Combined Analysis of Genome Scans of Dutch and Finnish Families Reveals a Susceptibility Locus for High-Density Lipoprotein Cholesterol on Chromosome 16q

Päivi Pajukanta,[1] Hooman Allayee,[1] Kelly L. Krass,[2,3] Ali Kuraishy,[1] Aino Soro,[1,7] Heidi E. Lilja,[1,8] Rebecca Mar,[1] Marja-Riitta Taskinen,[7] Ilpo Nuotio,[9] Markku Laakso,[10] Jerome I. Rotter,[1,3,4,6] Tjerk W. A. de Bruin,[11] Rita M. Cantor,[1,4] Aldons J. Lusis,[1,2,3,5] and Leena Peltonen[1,8]

Departments of [1]Human Genetics, [2]Microbiology and Molecular Genetics, [3]Medicine, and [4]Pediatrics and [5]Molecular Biology Institute, David Geffen School of Medicine at the University of California–Los Angeles, and [6]Division of Medical Genetics, Steven Spielberg Pediatric Research Center and Cedars-Sinai Research Institute, Los Angeles; [7]Department of Medicine, Helsinki University Central Hospital, and [8]Department of Molecular Medicine, National Public Health Institute, and Department of Medical Genetics, University of Helsinki, Helsinki; [9]Department of Medicine, Turku University Central Hospital, Turku, Finland; [10]Department of Medicine, Kuopio University Central Hospital, Kuopio, Finland; and [11]Department of Medicine and the Cardiovascular Research Institute Maastricht, Academic Hospital, Maastricht, the Netherlands

Several genomewide screens have been performed to identify novel loci predisposing to unfavorable serum lipid levels and coronary heart disease (CHD). We hypothesized that the accumulating data of these screens in different study populations could be combined to verify which of the identified loci truly harbor susceptibility genes. The power of this strategy has recently been demonstrated with other complex diseases, such as inflammatory bowel disease and asthma. We assessed the largely unknown genetic background of CHD by investigating the most common dyslipidemia predisposing to CHD, familial combined hyperlipidemia (FCHL), affecting 1%–2% of Western populations and 10%–20% of families with premature CHD. To be able to perform a combined data analysis, we unified the diagnostic criteria for FCHL and its component traits and combined the data from two genomewide scans performed in two populations, the Finns and the Dutch. As a result of our pooled data analysis, we identified three chromosomal regions, on chromosomes 2p25.1, 9p23, and 16q24.1, exceeding the statistical significance level of a LOD score >2.0. The 2p25.1 region was detected for the FCHL trait, and the 9p23 and 16q24.1 regions were detected for the low high-density lipoprotein cholesterol (HDL-C) trait. In addition, the previously recognized 1q21 region also obtained additional support in the other study sample, when the triglyceride trait was used. Analysis of the 16q24.1 region resulted in a statistically significant LOD score of 3.6 when the data from Finnish families with low HDL-C were included in the analysis. To search for the underlying gene in the 16q24.1 region, we investigated a novel functional and positional candidate gene, helix/forkhead transcription factor (*FOXC2*), by sequencing and by genotyping of two single-nucleotide polymorphisms in the families.

Introduction

Coronary heart disease (CHD) is the leading cause of death in Western societies. Unfavorable serum lipid levels, such as high serum total cholesterol (TC), high serum triglycerides (TGs), and low high-density lipoprotein cholesterol (HDL-C), are well-known risk factors for atherosclerosis and CHD. Consequently, familial dyslipidemias featuring these lipid disturbances predispose the affected family members to CHD. Familial combined hyperlipidemia (FCHL [MIM 144250]) is one of the most common familial dyslipidemias, with a population prevalence of 1%–2% (Goldstein et al. 1973). Although it has been evident for 30 years that FCHL has a strong genetic component (Goldstein et al. 1973; Nikkilä and Aro 1973), the actual underlying genes have not yet been identified. Several encouraging linkage findings have been reported in different study samples (Pajukanta et al. 1998, 1999; Aouizerat et al. 1999*a*, 1999*b*), but the identification of causative allelic variants has not proceeded as rapidly. The slow process of gene identification in complex traits is most likely caused by several factors, such as locus and allelic heterogeneity, as well as by difficulties in assessing the complex phenotypes, which

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Address for correspondence and reprints: Dr. Päivi Pajukanta, David Geffen School of Medicine at the University of California–Los Angeles, Department of Human Genetics, Gonda Neuroscience and Genetics Research Center, 695 Charles E. Young Drive South, Los Angeles, CA 90095-7088, e-mail: ppajukanta@mednet.ucla.edu; and Dr. Leena Peltonen, Biomedicum Helsinki, Haartmanink. 8, 00250 Helsinki, Finland, e-mail: Leena.Peltonen@ktl.fi

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can consequently weaken the crucial phenotype-genotype link (Weiss and Terwilliger 2000).

The sequencing of the human genome (International Human Genome Sequencing Consortium 2001; Venter et al. 2001) has provided the essential tools for fine mapping the loci for complex traits. In addition, novel approaches identifying the causal variants among those in an associated haplotype are currently under study (Daly et al. 2001; Johnson et al. 2001; Reich et al. 2001; Rioux et al. 2001; Gabriel et al. 2002). Haplotype-based methods, based on association between causal mutations and the ancestral haplotypes on which they arose, provide a powerful approach to disease gene mapping. Extended families with FCHL with refined phenotypic data would be well-suited for these analytic approaches. However, the first challenge in fine mapping is to verify those identified regions that have the highest statistical likelihood of harboring causative genes. Furthermore, the study samples originating from different populations are likely to provide different disease-associated allelic variants and their haplotypes with varying lengths of linkage disequilibrium (LD) (Pritchard et al. 2001; Gabriel et al. 2002; Van Eerdewegh et al. 2002), which can be considered as an additional advantage when utilizing study samples from different populations.

The purpose of the present study is to combine genome-scan data of two study samples, one originating from the genetically isolated population of Finland (de la Chapelle 1993; Peltonen et al. 2000) and the other from the more mixed population of the Netherlands. We report here the detailed combined data analysis of two genome scans for FCHL and its four component traits: serum TGs, TC, apolipoprotein B (apoB), and HDL-C. After unifying the diagnostic criteria for FCHL and its component traits, the most solid evidence for linkage was obtained between a region on chromosome 16q24.1 and the HDL-C trait. Importantly, this study also shows which of the other loci identified earlier in separate genome scans (Aouizerat et al. 1999*b*; Pajukanta et al. 1999) exhibited additional evidence for linkage in the other study population.

Material and Methods

A total of 560 genotyped individuals from Dutch and Finnish families were included in the study. Each study subject provided written informed consent prior to participating in the study. All samples were collected in accordance with the Helsinki Declaration, and the ethics committees of the participating centers approved the study design.

Dutch and Finnish Families with FCHL

Eighteen extended Dutch families with FCHL were ascertained through probands recruited from the lipid clinic of the Utrecht Academic University Hospital, as described elsewhere (Dallinga-Thie et al. 1997). These families were included in the original Dutch genomewide scan for FCHL (Aouizerat et al. 1999*b*). The probands met the following criteria: (1) a primary combined hyperlipidemia with varying phenotypic expression, including a fasting plasma TC >6.5 mmol/liter or >90th percentile for age, defined according to tables from the Lipid Research Clinics, and fasting plasma TGs >2.3 mmol/liter; (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 (MI) or cardiovascular disease before age 60 years. Exclusion criteria for the probands were diabetes, BMI >30, tendon xanthomas, or type III hyperlipidemia (apoE2/E2). Of these 18 Dutch families with FCHL, 13, comprising 173 genotyped individuals, fulfilled the strict unified diagnostic criteria defined below, and only these families were analyzed in this study.

A total of 35 extended Finnish families with FCHL were recruited in the Helsinki, Turku, and Kuopio University Central Hospitals, as described elsewhere (Pajukanta et al. 1998, 1999). These families were included in the original Finnish genomewide scan for FCHL (Pajukanta et al. 1999). A total of 168 individuals were genotyped, of which 135 were affected with FCHL. The "unaffected" individuals were genotyped only to increase phase information and were treated as if their phenotype were unknown in the statistical analysis, because of the nondeterministic genotype-phenotype relationship hypothesized for this complex phenotype (see the "Statistical Analyses" subsection). The inclusion criteria for the probands with FCHL were as follows: (1) serum TC and/or TGs >90th age/sex-specific Finnish population percentiles—however, if the proband had only one elevated lipid trait, a first-degree relative had to have the combined phenotype (Pajukanta et al. 1998); (2) age >30 years and <55 years for men and <65 years for women; and (3) >50% stenosis in one or more coronary arteries, as assessed by coronary angiography. Exclusion criteria for the probands with FCHL were type 1 diabetes mellitus, hepatic or renal disease, and hypothyroidism. Familial hypercholesterolemia was excluded from each pedigree by determining the low-density lipoprotein (LDL)–receptor status of the proband, by the lymphocyte culture method (Cuthbert et al. 1986). Provided that the above-mentioned criteria were fulfilled, families with at least two affected members were included in the study, and all the accessible family members were examined. Family members with FCHL were scored as "affected" if they had combined hyper-

lipidemia phenotype IIb or if they had high TC (phenotype IIa) or high TGs (phenotype IV), when the Finnish age/sex-specific 90th percentiles for TC and TGs were used. The criteria used are fully comparable to the original criteria by Goldstein et al. (1973) and aim to focus on families with combined hyperlipidemia phenotype IIb.

Finnish Families with Low HDL-C

The low–HDL-C study sample was used to increase linkage information in the 2p25.1, 9p23, and 16q24.1 regions identified in the combined data analysis of the Dutch and Finnish families with FCHL (see the "Results" section). This study sample was collected in Helsinki and Turku University Central Hospitals in Finland, as described in detail elsewhere (Lilja et al. 2002; Soro et al. 2002). A total of 219 individuals, of whom 104 were affected, from 25 families with well-defined low HDL-C were included in the study. Inclusion criteria for the probands with low HDL-C were an age of 30–60 years for men and women, HDL-C levels <10th age/sex-specific Finnish population percentile, and CHD verified by either coronary angiography (>50% stenosis in one or more coronary arteries) or MI. Additional lipid criteria for the probands were total TC <6.3 mmol/liter in men and <6.0 mmol/liter in women, and TGs <2.3 mmol/liter in both sexes. The affected family members were ascertained for low HDL-C through use of the 10th Finnish age/sex-specific population percentiles. These families with low HDL-C have been screened for ABC1 mutations, as described elsewhere (Lilja et al. 2002; Soro et al. 2002), but no segregating mutations were found. Low cholesterol efflux has not been excluded.

Biochemical Analyses and Classification of Affection Status

Serum TC, TGs, apoB, and HDL-C were measured in the Finnish families with FCHL and low HDL-C, as described elsewhere (Pajukanta et al. 1998, 1999). The data collection, laboratory measurements, and phenotype determinations for the Finnish families with low HDL-C and FCHL were performed in the same center, making the clinical and biochemical data in these two samples fully compatible. In the Dutch families with FCHL, lipids and apolipoproteins were quantified by methods described elsewhere (Castro Cabezas et al. 1993; Dallinga-Thie et al. 1996). Probands or hyperlipidemic relatives who used lipid-lowering drugs were studied after their lipid lowering treatment was withheld for 3 wk in the Dutch study sample and for 4 wk in the Finnish study samples.

To maximize the similarity of ascertainment between the two FCHL cohorts, we used the age/sex-specific 90th population percentiles of TC, TGs, and apoB, as well as the 10th percentile of HDL-C, to classify both the Dutch and Finnish families. Accordingly, the Dutch families were first redefined using the same diagnostic criteria outlined above for the Finnish families. Because the age/sex-specific lipid percentiles are publicly available only for the Finnish population (Porkka et al. 1994; Vartiainen et al. 1994), the Finnish percentiles were also used to classify the Dutch families. The cutoffs for the Finnish 90th age/sex-specific percentile values for TC, TGs, and apoB are available from the National Public Health Institute, Finland, Web site, and the cutoffs for the Finnish 10th age/sex-specific percentile values for HDL-C have been published elsewhere (Lilja et al. 2002). Since the lipid levels in the Finnish population are, on average, somewhat higher than the Dutch, especially TGs (T.W.A.dB., unpublished data), the Finnish criteria used for the Dutch families can be considered more stringent than if population averages from the Netherlands were used, resulting in a conservative analysis of the Dutch families with FCHL. Of the 18 Dutch families with FCHL, 13, comprising 173 genotyped individuals, matched the strict criteria defined above, and only these families were analyzed in this study. In the Dutch families with FCHL, the numbers of individuals classified as affected with FCHL, elevated apoB, elevated TGs, elevated TC, and low HDL-C were 55, 72, 47, 56, and 37, respectively. In the Finnish families with FCHL, the corresponding numbers were 135, 107, 96, 107, and 64. The original genotyping strategy and numbers of affected individuals differed among the Finnish and Dutch FCHL samples. In the Finnish families with FCHL, 135 of the 168 genotyped individuals were affected with FCHL (Pajukanta et al. 1999). The total number of individuals in these 35 families is 501, resulting in 27% affected subjects with FCHL. In the Dutch study sample, 173 of 373 individuals were genotyped, and 55 of these 173 were affected with FCHL (15%), since the genotyping strategy for the Dutch sample concentrated on the affected individuals and their first-degree relatives in the available nuclear families (Aouizerat et al. 1999*b*). Combining these samples through use of the strict Finnish criteria has made the study samples more consistent, but the effect of this difference in genotyping strategies on the analytic results is not clear.

Although LDL-C is an important component of FCHL, it was not included in the diagnostic criteria when ascertaining these Dutch or Finnish families with FCHL or in the original study, by Goldstein et al. (1973), describing the FCHL disorder. One reason is the significant hypertriglyceridemia associated with FCHL. The Friedewald formula is not recommended when TGs are >400 mg/dl—that is, 4.4 mmol/liter—which is often the case with hypertriglyceridemic family members. In addition, the population percentile points of LDL-C could not be estimated when including

this factor, since we currently don't have population percentiles for LDL-C.

We recognize that quantitative traits usually provide more linkage information than binary ones. However, ascertainment of these families with FCHL via a proband and an additional first-degree relative with high TC or TGs and the genotyping strategy of the Finnish families with FCHL, which was designed to maximize the information obtained from the affected subjects with only necessary unaffected relatives genotyped for phase information, resulted in limited variation in serum TC and TG levels for effective QTL analysis. Since these constraints would allow very little power to be gained from a continuous trait analysis, we chose to limit our analyses to binary traits, as explained in the "Statistical Analyses" subsection.

Genotyping of the Markers and Sequencing of the FOXC2 *Gene*

In the two genome scans, ~385 microsatellite markers included in the Weber screening set version 6 (Sheffield et al. 1995) and spaced, on average, 10.0 cM apart were genotyped. The separate genome scans previously conducted in the Dutch and Finnish families with FCHL have been reported elsewhere (Aouizerat et al. 1999*b*; Pajukanta et al. 1999). In addition, to maximize the linkage information in potentially important regions, we genotyped the peak markers in the missing study sample in cases where the marker was genotyped in only one study sample. Furthermore, two additional markers for chromosome 16q were genotyped in both FCHL study samples. Thus, markers D1S104, D1S2844, D2S243, D16S518, D16S422, and D16S505 were genotyped in the Dutch families with FCHL; markers D15S655, D16S516, and D16S507 were genotyped in the Finnish families with FCHL; and markers D16S3096 and D16S3040 were genotyped in both FCHL study samples. The fluorescently labeled PCR products were electrophoretically separated on an automated DNA sequencer (ABI 377XL; Perkin Elmer), with analysis by Applied Biosystems Genescan 2.1 software (Perkin Elmer). The order of the markers for the regions on 2p25.1, 9p23, and 16q24.1 was based on the genetic maps of the Marshfield Medical Research Foundation (Center for Medical Genetics Web site).

The entire human winged helix/forkhead transcription factor gene (*FOXC2* [MIM 602402]) and a 760-bp region upstream from the 5′ end of the *FOXC2* gene were sequenced in the probands of the families contributing to the linkage signal (see the "Results" section). The 760-bp region was selected by comparing the 10-kb sequence from both ends of the *FOXC2* gene between mouse and human, using the VISTA program (VISTA Home Page). The samples were amplified by PCR for the automated DNA sequencer ABI 377XL (Perkin Elmer). Purification of the PCR product was performed with exonuclease I (Amersham Life Sciences) and shrimp alkaline phosphatase (Amersham Life Sciences). Sequencing was performed in both directions, according to the Big Dye Terminator Cycle Sequencing protocol (PE Biosystems), with minor modifications. Sequencing Analysis software, version 3.2 (PE Biosystems), was used to perform the initial base calling of the sequencing traces. Sequence contigs were assembled through use of Sequencer software (GeneCodes). The dbSNP (dbSNP Home Page) and Celera (Celera Web site) databases were also used to find polymorphisms in the *FOXC2* gene. We applied a pyrosequencing technique for SNP genotyping, using the PSQ96 instrument and the SNP Reagent kit (Pyrosequencing AB), as described elsewhere (Pielberg et al. 2002). Primers for PCR were designed through use of the Primer3 program, available at the Whitehead Institute for Biomedical Research Web site. Detection primers for pyrosequencing were designed through use of the SNP Primer Design Software, version 1.01 (Pyrosequencing AB). Oligo Analyzer 2.5, available at the Integrated DNA Technologies Web site, was used to calculate the melting temperature of the primers and to check all the primers for primer dimers and hairpins, to prevent possible background signals in the SNP genotyping.

Statistical Analyses

Statistical analyses were conducted according to the strategy described below. The genomewide set of markers genotyped earlier in the Finnish and Dutch families with FCHL (Aouizerat et al. 1999*b*; Pajukanta et al. 1999) were analyzed with five binary traits—FCHL and its component traits TC, TGs, apoB, and HDL-C—through use of the unified diagnostic criteria described in detail above. The Finnish families with low HDL-C were analyzed only for the low–HDL-C trait and only in the three regions on 2p25.1, 9p23, and 16q24.1 identified in the combined analysis of the Dutch and Finnish families with FCHL. The purpose was to increase the linkage information in potentially interesting regions. In the Dutch and Finnish families with FCHL, the FCHL component traits—TC, TGs, apoB, and HDL-C—were analyzed in addition to the FCHL trait, to identify any loci predisposing to one of those traits individually that might not be involved in the more extreme FCHL phenotype. Two-point linkage analyses using parametric and nonparametric affected-sib-pair (ASP) analyses were performed for the genomewide set of markers in the combined sample of the Finnish and Dutch families with FCHL. Multipoint analyses were performed only for the three regions identified by two-point LOD scores >2.0. Multipoint analysis was restricted to these regions because of possible undetected problems of genotyping and

map errors. For the 16q region, a parametric four-point linkage analysis was also performed by analyzing trios of adjacent markers with the Mlink program (Lathrop et al. 1984). In this analysis, the tightly linked markers are placed in a fixed order, and the disease locus is allowed to vary outside the marker map. This strategy was used as an alternative to multipoint analysis with flanking markers (moving the disease across the map), because of the latter method's known propensity for false exclusions (Risch and Giuffra 1992). The main purpose was to score meioses uninformative for one of the markers for adjacent markers (analogous to the previous studies [Terwilliger and Ott 1993], with the benefit of controlling for the intermarker recombination fractions), thus allowing all meioses on the pedigrees to be scored in this analysis (Pajukanta et al. 1998). Affecteds-only parametric and nonparametric analyses were employed to better model the unknown mode of inheritance of FCHL. To circumvent problems of incomplete penetrance and genetic ambiguity of the "unaffected" phenotype, we used an affecteds-only strategy, by coding the family members as either "affected" or "unknown" on the basis of the age/sex-specific 90th percentile thresholds for TC, TGs, and apoB and the 10th percentile threshold for HDL-C. The unaffected subjects were genotyped only to increase phase information and were treated as if their phenotypes were unknown (Pajukanta et al. 1998, 1999). The parametric linkage analyses were performed with both dominant and recessive modes of inheritance, with gene frequencies of 0.6% and 10.95%, respectively, reflecting an estimated population prevalence of ~1%–2% for FCHL (Goldstein et al. 1973).

The Mlink program of the Linkage package (Lathrop et al. 1984), version Fastlink 4.1P (Cottingham et al. 1993; Schäffer et al. 1994), was used, with the help of the Analyze package (Göring and Terwilliger 2000*a*), to perform the parametric linkage analyses. The ASP analysis was performed using the Sibpair program (Kuokkanen et al. 1996) of the Analyze package (Göring and Terwilliger 2000*a*). For each marker, the allele frequencies were estimated from all individuals by the Downfreq program (Göring and Terwilliger 2000*b*). The whole genomewide scan of each trait was run in a single computer analysis through use of the Autoscan program, available at the authors' Web site (UCLA Human Genetics Autoscan 1.0 Web site). Multipoint analyses were performed with the SimWalk2 program, version 2.82 (Sobel and Lange 1996). In the nonparametric linkage analysis (NPL) of the SimWalk2 program, statistic A is most powerful at detecting linkage to a recessive trait, statistic B is most powerful at detecting linkage to a dominant trait, and statistics C and D are more general statistics indicating whether a few founder alleles are overrepresented among the affected individuals. The Mendelian errors were checked with the PedCheck program (O'Connell and Weeks 1998). Genetic heterogeneity was tested using the Homog program (Ott 1991) of the Analyze package (Göring and Terwilliger 2000*a*). The SNPs in the *FOXC2* gene were tested for association through use of the gamete competition test (Sinsheimer et al. 2000). The gamete competition test (Sinsheimer et al. 2000) is a genetic application of the Bradley-Terry model, originally designed for the ranking of sports teams. It provides a parametric extension of the transmission/disequilibrium test and views transmission of marker alleles to affected children as a contest between the alleles, making effective use of full pedigree data. Furthermore, the gamete competition model readily extends to two linked markers (Sinsheimer et al. 2000), enabling simultaneous analysis of multiple SNPs in a gene.

Results

We performed a combined data analysis of Finnish and Dutch FCHL genome scans, through use of unified diagnostic criteria for the analyzed phenotypic lipid traits. In addition to the FCHL trait, four component traits of the disorder—TGs, TC, apoB, and HDL-C—were analyzed. For each chromosome, figure 1 summarizes the results of the two-point linkage analysis with the FCHL trait in the Finnish, Dutch, and combined study samples. In addition, the complete two-point linkage results for all markers are available, at the authors' Web site (UCLA Human Genetics Web site), for all five lipid traits. For comparison among the methods, *P* values have been included in parentheses for all results presented in the following paragraphs of this section.

Regions Where the Combined Data Analysis Provided LOD Scores >2.0

The combined data analysis provided evidence of linkage to three regions, on 2p25.1, 9p23, and 16q24.1, resulting in LOD scores of 2.2 ($P = .0007$), 2.1 ($P = .0009$), and 3.6 ($P = .00002$), respectively. The 2p25.1 region was detected for the FCHL trait, and the 9p23 and 16q24.1 regions were detected for the low–HDL-C trait (tables 1 and 2).

On chromosome 16q, the highest two-point results for low HDL-C were 0.8 ($P = .03$), 1.1 ($P = .01$), and 1.7 ($P = .003$), in the Dutch, Finnish, and combined study samples, respectively (table 1). The multipoint results ($-\log_{10}$[*P* value]) of the NPL of the SimWalk2 program for each study group with the low HDL-C trait were 2.1 ($P = .007$), 1.1 ($P = .07$), and 2.7 ($P = .002$), respectively (table 1). The parametric multipoint analysis also provided support for low HDL-C in the combined data analysis of the Finnish and Dutch families with FCHL, with a location score of 3.2 ($P = .0001$) when allowing for heterogeneity (α value = 0.75) and with a location

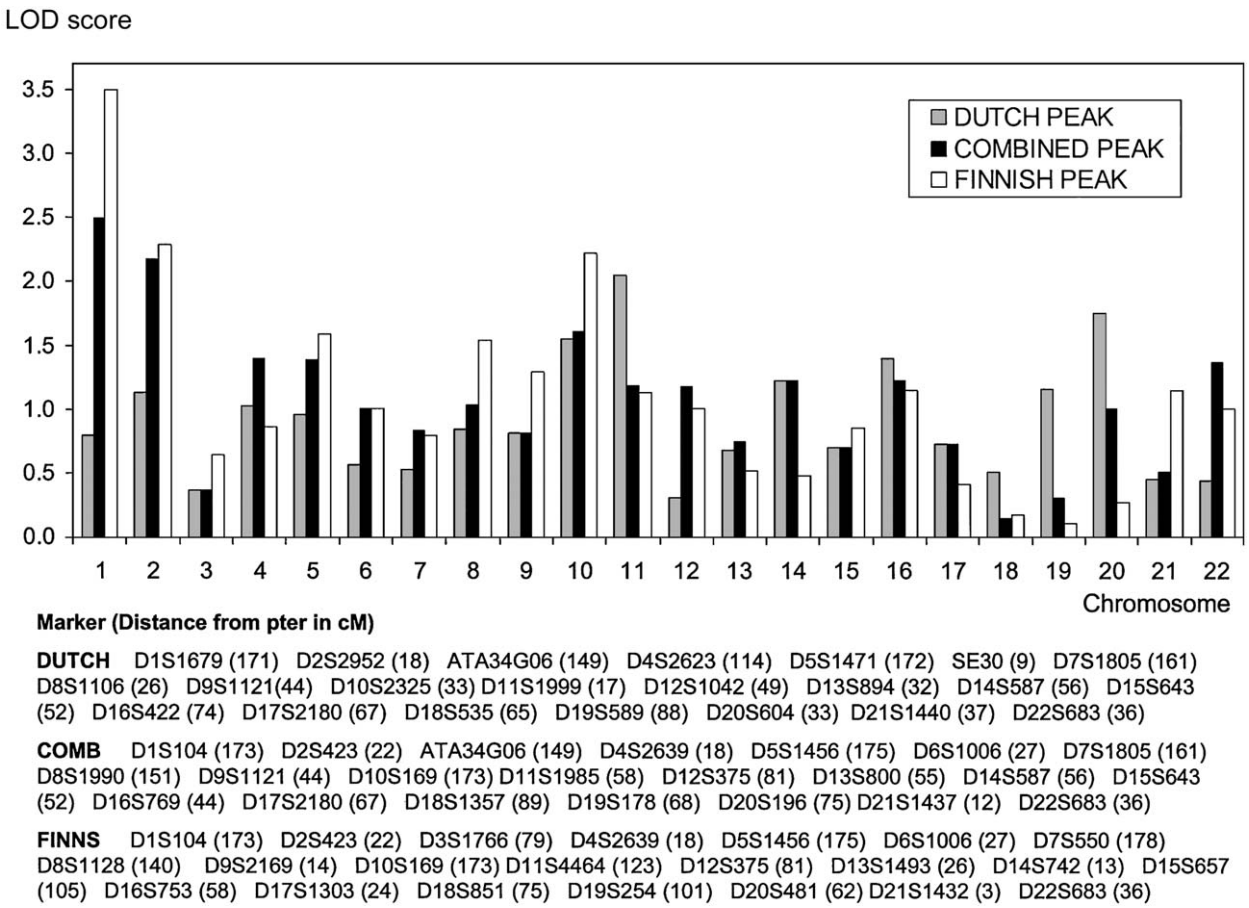

Figure 1 Two-point maximum LOD scores (from parametric linkage analysis with either a dominant or recessive mode of inheritance or from ASP analysis) obtained for each chromosome in different study samples, using the FCHL trait. Gray columns show the two-point results for the Dutch families with FCHL, white columns show the results for the Finnish families with FCHL, and black columns show the results for the combined data analysis of the Dutch and Finnish families with FCHL. The marker resulting in the highest LOD score on each chromosome is indicated for each study group, and its position from pter (in cM) is given in parentheses. The complete genomewide two-point results are available for all five traits at the authors' Web site.

score of 2.2 under homogeneity ($P = .0007$), suggesting a more recessive type of inheritance model for the trait (fig. 2). Furthermore, since the three regions on 2p25.1, 9p23, and 16q24.1 were detected with either the HDL-C trait or a trait known to be closely correlated with HDL-C, we also analyzed these three regions by combining the genomewide scan data from Finnish families with low HDL-C (Soro et al. 2002). These Finnish families with low HDL-C were analyzed only for the low–HDL-C trait and only in the three regions on 2p25.1, 9p23, and 16q24.1 identified in the combined analysis of the Dutch and Finnish families with FCHL. When the data from the families with low HDL-C were included in the analysis, the 16q24.1 region produced a nonparametric multipoint score of 3.0 ($P = .0009$), by means of the NPL analysis of the SimWalk2 program, and a parametric multipoint LOD score of 3.4 (α value = 0.50) ($P = .00007$), by means of a parametric location score analysis from the SimWalk2 program for the low–HDL-C trait (table 1 and fig. 2). For $\alpha = 1.00$, the largest overall location score was 2.3 ($P = .0006$). This result suggests that there is locus heterogeneity among the families, with approximately half of them exhibiting linkage to this chromosomal region. Figure 2 shows the parametric multipoint results for chromosome 16q24.1, when the HDL-C trait is used for each study sample. The LOD scores of the other regions on 2p25.1 and 9p23 remained <2.0 when the families with low HDL-C were included in the analysis.

A parametric four-point linkage analysis was also performed on 16q24.1, by analyzing trios of adjacent markers and the HDL-C trait in the combined analysis of the Finnish and Dutch families with FCHL and Finnish families with low HDL-C, through use of the low–HDL-C trait. The maximum four-point LOD score of 3.6 (recombination fraction [θ] = 0.08) ($P = .00002$) was ob-

Table 1

Two-Point and Multipoint Results of the Chromosome 16q24.1 Region for the HDL-C Trait in Dutch and Finnish Families with FCHL, in the Combined Analysis of Families with FCHL, and in the Combined Analysis of Families with FCHL and Low HDL-C

Marker	Distance from D16S518 (cM)	Dutch[a]		Finns[b]		Combined[c]		Combined + Families with HDL-C[d]		
		LOD	MP	LOD	MP	LOD	MP	LOD	MP	PMP
D16S518	.0	.2 (.2)	1.0 (.1)	1.1 (.01)	.9 (.1)	1.3 (.007)	1.4 (.04)	1.3 (.007)	1.7 (.02)	3.4 (.00007)
D16S3096	4.4	.5 (.06)	2.1 (.007)	1.0 (.02)	1.1 (.07)	1.5 (.004)	2.7 (.002)	.9 (.02)	3.0 (.0009)	3.4 (.00007)
D16S516	5.3	.6 (.05)	2.0 (.01)	.1 (.2)	1.1 (.08)	.6 (.05)	2.3 (.005)	.6 (.05)	2.9 (.001)	2.9 (.0002)
D16S3040	9.4	.4 (.09)	1.6 (.02)	.0 (.5)	.8 (.2)	.2 (.2)	1.7 (.02)	.4 (.09)	2.5 (.003)	2.4 (.0008)
D16S507	10.1	.6 (.05)	2.0 (.01)	1.0 (.02)	.8 (.1)	1.7 (.003)	2.0 (.01)	1.7 (.003)	2.7 (.002)	2.7 (.0004)
D16S505	13.9	.5 (.06)	1.8 (.02)	.0 (.5)	.6 (.3)	.3 (.1)	1.5 (.03)	1.5 (.004)	2.5 (.003)	2.0 (.002)
D16S3091	16.0	.1 (.2)	.7 (.2)	.1 (.2)	.3 (.5)	.1 (.2)	.7 (.2)	1.4 (.006)	1.5 (.03)	.6 (.07)
D16S422	16.1	.8 (.03)	.7 (.2)	.0 (.5)	.1 (.7)	.5 (.06)	.4 (.4)	.5 (.06)	1.2 (.07)	.4 (.1)
D16S402	18.5	.7 (.04)	1.0 (.09)			.7 (.04)	.6 (.3)	.5 (.06)	1.1 (.08)	.3 (.2)
D16S3061	26.4							.0 (.50)	.6 (.2)	.0 (.6)

Note.—For comparison among the methods, *P* values have been included for LOD scores, location scores, and $-\log_{10}(P \text{ value})$ scores (in parentheses). LOD = the two-point maximum LOD score of the parametric linkage analysis using a recessive mode of inheritance; MP = nonparametric multipoint results ($-\log_{10}[P \text{ value}]$) of the NPL analysis using the SimWalk2 program, statistic C. The results of the other statistics of NPL analysis were consistent with those of statistic C. PMP = parametric multipoint location scores using the SimWalk2 program with a recessive mode of inheritance and allowing for heterogeneity.

[a] Study sample of the Dutch families with FCHL.

[b] Finnish families with FCHL.

[c] Dutch and Finnish families with FCHL.

[d] Dutch and Finnish families with FCHL as well as the Finnish families with low HDL-C.

served with markers D16S507-D16S505-D16S3091, suggesting that the most significant evidence of linkage is obtained in this 6-cM region. Furthermore, the highest two-point LOD score, 2.0 ($P = .001$), for 16q24.1, was obtained in the ASP analysis with marker D16S505, which is located in the middle of this trio.

The regions on chromosomes 2p25.1 and 9p23 showed suggestive evidence of linkage in the pooled data analyses, with the maximum LOD scores 2.2 ($P = .0007$) and 2.1 ($P = .0009$), respectively, when the FCHL and low HDL-C traits were used (table 2). The addition of the Finnish families with low HDL-C did not improve these results. In the case of the 2p25.1 region (table 2), the most significant result of the combined study sample was obtained with the FCHL trait and marker D2S423 (LOD score 2.2 [$P = .0007$]). However, in the Dutch study sample, the segregating trait was TGs, resulting in a LOD score of 2.3 ($P = .0006$) with marker D2S2952, located 4.2 cM from D2S423. In the Finns, the trait with the strongest linkage was HDL-C, yielding an ASP LOD score of 3.4 ($P = .00004$) with marker D2S423. Thus, the combined data analysis did not result in statistically significant evidence for linkage, although both study samples showed some evidence for linkage with chromosomal region 2p25.1.

Results for Other Chromosomal Regions Previously Linked with FCHL, in Either the Dutch or Finnish Families with FCHL

To date, two genomewide scans have been published for FCHL. Five loci, on chromosomes 1q21, 2q31, 10p11.2, 10q11.2-10qter, and 21q21, were identified in the Finnish studies (Pajukanta et al. 1998, 1999), and four loci, on chromosomes 2p, 11p, 16q, and 19q, were identified in the Dutch scan (Aouizerat et al. 1999*b*). The regions on 1q21, 2p, 11p, and 16q were detected for the FCHL trait; those on 2q31 and 10p11.2 were detected for TGs; that on 10q11.2-10qter was detected for TC; and that on 21q21 was detected for apoB. In addition, regions on 2p, 8q, 16q, and 20q have been identified for low HDL-C in the Finnish families with FCHL (Soro et al. 2002), as well as a region on 1p for apoB in the Dutch families with FCHL (Allayee et al. 2002). Of these regions, those on chromosomes 2p and 16q could be detected in the combined data analysis of the present study when the low–HDL-C and FCHL traits were used, as described above. In addition, chromosomal regions on 1q21 and 2q31 were detected, with some statistical significance, when the TG trait in the Dutch study sample was used as well, although the combined data analysis did not provide statistically significant evidence for linkage. These results are described in detail below.

On chromosome 1q21, the highest two-point LOD score in the Dutch families with FCHL was 1.8 ($P = .002$), detected with the TG trait and with marker D1S1679, as reported elsewhere (Allayee et al. 2002). Marker D1S1679 is located <5 cM centromeric from the peak linkage markers of the Finnish study sample, D1S104 and D1S1677. In the current study, these peak linkage markers of the Finnish study sample were also

Table 2

Two-Point and Multipoint Results of the 2p25.1 and 9p23 Regions

Linkage and Marker	Distance from First Marker (cM)	Dutch[a]		Finns[b]		Combined[c]		
		LOD	ASP	LOD	ASP	LOD	ASP	MP[d]
2p25.1 region and FCHL trait:								
D2S2952	0	.6	1.1 (.01)	1.0 (.02)	.6	1.7 (.003)	1.6 (.004)	1.9 (.01)
D2S423	4.22	.2	.4	2.3 (.0006)	.8	2.2 (.0007)	1.2 (.009)	1.5 (.03)
D2S1400	9.72	.0	.6	.1	.1	.1	.4	1.2 (.06)
2p25.1 region and TG trait:								
D2S2952	0	.8	2.3 (.0006)	.5	.3	1.3 (.007)	1.6 (.003)	1.8 (.02)
D2S423	4.22	.6	1.0 (.01)	.7	.4	1.2 (.009)	1.4 (.005)	1.6 (.03)
D2S1400	9.72	.8	1.7 (.003)	.5	.6	1.2 (.009)	1.8 (.002)	1.4 (.04)
2p25.1 region and HDL-C trait:								
D2S2952	0	.0	.0	1.2 (.009)	.7	.2	.2	.9
D2S423	4.22	.0	.0	2.6 (.0003)	3.4 (.00004)	1.2 (.009)	1.4 (.005)	1.2 (.07)
D2S1400	9.72	.7	.0	.7	.4	1.2 (.009)	.2	1.0 (.1)
9p23 region and HDL-C trait:								
D9S2169	0	.3	.0	.8	.2	1.0 (.02)	.1	1.4 (.04)
GATA175H06	3.83	.6	.6	.0	.0	.4	.3	1.6 (.03)
D9S921	7.65	1.7 (.003)	.1	.1	.2	2.1 (.0009)	.4	1.8 (.01)
D9S925	18.01	.0	.0	.0	.0	.0	.0	1.0 (.1)

Note.—The 2p25.1 region is shown with three phenotypes to demonstrate the effect of the phenotype. LOD = the two-point maximum LOD score of the parametric linkage analysis using a recessive mode of inheritance; ASP = the LOD score of the two-point affected sib-pair analysis. For comparison among the methods, P values have been included for LOD scores and $-\log_{10}(P$ value) scores >1.0 (in parentheses). The LOD scores detected in the parametric (LOD) and nonparametric (ASP) two-point analyses are directly comparable.

[a] Study sample of the Dutch families with FCHL.

[b] Finnish families with FCHL.

[c] Dutch and Finnish families with FCHL.

[d] Nonparametric multipoint results ($-\log_{10}[P$ value]) of the NPL analysis using the SimWalk2 program. SimWalk statistic A was used for the analysis of linkage between the 2p25.1 region and both the FCHL and TG traits; statistic D was used for the analysis of linkage between the 2p25.1 region and the HDL-C trait; and statistic B was used for the analysis of linkage between the 9p23 region and the HDL-C trait. Although the best-fitting statistics for each region and trait are shown here, the results of all statistics in the NPL analysis were consistent with these.

genotyped and analyzed in the Dutch study sample (table 3). In the Dutch study sample, they resulted in maximum two-point LOD scores of 0.5 ($P = .06$). The overall multipoint NPL result for TGs was 1.1 ($P = .09$) in the Dutch study sample. However, the combined data analysis resulted in a lower LOD score of 2.8 ($P = .0002$) for TGs, when compared with the result in the Finnish families with FCHL (LOD score of 3.1 [$P = .00008$]) (table 3). It is worth noting that, in the Finnish study sample, the strongest signal for this region was detected with the FCHL trait (Pajukanta et al. 1998).

On chromosome 2q31, the inclusion of the Dutch families resulted in LOD scores of ~1.0 with consecutive markers when the TG trait was used, shifting the region 10 cM toward the telomeric end of the chromosome. Accordingly, in the Dutch study sample, the highest two-point LOD score, 1.3 ($P = .007$), was obtained with marker D2S1649, located 5 cM telomeric from the Finnish peak linkage markers. In the Finnish study sample, the strongest signal was also detected with the TG trait (ASP LOD score 2.2 [$P = .0007$]).

On chromosome 10p11.2, the highest two-point LOD score, of 3.3 ($\theta = 0.0$; $P = .00005$), was obtained with marker D10S1220 and the TG trait in the combined data analysis. In the separate analyses, the Dutch study sample provided a LOD score of 0.5 ($\theta = 0.0$; $P = .06$), and the Finnish families with FCHL provided a LOD score of 2.6 ($\theta = 0.0$; $P = .0003$). Further, when the additional Finnish families with FCHL, genotyped for this region in the dense mapping of the original genome scan (Pajukanta et al. 1999), were included in the current study, we obtained a LOD score of 3.9 ($\theta = 0.0$; $P = .00001$) with D10S1220 for TGs. However, the adjacent markers gave two-point LOD scores <1.0 for this region in the combined analysis, as did the multipoint analysis.

The chromosome 11p region resulted in a two-point maximum LOD score of 2.1 ($P = .0009$) with marker D11S1999 in the Dutch study sample when the FCHL trait was used, whereas, in the Finnish study sample, the markers for the 11p region did not yield two-point LOD scores >0.5 with any of the five investigated traits. Analysis of the apoB region on 1p that was recently identified in the Dutch families with FCHL (Allayee et al. 2002)

resulted in reduced evidence for linkage with the apoB trait when the Finnish families with FCHL were included. The LOD score with the peak marker D1S1665 and the apoB trait decreased from 3.8 ($P = .00001$) in the Dutch study sample to 2.0 ($P = .001$) in the combined study sample, and the adjacent markers also remained nonsignificant. In addition, no other regions with LOD scores >2.0 were detected for the apoB or TC trait in the combined analysis of the Dutch and Finnish families with FCHL, and none of the other regions detected earlier in the Finnish or Dutch families with FCHL provided additional support for linkage in the combined data analysis.

Table 3

Two-Point Linkage Results for Chromosome 1q21 in the Dutch Families with FCHL, Finnish Families with FCHL, and the Combined Analysis, Using the TG Trait

Marker	Distance from D1S1679 (cM)	LOD/ASP[a]		
		Dutch	Finns	Combined
D1S1679	0	1.8/.4	.2/.2	1.9/.6
D1S2844	4.2	.4/.2	1.2/1.3	1.3/1.4
D1S104	4.8	.5/.1	3.1/2.2	2.8/1.9
D1S1677	4.8	.0/.5	.4/1.0	.1/1.2
D1S426	7.0	.0/.0	.9/1.0	.5/.9

[a]LOD = the highest two-point maximum LOD score of the parametric linkage analysis using a dominant mode of inheritance; ASP = the LOD score of the two-point ASP analysis. The LOD scores produced by both methods are directly comparable.

The FOXC2 *Gene Resides on Chromosome 16q24.1*

An interesting positional candidate gene residing in the linked 16q region is the human winged helix/forkhead transcription factor gene, *FOXC2,* which has just recently been shown to play a role in hypertriglyceridemia, obesity, and diet-induced insulin resistance in mice (Cederberg et al. 2001). Since *FOXC2* is a small gene (1,506 bp) with only one exon, we sequenced the entire gene, as well as 750 bp of the 5′ flanking region. It is noteworthy that this 750-bp region was found to be highly conserved between mouse and human (fig. 3). The region may thus represent the promoter or another potentially functionally important region of the *FOXC2* gene. For sequencing, we selected the probands of the families that provided a LOD score >0.5 in the multipoint location score analysis for chromosome 16q24.1. Consequently, 17 probands were included in the sequence analysis from four Dutch families

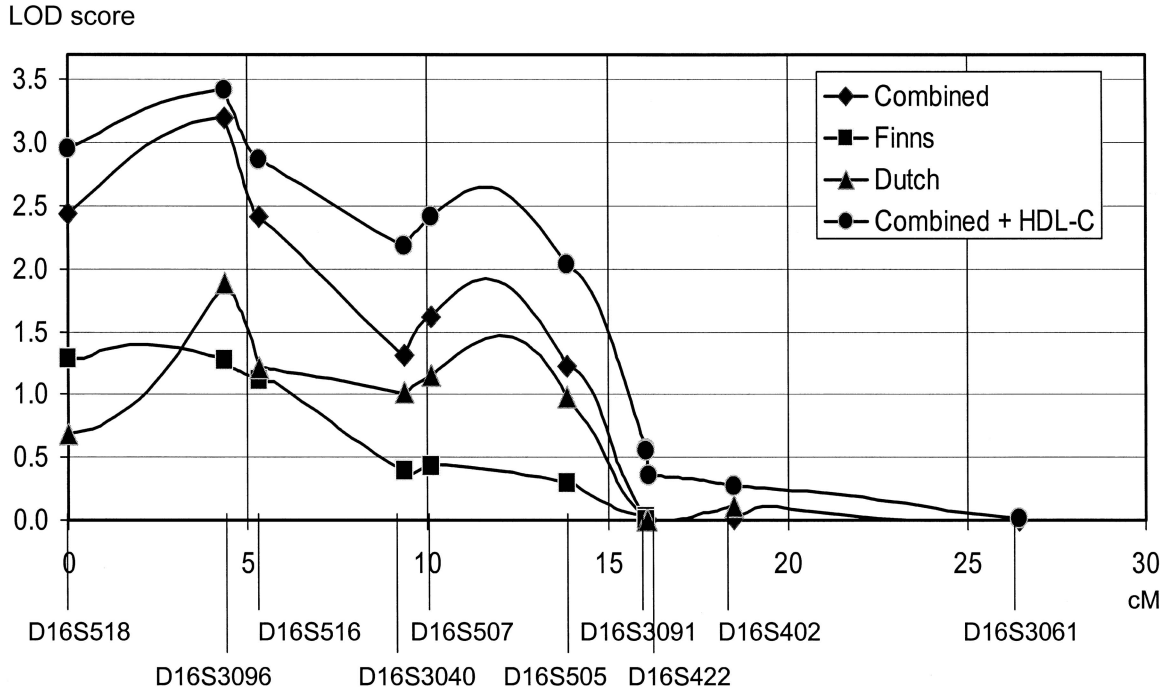

Figure 2 Parametric multipoint results for chromosome 16q24.1, obtained using the HDL-C trait and a recessive mode of inheritance. The analysis was performed using the "Location Score" option of the SimWalk2 program and allowing for heterogeneity. Dutch = the study group of the Dutch families with FCHL; Finns = the Finnish families with FCHL; combined = the Dutch and Finnish families with FCHL; combined + HDL-C = the Dutch and Finnish families with FCHL, as well as the Finnish families with low HDL-C. The α values for the peak location scores of the Dutch, Finns, combined, and combined + HDL-C families were 0.80, 0.70, 0.75, and 0.50, respectively.

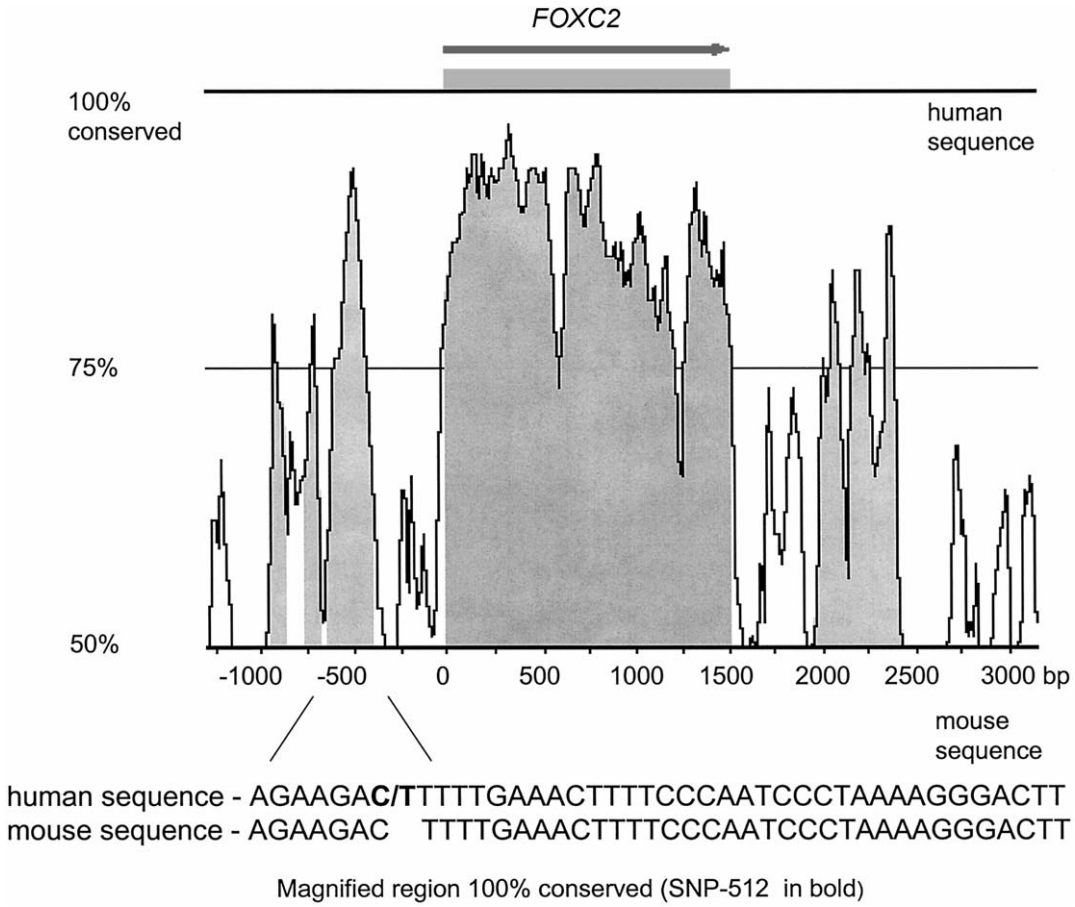

Figure 3 Conservation between mouse and human sequences in the genomic region of the *FOXC2* gene. The SNP located in the putative promoter of the *FOXC2* gene (−512) is shown in detail. The comparison between the genomic sequences of human and mouse was performed using the VISTA program.

with FCHL, five Finnish families with FCHL, and eight Finnish families with low HDL-C. Although no coding variants were identified, two variants were found in the conserved 5′ region upstream of the *FOXC2* gene (fig. 3): nine probands had a T→C variation (eight heterozygotes and one rare-allele homozygote) at position −512, upstream from *FOXC2,* and two probands had a G→T variant (two heterozygotes) at position −350. We selected the more common –512 SNP, located in a region that is well conserved between mouse and human, and another SNP (hCV2849496), found using the Celera database, for genotyping in the families. The latter SNP (hCV2849496) is located 6,452 bp downstream from the 3′ end of the *FOXC2* gene. Given the small sample size when independent affected individuals are selected from these extended families with FCHL and low HDL-C, we chose to test the SNP associations by using the gamete competition test only with haplotype information. Thus, to obtain more information for the association analysis, we employed the gamete competition test to test for association in a combined analysis of the two SNPs simultaneously. As a result, evidence for association ($P = .005$) was obtained with the TG trait, suggesting that the allelic variants of *FOXC2* may regulate the TG and/or HDL-C levels in the families with FCHL. We found that the haplotype combination 1-1 (i.e., the common allele of the −512 SNP and the common allele of the hCV2849496 SNP) was more often transmitted to the affected subjects in the combined analysis of all families. With the low–HDL-C trait, the corresponding *P* value remained nonsignificant ($P > .05$).

Discussion

We performed a combined data analysis of Dutch and Finnish genomewide scans for FCHL to identify the regions most likely to harbor allelic variants for this complex lipid disorder. Three regions, on chromosomes 16q24.1, 2p25.1, and 9p23, with maximum LOD scores of 3.6, 2.2, and 2.1, respectively, were detected in which

the evidence for linkage emerged from the combined study sample (tables 1 and 2; fig. 2). The 9p23 and 16q24.1 regions were both detected for the low–HDL-C trait, and the 2p25.1 region was detected for the FCHL trait. In addition, the previously identified FCHL region on 1q21 (Pajukanta et al. 1998) also showed some evidence for linkage of TGs in the Dutch study sample, although the combined data analysis did not provide additional support, as is discussed in detail below.

In the earlier separate genome scans, five loci, on chromosomes 1q21, 2q31, 10p11.2, 10q11.2-10qter, and 21q21, were identified in the Finnish families with FCHL (Pajukanta et al. 1998, 1999), and four loci, on chromosomes 2p, 11p, 16q, and 19q, were identified in the Dutch families with FCHL (Aouizerat et al. 1999*b*; reviewed by Eurlings et al. 2001). In addition, regions on 2p, 8q, 16q, and 20q have been detected for low HDL-C in the Finnish families with FCHL (Soro et al. 2002), and a region on 1p has been detected for apoB in the Dutch families with FCHL (Allayee et al. 2002). To date, these are the published genomewide scans for FCHL. The combined data analysis of the present study provided significant evidence for linkage, especially with the 16q24.1 region, resulting in a multipoint LOD score of 3.6 for low HDL-C. In the earlier Dutch FCHL genome scan, the multipoint LOD score for this same region was 1.4, the peak occurring within this very same interval over marker D16S402 for FCHL (Aouizerat et al. 1999*b*). In the Finnish families with FCHL, this region previously resulted in a two-point LOD score of 1.2 with marker D16S2624 when the apoB trait was used and in a two-point LOD score of 1.0 with marker D16S518 when the low–HDL-C trait was used (Pajukanta et al. 1999; Soro et al. 2002). In the Finnish families with low HDL-C, the highest two-point LOD score for this region was 1.9, with marker D16S3091 (Soro et al. 2002), but the multipoint NPL score for low HDL-C remained <1.5 in the Finnish families with FCHL and low HDL-C (Soro et al. 2002). The 9p23 region previously resulted in a two-point LOD score of 1.3 with the FCHL trait in the Finnish families with FCHL (Pajukanta et al. 1999). In the current study, the addition of the Dutch families provided some support for this region (table 2).

Interpretation of the results of the combined data analysis for the 1q21 and 2p25.1 regions is more complex. Chromosome 1q21 previously provided a two-point maximum LOD score of 3.5 and a parametric three-point LOD score of 5.9 with the FCHL trait in the Finnish families with FCHL (Pajukanta et al. 1998), whereas, in the Dutch families, the TG trait produced the highest LOD score, 1.8, with marker D1S1679, residing in a region close to the Finnish peak linkage markers, and the results of the combined linkage analysis remained lower than in the Finnish families with FCHL separately (table 3). The 2p25.1 region provides a similar kind of example, with the HDL-C trait producing the strongest evidence for linkage in the Finnish and the TG trait in the Dutch families with FCHL with different markers (table 2). Thus, this region did not result in statistically significant evidence for linkage in the combined data analysis, although both study samples provided support for this region separately. These somewhat ambiguous results most likely reflect several underlying features of the data, such as the original ascertainments of the two study samples, potentially different causative variants and the ancestral haplotypes on which they arose in the Finnish and Dutch populations, and varying marker informativeness combined with unavoidable genotyping errors. Because of these factors, it is not unusual that two study samples do not peak at the very same location (Lander and Shork 1994; Roberts et al. 1999; Ekelund et al. 2000). Consequently, we interpret this to imply that the 1q21 and 2p25.1 regions are candidates for fine-mapping efforts using larger study samples. Furthermore, the 1q21 region has been replicated in families with FCHL from other populations (Coon et al. 2000; Pei et al. 2000). Importantly, the same markers in the 1q21 region have also been linked to type 2 diabetes in several earlier studies (Hanson et al. 1998; Elbein et al. 1999; Vionnet et al. 2000; Wiltshire et al. 2001), including a Finnish study sample (Watanabe et al. 2000). This may indicate that there are one or more genes in this region, the different variants of which are causative for FCHL and type 2 diabetes. This is a plausible explanation, since the FCHL phenotype clearly overlaps with type 2 diabetes. Interestingly, the 1q21 region was also recently linked to lipoprotein a in families with CHD of western European origin (Broeckel et al. 2002).

The purpose of the present study was to combine genome-scan data to accelerate the slow process of gene identification in complex lipid traits. This strategy was successfully used in previous studies with other complex traits (Cox et al. 2001; Perola et al. 2001; Fisher et al. 2002; Wu et al. 2002), and it even led to gene identification in recent studies of asthma (Van Eerdewegh et al. 2002) and inflammatory bowel disease (Hugot et al. 2001; IBD International Genetics Consortium 2001; Ogura et al. 2001). In the present study, the obtained results were encouraging, especially in the case of overlapping linkage signals in the 16q24.1 region. Given the expected difficulties in replicating and verifying the results of a genomewide scan, the complete results available at our Web site may also help other research groups to identify regions showing evidence for linkage in an even larger number of study populations. On the other hand, the complexity of our results, as exemplified by some of the regions (see above)—that is, nonadditive

LOD scores, different informative traits, varying peak locations, and relatively weak signals—reflects well the problems of complex traits (Risch and Giuffra 1992; Lander and Shork 1994; Göring and Terwilliger 2000*a*), as well as problems associated with genome scans in general, including inevitable map and genotyping errors. In addition, population-based differences are likely to explain part of the picture, although similar problems can also be seen even when the study samples are collected from the same population, as was demonstrated by the results of three Finnish type 2 diabetes genome scans (Mahtani et al. 1996; Ghosh et al. 2000; Watanabe et al. 2000). More refined phenotyping and detailed haplotype mapping is needed to clarify these partially overlapping linkage signals across different traits.

In the linked 16q region, we investigated one obvious positional and functional candidate gene, the *FOXC2* gene. This gene has been proposed to work as an antithrifty gene in hypertriglyceridemia, obesity, and diet-induced insulin resistance (Cederberg et al. 2001). On the one hand, thrifty genes are suggested to conserve energy during periods of famine, and, on the other hand, they constitute a risk for developing obesity-related conditions, such as type 2 diabetes, when energy is abundant. Although no coding variants were identified in our sequence analysis of the *FOXC2* gene, we found some evidence of association with an SNP haplotype including the SNP located in the middle of a well-conserved putative promoter or a regulative element of *FOXC2*. However, caution is needed when interpreting this result, given the amount of multiple testing. Functional studies of these variants and further studies in other study samples with additional SNPs are warranted to obtain a clearer picture of the possible role of *FOXC2* in FCHL. Furthermore, we have not excluded the possibility that the observed linkage (and association) at this locus may be caused by another nearby gene.

In this study, both parametric and nonparametric linkage analyses were performed, and, for the fine-mapping regions, multipoint analyses were performed in addition to the two-point analyses. We recognize that an important question arises as to whether the LOD scores obtained by these different analyses are comparable (Nyholt 2000). The two-point LOD scores were obtained by affecteds-only parametric linkage and nonparametric ASP analyses, using the Mlink and Sibpair programs of the Analyze package (Göring and Terwilliger 2000*a*). The multipoint results were obtained by parametric location score and NPL analyses through use of the SimWalk program (Sobel and Lange 1996). The LOD scores of the parametric and nonparametric two-point analyses, as well as the LOD scores of the parametric multipoint location score analyses that are conducted under the assumption of homogeneity, are comparable (Sobel and Lange 1996; Göring and Terwilliger 2000*a;* Nyholt 2000). The results from the multipoint NPL analysis represent $-\log_{10}(P \text{ value})$ scores, not LOD scores. These $-\log_{10}(P \text{ value})$ scores and the LOD scores of the parametric multipoint location score analysis permitting heterogeneity are not directly comparable to the others. However, we converted the results to *P* values, and the *P* values are provided along with the LOD scores and $-\log_{10}(P \text{ value})$ scores, to make the results comparable. The *P* values for LOD scores detected under homogeneity were estimated according to the method proposed by Nyholt (2000), and the *P* values for LOD scores detected under heterogeneity were estimated according to the method proposed by Chiano and Yates (1995). The output of the SimWalk program also provides *P* values, as does the two-point ASP analysis using the Sibpair program, and these *P* values are given for these nonparametric methods.

Five binary traits—FCHL and its component traits TGs, TC, ApoB, and HDL-C—were analyzed because of their importance in FCHL (Goldstein et al. 1973; Brunzell et al. 1983). We recognize that this number of tested traits raises the question of multiple testing. However, these five traits are closely correlated, making it difficult to compute a global *P* value for multiple testing using, for example, the Bonferroni correction. In general, some guidelines have been suggested for encouraging or positive LOD scores in genomewide scans (Lander and Kruglyak 1995). Results can be interpreted on the basis of the number of times that one would expect to see such a result at random in a complete genome scan. A pointwise threshold of a LOD score of 3.3 was proposed for significant linkage, a threshold of 1.9 was proposed for suggestive linkage, and a threshold of 0.59 was proposed for nominal linkage. These guidelines have, however, been challenged by others (Morton 1998). In addition, with the denser map regions, the increased marker density and information content also require an increased LOD-score threshold (Wiltshire et al. 2002). Thus, for a complex disorder such as FCHL, posing a critical value for a positive LOD score is difficult. A commonly accepted approach is replication in an independent study sample. In the present study, both study samples, the Dutch and the Finnish, supported the 16q region, although the interpretation of the results on 2p and 9p is much less straightforward.

In conclusion, the goal of this study was to identify chromosomal loci for FCHL and related lipid traits TG, TC, apoB, and HDL-C in a combined data analysis of study samples from two populations. A locus on chromosome 16q24.1, previously seen with suggestive statistical significance in the separate genome scans, provided the most solid evidence for linkage, with a maximum multipoint LOD score of 3.6 for the low–HDL-C trait. In addition, loci on chromosomes 2p25.1, 9p23, and 1q21 provided suggestive evidence of linkage

in the combined data sets and should be targeted for further analysis.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Autoscan 1.0, http://www.genetics.ucla.edu/software/autoscan/index.html

Celera, http://www.celera.com

Center for Medical Genetics, Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/

dbSNP Home Page, http://www.ncbi.nlm.nih.gov/SNP/

Integrated DNA Technologies, http://www.idtdna.com/ (for Oligo Analyzer 2.5)

National Public Health Institute, Finland, http://www.ktl.fi/molbio/wwwpub/fchl/genomescan/link4.html (for age/sex-specific Finnish population percentiles of lipids)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *FOXC2* and FCHL)

UCLA Human Genetics, http://www.genetics.ucla.edu/labs/pajukanta/fchlcomb/ (for complete results of the linkage analyses)

VISTA Home Page, http://www-gsd.lbl.gov/vista/

Whitehead Institute for Biomedical Research, http://www-genome.wi.mit.edu/genome_software/other/primer3.html (for the Primer3 program)

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