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Families with Familial Combined Hyperlipidemia and Families Enriched for Coronary Artery Disease Share Genetic Determinants for the Atherogenic Lipoprotein Phenotype

Hooman Allayee,[1] Bradley E. Aouizerat,[1] Rita M. Cantor,[2] Geesje M. Dallinga-Thie,[3] Ronald M. Krauss,[4] Christopher D. Lanning,[2] Jerome I. Rotter,[2] Aldons J. Lusis,[1] and Tjerk W. A. de Bruin[3,5]

[1]Department of Microbiology and Molecular Genetics, Department of Medicine, and Molecular Biology Institute, University of California, and [2]Division of Medical Genetics, Departments of Medicine and Pediatrics, Cedars-Sinai Research Institute, Los Angeles; [3]Department of Medicine, University Hospital, Utrecht, The Netherlands; [4]Life Sciences Division, Lawrence Berkeley Laboratory, University of California, Berkeley, Berkeley; and [5]Departments of Medicine and Endocrinology, University Hospital, Maastricht, The Netherlands

Summary

Small, dense LDL particles consistently have been associated with hypertriglyceridemia, premature coronary artery disease (CAD), and familial combined hyperlipidemia (FCH). Previously, we have observed linkage of LDL particle size with four separate candidate-gene loci in a study of families enriched for CAD. These loci contain the genes for manganese superoxide dismutase (MnSOD), on chromosome 6q; for apolipoprotein AI-CIII-AIV, on chromosome 11q; for cholesteryl ester transfer protein (CETP) and lecithin:cholesterol acyltransferase (LCAT), on chromosome 16q; and for the LDL receptor (LDLR), on chromosome 19p. We have now tested whether these loci also contribute to LDL particle size in families ascertained for FCH. The members of 18 families (481 individuals) were typed for genetic markers at the four loci, and linkage to LDL particle size was assessed by nonparametric sib-pair linkage analysis. The presence of small, dense LDL (pattern B) was much more frequent in the FCH probands (39%) than in the spouse controls (4%). Evidence for linkage was observed at the MnSOD ($P = .02$), CETP/LCAT ($P = .03$), and apolipoprotein AI-CIII-AIV loci ($P = .005$) but not at the LDLR locus. We conclude that there is a genetically based association between FCH and small, dense LDL and that the genetic determinants for LDL particle size are shared, at least in part, among FCH families and the more general population at risk for CAD.

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Address for correspondence and reprints: Dr. Aldons J. Lusis, Department of Medicine, Division of Cardiology 47-123, Center for the Health Sciences, UCLA School of Medicine, Los Angeles, CA 90095. E-mail: jlusis@medicine.medsch.ucla.edu

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Introduction

Familial combined hyperlipidemia (FCH [MIM 144250]), the most common genetic dyslipidemia in man, affects 1%–2% of the population and occurs in 10%–20% of premature myocardial-infarction survivors (Goldstein et al. 1973). It is believed that the primary metabolic defect in FCH leads to hepatic overproduction of apolipoprotein B-100–containing lipoproteins—namely, very-low-density lipoprotein (VLDL) and LDL—resulting in elevated plasma cholesterol and/or triglycerides levels (Cortner et al. 1991; Venkatesan et al. 1993). FCH was first postulated to segregate as an autosomal dominant trait, but there is increasing evidence that it is an oligogenic disorder with a complex pattern of inheritance (Williams and Lalouel 1982; Cullen et al. 1994; Jarvik et al. 1994; Bredie et al. 1996). The primary genetic factors underlying FCH have yet to be identified.

Frequently, but not always, certain other traits also occur in patients with FCH, although they are not considered as criteria for the diagnosis of disease. These traits include reduced levels of HDL (Grundy et al. 1987; Castro Cabezas et al. 1993) and a preponderance of small, dense LDL particles (Austin et al. 1990*a;* Jarvik et al. 1994). The presence of such dense LDL particles, which occurs in combination with increased plasma triglycerides and apolipoprotein B (apoB) concentrations and with reduced HDL-cholesterol levels, has been termed the "atherogenic lipoprotein phenotype" (ALP) and is independently associated with an increased risk for coronary artery disease (CAD) (Austin et al. 1988*a*). Moreover, it has been suggested that hyperapobetalipoproteinemia (hyperapoB), defined as an increased apoB:LDL-cholesterol (apoB:LDL-C) ratio, reflects the presence of FCH in affected individuals (Sniderman et al. 1980). However, it is unclear whether the associations

Table 1

Oligonucleotides and Conditions Used for Radiation-Hybrid Mapping of Candidate Genes

Candidate Gene	Sequence[a]	Annealing Temperature (°C)	Product Size (bp)	Reference
CETP:				
Forward	5′-TCATGAACAGCAAAGGCGTGAGCCTCTTCG-3′	58	180	Sakai et al. (1995)
Reverse	5′-AGCCAAGCTGGTAGAGGCCCCTCTGTCTGT-3′			
LCAT:				
Forward	5′-CGACAGCCCAGTGCCGCTTTCTCTG-3′	56	206	Skretting and Prydz (1992)
Reverse	5′-GGCTTACCGAGGATGACGGGCCGTG-3′			
MnSOD:				
Forward	5′-CGACCTGCCCTACGACTACG-3′	58	549	…
Reverse	5′-CACACCTACCTGAGAGACCAAACA-3′			

[a] For LCAT and CETP, previously published oligonucleotides were used for radiation-hybrid mapping; for MnSOD, new oligonucleotides were designed from the genomic sequence, by means of the OLIGO 4.0 program.

between these traits and FCH are the result of common underlying metabolic defects or are separate entities.

In previous studies with families enriched for CAD, we have obtained evidence of linkage of the presence of small, dense LDL particles to four separate candidate-gene loci: the LDL receptor (LDLR) gene on chromosome 19p, the apoAI-CIII-AIV gene cluster on chromosome 11q, the cholesteryl ester–transfer protein/lecithin:cholesterol acyltransferase (CETP/LCAT) locus on chromosome 16q, and the manganese superoxide dismutase (MnSOD) locus on chromosome 6q (Nishina et al. 1992; Rotter et al. 1996). In the present study, we sought to test whether these same loci contribute to either LDL particle size or related phenotypes in families with FCH. The results indicate that the MnSOD, CETP/LCAT, and AI-CIII-AIV loci exhibit evidence of linkage, whereas the LDLR locus failed to show significant evidence of linkage. Furthermore, the presence of small, dense LDL particles was 10-fold greater in FCH probands than in spouses, strengthening the frequently observed association between FCH and ALP. These results provide supportive evidence that the MnSOD, CETP/LCAT, and AI-CIII-AIV loci contribute to LDL particle size, and they suggest that ALP and FCH share a subset of their genetic determinants.

Subjects and Methods

Ascertainment of FCH Families

Eighteen unrelated Dutch Caucasian index FCH patients were ascertained through family members recruited from the Lipid Clinic of University Hospital (Utrecht). These subjects met the criteria described elsewhere (Goldstein et al. 1973; Brunzell et al. 1983; Castro Cabezas et al. 1993): first, a primary hyperlipidemia with varying phenotypic expression, including a fasting plasma cholesterol concentration >250 mg/dl (6.5 mmol/liter), or above the 95th percentile for age (defined according to the tables from the Lipid Research Clinics), and/or fasting plasma triglyceride concentration >200 mg/dl (2.3 mmol/liter) and elevated plasma apoB concentrations, exceeding, by $\geqslant 2$ SDs, the mean for age-adjusted levels; second, at least one first-degree relative with a hyperlipidemic phenotype different from that of the proband; third, a positive family history of premature CAD, defined as myocardial infarction or cerebrovascular disease at age <60 years in either at least one blood-related subject or the index patient; and fourth, absence of xanthomas. Exclusion criteria included diabetes, familial hypercholesterolemia (absence of isolated elevated plasma LDL-C levels and tendon xanthomas), and type III hyperlipidemia (apoE2/E2 genotype). All subjects gave informed consent, and the study protocol was approved by the Human Investigation Review Committee of University Hospital (Utrecht).

Relatives were assigned the FCH phenotype when they met the following criteria: plasma cholesterol levels >250 mg/dl, plasma triglycerides >200 mg/dl, and/or plasma apoB levels above the 75th percentile. Under these criteria, there were 151 affected individuals and 176 unaffected relatives. The spouses ($n = 154$) represented a common, environment-, nutrition-, and age-matched control group for the probands and their hyperlipidemic relatives.

Biochemical Analyses

Venous blood was drawn after an overnight fast of 12–14 h and abstention from alcohol consumption for $\geqslant 48$ h. Plasma was prepared by immediate centrifugation and was stored at −70°C until the analysis. Lipids and apolipoproteins were quantified according to methodology described elsewhere (Castro Cabezas et al. 1993; Dallinga-Thie et al. 1997). LDL subclass distributions were analyzed after nondenaturing gradient gel electrophoresis of plasma in 2%–16% polyacrylamide gradient gels with lipid staining and densitometric scan-

ning, as described elsewhere (Nichols et al. 1986; Austin et al. 1988*a,* 1988*b,* 1990*b*). LDL particle diameters were calculated from the calibration curves, by means of protein size standards (Nichols et al. 1986), and the diameter of the peak of greatest amplitude for each sample was designated the "peak particle diameter" (PPD). Qualitative LDL subclass pattern was assigned by criteria described elsewhere (Austin et al. 1988*a,* 1988*b,* 1990*b*). According to the broad definition of the ALP, we have included intermediate-pattern individuals with pattern B individuals and have defined this group collectively as being affected with ALP (Rotter et al. 1996).

Radiation-Hybrid Mapping

Polymorphic microsatellite markers for candidate genes were ascertained through radiation-hybrid mapping (Walter et al. 1994) using the Genebridge 4 Radiation Hybrid screening panel (Research Genetics). In brief, oligonucleotides corresponding to a region of each candidate gene (Skretting and Prydz 1992; Sakai et al. 1995) were used to screen the radiation-hybrid panel by means of PCR amplification of hybrid-cell-line DNA (table 1). The data were entered into a software program at the Whitehead Institute/MIT Center for Genome Research, for mapping. Polymorphic microsatellites anchored on both a genetic and radiation-hybrid map were chosen so as to flank the candidate gene in the smallest interval possible. These markers were then used for subsequent linkage studies. Reaction conditions were as follows: 50 ng of the cell-line DNA, 0.70 μM of each oligonucleotide, 1.5 mM $MgCl_2$, 100 μM of each dNTP (Gibco BRL), and 0.5 U of *Taq* polymerase (Gibco BRL) and 35 cycles at 94°C for 30 s, 56°C or 58°C for 30 s, and 72°C for 45 s. PCR products were resolved on 2% agarose gels.

Genotyping

DNA was isolated from peripheral blood cells and was typed for polymorphic markers at or flanking the following candidate genes: the apoAI-CIII-AIV gene cluster, CETP, LCAT, LDLR, and MnSOD. Table 2 provides a description of the polymorphic markers used in this study. Microsatellites near CETP, LCAT, and MnSOD were identified through radiation-hybrid mapping. A previously identified intragenic marker was used for the LDLR gene (Zuliani and Hobbs 1990). The apoAI-CIII-AIV gene cluster was genotyped according to three RFLPs located upstream of the apoAI gene (*Xmn*I and *Msp*I) and within the 3′ UTR of exon 4 of the apoCIII gene (*Sst*I). These RFLPs have been described in detail elsewhere (Dallinga-Thie et al. 1996).

In total, 481 individuals were genotyped for linkage analysis. Reaction conditions for microsatellite analysis were as follows: 75 ng of genomic DNA, 0.34 μM of each primer (the forward primer was end-labeled with ^{32}P), 200 μM of each dNTP (Gibco BRL), 3.0 mM $MgCl_2$, and 0.5 U of *Taq* polymerase (Gibco BRL). PCR was performed in 96-well plates, with 25 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s. PCR products were resolved on 5% polyacrylamide gels, blotted with Whatman paper, and exposed to film overnight. Genotypes were then assigned to each individual, by two independent observers.

The presence or absence of the RFLPs within the apoAI-CIII-AIV gene was determined as described elsewhere (Dallinga-Thie et al. 1996). In brief, the region of DNA encompassing the restriction site was PCR amplified by means of site-specific primers and incubated with the appropriate enzyme at 37°C for 1 h, and the products were resolved on 3% agarose gels. The alleles of the RFLPs (1 if the restriction site was present, 2 if it was not present) were constructed into haplotypes to increase the informativeness of the locus and then were used for linkage analysis.

Statistical Analysis

Statistical differences in mean trait values between groups were assessed by unpaired Student's *t*-test. Differences in the ALP frequency distribution between groups were assessed by Fisher's exact test.

Nonparametric Sib-Pair Linkage Analysis

The methodology of robust sib-pair analysis was used to test whether there is linkage between qualitative and quantitative traits and a polymorphic marker(s) at each of the candidate-gene loci (Haseman and Elston 1972; Amos et al. 1989). By means of the SIBPAL subprogram

Table 2

Candidate Genes and Linked Polymorphic Markers Assessed for Linkage to ALP

Candidate Gene and Marker[a]	Heterozygosity	Chromosome	Reference
AI-CIII-AIV:			
XMS	.41	11	Dallinga-Thie et al. (1996)
CETP/LCAT:			
D16S408	.69	16	
D16S514	.82		
D16S400	.61		
D16S496	.74		
LDL-R:			
LDLR	.48	19	Zuliani and Hobbs (1990)
MnSOD:			
D6S1007	.60	6	
D6S1008	.77		

[a] Markers for CETP, LCAT, and MnSOD were ascertained through radiation-hybrid mapping; a previously reported microsatellite was used for LDL-R; and XMS = haplotype of *Xmn*I, *Msp*I, and *Sst*I restriction sites within the apoAI-CIII-AIV gene cluster.

Table 3

Clinical Characteristics of FCH-Family Members

Variable	Hyperlipidemic Individuals (n = 151)[a]	Normolipidemic Individuals (n = 176)	Spouse Controls (n = 154)
Age (years)	47 ± 15	31 ± 13	49 ± 16
Cholesterol (mg/dl)	281 ± 72	190 ± 32	220 ± 41
LDL-C (mg/dl)	183 ± 46	120 ± 29	144 ± 38
HDL-C (mg/dl)	43 ± 11	48 ± 12	48 ± 12
Triglycerides (mg/dl)	210 ± 107	112 ± 47	142 ± 78
apoB (mg/dl)	143 ± 27	91 ± 20	110 ± 27
apoB:LDL-C ratio[b]	175 ± 56	164 ± 23	167 ± 37

NOTE.—Values are expressed as mean ± SD.

[a] Includes the 18 probands and 133 hyperlipidemic relatives.

[b] Plasma apoB:LDL-C ratio multiplied by 1,000. The ratio is a surrogate marker for hyperapoB.

in the S.A.G.E. package (S.A.G.E. 1997), all quantitative traits were evaluated for linkage with marker loci, by regressing the squared trait difference of sib pairs on the proportion of alleles shared identical by descent (IBD). If there is no linkage between the trait and the marker locus, the squared trait difference is expected to be relatively constant, regardless of the proportion of marker alleles shared IBD. Hence, the slope of the regression line should not be significantly different from zero. In the presence of linkage, however, the squared trait difference is expected to be decreased when the siblings share a greater proportion of marker alleles, resulting in a significant negative slope of this regression line (Haseman and Elston 1972). Because sib pairs in the same sibship may be nonindependent statistically, significance levels are calculated by means of effective df, which reflects the number of independent sib pairs in the analyses (S.A.G.E. 1997).

For the qualitative trait, ALP, the linkage analysis was conducted only with sib pairs concordant for the presence of the ALP trait. The mean proportion of alleles shared IBD by the affected sib pairs was calculated and then tested to assess whether it was significantly >.50, the expected proportion of marker sharing in the absence of linkage between the trait and the marker. Since we were testing specific hypotheses with respect to each of four candidate genes, we set the threshold for significant evidence of linkage at $P < .05$ for each of these loci.

Results

Study Subjects

The clinical characteristics of the FCH probands, their normolipidemic and hyperlipidemic relatives, and the spouse controls are summarized in table 3. The hyperlipidemic relatives and probands were characterized by higher levels of total plasma cholesterol, triglycerides, LDL-C, and apoB and lower HDL levels, compared with those in the normolipidemic relatives and spouse controls. However, since the FCH phenotype typically is not manifested until the 2d decade of life, it cannot be excluded that some of the normolipidemic relatives have yet to fully express the disease trait. In addition, the apoB:LDL-C ratio also was higher in the hyperlipidemic relatives than in the normolipidemic relatives and spouse controls (table 3).

Identification of Informative Genetic Markers for Candidate Genes

In a previous study, we had assessed linkage of the MnSOD and CETP gene loci to LDL particle size by using either RFLPs or a microsatellite marker that mapped 6 cM from CETP (Rotter et al. 1996). In the present study, we precisely placed the CETP, LCAT, and MnSOD genes on their respective chromosomes, relative to previously mapped microsatellites markers, using the radiation-hybrid system. The advantage of this approach is twofold: first, microsatellites that are anchored on both the physical radiation-hybrid map and a genetic map can be chosen so as to flank the candidate gene in a small interval, thereby providing the best assessment of linkage; second, microsatellites have high heterozygosity indices and therefore are particularly informative for linkage studies. For the CETP, LCAT, and MnSOD genes, two flanking microsatellites were chosen, with heterozygosity indices of .61–.82. The CETP and LCAT genes mapped in close proximity (<10 cM) to each other, and the linkage results could not, with absolute certainty, be assigned to one gene or the other. Therefore, in the present report, this candidate region is referred to as the "CETP/LCAT locus" and will be considered as one locus.

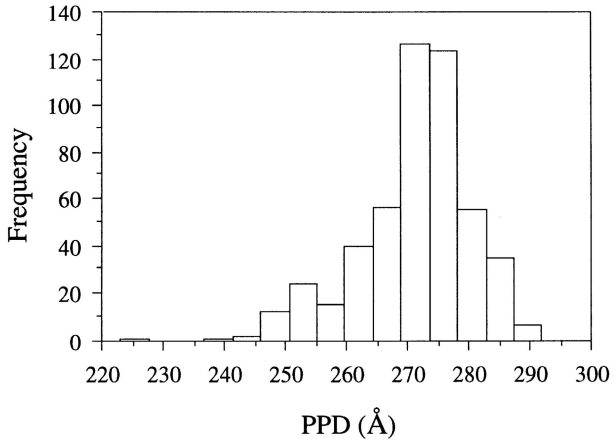

Figure 1 Distribution of PPD in study population

Table 4

Distribution of LDL Subclass Patterns and Their PPDs in the Study Population

LDL Subclass	No. (%)	PPD ± SD (Å)
Pattern A	385 (85)	274 ± 6
Intermediate pattern	21 (5)	261 ± 2
Pattern B	45 (10)	251 ± 6

Analysis of Linkage of Four Candidate Genes to LDL Particle Size

The distribution of peak LDL particle size in the FCH families, as measured by gradient gel electrophoresis, is shown in figure 1. Table 4 shows both the distribution of the PPD for each subclass pattern and the number of individuals in each category. A total of 451 family members were phenotyped for plasma LDL particle size. Significantly, there was a 10-fold–higher prevalence of ALP in the FCH probands than in the spouse controls (39% vs. 4%; $P < .0001$) (table 5), consistent with the concept that FCH and ALP exhibit common genetic and environmental determinants (Austin et al. 1990*a*; Jarvik et al. 1994). A similar trend also was observed when all family members were examined, with ALP being present in 25% of the affected, hyperlipidemic relatives and in only 3% of the unaffected relatives (table 5).

We conducted quantitative linkage analysis on two measures of LDL particle size: PPD and apoB:LDL-C ratio. A qualitative sib-pair linkage analysis with ALP as a discrete trait also was performed. Since each LDL particle has one apoB-100 apolipoprotein, the amount of LDL apoB is reflective of the number of LDL particles. Therefore, the apoB:LDL-C ratio can be interpreted as a measure of LDL particle size, in which individuals with pattern B have a ratio greater than that in individuals with pattern A. In our FCH population, we utilized measurement of total apoB in plasma as a surrogate for LDL apoB, and an inverse relationship between PPD and the apoB:LDL-C ratio was observed ($r = -.36$) (fig. 2). High apoB:LDL-C ratios were defined arbitrarily as those above the 95th percentile in the spouse controls, and the highest apoB:LDL-C ratios were found in individuals with LDL pattern B (fig. 2).

In the quantitative analysis, we obtained evidence of linkage of PPD and the apoB:LDL-C ratio to the CETP/LCAT locus (see table 6) but found no evidence of linkage of these measures to any of the other three loci tested. With the qualitative trait, ALP, significant evidence for linkage was observed, with both markers, at the MnSOD gene locus, as well as with the haplotype of the RFLPs within the apoAI-CIII-AIV gene cluster (table 7). However, we did not observe evidence of linkage to the LDLR locus.

Discussion

Small, dense LDL particles are a characteristic feature of ALP and hyperapoB, complex phenotypes associated with increased incidence of CAD. Previous linkage studies of families from general populations and of families enriched for CAD have provided evidence for the involvement of four loci contributing to small, dense LDL particles (Nishina et al. 1992; Rotter et al. 1996). Another disorder that has been associated with small, dense LDL is FCH, a phenotype of "mixed hyperlipidemia" characterized by hypercholesterolemia, hypertriglyceridemia, or both and by a complex pattern of inheritance with a dramatic familial aggregation. It has, in fact, been speculated that ALP, hyperapoB, and FCH are manifestations of the same underlying metabolic syndrome (Sniderman et al. 1980; Kwiterovich 1993). To further investigate the relationship between these disorders, we have examined the association between ALP and FCH and have tested whether the four loci previously shown to contribute to small, dense LDL particles in families ascertained for CAD also contribute to the phenotype in FCH families. Our results support the involvement of loci containing the genes for MnSOD, CETP/LCAT, and apoAI-CIII-AIV—but not the involvement of the locus containing the gene for LDLR—in FCH-associated ALP. The results also demonstrate that there is an enrichment of ALP in FCH-affected individuals, compared with controls. These two lines of data further strengthen the notion that these complex traits are related.

Austin et al. (1988*b*) initially postulated that the ALP in normolipidemic families is under the influence of a major gene, a hypothesis that has also been used to explain LDL particle size in FCH families (Austin et al.

Table 5

Association between ALP and FCH Affection Status

	No. (%) of Individuals with ALP		
ALP Status	Spouses	Probands	Total
Absent	138 (96)	11 (61)	149
Present	6 (4)*	7 (39)*	13
Total	144	18	162
	Unaffected Relatives[a]	Affected Relatives	
Absent	292 (97)	110 (75)	402
Present	9 (3)	36 (25)	45
Total	301	146	447

[a] Spouse controls and normolipidemic relatives were included within the group of unaffected relatives, since there were no differences in their mean PPDs.

* $P < .0001$ (difference between probands with ALP and spouse controls with ALP, by Fisher's exact test). Under the broad definition of ALP, individuals with an intermediate pattern were grouped with pattern B individuals.

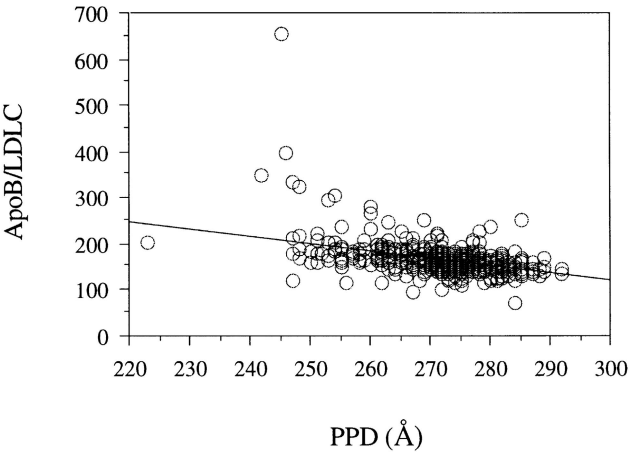

Figure 2 Regression plot of PPD and apoB:LDL-C ratio in study population.

1990*a;* Jarvik et al. 1994; Bredie et al. 1996). Using parametric linkage analysis and assuming incomplete penetrance, Nishina et al. (1992) obtained evidence of linkage to the LDLR gene. This finding was confirmed by a subsequent study using nonparametric analysis in an independent series of families enriched for CAD; however, three additional loci—the apoAI-CIII-AIV locus, the CETP gene locus, and a chromosome 6 locus containing the MnSOD gene—also were found to be linked to LDL particle size, suggesting that ALP is under multigenic control (Rotter et al. 1996).

In the present study, we have obtained evidence supporting the linkage of the CETP/LCAT, MnSOD, and apoAI-CIII-AIV gene loci to LDL particle size. Since the CETP and LCAT genes map very close to each other, it is possible that our previous study (Rotter et al. 1996) detected linkage with the LCAT gene locus. The results of our present study, taken together with our previous findings, suggest that this region of chromosome 16 contains a genetic determinant of LDL particle size. In the population in the present study, we did not observe linkage with the LDLR gene locus (Nishina et al. 1992; Rotter et al. 1996). It is possible that the underlying defect in FCH alters LDL metabolism in such a way that ALP in FCH families results from pathways not involving the contribution of the LDLR gene locus. Alternatively, the effects of the LDLR gene locus may be obscured by the FCH trait itself. Interestingly, in a recent follow-up study of the original families in which linkage to this locus had been demonstrated, no mutations in the coding sequence of the LDLR gene were found, raising the possibility that the observed effect of this locus is due to a nearby gene rather than to the LDLR gene itself (Naggert et al. 1997).

The present study's observation of linkage of ALP with the apoAI-CIII-AIV gene cluster is important for two reasons: first, it supports the role of the apoAI-CIII-AIV gene cluster as a genetic determinant of LDL particle size, as observed by Rotter et al. (1996); and, second, we and others have demonstrated evidence for linkage of the apoAI-CIII-AIV gene cluster to FCH (Wojciechowski et al. 1991; Dallinga-Thie et al. 1997). Thus, since the apoAI-CIII-AIV gene cluster demonstrates linkage to both FCH and ALP, it provides evidence for a common genetic basis for these traits, which has been suggested elsewhere (Kwiterovich 1993). Expression of the apoCIII gene in this cluster has been shown to be a determinant of plasma VLDL triglyceride levels in mice (Weinstock et al. 1997). In humans, the apoCIII gene affects plasma apoCIII concentrations, and the elevated apoCIII plasma concentrations in FCH subjects predict impaired postprandial lipemia (Castro-Cabezas et al. 1993; Dallinga-Thie et al. 1996). Furthermore, it is known that, in general, plasma triglyceride levels are a determinant of LDL pattern B as well as ALP (Austin et al. 1988*a;* Bredie et al. 1997). Therefore, the involvement of the apoCIII gene in ALP and FCH is not only evident from the linkage results but biologically plausible as well.

LDL particle size presumably is influenced by factors governing the synthesis, processing, and cellular uptake of VLDL and its metabolic products. The activities of CETP and LCAT are dependent on the size of the pool of triglyceride-rich lipoproteins, a concept that has been employed to explain the well-known association between hypertriglyceridemia and reduced HDL levels (Tall 1986). Other observations indicate that, in some human populations, hypertriglyceridemia is associated with elevated levels of small, dense LDL. Given the evidence for linkage between the CETP/LCAT gene locus and LDL particle size, a similar lipid-exchange mechanism mediated by CETP and LCAT may explain the generation of small, dense LDL particles in the presence of an expanded pool of triglyceride-rich lipoproteins.

A biological basis for the linkage of the MnSOD gene locus with LDL particle size is more speculative. Small, dense LDL particles have been shown to be more susceptible to oxidation (de Graaf et al. 1991; Tribble et al. 1992). It is conceivable that MnSOD influences li-

Table 6

Significant Results of Quantitative Sib-Pair Linkage Analysis for PPD and apoB:LDL-C Ratio: CETP/LCAT Locus

	Candidate-Gene Region	Marker	Effective df	*P*
PPD	CETP/LCAT	D16S496	132	.035
apoB:LDL-C ratio	CETP/LCAT	D16S514	146	.031

Table 7

Results of Sib-Pair Linkage Analysis for ALP

Candidate Gene	Marker	No. of Affected Sib Pairs	Mean Allele Sharing	P[a]
AI-CIII-AIV	XMS	32	.60	.005
MnSOD	D6S1007	29	.62	.036
	D5S1008	15	.64	.020
LDL-R	LDLR	25	.53	NS

[a] NS = not significant ($P > .10$).

poprotein metabolism by effects on mitochondrial fatty-acid oxidation or acylation. For example, the high fatty-acid flux that occurs in FCH could result in an elevated oxidative burden, leading to increased superoxide production (Bredie et al. 1997). It also is noteworthy that MnSOD confers resistance to tumor necrosis factor (TNF) and that TNF down-regulates lipoprotein lipase (LPL) expression (Wong et al. 1989). Thus, the decreased expression of LPL observed in a high fraction of FCH patients could result from cytokine-mediated effects possibly involving MnSOD.

In the families in the present study, a low (4%) prevalence of ALP or LDL pattern B was observed among unaffected relatives, resulting in a very high (96%) specificity of LDL pattern B for the diagnosis of FCH, although sensitivity was only moderate. Austin et al. (1990*a*) reported a 29% prevalence of ALP (also measured as small, dense LDL [pattern B]) in a FCH population comprising 234 affected and unaffected relatives. Similarly, Jarvik et al. (1994) found a 34.4% prevalence of small, dense LDL in four FCH pedigrees ($n = 192$). However, in the population of FCH families that we studied, the prevalence of ALP was only 15%. A reasonable explanation for the observed differences is that the penetrance of LDL pattern B is dependent on and can be affected by genetic factors associated with the FCH phenotype, population differences, and/or environmental influences such as diet. Thus, the lack of observed linkage with the LDLR locus also may be attributed to the lower prevalence of ALP in the population that we studied, which reduced the number of affected sib pairs in the analysis.

As a surrogate marker for hyperapoB, the apoB:LDL-C ratio demonstrated an inverse relationship with LDL PPD in FCH relatives, and the highest ratios were found among subjects with small, dense LDL particles (pattern B). When the ratio was converted to a discrete variable, with the 95th percentile as a cutoff point, it was observed to be independent of the presence of FCH, consistent with the original definition of hyperapoB (data not shown) (Sniderman et al. 1980). Thus, our results suggest that the apoB:LDL-C ratio, although an informative measure of LDL particle size, does not appear to be diagnostic for FCH, a finding consistent with a report showing the independent segregation of apoB and LDL particle size in FCH (Jarvik et al. 1994).

In conclusion, our results indicate that three candidate loci contributing to small, dense LDL particles in the general population also contribute to this trait in FCH families. Thus, our results support the hypothesis that LDL particle size is under multigenic control. We also demonstrated that the prevalence of small, dense LDL particles is 10-fold higher in affected FCH individuals. Taken together, these results provide new insights into the frequently observed relationship between FCH and the small-dense-LDL trait, both of which significantly predispose to CAD.

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Electronic-Database Information

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

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for FCH [MIM 144250])
Whitehead Institute for Genome Research, http://www.genome.wi.mit.edu (for mapping program)

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