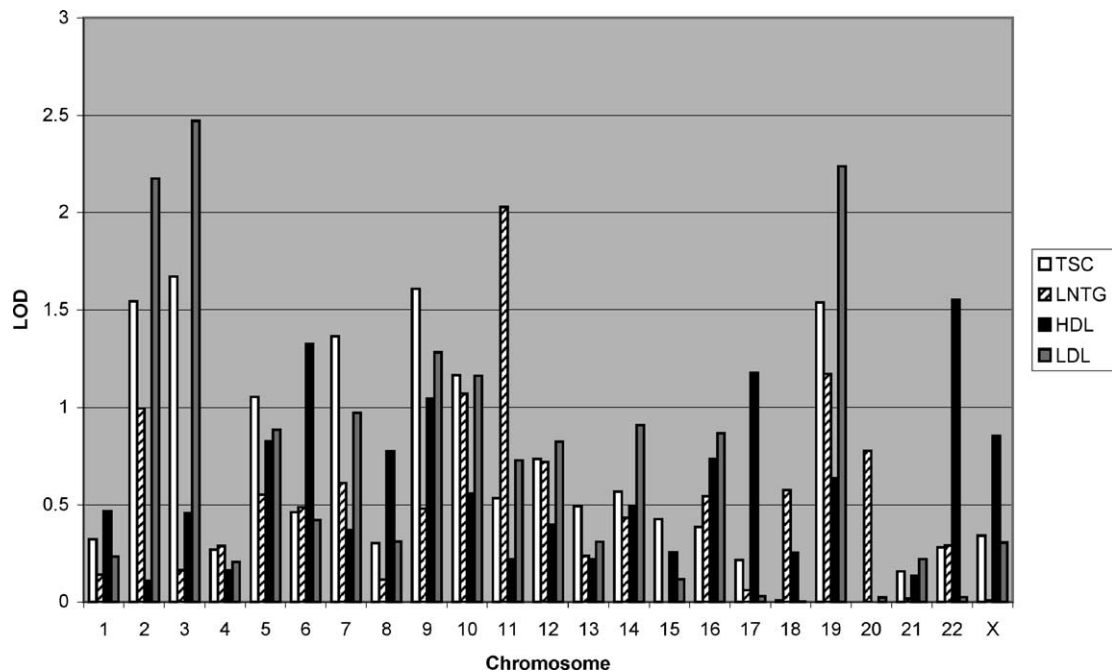


Fig. 1. Peak LOD scores by lipid trait and chromosome.

($P = 0.001$) at chromosome 11q23 near marker D11S1345. An additional peak for triglycerides was observed near marker D11S902 at 11p15 (LOD = 1.70; $P = 0.003$). Suggestive linkage (LOD = 1.56; $P = 0.004$) was seen for HDL-C near marker D22S315 on chromosome 22q11-q13. A subsequent sex-stratified analysis of HDL-C revealed, in males only, a LOD score of 2.63 at chromosome 9q21 near marker D9S167, 22 cM upstream from the original HDL-C

peak of LOD = 1.05 (1.18 in females) near marker D9S287 at 9q22.

Adjusting for BMI or diabetes did not appreciably alter the genome scan results, although some peaks decreased modestly in size; for example, the peak LDL-C level LOD scores on chromosomes 2, 3 and 19 decreased by 0.34 to 1.83, by 0.47 to 2.00, and by 0.09 to 2.14, respectively, when adjusting for BMI and by 0.49 to 1.68, by 0.68 to

Table 2

Multipoint linkage analysis peaks with LOD ≥ 1.5 ($P < 0.005$) (LOD ≥ 2.0 ($P < 0.0012$) emphasized in bold)

Chromosome location	Distance (cM)	Closest marker(s)	Trait	LOD (P -value)	Positional candidate genes ^a (falling within 1-LOD support interval)	Previously reported by
2p23	32	D2S312/D2S220	LDL-C	2.17 (0.0008)	APOB, LPIN1, ABCG5, ABCG8	
2p23	33	D2S312/D2S220	TSC	1.54 (0.004)		
3p25	25	D3S1263	LDL-C	2.47 (0.0004)	PPARG	
3p25	25	D3S1263	TSC	1.67 (0.003)		
9q34	131	D9S290	TSC	1.61 (0.003)	CEL, RXRA	
11p15	30	D11S902	LNTG	1.70 (0.003)	ABCC8 (SUR1)	Aouizerat et al. [54] (FCHL, LOD = 2.6 at 11p14)
11q23	135	D11S1345	LNTG	2.03 (0.001)	APOC3, APOA1, APOA4, APOA5	
19p13	27	D19S221	LDL-C	2.15 (0.0008)	LDLR	Imperatore et al. [8] (TSC, peak LOD = 3.89 at 19pter, LOD \approx 3 at 19p13)}
	39	D19S433		2.23 (0.0007)		
19p13	40	D19S433	TSC	1.54 (0.004)		
22q11-13	17	D22S315	HDL-C	1.55 (0.004)	ADRBK2 (BARK2), HCF2	

^a Candidate genes: ABCC8 (SUR1): ATP-binding cassette, subfamily C (sulfonylurea receptor); ABCG5: ATP-binding cassette, subfamily G, member 5; ABCG8: ATP-binding cassette, subfamily G, member 8; ADRBK2 (BARK2): β -adrenergic receptor kinase 2; APOA1: apolipoprotein A1; APOA4: apolipoprotein A4; APOA5: apolipoprotein A5; APOB: apolipoprotein B; APOC3: apolipoprotein C3; CEL: carboxyl-ester lipase; HCF2: heparin cofactor II; LDLR: low density lipoprotein receptor; LPIN1: lipin 1; PPARG: peroxisome proliferator-activated receptor- γ ; RXRA: retinoid X receptor- α .

1.79, and by 0.38 to 1.85 when adjusting for diabetes status (which also slightly decreased the sample size). Similarly, the LNTG LOD score on chromosome 11q decreased by 0.17 to 1.86 and by 0.76 to 1.27 with adjustment for BMI and diabetes status, respectively.

The results of our power simulation indicated that our sample provided good power (78%) to detect linkage (at LOD scores >3.0) for a QTL accounting for 30% or more of the total trait variance. For a QTL accounting for 25% of the variance, power would be 52% to detect a LOD score greater than 3.0 and 76% for a LOD score greater than 2.0. The power was much lower for a QTL accounting for only 20% of the variance (32% for a LOD score > 3.0 and 52% for a LOD score > 2.0).

4. Discussion

Serum lipid levels are substantially heritable in the OOA, as in other populations (summarized in [22]). Despite evidence for a strong genetic component to normal lipid level variation, the specific genes involved are largely unknown. In this study, no loci were significant at the genome-wide level of statistical significance (LOD ≥ 3), possibly reflecting the genetic and biochemical complexity of the traits and the relatively small effect of individual genes even in this relatively homogeneous population. Also, despite the large number of relative pairs in our study, the power to detect “significant” QTL effects accounting for as much as 20–25% of total phenotypic variance was relatively low.

The highest LOD score we observed was 2.47 for LDL-C levels at 3p25 (LOD = 1.67 for TSC), the location of peroxisome proliferator-activated receptor- γ (PPARG), a key regulator of genes involved in lipid metabolism [28,29]. PPARG is located less than one megabase (Mb) away from D3S1263, the most closely linked marker on 3p. Interestingly, suggestive evidence for linkage of TSC was observed at 9q34 (LOD = 1.61; LOD = 1.28 for LDL-C) near retinoid acid receptor- α (RXRA, 6 Mb from D9S290), which heterodimerizes with PPARG [28,29]. Also on chromosome 9q34 (4 Mb from D9S290) is the gene for carboxyl-ester lipase, a pancreatic enzyme that hydrolyzes cholesteryl esters [30].

Suggestive evidence of linkage to LDL-C (LOD = 2.17) and total cholesterol (LOD = 1.54) was seen at 2p23, near marker D2S220, which is located 200 kilobases (kb) from apolipoprotein B (APOB), the major lipoprotein component of chylomicrons and LDL-C particles. A rare Arg3500Gln APOB variant has been reported in patients with hypercholesterolemia due to reduced LDL-C clearance and is associated with increased risk of ischemic heart disease [31,32]. The fld mouse, which has increased susceptibility to atherosclerosis, is a double knockout for the lipin (LPIN1) gene [33], whose human homolog is found at 2p21 (3 Mb from D2S312, the marker closest to our LDL-C peak). Also at 2p21 are the ATP-binding cassette genes ABCG5 and

ABCG8, mutations in which have been shown to cause sitosterolemia, a rare disorder characterized by hyperabsorption of plant sterols and cholesterol and inefficient removal of these lipids by the liver for excretion in bile, resulting in elevated plasma plant sterols and premature coronary artery disease [34–37]. Persons affected with sitosterolemia may in addition have depressed activity of enzymes involved in cholesterol biosynthesis [38,39]. Sequencing of 9 unrelated sitosterolemia probands by Berge et al. revealed one mutation in the AGCG5 gene and six different mutations in the ABCG8 gene. One of the probands was a 13-year-old Amish boy who was homozygous for a missense mutation in ABCG8, G574R, not found in any of the other probands [34]. Genotype analysis in our Amish participant samples revealed that 3% were heterozygous for the G574R ABCG8 variant; there were no homozygotes. The G547R variant does not account for the linkage observed and was not associated with altered levels of TSC, LDL-C, HDL-C, or triglycerides.

Our peak LOD scores on chromosome 19 for LDL-C (LOD = 2.23 at 39cM and LOD = 2.15 at 27cM on two overlapping signals) and TSC (LOD = 1.54 at 40cM), map to the same region, 19p13, as the low density lipoprotein receptor (LDLR), the causative gene in familial hypercholesterolemia [40]. The LDL-C peak at 27cM is closest to marker D19S221, which is ~ 1.5 Mb from LDLR. In the Pima Indians, Imperatore et al. also showed linkage of TSC near LDLR, although their peak LOD score (3.89) was at the tip of 19p [8]. Finally, a weak confirmation of Reed et al.’s two-point linkage at 10q for TSC was seen in our multipoint scan with a peak LOD score of 1.17 at 141cM (not shown in table) [41].

The highest LOD score observed for ln-triglycerides was 2.03 at chromosome 11q23, occurring near candidate genes apolipoproteins A1 (APOA1), A4 (APOA4), A5 (APOA5) and C3 (APOC3) (all 4.5 Mb from D11S1345). APOA1 is the major lipoprotein in HDL-C and promotes cholesterol efflux from cells, and its variation has been implicated in hypoalphalipoproteinemia (a low HDL-C phenotype) [42]. Variations in the entire cluster, including the recently discovered APOA5 [43–45], have shown association with hypertriglyceridemia [46]. Interestingly, a LOD score for ln-triglycerides of nominal significance ($P < 0.05$, LOD = 1.17, not shown in table) was found at chromosome 19q13, the location of another cluster of apolipoproteins, including APOC1 and APOC2 as well as APOE. Of this cluster, APOC2 has been shown to be an activator of lipoprotein lipase and the gene modified in a hypertriglyceride disorder that mimics LPL deficiency [47]. In addition, the strongest linkage in a lipid genome scan of Utah Mormons (LOD = 3.16) was to triglyceride levels in this region [18]. An additional peak (LOD = 1.70) for ln-triglycerides was observed at 11p15, a region near the Dutch familial combined hyperlipidemia peak (LOD = 2.6) [7] and containing ATP-binding cassette, subfamily C, member 8 (ABCC8, 36 KB from closest linked marker D11S902), a potassium

ion channel that regulates insulin secretion and is also known as the sulfonylurea receptor. A recent study observed lower triglyceride levels in subjects with type 2 diabetes when treated with sulfonylurea agents if they carried at least one copy of an intron 16 T → C polymorphism in this gene vs. those with the TT genotype [48].

The highest LOD score for HDL-C was 1.55 at 22q11-q13. While there do not appear to be genes with known direct roles in lipid level variation in this region, genes with potential indirect roles include β -adrenergic receptor kinase 2 (ADRBK2 or BARK2, 40 kb from D22S315), which promotes phosphorylation and desensitization of G-protein coupled receptors [49,50], and heparin cofactor II (HCF2, 5 Mb from D22S315), a thrombin inhibitor [51]. We also observed a LOD score of 0.83 for HDL-C near the NHLBI Family Heart Study's peak (LOD = 3.64) for HDL-C on chromosome 5 [52]. Interestingly, a subsequent sex-specific HDL analysis revealed in males a relatively large signal (LOD = 2.63) on chromosome 9q21, suggesting the presence of a sex-limited factor involved in HDL control in that region, which was also suggestively linked (LOD = 1.90) to triglyceride (but not HDL) levels in western European families [53].

In conclusion, while our genome-wide scan for variation in lipid levels failed to produce any QTLs significant at the genome-wide level (LOD \geq 3.0), we observed several loci with suggestive linkages to lipid QTLs, some novel and some providing support for linkages previously reported by other groups. Several of these linked regions harbor genes with well-established functional and clinical data supporting their role in lipid metabolism, including lipoproteins, PPAR γ and the LDL-C receptor. Further analyses of these regions will be required to identify the causative gene variants in these positional candidate genes or in other genes in these regions previously not known to affect serum lipid levels.

Electronic database information

The Genome Database: <http://www.gdb.org/>.

Marshfield Comprehensive Human Genetic Maps: http://www.marshfieldclinic.org/research/genetics/Map_Markers/maps/IndexMapFrames.html

NCBI UniSTS: <http://www.ncbi.nih.gov/entrez/query.fcgi?db=unists>.

Online Mendelian Inheritance in Man: <http://www.ncbi.nlm.nih.gov/omim>.

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