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Evidence of Linkage of Familial Hypoalphalipoproteinemia to a Novel Locus on Chromosome 11q23

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Coronary heart disease (CHD) accounts for half of the 1 million deaths annually ascribed to cardiovascular disease and for almost all of the 1.5 million acute myocardial infarctions. Within families affected by early and apparently heritable CHD, dyslipidemias have a much higher prevalence than in the general population; 20%-30% of early familial CHD has been ascribed to primary hypoalphalipoproteinemia (low HDL-C). This study assesses the evidence for linkage of low HDL-C to chromosomal region 11q23 in 105 large Utah pedigrees ascertained with closely related clusters of early CHD and expanded on the basis of dyslipidemia. Linkage analysis was performed by use of 22 STRP markers in a 55-cM region of chromosome 11. Two-point analysis based on a general, dominant-phenotype model yielded LODs of 2.9 for full pedigrees and 3.5 for 167 four-generation split pedigrees. To define a localization region, model optimization was performed using the heterogeneity, multipoint LOD score (mpHLOD). This linkage defines a region on 11q23.3 that is ~10 cM distal to—and apparently distinct from—the ApoAI/CIII/AIV gene cluster and thus represents a putative novel localization for the low HDL-C phenotype.

Introduction

Cardiovascular disease remains the most common cause of death in the United States, accounting for nearly one third of all mortality. Coronary artery disease (CAD) or coronary heart disease (CHD) accounts for half of the 1 million deaths annually ascribed to cardiovascular disease and for almost all of the 1.5 million acute myocardial infarctions. Studies of early CHD sib pairs have identified several risk factors that contribute to CHD, including family history, obesity, smoking, diabetes, hypertension, dyslipidemia, and physical inactivity (Goldstein et al. 1973; Williams et al. 1990).

Within families having early and apparently heritable CHD, dyslipidemias are much more common than in the general population. The most prevalent familial dyslipidemias associated with CHD are hyperbetalipoproteinemia (MIM 144010), hypoalphalipoproteinemia (MIM 205400, 604091), combined hyperlipidemia (MIM 144250, 602491), high Lp(a) (MIM 152200), hypertriglyceridemia (MIM 144600, 145750), and hypercholesterolemia (MIM 143890, 603776) (Genest et al. 1992).

About 20%–30% of early familial CHD is ascribed to hypoalphalipoproteinemia, or primary low HDL cholesterol (HDL-C). Although not initially recognized as a predisposing dyslipidemia, extensive epidemiological work has implicated low HDL-C levels in increased risk of cardiovascular disease, and low HDL-C is now considered a true dyslipidemic syndrome (Warnick and Wood 1995).

About half of the variation in HDL-C levels appears to be genetically determined (Friedlander et al. 1986; Hunt et al. 1989; Perusse et al. 1989; Prenger et al. 1992). Defects in several genes are known to cause low HDL-C, including apolipoprotein AI (ApoAI) (MIM 107680) (Franceschini et al. 1980; Utermann et al. 1982; von Eckardstein et al. 1989; Funke et al. 1991; Romling et al. 1994; Breslow 1995; Tall and Breslow 1996; Huang et al. 1998; Yamakawa-Kobayashi et al. 1999), apolipoprotein B (ApoB) (MIM 107730) (Peacock et al. 1994), lipoprotein lipase (LPL) (MIM 238600) (reviewed by Fisher et al. 1997), lecithin:cholesterol acyltransferase (LCAT) (MIM 245900) (reviewed by Kuivenhoven et al. 1997), and—most recently—ATP-binding cassette transporter 1 (ABC1) (MIM 205400, 600046) (Bodzioch et al. 1999; Brooks-Wilson et al. 1999; Rust et al. 1999; Lawn et al. 1999). However, in aggregate, these known genetic defects account for only a small proportion of individuals with

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low HDL-C. Some studies have shown association of HDL-C levels with variations at the hepatic triglyceride lipase (MIM 151670) (reviewed by Connelly [1998]), ApoAI/CIII/AIV (MIM 107680) (Cohen et al. 1994), apolipoprotein CII (MIM 207750) (Bu et al. 1994), apolipoprotein J (MIM 185430) (Nestlerode et al. 1999), paraoxonase (MIM 168820) (Boright et al. 1998), beta-glucocerebrosidase (MIM 230800) (Pocovi et al. 1998), cholesteryl ester transfer protein (CETP) (MIM 118470) (reviewed by Yamashita et al. 1996) and scavenger receptor BI (SR-BI) (MIM 601040) (Acton et al. 1999) loci. In most cases, the relevance of these loci to dyslipidemias in the general population remains uncertain. A recent quantitative-trait analysis suggested two loci controlling HDL-C levels, on chromosomes 8 and 15, but the underlying genes have not yet been identified (Almasy et al. 1999). Thus, the genetic causes of common low HDL-C remain poorly understood.

This study assesses the evidence for linkage of the hypoalphalipoproteinemia trait to chromosomal region 11q23 in 105 large Utah pedigrees ascertained with closely related clusters of early CHD and expanded on the basis of dyslipidemia. Both two-point and multipoint linkage analyses were performed. A MOD-score approach (Clerget-Darpoux et al. 1986) was used to determine appropriate multipoint phenotype models. The locus we have detected appears to be distinct from the ApoAI/CIII/AIV gene cluster.

Material and Methods

Family Recruitment and Development

All the families in this study were ascertained by the Cardiovascular Genetics Research Clinic (University of Utah). Most of the families for the CHD project were identified through familial aggregation of early-onset CHD. In collaboration with Intermountain Health Care (IHC), we used the IHC cardiovascular-disease hospital discharge records for the years 1990–1995 as a source of probands. IHC discharge records were screened for cases of cardiovascular disease, as defined by myocardial infarction, balloon angioplasty, coronary bypass surgery, or other explicit symptoms. Early-onset disease was defined as prior to age 55 years for men and prior to age 60 years for women. This screen produced a total of 2,470 potential CHD probands for the 5-year period of record.

Probands were contacted by telephone and were given a brief oral questionnaire. Familial history of CHD was established by the occurrence of disease in relatives of the proband. All probands with a family history of CHD were invited to participate in the study by submitting a detailed medical history and contributing a blood sample for chemistry analysis and possible DNA testing. Blood samples were collected after 12–16 hour fasts. Lipids were measured as described elsewhere (Wu et al. 1989). Full informed consent was obtained at all stages of the study.

Probands with family history of CHD who also exhibited dyslipidemia, on the basis of the fasting blood sample, were subsequently developed into extended pedigrees. This expansion did not follow strict sampling rules. Generally, pedigrees were expanded until a sizeable clustering of dyslipidemic relatives was acquired; sampling did not extend to all identified relatives. Since phenotypic status could only be determined after sampling, based on the lipid profile, affected individuals could not be preferentially sampled. At early stages of the analysis, however, affected individuals were preferentially genotyped. In some cases, relatives living out of state were sampled at local clinics, and the blood samples were transferred to the central study site for analysis. Eventually, all sampled members of informative pedigrees were genotyped for at least a subset of markers in the linked region. At later stages of the analysis, some attempt was made to further extend pedigrees showing potential linkage to the region, following the best segregating haplotype. Although the primary focus of this study was low HDL-C, probands with other forms of dyslipidemia, such as familial combined hyperlipidemia, were also developed into extended pedigrees. To some extent, sampling was dependent on available genealogical information. Most pedigrees, however, could be extended to at least three generations (see Results for a full description of the pedigree resource).

In addition to the pedigree set identified from the IHC coronary-disease probands, a further set of dyslipidemic pedigrees was made available by the Cardiovascular Genetics Research Clinic at the University of Utah. These additional pedigrees were developed over the past decade by use of various ascertainment criteria, including dyslipidemia, stroke, and hypertension. An attempt was made to restrict the pedigrees used in this study to those with dyslipidemia as the major familial phenotype, disregarding in particular those pedigrees with significant morbid obesity. A total of 105 pedigrees were included in this study.

Phenotype Definition

Individual phenotypes were encoded as semiquantitative traits. Fasting plasma lipid levels were compared to the LRC national distribution profiles (Lipid Research Clinics 1980) and were assigned percentiles according to age and sex by use of the national data set. It would be inappropriate to use our family data set itself for determining lipid distributions, since the data set is subject to a strong ascertainment bias for clustered dyslipidemia. Average values were used for those subjects

with multiple blood draws. On the basis of these percentiles, four phenotypic liability classes were defined: liability class 1, strongly affected, HDL-C ⩽ 10th percentile; liability class 2, weakly affected, HDL-C 10th–25th percentile; liability class 3, weakly unaffected, HDL-C 25th–50th percentile; and liability class 4, strongly unaffected, HDL-C > 50th percentile. Individuals with no phenotypic information were coded as unknown.

Because of the sensitivity of circulating plasma lipid levels to various confounding factors, some corrections were applied to the raw percentiles. Diabetic individuals were classed as weakly affected (liability class 2), regardless of the actual HDL-C concentration at the time of measurement. Birth control and hormone replacement in females are both potential confounders; however, the effects are not thoroughly understood, and, therefore, no correction was made for these medications. Individuals with hypothyroidism were treated normally if they were currently taking synthetic thyroid hormone. No corrections were made for obesity in the subjects or for alcohol use; significant alcohol use is rare in the subjects comprising this data set.

We chose not to use a quantitative-trait model for linkage analysis, for several reasons. First and foremost, the control-population data are presented by the LRC in the percentile format. In addition, many of the individuals in the study are on potent medications, whose quantitative effects on fasting HDL cholesterol are uncertain. Finally, complicating medical conditions such as diabetes are also difficult to model quantitatively.

Genetic Markers and Genotyping

Genomic search markers (di-, tri-, and tetranucleotide repeats) are described in public databases such as Marshfield and Généthon. In some cases, PCR primer sequences were moved or modified to optimize marker performance. Markers MYR0018, MYR0020, and MYR0021 are all dinucleotide-repeat markers developed at Myriad Genetics on the basis of genomic sequencing in the region. PCR primers for these markers are as follows: MYR0018, TTCACAGGGAGCTATGAGAGTA and *GTTT*CAGGGAGTATTTTAGGTAGC; MYR0020, TGGGCTAGTTCATCTTTAAGGTAGGT and *GTTT*AGTGAATACTGAGCAATACATCAAGCT; and MYR0021, GCATAATGTTAGCCGTCAATCT and *GTTT*TGATACTTCCTTGGGTCTGTTC. For each marker, the *GTTT* at the 5′ end of the second primer is not part of the genomic sequence but was included to reduce the variability of addition of nontemplated nucleotides at the 3′ end of the labeled products (Brownstein et al. 1996).

DNA was prepared from blood samples according to standard methodologies. PCR products were analyzed on ABI 377 fluorescent sequencing machines. Of phenotyped individuals, 90% (3116 of 3467 in liability classes 1, 2, 3, and 4) were eventually genotyped, with the exception of those few individuals for whom DNA extraction following blood draw failed. Genotyping was not restricted only to affected individuals. All individuals were genotyped for a central set of 17 of 18 markers between positions 11.0 and 42.6 on the local genetic map. For the four markers further removed from the peak of LOD, a subset of individuals from the most informative pedigrees was genotyped. Inheritance of all alleles was verified by use of the Pedcheck software package (O'Connell and Weeks 1998). Allele frequencies were calculated by pooling data from the total set of genotyped individuals. The large number of independent pedigrees and married-in spouses in the sample set allowed for an accurate determination of allele frequencies in this population. Note that, although the genotypes of related individuals are correlated, the resulting estimated allele frequencies from the complete data set are, in the strictest statistical sense, unbiased. Moreover, allele frequencies estimated by use only of the founders (including spouses) were essentially similar to those estimated by use of the complete data set.

Building the Genetic Marker Map

The microsatellite markers used in subsequent analyses were mapped by use of the genotyping in the CHD pedigree set. The 105 large pedigrees were split into 593 independent, CEPH-structured pedigrees (nuclear families with grandparents, where available and typed). This yielded a mapping resource with 3,916 meioses, which compares favorably to the resource used by Généthon, comprising 8 large CEPH pedigrees with 186 meioses (Dib et al. 1996). Initial marker order was obtained from the Center for Medical Genetics (Broman et al. 1998). Confidence in order was assessed by use of the "flips5" option of CRI-MAP (Lander and Green 1987), in which each quintuple of adjacent markers are permuted and the likelihood of the map order is calculated. If a different order had a higher likelihood, CRI-MAP was run with this new order. With the maximum-likelihood order, haplotypes were determined with the "chrompic" option of CRI-MAP. These haplotypes were used to identify possible data errors (Litt et al. 1995). After correction of data errors, the mapping procedure was repeated. The final framework map (table 2) was based on criteria of a minimum of two crossovers and >1000:1 odds in favor of the given order of adjacent markers (Litt et al. 1995).

Linkage Analysis

Pairwise linkage analyses were performed with the FASTLINK modifications (Cottingham et al. 1993;

Table 1

Description of the HDL-C Pedigrees Studied

Pedigrees	No. of Individuals	HDL-C Liability Class				
		1	2	3	4	Unknown
Full (n = 105)	4,547 (3,116)	842 (755)	1,009 (898)	702 (631)	914 (830)	1,096 (3)
Split (n = 167)	4,363 (3,057)	836 (753)	997 (886)	681 (615)	883 (802)	966 (1)

Note.— Numbers in parentheses are counts of genotyped individuals in each liability class. Most of the individuals whose phenotypes are unknown were not sampled or genotyped. Liability classes are defined with respect to the LRC national lipid frequency distribution tables, not according to the lipid distributions in the data set itself (see Material and Methods).

Schaffer et al. 1994) of LINKAGE (Lathrop and Lalouel 1984; Lathrop et al. 1984, 1986). Multipoint linkage analyses were performed with MCLINK (Thomas et al., in press), which implements a Gibbs sampling, Monte Carlo, Markov chain algorithm to generate multipoint inheritance matrices (haplotypes) from the genotyping data. Phenotype data are then incorporated to evaluate multipoint LOD scores (mpLOD). Heterogeneity analyses used the admixture test of Smith (1961), in which $\hat{\alpha}$ (estimated proportion of linked pedigrees) and $\hat{\theta}_1$ (estimated recombination fraction for the linked pedigrees) are jointly varied, and a maximum LOD score in the presence of heterogeneity (HLOD for pairwise LOD) is calculated. For multipoint data, $\hat{\theta}_1$ is not estimated; rather, the map position is considered an independent variable (mpHLOD).

The rules by which pedigrees were ascertained and expanded made segregation analysis inappropriate, requiring the specification of phenotype models on a more ad hoc basis. The initial phenotype model assumed a major gene with dominant mode of inheritance and modest frequency (.01) and penetrances such that, for increasing HDL-C concentrations, the penetrance for gene carriers and the probability of being a gene carrier decreased. Model optimizations (MOD scores) were evaluated for mpLOD scores. Five model parameters were varied, considering only a dominant mode of inheritance: the gene frequency and one value in each liability class; this value was the penetrance of the non–gene carriers for liability classes 1 and 2 (the first penetrance) and the penetrances of the carriers for liability classes 3 and 4 (the second and third penetrances). This simplification, which essentially varied the penetrance ratios between non–gene carriers and carriers, was possible because each liability class defines either all affecteds or all unaffecteds. In all cases, the monotonic constraints for penetrance and carrier probability described for the initial model were maintained. In an attempt to reduce the effect of intrafamilial heterogeneity in the set of large pedigrees, the pedigrees were also split into smaller, independent pieces defined by a maximum of four generations and a minimum of three individuals in liability classes 1 and 2. This splitting was done without use of linkage evidence. Pedigrees four generations or fewer in depth were left intact by the splitting algorithm.

Results

Pedigrees in this study are of northern European descent. Table 1 describes the final pedigree resource used in the genetic analyses and includes counts of phenotyped and genotyped individuals in each phenotypic class. Each of the 105 pedigrees has at least 3 genotyped individuals whose HDL-C levels place them in liability class 1 or 2, with an average of 15.7 such individuals per pedigree. The largest pedigree comprises 224 individuals, 65 whom are affected; the pedigree with the greatest number of affecteds comprises 177 individuals, 101 affected, and 97 affected and genotyped. The resource is strongly enriched for individuals with low HDL-C: 24.3% of phenotyped individuals have HDL-C levels which place them in liability class 1 (10% is the expected population average based on LRC distributions), 53.4% are in liability classes 1 or 2 (25% is the expected population average). There is also an enrichment for other dyslipidemic phenotypes. For example, 38.8% of individuals in liability class 1 for HDL-C are also in the 95th or greater percentile for triglyceride levels.

In the first stage of the project, linkage to 13 candidate loci was assessed (apolipoprotein AI/CIII/AIV [MIM 107680], apoAII [MIM 107670], apoB [MIM 107730], apoCI/CII/E [MIM 107710], apoD [MIM 107740], lecithin:cholesterol acyltransferase [MIM 245900], apoH [MIM 138700], apolipoprotein regulatory protein I [MIM 107773], cholesteryl ester transfer protein [MIM 118470], HDL-binding protein [vigilin] [MIM 142695], insulin receptor [MIM 147670], LDL receptor [MIM 143899], and Lp(a) [MIM 238600]). For each locus, 1–3 genetic markers were typed in three multigenerational kindreds (K604, K720, and K610; K610 was subsequently eliminated from the study). Preliminary evidence was observed only for linkage near the ApoAI/CIII/AIV gene cluster on chromosome 11q23. Further sampling in the linked pedigrees and in additional pedigrees, together with the use of more genetic

markers, quickly shifted the region of interest distal to the apolipoprotein gene cluster. The markers used for this study reflect this, with several around the ApoAI/CIII/AIV cluster, but with a higher density distal. By the conclusion of the study, 90% of sampled individuals were genotyped. Because genetic analyses were ongoing during the extended sampling phase, rather than presenting all intermediate stages only the linkage results for the final data set will be presented here.

Pairwise maximum LOD scores (Z_{max}), using the initial phenotype model, are shown in table 2. The marker order corresponds well with published genetic maps (Broman 1998), although we were able to confidently order several markers which were previously unordered and to place the three novel MYR markers in the map. The highest pairwise Z_{max} for full pedigrees is 2.91 and occurs at D11S925. Four pedigrees show linkage to D11S925 with Z_{max} >1.0, and 12 pedigrees are linked between D11S924 and D11S933 (a 9.8-cM region) with Z_{max} >1.0 (table 3); the maximum LOD score exhibited by a single pedigree (K3311) is 2.41 at MYR0021. K3311 was not one of the original three pedigrees searched for linkage but was ascertained through a coronary disease sib trio in which all three siblings shared a haplotype in the region of linkage. Heterogeneity analysis yielded no evidence for an admixture of linked and unlinked pedigrees ($\hat{\alpha} = 1$).

The value of θ at which Z_{max} occurs ($\hat{\theta}$) is >.34 for all markers in the region. This could be the result of locus heterogeneity, allelic heterogeneity, or model misspecification. Locus heterogeneity is identified by the admixture test if the gene frequency is sufficiently small relative to the pedigree size; that is, there is little intrafamilial heterogeneity caused by multiple loci segregating within a single pedigree. Because the admixture test provided no evidence for locus heterogeneity, we split the pedigrees into four-generation independent pieces to assess the contribution of intrafamilial heterogeneity. The pairwise LOD scores for these split pedigrees are also shown in table 2. Although having only a slight effect on $\hat{\theta}$, and no effect on admixture evidence, the LOD score attained a significant value of 3.48. Fifteen split pedigrees are linked between D11S924 and D11S933 with Z_{max} >1.0, with a single-pedigree maximum LOD score of 2.95 for K3311 (table 3).

Multipoint analyses were performed to identify a localization region and to further investigate phenotype modeling. The initial phenotype model gave low mpLOD scores for both full pedigrees and splits: 0.97 and 0.88, respectively, with $\hat{\alpha} = 1$. Model optimization was performed using mpHLOD as the dependent variable and the entire map interval between D11S1995 and D11S2359 (55.2 cM). The optimized model for the full pedigrees generated a maximum mpLOD of 3.69 and maximum mpHLOD of 4.68 ($\hat{\alpha} = .68$), both at map position 32.5 cM on the region map (figure 1*a*). Optimization for the split pedigrees yielded a slightly different result (figure 1*b*), with maximum mpLOD of 3.89 (map position outside of the marker map) and maximum mpHLOD of 5.19 ($\hat{\alpha} = .36$, map position 33.0 cM). There are two major differences between the initial phenotype model and the optimized models (table 4); the optimized models have a higher gene frequency and have a higher penetrance for non–gene carriers than carriers in liability class 2; that is, individuals in liability class 2 are more likely to be non–gene carriers than carriers.

Phenotype model misspecification in the initial model was identified by a high $\hat{\theta}$ at Z_{max}. To evaluate the optimized models in the same way, pairwise LOD scores were calculated using these models. For the full pedigrees, the highest pairwise Z_{max} was 2.79, with $\hat{\theta} = .05$; split pedigrees had the highest pairwise $Z_{max} = 2.25$, with $\hat{\theta}$ .21. Heterogeneity analysis yielded $\hat{\theta}_1 = .04$ (HLOD = 2.82, $\hat{\alpha} = .87$) for full pedigrees, and $\hat{\theta}_1 = 0$ (HLOD = 2.99, $\hat{\alpha} = .33$) for the splits. Thus, the models optimized for mpHLOD retained power to detect linkage when used for pairwise linkage analyses. A further confirmation that the optimized models describe the linked pedigrees is provided by analyzing only those pedigrees

Table 2

Pairwise LOD Scores

		Full Pedigrees		Split Pedigrees	
Marker	Position[a]	Z_{max}	$\hat{\theta}$	Z_{max}	$\hat{\theta}$
D11S1995	.0	.00	.50	.01	.49
D11S1366	4.5	.01	.48	.08	.44
D11S2000	11.0	.12	.47	.11	.47
D11S1998	23.3	.08	.42	.15	.40
D11S924	25.4	1.12	.39	1.37	.37
D11S925	27.6	2.91	.35	3.48	.33
D11S4089	27.8	.77	.42	1.52	.39
D11S4167	29.7	.90	.42	.97	.41
D11S1353	32.5	.95	.41	1.48	.39
MYR0020	33.1	.61	.37	1.27	.34
D11S1328	33.8	.29	.44	.58	.40
MYR0021	34.0	.23	.45	1.53	.37
MYR0018	34.1	.55	.42	1.31	.37
D11S933	35.2	2.07	.37	1.34	.38
D11S4158	35.7	.34	.45	.98	.41
D11S1896	37.1	.48	.41	1.36	.36
D11S934	37.6	.98	.42	1.84	.38
D11S4151	37.8	.46	.43	1.63	.38
D11S4111	39.9	.85	.41	1.16	.39
D11S912	42.6	.97	.39	1.61	.36
D11S1304	51.0	.00	.50	.00	.50
D11S2359	55.2	.07	.45	.05	.43

[a] Positions are in Kosambi cM relative to D11S1995, which is actually at 93.1 cM on the Marshfield chromosome 11 sex-averaged genetic map.

Table 3

Strongly Linked Pedigrees

Pedigree ID	No. Affected[a]	No. Phenotyped	No. in Pedigree	Maximum LOD in Region[b]	Map Position of LOD[c] (cM)
Full pedigrees:					
3311	26	124	147	2.41	34.0
1001	9	40	58	1.86	33.1
1277[d]	17	36	39	1.77	27.6
612[d]	24	53	63	1.57	35.2
726	15	57	70	1.47	27.8
3526[d]	15	59	74	1.34	33.1
3216[d]	9	41	47	1.29	27.6
3101[d]	12	68	73	1.19	35.2
506	18	113	130	1.08	35.7
794[d]	4	17	24	1.07	27.6
3437[d]	6	26	31	1.06	25.4
3565[d]	7	16	19	1.01	25.4
Split pedigrees:[e]					
331102	22	108	128	2.95	32.5
117302	9	37	39	1.57	34.1
7300102	6	21	28	1.26	25.4
306502	6	21	28	1.26	25.4
348001	5	15	24	1.25	33.1
343401	8	25	31	1.14	25.4
72601	9	30	32	1.11	27.8

[a] Individuals in liability class 1: HDL-C $\leq$ 10th percentile.
[b] Maximum pairwise LOD scores between markers D11S924 and D11S933.
[c] Position of the marker at which the maximum LOD score was found, relative to D11S1995.
[d] The split pedigree is identical to the full pedigree.
[e] The originating full pedigree ID is determined by deleting the last two digits of the split ID.

identified as linked. Using the criterion suggested by Ott (1983) of $Z_{max} > 0$ and considering the entire 55.2-cM interval, 73 full pedigrees and 110 split pedigrees were selected. The mpLOD and mpHLOD curves (figure 1*c* and *d*) for the linked pedigrees show little or no evidence of heterogeneity near the peak of LOD.

The linkage confidence interval may be determined from the mpLOD plots in figure 1. Using the recommendation of $Z_{max}-1$ by Conneally et al. (1985), the HDL-C locus maps to 11q23.3 between D11S4167 and MYR0021 (a 4.3-cM interval) (figure 1*d*). This linkage is at least 10 cM distal to the apoAI/CIII/AIV cluster, which maps between D11S2000 (11.0 cM in figure 1) and D11S1998 (23.3 cM in figure 1) (Broman et al. 1998), and represents a putative novel locus governing serum HDL-C concentration.

To assess whether our conclusions regarding region localization and ApoAI/CIII/AIV exclusion are artifacts of the use of phenotype models optimized for peak mpHLOD score, two other optimization strategies were used. Independently optimizing at each position in the interval shows that the decrease in mpHLOD on each side of the peak at position 32.5 cM (figure 2*a* and *b,* dashed curves) gives nearly identical results as use of the optimized model optimized at the peak (figure 1*a* and *b,* dashed curves). To determine whether there is a phenotype model which indicates linkage to the ApoAI/CIII/AIV cluster, rather than the peak at 32.5 cM, model optimization was performed exclusively in the 12-cM interval defining the ApoAI/CIII/AIV cluster. The model which yielded the maximum mpHLOD in this interval was then applied to the entire region. Although there is some attenuation of the mpHLOD score at the peak (figure 2*a* and *b,* solid curves), the peak is still at position 32.5 cM and >2 LOD units greater than the maximum mpHLOD in the ApoAI/CIII/AIV interval.

Discussion

In the present study, we have identified a putative novel locus on chromosome 11q23.3 contributing to familial, low HDL-C. By use of a resource of large, multiplex Utah pedigrees ascertained through cases of early-onset CHD, preliminary evidence from a scan of candidate genes indicated linkage near the ApoAI/CIII/AIV cluster. Addition of markers, pedigrees, and individuals within pedigrees localized the linkage to a 4.3-cM interval between D11S4167 and MYR0021 (0.2 cM distal to

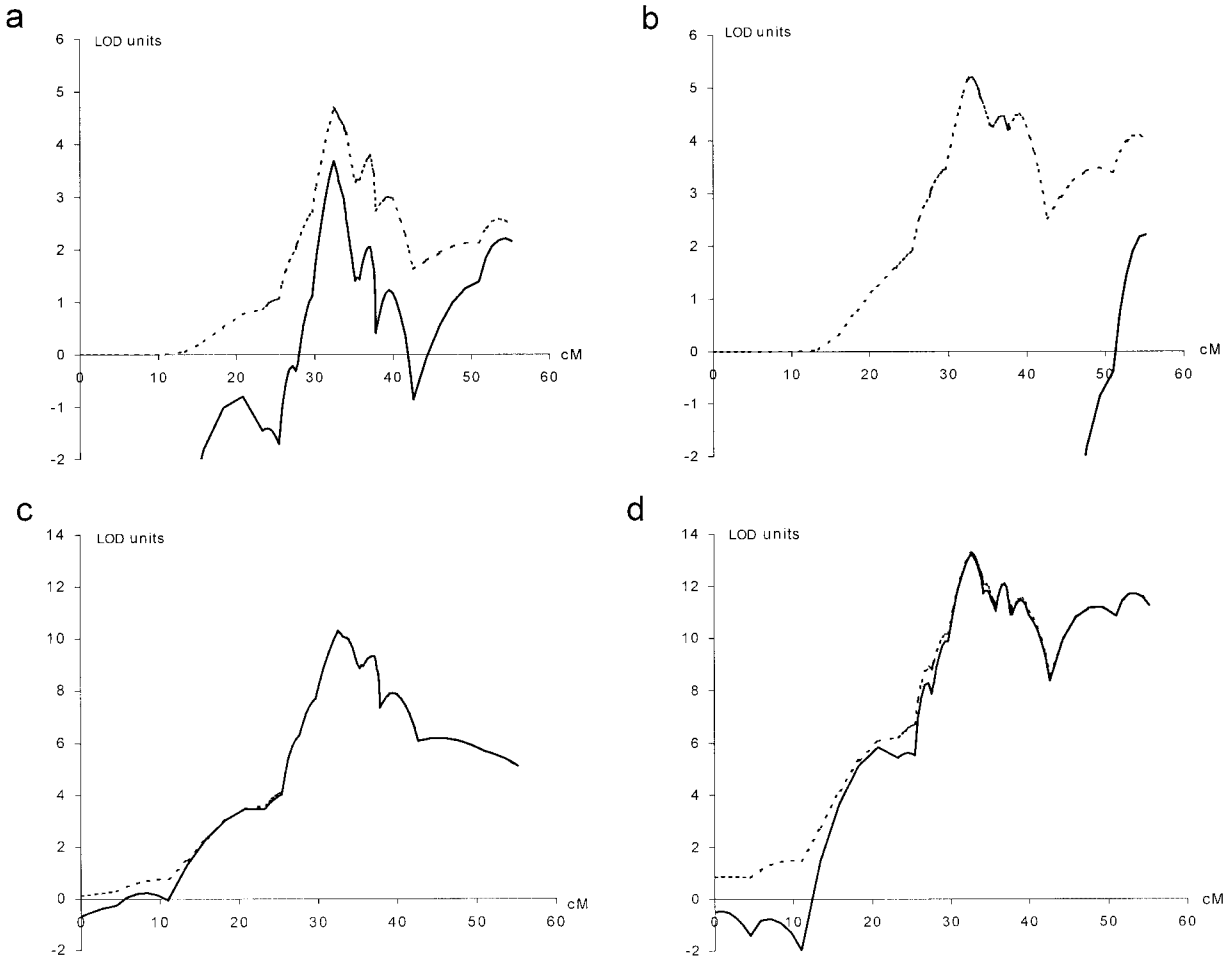

Figure 1 Multipoint LOD scores for 22 markers in 11q23.3 using phenotype models optimized for mpHLOD. *Solid curves,* mpLOD scores; *broken curves,* mpHLOD scores. Within each panel, the phenotype model used for analysis was the one yielding the peak mpHLOD score. *a,* 105 full pedigrees. *b,* 167 split pedigrees. *c,* 73 linked, full pedigrees analyzed with the optimized model from *a*. *d,* 100 linked, split pedigrees analyzed with the optimized model from *b*.

D11S1328), ~10 cM distal to the ApoAI/CIII/AIV cluster. We found no evidence for linkage directly over this apolipoprotein gene cluster.

Pairwise linkage results are robust to phenotypic model misspecification (Ott 1977). Under the correct mode of inheritance, this misspecification may cause a loss of power to detect linkage and an inflated $\hat{\theta}$, but the type I–error rate is unchanged. On the basis of the observation that $\hat{\theta}$ to every marker in the localization region was >.3, the initial phenotype model was not accurately specified. Nonetheless, a significant LOD score of 3.0 was nearly achieved by full pedigrees (2.91) and was exceeded by splits (3.48). Applying a two-test Bonferonni correction to these scores still yields a significant LOD score of 3.19 and is clearly conservative in that the data sets are not independent; many of the "split" pedigrees are identical to the full pedigrees.

Multipoint linkage results, on the other hand, are not robust to model misspecification (Risch and Giuffra 1992); the inflated $\hat{\theta}$ in pairwise analysis is seen as a negative mpLOD score at the actual locus position. To use multipoint results to localize the locus identified by pairwise analyses, we performed model optimization by maximizing the mpHLOD. We chose the heterogeneity measure (rather than the mpLOD) because an assumption of $\alpha = 1$ was unrealistic for a phenotype likely to be affected by multiple loci. Although the statistical characteristics of the mpHLOD are not as well behaved as those of the HLOD, our goal at this stage was not to demonstrate significant linkage but to find a phenotype model that allows locus localization. We chose to use a phenotype model based on a small number of liability classes (four HDL-C levels, standardized to age/sex population norms) rather than a quantitative model based on two or three Gaussian distributions (see Material and Methods for explanation). The models de-

Table 4

Phenotype Models

Model and Liability Class	Penetrance of Genotype		
	AA	Aa	aa
Initial:[a]			
1	.025	.500	.500
2	.150	.350	.350
3	.200	.800	.800
4	.200	.800	.800
Optimized for full pedigrees:[b]			
1	.079	.500	.500
2	.429	.350	.350
3	.200	.654	.654
4	.200	.654	.654
Optimized for split pedigrees:[c]			
1	.074	.500	.500
2	.577	.350	.350
3	.200	.771	.771
4	.200	.771	.771

[a] Frequency of allele a = .010.
[b] Frequency of allele a = .487.
[c] Frequency of allele a = .349.

rived from mpHLOD optimization met our requirements for suitability: (1) improved multipoint scores (mpHLOD of 4.68 for full pedigrees, 5.19 for split pedigrees), (2) multipoint LOD traces that did not exclude the linkage region as defined by pairwise analyses, and (3) pairwise analyses with $\hat{\theta}_1 = 0$ and $\hat{\alpha} < 1$. When the optimized models and a $Z_{max}-1$ criterion are used, a localization region of 4.3 cM is found. This region is consistent between full pedigrees and splits and between the complete data set and linked pedigrees.

Support that this linkage and the ApoAI/CIII/AIV cluster are distinct is provided by a number of observations. The pairwise linkage results show no evidence of linkage to the two markers flanking the cluster, D11S2000 and D11S1998. Localization scores, based on optimized models and either full pedigrees or split pedigrees, exclude the region defined by the two cluster-flanking markers by at least 4 LOD units. The shape of the mpHLOD curve is quite similar whether the generating phenotype model was derived by maximizing the peak mpHLOD score or was independently derived at each position; in both cases, the ApoAI/CIII/AIV interval is excluded relative to the peak between D11S4167 and MYR0021. And, even if the mpHLOD analysis model is optimized only in the ApoAI/CIII/AIV interval, this peak is strongly favored. Finally, we have screened all exons of the apolipoprotein AI gene by direct sequencing in a search for mutations, using samples from a set of the linked pedigrees (including K3311, the most strongly linked). No potential mutations were observed (S. Wagner, personal communication).

It is important to note that phenotype models developed by maximizing the pairwise HLOD did not meet the requirements of a suitable localization model, yielding inappropriate values of $\hat{\theta}_1$ and $\hat{\alpha}$. The maximum HLOD, maximizing each marker separately, was found at D11S925, the peak of the pairwise LOD scores. For full pedigrees , $\hat{\theta}_1 = .38$ and $\hat{\alpha} = 1$ (HLOD = 3.93); for splits, $\hat{\theta}_1 = .35$ and $\hat{\alpha} = 1$ (HLOD = 4.63).

A four–liability class phenotype is not the most parsimonious model, in terms of number of liability classes. Table 4 shows that the optimized models treat liability classes 3 and 4 identically. The number of liability classes in the optimized models may be reduced further with little loss in power. A one–liability class model, in which all phenotyped individuals in liability class 1 are considered affected and all other phenotyped individuals are considered unaffected, yields mpHLOD scores of 4.23 ($\hat{\alpha} = .65$) for full pedigrees and 4.52 ($\hat{\alpha} = .32$) for splits at the same map position as the four–liability class model.

Several linkages have been proposed near 11q23 by means of phenotypes potentially related to low HDL-C. Evidence for linkage to 11q23 was observed in the Finnish study of familial combined hyperlipidemia (Pajukanta et al. 1999), specifically to marker D11S4464, by means of either simple high total cholesterol or apoB levels. This marker, although not shown on our genetic map, is within the region of maximum linkage reported in this study, on the basis of physical and genetic mapping (S. Wagner, personal communication). The Finnish linkage to 11q23, with a maximum LOD score of 1.8, did not achieve suggestive statistical significance (LOD 1.9) (Lander and Kruglyak 1995). In light of our results, it should potentially be reevaluated, perhaps with HDL-C concentration as a phenotype.

Two genomic scans have identified suggestive linkages near 11q23 by use of measures of obesity as a phenotype. Hanson et al. (1998) found their strongest linkage to body mass index (BMI) and type II diabetes among Pima Indians between genetic markers D11S4464 and D11S912, coinciding with the peak of linkage reported in this study. Likewise, Jaquish et al. (1998) found the strongest linkage to BMI in the Framingham study between markers D11S1998 and D11S912.

Because there is a known relationship between obesity and dyslipidemia, we felt that it was important to test whether our linkage was a direct result of an obesity phenotype. Therefore, we reexamined our pedigrees for any relationship between obesity and hypoalphalipoproteinemia and for linkage of obesity to our genetic markers. A number of lines of evidence indicate that obesity is not responsible for the low HDL-C linkage reported here. (1) There are relatively few obese individuals in the data set: 200 with body mass index (BMI) >35 (morbidly obese), representing 6.3% of the individuals with height/weight information; and 215 with

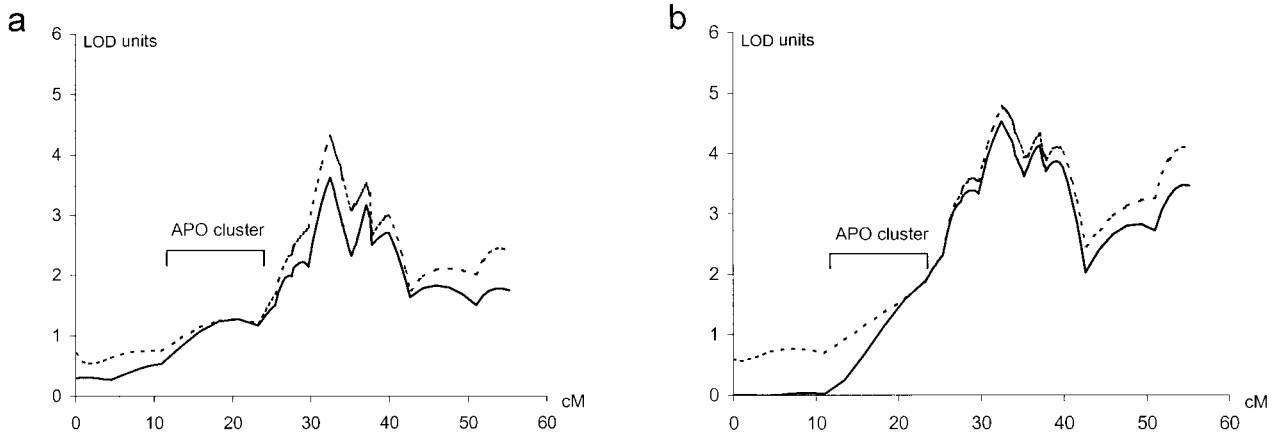

Figure 2 Comparison of mpHLOD scores for phenotype models optimized for the region containing the ApoAI/CIII/AIV cluster and models optimized independently at each map position. Solid curves were derived using the phenotype model yielding the maximum mpHLOD score in the cluster region, broken curves are mpHLOD scores independently maximized. *a,* 105 full pedigrees. *b,* 167 split pedigrees.

BMI between 32 and 35 (moderately obese), representing 6.7%. (2) The correlation between BMI and HDL-C level is low ($\rho = -.20$). (3) There is little evidence of linkage in this region when our data set and an obesity phenotype are used. A qualitative analysis model (likely gene carrier if BMI > 35) yielded pairwise maximum HLODs of 0.34 for full pedigrees and 0.31 for splits. A bimodal quantitative model (Adams et al. 1993) yielded HLODs of 0.91 for both full pedigrees and splits. Such LOD scores are below a suggestive significance level. (4) None of the pedigrees which gave linkage evidence (LOD > 0.5) with an obesity phenotype gave linkage evidence based on HDL-C levels. Although a single gene may be responsible for the obesity phenotype reported by others and for the low HDL-C phenotype reported here, our results offer no support for this hypothesis and are consistent with independent loci.

In contrast to these results, three genomic scans for loci regulating HDL-C levels failed to show linkage to our region. Almasy et al. (1999) report linkages on chromosomes 8 and 15 among Pima Indians, Imperatore et al. (1998) to chromosome 3 among Pima Indians, and Cupples et al. (1998) to chromosome 6 among Framingham study participants. It is difficult to interpret the lack of concordance among these studies, given the differences in methodology and in the populations being studied.

On the other hand, numerous studies have attempted to examine association or linkage between genetic markers in the apolipoprotein AI/CIII/AIV cluster and dyslipidemia. Some investigators report finding strong association to this region (Wojciechowski et al. 1991; Dammerman et al. 1993; Cohen et al. 1994); others find weaker association (Kaprio et al. 1991; Peacock et al. 1994; Kamboh et al. 1999), whereas some find little or no association (Mahaney et al. 1995; Wijsman et al. 1998). Clearly, mutations in the apolipoprotein AI gene itself have potential for predisposition to dyslipidemia (Breslow 1995; Tall and Breslow 1996; Yamakawa-Kobayashi et al. 1999). It remains uncertain whether the other reported associations involve causal genetic variants within this apolipoprotein gene cluster itself. Potentially, some of these other associations might be due to variations at the locus reported in this study. Molecular cloning in the linked region described in this report—and, ultimately, identification of the predisposing gene—should permit clarification of these effects.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, http://www.marshmed.org/genetics/Map_Markers/maps/indexmap.html (for markers used for genotyping)

Généthon, http://www.genethon.fr (for markers used for genotyping)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for familial combined hyperlipidemia [MIM 144250, 602491], familial hypercholesterolemia [MIM 143890, 603776], hyperbetalipoproteinemia [MIM 144010], high Lp(a) [MIM 152200], hypertriglyceridemia [MIM 144600, 145750], hypoalphalipoproteinemia [MIM 604091], apolipoprotein A1 [MIM 107680], apolipoprotein AII [MIM 107760], apolipoprotein B [MIM 107730], apolipoprotein CI [MIM 107710], apolipoprotein CII [MIM 207750], apolipoprotein D [107740], apolipoprotein H [MIM 138700], apolipoprotein J [MIM 185430], apolipoprotein regulatory protein [MIM 107773], ABC1 [MIM 205400, 600046], beta-glucocerebrosidase [MIM 230800], cholesteryl ester transfer protein [MIM 118470], hepatic lipase [MIM 151670], insulin receptor [MIM 147670], lecithin:cholesterol acyltransferase [MIM 245900], lipoprotein lipase [MIM 238600], low density lipoprotein receptor [MIM 143890], paraoxonase [MIM 168820], scavenger receptor B1 [MIM 601040], and vigilin [MIM 142695])

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