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A Common Genetic Mechanism Determines Plasma Apolipoprotein B Levels and Dense LDL Subfraction Distribution in Familial Combined Hyperlipidemia

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Summary

Familial combined hyperlipidemia (FCH) is a common lipid disorder characterized by elevations of plasma cholesterol and/or triglyceride in first-degree relatives. A predominance of small, dense LDL particles and elevated apolipoprotein B (apoB) levels is commonly found in members of FCH families. Many studies have investigated the genetic mechanisms determining individuals' lipid levels, in FCH families. Previously, we demonstrated a major gene effect on LDL particle size and codominant Mendelian inheritance involved in determination of apoB levels in a sample of 40 well-defined FCH families. An elevation of apoB levels is associated metabolically with a predominance of small, dense LDL particles in FCH. To establish whether a common gene regulates both traits, we conducted a bivariate genetic analysis to test the hypothesis of a common genetic mechanism. In this study, we found that 66% of the total phenotypic correlation is due to shared genetic components. Further bivariate segregation analysis suggested that both traits share a common major gene plus individual polygenic components. This common major gene explains 37% of the variance of adjusted LDL particle size and 23% of the variance of adjusted apoB levels. Our study suggests that a major gene that has pleiotropic effects on LDL particle size and apoB levels may be the gene underlying FCH in the families we studied.

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Introduction

Familial combined hyperlipidemia (FCH; MIM 144250) is the most common form of lipid disorder, with an estimated population frequency of 1%–2%. Lipid profiles in FCH families are characterized by elevated concentrations of total plasma cholesterol and/or triglyceride in first-degree relatives; this disorder is therefore also known as "multiple-type hyperlipidemia." Other characteristics of FCH include elevated plasma apolipoprotein B (apoB) levels, decreased HDL cholesterol concentration, and a predominance of small, dense LDL particles (Grundy et al. 1987; Kwiterovich et al. 1987; Austin et al. 1990).

Originally, FCH was thought to be a single-gene disorder with a major effect on triglyceride levels and a secondary effect on cholesterol levels (Goldstein et al. 1973). Several phenotypes, which include hyperapobetalipoproteinemia (Sniderman et al. 1980, 1982), LDL subclass pattern B (Austin and Krauss 1986; Austin et al. 1988), familial dyslipidemic hypertension (FDH) (Hunt et al. 1989; Williams et al. 1993), and syndrome X (Reaven 1988), have been proposed to have a relationship with FCH. These dyslipidemic syndromes appear related to each other through the presence of small, dense LDL particles. The diverse metabolic and biochemical defects suggest that the genetic basis of FCH is heterogeneous (Kwiterovich 1993).

The cause of the mixed hyperlipidemia in FCH is unknown and may vary among families. From a metabolic point of view, there are two possible underlying defects: (1) overproduction of apoB, and (2) delayed clearance of triglyceride-rich lipoproteins (Kwiterovich 1993). FCH is thought to be caused by hepatic overproduction of apoB, which is channeled into a pool of very low–density lipoprotein (VLDL) particles, resulting in elevated levels of other apoB-containing lipoproteins, which include intermediate density lipoprotein (IDL) and LDL (Kissebah et al. 1981; Grundy et al. 1987; Venkatesan et al. 1993). This "metabolic channeling"

may explain the apparent increase in production of apoB associated with a predominance of small, dense LDL (Krauss 1994; de Graaf et al. 1993). Delayed clearance of triglyceride-rich lipoproteins in FCH can be in part caused by impaired lipoprotein lipase activity (Babirak et al. 1989, 1992). Although high apoB levels and small, dense LDL particles are strongly associated with an increase in plasma triglyceride concentrations in FCH families, several studies have suggested distinct genetic mechanisms (Beaty et al. 1992; Hokanson et al. 1995) for expression of elevated apoB levels and hypertriglyceridemia.

Previously, we have investigated the genetic mechanisms of the distribution of dense LDL subfraction profiles (i.e., distribution of LDL density) and plasma apoB levels in 40 well-defined Dutch FCH families, and a major gene effect was found for each trait (Bredie et al. 1996, 1997). Since the distribution of LDL subfractions is metabolically related to apoB levels, we here extend our previous findings to investigate a common genetic mechanism controlling both traits in these families.

Material and Methods

Study Population

The study population has been described elsewhere (Bredie et al. 1996, 1997). In brief, families were ascertained through probands exhibiting both cholesterol and triglyceride concentrations >90th percentile, adjusted for age and gender. Families were included when a multiple-type hyperlipidemia with levels of total cholesterol and/or triglyceride >90th percentile was present. If the body mass index (BMI) exceeded 30 kg m^{-2} or alcohol consumption was more than two drinks (beer, wine) per day (equivalent to 20 g of alcohol), the 95th percentile of cholesterol and triglyceride was used. Thus, at least one first-degree relative exhibited hypercholesterolemia or hypertriglyceridemia, in addition to the proband. Families were excluded when probands had any underlying diseases causing hyperlipidemia, probands were homozygous for the apoE2 allele, or any first-degree family member had tendon xanthomata. All individuals were Caucasians above the age of 10 years. The study protocol was approved by the ethics committee of the University Hospital of Nijmegen.

Measurement of Lipid and LDL Subfraction

A detailed description of lipid assays has been given previously (Bredie et al. 1996; 1997). In short, total plasma apoB concentrations were determined by immunonephelometry (Lopes-Virella et al. 1980).

LDL subfractions were detected by single spin density gradient ultracentrifugation, according to a method described earlier (Swinkels et al. 1989). After ultracentrifugation the LDL subfractions were visible as distinct bands in the middle of the tube. Up to five LDL subfractions could be distinguished, concentrated in the following density ranges: LDL1 (1.030–1.033 g ml^{-1}), LDL2 (1.033–1.040 g ml^{-1}), LDL3 (1.040–1.045 g ml^{-1}), LDL4 (1.045–1.049 g ml^{-1}), and LDL5 (1.049–1.054 g ml^{-1}). The ultracentrifugation tubes containing the LDL subfractions stained with Coomassie Brilliant Blue R were placed in a specially designed rack and photographed. Accurate documentation of the different LDL subfraction patterns was obtained by scanning the obtained slides in triplicate on an LKB 2202 ultrascan laser densitometer (Pharmacia LKB, Uppsala, Sweden). The mean peak heights (h1–h5) of the LDL subfractions (LDL1–LDL5) on the three scans were used to calculate the variable K as a continuous variable that best describes each individual LDL subfraction pattern (de Graaf et al. 1992). The relative contribution of each LDL subfraction, expressed as the percent contributed at each peak height (%h1–%h5) relative to the total LDL subfraction profile [total LDL (100%) = %h1 + %h2 + %h3 + %h4 + %h5] was calculated. The relative peak heights of LDL3 and the less-frequently occurring LDL4 and/or LDL5 were added to give $\%h3' = (\%h3 + \%h4 + \%h5)$, where LDL(100%) = LDL1(%h1) + LDL2(%h2) + LDL3(%h3′). When a subfraction profile was characterized by a predominance of buoyant LDL particles ($h1–h3 > 0$), variable K was calculated by $K = (\%h1–\%h3')/(\%h2–\%h3' + 1)$. In the case of a predominance of heavy, dense LDL subfractions ($h1–h3 < 0$), variable K was calculated by: $K = (\%h1–\%h3')/(\%h2–\%h1 + 1)$. A negative K value reflects a dense subfraction profile, $K = 0$ reflects an intermediate subfraction profile, and a positive K value reflects a complete buoyant profile.

Statistical Analysis

To minimize other covariate effects on total plasma apoB levels and variable K, crude phenotypic values (including probands and all other family members) were adjusted for gender, age, BMI, and smoking by linear regression. The total sample mean was then added to the residual value from each individual as the adjusted value. The Spearman correlation coefficients between apoB and variable K were calculated before and after adjustment for covariates, to depict their relation.

Segregation Analysis

Bivariate segregation analysis of adjusted apoB and K values was carried out by the computer program PAP (version 4; Hasstedt 1994). For a trait under the control of a single major gene, we assume that phenotypic variation can be accounted for by a major gene effect, residual additive polygenes, and random environmental

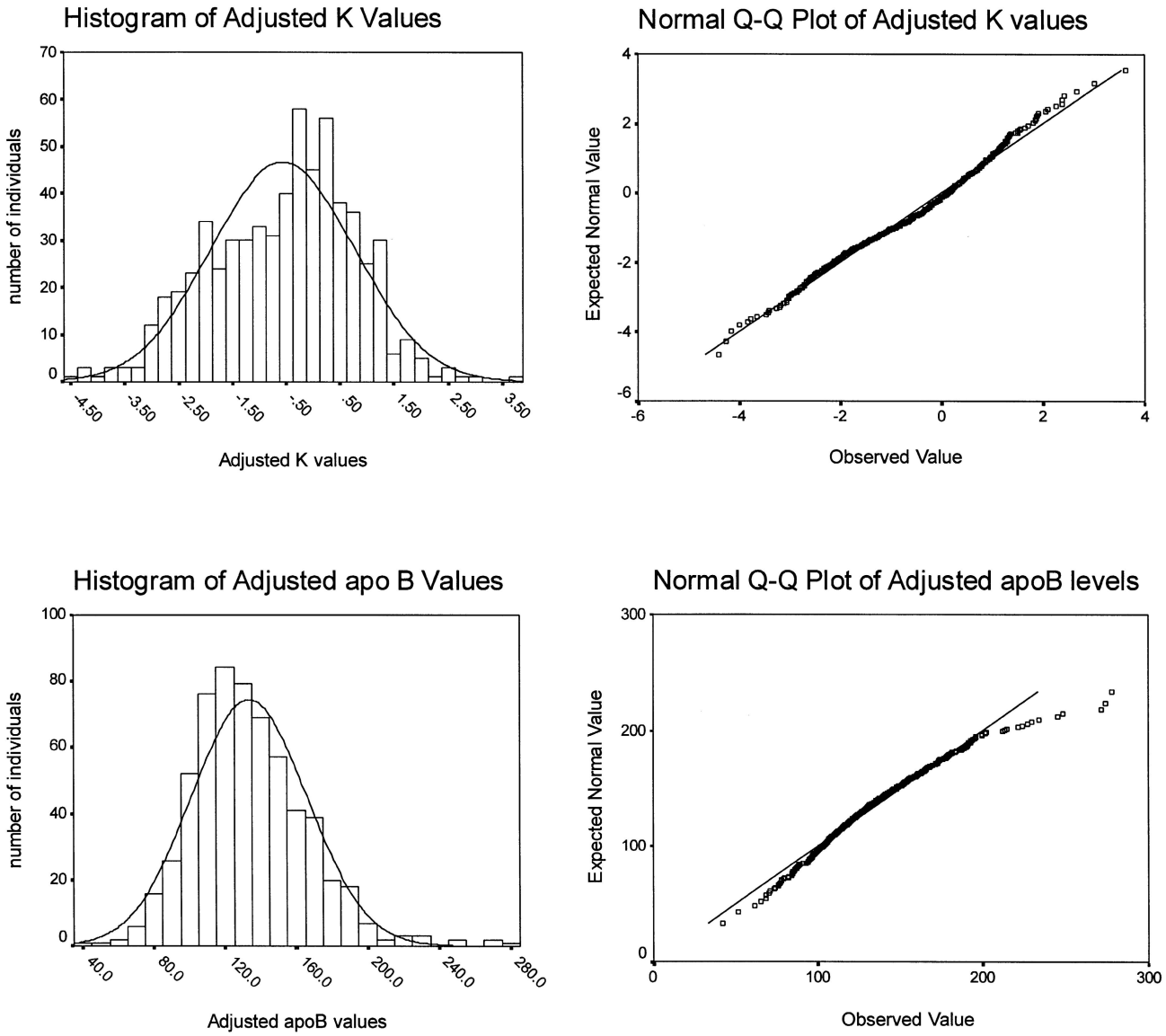

Figure 1 Histograms and Q-Q plots of adjusted variable *K* and apoB levels

factors for which we failed to adjust in the linear regression models. Among the tested models, unobservable genotypes are the products of two alleles, "A" and "B," at a single major gene. The B allele is associated with higher levels of the trait, and A is associated with lower levels. Thus, the general model includes three normal distributions, denoted AA, AB, and BB, which are assumed to occur in Hardy-Weinberg proportions in the general population (i.e., P_A^2, $2P_AP_B$, and P_B^2 for each genotype, where P_A is the frequency of A and $P_B = 1 - P_A$). Three means (μ_{AA}, μ_{AB}, and μ_{BB}) corresponding to each genotype are estimated, along with a single common variance (Φ^2). The within-genotype variance can be further partitioned into a component representing residual polygenic effects (PG, also known as h^2) and individual specific environmental effects plus measurement error (E, also known as $1 - h^2$). Three arbitrary transmission parameters (τ_{AA}, τ_{AB}, and τ_{BB}) represent the probability that an individual of a given genotype transmits an A allele to an offspring, and these are also estimated under the most general single gene model.

Blangero and Konigsberg (1991) extended univariate segregation analysis to multivariate segregation analysis and implemented the maximum likelihood methods in a modified version of PAP. When a second trait is incorporated into the model, the assumptions concerning number of alleles per locus, Hardy-Weinberg equilibrium, relationship of alleles to higher and lower levels

of the trait, and a single within-genotype variance are the same as assumed above for the first trait. We used bivariate models to estimate parameters corresponding to apoB and variable *K*, simultaneously, as well as their residual correlations from additive polygenes (ρ_G) and environmental (ρ_E) factors. We postulated that the distribution of LDL subfraction profiles represented by variable *K* is determined by an unobserved major gene (α_1G), residual additive polygenic background (α_2PG), and a residual nongenetic component (α_3E); therefore, variable *K* values are the sum of $\alpha_1 G + \alpha_2 PG + \alpha_3 E$, with corresponding variance components $\sigma^2_{total} = \sigma^2_{\alpha 1G} + \sigma^2_{\alpha 2PG} + \sigma^2_{\alpha 3E}$. Similarly, for the second trait, apoB levels are the sum of $\beta_1 G + \beta_2 PG + \beta_3 E$, where the same underlying genetic factors (G and PG) exert different effects (i.e., α and β) on variable *K* and apoB levels, respectively. Path analysis can be used to describe the correlation between two traits in the same individual. As reviewed by Lynch and Walsh (1998), the genetic and environmental correlations can be derived from the path analysis, which calculates phenotypic correlation (ρ_P) by summing the appropriate products of path and correlation coefficients:

$$\rho_p = \rho_G \sqrt{h_1^2 h_2^2} + \rho_E \sqrt{(1 - h_1^2)(1 - h_2^2)}.$$

When bivariate models do not incorporate a major gene effect, h^2 represents the proportion of the phenotypic variance accounted for by the total additive genetic effects, and ρ_G reflects the total genetic correlation between two traits. When a major gene effect is incorporated into models, h^2 represents the proportion of the phenotypic variance accounted for by the residual additive polygenes and ρ_G represents genetic correlation from the shared residual additive polygenes.

Since families were ascertained via two known affected individuals, ascertainment correction was undertaken by conditioning phenotypes of family members on those of the affected probands plus one extra affected individual per kindred. No transformation was performed for either trait, since the coefficients of skewness for variable *K* and apoB were −0.23 and 0.68, respectively. Figure 1 shows a histogram and a QQ plot, which do not seriously violate normality, for each trait. Furthermore, we included the environmental model in the segregation analysis, which reduced the possibility that skewedness alone leads to false detection of a major gene (Demenais et al. 1986).

Model Hypothesis and Comparison

We first tested the total genetic correlation between these two traits. In table 1, we fit the data to model 1, which was a polygenic model without the genetic correlation, and to model 2, which was another polygenic model with the genetic correlation. Comparison between these two models indicated the significance of the total genetic correlation. Then we further explored the source of the genetic correlation by modeling a major gene effect—that is, we assumed that the significant genetic correlation was caused by a common major gene. For one-gene Mendelian models, the modes of transmission can be dominant, recessive, or codominant for each trait. Thus there are nine possible combinations in one-gene bivariate models. A one-factor environmental model (model 6) was considered an alternative model in which three transmission parameters were equal to the frequency of a major environmental factor, P_L. A one-gene general bivariate model (model 7) in which three transmission parameters were free to be estimated was fit as the reference model. We also tested a two-gene Mendelian model (model 8) in which each trait has its corresponding single major gene. We compared the fit of this two-gene model with those from the one-gene general model and the best-fitting one-gene bivariate Mendelian model, to further test the hypothesis of a common major gene.

Two criteria were employed to compare these models. For hierarchical models, the likelihood ratio test (LRT) was used. Twice the difference in log likelihoods ($-2 \ln L$) between a restricted model and an unrestricted model is asymptotically distributed as a χ^2 statistic, with degrees of freedom equal to the difference in the number of parameters fit under the two models. The most parsimonious model is the one requiring the fewest estimated parameters while giving a log likelihood not significantly smaller than the most general model. When comparing nonhierarchical models, however, the Akaike's Information Criterion (AIC) was used (Akaike 1974). The AIC is equal to $-2 \ln L + 2n$, where n is the number of parameters estimated in the models. By this criterion, the most parsimonious model is the one with the smallest AIC score.

Results

Sample

There were 40 multigenerational families (two to four generations) in which 623 individuals had data on adjusted variable *K* and 607 individuals had adjusted apoB values. Our previous study (Bredie et al. 1997) showed possible etiologic heterogeneity for apoB inheritance in this population. One family containing 12 individuals (in two of whom apoB data were missing) was found to strongly indicate an environmental transmission model, and thus this family was excluded for segregation analysis of apoB. In the bivariate analysis, apoB values in this family were coded as missing. Therefore, apoB

Table 1

Bivariate Segregation Analysis of Variable *K* and apoB Levels

			Variable *K*					apoB Levels									
Model[a]	P_L	P_A	μ_{LL}	μ_{HL}	μ_{HH}	σ_K	h_K^2	μ_{AA}	μ_{AB}	μ_{BB}	σ_{apoB}	h_{apoB}^2	ρ_G	ρ_E	$-2\ln L$	AIC	LRT (*P* Value)[b]
1. Sporadic I	[1]	…[c]	−.32	$=\mu_{LL}$	$=\mu_{LL}$	1.29	.33	126.45	$=\mu_{AA}$	$=\mu_{AA}$	28.66	.23	[0]	−.51	9199.4	9213.4	$x_9^2 = 72.8$ (<.001)
2. Sporadic II	[1]	…[c]	−.13	$=\mu_{LL}$	$=\mu_{LL}$	1.31	.44	123.43	$=\mu_{AA}$	$=\mu_{AA}$	29.03	.34	−.81	−.27	9167.8	9183.8	$x_8^2 = 41.2$ (<.001)
3. Rec-Cod	.98	…[c]	−.03	$=\mu_{LL}$	−1.90	1.22	.41	123.80	123.47	234.76	28.62	.37	−.81	−.33	9140.3	9164.3	$x_4^2 = 13.7$ (.01)
4. Cod-Cod I	.60	…[c]	.59	−.06	−1.84	.94	.13	113.64	120.82	155.39	23.65	.09	.26[d]	−.17	9132.3	9158.3	$x_3^2 = 5.7$ (.13)
5. Cod-Cod II	.60	…[c]	.58	−.05	−1.83	.94	.14	113.97	120.60	155.24	23.73	.11	[0]	−.15	9132.5	9156.5	$x_4^2 = 5.9$ (.21)
6. Environmental[e]	.89	…[c]	.26	−1.02	−.74	1.23	.45	125.07	112.63	203.85	27.18	.42	−.72	−.15	9153.8	9179.8	$x_3^2 = 27.2$ (.001)
7. One-gene general[f]	.71	…[c]	.58	−.33	−1.96	.94	.12	110.38	125.51	160.13	21.88	.05	.81[d]	−.11	9126.6	9158.6	…
8. Two-gene	.74	.42	−.12	.11	−2.03	1.18	.43	116.90	114.87	201.14	26.04	.25	−.99[g]	−.39	9150.5	9178.5	…

Note.—Brackets indicate that the value was fixed.

[a] Rec = recessive; and cod = codominant.

[b] LRT = likelihood-ratio test. *P* values were from comparisons between each restricted model (models 1–6) and the one-gene general model (model 7).

[c] We assumed the second gene for apoB levels is the same as the first gene for *K* values.

[d] Both parameters are not significant because of large standard errors (.26 ± .84 and .81 ± 1.61).

[e] The three transmission parameters are equal to P_L.

[f] The three transmission parameters are .90, .39, and .08.

[g] The parameter was fixed by the maximum-likelihood algorithm.

analysis was based on 597 individuals. 571 individuals had data on both traits.

Adjustment for Covariates and the Spearman Correlation

Environmental factors included in the multiple linear regression analysis accounted for 24% of the variance of variable *K* (Bredie et al. 1996). For apoB, the environmental factors explained 36% of the variance of apoB (Bredie et al. 1997). The crude Spearman correlation coefficient between two traits was −0.65, and the coefficient decreased to −0.47 after adjustment for environmental factors.

Segregation Analysis

The total genetic component for each trait was estimated to be 0.44 for adjusted *K* and 0.34 for adjusted apoB on the basis of model 2 (table 1) (these estimates are identical to those obtained from univariate models, data for which are not shown). Model 2 fit much better than model 1 ($p < 0.0001$). This suggested significant genetic correlation between these two traits. We further calculated the total genetic and environmental correlations between these two traits on the basis of parameters in model 2 using the formula mentioned in the Material and Methods section (Lynch and Walsh 1998). The total genetic correlation was −0.31, the environmental correlation was −0.16, and the sum of them was equal to a total phenotypic correlation (i.e., the Spearman correlation) of −0.47. It should be noted that a predominance of small, dense LDL particles is associated with high apoB levels. The negative value for the correlation indicates this inverse association.

As mentioned above, we tested nine different one-gene bivariate Mendelian models. In table 1, we present only informative models. Our previous report of variable *K* (Bredie et al. 1996) showed that neither the codominant nor the recessive model could be rejected, although the recessive model was more parsimonious than the codominant model. Analysis of apoB (Bredie et al. 1997) showed that the codominant model fit these data best. Therefore, we were particularly interested in two types of bivariate models: one recessive for variable *K* and codominant for apoB (model 3) and the other codominant for both traits (models 4 and 5). After comparison of the one-gene models 1–6 with the one-gene general model 7, all were rejected except models 4 and 5. Note that the estimated genetic correlation in model 4 reversed to a positive value of 0.26 but with a large standard error of 0.84. This suggested that the residual genetic correlation beyond the common major gene is negligible. Therefore, we further tested a similar model with the residual genetic correlation fixed at zero (model 5). In comparison of the likelihoods between models 4 and 5, model 5 fit as well as model 4, and the parameters in both models were similar. Model 5 was considered more parsimonious than model 4. Model 8 includes two independent genes, and each gene has its single correspondent trait. Thus, it is conceptually different from one-gene models. The AIC scores were used to compare model 8 with model 5 and with the one-gene general model 7. Model 8 had a worse fit as judged by the AIC scores. Thus model 5 had the best fit among all the competing models. We concluded that a common major gene accounted for most genetic components shared between both traits.

For Mendelian models of inheritance, the variance due to a major gene (Boerwinkle and Sing 1986) is computed as

$$\sigma^2_{\mathrm{ML}} = \sum_{i=l}^{I} f_i(\mu_i - \mu)^2 - \frac{I-1}{n}\sigma^2_T,$$

where μ is the overall mean, μ_i is the estimated mean of the ith genotype (i= 1, 2, 3, .., I), f_i is its genotypic frequency, n is the total number of individuals, and $\sigma^2{}_T$ is the total variance of phenotypic values. The ratio $\sigma^2_{\mathrm{ML}}/\sigma^2_T$ provides the major gene contribution to the phenotypic variance. In the best-fitting model 5, the major gene explained 37% of the variance in the adjusted *K* values and 23% of that in adjusted apoB levels. The residual genetic correlation of zero in model 5 indicated distinct residual polygenes for each trait. The residual polygenes for variable *K* explained 7% of the variance in variable *K* values (as calculated by $h^2_K\sigma^2_K/\sigma^2_T$, parameters from model 5), and the residual polygenes for apoB also explained 7% of the variance of apoB levels. The total environmental correlation in model 5 is equal to −0.13, which was close to the original estimate in model 2.

Discussion

Evidence of a common genetic mechanism controlling both apoB levels and distribution of LDL subfraction profiles (represented by variable *K*) in FCH families is supported from the present bivariate segregation analysis. The best-fitting model has a common major gene with codominant alleles for both traits, plus distinct polygenes for each trait. The significant decrease in the residual genetic correlation in model 5 supports the hypothesis that a common major gene regulates these two traits.

In the univariate analysis of variable *K*, the recessive model was considered more parsimonious than the codominant model, although neither could be rejected when compared to the most general model (Bredie et al. 1996). However, both the recessive and codominant models provided similar information—predicting a

dense LDL subfraction profile in ~17% of the population and either an intermediate or a buoyant subfraction profile in the other 83% of the population. Thus, codominant inheritance of variable *K* in this present bivariate analysis was not in disagreement with our previous study (Bredie et al. 1996). Further comparison between the estimated parameters in our best-fitting bivariate model and those in the best-fitting univariate models of variable *K* and apoB (Bredie et al. 1996, 1997) showed they are similar in terms of allele frequency and genotypic means.

On the basis of a larger major gene effect on variable *K* than apoB and the consistent mode of transmission between both traits, we hypothesized that the major gene has a primary effect on LDL heterogeneity with a carryover effect on apoB levels. This hypothesis is in concert with the observation that the distribution of LDL particle sizes was less affected by environment than were the apoB levels (Hokanson et al. 1993). Hokanson et al. (1995) also reported that the primary increase in plasma apoB levels in the FCH patients was accounted for by significant increases in apoB in denser LDL particles. This may also suggest that increasing the number of denser LDL particles results in elevated apoB levels. Analysis of the chemical properties of lipoproteins showed that not only LDL particles but also VLDL and IDL from FCH patients had higher lipoprotein mass than controls (Hokanson et al. 1995). Since only one apoB molecule is present on each LDL and VLDL particle, it is reasonable to hypothesize that a defective gene (or genes) that increases assembly or formation of small, dense lipoproteins leads to an increased number of particles and thus results in elevated apoB levels. If this biological model is accurate, the bivariate model presented here truly reflects the relation between LDL particles and apoB levels.

However, both Jarvik et al. (1994) and Austin et al. (1992) reported distinct genetic mechanisms for LDL subclass phenotype (a dichotomized phenotype of LDL particle size) and apoB levels in FCH families. These conflicting results could have several possible causes. Jarvik et al. (1994) first conducted a segregation analysis of apoB concentration and then calculated the probability of each individual's genotype under the best-fitting model of apoB concentrations. They found no association between these predicted apoB level genotypes and LDL subclass phenotypes. However, the assignment of individual's genotype was made on the basis of an arbitrary cutoff point of genotype probability, which could lead to unknown degrees of misclassification. This problem can be more serious if more than one underlying major gene determines apoB levels. A similar method has been applied to our data set, leading to a conclusion of separate genetic mechanisms for variable *K* and apoB levels (data not shown). Although Jarvik et al. (1994) justified their conclusions by showing a major gene in segregation analysis of LDL subclass–adjusted apoB, the adjustment by regression models that used average information could leave significant residual effects. We have repeated a similar strategy in our data and also found a major effect on apoB-adjusted variable *K* (data not shown). In other words, we used these indirect methods in our data set and found that the results were the opposite of those from the direct bivariate analysis. Austin et al. (1992) used commingling analysis of apoB in FCH families and reported that the gene regulating LDL subclass phenotype could not explain apoB levels. Their conclusions were made on the basis of a bimodal distribution of apoB levels in a subset of 54 individuals who had LDL subclass B (i.e., small, dense LDL particles). In the Austin et al. (1992) analysis, individuals with LDL subclass B were selected from 78 nuclear families; therefore, the family structure was broken and information from other relatives could not be incorporated into the subset analysis. Furthermore, because of the small sample size, their results were more subject to sample variation. Jarvik et al. (1994) found a major gene effect on apoB levels in individuals with LDL subclass A; Austin et al. (1992) did not find similar results in such individuals. The etiology of elevated apoB levels can be heterogeneous (Coresh et al. 1993; Bredie et al. 1997). The conflicting results of the present study and the two previous studies (Austin et al. 1992; Jarvik et al. 1994) may reflect the complexity of the genetic mechanisms for these traits.

There are some limitations in our study. We did not test gene-environment interaction in the segregation models. Our sample was a highly selected population, which makes adequate ascertainment correction unlikely. Therefore, the interpretation of our results needs to be conservative.

Our study, which used a more direct statistical approach and a larger sample size, supported the hypothesis of a common genetic mechanism for apoB levels and LDL subfraction profiles. Furthermore, our results are more consistent with results from metabolic studies. For future genetic studies of FCH, especially linkage analyses, use of a subset of families in which individuals have a predominance of small, dense LDL particles as well as high apoB levels may be able to minimize etiologic heterogeneity.

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Electronic-Database Information

Accession number and URL 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])

References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Automatic Control AC 19:719–723

Austin MA, Brunzell JD, Fitch WL, Krauss RM (1990) Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. Arterioscler Thromb 10: 520–530

Austin MA, Horowitz H, Wijsman E, Krauss RM, Brunzell J (1992) Bimodality of plasma apolipoprotein B levels in familial combined hyperlipidemia. Atherosclerosis 92:67–77

Austin MA, King M-C, Vranizan KM, Newman B, Krauss RM (1988) Inheritance of low-density lipoprotein subclass patterns: results of complex segregation analysis. Am J Hum Genet 43:838–846

Austin MA, Krauss RM (1986) Genetic control of low-density lipoprotein subclasses. Lancet 2:592–595

Babirak SP, Brown BG, Brunzell JD (1992) Familial combined hyperlipidemia and abnormal lipoprotein lipase. Arterioscler Thromb 12:1176–1183

Babirak SP, Iverius PH, Fujimoto WT, Brunzell JD (1989) Detection and characterization of the heterozygote state for lipoprotein lipase deficiency. Arteriosclerosis 9:326–334

Beaty TH, Prenger VL, Virgil DG, Lewis B, Kwiterovich PO Jr, Bachorik PS (1992) A genetic model for control of hypertriglyceridemia and apolipoprotein B levels in the Johns Hopkins colony of St. Thomas Hospital rabbits. Genetics 132:1095–1104

Blangero J, Konigsberg LW (1991) Multivariate segregation analysis using the mixed model. Genet Epidemiol 8:299–316

Boerwinkle E, Sing CF (1986) Bias of the contribution of single-locus effects to the variance of a quantitative trait. Am J Hum Genet 39:137–144

Bredie SJH, Kiemeney LA, de Haan AFJ, Demacker PNM, Stalenhoef AFH (1996) Inherited susceptibility determines the distribution of dense low-density lipoprotein subfraction profiles in familial combined hyperlipidemia. Am J Hum Genet 58:812–822

Bredie SJ, van Drongelen J, Kiemeney LA, Demacker PN, Beaty TH, Stalenhoef AF (1997) Segregation analysis of plasma apolipoprotein B levels in familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol 17:834–840

Coresh J, Beaty TH, Kwiterovich PO Jr (1993) Inheritance of plasma apolipoprotein B levels in families of patients undergoing coronary arteriography at an early age. Genet Epidemiol 10:159–176

de Graaf J, Hendriks JC, Demacker PN, Stalenhoef AF (1993) Identification of multiple dense LDL subfractions with enhanced susceptibility to in vitro oxidation among hypertriglyceridemic subjects: normalization after clofibrate treatment. Arterioscler Thromb 13:712–719

de Graaf J, Swinkels DW, de Haan AFJ, Demacker PNM, Stalenhoef AFH (1992) Both inherited susceptibility and environmental exposure determine the low-density lipoprotein-subfraction pattern distribution in healthy Dutch families. Am J Hum Genet 51:1295–1310

Demenais F, Lathrop M, Lalouel JM (1986) Robustness and power of the unified model in the analysis of quantitative measurements. Am J Hum Genet 38:228–234

Goldstein JL, Schrott HG, Hazzard WR, Bierman EL, Motulsky AG (1973) Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J Clin Invest 52:1544–1568

Grundy SM, Chait A, Brunzell JD (1987) Familial combined hyperlipidemia workshop. Arteriosclerosis 7:203–207

Hasstedt SJ (1994) Pedigree analysis package (PAP), revision 4.0. University of Utah, Salt Lake City

Hokanson JE, Austin MA, Zambon A, Brunzell JD (1993) Plasma triglyceride and LDL heterogeneity in familial combined hyperlipidemia. Arterioscler Thromb 13:427–434

Hokanson JE, Krauss RM, Albers JJ, Austin MA, Brunzell JD (1995) LDL physical and chemical properties in familial combined hyperlipidemia. Arterioscler Thromb Vasc Biol 15:452–459

Hunt SC, Wu LL, Hopkins PN, Stults BM, Kuida H, Ramirez ME, Lalouel JM, et al (1989) Apolipoprotein, low density lipoprotein subfraction, and insulin associations with familial combined hyperlipidemia: study of Utah patients with familial dyslipidemic hypertension. Arteriosclerosis 9: 335–344

Jarvik GP, Brunzell JD, Austin MA, Krauss RM, Motulsky AG, Wijsman E (1994) Genetic predictors of FCHL in four large pedigrees: influence of apoB level major locus predicted genotype and LDL subclass phenotype. Arterioscler Thromb 14:1687–1694

Kissebah AH, Alfarsi S, Adams PW (1981) Integrated regulation of very low density lipoprotein triglyceride and apolipoprotein-B kinetics in man: normolipemic subjects, familial hypertriglyceridemia, and familial combined hyperlipidemia. Metabolism 30:856–868

Krauss RM (1994) Heterogeneity of plasma low-density lipoproteins and atherosclerosis risk. Curr Opin Lipidol 5: 339–349

Kwiterovich PO Jr (1993) Genetic and molecular biology of familial combined hyperlipidemia. Curr Opin Lipidol 4: 133–143

Kwiterovich PO Jr, White S, Forte T, Bachorik PS, Smith H, Sniderman A (1987) Hyperapobetalipoproteinemia in a kindred with familial combined hyperlipidemia and familial hypercholesterolemia. Arteriosclerosis 7:211–225

Lopes-Virella MF, Virella G, Evans G, Malenkos SB, Colwell JA (1980) Immunonephelometric assay of human apolipoprotein AI. Clin Chem 26:1205–1208

Lynch M, Walsh B (1998) Appendix A2: path analysis. In:

Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, Massachusetts, pp 829–830

Reaven GM (1988) Banting lecture 1988: role of insulin resistance in human disease. Diabetes 37:1595–1607

Sniderman A, Shapiro S, Marpole D, Skinner B, Teng B, Kwiterovich PO Jr (1980) Association of coronary atherosclerosis with hyperapobetalipoproteinemia (increased protein but normal cholesterol levels in human plasma low density [beta] lipoproteins). Proc Natl Acad Sci USA 77:604–608

Sniderman AD, Wolfson C, Teng B, Franklin FA, Bachorik PS, Kwiterovich PO Jr (1982) Association of hyperapobetalipoproteinemia with endogenous hypertriglyceridemia and atherosclerosis. Ann Intern Med 97:833–839

Swinkels DW, Demacker PN, Hendriks JC, van 't Laar A (1989) Low density lipoprotein subfractions and relationship to other risk factors for coronary artery disease in healthy individuals. Arteriosclerosis 9:604–613

Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J (1993) Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglyceride levels and indicate a metabolically and biochemically coherent basis for familial combined hyperlipidemia. Arterioscler Thromb 13: 1110–1118

Williams RR, Hunt SC, Hopkins PN, Wu LL, Hasstedt SJ, Berry TD, Barlow GK, et al (1993) Genetic basis of familial dyslipidemia and hypertension: 15-year results from Utah. Am J Hypertens 6:319S–327S