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Report

Linkage of a Gene for Familial Hypobetalipoproteinemia to Chromosome 3p21.1-22

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Familial hypobetalipoproteinemia (FHBL) is an apparently autosomal dominant disorder of lipid metabolism characterized by less than fifth percentile age- and sex-specific levels of apolipoprotein β (apoβ) and low-density lipoprotein–cholesterol. In a minority of cases, FHBL is due to truncation-producing mutations in the apoβ gene on chromosome 2p23-24. Previously, we reported on a four-generation FHBL kindred in which we had ruled out linkage of the trait to the apoβ gene. To locate other loci containing genes for low apoβ levels in the kindred, a genomewide search was conducted. Regions on 3p21.1-22 with two-point LOD scores >1.5 were identified. Additional markers were typed in the region of these signals. Two-point LOD scores in the region of D3S2407 increased to 3.35 at Ø = 0. GENEHUNTER confirmed this finding with an nonparametric multipoint LOD score of 7.5 (*P* = .0004). Additional model-free analyses were conducted with the square root of the apoβ level as the phenotype. Results from the Loki and SOLAR programs further confirmed linkage of FHBL to 3p21.1-22. Weaker linkage to a region near D19S916 was also indicated by Loki and SOLAR. Thus, a heretofore unidentified genetic susceptibility locus for FHBL may reside on chromosome 3.

Strong arguments may be made for studying the genetic causes of low cholesterol. First, individuals with low cholesterol levels live longer than do those with high cholesterol levels (Anderson et al. 1987), and there is good evidence that genetic factors are important in determining low cholesterol levels (Hobbs et al. 1989; Welty et al. 1998; Wu et al. 1999). Second, low cholesterol levels may increase the risk for development of fatty livers, at least in selected subjects (Tarugi et al. 1996; Ahmed and Keefe 1998). By contrast, high cholesterol is a well-established risk factor for atherosclerotic coronary artery disease, along with high blood pressure, diabetes mellitus, smoking, and a sedentary lifestyle (Havel and Kane 1995*a,* 1995*b*). Although genetic factors are important in determining both high and low cholesterol levels, much more is known about the genetic determinants of elevated plasma cholesterol concentrations than about the factors that determine very low cholesterol levels.

To identify "low cholesterol"–associated genes, we have studied families with familial hypobetalipoproteinemia (FHBL), a disorder of lipid metabolism defined by less than fifth percentile age- and gender-specific levels of apolipoprotein B (apoβ) (MIM 107730) and low-density lipoprotein (LDL) cholesterol (Havel and Kane 1995*a,* 1995*b;* Schonfeld 1995). More than 35 different truncation-specifying mutations of the apoβ gene (chromosome 2p23-24) have been identified that cosegregate with FHBL (Farese et al. 1992; Schonfeld 1995), and the syndrome has been reproduced in mice. Nevertheless, the genetic etiology of FHBL in the overwhelming majority of kindreds is not well understood (Welty et al. 1998; Wu et al. 1999). Fazio et al. (1991) and Pulai et al. (1998) have reported on two FHBL kindreds in which the trait is not linked to the apoβ gene. This suggested that hitherto unreported susceptibility gene(s) might be responsible for the low apoβ levels in these kindreds.

We now report on a genomewide search undertaken

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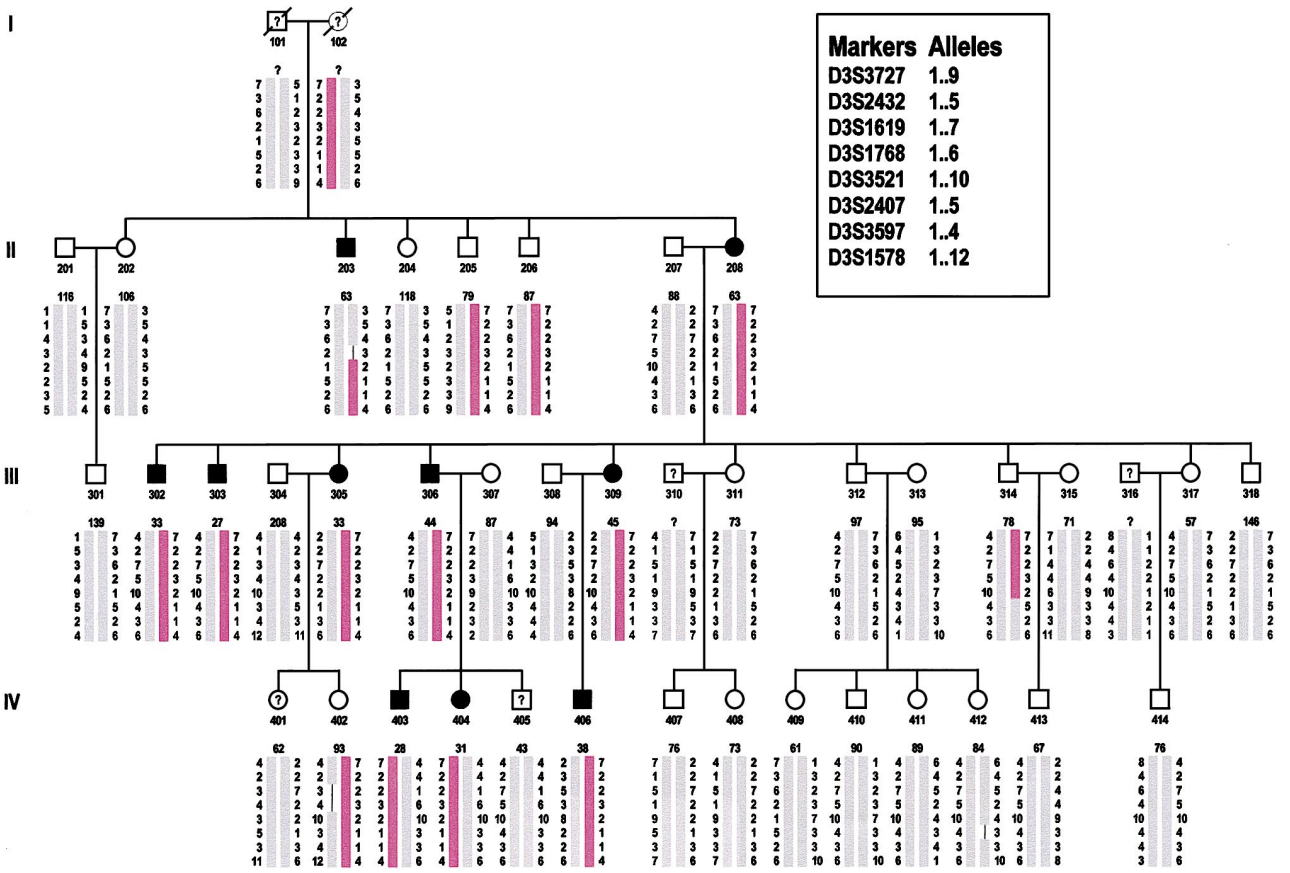

Figure 1 FHBL kindred, showing haplotypes consisting of eight markers on chromosome 3p. Solid symbols denote affected subjects. Question marks denote borderline apoβ level. The magenta-colored bars represent the region segregating with affected FHBL subjects.

to find other susceptibility loci for FHBL in the family reported by Pulai et al. (1998). Ascertainment of this family occurred during screening of various St. Louis–area volunteer populations for potential probands with FHBL. Probands provided information on the structures of their kindreds, members of which were then invited to participate. The protocol was approved by the Human Studies Committee (institutional review board) of the Washington University Medical Center. Venous blood plasmas were analyzed in the Core Laboratory for Clinical Studies, which is Centers for Disease Control standardized. Very-low-density-lipoprotein (VLDL), LDL, and high-density lipoprotein (HDL) were isolated by ultracentrifugation (Manual of Laboratory Operations 1982) and dextran sulfate precipitation, and their triglyceride and cholesterol contents were assessed by enzymatic methods. Total plasma apoA1 and apoβ were determined by immunonephelometry (Contois et al. 1996).

Genomic DNA was extracted from peripheral blood leukocytes by use of the Gentra Puregene DNA extraction kit. In the first round of genotyping, 387 markers consisting of polymorphic tetra-, tri-, or dinucleotide markers distributed over the genome at an average spacing of 10 cM were typed at the Marshfield Medical Research Foundation, Marshfield, WI, by use of screening set 8. Additional markers around loci of interest were subsequently typed. PCR amplifications and sizing of alleles were carried out with standard procedures.

The FHBL family in this study consisted of 38 genotyped individuals (fig. 1). For the initial analysis, pedigree members with less than fifth percentile age- and sex-specific apoβ levels were considered affected. On the basis of this criterion, 10 individuals were designated affected. Individuals 401 and 405 in the fourth generation had borderline apoβ levels and were considered unknown. The average apoβ level was 41 mg/dl (standard deviation 14) for the 10 affected individuals and 94 mg/dl (std = 32) for those unaffected. LDL cholesterol (44 vs. 110 mg/dl) and total cholesterol levels (85 vs. 182 mg/dl) also were significantly lower in affected members ($P < .0001$). Mean ages and body mass indexes (BMI) were not significantly different in affected and unaffected subjects.

Table 1

Summary of Results

	Maximum Score (Location) Given by			
Chromosome	MLINK LOD	GENEHUNTER NPL	Loki	SOLAR LOD
3	3.3 (68 cM)	7.5 (68 cM)	2.0 (68 cM)	3.8 (68.5 cM)
19	1.7 (52 cM)	1.8 (16 cM)	2.0 (30 cM)	1.7 (30.0 cM)
20	1.8 (2 cM)	6.2 (0 cM)	.5 (18 cM)	.9 (25.0 cM)

Two-point LOD scores were computed for all markers with the FASTLINK program (Cottingham et al. 1993), under the assumption of an autosomal dominant mode of transmission with reduced penetrance (75%) and a gene frequency of .001. Genetic parameters were obtained from segregation analysis with the computer program Pedigree Analysis Package, or PAP (Hasstedt 1994). If a LOD score was >1.5, additional markers were typed in the region of the signal, and updated two-point LOD scores were computed. To avoid any potential uncertainty in the exact mode of inheritance of FHBL, the data were also analyzed with the model-free multipoint allele-sharing method implemented in GENEHUNTER (Kruglyak et al. 1996). We used linkage maps provided by the Marshfield Medical Research Foundation's Web site. All marker allele frequencies were computed using ILINK from the LINKAGE suite of programs (Lathrop et al. 1984).

Dichotomizing a quantitative phenotype via a somewhat arbitrary cutoff can introduce an element of uncertainty in the diagnostic classification and potentially causes a loss of information in the analytic phase. Accordingly, additional investigations were performed with the quantitative phenotype, using multipoint linkage methods implemented in the computer programs Loki (Heath 1996) and SOLAR (Almasy and Blangero 1998). Age and gender were included as covariates in both analyses. Loki, a promising newly proposed method for oligogenic quantitative traits, permits joint analysis of more than one chromosome at a time. It requires neither an exact specification of the genetic model nor a designation of the exact number of quantitative trait loci (QTLs) hypothesized to be in the model. Loki will jointly estimate the number of QTLs affecting the trait and perform linkage analysis. Although Loki does not provide traditional LOD scores, regions that have a high probability of containing a QTL can be identified by counting the number of QTLs placed within each centimorgan of the genome and comparing this value to the expected number under the hypothesis of no linkage. The Loki score is defined to be the base 10 logarithm of this ratio. Preliminary research has suggested that data sets with Loki scores >2 generally produce LOD scores >3 (E. Wijsman, personal communication). Loki has successfully identified two known genes in an Alzheimer data set (Daw et al. 1999) and the trait genes in simulated data (Heath 1996). The SOLAR computer package conducts linkage analysis under the variance components framework, providing LOD scores for extended pedigrees. Multivariate normality of the trait is assumed. We report two-point SOLAR LOD score results. By use of the program Simwalk2, haplotypes were constructed in those chromosomal regions giving the strongest signal (Sobel and Lange 1996).

The initial genome scan resulted in two-point LOD scores >1.5 at markers D3S1768 (Z_{max} = 2.3; θ = 0), D19S433 (Z_{max} = 1.73; θ = 0), and D20S103 (Z_{max}=1.8; θ = 0). Additional markers were typed in the regions surrounding these markers. Chromosome 3 markers provided the most significant increase, to Z_{max} = 3.28 (θ = 0), at the newly typed D3S2407. LOD scores did not increase for any of the additionally typed markers on chromosomes 19 or 20 (table 1). Multipoint linkage analysis with GENEHUNTER, using the dichotomous phenotype, yielded a maximum nonparametric multipoint LOD (NPL) score of 7.5 ($P < .001$) close to D3S2407 at 68 cM on our map. The maximum NPL score for chromosomes 19 was 1.81 ($P = .02$) at 16 cM, not consistent with the initial maximum LOD score of 1.73 at 52 cM on the sex-averaged map (at D19S433). The chromosome 20 maximum NPL score of 6.2 ($P < .005$) was at 0.0 cM, ~4 cM from the location of the maximum 2-point LOD score at marker D20S103.

For the Loki and SOLAR analyses, the phenotype was defined to be the square root of the apoβ level, to reduce kurtosis. To determine whether there were regions of chromosome with evidence for quantitative trait loci (QTL), we computed the Loki score jointly, using chromosome 3, 19, and 20 markers. Because Loki is a Markov chain–Monte Carlo (MCMC) method, analyses were repeated multiple times, each with different starting values. We present results that are representative of all Loki runs. Figure 2 displays a histogram of map position versus Loki score for chromosome 3. Chromosome 3 showed strong support for linkage in the region of D3S2407 at ~68 cM on the Marshfield sex-averaged map (table 1). The ratio of the observed to expected number of QTLs was almost 100:1, or ~2.0 on the base 10 logarithmic scale. If preliminary research on Loki scores is correct, the LOD score would be >3.0. Chro-

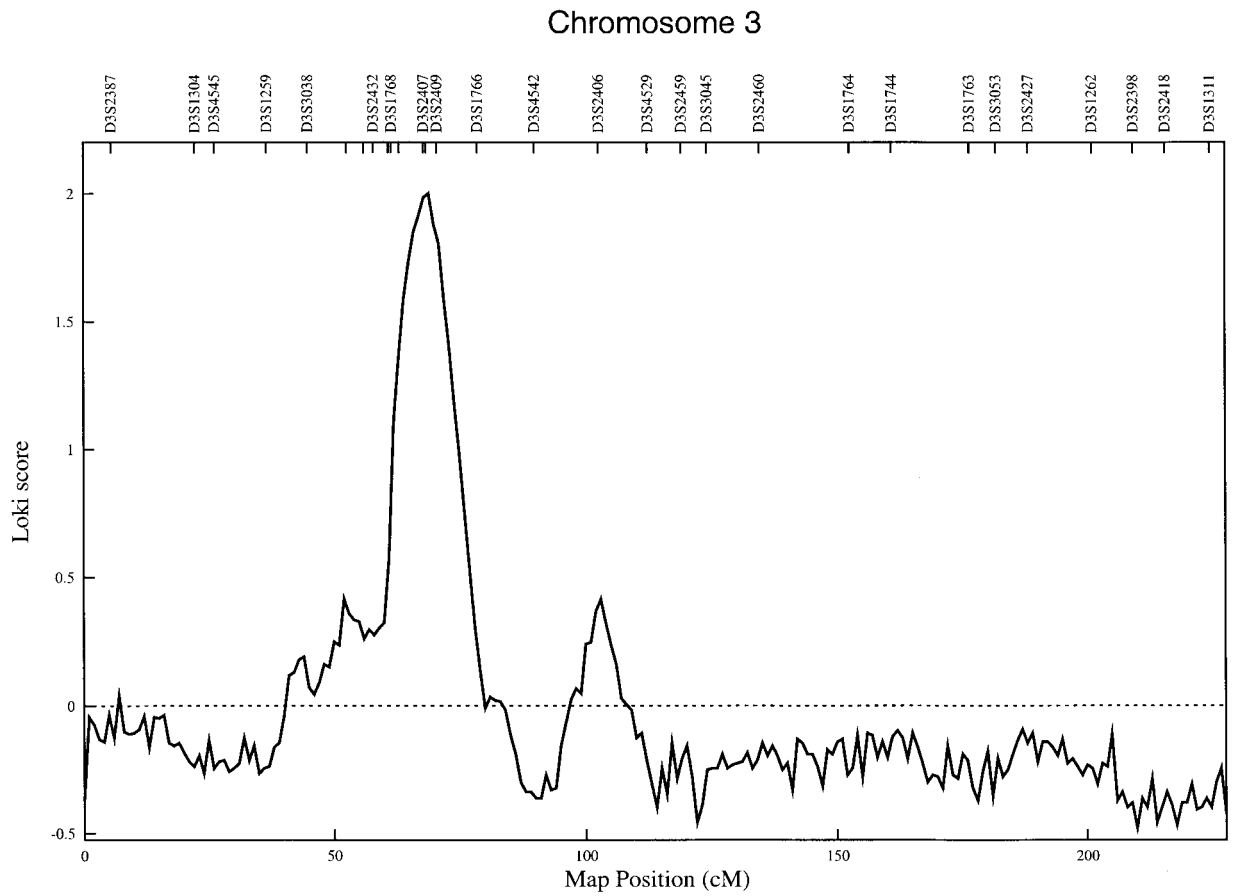

Figure 2 Histogram of map location versus Loki score for chromosome 3p. The Loki score is the logarithm_{10} of the ratio of observed number of QTLs versus the expected number of QTLs in each centimorgan.

mosomes 19 and 20 analyses produced much broader regions for the maximum Loki scores than did chromosome 3 (data not shown). Evidence for linkage to chromosome 19 was greatest at ~30 cM, where the Loki score was 0.84, or a ratio of observed to expected QTLS of almost 7. Note that, on the basis of the dichotomous phenotype, the two-point MLINK LOD scores were <-2 at markers D19S586 and D19S916, located at ~31 cM (table 1). The maximum number of QTLs placed on chromosome 20 was generally only two to three times the expected number, producing Loki scores in the range of 0.5, and the map position with the maximum number of QTLs was less consistent than were those on chromosome 3 and 19. Results from the two-point analysis with SOLAR were most consistent with the chromosome 3 and 19 locations identified with Loki (table 1). The maximum SOLAR LOD score of 3.8 was found on chromosome 3 at D3S3597, located at 68.5 cM, ~0.5 cM from D3S2407. The maximum SOLAR LOD score for chromosome 19 was 1.65 at marker D19S916, located at ~30 cM. The chromosome 20 maximum SOLAR LOD score was 0.86 at 25 cM for marker D20S470. Because of the low LOD scores obtained from Loki and SOLAR, no further analyses were performed on chromosome 20.

Haplotypes were constructed for the family with eight markers spanning ~12.7 cM on chromosome 3 (fig. 1). A common haplotype between D3S3521 and D3S1578 was observed in all affected pedigree members. This haplotype was also carried by the two brothers, subjects 205 and 206, in generation II and subject 402 in generation IV, none of whom was scored as affected. Individual 205, a 74-year-old male, has an apoβ level of 79 mg/dl, (fifth percentile for his age and gender = 74 mg/dl). Individual 206 is a 60-year-old male with an apoβ level of 87 mg/dl, placing him in the lower 25th percentile for his age and sex. Subject 402 is a 14-year-old female with apoβ level of 93 mg/dl (>25th percentile). There was a recombination in unaffected individual 314 (78 mg/dl; >25th percentile) between D3S3521 and D3S2407, which suggests that the FHBL susceptibility gene is located in the 3-cM region between D3S2407

and D3S1578. A shared haplotype among all but one affected subject was observed for chromosome 19 markers in an ~11-cM sex-averaged region, 20.7–31.3 cM, near the area producing the most significant quantitative Loki and SOLAR scores. This haplotype, however, was also shared by seven individuals with apoβ levels greater than the fifth percentile for their age and sex.

Our analyses strongly suggest that chromosome 3 contains a susceptibility region for FHBL in this kindred. This was borne out not only by the positive LOD scores obtained for the dichotomous phenotype but by analyses in which apoβ level defined a quantitative phenotype. Both the Loki and SOLAR programs identified the same region to be harboring a FHBL trait locus. Haplotype analysis supports that this susceptibility locus lies in the 3-cM region between D3S2407 and D3S1578. A region on chromosome 19 was suggested in the two-point analyses as a possible location for a FHBL gene; however, the highest scores obtained by quantitative analyses were not in the same area. Further data are required to either confirm the chromosome 19 finding or identify it as a false positive. An initial weak signal on chromosome 20 did not obtain support from analyses using the quantitative phenotype.

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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 (for screening sets of markers and genetic map)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/htbin-post/omim/dispmim?107730 (for apolipoprotein B [MIM 107730])

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