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A Genome Scan for Familial Combined Hyperlipidemia Reveals Evidence of Linkage with a Locus on Chromosome 11

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Summary

Familial combined hyperlipidemia (FCHL) is a common familial lipid disorder characterized by a variable pattern of elevated levels of plasma cholesterol and/or triglycerides. It is present in 10%–20% of patients with premature coronary heart disease. The genetic etiology of the disease, including the number of genes involved and the magnitude of their effects, is unknown. Using a subset of 35 Dutch families ascertained for FCHL, we screened the genome, with a panel of 399 genetic markers, for chromosomal regions linked to genes contributing to FCHL. The results were analyzed by use of parametric-linkage methods in a two-stage study design. Four loci, on chromosomes 2p, 11p, 16q, and 19q, exhibited suggestive evidence for linkage with FCHL (LOD scores of 1.3–2.6). Markers within each of these regions were then examined in the original sample and in additional Dutch families with FCHL. The locus on chromosome 2 failed to show evidence for linkage, and the loci on chromosome 16q and 19q yielded only equivocal or suggestive evidence for linkage. However, one locus, near marker D11S1324 on the short arm of human chromosome 11, continued to show evidence for linkage with FCHL, in the second stage of this design. This region does not contain any strong candidate genes. These results provide evidence for a candidate chromosomal region for FCHL and support the concept that FCHL is complex and heterogeneous.

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Introduction

Familial combined hyperlipidemia (FCHL; MIM 144250), with a frequency of ~1% in U.S. and European white populations, is the most common genetic lipid disorder observed in patients with coronary heart disease (CHD) and in their relatives. It occurs in 10%–20% of patients with premature CHD and is characterized by elevated concentrations of plasma triglycerides (>90th percentile) and/or plasma cholesterol (>90th percentile) in probands and their affected relatives (Goldstein et al. 1973; Nikkila and Aro 1973; Rose et al. 1973; Brunzell et al. 1983). Although FCHL was first described >25 years ago, its primary etiology remains unknown. The disease was first postulated to have a Mendelian-dominant pattern of transmission, but subsequent segregation analyses suggested a more complex inheritance pattern (Iselius 1981; Williams and Lalouel 1982; Cullen et al. 1994). To date, hypotheses regarding the underlying mode of inheritance of FCHL have been made on the basis of segregation analyses and remain to be corroborated by identification of the contributing loci and genes. Lipoprotein metabolism in patients with FCHL is characterized by an overproduction of hepatic apolipoprotein (apo) B–containing lipoproteins, resulting in increased plasma levels of LDL and of very-low-density lipoproteins (VLDL) (Kissebah et al. 1981; Cortner et al. 1991; Venkatesan et al. 1993; Aguilar-Salinas et al. 1997). Defects in lipoprotein clearance (Cabezas et al. 1993; Yang et al. 1996), glucose intolerance (Vakkilainen et al. 1998), reduced peripheral triglyceride synthesis (Cianflone et al. 1995), insulin resistance (Aitman et al. 1997), and reduced adipocyte lipolysis (Reynesdottir et al. 1995) have also been implicated.

Candidate-gene studies have provided evidence that common variations of several genes, including lipoprotein lipase (LPL) and the apoAI-CIII-AIV cluster, can influence lipid levels in affected individuals, but none of these appear to be a primary genetic determinant (Wojciechowski et al. 1991; Dallinga-Thie et al. 1996, 1997;

Yang et al. 1996; Pajukanta et al. 1997; Aouizerat et al., in press). Recent studies of the Finnish isolate revealed a locus near the candidate gene apoAII, on human chromosome 1, that appears to contribute to FCHL (Pajukanta et al. 1998). Strong support for this locus comes from the mapping of a gene for combined hyperlipidemia to a region of conserved synteny on mouse chromosome 3 (Castellani et al. 1998).

Linkage studies of FCHL pose a considerable challenge, partly because the disorder lacks unequivocal diagnostic criteria. The original diagnosis of FCHL was given on the basis of premature CHD and evidence of three different lipid phenotypes among the first-degree family members: elevated cholesterol (phenotype IIA), elevated cholesterol and triglycerides (phenotype IIB, combined hyperlipidemia), or elevated triglycerides (phenotype IV) (Goldstein et al. 1973; Nikkila and Aro 1973). In addition, hyperlipidemia is quite variable among affected individuals and can also vary, over time, for an individual. Hyperlipidemia is age dependent, since children usually exhibit only modest elevations of cholesterol and triglycerides. Compounding these difficulties is the lack of a genetic model that adequately reflects the clear familial inheritance of the disease. These problems make it difficult to apply traditional model-based linkage approaches, which have been used successfully for mapping genes causing monogenic disorders (Hanis et al. 1996).

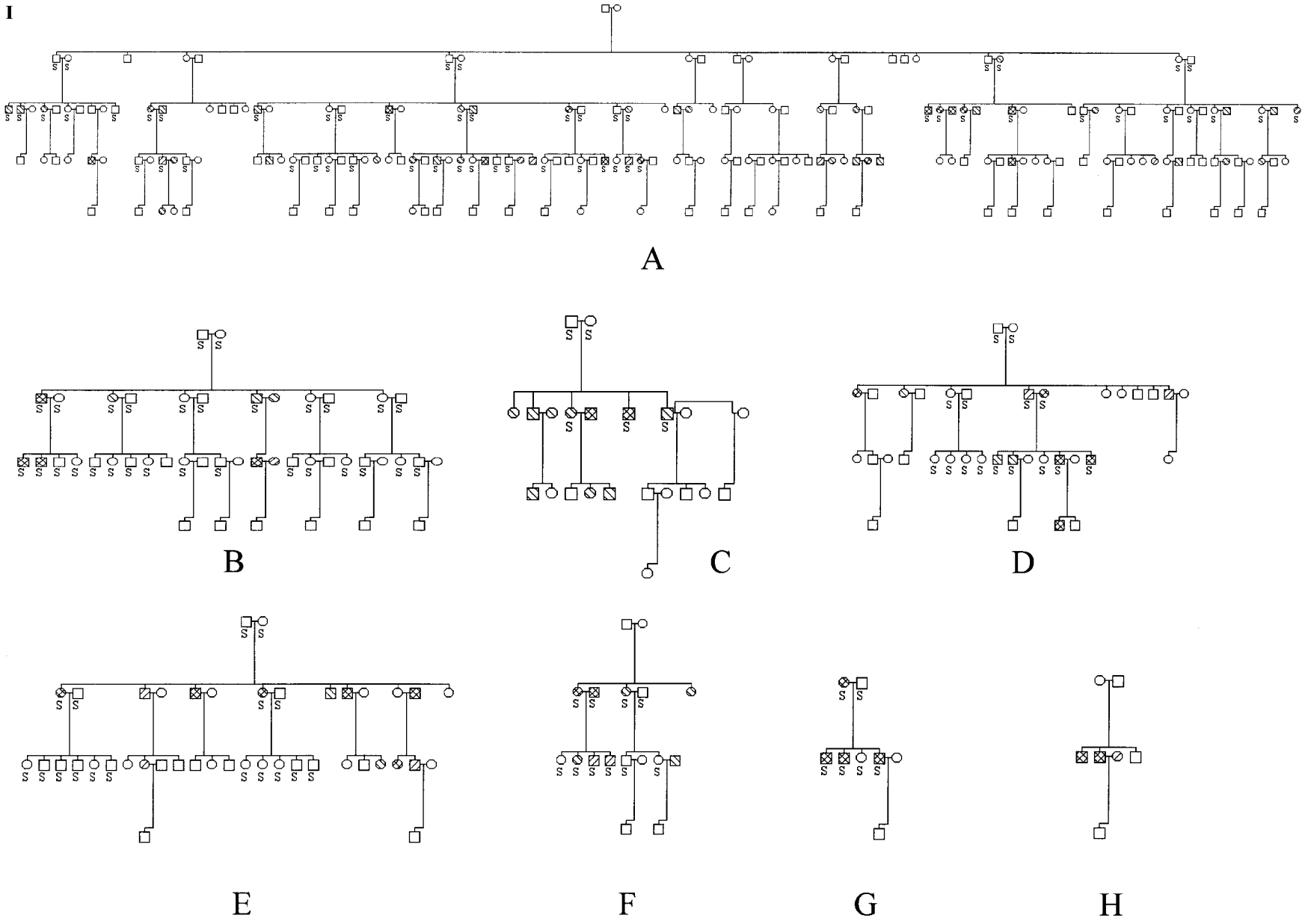
In the present study, we report the results of a genomewide screen for FCHL-susceptibility genes, using nonparametric-linkage approaches and a two-stage design. In stage 1, a complete multipoint genome scan was done in 240 individuals in nuclear families from 18 Dutch FCHL families, with use of 399 genetic markers spanning ~10-cM intervals. Four regions with suggestive LOD scores between 1.0 and 3.0 were identified. These four loci were examined subsequently in 782 individuals, extending the number of pedigrees to 35. Three of the loci exhibited only suggestive or no evidence for linkage in the stage 2 analysis. However, a region on the short arm of chromosome 11 continued to exhibit evidence for linkage when the additional sampled families were analyzed. Since only modest LOD scores were observed for any loci in this relatively large family study, the data are not consistent with a model in which the disease is due to a single major gene. Thus, these results also provide strong evidence for the genetic complexity of FCHL, supporting previous segregation analyses. The novel FCHL locus on chromosome 1q21-q23, identified in the Finnish isolate (Pajukanta et al. 1998), did not exhibit strong evidence for linkage in the present study of the Dutch outbred population. These observations imply that FCHL not only is multigenic but also is heterogeneous in nature.

Families and Methods

Ascertainment of FCHL Families

Dutch FCHL families were ascertained as described elsewhere (Dallinga-Thie et al. 1996, 1997) and exhibit remarkable similarity with the original description of FCHL (Goldstein et al. 1973). The probands with FCHL were identified through a lipid-out patient clinic in a tertiary academic referral center. Although all are of Dutch descent, the recruited FCHL families are not from a single geographic area in the Netherlands. Initially, 18 unrelated probands with FCHL were recruited through the Lipid Clinic of the Utrecht University Hospital; subsequently, 17 additional probands were identified. The probands met three criteria, as follows: (1) primary hyperlipidemia, with plasma cholesterol>250 mg/dl (>6.5 mmol/liter), triglycerides >200 mg/dl (>2.3 mmol/liter), and apoB concentrations exceeding, by 2 SD, the mean for age-adjusted levels; (2) at least one first-degree relative with a different hyperlipidemic phenotype; and (3) a positive family history of premature CHD, defined as myocardial infarction or cardiovascular disease with age at onset <60 years. Exclusion criteria for probands included diabetes, obesity (body-mass index [BMI] >28), familial hypercholesterolemia (tendon xanthomas), and/or type III hyperlipidemia (apoE2/E2). Low HDL-cholesterol level was not used as a trait for ascertainment, since it was observed in only a subset of the FCHL families. All subjects gave informed consent, and the study protocol was approved by the Human Investigation Review Committee of the University Hospital, Utrecht.

The pedigree structures and clinical characteristics of the 35 FCHL families are shown infigure 1and table 1, respectively. Relatives and spouses of probands were recruited without bias with regard to affectation status, and >95% of all available living relatives of probands were studied. Hyperlipidemic family members (n = 253) were assigned an FCHL phenotype on the basis of presenting with plasma cholesterol and/or plasma triglycerides >90th percentile and/or apoB >75th percentile, for age-matched and gender-matched controls. Family members who did not meet these criteria were classified as normolipidemic (n = 298). A spouse group (n = 232) provided unrelated controls matched for environment, nutrition, and age. These criteria are likely to provide reasonable estimates of normal values for the defining diagnostic criteria (i.e., levels of total cholesterol, triglycerides, LDL-cholesterol, apoB, and HDL-cholesterol). The age of probands was ~51 years (range 27–76 years); 24% were female, as compared with 45% of affected individuals identified in the entire sample. The large proportion of male probands is in part the result of an ascertainment bias, since CHD affects men earlier than women, regardless of the underlying etiol-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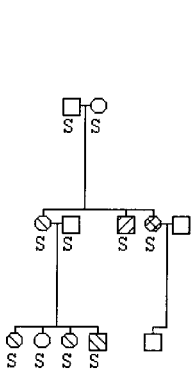

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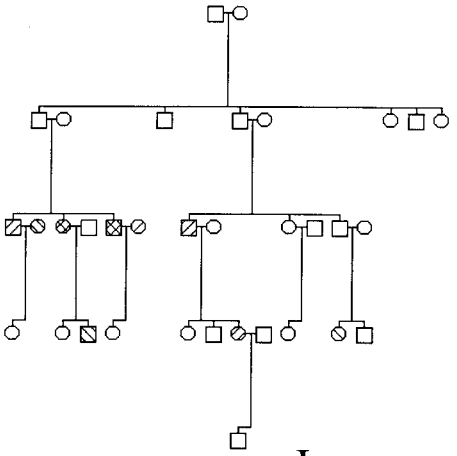

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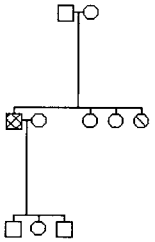

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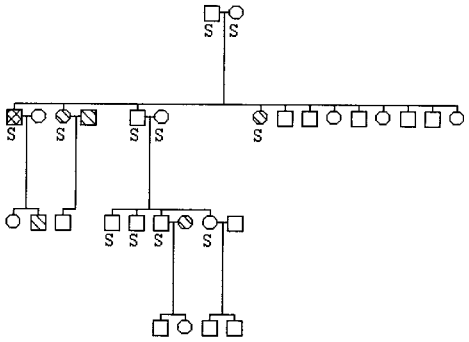

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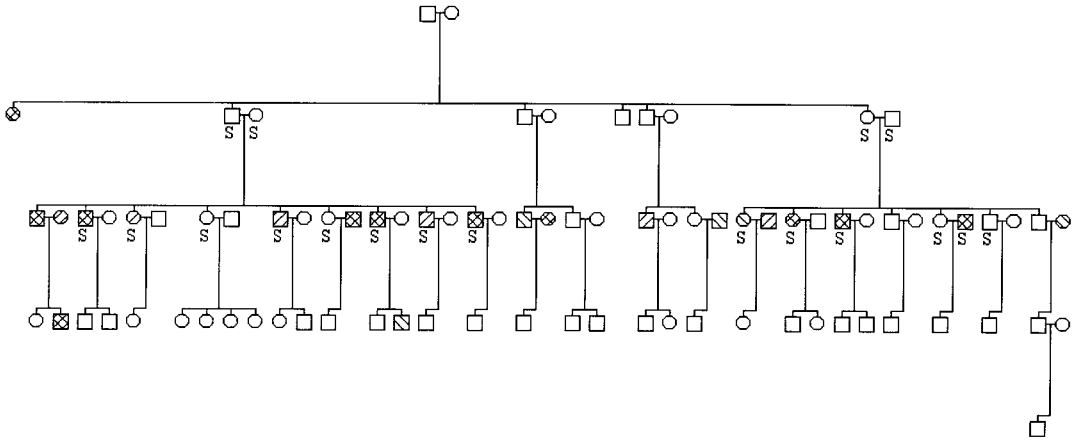

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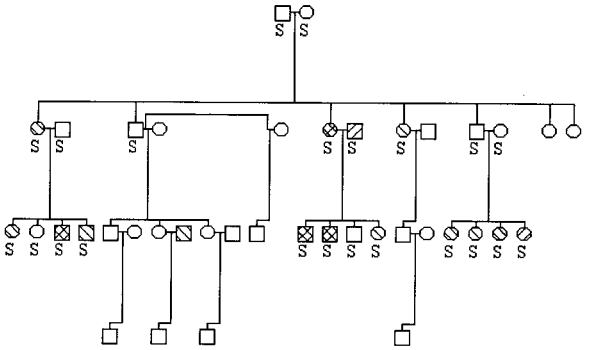

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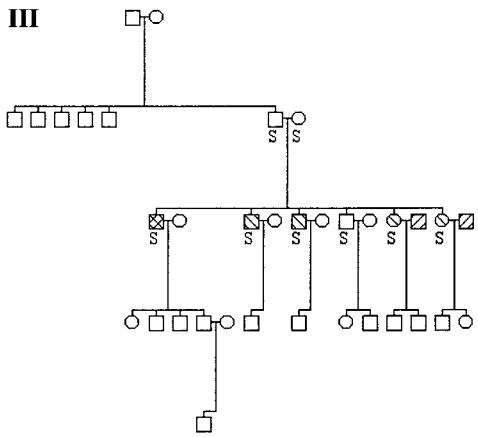

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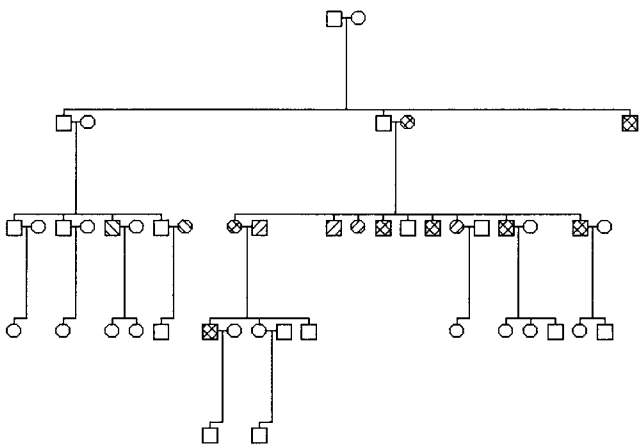

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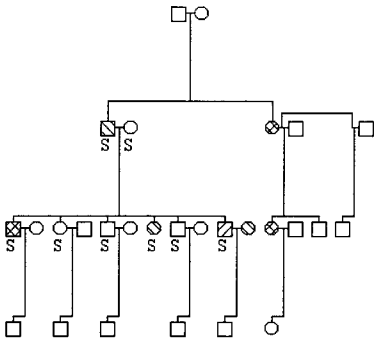

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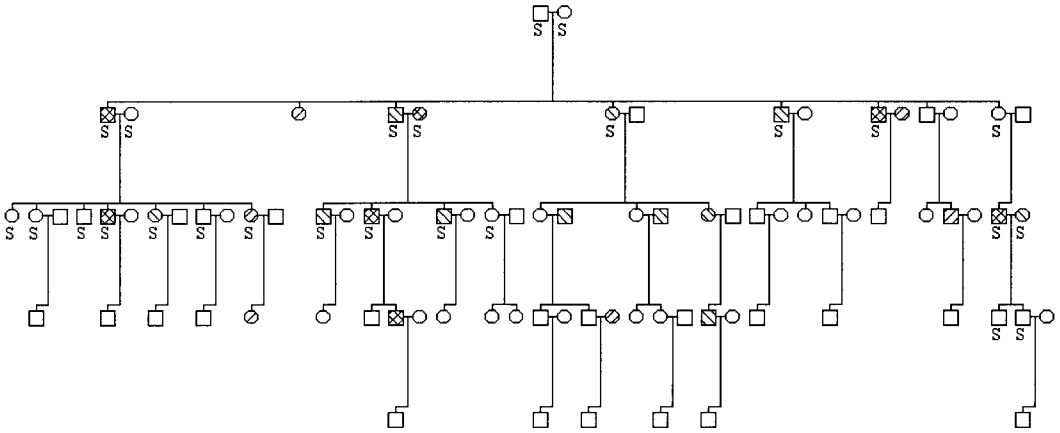

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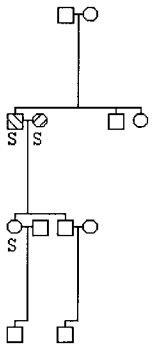

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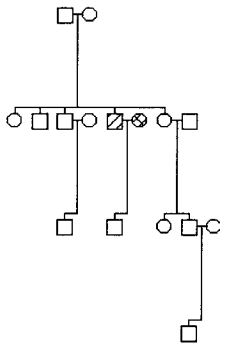

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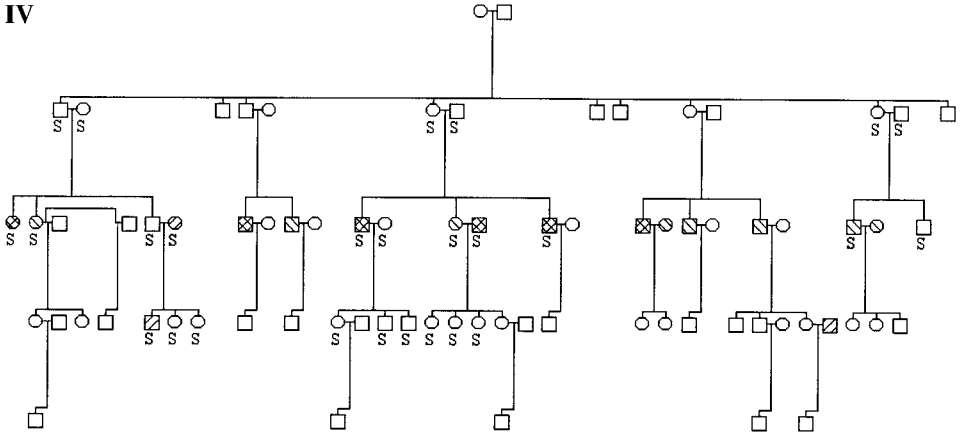

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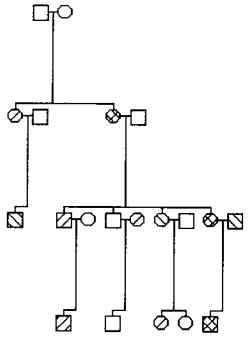

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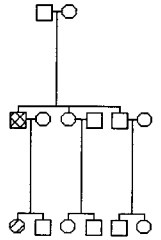

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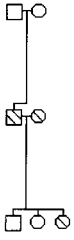

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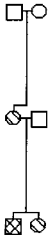

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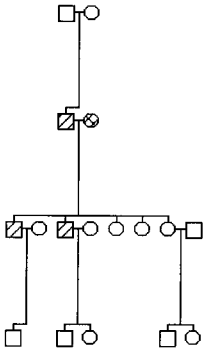

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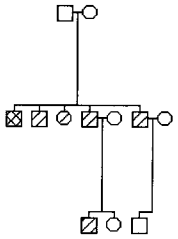

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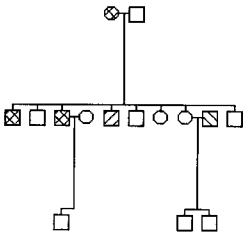

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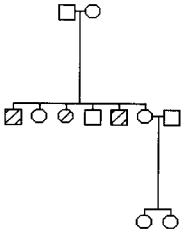

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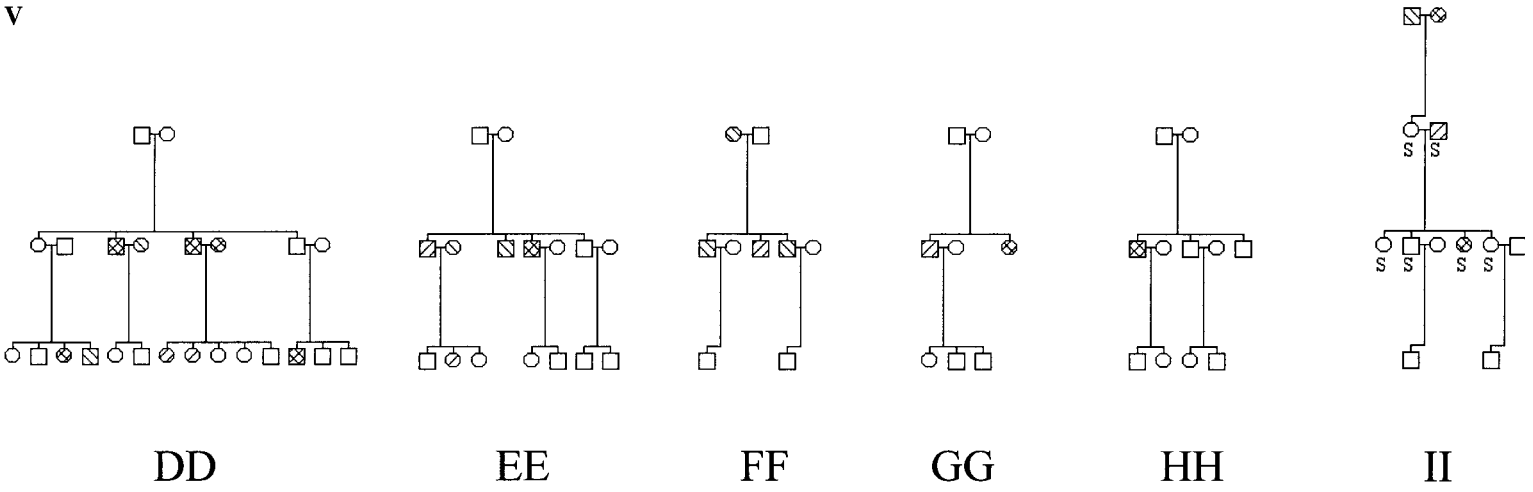

Figure 1 Pedigree structures of the 35 Dutch FCHL families. Hypertriglyceridemic individuals (i.e., with plasma triglyceride levels >90th percentile) are denoted by upward diagonal hatching. Hypercholesterolemic individuals (i.e., with plasma cholesterol levels >90th percentile) are indicated by downward diagonal hatching. Individuals who are both hypercholesterolemic and hypertriglyceridemic are denoted by a cross-hatching. Individuals genotyped in the stage 1 genome scan are indicated by an "s" below the symbol.

Table 1

Clinical Characteristics of the FCHL Families

Variable	Mean ± SD in Hyperlipidemic Individuals[a] (n = 253)	Mean ± SD in Normolipidemic Individuals (n = 298)	Mean ± SD in Spouse Controls (n = 231)
Age (years)	49 ± 16	34 ± 15	48 ± 15
Lipids (mg/dl):			
Triglycerides	243 ± 143	116 ± 43	144 ± 91
Cholesterol	281 ± 90	191 ± 31	219 ± 40
LDL-cholesterol	179 ± 53	122 ± 29	144 ± 38
apoB	131 ± 30	84 ± 22	102 ± 8
apoCIII	11.8 ± 4.2	7.4 ± 2.5	8.7 ± 3.2

[a] Includes the 35 probands and 218 hyperlipidemic individuals.

ogy. The hyperlipidemic relatives had characteristics similar to those of the probands, including a male preponderance (55%), as well as higher values for BMI and waist-hip ratio than were seen in normolipidemics (Dallinga-Thie et al. 1996). Normolipidemic relatives were younger than the hyperlipidemic relatives, and it is likely that the FCHL phenotype had not yet been fully expressed in some normolipidemic individuals. Although children with FCHL exhibit somewhat elevated plasma lipid values, the age at onset of the FCHL phenotype has been assumed to be >20 years (Goldstein et al. 1973; Castro Cabezas et al. 1992).

Analytic Methods

Venous blood was drawn after an overnight fast of 12–14 h, and plasma was prepared by immediate centrifugation. Lipids and apo were quantified by use of methods described elsewhere (de Bruin et al. 1991; Cabezas et al. 1993; Dallinga-Thie et al. 1996). Probands or hyperlipidemic relatives who used lipid-lowering drugs were sampled after their lipid-lowering treatment was withheld for 3 wk. Only these lipid values and other quantitative measurements were used in this study. Hormone-replacement therapy showed little effect on lipid traits in this Dutch FCHL sample and therefore was not considered a confounding factor (T. W. A. de Bruin, unpublished data). Alcohol use was not controlled for, beyond the subjects' abstention from alcohol consumption for ⩾48 h prior to having blood drawn.

Genotyping

In the present study, DNA from 782 subjects (Dallinga-Thie et al. 1996, 1997) was used for analysis. DNA was isolated from 10 ml EDTA-augmented blood, by use of standard procedures (Weber and May 1989). Genotyping was performed either manually, with use of radiolabeled PCR products (Allayee et al. 1998), or with the aid of a scanning fluorescent detector, with use of fluorescently labeled PCR products (Yuan et al. 1997). For fluorescent genotyping, the final PCR reaction conditions were as follows: 100 μM each of dGTP, dATP, dTTP, and dCTP; 0.075 μM each PCR primer (Research Genetics; forward primer labeled with fluorescent dye); 45 ng genomic DNA template; and 0.12 U Ampli*Taq* Polymerase (Boehringer Manheim), in a final volume of 4 μl. Amplified products were resolved by electrophoresis with 6.0% polyacrylamide gels containing 7.7 M urea, 89 mM Tris, 89 mM borate, and 2.5 mM EDTA and were visualized by a scanning fluorescence detector (Rusch et al. 1996). The 399 markers used in the construction of the genome-screen linkage map constituted the Weber 6 screening set (Marshfield Medical Research Foundation).

Linkage Analysis

Since FCHL is thought to have a complex pattern of inheritance, we analyzed data from the genome screen by using nonparametric linkage (NPL) methods that do not require assumptions regarding the mode of inheritance (Ott 1991). The primary screen was done with use of the multipoint linkage analysis program MAPMAKER/SIBS, which uses information from multiple linked markers to calculate allele sharing at and between markers determined on the basis of the most likely genotypes (Kruglyak and Lander 1995). Significance of the test statistic for excess allele sharing among affected sibling pairs is expressed as a LOD score (Ott 1991; Kruglyak and Lander 1995). In addition, this analytic methodology mitigates, in part, the potential effect of markers with low heterozygosity indices, since the informativeness of a locus is also calculated with linked markers.

In stage 2, regions with suggestive LOD scores (i.e., between 1.0 and 3.0) were examined in 782 individuals, extending the number of pedigrees to 35. Polymorphic markers at the four loci detected in stage 1 were typed in the second set of individuals (with use of the peak marker from the stage 1 scan and an additional closely linked marker), and P values, calculated by two-point allele-sharing methods (Haseman and Elston 1972; Elston 1997), for the entire sample were compared with those of the initial sampled pedigrees in stage 1. The recent observation that a particular set of markers for genomewide scanning will have different informativeness among ethnic groups suggests that use of allele frequencies from the study population, rather than published frequencies, is appropriate (Hegele et al. 1998). Therefore, spousal genotype data were used to calculate allele frequencies for markers analyzed in stage 2. Although the allele frequencies observed in the Dutch FCHL sample varied slightly from those distributed through the CEPH database (Généthon), they were not substantially different. None of the markers' heterozygosity indices differed >.15 from the CEPH panel, and

>84% of the markers varied <.05. In addition, spousal genotypes were used to confirm Mendelian inheritance for marker alleles. Families with bilineal inheritance (i.e., a spouse with an expressed Fredrickson type IIB hyperlipidemic profile) were excluded from statistical analysis.

To assess linkage in the sample of clinically concordant affected sibling pairs, we calculated the mean allele sharing at the loci of interest and tested each allele for a significant difference from the expected value of random allele sharing (.50), by using the SIBPAL subprogram of the SAGE package (Elston 1997). A significant increase in allele sharing (>.50) was taken as evidence for linkage. The advantage of an initial analysis that uses only affected sibling pairs is that each person in the analysis is assumed to have the disease gene of interest, and other apparently unaffected individuals who may have the gene but who have not expressed the trait do not confound the results. Because a specific hypothesis was being tested with each of these loci, a $P < .05$ was chosen to support our further analysis of the data. In addition to single-marker analyses, haplotypes derived from combinations of markers from the selected candidate regions were also used, to maximize the linkage information available in the extended stage 2 sample.

When the sample of affected sibling pairs in the selected candidate regions exhibited evidence for linkage, we extended the analyses to include two additional groups of sibling pairs, one in which both were unaffected (clinically concordant unaffected sibling pairs) and another in which one was affected and the other was unaffected (clinically discordant sibling pairs). At linked markers, both concordant affected and concordant unaffected sibling pairs are expected to demonstrate increased sharing of marker alleles identical by descent (IBD), whereas clinically discordant pairs should exhibit decreased sharing. However, both concordant unaffected and discordant sibling pairs should show weaker evidence for linkage than do affected sibling pairs, because some unaffected siblings may carry the disease gene but not express the phenotype, because of a lack of other genetic and/or environmental factors that contribute to the development of the disease (Thomson 1994).

Data from all three groups of sibling pairs were also combined into a linear-regression analysis with use of SIBPAL, which regresses the trait differences (disease status) between sibling pairs versus the proportion of marker alleles that they share IBD. A significantly negative regression-line slope indicates that those who are phenotypically alike tend to share more alleles, whereas those who are discordant tend to exhibit less allele sharing, thus providing evidence for linkage. In the absence of linkage, the slope of the regression line is not expected to be significantly different from zero. The linear-regression analysis was also done with haplotypes of markers from selected candidate regions.

The quantitative traits associated with FCHL (levels of total plasma triglycerides, LDL-cholesterol, apoB, apoCIII, and HDL-cholesterol) were analyzed in a similar fashion. All quantitative analyses were done initially on untransformed data, and logarithmically transformed data were analyzed when the quantitative-trait distribution was skewed. The effects of gender and age on plasma LDL-cholesterol, triglyceride, apoB, apoCIII, and HDL-cholesterol levels accounted for a small amount of the variability in the traits ($r^2 < 10\%$). Accordingly, they were not used as covariates.

In an attempt to define criteria that better reflect FCHL affectation status, two new quantitative phenotypes were assessed as intermediate phenotypes. One, the ratio of total triglycerides to apoCIII, has been shown to be decreased in individuals with FCHL (van Barlingen et al. 1996). To better represent the source of elevated cholesterol in individuals with FCHL, non-HDL–cholesterol levels were used as another intermediate phenotype.

Assessing the Degree of Heterogeneity at the Chromosome 11 Locus

To test for genetic heterogeneity, both an affected-sibling-pair analysis (which disregards parental phenotype) and a parametric LOD-score analysis (which uses parental phenotypic information) were done, with a peak marker (D11S1324) at the chromosome 11 locus. The GENEHUNTER program calculates an NPL statistic and the corresponding relative informativeness of the genotypic data for the families (Kruglyak et al. 1996; Kruglyak and Lander 1998). The NPL statistic is a model-free measure of allele sharing (a Z score). A parametric LOD-score analysis was also performed on the component nuclear families as if they were independent, with use of the LINKAGE package programs (v. 5.1, updated by J. Ott; Lathrop et al. 1984; Ott 1991) and the POLYPREP program (Terwillinger and Ott 1994) with FASTLINK (v. 2.3P; Lathrop et al. 1984, 1986; Cottingham et al. 1993; Schaffer et al. 1994). In our analysis, a LOD score was first calculated with the assumption of homogeneity at the locus, and a second LOD score was calculated with the assumption of heterogeneity. To circumvent problems of incomplete penetrance and the genetic ambiguity of an "unaffected" phenotype, we used an affecteds-only strategy by coding the subjects as either "affected" or "unknown" (Pajukanta et al. 1997). POLYPREP (Terwillinger and Ott 1994), which contains the program HOMOG, was used to test for possible heterogeneity for FCHL at marker D11S1324.

Table 2

Maximum Multipoint LOD Scores for Each Chromosome in the Genome Scan for FCHL

Chromosome	Maximum LOD Score[a]	Nearest Marker[b]	Distance from pter (cM)	Information Content	Allele Sharing
1	1.0	D1S1597	21	.7	.52
2	2.6	D2S2952	16	.7	.62
3	.7	D3S1766	84	.6	.56
4	.6	D4S2639	21	.8	.53
5	.7	D5S1505	140	.8	.54
6	.8	D6S1021	111	.8	.50
7	.1	D7S513	14	.8	.56
8	.3	D8S1106	24	.6	.54
9	.2	D9S1121	33	.8	.52
10	.8	D10S1213	155	.5	.53
11	2.6	D11S1985	62	.6	.55
12	.6	D12S2078	150	.6	.50
13	.5	D13S800	53	.7	.59
14	.6	D14S306	36	.6	.53
15	.7	D15S655	75	.5	.53
16	1.4	D16S402	106	.8	.53
17	.1	D17S2180	100	.8	.48
18	.6	D18S1357	90	.8	.52
19	1.3	D19S589	88	.5	.52
20	.3	D20S171	90	.7	.54
21	.1	D21S1432	0	.6	.52
22	.1	D22S420	0	.5	.53
X	.9	DXS6800	93	.5	…

[a] Maximum multipoint LOD scores were calculated with use of the MAPMAKER/SIBS program.

[b] Information for individual markers in the genome scan is provided at the Marshfield Center for Human Genetics Website.

Results

Study Design

Previous candidate-gene studies have failed to identify the genetic factor(s) that can account for overproduction of VLDL, the metabolic hallmark of FCHL. To map the principal genes involved and to examine the complexity of the disease, a genome scan was done in families ascertained for FCHL. Since previous modeling has suggested that FCHL is a complex disorder (Williams and Lalouel 1982), we primarily have used nonparametric (model-free) strategies for analyzing linkage data. Because a genome scan involves multiple testing, it is likely that some loci exhibiting modest LOD scores (1.0–3.0) will result by chance alone. To address this possibility, a two-stage study design was adopted.

In stage 1, a complete genome scan was done in 240 individuals in nuclear families from 18 Dutch FCHL families and then was analyzed with use of nonparametric multipoint methods (MAPMAKER/SIBS program; Kruglyak and Lander 1995). In stage 2, regions with suggestive LOD scores were examined by genotyping polymorphic markers at the loci detected in stage 1 in 17 additional families, thus extending the number of pedigrees to 35 (782 individuals). *P* values were then calculated by use of two-point allele-sharing methods (Elston 1997) for the entire sample and were compared with those of the initial sampled pedigrees in stage 1. Loci harboring FCHL genes are expected to attain or improve the stage 1 level of significance for the region, whereas loci observed by chance in stage 1 are expected to exhibit reduced significance.

Stage 1 Analysis

The complete linkage map consisted of 399 markers spanning ~10-cM intervals (Yuan et al. 1997). All 22 autosomes and the X and Y chromosomes were typed, and the heterozygosity of the markers averaged .82 in the families analyzed. Table 2 presents the maximum LOD score obtained for each chromosome, along with the marker nearest to the peak. In the initial scan, no locus with a LOD score >3.0 was identified; two loci (on chromosomes 2 and 11) had LOD scores >2.0, and two other loci (on chromosomes 16 and 19) had LOD scores >1.0. Figure 2 shows the multipoint analyses of the four chromosomes exhibiting suggestive LOD scores. All four loci with LOD scores >1.0 were examined further. A locus for FCHL identified in the Finnish isolate (Pajukanta et al. 1998) did not show evidence for linkage in the Dutch pedigrees, although a modest peak for tri-

glyceride levels (LOD = 1.0) was observed (data not shown).

We also tested for linkage several quantitative traits associated with FCHL, including total plasma levels of triglycerides, LDL-cholesterol, apoB, apoCIII, and HDL-cholesterol, as well as two empirically derived intermediate phenotypes, the triglyceride-to-apoCIII ratio and non-HDL-cholesterol levels (described in Families and Methods). Analysis of these component quantitative traits and derived phenotypes in the genome scan failed to produce LOD scores > 1.0 that were coincident with any of the four suggestive regions identified for the qualitative FCHL phenotype (data not shown). This finding is consistent with the concept that the combined parameters for the disorder may be a better diagnostic indicator than individual lipid or apo levels.

Stage 2 Analysis

The suggestive loci identified in the multipoint genome scan were further examined in the entire set of pedigrees comprising 35 families and 782 individuals. At each of the four loci, the peak marker and an additional closely linked marker were typed in all family members (table 3). A direct comparison of the results from the initial genome-scan linkage analysis with the results from the eight markers typed in the entire population was not possible. The MAPMAKER/SIBS program evaluates multipoint linkage at a locus by using marker information along the whole chromosome, which was available only for the 240 individuals in the genome scan.

Table 3

Two-Point Linkage Analyses in Regions Showing Multipoint LOD scores > 1.0 in the Genome Scan

Chromosome and Marker	Stage 1			Stage 2		
	No. of ASP[a]	Allele Sharing	*P*	No. of ASP[a]	Allele Sharing	*P*
2:						
D2S2952	79	.62	.0005	206	.51	.23
D2S319	…	…	…	162	.48	.82
11:						
D11S1985	81	.55	.06	116	.56	.02
D11S1324	…	…	…	181	.55	.01
16:						
D16S402	77	.53	.21	151	.56	.01
D16S516	…	…	…	167	.51	.40
19:						
D19S589	81	.52	.25	179	.53	.10
D19S927	…	…	…	200	.51	.01

[a] ASP = affected sibling pairs. The number of informative sibling pairs (of the possible 115 ASP genotyped) is given.

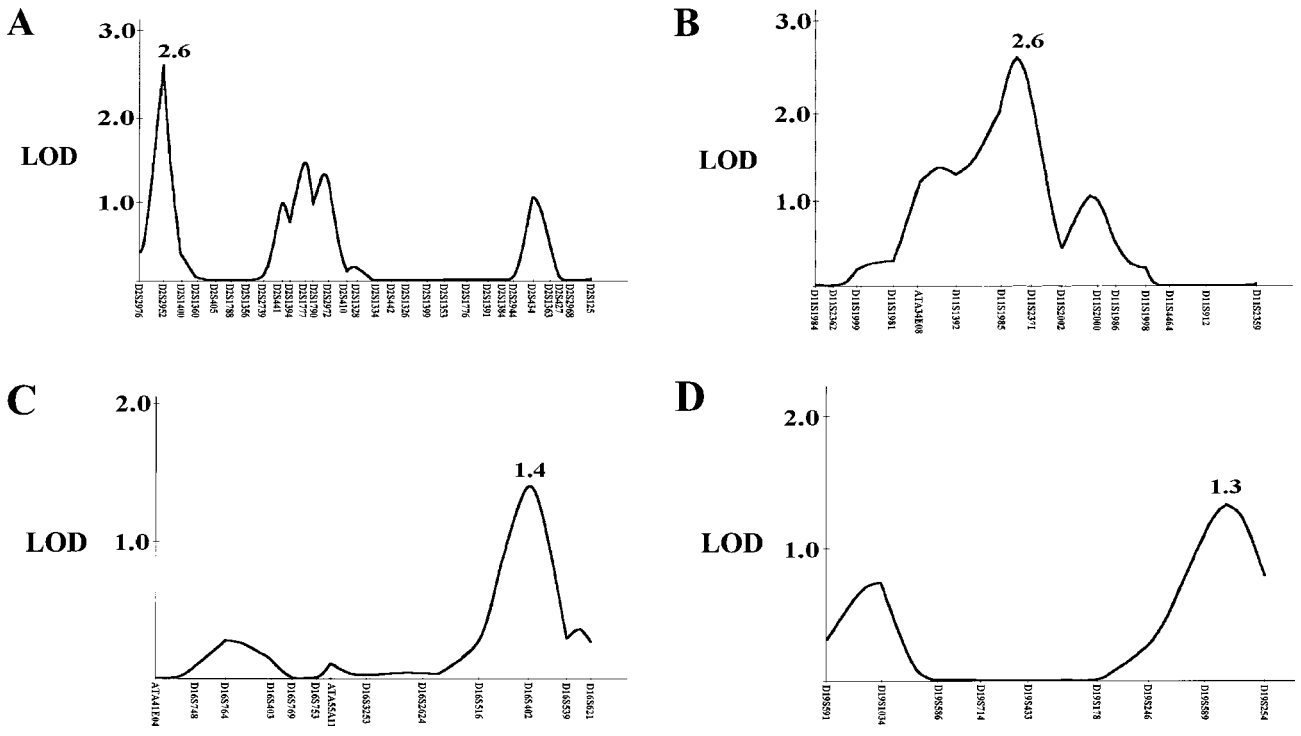

Figure 2 Potentially linked loci (n = 240) identified in the genome scan of 18 FCHL families. Multipoint plots for chromosomes 2, 11, 16, and 19 (*A, B, C,* and *D,* respectively) were obtained with use of the MAPMAKER/SIBS program. The chromosome length spanned by genetic markers on chromosomes 2, 11, 16, and 19 are 294, 147, 124, and 94 cM, respectively. The locations of genetic markers typed for each chromosome are indicated at the bottom of each plot. Intrachromosomal genetic distances and additional genetic marker information are available at the Marshfield Center for Human Genetics Website.

Therefore, a two-point linkage analysis was used to examine the extended population (Elston 1997). The peak LOD-score marker within each of the four loci identified by the genomewide scan was first reanalyzed with use of two-point allele-sharing estimates in the affected sibling pairs. We evaluated the entire population for linkage by using the same two-point method, which made it possible to compare the results from the genome scan directly with the results from the entire population.

Table 3 shows the two-point linkage results for markers typed at these loci. For each marker, the mean allele sharing and the result of the test for its difference from .50 is given, expressed in terms of a *P* value. When extended from the initial sample, the locus on chromosome 2 exhibited a decrease in allele sharing and a nonsignificant linkage test for both markers (table 3). In the extended pedigrees, the degree of allele sharing among affected siblings at the chromosome 16 locus was significant at one marker (D16S402; $P = .01$) but not at the other marker (D16S516; $P = .40$). Similarly, at the chromosome 19 locus, one marker yielded evidence for linkage (D19S927; $P = .01$), whereas the peak LOD-score marker did not exhibit significant linkage (D19S589; $P = .10$). In contrast, at the chromosome 11p locus, the peak marker yielded evidence for linkage (D11S1985; $P = .02$), as did the other nearby marker not typed in the genome scan (D11S1324; $P = .01$). On the basis of these data, the chromosome 11 locus was further examined for evidence of linkage and potential genetic heterogeneity.

Evaluating Evidence for an FCHL Locus on Chromosome 11

To further evaluate the chromosome 11 locus, we typed additional markers to better define the peak location of the putative FCHL gene. In addition to an affected-sibling-pair analysis, we also performed a linear-regression analysis with these markers, using all available sibling pairs. As shown in table 4, the peak linkage in both analyses was observed with marker D11S1324. To maximize the informativeness of the region, we also constructed haplotypes by using the markers surrounding the peak (D11S1324, D11S1392, and D11S1985) and tested the haplotypes for linkage by using both the mean-allele-sharing test and the regression test (table 4). The affected sibling pairs demonstrated a significant mean allele sharing of .56, and the two-point *P* values increased in significance for the haplotype ($P = .004$), compared with the individual markers (table 4). When all available sibling pairs were used in the linear-regression analysis, evidence for linkage with the haplotype was also observed ($P = .002$; fig. 3). This *P* value corresponds to a LOD score of ~1.9. The fact that it is lower in magnitude than the peak LOD score of 2.6 observed, for chromosome 11, in the original scan presumably relates to the fact that it is based on a haplotype of three markers rather than on the increased power of a complete multipoint analysis. Furthermore, the clinically discordant sibling pairs (one affected and one unaffected) exhibited a significantly decreased mean allele-sharing of .44 ($P = .007$) for the haplotype, consistent with linkage of FCHL to this region.

The possibility exists that FCHL is genetically heterogeneous (Williams and Lalouel 1982; Aouizerat et al. 1999*a*). Using the peak marker at the chromosome 11 locus, D11S1324, we analyzed individual families to test for heterogeneity. The families were initially analyzed nonparametrically with GENEHUNTER (Kruglyak et al. 1996; Kruglyak and Lander 1998), which, analogous to SIBPAL, assesses the degree of allele sharing, among affected sibling pairs, at a marker locus. An advantage of GENEHUNTER for these nonparametric analyses is that it provides an estimate of the relative informativeness, for each family, at the marker locus. No evidence of genetic heterogeneity was observed at the chromosome 11 locus (data not shown). However, because nonparametric approaches have relatively low power for detection of linkage and heterogeneity (Ott 1991), a parametric analysis was also done. Parametric analyses require the specification of a mode of inheritance, disease-allele frequency, and degree of penetrance. For the purposes of these analyses, the mode of inheritance was assumed to be dominant, as originally suggested for FCHL (Goldstein et al. 1973), and on the basis of a population prevalence of 1%—2%, the disease-allele frequency was set at 0.6%. We adopted an affecteds-

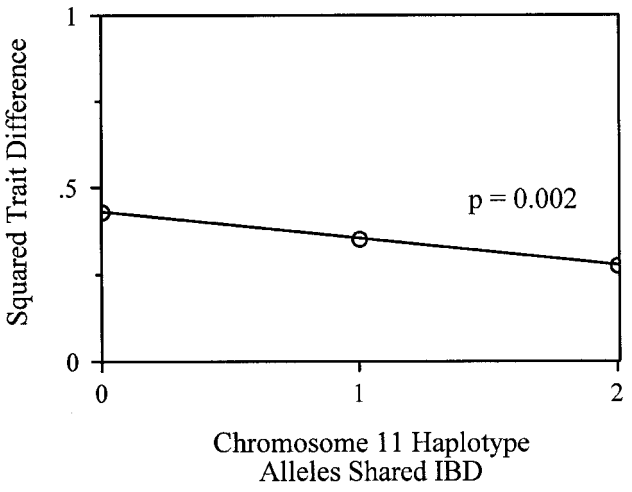

Figure 3 Linear-regression analysis, for chromosome 11 haplotypes with FCHL. Negative regression of squared-trait differences for FCHL versus the number of marker alleles shared IBD are shown. The number of sibling pairs sharing zero, one, or two alleles IBD is 151, 289, and 146, respectively.

Table 4

Two-Point Sibling-Pair-Linkage Analysis Results, for Chromosome 11 Markers and FCHL

		ASP[b] Analysis			LR[c] Analysis	
Marker	Distance[a] (cM)	No. of ASP	Allele Sharing	*P*[d]	Edf[e]	*P*[d]
D11S1999	17	159	.52	.25	221	.05
D11S1981	29	162	.52	.16	229	.44
ATA34E08	37	165	.54	.05	245	.04
D11S1324	41	181	.55	.01	247	.002
D11S1392	50	208	.54	.02	266	.06
D11S1985	65	116	.56	.02	183	.01
D11S2371	75	160	.51	.40	221	.25
D11S2002	85	131	.52	.17	218	.61
Haplotype[f]	...	219	.56	.004	291	.002

[a] From pter of chromosome 11.
[b] ASP = affected sibling pairs.
[c] LR = linear regression.
[d] Significant *P* values are underlined.
[e] Edf = effective df.
[f] The haplotype was constructed with use of markers D11S1324, D11S1392, and D11S1985.

only approach, and therefore the penetrance was set at 100%. A LOD-score–based statistic, generated by POLYPREP, was used to test for heterogeneity of FCHL at this locus. The total LOD score for this analysis with the assumption of heterogeneity did not deviate significantly from the original parametric analysis with the assumption of homogeneity (ΔLOD < 0.12; data not shown), thus providing no significant evidence of heterogeneity at marker D11S1324.

Discussion

The molecular and genetic factors contributing to FCHL have remained largely unknown since the discovery of the trait, >25 years ago. Originally, FCHL was postulated to exhibit an autosomal dominant Mendelian pattern of inheritance (Goldstein et al. 1973), but subsequent analyses have suggested a more complex pattern (Williams and Lalouel 1982; Jarvik et al. 1993; Cullen et al. 1994; Bredie et al. 1997*b* Aouizerat et al. 1999*a*). Physiological and molecular studies have provided evidence that abnormalities associated with FCHL include overproduction of VLDL, altered lipid metabolism in peripheral tissues, and reduced catabolism of triglyceride-rich lipoproteins. Candidate-gene studies have identified possible modifier genes for FCHL, including variations of the LPL gene on chromosome 8 (Yang et al. 1996) and the apoAI-CIII-AIV gene cluster on chromosome 11 (Dallinga-Thie et al. 1997). We now report a genome scan for FCHL, done in a series of Dutch pedigrees. The results provide evidence for at least one locus that may harbor a gene(s) for FCHL. Furthermore, these results also support the notion that the genetic architecture of the disease is complex, which is consistent with previously reported segregation analyses.

Our comprehensive genome screen identified two loci with LOD scores >2.0 and two loci with LOD scores 1.0–2.0. Because of the extensive number of linkage tests involved, we assumed that at least one of these loci was likely to have occurred by chance. Therefore, we typed markers at each of these four loci in 542 additional family members to test which, if any, would continue to exhibit evidence of linkage. The chromosome 2 locus failed the test of replication. The chromosome 16q and 19q results were suggestive and merit further investigation. However, the results for chromosome 11p were supportive, since the *P* values of markers typed in the region improved with the additional families examined. Moreover, typing additional markers in the region allowed haplotype analysis, which provided stronger evidence for linkage. The only obvious candidate gene related to lipid metabolism, identified in the 11p region, is that for the oxysterol-binding protein (OSBP; Entrez Database), which binds a range of biologically active sterols, including 25-hydroxysterol (Dawson et al. 1989; Levanon et al. 1990). However, recent studies of the role of OSBP in cellular cholesterol metabolism suggest that it is unlikely to underlie the FCHL trait (Ridgway et al. 1998; Storey et al. 1998; Lagace et al. 1999).

There was no evidence for genetic heterogeneity in the nonparametric analysis when the chromosome 11 locus (D11S1324) was tested. It is possible that the variability of these pedigree structures (fig. 1) may not permit its detection in the nonparametric analysis. An additional factor is the relative informativeness of the genetic marker (D11S1324), which varied among families (data not shown). The results from an adoption of a parametric approach, which is not vulnerable to these factors, also did not reveal evidence of genetic heterogeneity. However, these results are dependent on the accuracy of estimated parameters, such as disease-allele frequency, recombination fraction, and penetrance, none of which are easily assigned to a complex trait such as FCHL. Genetic heterogeneity is also revealed by the comparison of our results with those seen in a separate FCHL population. In a previous study on the Finnish isolate, strong evidence was provided for a major FCHL locus on human chromosome 1q21 (Pajukanta et al. 1998). However, this locus, identified on 1q21 (peak markers D1S104 and D1S1677), did not show significant evidence of linkage in the Dutch pedigrees reported here.

Complex segregation analyses have been applied to estimate the number of genes influencing FCHL, and evidence has been presented suggesting a model with only one or two major loci affecting serum triglyceride levels and/or apoB levels (Cullen et al. 1994; Jarvik et

al. 1994). Our results are consistent with a more complex pattern of inheritance involving multiple genetic factors or extensive heterogeneity. No locus with a LOD score >3.0 was observed; therefore, it is highly unlikely that FCHL results from a single major predisposing gene in this Dutch population. Similarly, no locus with a LOD score >3.0 was observed for any of the primary quantitative traits used in the diagnosis of FCHL, including levels of plasma triglycerides, cholesterol, apoB, and apoCIII (data not shown). Furthermore, these component quantitative traits failed to reveal evidence for linkage in regions identified by the analysis of the qualitative FCHL phenotype. This is best exemplified in the chromosome 11p candidate region, suggesting that the individual quantitative measurements may not accurately encompass the FCHL trait and that the qualitative FCHL phenotype may be a more accurate means of diagnosis.

An issue confounding the genetic analysis of FCHL is its mode of inheritance. The very large empiric risk (approaching 50% in some cases) to first-degree relatives has suggested, to some investigators, an autosomal dominant mode of inheritance. Although a number of segregation analyses have yielded results inconsistent with the dominant model, it does remain a possibility (Williams and Lalouel 1982; Jarvik et al. 1994; Bredie et al. 1996; Bredie et al. 1997*b*; Juo et al. 1998). One of the potential inferences from these results is that autosomal dominant inheritance is very unlikely as a potential explanation for FCHL in the Dutch population. We did not observe a LOD score ≥ 3.0, using a large set of affected sibling pairs and a 10-cM-density genome scan. Linkage analysis for an autosomal dominant locus, contributing a risk to siblings (λ_s) of 3.0, should yield a LOD score of 3.3, given the large number of sibling pairs and the extensive pedigrees in the present study (Hauser et al. 1996). Yet, given both the empiric risk to sibs and the population frequency, FCHL has a sibling-risk estimate of $\lambda_s > 20$. Thus the results reported here contribute to the evidence that FCHL is complex, since traits exhibiting Mendelian inheritance would be expected to yield a highly significant LOD score at one locus. These results indicate that FCHL may develop from the additive effects of many genes, a high degree of genetic heterogeneity, or gene-gene and gene-environment interactions.

To conclude, the results of this genomewide scan provide evidence for a novel region, on chromosome 11, contributing to the FCHL phenotype in 35 Dutch families. At this time, chromosome 11 should still be considered a hypothesis-generating locus, and additional families should be studied to provide definitive evidence. It is now clear that FCHL is complex and heterogeneous, emphasizing the need for a variety of approaches, both biochemical and genetic, and for studies of multiple populations.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Entrez Database Browser, http://www.ncbi.nlm.nih.gov/Entrez/ (for mapping information on OSBP)
Généthon, http://www.genethon.fr/genethon_en.html (for CEPH database)
Marshfield Center for Human Genetics, http://www.marshmed.org/genetics/ (for genome screen marker information)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for FCHL [MIM 144250])

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