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Genomewide Scan for Familial Combined Hyperlipidemia Genes in Finnish Families, Suggesting Multiple Susceptibility Loci Influencing Triglyceride, Cholesterol, and Apolipoprotein B Levels

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Summary

Familial combined hyperlipidemia (FCHL) is a common dyslipidemia predisposing to premature coronary heart disease (CHD). The disease is characterized by increased levels of serum total cholesterol (TC), triglycerides (TGs), or both. We recently localized the first locus for FCHL, on chromosome 1q21-q23. In the present study, a genomewide screen for additional FCHL loci was performed. In stage 1, we genotyped 368 polymorphic markers in 35 carefully characterized Finnish FCHL families. We identified six chromosomal regions with markers showing LOD score (*Z*) values >1.0, by using a dominant mode of inheritance for the FCHL trait. In addition, two more regions emerged showing $Z > 2.0$ with a TG trait. In stage 2, we genotyped 26 more markers and seven additional FCHL families for these interesting regions. Two chromosomal regions revealed $Z > 2.0$ in the linkage analysis: 10p11.2, $Z = 3.20$ ($\theta = .00$), with the TG trait; and 21q21, $Z = 2.24$ ($\theta = .10$), with the apoB trait. Furthermore, two more chromosomal regions produced $Z > 2.0$ in the affected-sib-pair analysis: 10q11.2-10qter produced $Z = 2.59$ with the TC trait and $Z = 2.29$ with FCHL, and 2q31 produced $Z = 2.25$ with the TG trait. Our results suggest additional putative loci influencing FCHL in Finnish families, some potentially affecting TG levels and some potentially affecting TC or apoB levels.

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Introduction

Familial combined hyperlipidemia (FCHL) is a common dyslipidemia affecting ~1%–2% of the populations in Western societies (Grundy et al. 1987). FCHL is characterized by elevated serum total cholesterol (TC), elevated triglycerides (TGs), or both, with affected individuals aggregating in families (Goldstein et al. 1973; Nikkilä and Aro 1973). A consistent metabolic finding in FCHL patients is increased apolipoprotein B (apoB) concentration, although it is not clear whether an increased production or decreased clearance of apoB-containing lipoproteins predominates (Castro Cabezas et al. 1993; de Graaf and Stalenhoef 1998). Other metabolic abnormalities in FCHL include glucose intolerance (Vakkilainen et al. 1998), insulin resistance (Aitman et al. 1997), and decreased adipocyte lipolysis (Reynisdottir et al. 1995; Arner 1997). It is thus evident that FCHL shares many features of the metabolic syndrome (Reaven 1988). As a consequence of these lipid disturbances, FCHL patients are prone to early-onset coronary heart disease (CHD). In fact, FCHL is one of the most common familial dyslipidemias associated with premature CHD (Genest et al. 1992).

The genetic component in combination with environmental factors is predicted to play an important role in the etiology of FCHL. However, difficulties resulting from genetic heterogeneity, unknown mode of inheritance, and lack of standardized diagnostic criteria for FCHL have hampered studies aiming to reveal the molecular defect behind this disorder. By using the candidate-gene approach, researchers have investigated genes encoding proteins that may be involved in FCHL pathophysiology. To date, the lipoprotein lipase gene (Gagne et al. 1994; Reymer et al. 1995; Yang et al. 1995; Marcil et al. 1996) and the apolipoprotein AICIIIAIV gene cluster (Wojciechowski et al. 1991; Dallinga-Thie et al.

1997; Wijsman et al. 1998) have been studied most thoroughly. However, contradictory data for the involvement of these candidate genes have emerged. In addition to inconsistent diagnostic criteria, population-based factors may explain some of the contradictions with respect to FCHL-candidate genes, because the alleles have different frequencies in different populations, and environmental risk factors may also differ substantially among populations (Kaufman et al. 1986). To summarize the candidate-gene studies, none of the genes examined seems to explain the major genetic component of FCHL.

Genomewide scans are widely used for systematic screening to identify possible susceptibility loci. In this approach, no a priori knowledge of disease pathophysiology is presumed. To date, genomewide scans have been done for a number of different complex diseases (e.g., see Ginns et al. 1996; Hanis et al. 1996; Sawcer et al. 1996) but not for FCHL. Our analysis of 10 chromosomal regions containing candidate genes revealed the first novel FCHL locus on chromosome 1q21-q23, in the vicinity of the gene encoding apolipoprotein AII (Pajukanta et al. 1998). However, the most obvious candidate genes in this region (apolipoprotein AII and the selectin gene cluster) were positionally excluded, and, most probably, this FCHL locus represents a novel, still-unknown gene. To search for additional major loci modifying susceptibility to FCHL, we performed a genomewide scan in essentially this same set of Finnish families.

Subjects and Methods

Pedigrees

As part of the European Multicenter Study on Familial Dyslipidemias (EUFAM) study (Porkka et al. 1997), 35 Finnish FCHL families were selected for genetic analyses in stage 1, and 7 more were included in stage 2. Individuals from these 42 Finnish FCHL families were recruited in the Helsinki (23 families), Turku (11 families), and Kuopio (8 families) university hospitals as a part of the EUFAM study (Pajukanta et al. 1998). The inclusion criteria for probands were the following: age 30–55 years for men and 30–65 for women, premature CHD confirmed by either angiography (38 probands) or myocardial infarction (MI) (4 probands), and serum TC and/or serum TGs equal to or greater than the age-sex–specific 90th percentile. The CHD events were validated on the basis of the medical records. At least two physicians at the university hospitals confirmed the CHD diagnosis of every proband, on the basis of either angiography (>50% stenosis in one or more coronary arteries) or MI. MI was diagnosed on the basis of the following criteria: (1) typical clinical symptoms; (2) definite electrocardiography findings, according to Minnesota coding (World Health Organization criteria) (Rose et al. 1982); and (3) elevated levels of the creatine kinase (CK) enzyme and its cardiac isoenzyme, CK-MB. Individuals with type I diabetes, hypothyreosis, hepatic or renal disease, or an LDL-receptor defect were excluded as probands. Details of the family collection have been reported elsewhere (Porkka et al. 1997; Pajukanta et al. 1998). We selected families in which, besides premature CHD, either the proband or a first-degree relative had the combined phenotype IIB. As in our earlier study (Pajukanta et al. 1998), family members were considered to be affected with FCHL if they had either (*a*) both elevated TC and elevated TGs (combined lipid phenotype) or (*b*) either high TC (phenotype IIA) or high TGs (phenotype IV) (i.e., above the age-sex–specific 90th percentiles). In addition (in extended pedigrees), it was required that each individual with only elevated TC or elevated TGs also had a first-degree relative with the combined phenotype IIB, in order to be scored as affected for the purpose of this analysis. This strategy has been adopted to allow more certainty about the FCHL diagnosis; elevation of only one lipid class (TC or TGs) is common because of several environmental causes, as well as because of genetic causes other than FCHL. This strategy results in the ascertainment of a combined phenotype of FCHL.

The study design was approved by the ethical committees of the participating centers, and all samples were collected in accordance with the Helsinki declaration. All participants gave their informed consent.

Biochemical Analysis

All blood samples were taken after an overnight fast. The lipid measurements were performed in the laboratories of the Helsinki and Kuopio university hospitals. Serum TC and TGs were measured by enzymatic methods (Boehringer-Mannheim and Hoffman-La Roche). Serum apoB was measured by immunoturbidimetric methods (Orion Diagnostica and Kone Instruments). Lipid-lowering medication was interrupted for 4 wk before blood sampling. The lipid criteria used for classification of study subjects ages $\geq$25 years were derived from a large Finnish population study, FINMONICA (n = 6,022) (Vartiainen et al. 1994). The percentile values for 60–65-year-olds were applied to all individuals >60 years old. The cutoff for the upper 90th percentile of each phenotype distribution for subjects <25 years old was derived from the follow-up samples in 1986 of another large Finnish population study, the Cardiovascular Risk Factors in Young Finns study (n = 2,236) (Porkka et al. 1994). Lipid-assay differences between centers were quantified in small subsamples (n = 50–215) by linear regression analysis, and the levels were unified accordingly. The age-sex–specific 90th percentiles for TC, TGs, and apoB for the Finnish population

will be available on the National Public Health Institute of Finland, Department of Human Molecular Genetics Webpage. Familial hypercholesterolemia was excluded from each pedigree by determination of the LDL-receptor status of the proband, by the lymphocyte-culture method (Cuthbert et al. 1986). Furthermore, in clinical examination, none of the participants had tendon xanthomas. In the studied FCHL families, one to three lipid measurements (the median being one measurement) were obtained from each study subject for the lipid phenotyping. For classification purposes, the highest untreated lipid measurement was used.

Stage 1 and Stage 2: Genotyping of the Markers

DNA was extracted from EDTA blood according to a standard procedure. In stage 1, a total of 368 microsatellite markers from a modified Weber screening set (version 6) (Sheffield et al. 1995) were typed in 168 individuals from 35 Finnish FCHL families, of which 135 individuals were affected with FCHL. The "unaffected" individuals were genotyped only to increase phase information and were treated as if their phenotypes were unknown in the statistical analysis, because of the nondeterministic genotype-phenotype relationship hypothesized for this complex phenotype (see the Statistical Analyses subsection, below). The average intermarker distance was 10.5 cM. Microtiter-well PCR amplification of the markers was automated by use of a pipetting robot (Biomek 2000; Beckman). The fluorescently labeled PCR products were electrophoretically separated on an automated laser fluorescence DNA sequencer ABI 377 (Perkin-Elmer), with GENESCAN (version 2.1) fragment-analysis software. The alleles were identified by the GENOTYPER program (version 2.0) (Perkin-Elmer). The interpretation of alleles was checked by two different individuals, to verify Mendelian segregation. Some of the markers, which did not yield satisfactory results after two PCRs and gel electrophoresis, were replaced by additional linked markers from either the Généthon marker map (Dib et al. 1996) or the Cooperative Human Linkage Center marker map (Sheffield et al. 1995).

In stage 2, further analysis was conducted on the five regions where the LOD score (Z) with the FCHL trait was >1.0, under the assumption of a dominant mode of inheritance. In addition, a denser marker map was studied in two further chromosomal regions where $Z > 2.0$ with a second trait, elevated TGs. A denser marker map for these initially interesting areas was analyzed by the typing of 26 new markers. Seven additional FCHL families (33 individuals) were included in stage 2 of the study. Altogether, 201 samples were genotyped for these 26 markers, by the ABI 377 sequencer. These additional samples were typed both for all of these new markers and for the markers producing the highest Z values in stage 1. The markers analyzed in stage 2 were selected and mapped on the basis of information derived from the genetic maps of the Cooperative Human Linkage Center and Généthon.

Statistical Analyses

Two-point pairwise linkage analysis was performed by the MLINK program of the linkage package (Lathrop et al. 1984), FASTLINK version 2.2 (Cottingham et al. 1993; Schäffer et al. 1994). The identity-by-descent (IBD) status in the affected sib pairs (ASPs) was analyzed by the SIBPAIR program (Kuokkanen et al. 1996). In both stages, the data were analyzed with both linkage analysis and ASP analysis. To control for the possibility of bilineal transmission, etiologic heterogeneity, and potential effects of inappropriate diagnostic classifications, Z analysis was also done on the component nuclear pedigrees as if they were independent. Both ASP analysis (which disregards parental-phenotype information) and Z analysis of these nuclear pedigrees (which uses parental-phenotype information) were done. To circumvent problems of incomplete penetrance and genetic ambiguity of the phenotype "unaffected," an affecteds-only strategy was used, by coding the subjects as either affected or unknown, on the basis of the age-sex–specific 90th percentile for the measured lipids. The unaffected family members were genotyped only to maximize the marker-locus genotype and phase information. In addition to the FCHL trait, we also performed linkage analyses treating elevated TC, TGs, or apoB separately, using age-sex–specific 90th-percentile thresholds for the affecteds, and again coded as unknown those individuals with values lower than the 90th percentile. These analyses were done to see whether we could identify any loci predisposing to one of those traits individually that might not be involved in the more extreme FCHL phenotype. As in our previous study (Pajukanta et al. 1998), we performed linkage analyses by assuming disease-allele frequencies of .006 under the dominant model and .1095 under the recessive model, on the basis of the proposed 1.0%–2.0% prevalence of FCHL (Grundy et al. 1987). No phenocopies were allowed for. This approach used in linkage analysis is a Z equivalent of model-free methods that reconstruct genotypes to make every meiosis potentially informative for linkage at the disease locus, as explained by Trembath et al. (1997) and Terwilliger (1998). Thus, this method is equivalent to model-free methods, even though linkage is used to perform the likelihood computations. We also conducted a multipoint analysis for the most positive markers identified in linkage analysis, using the same multipoint analysis strategy as we had adopted in our earlier study (Pajukanta et al. 1998). The multipoint analysis was not

performed with flanking markers moving the disease across the map, because of the known propensity for false exclusions in that method (Risch and Giuffra 1992). To avoid the known negative side effects of multipoint analysis, we performed a multipoint analysis in which the markers were placed in a fixed order and in which the disease locus was allowed to vary outside the map of markers (Terwilliger and Ott 1993), using the MLINK program. Using this method, we found that meioses uninformative for some markers can be scored for nearby markers, thus allowing all meioses in all families to be scored in the analyses.

For each marker, the allele frequencies were estimated from the total study sample by an allele-counting method. Genetic heterogeneity between the families was tested by the HOMOG program (Ott 1991; Kuokkanen et al. 1996).

Results

All the results obtained from the linkage analysis adopting an affecteds-only strategy and from the ASP analysis for all markers, with FCHL, TC, TGs, and apoB as phenotypic traits, are available on the National Public Health Institute of Finland, Department of Human Molecular Genetics Webpage.

FCHL Trait

In stage 1 of the genome scan, six markers, on chromosomes 1, 8–10, 21, and 22, gave pairwise $Z > 1.0$, by a dominant model for FCHL susceptibility in the linkage analysis (table 1). The chromosome 1 marker D1S1677, which produced $Z = 1.36$ ($\theta = .08$), overlapped the area, on chromosome 1q21-q23, that, in an earlier study (Pajukanta et al. 1998), we had identified as a novel FCHL locus. In that study, this same marker resulted in $Z = 0.91$ ($\theta = .10$). Of the families analyzed here, 31 of 35 in stage 1 had been included in our earlier study. Consequently, no further markers were genotyped in this area. The results of both the linkage analysis and the ASP analysis of these six chromosomal regions are given in table 1. The highest Z value was obtained on chromosome 10q11.2-10qter, with the marker D10S169 ($Z = 2.23$ [$P < .0007$]), in the ASP analysis (table 1).

In stage 2, the interesting regions of chromosomes 8–10, 21, and 22 were studied further. Additional markers ($n = 20$) were genotyped in the 35 families and in 7 additional families included in stage 2. The markers of the initial scan that produced the highest Z values in stage 1 were also genotyped in these seven families. Only two of these regions were further supported in stage 2 (table 1). On chromosome 10, marker D10S169 resulted in $Z = 2.29$ ($P < .0006$) in the ASP analysis (table 1), and the nearby marker D10S1651 resulted in $Z = 0.84$ ($P = .02$) in the ASP analysis. Furthermore, on chromosome 21, marker D21S1437 resulted in $Z = 1.29$ ($\theta = .16$) in the linkage analysis (table 1).

For chromosomes 8, 9, and 22, the Z values obtained in linkage analyses decreased in stage 2. None of the new markers in these regions resulted in $Z > 1.0$, suggesting that the initial $Z > 1.0$, in stage 1, could have been due to chance. The detailed results for these chromosomes in stage 2 are available on the National Public Health Institute of Finland, Department of Human Molecular Genetics Webpage.

Markers D8S1128 and D10S169 (as described above) also resulted in $Z > 1.0$ in the ASP analysis. In addition,

Table 1

Results of the Two-Point Linkage Analysis and ASP Analysis with the Markers Resulting in $Z > 1.0$ for the FCHL Trait

		Maximum Z[a] (θ) in Linkage Analysis/Z in ASP Analysis			
Chromosome	Marker	FCHL	TG	TC	apoB
Stage 1:					
1	D1S1677	1.36 (.08)/.82	.45 (.18)/1.02	.14 (.24)/.35	.00 (.50)/.00
8	D8S1128	1.54 (.14)/1.31	.58 (.16)/.21	.05 (.34)/.18	.06 (.32)/.03
9	D9S1122	1.11 (.12)/.70	.82 (.14)/.77	.12 (.26)/.05	1.18 (.12)/.74
10	D10S169	1.66 (.14)/2.23	.73 (.16)/.83	.92 (.16)/1.43	.94 (.16)/1.14
21	D21S1437	1.15 (.16)/.40	1.14 (.12)/1.29	.50 (.22)/.05	2.13 (.08)/.67
22	D22S683	1.00 (.22)/.98	.64 (.22)/.15	.47 (.24)/1.04	.31 (.28)/.79
Stage 2:[b]					
8	D8S1128	1.37 (.16)/1.07	.77 (.16)/.13	.14 (.32)/.29	.09 (.30)/.01
9	D9S1122	.81 (.16)/.46	.71 (.16)/.67	.03 (.32)/.00	.72 (.18)/.41
10	D10S169	1.61 (.14)/2.29	.45 (.20)/.54	1.53 (.14)/2.59	1.00 (.16)/1.42
21	D21S1437	1.29 (.16)/.46	1.03 (.14)/1.38	.71 (.20)/.12	2.24 (.10)/.57
22	D22S683	.78 (.24)/.57	.53 (.24)/.03	.37 (.28)/.70	.15 (.32)/.34

[a] For a dominant mode of inheritance.

[b] For chromosome 1, no further markers were genotyped in stage 2, since the area overlapped the novel FCHL locus region, 1q21-q23 (Pajukanta et al. 1998).

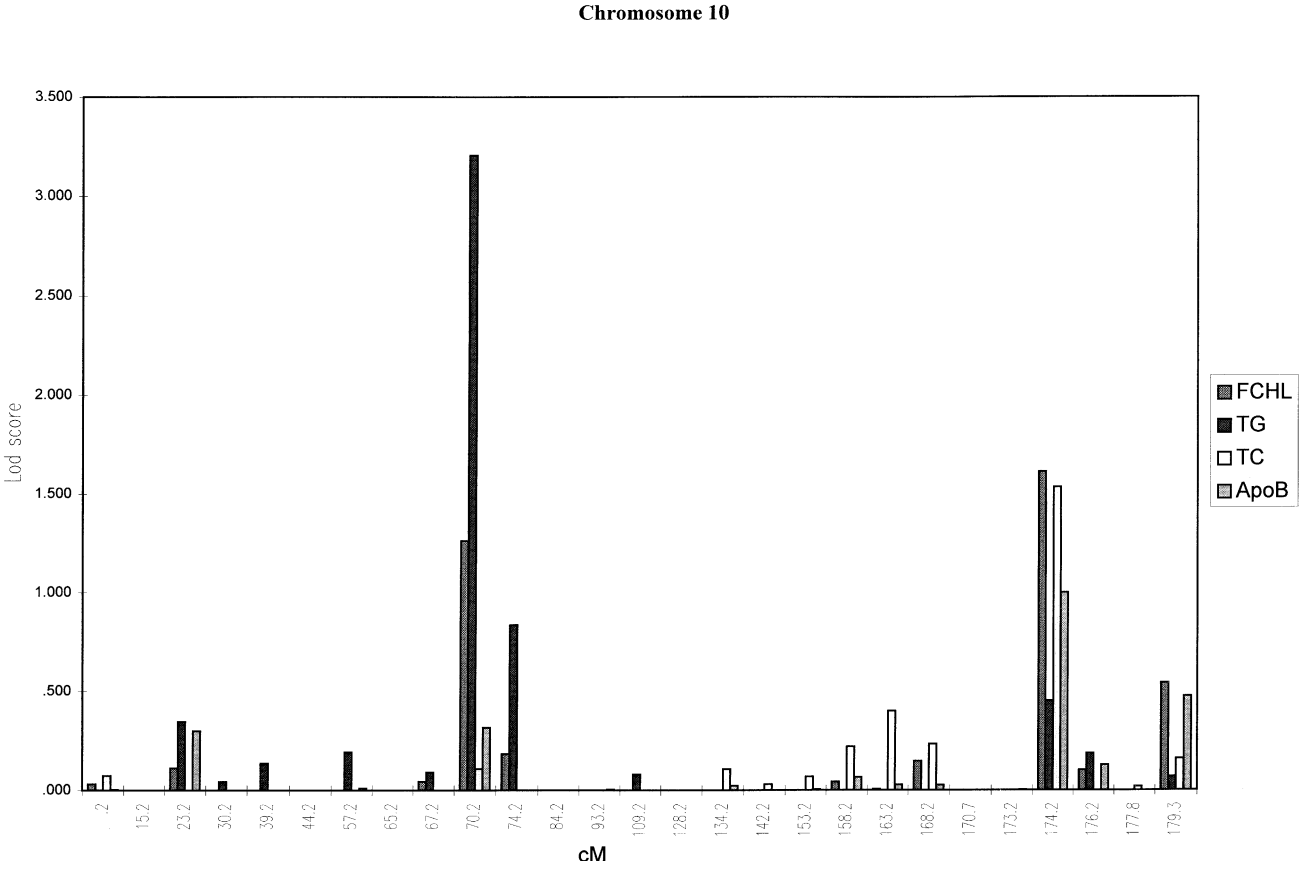

Figure 1 Results of two-point linkage analysis with a dominant mode of inheritance, for individual markers genotyped for chromosome 10, in stage 1 and 2, with traits FCHL, TC, TG, and apoB.

there were four other markers resulting in $Z > 1.0$ in the ASP analysis: D9S2169 ($Z = 1.30$ in ASP analysis and $Z = 0.74$ in linkage analysis [$\theta = .10$]), D11S4464 ($Z = 1.13$ in ASP analysis and $Z = 0.71$ in linkage analysis [$\theta = .22$]), D12S375 ($Z = 1.01$ in ASP analysis and $Z = 0.43$ in linkage analysis [$\theta = .18$]), and D16S753 ($Z = 1.15$ in ASP analysis and $Z = 0.85$ in linkage analysis [$\theta = .22$]). A recessive mode of inheritance was also tested, both for these markers and for all other markers on chromosomes 9, 11, 12, and 16, in two-point linkage analysis of the 35 FCHL pedigrees. Except for D9S1122 (which was also detected with a dominant model for the FCHL trait in the large pedigrees; see above), all the markers in these four chromosomes resulted in $Z < 1.0$ when a recessive mode of inheritance in the extended pedigrees was assumed. This can be expected, given the pedigree structure of the families that we studied, because, in our study material, a relatively small number of sib pairs but multiple vertical transmissions were available. Consequently, there are more relatives who could share, at most, one allele—and not two alleles—identical by descent. Furthermore, the vertical relationships, such as parent-child pairs, are not informative in the recessive analysis, because the recessive analysis assumes that every affected individual has two disease alleles, and therefore meioses to his/her children are uninformative for linkage. No further markers were genotyped in these regions.

Individual Specific Traits of High TC, High TG, and High apoB

The results of the analyses of the individual lipid traits, in stages 1 and 2, for the six regions potentially interesting with regard to FCHL are presented in table 1, together with the FCHL trait analyses. Figures 1 and 2 show the Z values for all markers typed on chromosomes 10 and 21, with each of the four traits, when a dominant mode of inheritance is assumed in linkage analysis. Other chromosomal regions where any of the individual traits resulted in $Z > 1.0$ in two-point linkage analysis and/or ASP analysis are presented in table 2.

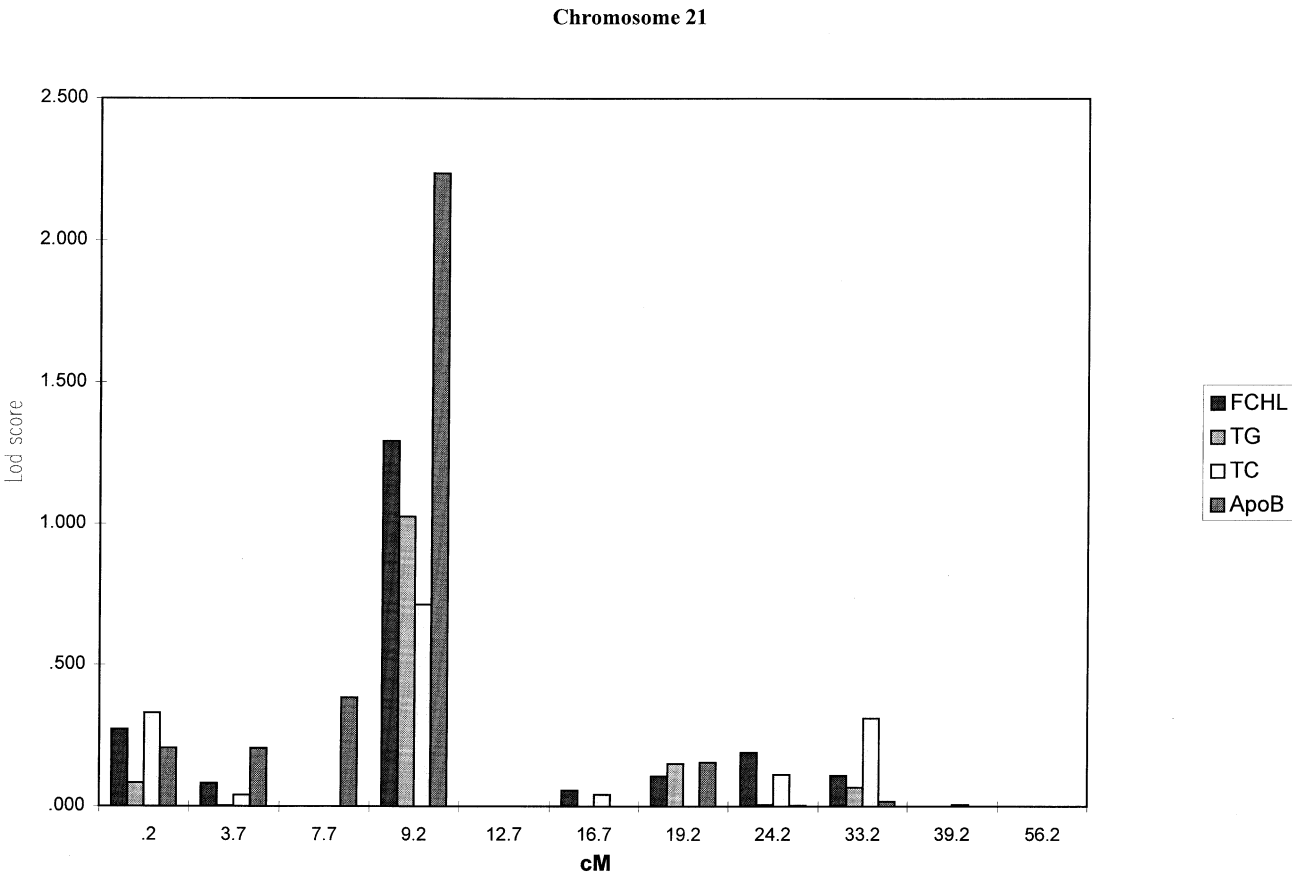

Figure 2 Results of two-point linkage analysis with a dominant mode of inheritance, for individual markers genotyped for chromosome 21, in stage 1 and 2, with traits FCHL, TC, TG, and apoB.

High TGs

In stage 1 of the genome scan, seven regions showed $Z > 1.0$ in linkage analysis, and one region did so in ASP analysis, with the TG trait (table 2). In two of these chromosomal regions $Z > 2.0$ was obtained: on chromosome 10, marker D10S1220 resulted in $Z = 2.57$ ($\theta = .00$) in linkage analysis, and, on chromosome 2, marker D2S1391 resulted in $Z = 2.02$ ($P = .001$) in ASP analysis. The results of the statistical analysis for the TG trait for the six regions identified with the FCHL trait are shown in table 1.

In stage 2, the inclusion of the seven additional families increased the Z value for marker D10S1220, from 2.57 ($\theta = .00$) to 3.20 ($\theta = .00$), in linkage analysis, in the affecteds-only strategy (table 3 and fig. 1). Worth noting is that these Z values were obtained at $\theta = .00$ and that no evidence of locus heterogeneity existed in these analyses. The nearby marker D10S1233 resulted in $Z = 0.84$ ($\theta = .10$). With the FCHL trait, marker D10S1220 resulted in $Z = 1.26$ ($\theta = .04$) in linkage analysis.

In stage 2, the Z value of marker D2S1391 increased from 2.02 ($P = .001$) to 2.25 ($P = .0006$), in ASP analysis (table 4). The most significant nearby marker (3.6 cM) was D2S148, with $Z = 0.78$ ($P = .029$) in ASP analysis (table 4).

High TC

In stage 1 of the genome scan, the highest Z value for the TC trait was obtained by ASP analysis in one of the six potentially interesting regions also identified for the FCHL trait: marker D10S169 exhibited $Z = 1.43$ ($P = .005$) in ASP analysis (table 1). In addition, in stage 1, the TC trait alone produced three different regions that, in both ASP analysis and linkage analysis, resulted in $Z > 1.0$ (table 2), although in neither of the analyses did any of the markers result in $Z > 2.0$. In stage 2, the Z value for marker D10S169 increased from 1.43 ($P = .005$) to 2.59 ($P < .0003$) with the TC trait, in ASP analysis (tables 1 and 4 and fig. 1).

High apoB

In stage 1 of the genome scan, the highest Z value for the apoB trait, 2.13 ($\theta = .08$), was obtained, with marker D21S1437 on chromosome 21, in linkage anal-

Table 2

Genome-Scan Markers Resulting in $Z > 1.0$ in Linkage Analysis or ASP Analysis, for Separate Traits

	Z		
Locus	Maximum[a]	Nuclear Family[b]	ASP
TG trait:			
D2S1326	1.395866	1.129947	.501995
D2S1391	1.22319	1.942121	2.023203
D5S408	1.086605	1.264405	1.033293
D7S1830	1.691056	1.659309	.843462
D10S1220	2.568114	1.386225	.496242
D13S800	1.910678	.883094	.706713
D14S742	1.064543	.885049	.405487
D6S1281	.263617	.415794	1.003509
TC trait:			
D6S1006	1.320385	.777908	.974036
D11S4464	1.06793	1.220498	1.26089
D12S1659	1.046737	.339792	.406268
D15S816	.013376	.558894	1.057962
D15S657	.996662	.980203	1.43362
DXS6804	.569403	1.558509	1.179271
apoB trait:			
D11S4464	1.07097	1.824949	1.610766
D15S153	1.05716	.306091	.397427
D16S753	1.170597	1.35869	1.321064
DXS6804	1.16265	1.690665	1.878295
D3S2398	.692005	.989974	1.009491
D6S1006	.498744	.552987	1.009014
D7S2201	.797234	.813216	1.101056
D15S816	.553682	.769874	1.128925
D16S2624	.230827	.969041	1.239194

[a] For a dominant mode of inheritance.
[b] For pedigrees divided into nuclear families.

ysis using the affecteds-only strategy, in one of the six regions that are interesting with regard to FCHL (table 1). In addition, in stage 1, the apoB trait alone resulted in four chromosomal regions showing $Z > 1.0$ in linkage analyses, and three of these four also showed $Z > 1.0$ in ASP analysis. Furthermore, five regions showed $Z > 1.0$ in ASP analysis only (table 2). None of the markers in these nine regions revealed markers resulting in $Z > 2.0$, in either of the analyses.

In stage 2, the Z value for the apoB trait, with the chromosome 21 marker D21S1437, increased from 2.13 ($\theta = .08$) to 2.24 ($\theta = .10$) in linkage analysis when the seven additional families were included (table 1 and fig. 2). The closest marker (at a distance of 1.5 cM), D21S1905, resulted in $Z = 0.38$, in linkage analysis (table 3).

Contribution of Individual FCHL Families to Linkage Findings

The results of the linkage analysis of the four potential susceptibility loci identified for independent traits in this study (loci for TGs, on chromosome 10p11.2 and on chromosome 2q31; a locus for apoB, on chromosome 21q21; and a locus for TC, on chromosome 10q11.2-10qter), as well as our earlier data on the FCHL locus on chromosome 1q21-q23 (Pajukanta et al. 1998), demonstrate that none of the individual families alone produced significant Z values (in the present study, the highest Z value obtained for an individual family was 0.64), but the total Z value for these Finnish families represents the sum of all families with no evidence for locus heterogeneity. Furthermore, there was no clear overlap between the families producing positive Z values in the chromosome 1 region and those doing so in other regions. The linkage data on all individual families, for the markers with the highest Z values in these regions, as well as phenotype information and lipid values for the five most informative pedigrees, are available on the National Public Health Institute of Finland, Department of Human Molecular Genetics Webpage.

Multipoint Analysis

Our study material consisting of extended FCHL families is likely to be most informative in an analysis such as the affecteds-only linkage analysis, which uses the data on vertical transmission of the trait that are available in these families. Therefore, to analyze whether we could extract more information from multiple markers,

Table 3

***Z* Values, in Linkage Analysis and ASP Analysis, for Chromosome 10 with the TG Trait and Chromosome 21 with the apoB Trait**

		$Z(\theta)$[b]		
Locus	Distance[a] (cM)	Maximum	Nuclear Family	ASP
TG trait:				
D10S1426	8	.19 (.22)	.00 (.50)	.00
D10S675[c]	2	.00 (.50)	.00 (.50)	.00
D10S1247[c]	3	.09 (.30)	.07 (.30)	.17
		1.76 (.08)[d]		
D10S1220[e]	4	3.20 (.00)	1.10 (.00)	.23
		3.13 (.01)[d]		
D10S1233[c]	10	.84 (.10)	.42 (.14)	.47
D10S1225		.00 (.50)	.00 (.50)	.00
apoB trait:				
D21S1432	3.5	.21 (.28)	.33 (.24)	.20
D21S1899[c]	4	.20 (.32)	.35 (.26)	.33
D21S1905[c]	1.5	.38 (.20)	.13 (.26)	.04
		2.39 (.12)[d]		
D21S1437[e]	3.5	2.24 (.10)	1.19 (.14)	.57
		.70 (.21)[d]		
D21S1922[c]	4	.00 (.50)	.03 (.36)	.02
D21S272[c]	2.5	.00 (.50)	.00 (.50)	.00
D21S1435		.16 (.26)	.11 (.28)	.12

[a] Between the marker and that in the row immediately below.
[b] Definitions are as in the footnotes to table 2.
[c] Genotyped for the dense marker map in stage 2.
[d] Multipoint Z, by MLINK, between the two adjacent markers.
[e] Genotyped in both stage 1 and stage 2.

Table 4

***Z* Values, in Linkage Analysis and ASP Analysis, for Chromosome 2 with the TG Trait and Chromosome 10 with the TC Trait**

		Z[b]		
Locus	Distance[a]	Maximum	Nuclear Family	ASP
TG trait:				
D2S1776	12.4	.44 (.22)	.69 (.14)	.52
D2S148[c]	3.6	1.08 (.16)	1.47 (.10)	.78
D2S1391[d]	3.3	1.95 (.08)	2.33 (.04)	2.25
D2S118[c]	4.5	.53 (.24)	.70 (.16)	.45
D2S117[c]	5.6	.46 (.22)	.25 (.24)	.21
D2S1384		.54 (.16)	.38 (.20)	.67
TC trait:				
D10S1223	5	.40 (.22)	.04 (.34)	.03
D10S1727[c]	2.5	.23 (.26)	.16 (.28)	.30
D10S1655[c]	2.5	.00 (.50)	.00 (.50)	.00
D10S1134[c]	1	.00 (.50)	.00 (.50)	.00
D10S169[d]	2	1.53 (.14)	2.74 (.04)	2.59
D10S1675[c]	1.6	.00 (.50)	.00 (.50)	.00
D10S1770[c]	1.5	.02 (.38)	.06 (.34)	.03
D10S1651[c]		.16 (.30)	.38 (.24)	.30

[a] Between the marker and that in the row immediately below.
[b] Definitions are as in the footnotes to table 2.
[c] Genotyped for the dense marker map in stage 2.
[d] Genotyped in both stage 1 and stage 2.

we conducted multipoint analysis with markers on 10p11.2 and 21q21, which, in the linkage analysis, showed potential evidence of linkage with the TG and apoB traits. To avoid the negative side effects of the multipoint analysis (Risch and Giuffra 1992), we conducted an analysis similar to that used in our earlier study, which had revealed the first novel FCHL locus on 1q21-q23 (Pajukanta et al. 1998; also see the Statistical Analyses subsection, above). Multipoint analysis did not significantly add information content on the families that we studied: for 10p11.2, the highest two-point Z value with the TG trait was 3.20 ($\theta = .00$), whereas the highest multipoint Z value for this region was 3.13 ($\theta = .01$); for 21q21, with the apoB trait, these values were 2.24 ($\theta = .10$) and 2.39 ($\theta = .12$), respectively (table 3).

Discussion

In the present study, we have performed a genomewide screen for loci involved in susceptibility to the FCHL phenotype, using 368 randomly spaced polymorphic markers in stage 1 and using 26 additional markers, in five interesting chromosomal regions, in stage 2. We identified four chromosomal regions suggesting a possible involvement in the etiology of FCHL, in addition to the earlier-characterized FCHL locus on chromosome 1q21-q23 (Pajukanta et al. 1998). The evidence for two loci emerged from linkage analysis using a dominant-inheritance model: a locus for susceptibility to elevated TGs, on chromosome 10p11.2 ($Z = 3.2$ [$\theta = .00$]), and a locus for susceptibility to elevated apoB levels, on chromosome 21q21 ($Z = 2.24$ [$\theta = .10$]). Two other potential regions were detected with the ASP analysis: a telomeric region of chromosome 10, with $Z = 2.59$ ($P < .0003$) with the TC trait (2.29 [$P < .0006$] with the FCHL trait) and a region on chromosome 2q31, with $Z = 2.25$ ($P = .0006$) with the TG trait. Because we performed the genome screen in essentially the same set of families in which we had characterized the first FCHL locus on 1q21-q23 (Pajukanta et al. 1998), our results would suggest that, in these FCHL families from the Finnish population, there is one major FCHL locus on 1q21-q23. The evidence presented here further suggests that multiple additional loci might affect the expression of different component traits that form the complex FCHL phenotype. However, it is evident that all four of these suggestive regions need to be further analyzed in other study samples, to clarify whether they truly harbor loci predisposing to FCHL, because multiple tests were performed, and the highest Z value in pairwise linkage analysis was 3.2 for the locus on chromosome 10p11.2.

The four potential susceptibility loci of individual traits identified show that different loci are identified with component traits forming the complex FCHL phenotype. There was no clear overlap between the individual families producing positive Z values in the chromosome 1 region identified in our earlier study (Pajukanta et al. 1998) and those that did so in the regions characterized in the present study. Furthermore, none of the individual families alone produced significant Z values, but the total Z value represents the sum of all families with no evidence for locus heterogeneity. We find that this is a reasonable result, because there are likely to be different genes involved in the different specific phenotypes, unrelated to the pathological combination of them. Furthermore, the fact that the traits are not 100% correlated indicates that some differences in the genetic basis of each trait probably exist. Our data also support the commonly suggested concept that FCHL is a heterogeneous disease with several loci affecting the complex phenotype (Cullen et al. 1994; Jarvik et al. 1994; Juo et al. 1998).

Linkage analysis of extended families has been, so far, the most successful locus-mapping method, for complex disease and simple disease alike (St. George-Hyslop et al. 1987; Hall et al. 1990; Pericak-Vance et al. 1991). FCHL was originally proposed to be inherited as an autosomal dominant disorder (Goldstein et al. 1973), which would be in accordance with the inheritance pattern in our FCHL families, because a dominant inheritance of a risk factor is the most consistent explanation for a genetic risk factor leading to correlations between phenotypes of relatives in different generations, such as was observed in these families. If the risk factor were

recessive, then, for risk, there would always be higher correlations within generations than between generations. However, in complex diseases such as FCHL, in which the interplay of multiple genes and environmental factors are necessary for the complicated phenotype to be expressed, it is evident that assumptions about the mode of inheritance represent oversimplifications. The disease aggregates in families yet does not segregate in a simple manner, making the determination of the genotype-phenotype relationships much more difficult than it is for simple monogenic traits. Therefore, a more complex polygenic background is also likely in FCHL, as has been suggested by metabolic (de Graaf and Stalenhoef 1998) and segregation studies (Cullen et al. 1994; Jarvik et al. 1994). Thus, to avoid oversimplifications concerning the genetic component, we performed both linkage analysis adopting an affecteds-only strategy and ASP analysis, in 35 extended Finnish FCHL families, in stage 1, and then in these 35 families and 7 additional families , in stage 2. The affecteds-only strategy was selected to minimize problems of incomplete penetrance, whereas the ASP analysis circumvents some of the errors incumbent in *Z* analysis, such as an incorrectly parameterized mode of inheritance for the complex disease. Two loci obtained with each analysis method were further supported by stage 2 of the genome scan, thus suggesting a potentially dominant-inheritance pattern for the loci on 10p11.2 and 21q21 and a recessive-inheritance pattern for loci on 10q11.2-10qter and 2q31. No significant evidence was found for locus heterogeneity, in any of the trait/marker combinations tested.

In all FCHL studies, the diagnosis of FCHL includes dyslipidemia in the proband, and in most reports—including the original ones by Goldstein et al. (1973) and Nikkilä and Aro (1973)—CHD in the family is required. Accordingly, in most studies so far, the families have been ascertained through clinical sources. Therefore, in the absence of further studies, the results of these investigations cannot be generalized to the population level.

The isolated Finnish population has well-established advantages for the mapping of monogenic diseases (Hästbacka et al. 1992; de la Chapelle 1993; Peltonen et al. 1995) that are caused by a characteristic single ancestral mutation. Such a strong founder effect is not expected for complex diseases, in which the susceptibility alleles are likely to be far more common. In the best case, the mutant allele and its corresponding set of haplotypes predate the founding of the Finnish population, with still potentially multiple haplotypes existing even in the founder population. In the worst case, multiple different mutations on different haplotype backgrounds may exist in the same gene even in this isolated population. However, in any case, because of the relatively small effective population size of the Finnish population, there is likely to be less allelic and locus heterogeneity here than in more mixed populations, making it potentially easier to map complex traits as well—although perhaps not as much so as is the case for rare monogenic recessive diseases.

Environmental factors have also been shown to differ substantially between populations (Kaufman et al. 1986). On the basis of the geographic, linguistic, and cultural factors that caused the Finnish genetic isolation and homogeneity of the population (de la Chapelle 1993; Peltonen et al. 1995), it is reasonable to expect smaller variations in both the environmental factors and life-style in Finland than is seen in a random sample from a more mixed population. This may be especially important in familial lipid disorders such as FCHL, in which an interplay between several environmental factors effecting lipid levels and multiple genes may act synergistically to lead to such complex disease phenotypes. In fact, genomewide scans using material collected from internal isolates of Finland have already been successful in the study of such other complex diseases as multiple sclerosis (Kuokkanen et al. 1996) and in type 2 diabetes (Mahtani et al. 1996). In the family material that we studied, which provides well-defined phenotype and diagnostic criteria (Pajukanta et al. 1998), we found some evidence for four potential susceptibility loci for FCHL, two of them having an impact mainly on TGs, one on apoB, and one on TC. These data would be consistent with earlier complex-segregation analyses, in which there is evidence for both a major locus affecting serum TGs in FCHL (Cullen et al. 1994) and a major locus affecting apoB levels (Jarvik et al. 1994).

The chromosome 10 finding in the present study is interesting because two potential candidate genes are initially positioned in this region, on 10p11.2. One of them is the fibronectin-receptor beta-subunit precursor gene (beta-1 integrin VLA4) (Argraves et al. 1987), which belongs to the adhesion receptors and interacts with the vascular cell–adhesion molecule 1 (VCAM1) on leukocytes. de Gruijter et al. (1991) have shown that FCHL patients exhibit a significantly increased cell adhesion to endothelial cells. Another candidate gene in this region is the glutamic acid decarboxylase 1 (GAD1) gene encoding a target for autoantibodies in people who later develop insulin-dependent diabetes (Baekkeskov et al. 1990). However, because biological evidence supporting the role of these genes in FCHL is currently lacking, further studies constructing a physical map over the linked region and looking for either intragenic polymorphisms of these candidate genes or any novel transcript in this region are warranted.

In conclusion, our data on four putative loci influencing serum lipid levels would suggest that these loci newly identified in the present study could modify the TG, TC, and apoB traits and, in combination with the

FCHL locus on 1q21-q23, result in the complex FCHL phenotype. However, further studies are warranted in this family material and in independent family sets, to clarify the true significance of these loci or any other FCHL locus.

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Electronic-Database Information

URLs for data in this study are as follows:

Cooperative Human Linkage Center, http://www.chlc.org
Généthon, http://www.genethon.fr/genethon_en.html
National Public Health Institute of Finland, Department of Human Molecular Genetics, http://www.ktl.fi/molbio/wwwpub/fchl/genomescan

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