

Locus for quantitative HDL-cholesterol on chromosome 10q in Finnish families with dyslipidemia

Heidi E. Lilja,* Elina Suviolahti,* Aino Soro-Paavonen,[†] Tero Hiekkalinna,* Aaron Day,[§] Kenneth Lange,[§] Eric Sobel,[§] Marja-Riitta Taskinen,[†] Leena Peltonen,^{1,*§,**} Markus Perola,* and Päivi Pajukanta^{1,§}

Department of Molecular Medicine,* National Public Health Institute, Helsinki, Finland; Department of Medicine,[†] Helsinki University Central Hospital, Helsinki, Finland; Department of Human Genetics,[§] David Geffen School of Medicine at the University of California Los Angeles, Los Angeles, CA; and Department of Medical Genetics,** University of Helsinki, Helsinki, Finland

Abstract Decreased HDL-cholesterol (HDL-C) and familial combined hyperlipidemia (FCHL) are the two most common familial dyslipidemias predisposing to premature coronary heart disease (CHD). These dyslipidemias share many phenotypic features, suggesting a partially overlapping molecular pathogenesis. This was supported by our previous pooled data analysis of the genome scans for low HDL-C and FCHL, which identified three shared chromosomal regions for a qualitative HDL-C trait on 8q23.1, 16q23.3, and 20q13.32. This study further investigates these regions as well as two other loci we identified earlier for premature CHD on 2q31 and Xq24 and a locus for high serum triglycerides (TGs) on 10q11. We analyzed 67 microsatellite markers in an extended study sample of 1,109 individuals from 92 low HDL-C or FCHL families using both qualitative and quantitative lipid phenotypes. These analyses provided evidence for linkage (a logarithm of odds score of 3.2) on 10q11 using a quantitative HDL-C trait. Importantly, this region, previously linked to TGs, body mass index, and obesity, provided evidence for association for quantitative TGs ($P = 0.0006$) and for a combined trait of HDL-C and TGs ($P = 0.008$) with marker D10S546. Suggestive evidence for linkage also emerged for HDL-C on 2q31 and for TGs on 20q13.32. Finnish families ascertained for dyslipidemias thus suggest that 10q11, 2q31, and 20q13.32 harbor loci for HDL-C and TGs.—Lilja, H. E., E. Suviolahti, A. Soro-Paavonen, T. Hiekkalinna, A. Day, K. Lange, E. Sobel, M-R. Taskinen, L. Peltonen, M. Perola, and P. Pajukanta. **Locus for quantitative HDL-cholesterol on chromosome 10q in Finnish families with dyslipidemia.** *J. Lipid Res.* 2004. 45: 1876–1884.

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Low serum HDL-cholesterol (HDL-C) and familial combined hyperlipidemia [familial combined hyperlipidemia

in Online Mendelian Inheritance in Man 144250 (FCHL OMIM)] are estimated to explain ~50% of familial dyslipidemias predisposing to premature coronary heart disease (CHD) (1). Both are typical complex disorders, influenced by several environmental and genetic factors. FCHL is characterized by increased levels of serum total cholesterol (TC) and triglycerides (TGs), or both, and also expresses low HDL-C as a component trait (2–4). It has been estimated that the genetic component accounts at least 50% of the variation in HDL-C levels (5). Although several genes have been identified for rare forms of the low HDL-C trait, the major loci affecting plasma HDL-C levels and explaining most of the HDL-C variation have not been identified (6).

We previously performed a genome-wide scan in Finnish low HDL-C families (3). The identified loci for low qualitative HDL-C were also tested in an independent study sample of Finnish FCHL families. In the pooled data analysis of these two study samples, evidence for linkage emerged for low HDL-C on 8q23.1, 16q23.3, and 20q13.32, the markers on 8q23.1 showing the most significant signal with a two-point logarithm of odds (LOD) score of 4.7 (3). All three of these loci have also been linked to HDL-C or to other CHD risk factors in other populations (7–14). The loci on 8q23.1 and 16q have been linked to quantitative HDL-C in Mexican Americans (7, 12). The locus on 20q12-13 seems to have a role in the development of other component traits of the metabolic syndrome, and the contribution of the same region to the development

Abbreviations: apoA-I, apolipoprotein A-I; BMI, body mass index; CHD, coronary heart disease; cm, centimorgan; FCHL, familial combined hyperlipidemia; HDL-C, high density lipoprotein-cholesterol; HDL2 and HDL3, high density lipoprotein cholesterol particles 2 and 3; LDLps, low density lipoprotein cholesterol particle size; QTL quantitative trait locus; TC, total cholesterol; TG, triglyceride.

¹ To whom correspondence should be addressed.
e-mail: leena.peltonen@ktl.fi (L.P.);
ppajukanta@mednet.ucla.edu (P.P.)

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of type 2 diabetes mellitus (MIM 125853) has been shown in several studies (8–11, 13, 14). The locus on 20q13 has also been linked to obesity (MIM 601665) (11). In addition to these three loci (8q23.1, 16q23.3, and 20q13), we have earlier identified loci for TGs on 10q11 (15) and for premature CHD on 2q31 and Xq24 (16). In these previous genome scans (3, 15, 16), our analysis strategy focused on the information obtained from the affected family members using dichotomized affection criteria, whereas in the present study, the aim was to use quantitative lipid phenotypes and to analyze these six chromosomal regions as potential quantitative trait loci (QTLs) for HDL-C and other lipid traits characteristic of CHD and the metabolic syndrome. For this purpose, we more than tripled the number of genotyped individuals and analyzed an extended study sample consisting of 1,109 genotyped family members from 92 multigenerational dyslipidemic families for a dense set of markers in each chromosomal region.

MATERIALS AND METHODS

Finnish low HDL-C and FCHL families

A total of 1,109 genotyped individuals from 92 dyslipidemic families were included in the study: 426 genotyped individuals from 39 Finnish low HDL-C families and 683 genotyped individuals from 53 Finnish FCHL families. Of the genotyped 1,109 individuals, 358 originated from the 54 families of the previous study (3) and the rest consisted of 426 additional family members included for these original 54 families as well as 325 family members for 38 new families genotyped in the present study. The families were recruited in the Helsinki and Turku University Central Hospitals, as described earlier (3, 15, 17, 18). Each study subject provided written informed consent before 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.

In the Finnish HDL-C families, there were 168 individuals affected by low HDL-C and 49 individuals affected by high TGs using the 10th and 90th age/sex-specific Finnish population percentiles for HDL-C and TGs. Inclusion criteria for the low HDL-C probands were age of 30–60 years for men and women, HDL-C levels below the 10th age/sex-specific Finnish population percentile, and CHD verified by either coronary angiography (>50% stenosis in one or more coronary arteries) or myocardial infarction (3). Additional lipid criteria for the probands were TC of ≤ 6.3 mmol/l in men and ≤ 6.0 mmol/l in women and TGs of ≤ 2.3 mmol/l in both genders. Exclusion criteria for the low HDL-C probands were type 1 and type 2 diabetes mellitus, hepatic or renal disease, and body mass index (BMI) > 30. If the criteria mentioned above were fulfilled, families with at least two affected members were included in the study and all accessible family members were examined. The affected family members were ascertained for low HDL-C using the Finnish age/sex-specific 10th population percentiles (19, 20). The age/sex-specific HDL-C and TG percentiles are publicly available for the Finnish population (18) (<http://www.ktl.fi/molbio/wwwpub/fchl/genomescan/link4.html>).

In the Finnish FCHL families, 214 individuals were affected by low HDL-C and 198 by high TGs using the 10th and 90th age/sex-specific Finnish population percentiles for HDL-C and TGs. The inclusion criteria for the probands of FCHL families were as follows: 1) FCHL lipid phenotype (serum TC and/or TGs > 90th

age/sex-specific Finnish population percentiles); 2) age of >30 years and <55 years for males and <65 years for females; and 3) at least 50% stenosis in one or more coronary arteries by coronary angiography. Exclusion criteria for the FCHL probands were type 1 diabetes mellitus, hypothyroidism, and hepatic or renal disease (15, 17).

Biochemical analyses and classification of the affection status

Serum TC and TGs were determined with an automated Cobas Mira analyzer (Hoffman-La Roche, Basel, Switzerland) by enzymatic methods (Hoffman-La Roche kits 0722138 and 0715166, respectively). Serum HDL-C was quantified by phosphotungstic acid/magnesium chloride precipitation procedures (Hoffman-La Roche kit 0720674). Concentrations of apolipoprotein A-I (apoA-I), apoA-II, and apoB were measured by immunoturbidimetric methods with commercial kits (Boehringer-Mannheim, Mannheim, Germany). HDL-C particles 2 and 3 (HDL2 and HDL3) were separated by ultracentrifugation, as described (21). LDL particle size (LDLps) was determined using native gradient gel electrophoresis, as described in detail by Vakkilainen et al. (22). BMI was calculated as weight/height². The clinical and biochemical data in the Finnish low HDL-C and FCHL families are fully compatible, because the data collection, laboratory measurements, and phenotype determinations for these two study samples were performed in the same laboratory at the Helsinki University Central Hospital. Patients who used lipid-lowering drugs were studied after their lipid-lowering treatment was withheld for 4 weeks.

Genotyping of the markers

A total of 67 microsatellite markers were genotyped for six regions (2q31, 8q23.1, 10q11, 16q23.3, 20q13.32, and Xq24) in 1,109 family members: 426 individuals from 39 low HDL-C families and 683 individuals from 53 FCHL families. On average, 11 markers were genotyped per locus. When selecting the markers, we aimed to cover ~ 10 Mb around peak markers in every selected chromosomal region to obtain an average intermarker distance of 1 Mb. The selection of the markers, as well as the order and distances of the markers for the six regions, were based on the genetic maps of the Decode map (23), Celera (<http://www.celera.com>), the Marshfield comprehensive human genetic map (<http://research.marshfieldclinic.org/genetics/>), and the Human Genome Browser Gateway (<http://www.genome.ucsc.edu>). The April 2003 version of the Human Genome Browser Gateway was used for chromosomal band determination. Therefore, the chromosomal bands of the loci analyzed in the current study differ slightly from the original bands reported in the previous publications (3, 15, 16).

Genotyping was performed at the Finnish Genome Center (Helsinki, Finland). The fluorescence-labeled PCR products were pooled into panels and the pooled samples were dialyzed before electrophoresis, which was performed on a MegaBace 1,000-capillary electrophoresis instrument (Molecular Dynamics). Alleles were defined by using Genetic Profiler version 1.1 software (Molecular Dynamics). The data were checked for Mendelian errors with Pedmanager (M. P. Reeve, unpublished data) (<http://www.genome.wi.mit.edu/ftp/distribution/software/pedmanager/>) and Pedcheck software (24).

Statistical analyses

Quantitative linkage analyses. To investigate the earlier identified regions using quantitative measures by variance component methods in extended families, QTL analyses were conducted using maximum likelihood-based approaches implemented in the

TABLE 1. Clinical characteristics of the 39 low HDL-C and 53 FCHL families

Variable	All Individuals (n = 1,395)			Proband (n = 92)			HDL-Affected Individuals (n = 407) ^a			TG-Affected Individuals (n = 248) ^b			Spouses (n = 290)		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
Age (years)	1,276	44.5	20.3	92	53.6	6.6	407	45.1	16.2	248	42.4	15.4	256	52.6	10.2
BMI (kg/m ²)	1,125	25.4	4.6	92	28.2	3.3	399	27.3	4.5	243	27.7	4.6	231	26.3	4.3
TC (mmol/l)	1,139	5.6	1.2	90	6.4	1.5	407	5.7	1.3	244	6.4	1.3	234	5.7	1.0
TGs (mmol/l)	1,142	1.8	1.4	91	3.4	2.5	406	2.4	1.9	245	3.4	2.1	234	1.5	0.8
apoB (mg/dl)	1,121	107.1	35.1	87	147.4	40.1	390	123.0	35.7	243	141.0	39.0	224	103.1	25.6
LDLps (nm)	688	26.3	1.4	61	25.0	1.3	266	25.6	1.4	171	25.4	1.4	126	26.8	1.2
HDL-C (mmol/l)	1,146	1.3	0.4	90	0.9	0.2	407	0.9	0.2	246	1.1	0.4	234	1.4	0.4
HDL2 (mmol/l)	833	0.7	0.3	80	0.5	0.2	314	0.5	0.2	198	0.6	0.3	178	0.8	0.3
HDL3 (mmol/l)	833	0.7	0.2	80	0.6	0.1	314	0.6	0.1	198	0.6	0.1	178	0.7	0.2
apoA-I (mg/dl)	1,123	137.0	24.8	87	119.7	18.5	390	118.3	15.6	243	134.3	25.7	224	144.9	24.9
apoA-II (mg/dl)	1,111	36.6	6.6	86	33.8	6.4	388	33.6	5.4	238	38.0	6.9	224	36.6	7.2

apoA-I, apolipoprotein A-I; BMI, body mass index; FCHL, familial combined hyperlipidemia; HDL-C high density lipoprotein-cholesterol; HDL2 and HDL3, high density lipoprotein-cholesterol particles 2 and 3; LDLps, low density lipoprotein cholesterol particle size; TC, total cholesterol; TG, triglyceride. The HDL2 and HDL3 cholesterol levels do not add up to total HDL-C because of the different measurement methods (see Materials and Methods).

^a Affected by the Finnish age/sex-specific 10th population percentiles of HDL-C.

^b Affected by the Finnish age/sex-specific 90th population percentiles of TGs.

computer program SOLAR version 1.7.4 (25). The significance of age, sex, and BMI as covariates was tested in our analyses of HDL-C and TGs. For HDL-C, sex and BMI were significant covariates. For TGs, age, sex, and BMI were significant. These significant covariates were included in the linkage analyses at the first stage. We also further investigated the relationship between TGs and HDL-C by including TGs as a covariate for the quantitative trait analyses of HDL-C, and vice versa, for 10q11. To correct for the study ascertainment, the proband status was scored as a dichotomized variable and included in the SOLAR analyses. Individual TG and apoB levels were log transformed to reduce skewness and kurtosis. No other transformations were needed before statistical analyses.

Qualitative linkage analyses. Qualitative analyses were performed using both parametric linkage and nonparametric affected sibpair analyses. Nonparametric analyses were used to better model the unknown mode of inheritance of the traits. For the parametric linkage analyses, the MLINK program of the linkage package (26) version FASTLINK 4.1P (27, 28) was selected. The identical-by-descent status of affected sibpairs was assessed with the help of the SIBPAIR program (29) of the ANALYZE package (30). The parametric linkage analyses were performed with dominant and recessive modes of inheritance. Gene frequencies of 0.4% and 8%, reflecting an estimated population prevalence of 1%, were used for the dominant and recessive modes of inheritance of the HDL-C trait, as described previously (3, 15, 17, 18). For TGs, gene frequencies of 0.6% and 11% were adopted for the dominant and recessive modes of inheritance (15, 17). Linkage analyses were also performed on the component nuclear families. To circumvent problems of incomplete penetrance and genetic ambiguity of the phenotype “unaffected,” we used the affecteds-only strategy and coded the subjects either affected or unknown based on their age/sex-specific 10th percentile of measured HDL-C and 90th percentile of TGs (3, 15). The unaffected individuals were genotyped to increase phase information and were treated as if their phenotype was unknown in statistical analyses. For each marker, the allele frequencies were estimated from all individuals with the DOWNFREQ program (31). Genetic heterogeneity between the families was examined using the HOMOG program (32).

Data for the 10q11 region were also analyzed with the QTL program MERLIN (33) to compare the results obtained with SOLAR with another QTL-based program. Furthermore, the most promising region, 10q11, was analyzed with a novel QTL association method included in the software package Mendel (34). Although the method and its implementation will be described at length elsewhere (K. Lange and E. Sobel, unpublished data), we can state the main ideas briefly here. These revolve around the “measured genotype” approach, which treats the genotypes at a candidate locus as linear predictors modifying the mean of an associated quantitative trait (35–37). The variance component context of measured genotype models allows one to control for random environment and polygenic background while testing for significant mean effects by maximum likelihood estimation and likelihood ratio tests. Mendel implements this strategy for a) multivariate traits, b) both random and ascertained pedigrees, and c) noncodominant markers. Ascertainment is handled by conditioning on proband trait values. Noncodominant markers are dealt with by imputing marker allele counts for each person conditional on the marker phenotypes observed throughout his or her pedigree. These counts may be fractional in the presence of missing data. In our view, the measured genotype approach to association testing is one of the most powerful approaches available. It does require normally distributed traits and can be con-

TABLE 2. Heritabilities of HDL-C and TGs in the study sample

Variable	Low HDL-C Families (n = 39)	FCHL Families (n = 53)	Combined Families (n = 92)
HDL-C	0.71	0.44	0.54
HDL-C adjusted with gender and BMI	0.59	0.53	0.57
HDL-C adjusted with gender, BMI, and TGs	0.35	0.45	0.51
TGs	0.36	0.45	0.44
TGs adjusted with age, gender, and BMI	0.67	0.54	0.49
TGs adjusted with age, gender, BMI, and HDL-C	0.30	0.48	0.45

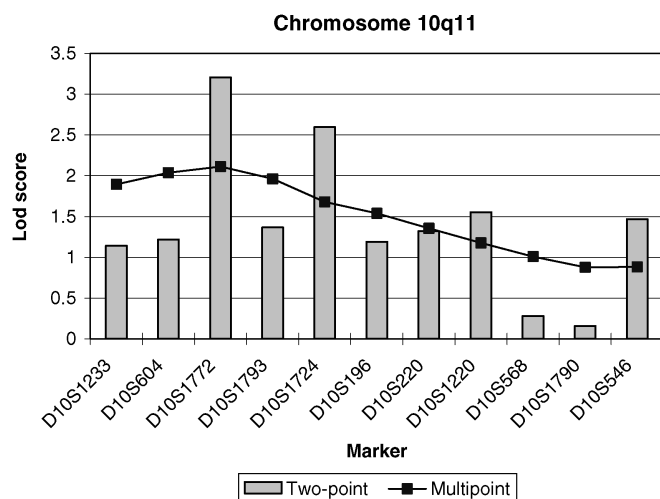


Fig. 1. Two-point and multipoint variance component analyses of the 92 dyslipidemic families for HDL-cholesterol in the 9.4 centimorgan region on chromosome 10q11. The distances between the markers are shown in Table 7.

fused by ethnic stratification. Fortunately, the latter problem can be largely ignored in the Finnish data. Another advantage in the use of variance component methodology as a linkage or association test is that it makes it possible to combine traits straightforwardly in a vector representation. It is possible to detect whether the putative trait locus is significantly linked or associated with a combined phenotype (i.e., jointly affecting the individual traits). If it is, this might indicate that the biological pathways leading to each of the individual phenotypes overlapped at the function of the gene at this location. This is compared with getting significant results only when analyzing the phenotypes separately, in which case the gene might be involved in each pathway in a different way.

RESULTS

We further investigated six chromosomal regions previously identified in our genome-wide scans of Finnish families ascertained for familial dyslipidemias and premature

CHD (3, 15, 16). We genotyped a total of 67 microsatellite markers, providing an average marker density of 1 centimorgan (cM), in an extended study sample of 1,109 genotyped individuals from 39 multigenerational low HDL-C families and 53 FCHL families. Both qualitative and quantitative analysis strategies were used. For qualitative traits, we used the age/sex-specific 10th and 90th Finnish population percentiles for HDL-C and TGs, respectively. For QTL analyses, HDL-C, TGs, TC, LDL, LDLps, HDL2, HDL3, apoA-I, apoA-II, apoB, and BMI were used. We analyzed the six loci in the combined sample of 92 low HDL-C and FCHL families as well as separately in the 39 low HDL-C families and 53 FCHL families. We also analyzed the loci separately in the new low HDL-C and FCHL families included in this study and in those low HDL-C and FCHL families that were included in the original genome-wide scans (3, 15) and extended here by genotyping all available family members. Clinical characteristics of the study subjects are presented in Table 1. Heritability estimates of HDL-C and TGs, calculated for each study sample (i.e., low HDL-C families, FCHL families, and their combination) are provided in Table 2. The results of all of the linkage analyses will be available at our website (<http://www.ktl.fi/mols/wwwpub.htm>).

Chromosome 10q11

The 10q11 region was originally identified for high TGs in a qualitative linkage analysis of our genome scan for FCHL (LOD 3.2) (15). In that study, neither the binary low HDL-C trait nor any quantitative traits were analyzed. In the present study, the variance component analyses for the quantitative HDL-C in 92 dyslipidemic families provided a two-point LOD score of 3.2 with marker D10S1772 (Fig. 1). We also analyzed the potential pleiotropic effect of this locus on HDL-C and TGs by including TGs as a covariate in addition to gender and BMI, and obtained a maximum LOD score of 2.5 for HDL-C with marker D10S1772. When the two study samples were analyzed separately, the HDL-C and FCHL families produced two-point LOD scores of 1.9 and 1.5, respectively, for marker D10S1772. In the FCHL families, a slightly higher two-

TABLE 3. Maximum LOD scores of the variance component analyses for HDL-C and TG traits obtained in the different sets of low HDL-C and FCHL families

Locus	54 Families of the Original Genome Scan ^a		38 New Families ^b		Combined Sample of 92 Families ^c	
	HDL-C	TG	HDL-C	TG	HDL-C	TG
8q23.1	D8S85 (0.5) ^d	D8S385 (0.1)	D8S1018 (0.8)	D8S200 (0.1)	D8S85 (0.6)	D8S385 (0.2)
10q11	D10S1772 (2.9)	D10S568 (0.2)	D10S1220 (1.4)	D10S220 (0.2)	D10S1772 (3.2)	D10S1220 (0.2)
16q23.3	D16S3040 (0.7)	D16S3091 (0.3)	D16S3061 (1.0)	0.0	D16S3061 (1.1)	D16S3091 (0.1)
20q13.32	D20S164 (0.3)	D20S173 (1.7)	D20S171 (1.8)	D20S164 (1.4)	D20S173 (1.3)	D20S173 (2.2)

LOD, logarithm of odds.

^a Study sample consisting of the 54 low HDL-C and FCHL families of the original low HDL-C genome scan (3) when genotyping all available family members in the present study ($n = 784$). The 10q11 locus was not analyzed with the quantitative or binary HDL-C trait in our previous study (15).

^b Study sample consisting of the 38 new low HDL-C or FCHL families genotyped in the present study ($n = 325$).

^c Combined study sample of 92 low HDL-C and FCHL families ($n = 1109$).

^d The maximum two-point LOD scores of the quantitative linkage analysis obtained with the SOLAR program are shown in parentheses.

TABLE 4. Comparisons of the previous qualitative two-point results with the current qualitative two-point results for chromosomes 8q32.1, 16q23.3, and 20q13.32 linked to the low HDL-C trait in the low HDL-C genome scan (3)

Locus	Original Low HDL-C Genome Scan (Soro et al., 2002)		Families of the Original Low HDL-C Genome Scan Extended Here by Genotyping All Available Family Members ^c		LOD Score of the 38 New Families		92 Finnish Low HDL-C and FCHL Families of the Present Study ^d			
	LOD Score ^a and the Peak Marker for the Low HDL-C Trait ^b		Low HDL-C Trait		Low HDL-C Trait		Low HDL-C Trait		High TG Trait	
8q23.1	4.7	D8S1132	3.1	D8S1132	0.1	D8S1132	1.6	D8S1132	0.9	D8S1046
16q23.3	2.2	D16S3091	1.4	D16S402	0.0	D16S3091	1.5	D16S402	1.1	D16S3091
20q13.32	1.9	D20S171	2.1	D20S164	0.2	D20S171	2.1	D20S164	2.4	D20S102

^a The maximum two-point LOD scores of the parametric linkage analysis or nonparametric affected sibpair analysis.

^b Previous combined study sample consisting of 54 Finnish low HDL-C and FCHL families analyzed for the low HDL-C trait (n = 358) (3).

^c Study sample consisting of the families of the original genome scan and their additional genotyped family members analyzed here for the low HDL-C trait (n = 784).

^d Combined study sample of 92 Finnish low HDL-C and FCHL families (n = 1,109).

point LOD score of 2.3 was obtained with marker D10S1220, 1.6 cM apart from D10S1772, whereas in the HDL-C families, the peak marker was D10S1772. Results obtained with the MERLIN program supported these findings: in 92 families, D10S1772 produced the highest two-point LOD score of 2.6 ($P = 0.0003$). Marker D10S1772 resulted in a LOD score of 2.9 in the original 54 families and in a LOD score of 3.2 in the 92 families. However, the 38 new families provided a LOD score of 1.4 alone for another marker, D10S1220, residing on 10q11 and thus also contributed to the quantitative multipoint result of the region. Thus, the analyses of the original and new families separately show that both data sets provide linkage information for the quantitative HDL-C trait, although the peak marker varies (Table 3). No significant evidence of linkage to this chromosomal region was observed for the dichotomized low HDL-C or high TG traits (Tables 4–6). Neither did any other tested quantitative trait show significant evidence for linkage to this locus. All results of the analyzed traits will be available at our website (<http://www.ktl.fi/mols/wwwpub.htm>).

We also tested the 10q11 region for association using a novel QTL association method included in the software package Mendel (34) (Table 7). Interestingly, in these analyses, evidence for association for quantitative TGs was

observed with D10S546, ~7 cM from D10S1772 ($P = 0.0006$) (Table 7). For HDL-C, the association analysis produced a P value of 0.02 with marker D10S1790, located in the close vicinity of D10S546 (0.1 cM, 889 kb) (Table 7). Furthermore, a combined analysis of quantitative HDL-C and TGs produced a P value of 0.008, again with marker D10S546 in the association analysis (Table 7).

Chromosome 20q13.32

The locus on 20q13.32 was originally identified for a binary low HDL-C (LOD 1.9) in the combined analysis of low HDL-C and FCHL families (3) (Tables 4–6). Here, in the extended study sample of 92 low HDL-C and FCHL families, both the quantitative and qualitative TG traits produced slightly higher two-point LOD scores of 2.2 and 2.4 for this region, with markers D20S173 and D20S102, 11 cM apart. The quantitative and qualitative HDL-C trait also showed some evidence for linkage (Tables 3–6). When the two study samples were analyzed separately, the 53 FCHL families produced LOD scores of 1.5 (with marker D20S164) and 2.1 (D20S102) for the qualitative HDL-C and TG traits, and the 39 low HDL-C families produced LOD scores of 1.1 (D20S94) and 0.9 (D20S100), respectively. For the quantitative HDL-C and TG traits, similar results were obtained (data not shown). When analyzing

TABLE 5. Comparisons of the previous qualitative two-point results with the current qualitative two-point results for chromosome 10q11 linked to the high TG trait in the FCHL genome scan (15)

Locus	Original FCHL Genome Scan (Pajukanta et al., 1999)		Families of the Original FCHL Genome Scan Extended Here by Genotyping All Available Family Members ^c		LOD Score of the 63 New Families		92 Finnish Low HDL-C and FCHL Families of the Present Study ^d			
	LOD Score ^a and the Peak Marker for the High TG Trait ^b		High TG Trait		High TG trait		Low HDL-C Trait		High TG Trait	
10q11	3.2	D10S1220	1.8	D10S1220	0.5	D10S1220	0.5	D10S1220	0.8	D10S1772

^a The maximum two-point LOD scores of the parametric linkage analysis or nonparametric affected sibpair analysis.

^b Previous study sample consisting of 42 Finnish FCHL families analyzed for the high TG trait (n = 301) (15).

^c Study sample consisting of the families of the original genome scan and their additional genotyped family members analyzed here for the TG trait (n = 581).

^d Combined study sample of 92 Finnish low HDL-C and FCHL families (n = 1,109).

TABLE 6. Comparisons of the previous qualitative two-point results with the current qualitative two-point results for the two loci on 2q31 and Xq24 identified earlier for premature CHD in Finnish premature CHD families (16)

Locus	Original Premature CHD Genome Scan (Pajukanta et al., 2000)		39 Low HDL-C Families of the Present Study				53 FCHL Families of the Present Study				92 Finnish Low HDL-C and FCHL Families of the Present Study ^c			
	LOD Score ^a and the Peak Marker for the Premature CHD Trait ^b		Low HDL-C Trait		High TG Trait		Low HDL-C Trait		High TG Trait		Low HDL-C Trait		High TG Trait	
2q31	3.7	D2S2313	2.7	D2S129	1.4	D2S2324	1.3	D2S2324	1.1	D2S2324	1.5	D2S129	1.5	D2S2324
Xq24	2.9	DXS1072	0.4	DXS1212	0.5	DXS8088	0.9	DXS1072	0.9	DXS8067	0.9	DXS8064	1.6	DXS8067

CHD, coronary heart disease.

^a The maximum two-point LOD scores of the parametric linkage analysis or nonparametric affected sibpair analysis.

^b Study sample consisting of 223 premature CHD families (n = 550) (16).

^c Combined study sample of 92 Finnish low HDL-C and FCHL families (n = 1,109).

the original and new families separately, we observed that although the new families did not show evidence for linkage to this region using the dichotomized approach (Tables 4–6), in the quantitative analyses these new families resulted in a LOD score of 1.4 with the TG trait and in a LOD score of 1.8 with the HDL-C trait (Table 3).

Chromosome 2q31

The locus identified earlier in the Finnish premature CHD families on 2q31 (LOD 3.7) (16) showed evidence for linkage in the 39 low HDL-C families of the present study with the dichotomized low HDL-C trait: a two-point LOD score of 2.7 was obtained with D2S129. The 53 FCHL families produced a LOD score of 1.3 for the low HDL-C trait with marker D2S2324 (Tables 4–6). The combined study sample of the 92 families resulted in a LOD score of 1.5 with D2S129. Quantitative traits did not provide any significant evidence for linkage to 2q31.

Chromosomes 8q23.1, 16q23.3, and Xq24

The regions on 8q23.1 and 16q23.3 did not provide additional evidence for linkage in the extended study sample of the 92 low HDL-C and FCHL families compared with the original scan results (Tables 4–6). When we analyzed the original families (3) in their extended form, including all available family members, a LOD score of 3.1 was obtained with the same peak marker, D8S1132, as in the original study (Tables 4–6), whereas the 38 new families provided no evidence of linkage (Tables 4–6). Quantitative traits did not provide any significant evidence for linkage (Table 3).

The 16q region, identified previously for low HDL-C in the combined analysis of low HDL-C and FCHL families (a LOD score of 2.2) (3) (Tables 4–6), provided a LOD score of 1.5 for the dichotomized HDL-C trait and a LOD score of 1.1 for the quantitative HDL-C trait in the extended study sample of 92 low HDL-C and FCHL families (Tables 3 and 4). The region on Xq24, detected originally for the CHD trait in the CHD families (16), resulted in a LOD score of 1.6 for high TGs in the 92 low HDL-C and FCHL families (Tables 4–6). With quantitative traits, no evidence for linkage to Xq24 was detected.

DISCUSSION

We have here addressed the linkage evidence of six chromosomal regions identified in our previous genome-wide scans for dichotomized lipid traits and premature CHD (3, 15, 16). We hypothesized that quantitative analyses of the lipid phenotypes that overlap between the FCHL and low HDL-C disorders could help further investigate the six regions. Accordingly, we genotyped 1,109 family members from 92 multigenerational Finnish FCHL and low HDL-C families to perform a powerful quantitative analysis. We also analyzed this extended study sample using binary traits to mimic the previous analyses (3, 15, 16). The obtained results supported three regions, 2q31, 10q11, and 20q, among which 10q11 also provided evidence for association ($P = 0.0006$). Importantly, all of these loci have been observed in other populations for traits closely related to the metabolic syndrome (3, 8, 10, 11, 14–16, 38–45), the increasingly common disorder that is strongly implicated in the growing worldwide epidemic

TABLE 7. Results of the association analyses of low HDL-C and FCHL families obtained by the novel association program for quantitative traits (K. Lange and E. Sobel, unpublished data)

Locus	Distance ^a	P^b		
		HDL-C Trait	TG Trait	Combined HDL-C and TG Trait
<i>centimorgan</i>				
D10S1233	0	n.s.	n.s.	n.s. ^c
D10S604	0.10	n.s.	n.s.	n.s.
D10S1772	2.28	n.s.	0.06	n.s.
D10S1793	2.38	n.s.	n.s.	n.s.
D10S1724	3.80	n.s.	n.s.	n.s.
D10S196	3.90	n.s.	n.s.	n.s.
D10S220	4.00	n.s.	0.06	n.s.
D10S1220	4.10	n.s.	n.s.	n.s.
D10S568	5.73	n.s.	n.s.	n.s.
D10S1790	9.31	0.02	n.s.	0.05
D10S546	9.41	n.s.	0.0006	0.008

^a Distance from the first marker.

^b Uncorrected P values.

^c n.s., nonsignificant.

of type 2 diabetes mellitus. Taken together, these loci may represent worthwhile targets for the metabolic syndrome.

We found significant evidence of linkage for quantitative HDL-C to 10q11. In our earlier genome-wide scan, this locus was linked to high TGs (15), a trait highly inversely correlated to HDL-C. The TG trait was also implicated here, especially in the association analysis. Evidence for an association on 10q11 was observed with marker D10S546 ($P = 0.0006$) using a novel quantitative association program (K. Lange and E. Sobel, unpublished data). This program also allowed us to analyze a combined phenotype of quantitative HDL-C and TGs, which provided some evidence for an association with the same marker ($P = 0.008$), suggesting that biological pathways leading to each of the individual phenotypes may overlap regarding the function of the underlying gene. Previously, this particular 10q11 region has been linked to obesity and BMI (38–40), implying that the underlying gene(s) contributes to various component traits of the metabolic syndrome. The 20q13.32 region was linked to TGs and HDL-C. Several previous studies have reported evidence for linkage between the 20q13 region and type 2 diabetes mellitus, obesity, and HDL-C in different populations (8, 10, 14, 41). However, the TG trait has not, to the best of our knowledge, been linked to 20q13.32 previously. The locus on 2q represents an independent replication: families ascertained for CHD produced the original linkage signal (16), whereas an independent sample of dyslipidemic families was analyzed here. This region has also been linked to type 2 diabetes mellitus (42) and, interestingly, to HDL-C (43, 44). Similarly, in the present study, 2q31 provided evidence for linkage to low HDL-C in the low HDL-C families. This replication thus confirms that the 2q31 region likely harbors a gene for low HDL-C.

The loci on 8q23.1, 16q23.3, and Xq24 did not obtain additional support in the extended study sample. This is not atypical for complex traits. Both FCHL and type 2 diabetes mellitus provide similar examples in which the second study cohort originating from the same population as the original one fails to show evidence for linkage for some of the identified regions (46, 47). It has been suggested that differences in family structures or in the frequency of a disease-causing allele within families could contribute to the power to detect linkage, especially in the case of small study samples (47–50). Furthermore, additional families and significant extension of existing families probably also pose a risk of introducing increased genetic heterogeneity within and between the families.

This study does not represent a typical replication attempt with an independent study sample. Rather, we aimed to apply a powerful quantitative approach by extending the genotyping to all families and family members. However, these families were ascertained for multiple dyslipidemic family members, which results in a somewhat restricted variation of the investigated traits and may have reduced the power of QTL analyses. Furthermore, the two inherently different approaches, dichotomized and quantitative, are expected to extract different genetic information from these families and thus can re-

sult in different linkage peaks, even in different genomic regions.

Low HDL-C and high TG are highly inversely correlated traits, potentially exhibiting at least a partially shared genetic background. We investigated this relationship by including TGs as a covariate for HDL-C, and vice versa, in the linkage analysis for the most significant signal, 10q11. The LOD score decreased from 3.2 to 2.5, suggesting that the incorporation of TGs as a covariate did not improve the signal-to-noise ratio in our study sample. The 10q11 locus, however, seems to have a pleiotropic effect on the HDL-C and TG levels.

The linked region on 10q11 between markers D10S1233 and D10S546 (9.4 cM, 11.5 Mb) contains three positional candidate genes. First, marker D10S546, which resulted in a P value of 0.0006 in the association analysis, is an intragenic marker of the protocadherin 15 gene (PCDH15), encoding an integral membrane protein that mediates calcium-dependent cell-cell adhesion (51), but is not known to be involved in dyslipidemias or CHD. Second, marker D10S1772, which produced a LOD score of 3.2, is an intragenic marker of gene KIAA1607, a lysosomal trafficking regulator. Although the exact function of this gene is unknown, it may be required for sorting of endosomal resident proteins into late multivesicular endosomes (52). Furthermore, this gene contains an ATP/GTP binding site motif A (P-loop), a domain that also exists in the ABCA1 gene, making KIAA1607 a highly potential candidate gene for HDL-C regulation. The third positional candidate gene is an RNA-binding protein apobec-1 complementation factor that is involved in the editing of the APOB mRNA (53). These three genes provide a starting point to identify the underlying gene(s) involved in the regulation of HDL-C and TG levels. ■■

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