Inheritance of Low Density Lipoprotein Subclass Patterns in Familial Combined Hyperlipidemia

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The inheritance of low density lipoprotein (LDL) subclass patterns was investigated in 234 members of seven large kindreds with familial combined hyperlipidemia (FCHL), a disorder characterized by elevated LDL cholesterol and/or triglyceride and increased coronary disease risk in families. Analysis of LDL subclass patterns by nonequilibrium gradient gel electrophoresis showed a predominance of small, buoyant LDL particles (pattern A) in 71% of the family members and a predominance of small, dense LDL particles (pattern B) in 29% of family members. Based on complex segregation analysis, pattern B appeared to be inherited as an autosomal trait with either a dominant or an additive mode of inheritance and a small, but significant, multifactorial inheritance component. The proposed allele for pattern B was common (frequency = 0.3), and reduced penetrance was observed among men under age 20 and among women under age 50. These results in these FCHL families are consistent with those from a previously reported population-based sample of families, in which pattern B showed an apparent dominant mode of inheritance. In that study, reduced penetrance was observed for men under age 20 and for premenopausal women, but a somewhat lower allele frequency was found for pattern B (0.25). In the FCHL family members, LDL subclass pattern B was associated with significantly increased plasma levels of apolipoprotein B and triglyceride and decreased high density lipoprotein cholesterol. In comparison with a group of controls, the FCHL family members with pattern A had similar mean triglyceride levels, but higher mean apolipoprotein B. Thus, in families with FCHL, a predominance of small, dense LDL particles appears to be inherited as a common, single-gene trait, which is closely associated with the higher plasma triglyceride levels found in these families. The increased plasma apolipoprotein B levels found in FCHL cannot, however, be accounted for by this proposed locus. (Arteriosclerosis 10:520–530, July/August 1990)

Familial combined hyperlipidemia (FCHL) is a disorder characterized by elevations of total plasma cholesterol and/or triglyceride in family members. Affected relatives have variable lipoprotein phenotypes, but familial aggregation of lipid elevations is always present. The primary defect in FCHL appears to be an overproduction of very low density lipoprotein (VLDL) or low density lipoprotein (LDL) apolipoprotein (apo) B, although VLDL particles in FCHL patients appear to be normal in composition. Risk of myocardial infarction (MI) is also increased in families with FCHL. In MI survivors under age 60, FCHL is estimated to be the most common form of hyperlipidemia. Although it was originally proposed that FCHL is a homogeneous single-gene disorder both within and between families, recent evidence suggests that FCHL is actually a heterogeneous condition. In particular, families with members who are heterozygotes for lipoprotein lipase deficiency7 and other families with cholesteryl ester hydrolase deficiency8 satisfy the criteria for FCHL.1

Elevated LDL cholesterol is believed to be a causative factor in the development of atherosclerosis and coronary heart disease (CHD).10-11-13 It is also well known that LDL particles are biochemically heterogeneous, as measured by variation in size and density.14-17 Gradient gel electrophoresis was used to identify two distinct LDL subclass patterns, denoted A and B. Individuals with LDL subclass pattern A have a predominance of large, buoyant LDL particles, while subjects with pattern B have a predominance of small, dense LDL particles. In a recent case-control study, it was demonstrated that LDL subclass pattern B was associated with increased risk of MI.18 In the same study, pattern B was associated with an atherogenic lipoprotein profile, including increases in plasma triglyceride and apo B and decreases in HDL cholesterol and apo A-I. Based on complex segregation analysis of data from a sample of primarily healthy families, it was also shown that LDL subclass patterns are apparently controlled by a single major genetic locus. Based on these studies, LDL subclass pattern B may be a genetic marker for increased risk of CHD. Thus, both FCHL and LDL subclass pattern B are familial conditions, are characterized by atherogenic lipo-
protein profiles, and are associated with increased risk of CHD. It has also been reported that subjects with FCHL have a predominance of LDL particles that are small and dense and have a decreased cholesterol to apo B ratio.20

The primary purpose of the present study is to investigate whether LDL subclass patterns are inherited as a single-gene trait in a sample of families with FCHL by using complex segregation analysis. To determine the relationship of LDL subclass patterns to the hyperlipidemia seen in FCHL, the distribution of plasma lipid and apo B levels is also evaluated among family members with the two LDL subclass patterns in comparison with a group of controls. The results of this study provide further evidence for both genetic control of LDL subclass patterns and a strong association between LDL subclass pattern B and increased plasma triglyceride levels.

Methods

Families

The study was based on data from 78 nuclear families in seven multigenerational kindreds. There were a total of 250 individual family members, 86 of whom married into the kindreds. One of the kindreds (identification number 41) was described in the initial report characterizing FCHL.6 Sixty-five relatives in three generations of this kindred were included in the present analysis. The remaining kindreds were ascertained through three patients who had disease.21 Both maternal and paternal families of the patients were then sampled and found to have FCHL. Since hyperlipidemia was detected in both parents of each of these patients, it was possible that the parents inherited a lipid abnormality from each parent.21 Therefore, the patients, their siblings, and offspring were not included in the analysis. The six parents of the original three patients were each considered to be the probands for the present analysis, and a total of 185 subjects were studied in these six kindreds, each of which was classified as having FCHL. The minimal definition of this condition in small families is the presence of hypercholesterolemia and hypertriglyceridemia in family members and at least one family member with plasma cholesterol above the 95th percentile in the absence of hypertriglyceridemia.8 For the large kindreds in this study, however, isolated hypercholesterolemia and isolated hypertriglyceridemia were common among family members. All family members were Caucasian.

A questionnaire was mailed to all relatives to collect demographic data and information on lifestyle, medications, and medical status. Data regarding pre- or postmenopausal status in women was, however, not available for the present analysis. Blood was drawn from the family members by their local physicians or by a local medical facility, and plasma was obtained by immediate low-speed centrifugation. These samples were shipped to the University of Washington by overnight mail on wet ice. Upon arrival in Seattle, one aliquot of the plasma was used for lipid and apo B determinations,22 and a second aliquot was mailed to Donner Laboratory in Berkeley, California, again by overnight mail on wet ice.

Analysis of Low Density Lipoprotein Subclass Patterns

At Donner Laboratory, multiple, discrete subclasses of LDL particles have been identified in normal subjects and have been characterized by using the techniques of analytic ultracentrifugation, density gradient ultracentrifugation, and gradient gel electrophoresis.15,16,23 Based on the latter technique, two distinct LDL subclass patterns, denoted pattern A and pattern B, have been described.18 Pattern A is characterized by a major peak of large, buoyant LDL subclasses and a minor peak of smaller, denser LDL. In contrast, pattern B has a major peak of small, dense LDL with a skewing of the curve toward the larger particle diameters. Thus, it is the distribution of LDL particles within the LDL density range that distinguishes these patterns. In general, the peak particle diameter from gradient gel electrophoresis for pattern A is greater than 255 Å, and in pattern B is 255 Å or less.

Nondenaturing gradient gel electrophoresis was performed on 2% to 16% polyacrylamide gradient gels by using lipid stain (oil red O) on whole plasma, as previously described.24 The gels were scanned with a Transidyne RFT Scanning Densitometer, and the particle diameters were calculated from calibration curves by using protein standards of known size.18 Figure 1 shows an example of a gradient gel and corresponding scans of the sample lanes. The scans were evaluated by three of the authors (M.A., R.K., and W.F.) to categorize the LDL subclass pattern of each subject without knowledge of the family structure. Of the 250 samples, 16 (6%) were excluded from the data analysis because of lack of consensus in the LDL subclass pattern assignment, resulting in a sample size of 234. A total of 167 (71%) subjects had LDL subclass pattern A, and 56 (24%) had pattern B. In addition, 11 (5%) scans had intermediate LDL subclass patterns, which had some of the characteristics of both LDL subclass patterns A and B.19 To maximize the sample size for the present analysis, these few samples were included in the group of subjects with LDL subclass pattern B. Previous work has demonstrated that this classification had little effect on the results of segregation analysis in primarily healthy families.19

Complex Segregation Analysis

Complex segregation of LDL subclass patterns in the families was investigated by using the mixed model with "pointers."25 The unit of analysis in this model is the nuclear family, and pointers are used to include individuals outside the nuclear family that lead to its ascertainment.26 For the present analysis, the ascertainment probability, 1, was set at 0.01. The mixed model assumes an underlying continuous liability scale for the trait under study, in this case, LDL subclass patterns. The model incorporates a major locus with two alleles, multifactorial inheritance (polygenic or cultural), and environmental effects. These effects are assumed to be additive on the liability scale so that individuals above a threshold value express the trait.27 In addition, liability classes can
Figure 1. An example of a 2% to 16% polyacrylamide gradient gel used to analyze low density lipoprotein (LDL) size and the corresponding optical density scans. The left lane shows standards of known size (STD) used to calibrate sample Lanes 1 to 4. Sample lane scans show peak particle diameters in angstroms within the very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and LDL size ranges. Only the LDL portions of the scans were used to evaluate LDL subclass patterns. Lane 1 demonstrates an intermediate LDL subclass pattern, Lanes 2 and 4 are examples of LDL subclass pattern B (peak particle diameters less than 255 Å with skewing to the left), while Lane 3 is an example of LDL subclass pattern A (predominance of large LDL and skewing to the right).

be defined with the probability of expressing the trait depending on age, gender, or other determinants.28 These classes are incorporated into the analysis, and penetrance values for subjects in each liability class are estimated. The parameters of the mixed model are H, multifactorial (polygenic or cultural) inheritance; D, dominance of the major locus; T, difference in the means between the homozygous genotypes on the liability scale expressed in standard deviation units; and Q, frequency of the susceptibility allele for the trait. Transmission probabilities T1, T2, and T3 are also incorporated into the model, and T2 is used to test for departure from Mendelian inheritance.29 Maximum-likelihood estimation is used, and likelihood ratio tests compare nested models using $\chi^2$ statistics. This analysis was based on likelihood values conditional on the phenotypes of parents and pointers.30 Segregation analysis calculations were performed with the computer program POINTER.28

Plasma Lipid and Apolipoprotein Analyses

The distributions of plasma levels of total cholesterol, triglyceride, LDL cholesterol, high density lipoprotein (HDL) cholesterol, and apo B were evaluated among the adult FCHL family members (age 18 and over). Ten subjects with triglyceride levels over 400 mg/dl were excluded from this part of the analysis, including eight subjects with LDL subclass pattern B, since hypertriglyceridemia may influence the composition of LDL particles.31 A total of 173 FCHL family members, including 119 subjects with LDL subclass pattern A and 54 subjects with LDL subclass pattern B, were included in the analysis. Lipid measurements were performed with standard methodology,32,33 and total plasma apo B was measured by radioimmunoassay.34 To determine the proportion of subjects with hyperlipidemia in the FCHL families associated with LDL subclass pattern phenotypes, the total cholesterol and triglyceride levels in family members were compared with the Lipid Research Clinics’ Visit 1 Prevalence Study 90th percentile values.35 In addition, the proportion of family members with elevated unadjusted apo B levels (above 130 mg/dl) by LDL subclass pattern was considered.

Comparisons of LDL cholesterol, HDL cholesterol, triglyceride, and apo B levels in the family members were performed by using a control group of 464 spouses of patients and spouses of patients’ relatives from 158 families participating in three separate studies of hyperlipidemia and CHD, not including the present family study.22 The patients in these three studies were 1) 67 consecutive patients age 60 or younger seen for coronary angiography at the University Hospital in Seattle between May and August, 1984; 2) 76 patients who participated in a study of the effect of drug treatment on coronary artery disease assessed by angiograms at the same hospital between March 1976 and May 1986; and 3) 15 patients with FCHL selected for large family size. The subjects included in the spouse control group for the present study were all Caucasian and were 18 years old or older. Nine control subjects with plasma triglyceride values over 400 mg/dl were excluded, resulting in a sample size of 455. In addition, three controls had missing data for LDL and HDL cholesterol determinations. The target population for these comparisons is thus members (both relatives and married-in subjects) of families demonstrating familial aggregation of lipid disorders and CHD. The spouses were considered an appropriate control group, since they share environmental and behavioral factors with relatives but do not share genes.
Quantitative comparisons of the lipid and apo B variables among groups were performed, adjusting for the potential confounding effects of age, gender, body mass index (BMI, \(\text{wt} / (\text{ht})^2\)), and smoking (packs per day), by using analysis of covariance.\(^{39}\) Specifically, models with apo B, triglyceride, LDL cholesterol, and HDL cholesterol as the dependent variables and with the covariates and a group variable (coded 0 for control, 1 for FCHL family members) as independent variables were used. Each of the covariates was associated with the dependent variables at \(p\) values of 0.10 and below. Heterogeneity of the regression of covariates between the control and FCHL groups was assessed using interaction terms. None of these terms were significant, with the exception of a group by age interaction term for apo B. The resulting equations were used to calculate values adjusted to those expected for nonsmoking men, age 50, with an average BMI of the control group. Reported \(p\) values comparing the controls and the FCHL family members are based on this analysis of covariance, including a partial \(F\) test for the group effect for apo B incorporating the interaction term. Additional analysis of covariance models were used for comparisons of the means of lipid and apo B variables for the family members with LDL subclass patterns A and B. However, no significant interaction terms were found for these models. Due to skewing of the distribution, triglyceride values were logarithmically transformed for statistical calculations. For ease of interpretation, however, values are reported in antilog units.

To assess the simultaneous associations of the lipid and apo B variables with the LDL subclass patterns among the FCHL family members, unconditional logistic regression analysis was performed.\(^{37}\) That is, LDL subclass pattern (coded 0 for A and 1 for B) was the dependent variable, and a series of lipid and apo B variables were used as independent variables in the models, in addition to the covariates described above. All calculations were performed with the SAS statistical analysis programs.\(^{38,39}\)

**Results**

**Complex Segregation Analysis**

Liability classes were defined for the segregation analysis based on the observed frequency distribution of LDL subclass patterns by gender and age in the 234 family members (Table 1). Overall, LDL subclass pattern B was present in 29% of the study subjects. The prevalence differed by gender and by age, consistent with previously reported results.\(^{18,40}\)

The segregation of LDL subclass patterns in the paternal side of kindred 2100 is shown in Figure 2. Of the two surviving siblings in generation II, one had LDL subclass pattern B. However, two spouses of deceased siblings also had this pattern (ages 69 and 68). Ten (53%) of the 19 individuals in generation III can be seen to have pattern B, and three-generation inheritance can be seen in generations II, III, and IV.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>LDL subclass pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pattern A (%)</td>
</tr>
<tr>
<td>All subjects</td>
<td>167 (71.4)</td>
</tr>
<tr>
<td>Men</td>
<td>76 (66.1)</td>
</tr>
<tr>
<td>Age &lt;20</td>
<td>30 (90.9)</td>
</tr>
<tr>
<td>Age ≥20</td>
<td>46 (56.1)</td>
</tr>
<tr>
<td>Women</td>
<td>91 (76.5)</td>
</tr>
<tr>
<td>Age &lt;50</td>
<td>73 (89.0)</td>
</tr>
<tr>
<td>Age ≥50</td>
<td>18 (48.6)</td>
</tr>
</tbody>
</table>

The values in parentheses are raw percentages.

Table 1. Frequency Distribution of Low Density Lipoprotein Subclass Patterns by Age and Gender

As can be seen, the model of no inheritance (#2) was rejected in comparison with the unrestricted model (#1) \((p<0.001)\). Both the multifactorial inheritance only model (#3) and the major gene only model (#4) were also rejected \((p<0.01)\). Thus, a mixed model, including both a major gene and a multifactorial component, was needed to accurately represent the family data. Although the general mixed model (#5) was rejected at the \(\alpha=0.05\) level in comparison with the unrestricted model (#1), the \(p\) value for the comparison was borderline \((p=0.041)\). The estimate of \(T=0.63\) in the unrestricted model (#1) indicates that strict Mendelian inheritance was not found.

The general mixed model (#5) iterated to a mode of inheritance between additive and dominant \((D=0.74)\) for the major locus, with a small, multifactorial component \((H=0.05)\). When a dominant mode of inheritance was set for the major locus (#6), the result was consistent with the general mixed model (#5). A similar result was seen when an additive mode of inheritance was assumed (#7). A recessive (#8) model was rejected in comparison with the general mixed model (#5).

Based on these likelihood statistics, the general mixed with a partially dominant mode of inheritance \((D=0.74)\) for the major locus provided the best fit to the family data. Both strictly dominant \((D=1.0)\) and strictly additive \((D=0.5)\) models were also consistent with the family data, each with similar likelihood values. The frequency of the proposed allele leading to LDL subclass pattern B \((Q)\) was common in all these models for the FCHL families, approximately 0.3. The estimates of the difference between the homozygous means \((T)\) were also similar for these models, between 2.0 and 2.5 standard deviations.
Figure 2. Four generations of the paternal side of kindred 2100 are shown with the age of each family member given. The solid symbols represent low density lipoprotein (LDL) subclass pattern B, and the striped symbols represent LDL subclass pattern A, as determined by gradient gel electrophoresis. The wave symbols represent samples in which the LDL subclass pattern could not be determined.

Legend
- LDL Subclass Pattern B
- LDL Subclass Pattern A
- Undetermined Pattern
- Not Sampled

on the liability scale. Thus, there was reasonable separation between the two homozygous genotypes. When the mode of inheritance was fixed at strictly dominant (D = 1.0) or additive (D = 0.5), nearly equal fit of the models to the family data was obtained. Therefore, the inheritance of LDL subclass patterns in these families with
FCHL appears to be controlled by a single locus with a significant multifactorial inheritance component.

Penetrance values for the liability classes are shown in Table 4 by genotype for the dominant, general, and additive mixed models (#6, #5, and #7, respectively). For the dominant model, the penetrance for men under age 20 with genotypes BB and AB was 0.17 and increased to 0.82 for men over age 20. The penetrance values varied in a similar way among women under age 50 and those age 50 and over. In all liability classes, the penetrance among AA genotype individuals was zero based on this model. When a general and additive mode of inheritance for the major locus was used, penetrance values again varied by gender and age. For both these models, penetrance was reduced considerably in men under age 20 and in women under age 50 with genotype AB, compared to those with genotype BB. In subjects with genotype AA, the prevalence of LDL subclass pattern B was zero in all liability classes for all models, with the exception of men age 20 and over and women age 50 and over in the additive model. Thus, LDL subclass pattern B apparently is rarely present in the absence of the allele for pattern B.

**Lipid and Apolipoprotein B Comparisons**

The fraction of FCHL family members with hyperlipidemia associated with the LDL subclass patterns is shown in Table 5 based on the Lipid Research Clinics’ 90th percentile age and gender specific values. Among the family members with LDL subclass pattern A, 8.4% had total cholesterol levels above the 90th percentile. In contrast, 27.8% of pattern B family members had hypercholesterolemia, a significant difference in proportions. The difference in the percentage of subjects with hypertriglyceridemia by LDL subclass pattern was even more substantial: 2.5% of pattern A subjects compared to 46.3% of pattern B subjects (p<0.0001). An increased frequency of unadjusted elevated plasma apo B levels was also found among the family members with LDL subclass pattern B: 61% vs. 22% in subjects with pattern A (p<0.001).

Adjusted mean plasma levels of apo B, triglyceride, LDL cholesterol, and HDL cholesterol are compared in the adult FCHL family members and the spouse controls in Figures 3 and 4, and probability values based on analysis of variance are given in Table 6. The mean apo B level was significantly higher among the FCHL family members compared to the spouse controls (mean±SD: 124±29 vs. 101±24, respectively, p=0.025 based on partial F test including the interaction term) (Figure 3). When the family members were stratified by LDL subclass pattern, the subjects with pattern B had a significantly higher mean value than subjects with pattern A (138±31 vs. 117±27, respectively, p<0.001) (Figure 3). In addition, both pattern A and pattern B subjects had a significantly higher mean apo B than the controls (Table 6). For triglyceride, the mean value for the FCHL family members was also significantly higher than the spouse controls (116±74 vs. 90±54, respectively, p<0.001). Again, when the FCHL family members were stratified by LDL subclass pattern, the pattern B subjects had a significantly higher adjusted mean value compared with the pattern A subjects (184±78 vs. 94±49, respectively, p<0.001). However, the mean adjusted triglyceride for pattern A subjects was very similar to the mean for the controls (94±49 vs. 90±54, respectively, p=0.490; Table 6).

As shown in Figure 4 and Table 6, no significant differences were seen for mean LDL cholesterol levels between any of the groups (133±33, 133±32, 139±39 for controls, pattern A, and pattern B subjects, respectively). Mean HDL cholesterol values were also very similar for all the FCHL family members compared to the controls (45.7±14.6 vs. 45.7±14.4, respectively). Pattern B FCHL family members did, however, have a significantly lower HDL cholesterol mean level than pattern A FCHL family members (40.8±14.9 vs. 47.9±13.9, respectively, p<0.001).

The results of the multivariate analysis of the association of lipid and apo B variables with the LDL subclass patterns among the FCHL family members are shown in Table 7 based on logistic regression. All models included the covariates age, gender, BMI, and smoking to control for the possible confounding effects of these variables. Model 1 includes triglyceride, apo B, and HDL cholesterol, but only triglyceride was significantly associated with LDL subclass patterns. The lack of significance for apo B and HDL cholesterol is not surprising, however, due to the strong correlation of these variables with triglyceride (r=0.58, p<0.001 for apo B; r=−0.44, p<0.001 for HDL cholesterol). Thus, there is likely to be considerable multicollinearity in this model. When apo B and HDL cholesterol were individually excluded in other models (not shown), similar coefficients and model x^2 values were found. When triglyceride was excluded, as seen in Model 2, both apo B and HDL cholesterol were both significantly associated with LDL subclass patterns. Thus, the multivariate analysis confirms that LDL subclass patterns, triglyceride, apo B, and HDL cholesterol are closely interrelated variables.

Thus, FCHL family members with LDL subclass pattern B appeared to have a lipid profile characterized by relative increases in apo B and triglyceride and decreased HDL cholesterol in comparison with family members with pattern A. Compared with the control group, apo B values were higher for FCHL family members with both pattern A and pattern B. Triglycerides were only higher for subjects with LDL subclass pattern B, not for those with pattern A, and HDL cholesterol values were similar for both groups.

**Table 2. Observed Segregation Ratios of Low Density Lipoprotein Subclass Patterns in Kindreds with Familial Combined Hyperlipidemia**

<table>
<thead>
<tr>
<th>Mating type</th>
<th>Number of matings</th>
<th>Pattern A</th>
<th>Pattern B</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A×A</td>
<td>21</td>
<td>40 (100%)</td>
<td>0 (0%)</td>
<td>40</td>
</tr>
<tr>
<td>A×B</td>
<td>14</td>
<td>21 (72%)</td>
<td>8 (28%)</td>
<td>29</td>
</tr>
<tr>
<td>B×B</td>
<td>7</td>
<td>6 (50%)</td>
<td>6 (50%)</td>
<td>12</td>
</tr>
</tbody>
</table>

The values in parentheses are raw percentages.

LDL SUBCLASS PATTERNS IN FAMILIAL COMBINED HYPERLIPIDEMIA  Austin et al. 525
Additive mixed model*

Subjects                      | Genotype |  |  |  |  | 
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant mixed model*</td>
<td>BB</td>
<td>AB</td>
<td>AA</td>
<td></td>
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<tr>
<td>Men</td>
<td>0.17</td>
<td>0.17</td>
<td>0.00</td>
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<tr>
<td>Age &lt;20</td>
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<tr>
<td>Women</td>
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<tr>
<td>Age &lt;50</td>
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<td>0.00</td>
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<tr>
<td>General mixed model†</td>
<td>0.94</td>
<td>0.06</td>
<td>0.00</td>
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<td>Men</td>
<td>1.00</td>
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<tr>
<td>Age &lt;50</td>
<td>0.71</td>
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<tr>
<td>Age ≥50</td>
<td>1.00</td>
<td>0.78</td>
<td>0.09</td>
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<tr>
<td>Additive mixed model‡</td>
<td>0.62</td>
<td>0.04</td>
<td>0.00</td>
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<tr>
<td>Men</td>
<td>0.99</td>
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<td>Age &lt;50</td>
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<tr>
<td>Age ≥50</td>
<td>1.00</td>
<td>0.78</td>
<td>0.09</td>
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</tr>
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</table>

*p<0.05, †p<0.01, ‡p<0.001.

Discussion

Complex segregation analysis based on this sample of kindreds with FCHL suggests that the LDL subclass pattern characterized by a predominance of small, dense LDL particles (pattern B) is controlled by a single major genetic locus and a small, but significant, multifactorial inheritance component. That is, a mixed model provided the best explanation for the familial aggregation of LDL subclass patterns. Dominant, partially dominant, and additive modes of inheritance for the major locus were consistent with the family data for LDL subclass pattern B, but a recessive mode of inheritance was rejected (p<0.01). The frequency of the allele leading to LDL subclass pattern B allele was common in these families (Q=0.3), and reduced penetrance for the pattern B allele was observed for men under age 20 and for women under age 50.

In the unrestricted model, in which all parameters are allowed to iterate to the best possible estimates, T2, the parameter used to test for strict Mendelian inheritance, was estimated to be 0.63. This value was significantly different from the expected value of 0.5, although the p value was borderline (p=0.041). This result is not, however, likely to invalidate the major locus model for two reasons. First, if multiple comparisons were taken into account in estimating p values, the hypothesis of T2=0.5 would not be rejected. Second, since FCHL is known to be a complex familial disease, it is possible that more than one major gene influencing LDL subclass patterns is operating in these kindreds.

The results of this study are remarkably similar to those recently reported based on a sample of primarily healthy kindreds, including 61 nuclear families. These families were Caucasian volunteers from a community-based sample. They were not selected (or excluded) based on the presence of cardiovascular disease or lipid disorders. The inheritance of LDL subclass patterns was found to be consistent with the presence of a single major locus alone, also based on complex segregation analysis. A dominant mode of inheritance for pattern B provided the best fit to the family data, but a somewhat lower allele frequency of 0.25 was found for pattern B. Among both sets of families, nearly full penetrance for pattern B was observed only for men age 20 and over and for postmenopausal women.

Although the uniformity of the results in these two studies is striking, the results of complex segregation analysis must be interpreted with caution. Common traits that are not genetically controlled can appear to fit a genetic model well. Since the POINTER program does not include a major environmental model, we were not able to test this hypothesis directly. In addition, ascertainment bias can have a substantial impact on the results of
Table 5. Percent of Familial Combined Hyperlipidemia Family Members with Elevated Total Cholesterol, Triglyceride, and Apolipoprotein B

<table>
<thead>
<tr>
<th>Plasma levels</th>
<th>LDL subclass</th>
<th>All subjects (n=173)</th>
<th>Pattern A (n=119)</th>
<th>Pattern B (n=54)</th>
<th>( \chi^2 ) for pattern A vs. pattern B</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>&gt;90th percentile*</td>
<td>14.5%</td>
<td>8.4%</td>
<td>27.8%</td>
<td>11.68</td>
<td>0.001</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>&gt;90th percentile*</td>
<td>16.2%</td>
<td>2.5%</td>
<td>46.3%</td>
<td>52.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apo B &gt;130 mg/dl</td>
<td>34.1%</td>
<td>21.9%</td>
<td>61.1%</td>
<td>25.48</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

*Based on Lipid Research Clinics' Prevalence Study Visit 1.

Table 6. Probability Values Comparing Means of Control and Familial Combined Hyperlipidemia Family Groups Based on Analysis of Covariance

<table>
<thead>
<tr>
<th>Groups compared</th>
<th>Apo B</th>
<th>TG*</th>
<th>LDL-C</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls vs. all FCHL family members</td>
<td>-0.025</td>
<td>&lt;0.001</td>
<td>0.516</td>
<td>0.866</td>
</tr>
<tr>
<td>FCHL family members: Pattern A vs. Pattern B</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.441</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Controls vs. Pattern A FCHL family members</td>
<td>&lt;0.001</td>
<td>0.490</td>
<td>0.958</td>
<td>0.050</td>
</tr>
<tr>
<td>Controls vs. Pattern B FCHL family members</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.234</td>
<td>0.027</td>
</tr>
</tbody>
</table>

*Triglyceride based on natural logarithm transformation. 
Apo = apolipoprotein, LDL-C = low density lipoprotein cholesterol, HDL-C = high density lipoprotein cholesterol, FCHL = familial combined hyperlipidemia.

Table 7. Logistic Regression Equations Associating Low Density Lipoprotein Subclass Patterns and Lipid and Apolipoprotein B Plasma Levels

<table>
<thead>
<tr>
<th>Variables</th>
<th>Regression coefficients (n=170*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.003</td>
</tr>
<tr>
<td>Gender (0=female, 1=male)</td>
<td>0.752</td>
</tr>
<tr>
<td>Body mass index (wt [kg]/ht[m]^2)</td>
<td>0.058</td>
</tr>
<tr>
<td>Smoking (packs/day)</td>
<td>-0.734</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>4.221†</td>
</tr>
<tr>
<td>Apo B (mg/dl)</td>
<td>0.008</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>-0.018</td>
</tr>
<tr>
<td>Model ( \chi^2 )</td>
<td>79.59</td>
</tr>
<tr>
<td>Degrees of freedom</td>
<td>7</td>
</tr>
<tr>
<td>( p ) value</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*There were no data for smoking in three subjects. †Based on natural logarithm units. 
‡\( p < 0.001 \), §\( p < 0.01 \). 
- Indicates that this variable was not included in the model.

segregation analysis. Although the POINTER program includes the ascertainment probability, \( \Pi \), this may not correct sufficiently for potential bias. However, the recruitment procedures for the two sets of families were completely different. The primarily healthy families are fairly representative of a community-based population, while the FCHL kindreds were highly selected based on characteristic lipid disorders and family size. Thus, it seems unlikely that the apparent presence of a major genetic locus with a dominant mode of inheritance is due to ascertainment bias. The multifactorial component in the FCHL kindreds could reflect additional genetic influences on LDL heterogeneity resulting from the selection procedure for FCHL.

Possible genetic control of LDL heterogeneity was first reported in 1975\(^4\) based on molecular weight mea-
The probability values for comparisons of mean values between family members combined, and in the FCHL family members with groups are given in Table 6.

In all familial combined hyperlipidemia (FCHL) family members with low density lipoprotein (LDL) subclass pattern A and pattern B, plasma apolipoprotein (apo) B and triglyceride levels were very similar to that of the control group (94 vs. 90 mg/dl, respectively), while the mean level for pattern B family members was considerably higher (184 mg/dl). Thus, the characteristic hypertriglyceridemia in the FCHL families may correspond to the increased triglyceride levels associated with LDL subclass pattern B.

However, the mechanism underlying this association is not understood, and it is conceivable that more than one mechanism is actually operating. That is, the proposed gene for pattern B may result in increased triglyceride levels, but it is also possible that small, dense LDL may be a consequence of hypertriglyceridemia, which is, in turn, due to other genetic or environmental causes. If so, it is feasible that both mechanisms are operating in the FCHL families. This possibility is consistent with the penetrance values based on the additive model. As shown in Table 4, genotype AA men age 20 and over and genotype AA women age 50 and over had probabilities of 0.05 and 0.09, respectively, for expressing LDL subclass pattern B in the absence of the proposed pattern B gene. Thus, some individuals in these families may be “phenocopies” in that they demonstrate the LDL subclass pattern B phenotype, but it results from a mechanism not associated with the proposed pattern B allele.

In contrast to the triglyceride results, the mean level of plasma apo B was significantly lower in the controls than in the FCHL family members with LDL subclass pattern A, which in turn was significantly lower than the mean level for FCHL family members with LDL subclass pattern B. Therefore, LDL subclass patterns were associated with significant differences in apo B levels, but this difference did not explain the increased plasma apo B levels in the FCHL families. Since elevated apo B is a characteristic feature of FCHL as a disorder, it is tempting to speculate that another major gene is responsible for this elevation, and that the proposed gene for LDL subclass pattern also contributes to the variation in apo B levels. Two recent studies using complex segregation analysis have suggested that a major genetic locus determines plasma apo B levels.
apo B levels. Alternatively, the difference in apo B levels would be seen if penetrance values for the proposed LDL subclass pattern B allele were underestimated based on the complex segregation analysis. How these possible mechanisms relate to hyperapobetalipoproteinemia, a condition in which a subset of coronary artery disease patients were found to have elevated apo B levels but normal LDL cholesterol levels, is not known.

Consistent with previous studies, mean HDL cholesterol levels were significantly lower in study subjects with pattern B compared with those with pattern A. HDL cholesterol levels were, however, not lower in the FCHL family members compared to the spouse controls. A previous report showed that the apo A-I/apo A-II ratio was reduced in FCHL, but only a borderline difference in HDL cholesterol level was found between patients and matched controls.

The apparent major locus inheritance of LDL subclass patterns in these families with FCHL and the association with small, dense LDL with increased triglyceride and apo B levels provides further evidence that LDL subclasses are significantly involved in genetically influenced mechanisms leading to the development of CHD. Identifying and mapping the proposed gene for LDL subclass patterns and elucidating the mechanisms responsible for the associated variations in lipid and apolipoprotein levels, will undoubtedly further the understanding of genetic susceptibility to atherosclerosis.

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Index Terms: low density lipoproteins • hyperlipidemia • triglycerides • apolipoproteins • genetics
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