Locus for Elevated Apolipoprotein B Levels on Chromosome 1p31 in Families With Familial Combined Hyperlipidemia

Hooman Allayee,* Kelly L. Krass,* Päivi Pajukanta, Rita M. Cantor, Carla J.H. van der Kallen, Rebecca Mar, Jerome I. Rotter, Tjerk W.A. de Bruin, Leena Peltonen, Aldons J. Lusis

Abstract—Familial combined hyperlipidemia (FCH), a common cause of premature coronary artery disease, is genetically complex and poorly understood. Recently, a major locus on chromosome 1q21-23 exhibiting highly significant linkage was identified in Finnish FCH families by use of a parametric analysis. We now report highly significant evidence of linkage (maximum LOD score 3.8, recombination fraction 0) of an important FCH phenotype, elevated apolipoprotein B (apoB) levels, to a distinctly separate locus on chromosome 1p31 in Dutch pedigrees. ApoB is the major protein on very low density and low density lipoproteins, and elevated apoB levels have been used as a surrogate trait for FCH. Additional microsatellite markers in the 1p31 region were genotyped, and evidence of linkage improved (maximum LOD score 4.7) in a multipoint analysis of two markers in the peak region. The leptin receptor gene resides within this locus and is involved in obesity and insulin/glucose homeostasis. However, there was no evidence of an association between leptin receptor and apoB levels, raising the possibility that another gene on this chromosomal region contributes to elevated apoB levels in this Dutch population. This is one of the first loci identified for apoB levels in humans and is the second major locus implicated in the genetic etiology of FCH. (Circ Res. 2002;90:926-931.)

Key Words: apolipoprotein B ■ genetics ■ hyperlipoproteinemia ■ coronary artery disease

Familial combined hyperlipidemia (FCH) (see Mendelian Inheritance in Man [MIM]-144250, which can be accessed online [OMIM] at http://www.ncbi.nlm.nih.gov/omim) is the most common major genetic lipid disorder in humans and accounts for 10% to 20% of premature coronary artery disease (CAD).1 The lipoprotein profile in FCH patients is characterized by increased levels of plasma VLDL and LDL, which have been postulated to result from either increased VLDL secretion, decreased lipoprotein clearance, insulin resistance, and/or altered fatty acid metabolism.2–4 Because FCH is not a monogenic disorder, it is also possible that FCH families have multiple or even different alterations in lipoprotein metabolism rather than just a single defect in one of these abnormalities.

To date, hypotheses regarding the underlying mode of inheritance of FCH, which was originally thought to be dominant, have been based on linkage and segregation analyses5–7 and remain to be corroborated by identification of the causative genes. A number of genetic studies have been conducted in various ethnic populations to identify susceptibility loci for FCH. Although several candidate genes have been implicated, these genes appear to have only subtle effects and most probably modify the elevated lipid phenotype rather than represent the primary cause.4 Two genome scans for FCH in Dutch and Finnish families have also been recently reported.8,9 These studies identified several loci in each FCH population, but there was no obvious overlap between the loci in the two studies. In particular, evidence for a major FCH locus was observed on chromosome 1q21-23 in the Finnish families.10 This locus has since also been observed with modest levels of significance in the US, Chinese, and German FCH populations as well11,12 but was not apparent in the initial analyses of Dutch data. Some explanation for this observation is provided by differences in the genetic background between the Finnish and Dutch populations. However, the use of different analytic methods can also contribute to these findings, because nonparametric affected sibling-pair linkage methods were used in the Dutch genome scan, whereas model-based parametric linkage analysis was used in the Finnish study.

In the present study, we have reanalyzed the chromosome 1 data in the Dutch families by using the same trait parameters and statistical methods used in the analysis of the Finnish families. The obtained results revealed a novel locus on the
Materials and Methods

Ascertainment and Diagnosis of FCH Families

Eighteen extended Dutch FCH families were ascertained through probands that were recruited from the Lipid Clinic of the Utrecht Academic University Hospital, as previously described. The probands met the following criteria: (1) a primary combined hyperlipidemia with varying phenotypic expression, including a fasting plasma cholesterol ≥6.5 mmol/L or ≥90th percentile for age defined according to tables from the Lipid Research Clinics, and fasting plasma triglycerides ≥2.3 mmol/L or ≥90th percentile for age; (2) at least one first-degree relative with a different hyperlipidemic phenotype; and (3) a positive family history of premature CAD defined as myocardial infarction or cardiovascular disease before 60 years of age. Exclusion criteria for the probands included diabetes, body mass index (BMI) >30, tendon xanthomas, or type III hyperlipidemia (apoE2/E2). Over 95% of the relatives and spouses of the probands were collected without regard to affection status. In addition to the FCH families, a case-control group of individuals from the Dutch population has also been collected. This panel consists of 121 randomly selected spouses from the entire cohort and 76 independent FCH probands. All subjects gave informed consent, and the study protocol was approved by the Human Investigation Review Committee of Utrecht and Maastricht Academic Hospitals, the Netherlands.

In the Finnish FCH study, the proband or a first-degree family member had elevated cholesterol and triglycerides (combined hyperlipidemia) above the age- and sex-specific 90th percentile for the Finnish population. If these criteria were met, then the family members were classified as being affected with the qualitative FCH trait if they had either cholesterol or triglyceride levels exceeding the age- and sex-specific 90th percentiles for Finns. This strategy was also applied to the extended members of the pedigree as well. Thus, extended family members beyond the proband and first-degree relatives were classified as affected only if they had elevated cholesterol and/or triglyceride levels as well as a first-degree relative with both hypercholesterolemia and hypertriglyceridemia. This scheme resulted in an enrichment of families exhibiting the combined hyperlipidemic phenotype rather than isolated high cholesterol or triglyceride levels alone. In the present study, the Dutch families were first redefined according to the same diagnostic criteria outlined above for the Finnish families. As previously conducted for the Finnish families, 8,10 the DOWNFREQ program was used to estimate allele frequencies of each marker from the total study population by using an allele-counting method. Genetic homogeneity of the families was tested by using the HOMOG program of the ANALYZE package.20,21

For fine mapping of the chromosome 1p locus, the MLINK program was used to conduct both 2-point and multipoint analyses. For the LEPR gene, two intragenic microsatellite markers22 were combined into a haplotype to increase the information content for linkage analysis, as described previously.23 The multipoint analyses were performed by placing tightly linked markers in a fixed order and allowing the disease locus to vary outside the marker map. The physical order of the markers in the region was determined by using the University of California, Santa Cruz, human genome working draft Web site (which can be accessed at http://genome.ucsc.edu). Genetic distances between markers were taken from the Marshfield Medical Research Foundation (which can be accessed online at http://research.marshfieldclinic.org/genetics). The heterozygosity indices for all fine-mapping markers are reported according to the CEPH database (Fondation Jean Dausset, which can be accessed online at http://www.ceph.fr) except for the LEPR haplotype, which was calculated from individuals in the Dutch families.

The transmission disequilibrium test (TDT) was performed with the haplotype of the intragenic LEPR microsatellites by using the TDTLIKE program version 2.1 of the ANALYZE package.24 It is a powerful likelihood-based test for linkage disequilibrium with affection status that is conservative when used to analyze multiple multiallelic markers. Differences in the allelic distribution of SNPs within the LEPR gene between the probands and spouses controls were calculated by a χ² test (SPSS, version 9.0, SPSS Inc).

Genetic Statistical Analyses

As previously conducted for the Finnish families, 2-point parametric linkage analysis was performed by using the MLINK program of the Linkage package,17 with FASTLINK version 2.218,19 and the help of the ANALYZE package.20 Linkage was assessed with the assumption of a dominant mode of inheritance and was based on the 1% to 2% population prevalence of FCH; the disease allele frequency for these analyses was set at 0.006. To circumvent problems of incomplete penetrance and ambiguity of the “unaffected” phenotype, linkage analysis was performed by using an “affecteds-only” strategy. Subjects were classified as either “affected” or “unknown” according to the Finnish age- and sex-specific 90th percentile lipid cutoffs, as described above. In total, four qualitative traits were analyzed: the discrete FCH trait and elevated plasma triglyceride, total cholesterol, and apoB levels. These three latter traits were dichotomized into qualitative traits by using age- and sex-specific 90th percentile cutoffs, similar to what was done previously for the Finnish families.8,10 The DOWNFREQ program was used to estimate allele frequencies of each marker from the total study population by using an allele-counting method. Genetic homogeneity of the families was tested by using the HOMOG program of the ANALYZE package.20,21

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Results

Previous genome scans for FCH in Dutch and Finnish families revealed linkage to several chromosomal regions, although there did not appear to be loci common to both populations. To assess whether adopting a different analytic approach would confirm previous loci and/or expose novel short arm of chromosome 1 associated with elevated apoB levels in Dutch FCH families.

Laboratory Analytical Methods

Venous blood was drawn after an overnight fast of 12 to 14 hours, and plasma was prepared by immediate centrifugation. Lipids and apolipoproteins were quantified by methods described elsewhere.14,15 Proband or hyperlipidemic relatives who used lipid-lowering drugs were studied after their lipid-lowering treatment was withheld for 3 weeks. Only these lipid values and other quantitative measurements were used in the present study. Alcohol use was not controlled for, other than the requirement that the subjects should abstain from alcohol consumption for at least 48 hours before having their blood drawn.
loci, the Dutch families were reanalyzed with the use of the same ascertainment criteria and statistical methods that were used to test for linkage in the Finnish families. Of the 18 Dutch FCH families available for analysis, 13 matched the diagnostic and family selection criteria used for the Finnish FCH families. We performed linkage analysis by using a dominant mode of inheritance and an affecteds-only approach, and tested four qualitative traits: the discrete FCH trait and elevated plasma triglyceride, cholesterol, and apoB levels (see Materials and Methods).

Analysis of the chromosome 1 data in the Dutch FCH families yielded suggestive evidence for linkage of elevated triglycerides (LOD score 1.8) and apoB (LOD 1.0) to D1S1679 on chromosome 1q21-23. This marker maps 5 cM proximal to the FCH locus identified in Finnish families by Pajukanta et al.10 There was no evidence for linkage of the other two traits to this locus (see Figure).

The most dramatic result in the analysis of the chromosome 1 data in the Dutch families was obtained on the short arm, 80 cM away from the 1q21-23 locus. We observed significant evidence of linkage (maximum LOD 3.8) of the qualitative elevated apoB trait with marker D1S1665 at a recombination fraction of 0. The next two centromeric markers, D1S1728 and D1S551, also yielded maximum LOD scores of 2.7 and 1.9, lending further support for linkage of elevated apoB to this region, although these results did not reach conventional criteria for significance. Evidence for linkage of the other three traits (FCH and elevated cholesterol and triglyceride levels) was not observed, but some evidence was obtained with elevated triglycerides (maximum LOD 1.4) and D1S2134, a marker that maps 26 cM distal to D1S1665 (Figure).

To more precisely map the location of the putative gene on 1p31 in the Dutch families, additional markers throughout the region were genotyped. As shown in Table 1, linkage analysis with these markers also yielded suggestive 2-point LOD scores. Based on the fine-mapping results, the most probable location of the FCH gene would be in the vicinity of D1S1665, inasmuch as this marker yielded the highest LOD score of all the markers and at a recombination fraction of 0. Next, we carried out multipoint analysis with D1S1665 and D1S481 and obtained a maximum LOD score of 4.7, with elevated apoB (Table 1). Although evidence of genetic heterogeneity between the families was not observed with the

<table>
<thead>
<tr>
<th>Marker (Heterozygosity Index)*</th>
<th>Distance, † cM</th>
<th>2-Point LOD Score, ‡</th>
<th>Multipoint LOD Score, ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1S3728 (0.63)</td>
<td>1.1</td>
<td>0.34 (0.24)</td>
<td></td>
</tr>
<tr>
<td>D1S203 (0.64)</td>
<td>4.7</td>
<td>0.29 (0.16)</td>
<td></td>
</tr>
<tr>
<td>D1S230 (0.82)</td>
<td>2.2</td>
<td>0.44 (0.24)</td>
<td></td>
</tr>
<tr>
<td>D1S3467 (0.85)</td>
<td>1.5</td>
<td>0.13 (0.32)</td>
<td></td>
</tr>
<tr>
<td>LEPR (0.65)</td>
<td></td>
<td>0.91 (0.16)</td>
<td></td>
</tr>
<tr>
<td>D1S219 (0.85)</td>
<td>2.5</td>
<td>0.43 (0.18)</td>
<td></td>
</tr>
<tr>
<td>GATA152F05 (0.77)</td>
<td>0.5</td>
<td>2.0 (0.02)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.2 (0.06)</td>
<td></td>
</tr>
<tr>
<td>D1S1665 (0.73)</td>
<td></td>
<td>3.8 (0)</td>
<td>4.7 (0)</td>
</tr>
<tr>
<td>D1S481 (0.89)</td>
<td>2.0</td>
<td>2.3 (0.04)</td>
<td>2.5 (0.1)</td>
</tr>
<tr>
<td>GATA193D02 (0.79)</td>
<td>0.5</td>
<td>2.1 (0.1)</td>
<td></td>
</tr>
<tr>
<td>D1S500 (0.68)</td>
<td>2.8</td>
<td>1.1 (0.08)</td>
<td></td>
</tr>
<tr>
<td>D1S1728 (0.58)</td>
<td>1.5</td>
<td>2.7 (0)</td>
<td></td>
</tr>
<tr>
<td>D1S551 (0.56)</td>
<td>4.7</td>
<td>1.9 (0.1)</td>
<td></td>
</tr>
</tbody>
</table>

*The heterozygosity indices of the markers are taken from the CEPH database.
†Distance between adjacent markers.
‡LOD scores >3.0 are indicated in bold.

HOMOG program (data not shown), we next assessed whether evidence of linkage on chromosome 1p31 was derived uniformly from all 13 families, because they varied markedly in size. Analysis of the multipoint results with D1S1665 and D1S481 for each family revealed that a single, large family contributed over half of the evidence for linkage, with a LOD score of 2.5, and that an additional 7 of the remaining 12 families contributed positively to the overall LOD score (Table 2).

On the basis of the role of leptin in obesity and insulin/glucose metabolism, the LEPR gene is a good positional and functional candidate gene located 3 cM distal to D1S1665. Because FCH individuals can exhibit insulin resistance and increased BMI, the contribution of the LEPR gene was examined by association analysis. We first performed a TDT in the families by using the haplotypes of the intragenic LEPR microsatellites. The results of this analysis did not reveal evidence of an association between the LEPR gene and affection status as defined by elevated apoB levels.
identifying FCH susceptibility genes, but the disease remains relatively uncharacterized at the molecular genetic level. Various candidate gene studies in several populations and two genome scans in Dutch and Finnish families have been reported for FCH. The latter studies identified several loci in each FCH population, but there was no obvious overlap between the loci in the two FCH populations, including the Finnish FCH locus on chromosome 1q21-23. We speculated that this might have been due to phenotypes that were not defined consistently across studies and/or to the application of different analytic methods. In the present study, we reanalyzed the chromosome 1 genotype data in the Dutch FCH families by using the same trait classification and statistical methods that were used in the analyses of the Finnish families. The power to detect linkage was increased by performing a model-based analysis and by using an affecteds-only strategy, which partially circumvented the ambiguity of incomplete penetrance, because the analysis was restricted to only those individuals who met the criteria for being affected. By use of this method, a major locus was previously identified on chromosome 1q21-23 with the FCH and elevated triglyceride traits in the Finnish study. In the Dutch population, we observed suggestive evidence of linkage to the 1q locus, with a LOD score of 1.8.

Significant evidence of linkage was observed with elevated apoB levels to a novel locus on chromosome 1p31 in the Dutch families. Affected FCH individuals typically exhibit higher plasma apoB levels compared with those of their unaffected relatives, but apoB has not been used as a diagnostic criterion for FCH in all studies. Because only one apoB molecule is found on VLDL and LDL particles, elevated plasma apoB can be thought of as a surrogate trait for FCH. Previous studies based on segregation analyses have also concluded that a major locus with large effects on apoB levels within FCH families may contribute to its etiologic heterogeneity. These results were subsequently corroborated in a different FCH population as well. Last, it has been reported that there is >80% concordance for estimating CAD risk in FCH families when apoB levels were used either alone or in conjunction with lipoprotein levels. These latter results are consistent with recent studies from our group demonstrating that common carotid artery intima-media thickness, a validated surrogate marker of asymptomatic atherosclerosis, is positively associated with plasma apoB concentrations in FCH subjects. Therefore, the identification of genes that potentially regulate apoB levels would be of particular interest for understanding not only how lipid metabolism is defective in FCH but also how such an alteration leads to increased premature CAD in FCH and perhaps in the general population.

Given the unknown genetic etiology of FCH, it has been difficult to speculate about the metabolic defect in FCH. Some studies have suggested that the hyperlipidemia in FCH individuals results from increased VLDL secretion by the liver, although these studies have not been conclusive. It is also possible that the FCH phenotype, at least in some families, could result from reduced LDL receptor activity in the presence of hypertriglyceridemia. This could not only

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**TABLE 2. Multipoint Linkage Results of Individual Families With Markers D1S1655 and D1S481 and ApoB Trait**

<table>
<thead>
<tr>
<th>Family</th>
<th>No. of Individuals</th>
<th>LOD Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>−0.1</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>−0.6</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>0.4</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>0.6</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>0.6</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>21</td>
<td>0.5</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>−0.3</td>
</tr>
<tr>
<td>Total</td>
<td>173</td>
<td>4.7</td>
</tr>
</tbody>
</table>

(P=0.32). To further evaluate the LEPR gene, we genotyped two SNPs in 76 unrelated FCH probands and 121 spouse controls from the Dutch population. The frequencies of the Lys109Arg and Lys656Asn polymorphisms were consistent with Hardy-Weinberg equilibrium, and similar to previous reports in another white population, the two SNPs were in strong linkage disequilibrium in this Dutch sample. As shown in Table 3, the frequencies of the Lys109Arg and Lys656Asn polymorphisms did not differ significantly between probands and spouses. When the allelic distributions of the polymorphisms were compared between the spouses and only those probands who had elevated apoB levels above the age- and sex-specific 90th percentile, the same results were obtained. In addition, there was no significant quantitative difference in mean apoB levels with either SNP in probands or spouses. Taken together, these results do not reveal evidence of an association between alleles in the LEPR gene and apoB levels in the present study sample.

**Discussion**

FCH is a common genetic lipid syndrome that substantially predisposes individuals to premature CAD. Although it appears to be a disorder of oligogenic etiology with an overlapping environmental component, segregation analyses indicate that some of the genes contributing to the disease are of large enough effect for loci to be identified by linkage, given a sufficient sample size. Considerable effort has focused on identifying FCH susceptibility genes, but the disease remains relatively uncharacterized at the molecular genetic level. Various candidate gene studies in several populations and two genome scans in Dutch and Finnish families have been reported for FCH. The latter studies identified several loci in each FCH population, but there was no obvious overlap between the loci in the two FCH populations, including the Finnish FCH locus on chromosome 1q21-23. We speculated that this might have been due to phenotypes that were not defined consistently across studies and/or to the application of different analytic methods. In the present study, we reanalyzed the chromosome 1 genotype data in the Dutch FCH families by using the same trait classification and statistical methods that were used in the analyses of the Finnish families. The power to detect linkage was increased by performing a model-based analysis and by using an affecteds-only strategy, which partially circumvented the ambiguity of incomplete penetrance, because the analysis was restricted to only those individuals who met the criteria for being affected. By use of this method, a major locus was previously identified on chromosome 1q21-23 with the FCH and elevated triglyceride traits in the Finnish study. In the Dutch population, we observed suggestive evidence of linkage to the 1q locus, with a LOD score of 1.8.

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**TABLE 3. Frequencies of LEPR Gene Polymorphisms in the Case-Control Panel**

<table>
<thead>
<tr>
<th></th>
<th>Lys109Arg</th>
<th>Lys656Asn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lys</td>
<td>Arg</td>
</tr>
<tr>
<td>FCH probands (n=76)</td>
<td>0.72</td>
<td>0.28</td>
</tr>
<tr>
<td>Spouse controls (n=121)</td>
<td>0.76</td>
<td>0.24</td>
</tr>
</tbody>
</table>
reduce the clearance of LDL particles from the circulation but, as reported recently, could also increase the secretion of apoB from the liver. In a preliminary examination of the linkage data on chromosome 19 (data not shown), we did not observe linkage with any of the four traits to the LDL receptor gene locus. Although reduced hepatic LDL receptor activity could still contribute to the FCH (and elevated apoB) phenotype in these families, such an effect would presumably result from the trans-acting effects of other gene(s). For example, autosomal recessive hypercholesterolemia is caused by mutations in an adaptor protein that is required for LDL receptor function.

Interestingly, the gene for this protein maps to chromosome 1p but is located ~50 cM distal to the apoB locus on 1p31.

Two major loci have now been identified for FCH. They are located on chromosome 1q21-23 in Finnish, Dutch, US white, German, and Chinese populations and on chromosome 1p31 in the current analysis of the Dutch FCH families. Both were identified by using parametric statistical analyses and are consistent with a dominant pattern of inheritance. Only weak evidence of linkage to chromosome 1p31 was observed with the Finnish families, and the Dutch pedigrees showed suggestive linkage to 1q21-23. These results support the concept that FCH is both a heterogeneous and oligogenic disease, resulting from multiple dominant-acting major genes with important contributions from modifier genes and environmental influences. It seems likely that other major genes will contribute to the disorder, inasmuch as the 1p and 1q loci do not show evidence of linkage in other large FCH pedigrees that we have studied (K.L. Krass and A.J. Luis, unpublished data, 2001). A model for family-specific mutations in FCH is also consistent with the observation that the hyperlipidemia within families exhibits a vertical transmission pattern that persists over numerous generations. A model in which multiple genes with small effect contribute equally to the trait within individual families, on the other hand, would be more consistent with a phenotype that would be diluted or lost in successive generations. Last, biochemical studies of FCH patients have also provided evidence of phenotypic heterogeneity; for example, some patients exhibit VLDL overproduction, whereas others appear to show decreased lipoprotein catabolism.

Although the recombination fraction estimate at which maximum LOD scores are obtained in the analysis of complex diseases does not necessarily reflect the actual distance to the disease locus, the linkage results on 1p31 implicate the location of the gene. This 2-cM peak region contains ~25 genes, but there are no biologically plausible candidates. This may be due to our poor understanding of the mechanisms contributing to the metabolic defect in FCH. Intriguingly, we previously demonstrated that this region, which contains the LEPR gene, segregates with plasma leptin levels and BMI in these FCH families. Leptin is a hormone involved in adiposity and insulin/glucose metabolism. These metabolic traits are often altered in FCH, and we have previously demonstrated that polymorphisms within the LEPR gene are associated with leptin levels. In the present study, however, a family-based association test and a case-control analysis did not reveal evidence of the contribution of the LEPR gene to affection status or apoB levels. This suggests that although the LEPR gene may underlie the linkage of leptin levels (and possibly BMI) to this locus, a different gene could explain the linkage with elevated apoB levels in this FCH sample. Because of the relatively small sample size in the family and case-control data sets, the interpretation of these negative results should only be considered preliminary at this time. Further dissection of this locus will likely require larger data sets (especially larger numbers of cases and controls) and the construction of dense SNP maps to test for linkage disequilibrium. This approach has been used with some success in other complex diseases, such as type 2 diabetes and Crohn’s disease. The identification of a gene influencing apoB levels may help not only to unravel the genetic complexity of FCH but also to shed light into the mechanisms involved in lipid metabolism.

Acknowledgments

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References


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