Linkage of a Candidate Gene Locus to Familial Combined Hyperlipidemia

Lecithin:Cholesterol Acyltransferase on 16q

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Abstract—Familial combined hyperlipidemia (FCHL) is a common lipid disorder characterized by elevated levels of plasma cholesterol and triglycerides that is present in 10% to 20% of patients with premature coronary artery disease. To study the pathophysiological basis and genetics of FCHL, we previously reported recruitment of 18 large families. We now report linkage studies of 14 candidate genes selected for their potential involvement in the aspects of lipid and lipoprotein metabolism that are altered in FCHL. We used highly polymorphic markers linked to the candidate genes, and these markers were analyzed using several complementary, nonparametric statistical allele-sharing linkage methodologies. This current sample has been extended over the one in which we identified an association with the apolipoprotein (apo) AI-CIII-AIV gene cluster. We observed evidence for linkage of this region and FCHL (P<0.001), providing additional support for its involvement in FCHL. We also identified a new locus showing significant evidence of linkage to the disorder: the lecithin:cholesterol acyltransferase (LCAT) locus (P<0.0006) on chromosome 16. In addition, analysis of the manganese superoxide dismutase locus on chromosome 6 revealed a suggestive linkage result in this sample (P<0.006). Quantitative traits related to FCHL also provided some evidence of linkage to these regions. No evidence of linkage to the lipoprotein lipase gene, the microsomal triglyceride transfer protein gene, or several other genes involved in lipid metabolism was observed. The data suggest that the lecithin:cholesterol acyltransferase and apolipoprotein AI-CIII-AIV loci may act as modifying genes contributing to the expression of FCHL. (Arterioscler Thromb Vasc Biol. 1999;19:2730-2736.)

Key Words: familial combined hyperlipidemia • lipid metabolism • genetics • lecithin:cholesterol acyltransferase

Familial combined hyperlipidemia (FCHL) is the most common genetic lipid disorder observed in patients with coronary artery disease and their relatives, with a frequency of ≈1% in the US white and European populations. FCHL occurs in 10% to 20% of patients with premature coronary artery disease. The disorder is characterized by elevated concentrations of plasma triglycerides and/or plasma cholesterol in probands and their affected relatives. Moreover, affected relatives exhibit multiple lipoprotein phenotypes, a predisposition to premature coronary artery disease, and a vertical pattern of transmission of hyperlipidemia. Early studies suggested that the familial aggregation of FCHL was consistent with a dominant, mendelian trait, but subsequent studies have suggested a more complex mode of inheritance. One characteristic of FCHL is the variability in lipoprotein profile, referred to as multiple lipoprotein phenotype, seen in affected relatives; thus, affected individuals may exhibit a Fredrickson type IIa, IIb, or IV hyperlipidemia. The fully expressed FCHL phenotype involves elevated levels of LDL, IDL, and VLDL. These particles have a precursor-product relationship, as the liver secretes VLDL, which then undergoes lipolysis to IDL and subsequently to LDL. The principal structural protein of these particles is apolipoprotein B (apoB), an integral membrane protein required for VLDL packaging. Elevated levels of apoB are a useful indicator of FCHL. Other traits frequently associated with FCHL include small, dense LDL particles, low HDL levels, insulin resistance, and elevated postprandial levels of free fatty acids.

The molecular mechanisms contributing to FCHL are largely unknown. Because hypertriglyceridemia is often the earliest manifestation of FCHL, it has been proposed that the primary defect(s) involves triglyceride metabolism, with secondary effects on cholesterol metabolism. Physiologi-
cal studies have suggested that patients with FCHL have increased secretion of VLDL triglycerides and associated apoB, raising the possibility that this is the primary metabolic defect. There is also substantial evidence that the FCHL phenotype results in part from defects in lipoprotein catabolism. In particular, about one third of FCHL patients exhibit decreased levels of lipoprotein lipase (LPL), the enzyme primarily responsible for lipolysis of chylomicrons and VLDL, although only a small fraction of this decrease in LPL levels appears to be due to mutations of the LPL gene. Furthermore, there is impaired removal of chylomicron remnants after an oral fat load, and this is associated with increased plasma apoCIII concentrations. We and others have observed significant evidence for linkage (and association) between specific alleles of the apoAI-CIII-AIV gene cluster and FCHL. A compelling hypothesis for FCHL is that it involves 1 or more primary gene defects affecting VLDL synthesis. These defects then act in combination with various secondary variations, most likely those affecting lipoprotein catabolism, such as the decrease in LPL enzyme activity or variations at the apoAI-CIII-AIV gene cluster.

A relatively small number of candidate genes for FCHL, such as apoB, LPL, and the apoAI-CIII-AIV cluster, have been previously examined, mostly by association studies. The results suggest that apoB gene variations do not contribute significantly to the trait and that the LPL and apoAI-CIII-AIV genes are likely to play a role as secondary or modifier genes. To further identify potential primary and secondary defects contributing to the expression of FCHL, we studied 14 separate candidate gene loci in 18 well-characterized FCHL families. Linkage results reveal a new locus that appears to contribute to the expression of this disorder, the lecithin:cholesterol acyltransferase (LCAT) gene locus on chromosome 16.

Methods

Ascertainment of FCHL Study Population

Eighteen unrelated FCHL probands were recruited through the Lipid Clinic of the Utrecht University Hospital. The probands met the following minimum criteria: (1) a primary hyperlipidemia with varying phenotypic expression: plasma cholesterol >250 mg/dL, plasma triglycerides >200 mg/dL, and/or apoB >75th percentile matched for age and sex; (2) at least 1 first-degree relative with a different hyperlipidemic phenotype; (3) a positive family history of premature coronary heart disease defined as myocardial infarction or cardiovascular disease before 60 years of age; and (4) absence of xanthomas. Exclusion criteria included diabetes, obesity, familial hypercholesterolemia (absence of tendon xanthomas), or type III hyperlipidemia (absence of apoE2/E2). All subjects gave informed consent, and the Human Investigation Review Committee of the University Hospital, Utrecht, approved the study protocol. Families were ascertained as previously described. The clinical and biochemical characteristics of the study population (n=481), which comprised 18 probands, their hyperlipidemic and normolipidemic relatives, and spouses, are summarized in Table 1. Over 95% of the relatives and spouses of probands were collected without regard to their FCHL status. In the present study, DNA samples from 481 subjects of the original data set (n=582) were available for molecular and linkage analysis. Hyperlipidemic family members (n=151) were assigned the FCHL phenotype based on plasma cholesterol >250 mg/dL and/or plasma triglycerides >200 mg/dL and/or apoB >75th percentile matched for age and sex. Additionally, normolipidemic relatives were younger than hyperlipidemic relatives and spouses. It is possible that a subset of these normolipidemic individuals have yet to express the FCHL phenotype. As previously reported, hyperlipidemic family members had higher plasma cholesterol, triglycerides, LDL cholesterol, and apoB concentrations than did normolipidemic relatives and spouses.

Genetic Markers for Candidate Genes

Genetic markers appropriate for testing specific candidate loci have been previously reported for a number of the genes studied herein. These include the apoAI-CIII-AIV gene cluster, apoAI, apoB, apoE, carboxyl ester lipase (CEL), cholesteryl ester transfer protein (CETP), hepatic lipase (HL), LCAT, LDL receptor (LDLR), LPL, manganese superoxide dismutase (MnSOD), microsomal triglyceride transfer protein (MTP), peroxisome proliferator-activated receptor (PPAR), and the VLDL receptor (VLDLR) (Table 2). Four candidate loci (CEL, CETP, HL, and LCAT) had not previously been mapped in detail; in these cases, polymorphic genetic markers were selected by employing a radiation hybrid mapping strategy. The genetic markers were then genotyped in 481 individuals of the 18 FCHL kindreds. DNA was isolated from EDTA-augmented blood by following standard procedures, and polymerase chain reaction amplification was conducted according to recommended protocols. ApoE genotypes were determined as described previously.

Linkage Methods

To assess the cosegregation of the FCHL phenotypes with genetic markers at the 14 candidate loci, we used several nonparametric linkage tests, ie, tests for which the mode of inheritance of the disease does not have to be hypothesized. At each candidate gene locus, the markers were tested for linkage individually. Initially, this was done using only affected sibling pairs. Positive linkage was followed by a linkage analysis of the data from all available sibling pairs in the sample. When significant evidence of linkage was observed for the individual marker, multiple markers in the same chromosomal region were typed and combined into haplotypes to maximize the linkage information.

The advantage of analyzing only affected sibling pairs for a disease with complex inheritance, such as FCHL, is that each analyzed individual is assumed to have 1 or more of the disease genes, and the results are not confounded by reduced penetrance. However, because multiple genes may contribute to a complex disorder such as FCHL, different combinations of such genes may

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**TABLE 1. Clinical Characteristics of the FCHL Families**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hyperlipidemic Individuals*</th>
<th>Normolipidemic Individuals</th>
<th>Spouse Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>151</td>
<td>176</td>
<td>154</td>
</tr>
<tr>
<td>Age, y</td>
<td>47±15</td>
<td>31±13</td>
<td>49±16</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>281±72</td>
<td>190±32</td>
<td>220±41</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dL</td>
<td>183±46</td>
<td>120±29</td>
<td>144±38</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>210±107</td>
<td>112±47</td>
<td>142±78</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>143±27</td>
<td>91±20</td>
<td>110±27</td>
</tr>
</tbody>
</table>

*The hyperlipidemic group includes the 18 probands and 133 hyperlipidemic relatives (n=151).
lead to clinical disease. This reduces the power of any linkage analysis, since in such a case not all affected pairs will demonstrate allele sharing at any given disease gene locus. Nevertheless, if any given locus contributes to the disease in a large proportion of the cases, affected sib pairs will share that gene in a greater proportion than expected by chance. Any 2 random sib pairs are expected to share a given allele 50% of the time. If there is linkage of the disease to a specific locus, then affected sib pairs are expected to exhibit a mean allele sharing significantly >0.5. We calculated the mean allele sharing at each locus of interest and tested each for a significant difference from the expected value of 0.5 by using the SIBPAL subroutine of the SAGE package.

When a candidate gene showed evidence for linkage, we extended the analyses to include 2 additional groups of sib pairs, 1 in which both sibs were unaffected (clinically concordant unaffected sibling pairs) and another in which 1 was affected and the other was not (clinically discordant sibling pairs). At linked markers, both clinically concordant affected and clinically concordant unaffected sib pairs are expected to demonstrate an increased sharing of marker alleles identical-by-descent, while clinically discordant pairs should exhibit decreased sharing below the expected value of 0.5. Clinically concordant unaffected and clinically discordant sib pairs may provide weaker evidence for linkage than affected sib pairs, as some unaffected siblings may carry the disease gene but not express it owing to the lack of other genetic and/or environmental factors that contribute to the development of the disease (ie, reduced penetrance).

Haplotype information can improve the significance level of a true linkage result by increasing the informativeness over that of single markers. Often, haplotype analysis allows the number of alleles shared identical-by-descent at a given marker to be assessed unambiguously. Because the result for $L_{\text{CAT}}$ was new and significant by our criteria, we typed an additional 3 markers and tested the haplotype for linkage to $F_{\text{CHL}}$. To incorporate information from all pairs, we used the Haseman-Elston linear regression method as programmed in the SIBPAL subroutine of SAGE. For each sibling pair, the squared difference in the disease status (affected pairs are coded as 1 and unaffected pairs are coded as zero) is regressed against their estimated proportion of haplotypes shared identical-by-descent. A significant negative regression, representing a greater proportion of shared haplotypes for those who are clinically concordant, is taken as evidence for linkage.

In addition to $F_{\text{CHL}}$, 4 related quantitative traits were assessed for linkage to the 14 candidate genes. The squared trait differences between sib pairs for levels of total plasma triglyceride, cholesterol, apoB, and apoCIII were regressed against the number of marker alleles that they share identical-by-descent for each locus. A significantly negative regression line was taken as evidence for linkage. We performed all quantitative analyses initially on the untransformed data; logarithmically transformed data were analyzed when the quantitative trait distribution was nonnormal. Because only candidate genes were tested in these analyses, a significance level of 0.001 was chosen as the criterion for indicating evidence for linkage.

### Results

Fourteen candidate loci were tested for linkage to $F_{\text{CHL}}$ in 18 $F_{\text{CHL}}$ families consisting of 481 individuals. The genes selected have been implicated in VLDL synthesis and secretion (apoB, MTP), lipolysis (CEL, HL, LPL), lipoprotein processing and remodeling (apoAI-CIII-AIV, CETP, LPL, HL, LCAT), HDL metabolism (apoAI, HL, LCAT, CETP, LPL), lipoprotein removal (apoE, LDLR, VLDLR), and cellular metabolism (PPAR)$\alpha$, MnSOD). Highly informative genetic markers (Table 1) either at or near the candidate loci were chosen on the basis of previous studies or after physical mapping of the candidate genes by radiation hybrid analysis (see Methods). By using a nonparametric sib-pair linkage methodology, 11 of the candidate gene loci did not exhibit significant evidence of linkage to the qualitative $F_{\text{CHL}}$ trait ($P > 0.05$, Table 3). Although not meeting the formal statistical threshold of $P < 0.001$, the MnSOD locus on chromosome 6 did yield suggestive evidence of linkage ($P < 0.006$), with a mean allele sharing of 0.57 (data not shown). Two candidate loci, the apoAI-CIII-AIV locus (chromosome 11) and the LCAT locus (chromosome 16), exhibited significant evidence of linkage (Table 3). Mean allele sharing in affected sibling pairs at the markers linked to apoCIII and LCAT was 0.62 and 0.59, respectively, signifi-

### Table 1. Candidate Genes and Polymorphic Markers Tested for Linkage With $F_{\text{CHL}}$

<table>
<thead>
<tr>
<th>Candidate Gene</th>
<th>Marker(s)</th>
<th>Genetic Distance, cm</th>
<th>Heterozygosity</th>
<th>Chromosome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoAI-CIII-AIV</td>
<td>HC3</td>
<td>0*</td>
<td>0.95</td>
<td>11</td>
<td>39</td>
</tr>
<tr>
<td>ApoAI</td>
<td>All</td>
<td>0*</td>
<td>0.76</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>ApoB</td>
<td>D2S1360</td>
<td>2</td>
<td>0.82</td>
<td>2</td>
<td>41</td>
</tr>
<tr>
<td>ApoE</td>
<td>E3/E4/E5</td>
<td>0*</td>
<td>0.43</td>
<td>19</td>
<td>41</td>
</tr>
<tr>
<td>CEL</td>
<td>D9S1838</td>
<td>1</td>
<td>0.89</td>
<td>9</td>
<td>42</td>
</tr>
<tr>
<td>CETP</td>
<td>D16S514</td>
<td>8</td>
<td>0.82</td>
<td>16</td>
<td>42</td>
</tr>
<tr>
<td>HL</td>
<td>D16S148</td>
<td>3</td>
<td>0.55</td>
<td>15</td>
<td>43</td>
</tr>
<tr>
<td>LCAT</td>
<td>D16S496</td>
<td>3</td>
<td>0.74</td>
<td>16</td>
<td>42</td>
</tr>
<tr>
<td>LDLR</td>
<td>LDLR</td>
<td>0*</td>
<td>0.48</td>
<td>19</td>
<td>44</td>
</tr>
<tr>
<td>LPL</td>
<td>LPL3*</td>
<td>0*</td>
<td>0.83</td>
<td>8</td>
<td>45</td>
</tr>
<tr>
<td>MnSOD</td>
<td>D6S1008</td>
<td>1</td>
<td>0.77</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td>MTP</td>
<td>MTP</td>
<td>0*</td>
<td>0.81</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td>PPAR$\alpha$</td>
<td>D22S275</td>
<td>3</td>
<td>0.82</td>
<td>22</td>
<td>42</td>
</tr>
<tr>
<td>VLDLR</td>
<td>VLDLR</td>
<td>0*</td>
<td>0.67</td>
<td>9</td>
<td>47</td>
</tr>
</tbody>
</table>

**Markers for CEL, CETP, LCAT, and MnSOD were ascertained through radiation hybrid mapping (42.48–50).** Previously reported microsatellites were used for the remainder of the candidate genes. Estimated genetic distances in centimorgans (cM) between polymorphic microsatellite markers and candidate genes are listed.

*Marker is located intragenic to the candidate gene.
TABLE 3. Nonparameteric Sib-Pair Linkage Analyses for the Qualitative FCHL Trait: Proportions of Alleles Shared in Affected Sib Pairs

<table>
<thead>
<tr>
<th>Candidate Gene</th>
<th>Number of Affected Sib Pairs</th>
<th>Mean Allele Sharing P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoAI</td>
<td>112</td>
<td>0.510</td>
</tr>
<tr>
<td>ApoB</td>
<td>56</td>
<td>0.530</td>
</tr>
<tr>
<td>ApoCIII</td>
<td>64</td>
<td>0.620</td>
</tr>
<tr>
<td>ApoE</td>
<td>158</td>
<td>0.530</td>
</tr>
<tr>
<td>CEL</td>
<td>95</td>
<td>0.460</td>
</tr>
<tr>
<td>CETP</td>
<td>114</td>
<td>0.510</td>
</tr>
<tr>
<td>HL</td>
<td>104</td>
<td>0.510</td>
</tr>
<tr>
<td>LCAT</td>
<td>93</td>
<td>0.594</td>
</tr>
<tr>
<td>LDLR</td>
<td>100</td>
<td>0.518</td>
</tr>
<tr>
<td>LPL</td>
<td>120</td>
<td>0.500</td>
</tr>
<tr>
<td>MnSOD</td>
<td>83</td>
<td>0.568</td>
</tr>
<tr>
<td>MTP</td>
<td>113</td>
<td>0.506</td>
</tr>
<tr>
<td>PPARα</td>
<td>102</td>
<td>0.546</td>
</tr>
<tr>
<td>VLDLR</td>
<td>71</td>
<td>0.532</td>
</tr>
</tbody>
</table>

In the analysis, the presence of FCHL in a family member was defined as plasma cholesterol levels >250 mg/dL and/or plasma triglyceride levels >200 mg/dL and/or apoB >75th percentile.

The molecular and genetic mechanisms contributing to the pathogenesis and expression of FCHL have remained largely unknown since the original delineation of the disorder by Goldstein and colleagues. The analyses presented herein have revealed a new locus showing significant evidence of linkage to FCHL: the LCAT locus on chromosome 16. In addition, the previously reported linkage of the apoAI-CIII-AIV gene cluster with FCHL was supported in an extended sample by using a highly polymorphic genetic marker. The MnSOD locus on chromosome 6 gave possible evidence for linkage to FCHL as well. Several analyses of the data provide linkage evidence for the LCAT locus: first, an increased allele sharing in affected sib pairs; second, a pattern of decreased sharing in discordant sib pairs and increased sharing in unaffected sib pairs expected with linkage; and third, additional support when additional markers and sibling pairs were included. In addition, consistent with the findings of several other studies, our data provide no support for linkage to 5 different loci, the apoAI, apoB, HL, LDLR, and LPL loci on chromosomes 1, 2, 15, 19, and 8, respectively. Furthermore, the MTP, VLDLR, and CETP loci on chromosomes 4, 9, and 16, respectively, do not appear to play an important role in the expression of FCHL in this population.

Recent findings of the FCHL-associated traits of triglycerides, total cholesterol, apoB, and apoCII levels for the LCAT, apoAI-CIII-AIV, and MnSOD chromosomal regions. Two of these markers demonstrated possible linkage to the constellation of FCHL-related traits: the LCAT locus on chromosome 16 was linked to the traits of cholesterol (P < 0.03) and apoCIII levels (P < 0.03), and the MnSOD locus on chromosome 6 was linked to the traits of cholesterol (P < 0.02), apoB levels (P < 0.02), and apoCIII levels (P < 0.03).

To further characterize the LCAT region, additional markers were typed, D16S514, D16S400, and D16S408, spanning, in addition to the original marker, a 12-cM distance. These markers were combined into a haplotype, thus allowing assessment of their inheritance as a group. As shown in the Figure, the allele sharing for the haplotype exhibited a pattern of excess sharing for both affected and unaffected discordant pairs as well as reduced sharing in clinically discordant pairs, providing additional support for linkage of the LCAT gene region to FCHL. Discordant disease status (1 affected and 1 unaffected) in the sib pair should result in decreased allele sharing, while the same disease status (either both affected or both unaffected) should result in increased sharing. The proportion of haplotypes shared identical-by-descent by affected pairs was 0.60, and the linear regression linkage analysis yielded a P value of 0.002.

**Discussion**

The LCAT gene region to FCHL. Discordant disease status (1 affected and 1 unaffected) in the sib pair should result in decreased allele sharing, while the same disease status (either both affected or both unaffected) should result in increased sharing. The proportion of haplotypes shared identical-by-descent by affected pairs was 0.60, and the linear regression linkage analysis yielded a P value of 0.002.

**Lecithin:Cholesterol Acyltransferase**

This enzyme is synthesized in the liver and circulates in the plasma as a component of HDL particles. The LCAT/CETP locus (the 2 genes are roughly 12 cM apart on chromosome 16) was shown previously to exhibit significant linkage with LDL particle size.27,58,65 Our data suggest that LCAT rather than CETP is the primary locus responsible for the observed linkage, based on the markers spanning the region. In addition, there is biological evidence supporting such a conclusion. Subjects with severe combined hyperlipidemia show significantly increased cholesterol ester mass transfer from HDL to VLDL but have reduced or even absent net mass cholesterol ester transfer from HDL to LDL.35 This phenomenon, which potentially contributes to small, dense LDL particle formation, is caused by the low affinity of CETP protein for the LDL from combined hyperlipidemia...
subjects as well as increased concentrations of the most active cholesterol ester acceptor, VLDL. Guerin et al.\textsuperscript{66} concluded that the biochemical characteristics of HDL and VLDL lipoproteins in combined-hyperlipidemia subjects were sufficient explanation for the altered composition rather than the plasma protein concentration of CETP itself. Several lines of biological evidence support this linkage as well. Transgenic rabbits overexpressing LCAT exhibit elevated levels of VLDL and LDL, similar to the phenotype in FCHL.\textsuperscript{67} In addition, fish-eye disease, a familial syndrome of LCAT deficiency, results in increased VLDL levels and hypertriglyceridemia, among other phenotypic abnormalities.\textsuperscript{49} Thus, LCAT may act as a modifier of the FCHL phenotype. Alternatively, a gene closely linked to LCAT must be considered. Whether the LCAT gene is in fact responsible for the linkage requires further testing in population or family-based association (linkage disequilibrium) studies.

**Apolipoprotein AI-CIII-AIV**

Previous association and linkage studies have implicated the apoAI-CIII-AIV gene cluster in FCHL.\textsuperscript{32–34} Recently, we carried out an association study confirming the involvement of the apoAI-CIII-AIV gene cluster in FCHL.\textsuperscript{32} Three restriction fragment length polymorphisms revealed that a minor allele was associated with elevated plasma cholesterol, triglycerides, LDL cholesterol, apoB, and apoCIII levels in FCHL probands and hyperlipidemic relatives versus controls.\textsuperscript{31} In addition, nonparametric sib-pair linkage analysis revealed significant evidence of linkage between these restriction fragment length polymorphisms in the gene cluster and the FCHL phenotype.\textsuperscript{32} Moreover, demonstration of 2 different susceptibility haplotypes in the gene cluster revealed a paradigm of complex genetic contribution to FCHL.\textsuperscript{32} A similar pattern was observed in a recent study of Finnish FCHL families, although the results did not attain statistical significance in that sample.\textsuperscript{68} The data reported here continue to support the previous linkage finding in our sample by using a more informative genetic marker (a tetranucleotide repeat within the apoCIII gene). Although there are fewer sib pairs available for analysis in this study compared with our previous study,\textsuperscript{52} the stronger linkage result with the gene cluster is likely due to the dramatic increase in the heterozygosity index of the microsatellite marker compared with the restriction fragment length polymorphism haplotypes (0.95 and 0.41, respectively) described previously.\textsuperscript{15} This finding is complemented by biochemical studies. Expression of the apoCIII gene has been shown to be a determinant of plasma VLDL triglyceride levels in mice.\textsuperscript{69} In humans, the apoCIII gene affects plasma apoCIII concentrations, and the elevated apoCIII concentrations in FCHL subjects predict impaired postprandial lipemia.\textsuperscript{31} Thus, the involvement of the apoCIII gene in FCHL expression is not only evident from the linkage results but biologically plausible as well.\textsuperscript{15}

**Manganese Superoxide Dismutase**

Although the results of our candidate gene analysis with markers linked to the MnSOD gene did not reach the criterion set for linkage, positive linkage results with related phenotypes in other samples suggest that this gene locus may have a role in FCHL. Previously, we reported significant linkage of the MnSOD locus with LDL particle size in a set of families enriched for coronary artery disease but without marked hyperlipidemia.\textsuperscript{98} We have also observed linkage of LDL particle size to this locus in FCHL families\textsuperscript{15} and in a sample of subjects undergoing dietary intervention.\textsuperscript{98} In the present study, the MnSOD gene was examined because the small, dense LDL trait is commonly associated with FCHL, and this gene has been correspondingly linked to LDL particle size.\textsuperscript{12,14,15} The mechanisms by which MnSOD, a mitochondrial enzyme responsible for the dismutation of superoxides generated as a by-product of cellular respiration, may influence lipid metabolism are yet unclear. Genetic inactivation of the mitochondrial form of superoxide dismutase in mice results in abundant hepatic lipid accumulation, diluted cardiomyopathy, and early neonatal death.\textsuperscript{70} MnSOD has been shown to influence cellular responses to tumor necrosis factor-\(\alpha\), which is a potent modulator of LPL expression.\textsuperscript{64} Reduced LPL activity is a frequent finding in FCHL patients.\textsuperscript{4,72} It is also conceivable that an increased incorporation of oxidized lipids, which accumulate intracellularly as a consequence of reduced clearance of superoxides by MnSOD, may affect the catabolism of VLDL.

**Evidence for an Oligogenic Model**

Our current understanding of FCHL is that it represents a complex metabolic syndrome, characterized by hepatic and possibly intestinal hypersecretion of lipoproteins, which in turn generates increased flux through the lipolytic cascade and receptor-mediated uptake routes. Therefore, each of the pathways involved is vulnerable to gene modifications that reduce the maximal functional capacity of the pathway under conditions of high lipoprotein flux caused by hepatic hypersecretion. In our view, major gene effects are expected to influence the regulation of hepatic lipoprotein secretion or the level of substrate supply to lipoprotein-secreting organs. Such major gene effects are expected to be present in all or the vast majority of FCHL subjects. Modifier gene effects can, at least in theory, be expected at the level of lipoprotein secretion, lipolysis, processing, remodeling, or cellular uptake, provided that the gene(s) involved plays a functional role in lipoprotein metabolism in FCHL. It is unlikely that LCAT is the primary determinant of FCHL because it would not be expected to influence VLDL assembly or secretion. Several kinetic studies have revealed a 2-fold increase in VLDL production, implicating a defect in VLDL synthesis as the primary cause.\textsuperscript{18–23} In addition, modifier loci, which may not significantly influence plasma lipid levels under normal circumstances, are likely to affect lipid levels under conditions of increased flux of VLDL in FCHL. The apoAI-CIII-AIV gene cluster and, less frequently, the LPL locus appear to be 2 such modifier loci, as identified previously.\textsuperscript{30,32} ApoAI-CIII-AIV and LCAT are presently considered response modifier genes. Although the present study has been performed on 1 of the largest collections of affected FCHL individuals, our ability to detect modifier gene effects was limited to those with at least an estimated 15% contribution (see below). Modifier genes are expected to be common in FCHL families, and combinations of modifier genes may prove to be frequent as well. Various combinations of modifier genes could have an impact on the expression of the multiple type hyperlipidemia, characteristic of FCHL.
Evidence from these analyses indicates that multiple genes contribute to the expression of FCHL. The FCHL genes, which can be detected by these linkage analyses, can be characterized by reference to the parameter known as lambda.2,3 Lambda represents the FCHL risk to the siblings of an affected individual compared with that of the general population. For FCHL, lambda may be as large as 25, calculated by dividing the nearly 50% risk to sibs by the 1% to 2% risk to the general population. Hauser et al4,5 have provided tables that can be used to estimate the minimum lambda of genes that can be identified in an affected sib-pair linkage analysis wherein the numbers of nuclear families and degrees of parental genotyping vary. Using $P < 0.001$ as the level of significance (a log of the odds score of 2.0) and this sample size of $\sim 100$ affected sib pairs, Table 2 of their article indicates we should be able to identify an FCHL gene with a lambda of 2.0 or larger 80% of the time. By using a multiplicative model for the genetic contribution to FCHL, such a gene could have a contribution of as little as 15% to this disorder.

In conclusion, we provide evidence herein, by linkage analysis in FCHL families, that 2 distinct loci, the apoAI-CIII-AIV and LCAT loci on chromosomes 11 and 16, respectively, may contribute to the expression of FCHL. These results illustrate the genetic complexity of this disorder and contribute to our understanding of this most common dyslipidemia predisposing to premature coronary artery disease.

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