

Multilocus Genetic Determinants of LDL Particle Size in Coronary Artery Disease Families

Jerome I. Rotter,¹ Xiangdong Bu,¹ Rita M. Cantor,¹ Craig H. Warden,² Jane Brown,¹ Richard J. Gray,¹ Patricia J. Blanche,³ Ronald M. Krauss,³ and Aldons J. Lusis²

¹Divisions of Medical Genetics and Cardiology, Departments of Medicine and Pediatrics, Cedars-Sinai Medical Center, and ²Departments of Medicine, Microbiology, and Molecular Genetics, University of California, Los Angeles, Los Angeles; and ³Life Sciences Division, Lawrence Berkeley Laboratory, University of California, Berkeley, Berkeley

Summary

Recent interest in atherosclerosis has focused on the genetic determinants of low-density lipoprotein (LDL) particle size, because of (i) the association of small dense LDL particles with a three-fold increased risk for coronary artery disease (CAD) and (ii) the recent report of linkage of the trait to the LDL receptor (chromosome 19). By utilizing nonparametric quantitative sib-pair and relative-pair-analysis methods in CAD families, we tested for linkage of a gene or genes controlling LDL particle sizes with the genetic loci for the major apolipoproteins and enzymes participating in lipoprotein metabolism. We confirmed evidence for linkage to the LDL receptor locus ($P = .008$). For six candidate gene loci, including apolipoprotein(apo)B, apoAII, apo(a), apoE-CI-CII, lipoprotein lipase, and high-density lipoprotein-binding protein, no evidence for linkage was observed by sib-pair linkage analyses (P values ranged from .24 to .81). However, in addition, we did find tentative evidence for linkage with the apoAI-CIII-AIV locus (chromosome 11) ($P = .06$) and significant evidence for linkage of the cholesteryl ester transfer protein locus (chromosome 16) ($P = .01$) and the manganese superoxide dismutase locus (chromosome 6) ($P = .001$), thus indicating multilocus determination of this atherogenic trait.

Introduction

Low-density lipoproteins (LDLs) are the major cholesterol-carrying lipoproteins in plasma. Elevated plasma LDL cholesterol level has been shown to be a major risk factor for coronary artery disease (CAD) (Kannel et al.

1971; Brown and Goldstein 1986; Castelli et al. 1986). It is well established that human plasma LDL varies in size, density, and lipid content (Adams and Schumaker 1969; Fisher et al. 1975; Krauss and Burke 1982). Multiple, discrete subclasses of LDL particles have been identified and characterized using density gradient ultracentrifugation and gradient-gel electrophoresis (Lindgren et al. 1972; Shen et al. 1981; Krauss and Burke 1982). Previous studies have suggested that there is up to a threefold higher risk of myocardial infarction for individuals with the phenotype that has been termed *LDL subclass pattern B*, characterized by a predominance of small, dense LDL particles, when compared with subjects with larger LDL particles (subclass pattern A) (Austin et al. 1988a, 1990b). Recent studies have also shown that the dense LDL particles are more susceptible to oxidation (de Graaf et al. 1991; Tribble et al. 1992). Although certain diets may also influence LDL particle size (Campos et al. 1991; Dreon et al. 1994), several lines of evidence, including both pedigree-segregation analysis and recombinant inbred mouse studies, support major gene control of LDL particle size (Austin et al. 1988b, 1990a; Jiao et al. 1990; de Graaf et al. 1992).

Studies in twins have afforded another approach to examine genetic influences on the LDL particle distribution. Heritability of LDL particle size, as assessed by relative concordance in monozygotic versus dizygotic twins, has indicated that genetic factors account for approximately half of the variation in LDL particle size in both men (Lamon-Favas et al. 1991) and women (Austin et al. 1993), with the remainder due to nongenetic (i.e., environmental) influences or stochastic variation. A number of such influences have been identified, including abdominal adiposity (Terry et al. 1989), presence of diabetes mellitus (Barakat et al. 1990; Feingold et al. 1992; Selby et al. 1993), use of progestin-containing oral contraceptives (de Graaf et al. 1992), and dietary factors.

Other studies have focused on identifying the genetic loci underlying the pattern B phenotype by performing linkage analysis of LDL subclass patterns and particle size to candidate genes. Initial analyses excluded linkage to apoB (La Belle et al. 1991) and apoE (Nishina et al.

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Address correspondence and reprints: Dr. Jerome I. Rotter, Division of Medical Genetics, SSB-3, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048.

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1992). Evidence has been presented that a gene controlling this atherogenic lipoprotein phenotype (LDL subclass pattern B) exhibits linkage to the LDL receptor locus on the short arm of chromosome 19 (Nishina et al. 1992).

In the present study, utilizing nonparametric quantitative sib-pair and newly developed relative-pair linkage methodologies, we tested for evidence for linkage of a gene or genes determining LDL particle size phenotype to the LDL receptor locus and other candidate gene loci, including the apoAI-CIII-AIV cluster, apoE-CI-CII cluster, apoAII, apoB, apo(a), cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL), and HDL binding protein. Nonparametric quantitative linkage methodology was felt to be the most appropriate method of analysis. In particular, nonparametric approaches do not require a prior assumption regarding the mode of inheritance, and quantitative analyses utilize the maximum available information, that is, the entire range of values of the quantitative trait rather than an arbitrary dichotomization of the data. As detailed below, we found evidence for involvement of four distinct chromosomal loci, which indicates multiple genetic determinants of this atherogenic phenotype.

Material and Methods

Clinical Studies

The study sample consisted of 25 CAD pedigrees ascertained through a proband and at least 1 other blood relative with documented (surgically or angiographically) CAD at Cedars-Sinai Medical Center in Los Angeles. Individuals studied included all family members >15 years of age willing to participate. There are 306 individuals in these 25 families, 278 of which were assayed for LDL particle size. All are Caucasian families, which minimizes interethnic variation in gene frequencies, both of marker and disease genes. The investigation was approved by the Cedars-Sinai Human Subjects Protection Committee.

Phenotyping

Ten milliliters of blood (overnight fasting) was drawn from each of the participating family members of these pedigrees. The blood was stored in tubes containing Na₂EDTA, 1.4 mg/ml. The plasma was separated at 4°C by low-speed centrifugation. Lipids and apolipoproteins were quantified by methods described elsewhere (Warden et al. 1993; Bu et al. 1994).

LDL subclass distributions were analyzed following nondenaturing gradient-gel electrophoresis of plasma in 2%–16% polyacrylamide gradient gels (Pharmacia) and densitometric scanning (Transidyne RFT scanning densitometer) as described elsewhere (Nichols et al. 1986; Austin et al. 1988a, 1988b, 1990b). The particle diameters

were calculated from calibration curves by using protein standards of known size (Nichols et al. 1986). Qualitative LDL subclass patterns were assigned by criteria described by Austin et al. (1988a, 1988b, 1990b).

Genotyping

DNA was isolated from peripheral blood cells (or transformed lymphocytes) and typed for polymorphisms at or near the following candidate genes: the apoAI-CIII-AIV gene cluster, apoAII, apoB, the apoE-CI-CII gene cluster, LPL, LDL receptor, CETP, HDL binding protein, Mn-SOD, and apo(a). These polymorphisms are described in table 1.

As regards genotyping at the apoCIII tetranucleotide repeat (Zuliani and Hobbs 1990b), novel primers were designed to amplify the apoCIII tetranucleotide repeat in a single step. Sequences were hC3F (AGGCAGGAG-AATGGGTTGAA) and hC3R (CGGGAGAGATGACAGAGTTG). PCR was carried out in 96 well plates in an MJ research thermocycler. Reaction conditions were 95°C for 1 min, 59°C for 30 s, and 72°C for 1 min, with 25 cycles.

Statistical and Linkage Analyses

The values of peak LDL particle size measured by gradient-gel electrophoresis were adjusted by multiple regression analysis for age, sex, and body mass index (BMI). These adjusted data and their log and square-root transformations were then analyzed for linkage. The methodology of robust sib-pair test of linkage was used to test the hypotheses as to whether there was evidence for linkage between a genetic locus controlling a quantitative trait of interest and a specific polymorphic marker locus (Haseman and Elston 1972; Amos et al. 1989). The actual sib-pair linkage analyses were performed by utilizing the SIBPAL subroutine program of SAGE, version 2.1 (SAGE 1992). Additional linkage analyses were performed on those sib pairs in whom an exact determination of the number of alleles shared identical by descent was possible from the pedigree structure. Since allele frequencies are not estimated with this approach, results of these analyses are likely to be conservative. Intra-class correlations between those sib pairs sharing exactly two and zero marker alleles identical by descent were also calculated. The ILINK subroutine program of LINKAGE 5.2 (Lathrop et al. 1984) was used to estimate the genetic distance between the polymorphic markers.

In addition to utilizing a robust linkage technology that minimizes assumptions regarding mode of inheritance, we have tried to avoid false-positive results by the following analytic measures: (a) when using this method, we exclude from our analyses those siblings with extreme trait values, i.e., greater than the mean +3 SD, but none occurred for this trait in this sample; (b)

Table 1**Candidate Gene Polymorphisms Typed in CAD Families**

Gene	Marker	Heterozygosity Index ^a	Chromosome	Reference
ApoAI-CIII-AIV gene cluster (A)	RFLPs detected by <i>Xba</i> IA, <i>Xba</i> IB, and <i>Xmn</i> I	<i>Xba</i> IA = .35; <i>Xba</i> IB = .41; <i>Xmn</i> I = .50	11	Reviewed by Mehrabian and Lusia (1992)
ApoAI-CIII-AIV gene cluster (B)	Simple-sequence tetranucleotide repeat within the apoC-III gene	.70-.95	11	Zuliani and Hobbs (1990b); Bu et al. (1994)
ApoAII	Simple-sequence repeat (CA) within the second intron	.74	1	Weber and May (1989)
ApoB	VNTR at the 3' end of the gene	.73	2	Boerwinkle et al. (1989)
ApoE-CII-CI gene cluster	Simple-sequence repeat (CA) within the apoCII gene	.80	19	Weber and May (1989)
apo(a)	Variable number of kringle repeats of gene	.94	6	Lackner et al. (1991)
Manganese SOD 2	RFLP detected by <i>Taq</i> I	.39	6	Xiang et al. (1987)
LDL receptor	Simple-sequence repeat (CA) within gene	.46	19	Zuliani and Hobbs (1990a)
CETP (A)	<i>Taq</i> I RFLP identified by Southern analysis with cDNA probe	.11	16	Drayna et al. (1987)
CETP (B)	<i>Taq</i> I RFLP identified by Southern analysis with cDNA probe	.47	16	Drayna et al. (1987)
D16S313	Simple-sequence repeat (CA) mapping 6 cM from the CETP gene	.57	16	Hudson et al. (1992)
HDL-binding protein (HDLBP)	RFLP detected by cDNA hybridization	.27	2	Xia et al. (1993); Bu et al. (1994)
LPL3GT	Microsatellite 3' to the LPL gene	.81	8	Wood et al. (1993)
LPL5GT	Microsatellite 5' to the LPL gene	.44	8	Wood et al. (1993)
LPL	<i>Hind</i> III RFLP identified by Southern analysis with LPL cDNA	.39	8	Heinzmann et al. (1988)

^a Percentage of unrelated individuals with two dissimilar marker alleles among all unrelated individuals.

without regard to their genotypes, we excluded those sib-pairs with very large squared trait differences, i.e., greater than the mean +3 SD; and (c) we used the more conservative unweighted least-squares option of SIBPAL for our sib-pair linkage analyses.

Assuming no genetic heterogeneity, a true linkage result, either from a LOD score method or nonparametric method, will hold in different families and/or in the same but extended families. In our data, we were able to explore the latter possibility. In this study, we also employed a quantitative relative-pair linkage approach. The two markers, the apoAI-CIII-AIV locus and the CETP locus, which yielded tentative evidence in favor of linkage with LDL particle size when the sib-pair linkage method was employed, were reanalyzed using all available relative pairs in the CAD families. This approach, suggested by Olson and Wijsman (1993), for the analysis of candidate genes, is analogous to the sib-pair method but can utilize other relative pairs such as uncle/aunt-niece/nephew or first-cousin pairs. All relative pairs are tested simultaneously for linkage, in one regression analysis. The squared difference in the trait is regressed on the number of marker alleles shared identical by descent.

Each class of pairs has a unique intercept estimated, which may be interpreted as the expected squared difference in the trait for that class of relative pairs when zero alleles are shared at the locus being tested. A common regression slope is estimated for all relative-pair classes, and this is the statistic that, when significantly negative, is interpreted as evidence for linkage. A common slope, as opposed to a separate one for each class of relative pairs, is estimated when testing for linkage of candidate genes, because, when the recombination fraction is zero, the expected values for the slopes of the regression lines for each class of relative pairs has the same value. Prior to this analysis, each relative pair was tested for identity-by-descent concordance at the marker locus by tracing the inheritance of the marker allele (or alleles, in the case of sib pairs) through all intervening relatives connecting the pair. Marker-allele frequencies were not employed to estimate a likelihood of identity by descent. Any relative pairs where missing data between them required marker allele frequencies to estimate the probability of marker concordance identical by descent were eliminated from the relative-pair analysis.

In initial analyses, when all relative pairs within a

Table 2

Mean (\pm SD) BMI, Serum Lipid, Lipoprotein, and Apolipoprotein Levels in the Members of 25 CAD Families

	Number	Mean (\pm SD)
BMI (kg/m ²)	306	25 (4)
Total cholesterol (mg/dl)	306	201 (42)
HDL-cholesterol (mg/dl)	306	55 (17)
LDL-cholesterol (mg/dl)	306	125 (38)
Triglyceride (mg/dl)	306	103 (70)
ApoB (mg/dl)	300	129 (35)
Lp(a) (mg/dl)	298	19 (24)
LDL particle size (\AA) ^a	278	267 (8)
LDL particle size (\AA) ^b	211	266 (8)

^a All family members.

^b Excluding males age \leq 20 years of age and females \leq 50 years of age.

pedigree were treated as independent, tests of linkage resulted in significant *P* values for the apo AI-CIII-AIV and CETP analyses. When these analyses were conducted accounting for the dependence in the family structures by using the generalized estimating equation (or, GEE) approach of Liang and Zeger (1986) as suggested by Olson and Wijsman (1993), more conservative results were obtained. To obtain these results, a program written by Karim and Zeger using the IML procedure of the SAS package of programs (1990) was employed.

Results

Descriptive Statistics

Mean (\pm SD) BMI (kg/m²), serum lipid, lipoprotein, and apolipoprotein levels, and peak LDL particle size in the members of the 25 CAD families are shown in table 2. The distribution of peak LDL particle sizes measured by gradient-gel electrophoresis in the 278 family members is shown in figure 1. In table 3, the distributions of LDL subclass patterns (A, B, and intermediate) and their corresponding mean particle sizes in all family members and in family members excluding males <20 years of age and females <50 years of age are indicated. Using the "broad definition" of pattern B (including intermediate patterns), the overall prevalence in this population (32%–36%) is similar to that other studies (Austin et al. 1988a, 1990b; Campos et al. 1992a) but somewhat less than that observed among patient populations with CAD (Austin et al. 1988a; Campos et al. 1992b).

Linkage of Gene(s) Controlling LDL Particle Size to LDL Receptor, ApoA1-C3-A4, CETP, and Mn-SOD Loci

We tested for linkage of the quantitative trait LDL particle size by using the robust nonparametric sib-pair

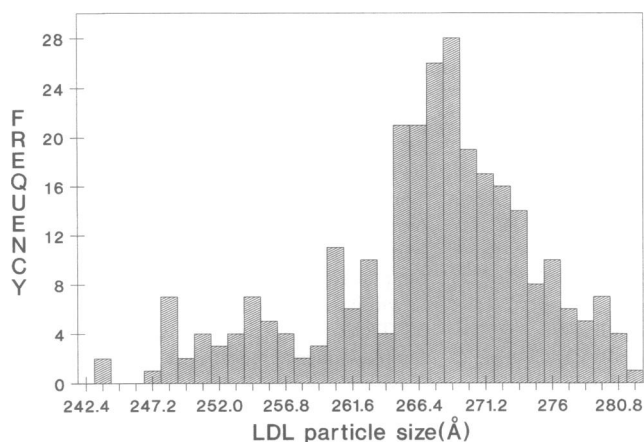


Figure 1 Distribution of LDL particle peak size (\AA) in the CAD families.

linkage method of Haseman and Elston (1972). The underlying basis for this approach is to compare the quantitative variation in a trait between siblings as a function of the number of marker alleles they share identical by descent. For selected markers, we endeavored to increase the sample size by incorporating additional relative pairs in our pedigrees, using a quantitative relative-pair linkage methodology proposed by Olson and Wijsman (1993). Remarkably, we observed evidence for linkage of a gene or genes determining LDL particle size to at least four distinct genetic loci.

We obtained evidence for linkage of a locus on chromosome 19p controlling LDL particle size with a three-allele dinucleotide repeat polymorphic marker within the LDL receptor gene locus (*P* = .008) (table 4). This finding was robust to both log and square-root transformation of LDL size. To illustrate this result, the regression of squared trait differences of peak LDL particle size (adjusted for age, sex, and BMI) versus the number of LDL receptor marker alleles shared identical by descent is shown in figure 2A. The negative slope of the

Table 3

Distribution of LDL Particle Size, Subclass Pattern vs. Particle Size

LDL SUBCLASS PATTERN	ALL FAMILY MEMBERS		RESTRICTED FAMILY MEMBERS ^a	
	No. (%)	LDL Size (\AA)	No. (%)	LDL Size (\AA)
Pattern A	187 (68)	270	138 (64)	270
Intermediate	50 (18)	261	43 (20)	261
Pattern B	38 (14)	251	36 (16)	251

^a Excluding those males <20 years of age and females <50 years of age.

regression line indicates evidence for linkage. To further examine the evidence of linkage on chromosome 19, we typed another informative microsatellite polymorphic marker (D19S199) on that chromosome. This D19S199 marker was 19 cM from the LDL receptor locus, as calculated with the ILINK program of LINKAGE 5.2 (Lathrop et al. 1984). Even at this distance, we observed a suggestive *P* value of .11 with 90 sib pairs.

There has been a long-term interest in the apoAI-CIII-AIV cluster locus on chromosome 11q as a risk factor for atherosclerosis. Suggestive evidence (65 sib pairs, *P* = .06; table 4) for linkage of LDL particle size with this locus was obtained by quantitative sib-pair linkage analysis using haplotypes constructed from three RFLPs (*XmnI*, *XbaI*, and *XbaII*). A more informative microsatellite polymorphism of the CIII gene was typed for further analysis using the quantitative relative-pair linkage method. This approach utilizes the information from all relative pairs, including uncle/aunt-nephew/niece, first-cousin, grandparent-grandchild, and sib pairs. Analysis of 102 relative pairs from the CAD families resulted in a negative slope (with an initial *P* < .007, when the dependence structure of relative pairs in the pedigrees was not taken into account) that was marginally significant when the dependencies in the pedigrees were taken into account (*P* < .06) (table 4).

CETP is an important enzyme in HDL and LDL cholesterol metabolism, and we have recently provided evidence for linkage of the CETP with quantitative HDL levels (Bu et al. 1994). Evidence for linkage was observed between a microsatellite polymorphism (D16S313) linked to the gene for CETP on chromosome 16p and a locus determining LDL particle size by the quantitative sib-pair linkage method (table 4). This result was robust when both log and square-root transformations of LDL size were applied. The distance between the D16S313 locus and CETP gene was estimated as 6.2 cM by the ILINK program of LINKAGE 5.2 (Lathrop et al. 1984). The estimated negative regression line of

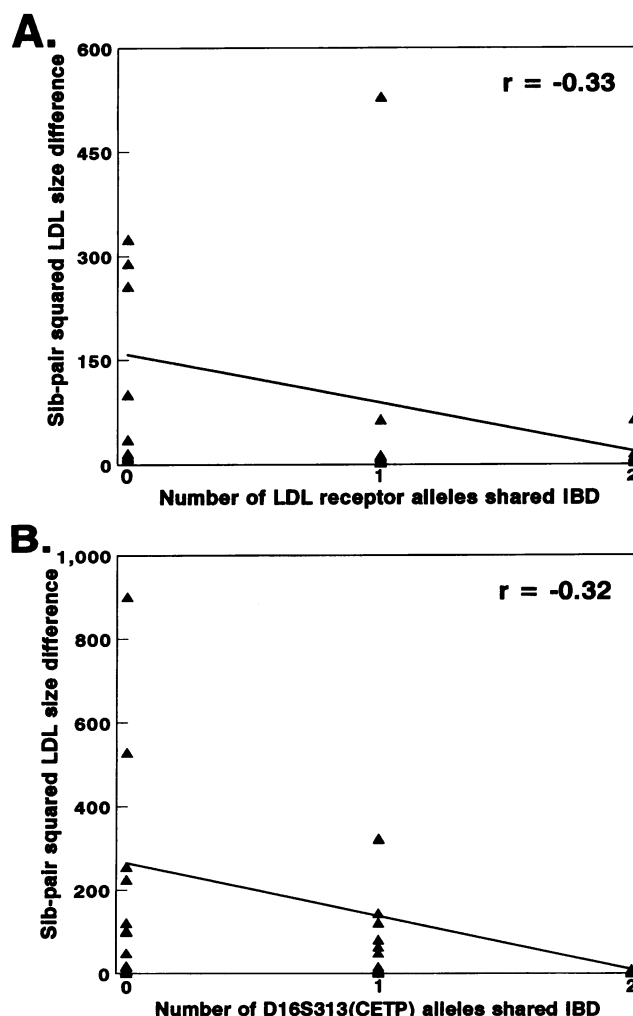


Figure 2 Negative regression of squared trait differences for age-, sex-, and BMI-adjusted peak LDL particle size versus the number of marker alleles shared identical by descent. *A*, LDL-receptor locus (*N* = 8, 18, and 14 sib pairs for sharing two, one, and zero alleles at LDL-receptor locus). *B*, D16S313 [CETP] locus (*N* = 4, 18, and 14 sib pairs for sharing two, one, and zero alleles out P16S313 [CETP] locus). The figures include only those sib pairs whose marker genotypes could be definitely determined to share two, one, or zero alleles identical by descent at the marker locus.

Table 4

Linkage Analysis, LDL Particle Size with LDL Receptor, ApoAI-CIII-AIV, D16S313 (CETP), and Mn-SOD Loci in CAD Families

GENETIC LOCI	SIB PAIR ^a		RELATIVE PAIR ^a	
	No.	<i>P</i> Value	No.	<i>P</i> Value
LDL Receptor	102	.008
ApoAI-CIII-AIV ^b	65	.06	96	.06
D16S313 (CETP)	87	.03	71	.01
Mn-SOD	55	.001

^a All sib pairs genotyped.

^b For relative pairs, a tetranucleotide repeat marker within the ApoCIII gene was employed.

squared trait differences for peak LDL particle size (adjusted for age, sex, and BMI) versus the number of D16S313(CETP) alleles shared identical by descent by the sibling pairs is shown in figure 2B. With this initial level of significance and the existence of a marker locus with multiple alleles allowing use of the relative-pair method, we extended the analysis to other relatives. Use of the quantitative relative-pair linkage method in 71 informative relative pairs from all 25 CAD families yielded stronger evidence for linkage (*P* = .01, corrected for the dependencies with the pedigrees; *P* = .001, uncorrected for such dependencies) (table 4).

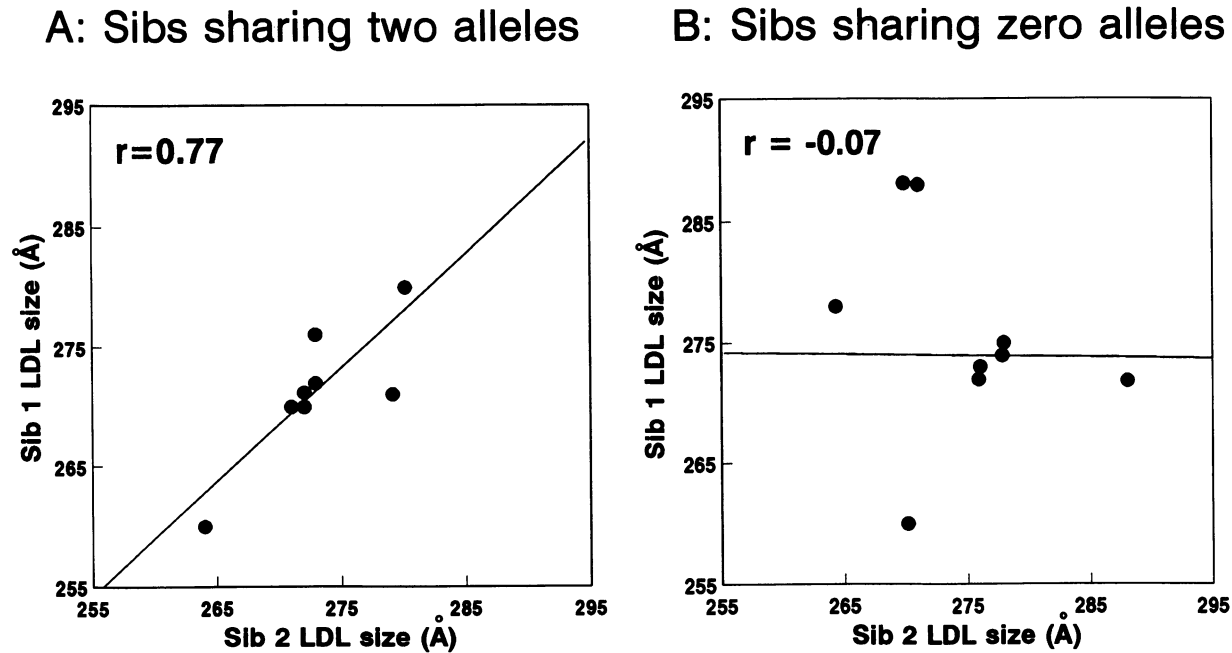


Figure 3 Intra-class correlations of LDL particle size for sib pairs sharing exactly two (panel A) and zero (panel B) LDL-receptor marker alleles identical by descent.

Finally, a *TaqI* RFLP marker at the manganese superoxide dismutase (Mn-SOD) gene locus on chromosome 6q was also linked to gene(s) determining LDL size ($P = .001$; table 4). This finding was also robust to different data transformations. Relative-pair linkage analysis was not performed, because of the relative uninformative-ness of this RFLP marker.

For other candidate gene loci, including apoB, apoAII, apo(a), apoE-CI-CII, lipoprotein lipase, and HDL-binding protein, no linkage was observed by sib-pair linkage analyses (P ranged from .24 to .81).

Correlation Analysis of LDL Particle Size

An alternative way to illustrate the linkage of a gene controlling a quantitative trait and a genetic marker locus is to compare the correlations among those sib pairs sharing exactly two marker alleles identical by descent with those pairs sharing zero alleles identical by descent. As expected from the linkage data, we obtained a strong positive correlation, $r = .77$, $P < .01$, for those sib-pairs sharing both LDL receptor alleles identical by descent and a negligible correlation, $r = -.07$, among those pairs sharing zero LDL receptor alleles identical by descent (see fig. 3). An analogous result was seen for the CETP locus.

Discussion

In this report, we have provided evidence for genetic control of the important atherosclerosis quantitative risk factor LDL particle size by four distinct chromosomal

loci. This was done by utilizing nonparametric linkage technology, including both quantitative sib-pair and relative-pair linkage analytic methods. Linkage to the LDL receptor locus on 19p is a confirmation of previous linkage report by Nishina et al. (1992). It should be emphasized that this was done in an entirely separate population consisting of CAD families, while the previous result was found with predominantly healthy families, and by an entirely distinct analytic methodology (LOD score linkage analysis in Nishina et al. [1992], nonparametric quantitative linkage here).

The three additional loci identified were the possible linkage of apoAI-CIII-AIV gene cluster on 11q, D16S313 (CETP) on 16p, and Mn-SOD on 6q. It should be noted that the effects of these loci were on quantitative variation in LDL particle size, which may not necessarily translate into an effect on the qualitative phenotype, that is, atherogenic lipoprotein phenotype B (Nishina et al. 1992). While these genetic loci have been identified by the use of polymorphic DNA markers that do not directly identify the causative mutations in the respective genes, it is intriguing that their protein products have connections to metabolic pathways for which there is evidence of possible involvement in the regulation of LDL particle size. Small, dense LDL have been shown to have reduced affinity for the LDL receptor (Nigon et al. 1991), and conceivably altered LDL receptor function or regulation could result in further impairment of plasma clearance of these LDL or their metabolic precursors.

Within the apoAI-CIII-AIV gene cluster, it has been demonstrated that apoCIII gene haplotypes are associ-

ated with variation in plasma triglyceride levels (Dammerman et al. 1993), which in turn could affect levels of small, dense LDL (Krauss 1987). One possible mechanism of such a genetic influence on triglyceride metabolism, supported by studies *in vitro* and in transgenic mice (Ito et al. 1990), is an effect of the gene on apoCIII levels. Such an effect has been suggested recently on the basis of an association of apoCIII gene variation with plasma apoCIII levels (Paul-Hayase et al. 1992). Increased apoCIII content may impair lipolysis of triglyceride-rich lipoproteins (Brown and Baginsky 1972; Krauss et al. 1973; Ito et al. 1990) and may also interfere with receptor-mediated clearance of the lipolytic remnant particles (Windler and Havel 1985). It is also of interest that some investigators have demonstrated association (Tybjærg-Hansen et al. 1993) and linkage (Wojciechowski et al. 1991) of polymorphisms in the apoAII-CIII-AIV cluster to familial combined hyperlipidemia, a disorder closely related to subclass pattern B (Krauss et al. 1983). Several studies have reported population associations of alleles at these loci with either hypertriglyceridemia or atherosclerosis (Doolittle et al. 1992; Dammerman et al. 1993). In addition, hypertriglyceridemia and insulin resistance are associated with dense LDL particle size (Reaven et al. 1993), and, in the few cases where serum insulin levels have been examined, they have been found to be associated or linked to the AII-CIII-AIV locus (Kamboh et al. 1991; Rotter et al. 1992; Cantor et al. 1993). Thus, an alternative hypothesis is that this locus would mediate its effect on LDL metabolism through the interrelationship between insulin resistance and triglyceride metabolism.

In the case of CETP, there is evidence for polydispersity and increased mass of small, triglyceride-rich LDL particles in patients with homozygous CETP deficiency (Sakai et al. 1991), and *in vitro* incubation of LDL with CETP results in conversion to a larger and more uniform size distribution (Lagrost et al. 1993). Furthermore, dense LDL particle size is associated with low HDL levels (Austin et al. 1990b), and we and others have provided evidence for the involvement of the CETP locus in determining HDL levels either by association or linkage analyses (Kodon et al. 1989; Heiba et al. 1993; Bu et al. 1994).

A mechanistic association of lipoprotein metabolism with Mn-SOD activity is much more speculative, but it is conceivable that defective function of Mn-SOD results in increased lipid hydroperoxides in plasma lipoproteins, with a concomitant increase in oxidative susceptibility, or otherwise alters lipoprotein metabolism in a manner leading to formation of small, dense, more oxidizable LDL (de Graaf et al. 1991; Tribble et al. 1992, 1994).

Some discussion regarding the statistical methods utilized here is warranted. The underlying basis of both the quantitative sib-pair and relative-pair linkage methods is

a comparison of the quantitative difference in a trait in the pairs as a function of the number of alleles they share identical by descent at a test locus (Haseman and Elston 1972; Amos et al. 1989; Olson and Wijsman 1993). When compared with the LOD score method, this approach has a major advantage of not requiring specification of a genetic model. This is particularly important for studying a complex trait such as LDL particle size, in which various modes of inheritance have been suggested by segregation analyses (Austin et al. 1988a, 1988b; de Graaf et al. 1992; Bu et al. 1992). A possible consequence of utilizing nonparametric linkage, however, is that relatively larger sample sizes may be required to achieve the same power that can be achieved with the LOD score approach. Our sample size of 25 pedigrees is minimal when the relative-pairs method is used due to the anticonservative nature of this test at small sample sizes (Olson 1994). The adoption of relative-pair linkage methods, however, in which information from other relatives besides siblings can be used, can improve the potential to identify linkage. Our finding of linkage of a gene or genes controlling LDL particle size to three different genetic loci (excluding the LDL receptor locus) should be considered preliminary, as should all first linkage reports, especially for complex phenotypes. We are encouraged by the consistency of the results utilizing both the sib-pair and relative-pair methods. The LDL receptor result may now be considered established, however, since it has been documented in two different studies with very different populations and statistical methods. We do feel that the other results have a favorable likelihood of confirmation, given (1) the robustness to different transformations of the quantitative traits and (2) the prior epidemiological and biochemical data that implicate these specific loci in various metabolic pathways contributing to variation in LDL particle size.

The findings described above lead to the hypothesis that several different genetic loci underlie the expression of the small, dense LDL phenotype, that these genes cumulatively account for the high prevalence of the trait in the general population, and that in any given family one or more of the loci are responsible for the major gene and additive effects identified by complex segregation analyses. Moreover, the results suggest that different genetically determined metabolic mechanisms may give rise to the dense LDL particles and that these differences, as well as gene-gene interactions, may result in variability of metabolic and pathological manifestations among affected individuals. Finally, the involvement of several different genes suggests several different points to intervene to prevent clinical CAD in those at genetic risk.

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