

Contribution of the hepatic lipase gene to the atherogenic lipoprotein phenotype in familial combined hyperlipidemia

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Abstract Familial combined hyperlipidemia (FCH) is a common genetic lipid disorder with a frequency of 1–2% in the population. In addition to the hypercholesterolemia and/or hypertriglyceridemia that affected individuals exhibit, small, dense LDL particles and decreased HDL-cholesterol levels are traits frequently associated with FCH. Recently, we reported that families with FCH and families enriched for coronary artery disease (CAD) share genetic determinants for the atherogenic lipoprotein phenotype (ALP), a profile presenting with small, dense LDL particles, decreased HDL-cholesterol levels, and increased triglyceride levels. Other studies in normolipidemic populations have shown that the hepatic lipase (HL) gene is linked to HDL-cholesterol levels and that a polymorphism within the HL promoter (–514C→T) is associated with increased HDL-cholesterol levels as well as larger, more buoyant LDL particles. In the present study, we tested whether the HL gene locus also contributes to ALP in a series of Dutch FCH families using nonparametric sibpair linkage analysis and association analysis. Evidence for linkage of LDL particle size ($P < 0.019$), HDL-cholesterol ($P < 0.003$), and triglyceride levels ($P < 0.026$) to the HL gene locus was observed. A genome scan in a subset of these families exhibited evidence for linkage of PPD (LOD = 2.2) and HDL-cholesterol levels (LOD = 1.2) to the HL gene locus as well. The –514C→T promoter polymorphism was significantly associated ($P < 0.0001$) with higher HDL-cholesterol levels in the unrelated males of this population, but not in unrelated females. No association was observed between the polymorphism and LDL particle size or triglyceride levels. Our results provide support that ALP is a multigenic trait and suggest that the relationship between small, dense LDL particles, HDL-cholesterol, and triglyceride levels in FCH families is due, in part, to common genetic factors.—Allayee, H., K. M. Dominguez, B. E. Aouizerat, R. M. Krauss, J. I. Rotter, J. Lu, R. M. Cantor, T. W. A. de Bruin, and A. J. Lusis. **Contribution of the hepatic lipase gene to the atherogenic lipoprotein phenotype in familial combined hyperlipidemia.** *J. Lipid Res.* 2000. 41: 245–252.

Supplementary key words familial combined hyperlipidemia • atherogenic lipoprotein phenotype • hepatic lipase • genetic linkage analysis

Familial combined hyperlipidemia (FCH, MIM-144250) is a common genetic lipid disorder that accounts for 10–20% of premature coronary artery disease (CAD) (1). Affected individuals exhibit increased plasma concentrations of either cholesterol, triglycerides, apolipoprotein B (apoB), or combinations thereof. In addition to the primary phenotype of hypercholesterolemia and/or hypertriglyceridemia, certain other traits are also frequently associated with FCH, although they are not considered as criteria for diagnosis. These include the presence of small, dense low density lipoproteins (LDL) and decreased levels of plasma high density lipoprotein (HDL) cholesterol (2–5). The combination of small, dense LDL particles, decreased plasma HDL-cholesterol, and increased triglyceride levels has been termed the atherogenic lipoprotein phenotype (ALP), an unfavorable lipid profile associated with an increased risk for CAD (6, 7). The association between FCH and these traits has led to the hypothesis that FCH and ALP are, in fact, related disorders, although the precise relationship between them is not well understood (8–13).

Recently, we analyzed a series of Dutch FCH pedigrees and observed a 10-fold enrichment of ALP in FCH probands compared to unaffected spouses (12). We also observed that individuals affected with FCH had a tendency to exhibit lower HDL-cholesterol levels than unaffected indi-

Abbreviations: apo, apolipoprotein; LDL, HDL, low and high density lipoprotein; FCH, familial combined hyperlipidemia; CAD, coronary artery disease; ALP, atherogenic lipoprotein phenotype; HL, hepatic lipase; PPD, peak particle diameter; PCR, polymerase chain reaction; i.b.d., identical-by-descent; edf, effective degrees of freedom.

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viduals, consistent with previous studies. Moreover, we demonstrated that the manganese superoxide dismutase, cholesteryl ester transfer protein/lecithin:cholesterol acyltransferase, and apolipoprotein AI-CIII-AIV gene loci, which we previously reported to be linked to small, dense LDL particles in CAD families, are also linked to ALP in FCH families (12, 14). These results indicated that ALP is a multigenic trait and raised the possibility that other loci could also be involved. One such locus may be the hepatic lipase (HL) gene. Cohen et al. (15) demonstrated significant evidence for linkage of HDL-cholesterol levels to the HL gene locus in a normolipidemic population using nonparametric sibpair linkage analysis. Subsequently, it was shown that a polymorphism within the HL promoter (-514C→T) conferred significantly higher HDL-cholesterol levels in men, but not in women (16). These results have been independently confirmed by other studies, and this polymorphism has also been associated with larger, more buoyant LDL particles as well as lower HL activity in CAD and normolipidemic populations (17–20). Furthermore, in a recent genome scan for HDL-cholesterol levels in normolipidemic families, evidence of linkage to the HL gene locus was also observed (20). Taken together, these findings make HL an attractive candidate gene for ALP.

In the present study, we tested whether the HL gene is linked to LDL particle size, HDL-cholesterol, and/or triglyceride levels in a panel of Dutch FCH families. The results demonstrate that the HL gene locus is linked to all three traits and that a HL promoter polymorphism is associated with HDL-cholesterol levels in men, but not women.

MATERIALS AND METHODS

Ascertainment of FCH families

Families were ascertained (by TdB) as previously described (21, 22). Initially, 18 unrelated FCH probands were recruited through the Lipid Clinic of the Utrecht University Hospital; subsequently, nine additional probands and their family members were also identified. Over 95% of all living relatives and spouses of the probands were recruited without bias with regard to affection status. All probands met the criteria described previously (1, 4, 23): first, a primary hyperlipidemia with varying phenotypic expression, including a fasting plasma cholesterol concentration >250 mg/dL (6.5 mmol/L) or >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/L) and/or elevated plasma apoB concentrations, exceeding the mean + 2 standard deviations 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 coronary artery disease, defined as myocardial infarction or cerebrovascular disease before the age of 60, in 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 cholesterol levels and tendon xanthomas), and type III hyperlipidemia (apoE2/E2 genotype).

Relatives were assigned the FCH phenotype based on plasma cholesterol and/or triglycerides exceeding the 90th percentile and/or apoB exceeding the 75th percentile for age-matched and gender-matched controls. Using these criteria, there were 234 af-

ected individuals (including the probands) and 269 unaffected relatives. The spouses (n = 217) represented a common, environment-, nutrition-, and age-matched control group for the probands and their hyperlipidemic relatives. All subjects gave informed consent and the study protocol was approved by the Human Investigation Review Committee of the University Hospital, Utrecht.

Biochemical analyses

Venous blood was drawn after an overnight fast of 12–14 h and abstention from alcohol consumption for at least 48 h. Probands or hyperlipidemic relatives who used lipid-lowering drugs were sampled after their lipid-lowering treatment was withheld for 3 weeks. Plasma lipids and apolipoproteins were quantified for the entire population (n = 720) as previously described (4, 21, 22). Due to unavailability of some samples, LDL particle size was determined for a subset of the population (n = 498) as reported previously (12). These latter individuals were drawn from the original 18 families collected. LDL subclass distributions were analyzed after nondenaturing gradient gel electrophoresis of plasma in 2% to 16% polyacrylamide gradient gels with lipid staining and densitometric scanning as described previously (6, 7, 24, 25). LDL particle diameters were calculated from the calibration curves using protein size standards (24) and the diameter corresponding to the peak of greatest amplitude for each sample was designated peak particle diameter (PPD). Qualitative LDL subclass patterns were assigned by criteria previously described (6, 7, 25). Pattern A is characterized by a major peak of large, buoyant LDL particles and a minor peak of small, dense particles. In contrast, pattern B has a major peak of small, dense LDL with a skewing of the curve towards the larger particle diameters. In general, the PPD from gradient gel electrophoresis for pattern A is greater than 255Å, and in pattern B is 255Å or less. According to the broad definition of ALP, we have included intermediate pattern individuals with pattern B individuals and collectively defined this group as affected with ALP (7, 14).

Genotyping

To assess linkage with the HL gene locus, tightly linked polymorphic microsatellite markers were chosen for genotyping. Specifically, these were D15S643 and D15S148, both linked to the HL gene at 0 cM according to the CEPH database. Genotyping was performed as previously described either manually, using radiolabeled PCR products (12), or with the aid of a scanning fluorescent detector, using fluorescently tagged PCR products (26–28). D15S148 was only genotyped in the subset of individuals phenotyped for LDL particle (n = 498), whereas D15S643 was genotyped in the entire data set (n = 720). For the genome scan, the 399 microsatellite markers used in construction of the linkage map comprised the Weber 6 screening set (Research Genetics, Huntsville, AL), and the genotyping was performed in the laboratory of Dr. James Weber at the Marshfield Medical Research Foundation (26, 27). The complete linkage map was previously constructed for 240 of the individuals from the original 18 Dutch FCH families, as described elsewhere (29). All 22 autosomes as well as the X and Y chromosomes were genotyped, and the average distance between adjacent markers was 10 cM (28).

The presence or absence of the polymorphism (C→T) at position -514 within the promoter of the HL gene was determined in the probands and spouses for whom DNA and phenotype data was available (n = 225), according to previously described methods (16). Briefly, site-specific primers were used to PCR amplify a 300 bp region of DNA encompassing the polymorphism. Next, 15 U of the enzyme *Nla*III in 30 μL of New England Biolabs buffer 4 was added directly to the PCR products and incubated at 37°C

for 2 h. The products were resolved on 2% agarose gels and visualized using ethidium bromide and UV light.

Linkage analyses

Because the modes of inheritance of the traits under analysis are unknown, robust sibpair analysis methods were used to test the hypothesis that there is linkage between ALP (quantitative and qualitative measures) and polymorphic markers at the HL gene locus (30, 31). Two-point linkage analyses of individual markers were performed using the SIBPAL subprogram of the S.A.G.E. package (32). PPD, HDL-cholesterol, and triglyceride values were assessed for non-normality and triglycerides were log transformed prior to all analyses. PPD, HDL-cholesterol, and triglycerides were evaluated for linkage with marker loci using the Haseman-Elston algorithm (30) by regressing the squared trait difference of sibling pairs on the proportion of alleles shared identical-by-descent (i.b.d.). 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 i.b.d. Hence, the slope of the regression line is expected to be zero. In the presence of linkage, however, the squared trait difference is expected to decrease when siblings share a greater proportion of marker alleles, resulting in a significant negative slope of this regression line (30). Because sibpairs in the same sibship may be nonindependent statistically, significance levels are calculated using effective degrees of freedom (edf), which reflects the number of independent sibling pairs in the analyses (32). For the ALP qualitative trait, defined as LDL pattern B, two-point linkage analysis was first conducted using only sibling pairs concordant for the presence of this trait. The mean proportion of alleles shared i.b.d. by these pairs was calculated and then tested to assess whether it was significantly greater than 0.50, the expected proportion of allele sharing in the absence of linkage. A linear regression analysis with ALP and all available sibpairs was then used to assess whether squared differences in ALP status varied in relation to the degree of allele sharing, and a significantly negative regression line slope is also considered evidence for linkage. For the majority of sibpairs, parental genotypes were available to calculate allele sharing i.b.d. In cases where parental information was not available, allele sharing identical by state was estimated using marker allele frequencies that were calculated from the unrelated individuals (i.e., the spouses and probands) of our Dutch population. As a specific hypothesis was being tested with respect to the HL gene locus, the level of significance in these two-point analyses was set at $P < 0.05$.

The genome scan for ALP was analyzed with the MAPMAKER/SIBS program using the genotyping data from the previously constructed linkage map (29) and the quantitative traits PPD, HDL-cholesterol, and triglyceride levels. This program utilizes information from multiple, linked markers to estimate allele sharing at and between markers based on the most likely genotypes (33). The test statistic for a correlation of allele sharing and squared trait differences among sibpairs is reported as a LOD score (34). A qualitative multipoint analysis with the discrete pattern B trait was not performed as there were comparatively few affected individuals in the genome scan data set ($n = 36$).

Statistical analysis

For association analyses of the HL promoter polymorphism, gender and genotypic differences in mean PPD, HDL-cholesterol, and triglyceride levels were initially assessed with a 2-way ANOVA using a measured genotype approach (35, 36). To ensure that all observations in the data were independent, only the probands and those marrying into the pedigrees, i.e., the spouses, were included in this analysis. Because the number of TT homozygotes was only 10, these individuals were included with the CT het-

TABLE 1. Clinical characteristics of the FCH family members

Variable	Hyperlipidemic Individuals ^a (n = 234)	Normolipidemic Individuals (n = 269)	Spouse Controls (n = 217)
Age (years)	48 ± 16	34 ± 16	48 ± 15
Cholesterol (mg/dl)	284 ± 91	191 ± 31	219 ± 41
LDL-cho. (mg/dl)	180 ± 53	121 ± 28	144 ± 38
HDL-cho. (mg/dl)	44 ± 12	48 ± 12	48 ± 13
Triglycerides (mg/dl)	243 ± 146	114 ± 43	144 ± 91
ApoB (mg/dl)	131 ± 30	83 ± 21	101 ± 28
PPD (Å)	265 ± 11	274 ± 7	273 ± 7

Values are expressed as mean ± SD.

^a Includes the 27 probands and 206 hyperlipidemic individuals.

erozygotes and considered as one group for the analysis. The significant ANOVA was followed by unpaired *t*-tests to assess genotypic differences within the male and female groups separately. The Statistical Analysis System (SAS) package of computer programs was used in these analyses (37).

RESULTS

The clinical characteristics of the hyperlipidemic relatives (including the probands), the normolipidemic relatives, and the spouse controls are summarized in **Table 1**. The hyperlipidemic relatives and probands were characterized by higher levels of total plasma cholesterol, triglycerides, LDL cholesterol, and apoB, and lower HDL-cholesterol levels as compared to the normolipidemic relatives and spouse controls. The hyperlipidemic individuals were also characterized by a smaller mean PPD than the normolipidemic and spouse groups.

To assess whether the HL gene locus was linked to the ALP, we conducted two-point linkage analyses with two microsatellite markers linked at 0 cM with HL. In the quantitative analyses, both D15S643 and D15S148 yielded evidence for linkage of PPD and triglyceride levels to the HL gene locus (**Table 2**). Evidence for linkage of HDL-cholesterol levels was observed only with D15S643, although the other marker, D15S148, yielded marginal results as well (**Table 2**). The negative regression plots of the squared trait difference versus marker allele sharing among sibpairs for these quantitative analyses is shown in **Fig. 1**. In the qualitative analyses of the discrete ALP trait (defined as LDL pattern B), D15S643 yielded an allele sharing estimate of 0.57 among the clinically concordant affected siblings,

TABLE 2. Quantitative sibpair linkage analysis results of ALP traits at the hepatic lipase gene locus

Trait	n	Marker	edf	P Value
PPD	498	D15S643	178	0.019
		D15S148	159	0.008
HDL-Cholesterol	720	D15S643	258	0.003
		D15S148	186	0.104
Triglycerides	720	D15S643	259	0.026
		D15S148	186	0.019

n, Number of subjects, see Methods; edf, effective degrees of freedom.

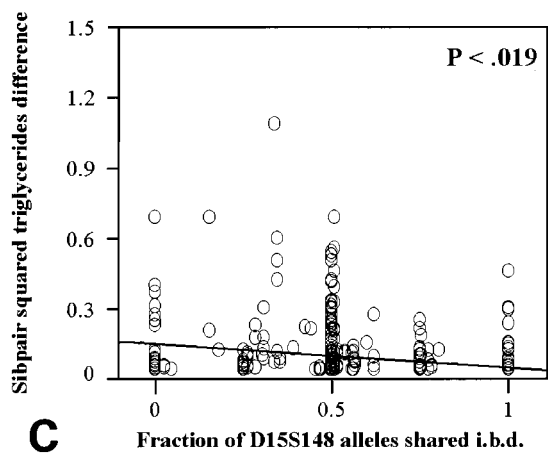
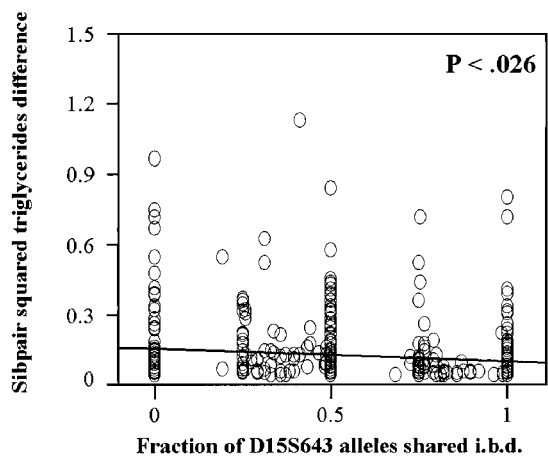
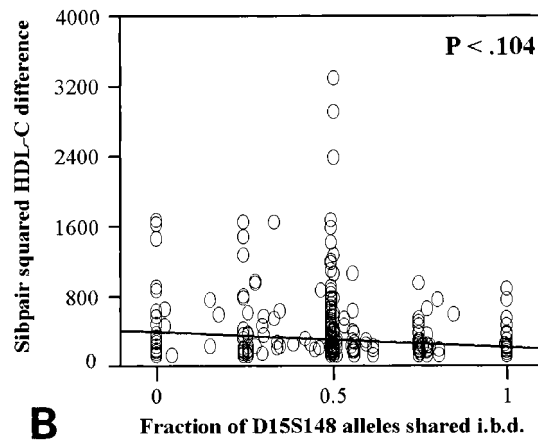
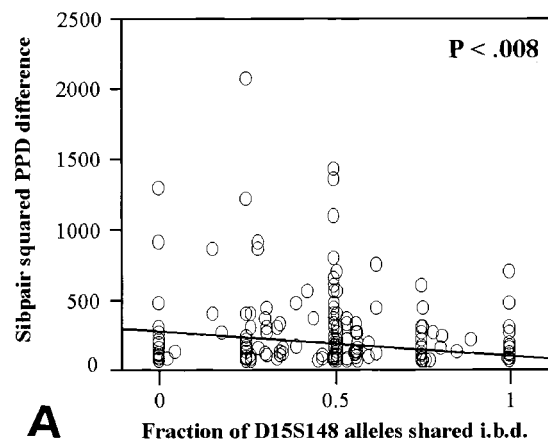
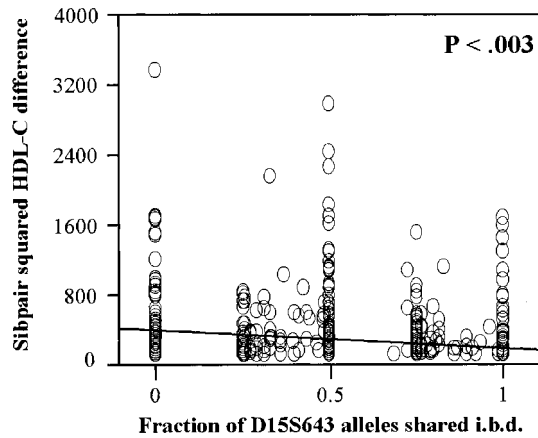
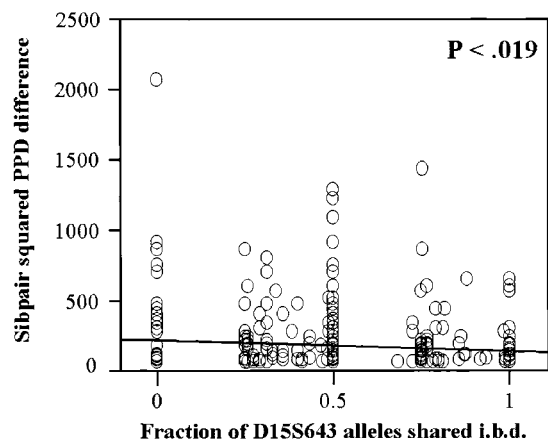


Fig. 1. Plots of sibpair squared trait differences versus estimated i.b.d marker allele sharing for PPD (A), HDL-cholesterol (B), and triglycerides (C) and regression lines.

greater than the expected 0.50, resulting in suggestive evidence for linkage ($P < 0.06$) to the HL gene locus. However, when all available sibpairs (affected and unaffected) were used in a Haseman-Elston linear regression analysis, evidence for linkage of ALP ($P < 0.035$) to D15S643 was observed. The linkage results with D15S148 did not reach statistical significance in these qualitative analyses ($P > 0.05$).

We next performed an association analysis with a HL promoter polymorphism ($-514C \rightarrow T$) to assess whether the linkage we observed at this locus could be due to the HL gene. These analyses were only conducted using the unrelated individuals of this population, i.e., the spouse controls and FCH probands. The frequency of the T allele in our Dutch population was calculated to be 0.21, slightly higher than a frequency of 0.15 reported in a Caucasian population elsewhere (16), and the allele frequencies were consistent with Hardy-Weinberg equilibrium (data not shown). A 2-way ANOVA identified significant effects due to both gender ($P < 0.001$) and genotype ($P < 0.024$) for HDL-cholesterol but not for PPD or triglycerides levels. Analyzing men and women separately with a t -test, a difference in HDL-cholesterol levels was observed in males, where the CT/TT group exhibited a significantly higher mean ($P < 0.0001$) than the CC homozygote group (48 ± 12 mg/dl vs. 40 ± 10 mg/dl, respectively; **Table 3**). The mean HDL-cholesterol levels of the 10 TT homozygotes were not higher than those of the CT heterozygotes (data not shown). In addition, no significant difference in HDL-cholesterol levels was observed in females (Table 3).

To further evaluate whether the HL gene locus contributes to ALP in FCH, we analyzed the data from a recently completed whole genome scan that was conducted on a subset of these families (29). The complete linkage map consisted of 399 markers spanning, on average, 10 cM intervals, and was carried out on 240 individuals from the original 18 FCH families. The MAPMAKER/SIBS multipoint linkage program was used to analyze the data for PPD, HDL-cholesterol, and triglyceride levels. In the complete genome scan, no locus with a LOD score greater than 3.0 was identified for either PPD, HDL-cholesterol levels, or triglyceride levels. However, several loci exhibited suggestive LOD scores ($1.0 < \text{LOD} < 3.0$) for these traits (**Table 4**). One locus, over the HL gene on chromo-

TABLE 3. Association of the HL promoter polymorphism ($-514C \rightarrow T$) and HDL-cholesterol levels in the unrelated individuals

	Males		Females	
	CC	CT/TT ^b	CC	CT/TT ^b
n	65	40	79	41
HDL-chol. (mg/dl)	40 ± 10	48 ± 12^a	50 ± 13	52 ± 16

Values are expressed as mean \pm SD.

^a CT/TT males had higher HDL-cholesterol levels compared to CC males ($P < 0.0001$, by unpaired t -test). Individuals who were either homozygous or heterozygous for the T allele were grouped together as there were only 10 TT homozygotes.

^b The means of the TT homozygotes were not higher than those of the CT heterozygotes.

TABLE 4. Genome scan loci exhibiting LOD scores greater than 1.0 with PPD, plasma HDL-cholesterol, or triglyceride levels

Chromosome	Peak Marker	Distance (cM) ^a	Trait	LOD Score ^b
1	D1S1678	228	Triglycerides	1.2
3	D3S1766	84	PPD	1.1
5	D5S1505	140	HDL-cholesterol	2.4
7	D7S3056	0	PPD	1.0
13	D13S793	81	PPD	1.0
15	D15S659	30	PPD	2.2
	D15S659	30	HDL-cholesterol	1.2
19	D19S1034	13	Triglycerides	1.0
	D19S246	75	PPD	1.6
20	D20S480	75	Triglycerides	1.1

^a The distance is given in cM from the p terminus of the chromosome according to genetic maps provided by the Marshfield Medical Research Foundation.

^b LOD scores obtained over the hepatic lipase gene locus are shown in bold.

some 15, demonstrated coincident LOD scores of 2.2 and 1.2 for PPD and HDL-cholesterol levels, respectively (**Fig. 2**). This locus yielded the highest LOD score for PPD and was the only one that demonstrated evidence of linkage to multiple ALP traits. Thus, the results of the genome scan support the linkage of LDL particle size and HDL-cholesterol levels to the HL gene locus in these FCH families.

DISCUSSION

The ALP, reduced HDL-cholesterol levels, and FCH are known risk factors for CAD. However, it is not certain whether the increased incidence of CAD in FCH patients is due solely to the increased plasma total cholesterol and/or

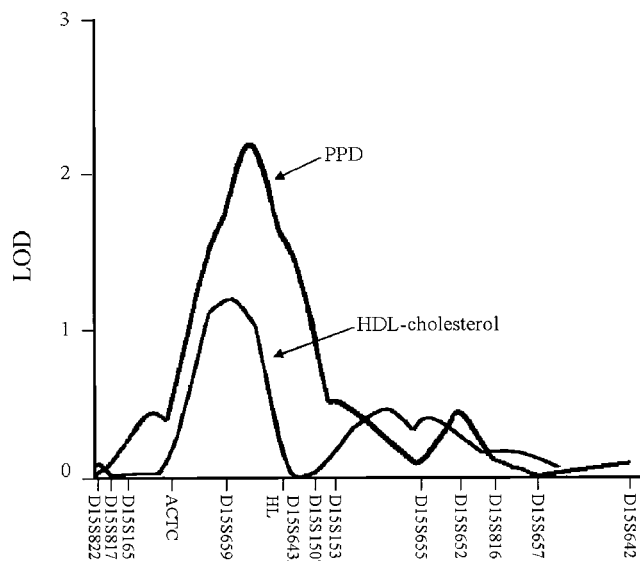


Fig. 2. Multipoint linkage analyses for PPD and HDL-cholesterol levels with chromosome 15 markers. The order of the markers along the chromosome is taken from maps provided by the Marshfield Medical Research Foundation. The genetic position of the HL gene relative to D15S643 is shown according to the recombination distance as taken from the CEPH database.

triglycerides or whether other contributing factors, such as the presence of small, dense LDL particles and/or depressed HDL-cholesterol levels, are also involved. In a recent study of this population, we observed linkage of ALP to three loci that had previously been linked to LDL particle size in CAD families (12, 14). These results supported a multigenic model for ALP and raised the possibility that other loci could also contribute to ALP or its associated quantitative traits. We now provide evidence that, in these FCH families, the HL gene locus is also linked to ALP's associated quantitative traits, PPD, HDL-cholesterol, and triglyceride levels.

Association studies have shown an inverse relationship between HL activity and plasma HDL-cholesterol levels (17, 18, 38). Moreover, transgenic mice over-expressing HL exhibited dramatically reduced HDL-cholesterol levels (39). This effect has been related to the phospholipase activity of HL (40). The hydrolysis of HDL lipid could, in turn, influence the turnover of the particles. HL also has triglyceride hydrolase activity and is involved in the direct hepatic uptake of triglyceride-rich lipoprotein remnants (41, 42). Furthermore, HL-deficient humans and mice exhibit larger, more buoyant LDL particles compared to their normal counterparts, consistent with a role for HL in the conversion of very low density lipoproteins (VLDL) to LDL (43, 44). In FCH, both hypersecretion of VLDL and the relative impaired elimination of triglyceride-rich lipoproteins could contribute to a higher flux of particles back to the liver. Therefore, in the presence of such an increased hepatic burden, the dual role of HL as both a phospholipase and a triglyceride hydrolase can lead to both decreased HDL-cholesterol levels and the formation of small, dense LDL particles.

Previous studies have indicated that a major proportion of the variation in plasma HDL-cholesterol levels is due to allelic variation in the gene encoding HL. In a large study of normolipidemic families, Cohen et al. (15) originally demonstrated significant evidence for linkage of HDL-cholesterol levels to the HL gene locus using nonparametric sibpair analysis. A subsequent study revealed that a polymorphism ($-514 C \rightarrow T$) in the HL promoter was associated with HDL-cholesterol levels in men (16). These results have now been confirmed in several studies, including normolipidemic and CAD populations (17, 19, 45, 46). Additionally, a recent genome scan of HDL-cholesterol levels in randomly ascertained families yielded significant evidence for linkage to the HL gene locus by using pedigree-based quantitative-trait linkage methods (20). In the present study, we also provided evidence for linkage of HDL-cholesterol to this locus, using both individual markers as well as a genome scan. Therefore, three separate studies in different populations have observed the segregation of the HL gene locus with HDL-cholesterol levels, strongly implicating the genetic contribution of this gene to HDL metabolism. Furthermore, it appears that the effect of the HL gene locus on HDL-cholesterol levels may not be obscured by a hyperlipidemic state as it occurs in both normolipidemic and FCH populations.

We did not directly assess whether or not the HL promoter polymorphism was associated with HDL-cholesterol

levels in the hyperlipidemic relatives of the probands because members of this sample are not statistically independent. However, the presence of a T allele conferred significantly higher HDL-cholesterol levels in the unrelated male spouses and probands, replicating previously reported observations. Although we did not have enough independent individuals to assess whether this effect also occurs in FCH affected individuals, these results, taken together with the linkage results, further support the role of HL in HDL metabolism. A molecular mechanism for the sex-specific effect is unknown, although the polymorphism could affect HL expression as it occurs within the promoter. In addition, the possibility remains that the observed association between the $-514 C \rightarrow T$ polymorphism and HDL-cholesterol levels is functionally due to another HL gene variation which is in linkage disequilibrium with this substitution. Unlike HDL-cholesterol levels, we did not observe a significant association between the promoter polymorphism and PPD or triglyceride levels. It is possible that the polymorphism has an effect on these traits that cannot be detected in this sample. Alternatively, the observed linkage of PPD and triglycerides to this locus could be due to another, as yet, unidentified HL gene variant or another nearby gene.

Previous studies have shown that HL activity and LDL particle size are inversely correlated in both normolipidemic men and those with CAD (19, 43, 47, 48). In the present study, we detected linkage of PPD, as well as the other ALP related traits, to the HL gene locus, consistent with these studies and the role of HL in lipid metabolism (49). Notably, the highest LOD score for PPD in the genome scan was observed over the HL gene locus. Additionally, this locus resulted in coincident evidence of linkage to HDL-cholesterol as well, demonstrating the strong effect this locus has on ALP in this FCH population. The fact that linkage to triglycerides was not observed could be due to the characteristics of the subset of individuals on which the genome scan was performed. Although these LOD scores did not reach conventional criteria for significance, i.e., $LOD > 3.0$, these data are consistent with the two-point results at the individual markers and lend support for the contribution of this locus to ALP.

Interestingly, evidence for linkage of ALP (pattern B phenotype) to the HL gene locus was not observed in two different studies by Austin et al. (50, 51), including a comprehensive linkage study of nine candidate genes. In the present study, we observed evidence for linkage of the quantitative trait, PPD, and the results with the qualitative ALP trait were suggestive as well. However, it is not surprising to obtain different results among genetic studies carried out on different populations as study parameters vary from one to the next. For example, the sample size, analytic strategy, heterozygosity indices of the microsatellite markers, or the methods of measuring the phenotypic trait could all be potential explanations for the different results obtained in these studies. Alternatively, it is possible that the HL locus influences PPD, HDL-cholesterol, and triglyceride levels in these Dutch FCH families, or as demonstrated previously, HDL-cholesterol levels alone in other normolipidemic families (2, 15, 20).

In conclusion, we have shown evidence that the HL gene locus contributes to ALP in FCH families. Taken together with our previous findings, the linkage results provide additional support that ALP is a multigenic trait. Interestingly, using a multivariate maximum likelihood-based approach, a recent study of FCH and familial hypertriglyceridemic families demonstrated evidence for pleiotropic genetic effects on ALP traits (52). Therefore, our results also suggest that, in FCH families, small, dense LDL particles, reduced HDL-cholesterol, and increased triglyceride levels may result from common genetic mechanisms and provide a unifying genetic explanation for the observed relationship between these traits. ■

We would like to thank the patients, relatives, and spouses for participating in this study. We gratefully acknowledge Patricia Blanche, Laura Holl, and the staff of the Lipoprotein Core Laboratory at Lawrence Berkeley National Laboratory for performing gradient gel electrophoresis analysis. This study was supported by the National Institutes of Health grant HL-28481 (A.J.L., J.I.R.), by a grant from the Laubisch Fund, UCLA (A.J.L.), by the Cedars-Sinai Board of Governors' Chair in Medical Genetics (J.I.R.), and by a grant from the Dutch Heart Foundation (D 91.101). T.d.B is a recipient of the PIONEER research grant of the Dutch Organization for Fundamental Research (NWO). R.M.K. was supported by Program Project Grant HL 18574 from the National Heart, Lung, and Blood Institute, a grant from the National Dairy Promotion and Research Board and administered in cooperation with the National Dairy Council, and the work was conducted, in part, at the Ernest Orlando Lawrence Berkeley National Laboratory through the U.S. Department of Energy under contract No. DE-AC05-76SF0098. B.E.A. was supported, in part, by the Warsaw Fellowship in Microbiology and The Sam and Rose Gilbert Fellowship. The results of the SIBPAL analysis were obtained by using the program package S.A.G.E., and were supported by U.S. Public Health Services Resource grant P41 RR03655 from the Division of Research Resources. Electronic Database Information: Accession numbers and URLs for data in this article are as follows: Foundation Jean Dausset-CEPH: <http://www.cephb.fr>; Marshfield Medical Research Foundation: <http://www.marshmed.org>; and Online Mendelian Inheritance in Man (OMIM): <http://www.ncbi.nlm.nih.gov/omim> (for familial combined hyperlipidemia).

Manuscript received 30 July 1999 and in revised form 22 November 1999.

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