Segregation Analysis of Plasma Apolipoprotein B Levels in Familial Combined Hyperlipidemia


Abstract
Familial combined hyperlipidemia (FCH) is a heritable lipid disorder that is associated with an increased risk of premature cardiovascular disease. An elevated plasma apolipoprotein (apo) B concentration is reported to be a diagnostic feature of the disorder. Recently we demonstrated a strong relation between plasma apoB concentrations and the cholesterol concentration in VLDL plus LDL, both elevated in FCH families. Therefore, examination of the inheritance of elevated plasma apoB levels in FCH families may reveal important information about the mechanism responsible for the aggregation of elevated plasma lipids in FCH. This study included 663 Dutch family members in 40 families ascertained through FCH probands. Plasma apoB concentration correlated significantly with apoB-related cholesterol both in the probands and the relatives (r=83 and r=90, respectively). Adjustment for age, sex, body mass index, and smoking habits accounted for 35.7% of the variation in apoB levels, and there was strong familial aggregation in adjusted apoB levels in these families. Complex segregation analysis was performed to determine the mechanism of inheritance behind this familial aggregation. The aggregation of elevated apoB levels was best explained by a major gene effect inherited by a codominant mechanism. Estimated mean apoB levels for the three supposed genotypes AA, AB, and BB were 111.5, 126.7, and 165.7 mg/dL, respectively, with relative frequencies of 43.5%, 44.9%, and 11.6%, respectively. In conclusion, despite assumed metabolic and genetic heterogeneity of FCH, there is clear evidence for a single gene effect on apoB concentrations in families ascertained through FCH. Linkage studies based on this analysis may further clarify the molecular basis of the apoB regulation in these families. (Arterioscler Thromb Vasc Biol. 1997;17:834-840.)

Key Words • lipoproteins • human genetics • apoB

Familial combined hyperlipidemia is the most common form of heritable lipid disorders and is associated with cardiovascular disease. This multiple phenotype hyperlipidemia is characterized by elevations of the total plasma cholesterol concentration and/or the plasma triglyceride concentration, consequently resulting in the presence of different lipid phenotypes in first-degree relatives. Furthermore, an elevated plasma apoB100 concentration, a decreased HDL concentration, and a preponderance of small, dense LDL particles are observed. Initially, FCH was thought to be inherited as a single gene disorder with a major gene effect on triglyceride levels. Recently this was supported by a segregation analysis in 55 British FCH families. Other studies, however, have shown FCH to be a more heterogeneous lipid disorder, comprising features of the insulin resistance syndrome, hyperapobetalipoproteinemia, and familial dyslipidemic hypertension, which in turn are all associated with the presence of small, dense LDL. Still, the absence of a specific metabolic or genetic marker complicates the diagnosis of FCH in individual patients.

In FCH, the hyperlipidemia is caused by hepatic overproduction of VLDL apoB100. Consequently, elevated levels of apoB-containing lipoproteins (i.e., IDL and LDL particles) are observed. A delayed clearance of the triglyceride-rich apoB-containing lipoproteins, in part caused by impaired lipoprotein lipase activity, may modify the lipid phenotypic expression. Elevated lipid levels in FCH reflect elevated VLDL and LDL cholesterol concentrations, which correspond to an increase in respective particle numbers. Since each VLDL and LDL particle always contains one apoB molecule per particle, a strong correlation can be expected in FCH between total plasma apoB and its related cholesterol in VLDL and LDL particles. On this basis, the individual plasma apoB concentration represents the actual VLDL and LDL concentrations, which may show some variability in time in FCH patients.

Studies have also shown the association between elevated apoB levels and cardiovascular disease, which is a feature of FCH as well. Some studies show that the apoB level is a better predictor of premature cardiovascular disease than the plasma lipids are. Complex segregation analyses demonstrated familial aggregation of elevated apoB levels in families presenting premature cardiovascular disease, families with familial hyperlipidemia, and healthy volunteers, but conclusions about the specific genetic mechanism were contradictory to date. This may be the reason that linkage studies could not identify conclusively the locus responsible for the elevation of apoB levels, although some gene markers showed some association. Before further linkage analyses with candidate genes can be undertaken, it is critical to have a better model of inheritance.

In the present study, segregation models were examined to explain the familial clustering of elevated apoB levels measured in individuals from 40 well-defined Dutch FCH families. Single gene effects and polygenic components were tested by using class D regressive
Apolipoprotein Levels

Measurement of Lipid, Lipoprotein, and Apolipoprotein Levels

VLDL was isolated from whole plasma by ultracentrifugation at \( \gamma = 1.006 \) g/mL for 16 hours at 36,000 rpm in a fixed rotor (TFT 45.6 rotor, Kontron), in a Beckman L7-55 ultracentrifuge (Beckman). HDL cholesterol was determined by the polyethylene glycol 6000 method. LDL cholesterol was calculated by subtraction of VLDL cholesterol and HDL cholesterol from total plasma cholesterol. Respective cholesterol and triglyceride concentrations were determined by enzymatic commercially available reagents (Boehringer Mannheim, catalog No. 237574 and Sera, Miles, catalog No. 6639, respectively). Total plasma apoB concentrations were determined by immunonephelometry. To achieve accurate results in relation to the Centers for Disease Control Standardization Program, obtained values were recalculated, on the basis of an exchange of sera with Dr S. Mar- covina (Northwest Lipid Research Laboratory, Seattle, Wash.), by the regression formula \( y = 0.70x + 143 \) (where \( y \) is the corrected plasma apoB value).

Statistical Analysis

Pearson correlation coefficients were calculated for the relation between crude plasma apoB values and apoB-related cholesterol in VLDL plus LDL. Subsequently, multiple linear regression was used to examine the association between the apoB level and age, sex, BMI, smoking habits, and alcohol intake in comparison with other reports. Based on the results of this analysis, those covariates that significantly influenced apoB level were selected. Given this regression model, predicted apoB levels were calculated and residual values were computed by subtracting each individual's apoB level from the predicted value. These residuals represent the fraction of the apoB level that is not predicted by the selected environmental variables but may represent the genetic contribution to the apoB level. Because these residuals can be positive or negative, the mean apoB level was added back in and this adjusted apoB level was used in further analysis.

Segregation Analysis

To investigate the role of genetic and environmental influences on apoB levels, a series of class D regressive models available in the SAGE program (release 2.2) was used. The models assume that variation among individuals for a quantitative trait is the result of a major gene effect and residual variation that may reflect both familial correlations and individual variation. A "general model" describes the distributions of apoB levels in terms of the independent contribution of a single factor with a major effect, residual familial correlations, and nongenetic effects. This general model emulates from two alleles at a single locus (denoted A and B), resulting in three possible "types" of individuals (AA, AB, and BB), termed "ousiotypes." The mean apoB value associated with each ousiotype is denoted \( \text{AA}_{\text{A}}, \text{AB}_{\text{A}}, \text{BB}_{\text{A}} \) and \( \text{AA}_{\text{B}}, \text{AB}_{\text{B}}, \text{BB}_{\text{B}} \), with a variance \( \sigma^2 \) assumed equal among all three types. The ousiotype effects may be attributable to a genetic or environmental factor. The parameter \( p \) is defined as the frequency of allele A; \( q = 1-p \) equals the frequency of allele B. Founder individuals are assumed to come from a population in Hardy-Weinberg equilibrium. Individuals characterized by the types AA, AB, and BB are assumed to transmit the allele A with probabilities \( \tau_{\text{AA}} \), \( \tau_{\text{AB}} \), and \( \tau_{\text{BB}} \), respectively. These transmission probabilities are used to calculate the probabilities of all three types for individuals whose parents are in the pedigree. In addition, the parameters \( \rho_{m-\text{p}} \), \( \rho_{m-\text{p}} \), \( \rho_{m-\text{p}} \), \( \rho_{m-\text{p}} \), \( \rho_{m-\text{p}} \), and \( \rho_{m-\text{p}} \) denote spouse, parent-offspring, parent-offspring, father-offspring, and sib-sib correlations, respectively. In this setting, the parent-offspring correlation provides an estimate of the polygenic heritability (h), i.e., \( h^2 = 2\rho_{m-\text{p}} ) \), \( \sigma^2 \) is the total variance and \( \sigma^2 \) is the variance conditional on the restricted model (assuming \( \rho_{m-\text{p}} = \rho_{m-\text{p}} \)). Hypotheses were tested by comparing the general model with various submodels in which certain parameters were restricted to specific values. The "major environmental model" restricts \( \tau_{\text{AA}} = \tau_{\text{AB}} = \tau_{\text{BB}} \), \( \rho_{m-\text{p}} = \rho_{m-\text{p}} \), while the recessive model restricts \( \rho_{m-\text{p}} = \rho_{m-\text{p}} \). Submodels containing \( \rho_{m-\text{p}} = \rho_{m-\text{p}} \) and \( \rho_{m-\text{p}} \) equal to zero are equivalent to restricting the "polygenic component" to zero. Restricted models are compared with the general model by using the likelihood ratio test. For nonhierarchical models, AIC...
was used to compare the fit of the models. For a given model, $AIC=-2\ln(L)+2 \cdot r$ (where $r$ is the number of parameters estimated in the model). The model with the lowest AIC is considered the most parsimonious model.34

Segregation analysis was performed on 40 families that included 825 individuals, although 162 individuals had missing data (these primarily included dead family members linking other relatives). In this study, no normalizing transformation was performed because analysis of untransformed data is easier to interpret and can be compared more easily with the published studies. In addition, normalizing transformations of a biologically skewed variable may lead to a large reduction in the power to detect a major gene when one exists.30 Families were selected through two affected probands. Consequently, the lipid inclusion criteria (ie, both plasma cholesterol and triglyceride levels) of probands versus nonprobands showed large differences (Table 1). Therefore, ascertainment correction was undertaken by conditioning the phenotypes of family members on those of the affected primary probands and that of one extra individual with elevated lipid levels per pedigree.33

### Results

#### Study Population

The study included 663 individuals aged between 10 and 88 years. The correlation between crude plasma apoB concentration and apoB-related cholesterol concentration (ie, VLDL plus LDL cholesterol), both for the 40 probands plus 40 additional subjects of each family and for the remaining relatives, is presented in Fig 1. In both groups, a significant correlation was observed ($r=0.83$, $P<.0001$ and $r=0.90$, $P<.0001$, respectively). Characteristics of the study population are presented in Table 1. BMI values varied between 13.6 and 40.9 kg/m²; crude apoB levels, between 60 and 298 mg/dL; and the adjusted apoB levels, between 42.3 and 277.7 mg/dL. Significant differences between probands and family members were seen in mean values of both crude and adjusted apoB levels.

#### Adjustment of the ApoB Level

Nonlipid covariates (ie, age, age², sex, BMI, smoking habits [0=not smoking, 1=one or more cigarettes per day], and alcohol consumption [0=no alcohol consumption, 1=one or more units per day]) were examined in association with plasma apoB level. Table 2 shows the covariates and their regression coefficients used in the adjustment of the apoB levels. Age² and alcohol consumption were excluded from the adjustment procedure because they did not contribute significantly. Although in this study sex was not significantly associated with apoB, it was included in the adjustment procedure to make our results more comparable with other studies. The variables combined explained 35.7% of the variation in apoB levels.

#### Segregation Analysis

In a first segregation analysis on the total study population, the data were consistent with two different models of inheritance: (1) a codominant major gene effect explaining 44% of the variance in apoB levels, with an insignificant polygenetic component of $h²=1\%$, and (2) an environmental model explaining 16% of the apoB level variance, with a polygenic component of $h²=38\%$ (data not shown). This ambiguous result may have been caused by either genetic heterogeneity among these 40 pedigrees, yielding a mixture of different mechanisms influencing apoB levels, or by extreme apoB levels in certain families or particular individuals.

To evaluate the extent to which each family supported one model over the other, the $-2\ln(L)$ scores of both an environmental model ($-2\ln(L|E)$) and a codominant model ($-2\ln(L|C)$) were calculated for each family. The ratio $-2\ln(L|E/L|C)$ measures the extent to which each family favored one or the other model.19,24,40,41 Families

### Table 1. Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Probands (n=80)</th>
<th>Family Members (n=583)</th>
<th>Total (n=663)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.7</td>
<td>3.8</td>
<td>24.4</td>
</tr>
<tr>
<td>Crude apoB, mg/dL</td>
<td>175.8</td>
<td>40.7</td>
<td>127.8</td>
</tr>
<tr>
<td>Adjusted apoB, mg/dL</td>
<td>156.9</td>
<td>43.1</td>
<td>130.7</td>
</tr>
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<td></td>
<td></td>
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</tbody>
</table>

BMI was missing for 6 probands and 8 family members; crude apoB levels were missing for 7 probands and 72 family members; and adjusted apoB levels were missing for 13 probands and 78 family members.
that favored an environmental model would have a $-2\ln(L_E/L_C)$ ratio <0, and families with a $-2\ln(L_E/L_C)$ >0 favored a codominant model. As shown in Fig 2, the distribution of the likelihood ratios was centered around zero, suggesting most families cannot discriminate definitively between these models. The procedure separated 19 families favoring an environmental model and 21 families favoring a codominant model. One family, however, seemed to give an extreme preference to the environmental model ($-2\ln(L_E/L_C)=-13.5$). This particular family (containing 12 subjects in three generations) included the individual with the highest adjusted apoB level and was excluded from further analysis.

The results of a second series of analyses testing the different genetic models on the 39 remaining pedigrees are shown in Table 3. All models assumed a single correlation among parents and offspring ($P_{po}$), which appeared to be equal to the sib-sib correlation ($P_{ss}$). Therefore, all submodels were assumed to have a single correlation for first-degree relatives ($P_{po}=P_{ss}$). The best-fitting one-distribution model (model 4) showed insignificant spouse correlations ($P_{po}=-0.14\pm0.07$) and significant residual familial correlations ($P_{po}=P_{ss}=0.15\pm0.03$). Using this familial correlation, the polygenic heritability was estimated to be $h^2=2P_{po} \cdot \sigma^2/\sigma^2 = 0.29$, which is compatible with published estimates from other studies. The non-Mendelian two-distribution model (model 6) showed a significant improvement of the $-2\ln(L)$, The three-distribution non-Mendelian model (model 7) gave no further improvement of the fit. Inclusion of a two-distribution Mendelian mechanism (model 9) showed a significant improvement of the $-2\ln(L)$ compared with model 4. The two-distribution Mendelian models (models 8 and 9) looked very similar to the two-distribution non-Mendelian models (models 5 and 6), with $P_{sp}=-0.11\pm0.10$ and $P_{po}=P_{ss}=0.22\pm0.05$. Extension of a two-distribution Mendelian model (model 9) to a three-distribution Mendelian model (model 11) revealed a significant improvement of the $-2\ln(L)$. The analysis presented a general model very similar to the best-fitting codominant model (model 11). Although the excluded family had a substantial influence on the $-2\ln(L)$ used to select the most parsimonious model, no major differences were seen in the parameter estimates. Comparison of the $-2\ln(L)$ scores of submodels against the general model (model 12) showed that models 1, 2, 3, 4, 7, 8, and 9 could be strongly rejected ($P<0.001$), while models 5, 6, and 10 could only be weakly rejected ($P<0.05$). Only model 11 could not be rejected. Rejected environmental models 5, 6, and 7 did not contradict the most parsimonious model (model 11), because they showed that variation in apoB levels was strongly explained by a genetic component ($P_{po}=P_{ss}=0.24\pm0.05$ to 0.25$\pm0.05$). Comparison of models 5, 6, and 7 with model 11 demonstrated that the significant familial correlation in apoB levels was absorbed by the inclusion of a codominant mechanism of inheritance. Therefore, the segregation of elevated apoB levels in 39 of the 40 FCH families was best explained as the result of a codominant major gene effect. Based on oustotype variance ($\sigma^2$), the codominant model could roughly account for $[\sigma^2(model 1)-\sigma^2(model 11)]/\sigma^2(model 1)=(821.4-442.6)/821.4=46\%$ of the genetic variance in plasma apoB.

**Table 2.** $\beta$ and SE for the Four Variables Used in a Multiple Linear Regression Model to Adjust the Crude Plasma ApoB Level

<table>
<thead>
<tr>
<th>Variables</th>
<th>$\beta$</th>
<th>SE</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.05</td>
<td>0.09</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>2.73</td>
<td>0.40</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sex, F</td>
<td>-3.27</td>
<td>2.74</td>
<td>0.2334</td>
</tr>
<tr>
<td>Smoking, $\geq$1 cigarette per day</td>
<td>13.55</td>
<td>3.00</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**Discussion**

In the present study, a segregation analysis was performed to examine genetic mechanisms that could explain the familial clustering of elevated apoB levels as observed in 663 individuals from 40 well-defined Dutch families. These families were ascertained through FCH, a common hyperlipidemia associated with premature cardiovascular disease. The observed Mendelian mechanism with a codominant mode of inheritance for the segregation of elevated plasma apoB levels in this study agrees very well with the reported inheritance of apoB levels in other populations containing families of patients with coronary artery disease and families with familial dyslipidemic hypertension. Such families probably also

![Fig 2. Relative likelihood of the environmental model $-2\ln(L_E)$ and the codominant model $-2\ln(L_C)$. The ratio $-2\ln(L_E/L_C)$ measures the extent to which each family supported one model over the other.](image-url)
contain patients who satisfy the criteria for FCH. On the other hand, the found mechanism of inheritance is not in contradiction to the previously reported major gene effect on plasma triglyceride levels,1,2 because VLDL particles, which carry most of the total triglycerides, show a consistent composition of lipids and apoB protein in FCH.8,11,12 Total plasma apoB was determined by immunonephelometry, and obtained values were adjusted for variation due to age, sex, and smoking habits before the segregation analyses.19,26 In the present study, BMI was also shown to be highly correlated with apoB levels.

Because a first segregation analysis could not discriminate conclusively between two models of inheritance, a more detailed analysis was performed on each family. In contrast to 39 other families, 1 family seemed to have an extreme preference for a two-distribution non-Mendelian model. Without the exclusion of this particular family, the segregation analyses yielded results that were not interpretable, ie, several models gave $-2 \ln (L)$ that were lower than the $-2 \ln (L)$ of the general model. This might have led to an incorrect interpretation that one clear segregation pattern does not exist for plasma apoB. Furthermore, the exclusion of this single outlying family in a second segregation analysis influenced only the $-2 \ln (L)$ scores and through these the selection of the most parsimonious model, whereas estimates of modelspecific parameters were remarkably consistent. Therefore, this family can be seen as an indication of rare heterogeneity, also observed in other family samples.23-25

Both our first and second segregation analyses showed a significant familial correlation of $P_{00}=P_{01}=0.24$ (model 7), which appeared not to be influenced by the excluded family. This estimated correlation is comparable to that reported by others (0.13 to 0.47).23,24 Although examined in completely different study populations, most studies reported a codominant model as the most parsimonious fit on their apoB data.20,22,23,25,31 A few studies, however, preferred a dominant or recessive model.19,23,26 The estimated relative frequency of the “low” apoB allele at 66% is in agreement with other reports.20,22,23,25,31 These consistent findings suggest that genetic control of apoB levels is comparable in subjects with different dyslipidemia phenotypes. Some of the remaining differences in the results may be explained by the large differences in ascertainment criteria of the analyzed study populations, since studies ascertained families through probands who suffered from CAD,19,21,25 who were patients of a lipid clinic,22,23 who were submitted to elective coronary angiography,24 or who had a coincident detection of CAD in the family.20,31 This study analyzed its sample after correction on ascertainment, because plasma apoB concentrations strongly correlated with plasma cholesterol and triglyceride levels. Although the influence of ascertainment bias on the segregation analysis is still a subject of debate, unadjusted analyses may indicate the existence of a major gene effect when one is absent or may bias toward a dominant expression when it actually should be a recessive one.42

FCH is caused by hepatic overproduction of VLDL apoB100, subsequently leading to elevated levels of apoB-containing IDL and LDL particles, of which the latter are predominantly smaller and denser compared with normolipidemic subjects.11,14 The finding of a genetic mechanism for elevated apoB levels in this FCH population suggests that there is genetic control of hepatic VLDL apoB production and that additional metabolic processes responsible for diminished clearance of apoB-containing particles, associated with mutations in the lipoprotein lipase gene9,10 and the apoAI/CHI/AIV gene cluster,3,4 are subordinate to this VLDL apoB overproduction. Thus, metabolic processes associated with VLDL apoB overproduction, such as increased delivery of free fatty acids to liver cells45 or dysregulation of the action of microsomal triglyceride transfer protein required for assembly and secretion of hepatic VLDL apoB,46 may have a genetic basis.

Recently, we demonstrated Mendelian inheritance of dense LDL subfraction profiles in these same FCH families.33 It appeared that the LDL subfraction profile was strongly related to the plasma apoB concentration, which raises the question whether the observed inheritance of the LDL subfractions more likely reflects the segregation of a trait in which affected subjects have elevated VLDL apoB overproduction. Thus, metabolic processes associated with VLDL apoB overproduction, such as increased delivery of free fatty acids to liver cells45 or dysregulation of the action of microsomal triglyceride transfer protein required for assembly and secretion of hepatic VLDL apoB,46 may have a genetic basis.

Recently, we demonstrated Mendelian inheritance of dense LDL subfraction profiles in these same FCH families.33 It appeared that the LDL subfraction profile was strongly related to the plasma apoB concentration, which raises the question whether the observed inheritance of the LDL subfractions more likely reflects the segregation of a trait in which affected subjects have elevated concentrations of apoB, as demonstrated in the present study. The few available reports, however, suggest that LDL subfraction profile phenotype and plasma apoB level genotype are separate Mendelian traits in FCH.2,21 Further analysis in which the LDL subfraction profile is considered independent of the genetic influence of apoB may elucidate this complicated issue.
In conclusion, there is a clear familial aggregation of elevated apoB concentrations that significantly correspond to the lipid phenotype in FCH families. Segregation analysis provides evidence that this aggregation is well explained by a single major gene effect with a codominant expression. Using these findings, linkage analysis can be used to explore the molecular defect involved in apoB regulation in FCH families.

Acknowledgments

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