Quantitative Trait Loci for Apolipoprotein B, Cholesterol, and Triglycerides in Familial Combined Hyperlipidemia Pedigrees

Rita M. Cantor, Tjerk de Bruin, Naoko Kono, Susan Napier, Atila van Nas, Hooman Allayee, Aldons J. Lusis

Objective—Familial combined hyperlipidemia (FCHL) is a genetically complex lipid disorder that is diagnosed in families by combinations of increased cholesterol, triglycerides, and/or apolipoprotein B (apoB) levels in patients and their first-degree relatives. Identifying the predisposing genes promises to reveal the primary risk factors and susceptibility pathways and suggest methods of prevention and treatment. As with most genetically complex disorders, a clinical definition of disease may not be the most useful phenotype for finding the complement of predisposing genes, and the quantitative traits used to define the disorder can provide important information. This is a report of a quantitative trait loci (QTL) analysis of FCHL.

Methods and Results—A full genome scan of 377 multi-allelic markers genotyped at ∼10 centimorgan (cM) intervals was conducted in 150 sibling pairs from 22 nuclear families in FCHL pedigrees. These data were analyzed by 2 multipoint QTL linkage methods using the nonparametric and Haseman–Elston procedures of the Genehunter software. Using a criterion of \( P < 0.001 \) by the nonparametric analysis, we found evidence of 2 apoB QTL at 1p21-31 \((P < 0.000009)\) and 17p11-q21 \((P < 0.000009)\), a total serum cholesterol QTL at 12p13 \((P < 0.0001)\), and a serum triglycerides QTL at 4p15-16 \((P < 0.0002)\). Using the criterion of \( P < 0.03 \) for at least 2 traits at the same locus, additional evidence for cholesterol \((P < 0.01)\) and a triglycerides \((P < 0.02)\) was observed at 17p11-21, as well as suggestive evidence for apoB \((P < 0.02)\) and triglycerides \((P < 0.01)\) at 4q34-35, and cholesterol \((P < 0.01)\) and triglycerides \((P < 0.02)\) and a binary FCHL trait \((\text{lod} = 1.5)\) at 16p12-13.

Conclusions—QTL analyses of the traits that define FCHL are effective for localizing disease-predisposing genes.

Key Words: FCHL ■ QTL ■ apolipoprotein B ■ cholesterol ■ triglycerides
full genome scans, extensive candidate gene analyses, and analyses of the FCHL diagnosis in large pedigrees and in nuclear families. Recently, very promising findings were generated by analyzing the FCHL diagnosis or a related binary trait derived from increases over age/sex-specific cutoff values. These include linkage to 1q in large Finnish pedigrees, followed by successful association analyses of a positional candidate gene, upstream transcription factor 1, replication of a linked region on 11p, and a combined analysis of Finnish and Dutch families, which showed linkage to 16q. Although some analyses of the quantitative lipid traits associated with FCHL have been included in these gene finding investigations, they have rarely been the main focus of the studies. For example, when analyzing FCHL-associated quantitative traits, Allayee et al found linkage of apoB to chromosome region 1p31 in a cohort of Dutch FCHL pedigrees when the trait was treated as binary. That is, if the individual exceeded the 90th percentile for their age and sex category, they were considered as affected. Using a similar analytic approach, Pajukanta et al treated high-density lipoprotein (HDL) cholesterol as a binary trait, and mapped a locus for HDL cholesterol to chromosome 16q. Almasy et al used the full quantitative distribution of HDL cholesterol and localized 2 putative genes influencing this trait in large pedigrees that were not ascertained for a specific lipid disorder, such as FCHL. Although the quantitative traits have been the focus of these analyses, we can hypothesize that against different genetic backgrounds (morbid or healthy), a different genetic cause in the same or different pathways may be operating to define one’s profile of lipid traits.

The linkage analyses we report herein were undertaken to identify the QTL for cholesterol, triglycerides, and apoB, which are the component traits contributing to a diagnosis of FCHL. We conducted these analyses in a sample of FCHL pedigrees containing affected and unaffected individuals, and in which the disease-defining quantitative traits show extensive variation that is not normally distributed. By searching for lipid QTL within FCHL families, we hypothesized genes that normally make small contributions to individual lipid profiles may have larger effects and act as major genes that are easier to detect in these FCHL families.

Materials and Methods

Study Sample

Pedigrees were ascertained in Maastricht, the Netherlands via probands with multiple lipid abnormalities associated with FCHL. The ascertainment criteria were: (1) a proband with a primary hyperlipidemia, including fasting total plasma cholesterol >6.5 mmol/L (250 mg/dL) and/or fasting plasma triglyceride concentrations >2.0 mmol/L (180 mg/dL); (2) multiple lipoprotein phenotypes within the family (Fredrickson classification IIa, IIb, or IV); and (3) a positive family history of premature cardiovascular disease, defined as the occurrence of a myocardial infarction or cerebrovascular accident before the age of 60 reported in medical records. Exclusion criteria were secondary causes of hyperlipidemia (renal or hepatic insufficiency, hypothyroidism, and medication), the apo E2/E2 genotype, and subjects with tendon xanthomas or a diagnosis matching familial hypercholesterolemia. To further reduce potential genetic heterogeneity, pedigrees from among those satisfying the aforementioned criteria were included in the current analyses only if the proband had levels of serum triglycerides and serum cholesterol exceeding age/sex-specific cutoff values and at least 1 first-degree relative had an increased age-specific and sex-specific serum triglyceride or serum cholesterol level. Specifically, the proband in each family included in these analyses exceeded the 90th percentiles for total serum cholesterol and triglyceride levels within the subject’s age and sex category according to the distributions from a large-scale Finnish population-based study. These percentiles may be more stringent than those for the Dutch, which are not available.

ApoB, cholesterol, and triglycerides levels are not normally distributed in these pedigrees, as illustrated in Figure 1. The means...
Identified those regions having 2 or more traits each with $P<0.03$ at the same locus.

**Additional QTL Analyses**
A second analytic approach, the Haseman–Elston (HE) test, which correlates allele-sharing ibd with the squares of raw trait differences in sibling pairs by regressing the squared differences against the allele sharing, is more vulnerable to the influences of non-normally distributed data. Multipoint HE analyses were also conducted with the Genehunter software, but the results were not used to identify the linked regions and were included for informational purposes only in those the regions first identified by the nonparametric analyses. One large sibship in the sample consisted of 13 siblings as well as additional sibsibs that contained their offspring. We conducted all of the analyses described, on this subsample alone, to infer which of the linkage signals derived primarily from this family.

**Linkage Analysis of ApoB and Triglycerides as a Discrete Trait**
To also investigate the binary phenotype of FCHL, we used the quantitative traits of apoB and triglyceride levels as suggested by the most recent workshop on FCHL to define a binary FCHL phenotype. Their consensus was that increased apoB and triglyceride levels, rather than the classical phenotype of combined elevated cholesterol and triglyceride levels, be applied in the diagnosis of FCHL. This was based on previous studies of the FCHL trait that showed impaired clearance of postprandial lipoproteins and increased very-low-density lipoprotein (VLDL) secretion lead to increased plasma levels of apoB and triglycerides, implying that apoB may be a more precise measure of increased VLDL. Thus, all pedigree members were assessed as to whether their apoB or triglyceride levels exceeded the 90th percentile for their age and sex categories, again using the Finnish distributions. Among the pedigrees, 20 contained at least 2 individuals who exceeded either of these criteria and they were included, resulting in 125 individuals who were classified as “affected.” A single-point parametric linkage analysis of the genome scan data was conducted using a dominant fully penetrant model for the inheritance of the putative disease gene.

**Results**
Table 2 summarizes the positive QTL results from the nonparametric linkage analyses as defined by $P<0.001$ for 1 trait, or $P<0.03$ for each of 2 or more traits. The table is organized vertically by the chromosome, and within that the trait names in alphabetic order. The first column gives the name of the trait, the second column gives the name of the marker closest to the linkage peak, and the third column gives the chromosome band(s) at which the QTL occurs. The fourth column gives the distance of the peak marker from the p-terminal end of the chromosome, the fifth column gives the $z$-score for the nonparametric analysis and the corresponding probability value, the sixth column gives the HE lod score, and the last column gives the nonparametric $z$-score and the corresponding probability value for the analysis of the largest family of 13 siblings and 3 nuclear families containing their offspring. Four QTL were identified by the criterion of $P<0.001$. They are at 1p21-31 ($P<0.000009$) and 17p11-21 ($P<0.000009$) for apoB, 12p13 ($P<0.00001$) for cholesterol, and 4p15-16 ($P<0.0002$) for triglycerides.

Detailed plots of the significant QTL as defined by $P<0.001$ by the multipoint nonparametric analysis of the full genome scan of apoB, total cholesterol, and triglycerides are presented in Figure 2. The QTL are displayed in both directions from the peak to the point where the linkage signal is zero. The nonparametric and HE linkage statistics are...
TABLE 1. Sibships in the FCHL Quantitative Trait Locus Analyses

<table>
<thead>
<tr>
<th>Sibship Size</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>8</th>
<th>13</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N of sibships</td>
<td>13</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>N of individuals</td>
<td>26</td>
<td>15</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>13</td>
<td>71</td>
</tr>
<tr>
<td>N of sibpairs</td>
<td>13</td>
<td>15</td>
<td>6</td>
<td>10</td>
<td>28</td>
<td>78</td>
<td>150</td>
</tr>
</tbody>
</table>

presented on the same axes, with the nonparametric \( z \)-score in solid black lines and the HE lod scores in dashed gray lines.

The results for regions showing \( >1 \) QTL, as defined by \( P<0.03 \) for each trait, are 4q34-35 for apoB (\( P<0.02 \)) and triglycerides (\( P<0.01 \)), 16p12-13 for cholesterol (\( P<0.01 \)) and triglycerides (\( P<0.02 \)), and 17p11-q21 shows linkage to cholesterol (\( P<0.008 \)) and triglycerides (\( P<0.02 \)) in addition to apoB. The traits are highly correlated in the probands of these sibships; the Spearman correlations are: 0.71 (\( P=0.0002 \)) for apoB and cholesterol, 0.42 (\( P=0.05 \)) for apoB and triglycerides, and 0.62 (\( P=0.002 \)) for cholesterol and triglycerides. Nonparametric analysis \( z \)-scores and probability values indicate that the largest sibship of 13 siblings and 3 nuclear families with their offspring can explain the linkage of triglycerides to 4p15-16. However, although linkage of apoB to 1p21-31 is also observed in the large family, other sibships in the sample also appear to contribute to this QTL. This same pattern is evident at 17p11-q21 for apoB. The QTL for cholesterol at 12p13 appears to derive very little of its linkage signal from this large family.

The parametric 2-point analyses of FCHL, when it was defined as a binary trait by either increased age/sex-specific apoB or triglyceride levels, yielded all but 2 lod scores \( <1.5 \). Both were in one 20-cM region of 16p12-13. Because only one of the markers yielded a high lod score of 4.0, we also conducted a multipoint analysis to include the linkage information from the surrounding markers. Those results were, as expected, more modulated, with a multipoint lod score of 1.5. In a parametric analysis of a binary trait, lack of evidence for linkage can occur either because there is no linkage to a region or because an incorrect model for the inheritance pattern of the FCHL genes was posed. We report these results, however, because the alternative definition of the FCHL trait is linked to a chromosome region that overlaps with the cholesterol and triglycerides QTL on 16p12-13 as defined by \( P<0.03 \) for both traits.

Discussion

The overwhelming success of gene-finding efforts for Mendelian diseases encourages a focus on genetically complex disorders such as FCHL. Recent reports indicate that a number of predisposing FCHL genes are on their way toward being identified and confirmed, but much remains to be performed before the genetics of this disorder is fully understood, and thoughts can turn to risk prediction and specific treatment targets. A successful example is the report of linkage\(^{10} \) and association of USF-1 in Finnish FCHL pedigrees,\(^{16} \) with support for linkage of triglycerides to this region found in Dutch FCHL families,\(^{18} \) as well as diabetes-related traits found in other independent cohorts.\(^{27-29} \) FCHL is a genetically complex disease, and the consensus is that innovative and more in-depth trait definitions and alternative analytic strategies are required to obtain insight. Toward that end, we examined individual FCHL-related quantitative traits in pedigrees to map their QTL. A model-free nonparametric statistical linkage procedure that makes no assumptions regarding trait normality and correlates the degree of allele sharing with the degree of trait sharing in the sibling pairs was used. The analyses provided evidence for apoB QTL on 1p21-31 and 17p11-q23, a cholesterol QTL on 12p13, and a triglycerides QTL on 4p15-16. Using a less stringent criterion, we found evidence of apoB and triglycerides QTL on 4q34-35, cholesterol and triglycerides QTL as well as linkage of a binary FCHL trait defined by increased apoB or serum triglycerides to 16p13-12, and all 3 quantitative traits to 17p11-21.

Although the criterion of \( P<0.001 \) was used for reporting linkage, 3 correlated quantitative traits and 1 bivariate binary trait have been analyzed separately, and multiple testing must be considered in the interpretation of these results. The QTL

TABLE 2. Linkage Peaks for ApoB, Cholesterol, and Triglycerides QTL in FCHL Sibships

<table>
<thead>
<tr>
<th>Quantitative Trait</th>
<th>Peak Marker</th>
<th>QTL Chromosome Band(s)</th>
<th>Marker Distance</th>
<th>Nonparametric ( z )-Score (( P ))</th>
<th>HE Lod</th>
<th>Nonparametric ( z )-Score (( P )), Large Sibship</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoB</td>
<td>D1S1627</td>
<td>1p21-31</td>
<td>139</td>
<td>4.3 (0.000009)</td>
<td>2.2</td>
<td>3.7 (0.0001)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>GATA70E01</td>
<td>4p15-16</td>
<td>35</td>
<td>3.6 (0.0002)</td>
<td>1.2</td>
<td>4.0 (0.00003)</td>
</tr>
<tr>
<td>apoB</td>
<td>D4S2417</td>
<td>4q34</td>
<td>182</td>
<td>2.1 (0.02)</td>
<td>0.5</td>
<td>2.5 (0.006)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
<td></td>
<td>2.3 (0.01)</td>
<td>1.0</td>
<td>2.8 (0.003)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>D12S372</td>
<td>12p13</td>
<td>6</td>
<td>3.7 (0.0001)</td>
<td>3.7</td>
<td>1.1 (NS)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>D16S2616</td>
<td>16p13</td>
<td>12</td>
<td>2.2 (0.01)</td>
<td>1.9</td>
<td>1.8 (0.04)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
<td></td>
<td>2.0 (0.02)</td>
<td>0.4</td>
<td>2.1 (0.02)</td>
</tr>
<tr>
<td>apoB</td>
<td>D17S1294</td>
<td>17p11-q21</td>
<td>51</td>
<td>4.3 (0.000009)</td>
<td>3.7</td>
<td>3.6 (0.0002)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td>2.4 (0.008)</td>
<td>0.7</td>
<td>1.7 (0.04)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
<td></td>
<td>2.0 (0.02)</td>
<td>0.5</td>
<td>1.3 (NS)</td>
</tr>
</tbody>
</table>

A binary trait defined by an age-specific and sex-specific increase in apoB or triglycerides only showed linkage to 16p12-13 with a multipoint lod of 1.5.
reported in Table 2 and Figure 2 are significant at the $P<0.001$ level, even if a stringent Bonferroni correction is applied. This is not true for the QTL identified by $P<0.03$ for 2 or more traits, so we report them but, without follow-up studies, interpret them as interesting results. In addition, we recognize that this 10-cM genome scan is only a first step in the gene identification process for FCHL. It can only localize the predisposing genes to wide chromosome regions of $\sim 35$ to 40 cM. However, we envision that reports, such as this one, will provide the basis for future collaborations to fine-map specific regions in combined study samples to localize the disease-predisposing genes with greater precision. As SNP technologies become more cost-effective, the individual risk genes will likely be discovered. This is a long process, and these initial investigations are necessary for future success. With this in mind, we examined the literature for other positive linkage signals in the QTL regions revealed by these analyses.

The apoB QTL at 1p21-31 with a peak at 139 cM near marker D1S1627 in this sample is $\sim 35$ cM from a linkage peak for apoB at marker D1S1665 found in an independent Dutch FCHL cohort from Utrecht. In that sample, apoB was treated as a binary trait, and those with an apoB level exceeding the 90th percentile for their age and sex were coded as affected in an “affecteds only” linkage analysis. Although it is tempting, the distance of 35 cM between the peaks may be too large to allow us to infer that these samples are mapping the same apoB gene.

We are most encouraged by the coincident location of QTL for apoB, cholesterol, and triglycerides within the 17p11-21 chromosomal region. Although the traits are correlated in this sample, the fact that all 3 have QTL in the same region may reflect a gene contributing to the increase in multiple lipid profiles associated with FCHL. A 2-point HE analysis of triglyceride levels in an independent sample of 75 obese families resulted in a peak z-score of 2.58 at $\sim 65$ cM, although the linked region ranged from 40 to 70 cM, and is consistent with our findings. Although it was not among the strongest results, linkage to this region with $P=0.014$ has also been reported for cholesterol in an independent cohort of British FCHL families. Because cholesterol-carrying lipoproteins have apoB as their structural apoprotein, and FCHL is characterized by elevated plasma levels of apoB containing VLDL and low-density lipoproteins (LDL), this is not unexpected. The putative gene in this region may have a pleiotropic effect on increased total serum cholesterol and

![Figure 2. Plots in bold black lines of nonparametric z-scores QTL defined by $P<0.001$ for apoB, cholesterol, and triglycerides in FCHL sibships. The HE lod scores for these data are plotted with dashed gray lines on the same axes. The distance along the horizontal axis is in centimorgans (cM) and the genotyped markers are indicated. The distance along the vertical axis is either nonparametric z-score or HE lod score.](http://atvb.ahajournals.org/)

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apoB within FCHL pedigrees. In addition, VLDL subclasses have direct impacts on the plasma concentrations of LDL subclasses in FCHL, specifically VLDL1 and small dense LDL, suggesting a VLDL–triglyceride pathway in FCHL. What is known about its biochemistry corresponds well with linkage and association results for apoA-CIII-AIV, apoAII, and apoB in FCHL. The USF-1 gene may also act in the VLDL–triglyceride pathway by stimulating lipogenesis, by fatty acid synthase, for export in VLDL, apoB, cholesterol, and triglycerides. 17q11 contains the gene for NOS2A, the inducible nitric oxide synthase, which we found to be downregulated in adipose tissue biopsy samples from FCHL subjects in comparison with age-, sex-, and body mass index-matched controls (data not shown). NOS2A acts in the liver and may have other less well-characterized functions than regulation of vascular tone.

The linkage found with apoB in the region of marker D1S1627 is of interest, because it was also found to be linked with plasma leptin concentrations that reflect adiposity in an independent cohort of Dutch FCHL subjects from Utrecht. It was suggested that the linkage may be caused by DNA variation in the leptin receptor (LEPR) gene, close to D1S1627. We also find that as adiposity, assessed by the waist-to-hip ratio, increases, FCHL subjects express higher plasma apoB concentrations than matched controls (data not shown). Thus, the linkage between plasma apoB concentrations and D1S1627 is consistent with the influence of the LEPR gene on apoB in FCHL. Extensive single nucleotide association studies would have to be conducted to pursue this possibility.

These findings illustrate that a QTL approach in combination with refined phenotypes will yield insight into novel genes and pathways responsible for FCHL and its associated risk of heart disease.

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