Quantitative Trait Linkage Analysis of Lipid-Related Traits in Familial Type 2 Diabetes

Evidence for Linkage of Triglyceride Levels to Chromosome 19q

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Macrovascular disease is a major complication of type 2 diabetes. Epidemiological data suggest that the risk of macrovascular complications may predate the onset of hyperglycemia. Hypertriglyceridemia, low levels of HDL cholesterol, and an atherogenic profile characterize the insulin resistance/metabolic syndrome that is also prevalent among nondiabetic members of familial type 2 diabetic kindreds. To identify the genes for lipid-related traits, we first performed a 10-cM genome scan using 440 markers in 379 members of 19 multiplex families ascertained for two diabetic siblings (screening study). We then extended findings for three regions with initial logarithm of odds (LOD) scores >1.5 to an additional 23 families, for a total of 576 genotyped individuals (extended study). We found heritabilities for all lipid measures in the range of 0.31 to 0.52, similar to those reported by others in unselected families. However, we found the strongest evidence for linkage of triglyceride levels to chromosome 19q13.2, very close to the ApoC2/ApoE/ApoC1/ApoC4 gene cluster (LOD 2.56) in the screening study; the LOD increased to 3.16 in the extended study. Triglyceride–to–HDL cholesterol ratios showed slightly lower LOD scores (2.73, extended family) in this same location. Other regions with LOD scores >2.0 included HDL linkage to chromosome 1q21-q23, where susceptibility loci for both familial type 2 diabetes and familial combined hyperlipidemia have been mapped, and to chromosome 2q in the region of the *NIDDM1* locus. Neither region showed stronger evidence for linkage in the extended studies, however. Our results suggest that genes in or near the ApoE/ApoC2/ApoC1/ApoC4 cluster on 19q13.2 may contribute to the commonly observed hypertriglyceridemia and low HDL seen in diabetic family members and their offspring, and thus may be a candidate locus for the insulin resistance syndrome. *Diabetes* 51:528–535, 2002

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ApoC1, apolipoprotein CI; ApoC2, apolipoprotein CII; ApoC4, apolipoprotein CIV; ApoE, apolipoprotein E; FCHL, familial combined hyperlipidemia; LOD, logarithm of odds; SOLAR, Sequential Oligogenic Linkage Analysis Routines; TG, triglyceride; WHO, World Health Organization.

Macrovascular disease is the major complication of type 2 diabetes (1), and lipid abnormalities are common among individuals with type 2 diabetes and their relatives (2,3). Both the lipid abnormalities and the risk of vascular disease likely predate the onset of glucose intolerance (4). The lipid abnormalities that characterize type 2 diabetes, high triglyceride (TG) levels and low levels of HDL cholesterol, also characterize the insulin resistance syndrome without diabetes (5,6). Insulin resistance also accompanies the common genetic disorder of familial combined hyperlipidemia (FCHL) (7), which is likewise characterized by elevated TG, low HDL cholesterol, and coronary artery disease.

Considerable data support genetic controls over lipoprotein metabolism (7–9), but known genetic variants cannot explain the observed lipid abnormalities in families at high risk for coronary disease. Consequently, many investigators have recently used the genome-scan approach to identify novel genes controlling lipids. Loci for FCHL have been identified and confirmed for chromosome 1q21–q23 (10,11) and suggested on 10p (12) and 11p (13). The chromosome 1q FCHL locus maps to the same region of chromosome 1 as a confirmed locus for type 2 diabetes (14–16). Loci controlling HDL cholesterol were proposed for 11q23 in Utah families (17) and for 8q and 15 in San Antonio Hispanic families. A major locus controlling TG levels was mapped to 15q in a second set of San Antonio Hispanic families ascertained for type 2 diabetes (18). In Pima Indians, Imperatore et al. (19) reported evidence for TG linkage on 2p and 3p (logarithm of odds [LOD] <2.0) and for HDL on 3q (LOD 2.64), but found the best evidence for linkage to total cholesterol on 19p (LOD 3.89). In unselected families, Shearman et al. (20) reported a gene for TG-to-HDL ratio on 7q32.3-qter. Although additional quantitative trait data are available from other recently published genome scans of type 2 diabetes (21,22), linkage analysis of lipoprotein phenotypes has not been reported. Results of additional candidate gene studies have been inconsistent (7). The available data suggest that multiple loci with heterogeneity and/or epistasis are likely. The genetic determinants of dyslipidemias, particularly in familial type 2 diabetes, thus remain to be defined.

In the present study, we used a staged genome-wide

TABLE 1
Numbers of relative pairs in screening and extended family studies

Relative pair	Number of pairs	
	Screening study	Extended study
Parent-offspring	369	536
Siblings	474	687
Grandparent-grandchild	83	110
Avuncular	917	1,239
Half-siblings	0	4
Grand avuncular	46	70
Half-avuncular	0	2
First cousins	1,015	1,316
First cousins, once removed	135	143
Second cousins	24	24
Total	3,063	4,132

Data are shown for triglyceride levels; somewhat fewer pairs were available for LDL, which could not be calculated in some individuals.

scan approach to test the hypothesis that a major gene controlling plasma lipids segregates in kindreds ascertained for familial type 2 diabetes. We examined TG, HDL cholesterol, total cholesterol, calculated LDL cholesterol, and two derived indexes of cardiovascular disease risk (TG-to-HDL ratio and LDL-to-HDL ratio) as continuous, quantitative traits in Utah Caucasian families ascertained for at least two diabetic siblings. We find the strongest linkage results for a locus controlling TG on chromosome 19q13.2. Evidence for linkage increases when additional families are studied and is also present in nondiabetic family members. The maximum LOD score is adjacent to the cluster of four strong candidate genes for hypertriglyceridemia: apolipoprotein E (ApoE), apolipoprotein CII (ApoC2), apolipoprotein CI (ApoC1), and apolipoprotein CIV (ApoC4).

RESEARCH DESIGN AND METHODS

Subjects. Families were ascertained for at least two siblings with known type 2 diabetes and no more than one parent known to have diabetes. All available family members, including parents of the proband sib pair, all available siblings, and offspring of both the proband sib pair and their diabetic siblings were tested if they were >18 years of age. All nondiabetic individuals underwent a standard 75-g glucose tolerance test with diabetes diagnosed by World Health Organization (WHO) criteria (23). Height and weight were obtained for all individuals, and age of diagnosis was ascertained for diabetic subjects. Lipid levels were determined using standardized methods in either the Cardiovascular Genetics Research Laboratory of the University of Utah (3,6) or at Medlantic Institute Penn Medical Laboratory (24). All measures were performed in plasma after a 12-h fast. LDL cholesterol was calculated from total cholesterol, HDL cholesterol, and triglycerides and was considered unknown for individuals with TG >4.52 mmol/l. Lipid levels for families only in the extension set were measured by Medlantic Institute's Penn Medical Laboratory (24). In the initial 19 families (referred to subsequently as "screening study"), genotypes were available for 407 individuals, including 379 for whom lipid measures were available. Of those individuals, 288 were nondiabetic by 1980 WHO criteria. At least 60 additional spouse controls were included for determination of allele frequencies. The "extended study" of 42 families included four simple sib pairs, but the remaining 19 new families were multigenerational, extended families. In the extended study, we tested a total of 610 individuals; lipid measures were available for 576 individuals, and 414 family members were nondiabetic. The extended study included 100 spouse controls for determination of allele frequencies. The number of relative pairs is shown in Table 1 for all available individuals in the data set from the screening set and the extension family set. Diabetes was diagnosed by glucose tolerance test or by treatment with insulin or oral hypoglycemic agents, as described elsewhere (25). Medication history was not used in the analysis, and all subjects were included regardless of antilipid medications. All subjects provided written informed consent under approval by the University of Utah Institutional Review Board, and all studies were conducted at the University of Utah General Clinical Research Center.

Genotyping. We tested 440 markers at a mean interval by our map of 9.2 cM for each of the 22 autosomes (14). Microsatellite markers were chosen from published maps (26–31) for heterozygosity >0.7. Most markers used in this study were dinucleotide repeats taken largely from Genethon maps (27,29), with additional markers at candidate genes for diabetes and from other maps (30,31). Most markers were amplified with a 5′ ^{32}P-labeled primer, separated on denaturing polyacrylamide gels, and detected by autoradiography as described previously (25,32). Markers were scored by two individuals, and discrepancies were resolved before analysis. Inheritance errors were resolved by review of original scores; unresolvable errors were scored as unknown. Markers run recently were labeled fluorescently. Infrared dyes were appended to an M13 primer. An M13 sequencing primer was appended to the standard amplification primer, and PCR was conducted with co-amplification of the labeled M13 primer and the amplification primers. Markers were detected on a LICOR GR4200 sequencer and scored semiautomatically with GeneImager software (Scanalytics, Billerica, MA).

Heritability estimates. We used variance-component analysis implemented in the SOLAR (Sequential Oligogenic Linkage Analysis Routines) computer program (33) to estimate the heritability of each tested trait both in the screening study and from the full phenotypic data available in the extended study. Heritability was estimated by including all available individuals, including diabetic family members, after correction for age and sex. No ascertainment correction was included.

Linkage analysis. We conducted quantitative trait linkage analysis of TG, total cholesterol, HDL cholesterol, TG-to-HDL ratio (20), and LDL-to-HDL ratio as a measure of cardiovascular risk. Each variable was adjusted for age and sex and transformed to reduce both skew and kurtosis to 0 before analysis (34). We used the multipoint variance-component approach to test linkage of each trait in all family members by maximum likelihood methods. In variance-component analysis, the variance is broken into locus-specific effects determined by the identity by descent relationships, the residual additive genetic effects, and individual-specific random environmental factors (18,33). We used the SOLAR computer program to calculate locus-specific identity-by-descent information for each pair of relatives with the multipoint extension, also implemented in SOLAR (33). Significance was expressed as LOD scores obtained by converting natural logarithm (ln) likelihood values into values of $\log_{10}$. Because TG may be elevated by poor glucose control, we tested TG linkage twice: first in all individuals, then only in nondiabetic family members, while considering the phenotype of diabetic family members as unknown. Distances were derived from multiple sources and confirmed using the ILINK program from actual family data (14). In contrast to our published dichotomous trait analysis of type 2 diabetes, where the first marker was considered to be at 0 cM, the maps for the current study estimate the location of the first marker as 5–10 cM from the telomere. Thus, distances are generally 5–10 cM greater than those published previously (14).

RESULTS

We conducted the screening study on 19 extended families for which full genotypic data were available. Table 1 summarizes the relative pairs, based on TG levels, for both screening and extended studies. We tested four measured traits related to diabetic dyslipidemia: TG in all members, TG in nondiabetic family members, total cholesterol, and HDL cholesterol. Additionally, we tested linkage of three derived measures of cardiovascular risk: LDL cholesterol, LDL-to-HDL ratio, and TG-to-HDL ratio. Heritabilities for these measures ranged from 0.31 for HDL to 0.52 for TG (Table 2). Figure 1 shows LOD score curves using our linkage map (14) for six traits: TG in all family members, total and HDL cholesterol, calculated LDL cholesterol, TG-to-HDL ratio, and TG-to-LDL ratio. In all, 37 contiguous regions had LOD >1.0 for at least one measured or derived trait (Fig. 1), and nine regions had LOD scores exceeding 1.5 (Fig. 1 and Table 3). TG tested in only nondiabetic individuals (data not shown) closely paralleled that in all individuals, albeit at generally lower LOD scores, with three exceptions. Possible linkage of TG in only nondiabetic family members was seen on chromosomes 8 (171–188 cM; LOD 1.53) and 21 (0–4 cM, LOD 1.12), but was not

TABLE 2
Heritability of lipid estimates from the extended study data

Variable	Extended study
Triglycerides	575 (0.52 ± 0.08)
Triglycerides in nondiabetic subjects	370 (0.52 ± 0.11)
Cholesterol	576 (0.47 ± 0.07)
LDL	550 (0.50 ± 0.07)
HDL	558 (0.34 ± 0.08)
TG-to-HDL ratio	556 (0.47 ± 0.08)
LDL-to-HDL ratio	550 (0.51 ± 0.08)

Data are n (h^2 ± SE). Heritability estimates are shown from variance-component analysis for all available data from extended study (42 family). Data from the screening study were nearly identical (not shown).

seen in all family members. The LOD on 19q at position 74–80 was 2.56 in all family members but only 1.38 in nondiabetic family members. The lower LOD scores when only nondiabetic individuals were tested were expected given the smaller sample size.

We focused on three regions with initial LOD >1.5: HDL linkage to chromosome 1q, which maps near the apolipoprotein AII (ApoA2) gene and in a region with putative genes for type 2 diabetes (10,14–16); HDL linkage to chromosome 2q in the region of *NIDDM1* (35) and calpain 10 (36); and TG linkage to the ApoC2/ApoE region of chromosome 19 (Fig. 2). Other regions were examined for all available data but were not specifically expanded and thus represent incomplete analyses. Of these three regions, analysis of the extended study data (see RESEARCH DESIGN AND METHODS) showed increased evidence for linkage only for TG on chromosome 19q, where the LOD score reached 3.16 at marker D19S178 (Fig. 2). The corresponding LOD score when diabetic individuals were excluded was 2.54, consistent with the loss of data. This peak of linkage is 3 cM from marker ApoC2 according to our map, but only 1.5 cM according the recent comprehensive maps (37). By current physical maps (38), D19S178 and the ApoC2/ApoE/ApoC1/ApoC4 gene cluster are separated by ~1.5 Mb.

Because errors in genetic distances might alter LOD scores when multiple markers in close proximity are analyzed using multipoint methods (39), we also performed a 2-point variance-component analysis using SOLAR. The peak LOD score was confirmed at 3.15 at marker D19S178, with nearby markers KLK, DM, HRC, and GYS1 showing 2-point scores of 1.99, 2.32, 1.02, and 1.15, respectively. These markers cover a physical distance from 48.6 Mb (D19S178) to 55.5 Mb (KLK1) on 19q13 (38). In contrast, the ApoC2 microsatellite polymorphism (49.8 Mb), which is in close physical proximity to the ApoE, ApoC1, ApoC2, and ApoC4 genes, showed a 2-point LOD score of only 0.45.

DISCUSSION

Macrovascular disease is the major complication of type 2 diabetes. Considerable previous data have suggested that the metabolic risk factors for macrovascular disease cluster in family members and probably predate the onset of hyperglycemia (4). Indeed, nondiabetic members of 16 of the families in the present study showed a 58% prevalence of lipid abnormalities (3). In the present study, we have used a variance-component approach to confirm that each of the major lipid traits is heritable in families ascertained for at least two diabetic siblings, with heritabilities in the same range as those for insulin resistance–related traits (14). These findings extend and confirm our earlier study (3) and other studies that have demonstrated insulin resistance–related traits in family members of diabetic individuals. Although such findings support the significance of shared genetic predisposition in controlling lipid-related traits, they do not necessarily indicate a major genetic locus controlling these traits. Genome scans offer one method to search for such loci. We used data from a genome scan conducted in our 19 largest families (screening study) to examine five traits that affect risk for macrovascular disease or, in the case of TG-to-HDL ratio, were shown by others (20) to be controlled by major genes. We then extended three of these regions to 19 additional multiplex families and 4 diabetic sibships (extended study) and examined all 42 families together. Within the screening study, no trait reached significance, with the highest LOD scores just over 2.0. Regions previously reported to be linked to lipid-related phenotypes were generally not replicated. These findings were not surprising. Even if a major gene were present, multiple interacting loci are likely to control each of these traits. Both complex disease and quantitative trait linkage studies in humans consequently have somewhat limited power, and replication of previously significant linkage can be difficult to achieve (39,40).

None of the nine regions with LOD scores >1.5 in our screening study corresponds to earlier reports. We found little evidence for linkage of TG to 15q (18), and although we found modest evidence for linkage to TG and TG/HDL ratio on chromosome 7, our peak linkage is at least 50 cM proximal to that reported by Shearman et al. (20). Large differences in map distances (total chromosome lengths of 150 cM for Shearman et al. and 236 cM in our study) between our studies might explain some of this discrepancy, and this region merits further analysis. In the recent study of Pima Indians (41), modest evidence for linkage of TG was shown to chromosomes 2p, 3, and 5q. In contrast, we have no evidence for linkage in these regions. We found three regions with modest evidence for linkage to HDL cholesterol, but none overlap with the regions identified by Almasy et al. (42) or with the regions of suggestive linkage in Pima Indians (chromosomes 3, 7, and 20). We also found no evidence for linkage of any lipid-related trait to the strong candidate regions on 11q (apolipoprotein AI, CIII, or AIV; ApoA1/ApoC3/ApoC4), hepatic lipase (15q21-q23), or cholesterol ester transfer protein (CETP) (16q21) (7). Finally, neither screening nor extended linkage data showed evidence for linkage of total cholesterol to 19p, where significant linkage was reported in the region of the LDL receptor (LDLR) in Pima Indians (41), although we found some evidence for linkage of TG-to-HDL ratio in this region (Fig. 2).

Several studies have recently reported linkage results for FCHL, a complex trait that is characterized by glucose intolerance and insulin resistance, and thus shares features of the insulin resistance/metabolic syndrome (12). One FCHL locus in Finnish families (10) and in a mouse model of FCHL (43) maps to the same region of chromo-

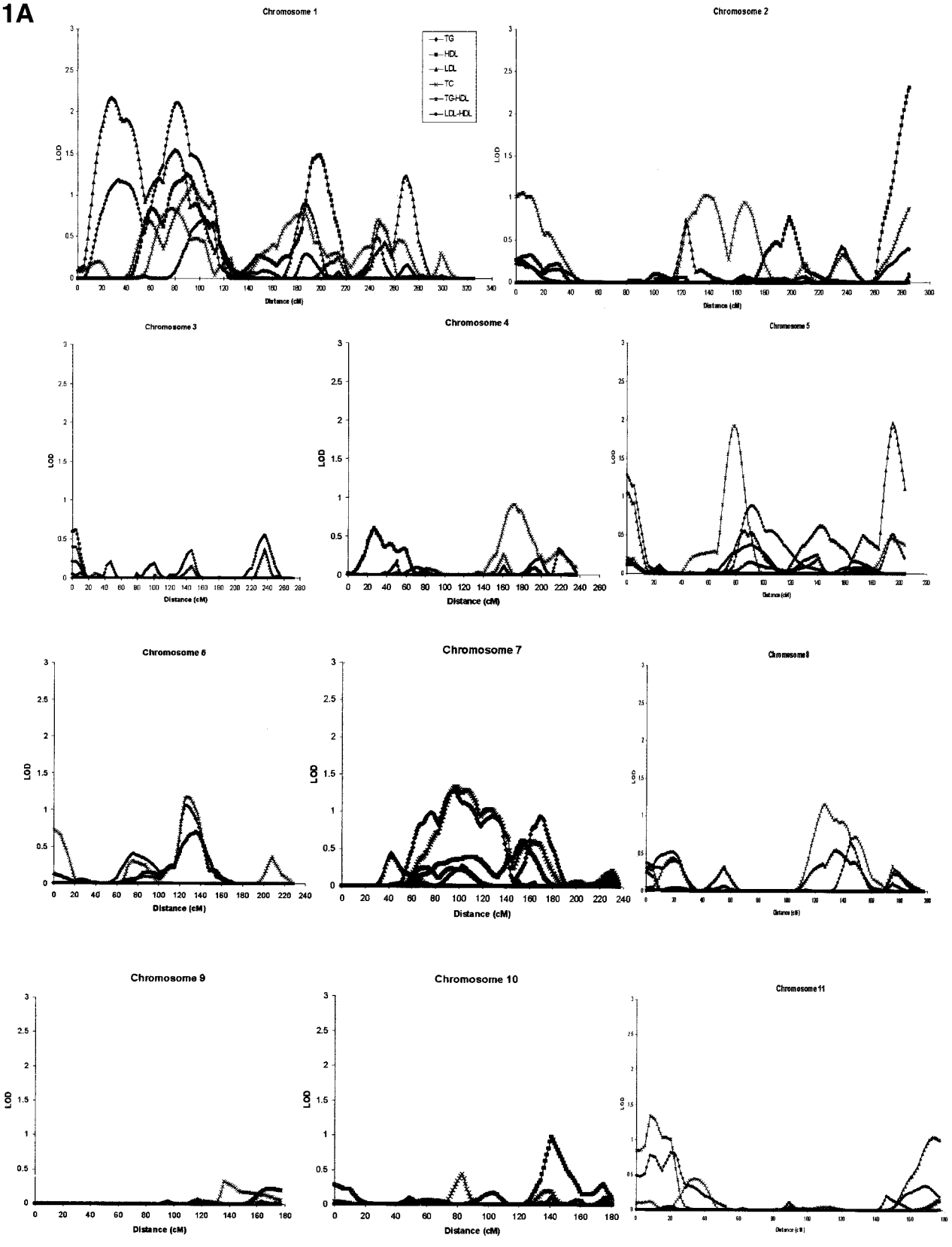

FIG. 1. LOD score tracings for the five lipid-related traits in the screening study. Distance is from the estimated *p*-telomere. Individual markers are not shown but are reported in Table 3 for LOD scores >1.5 and are available from the authors.

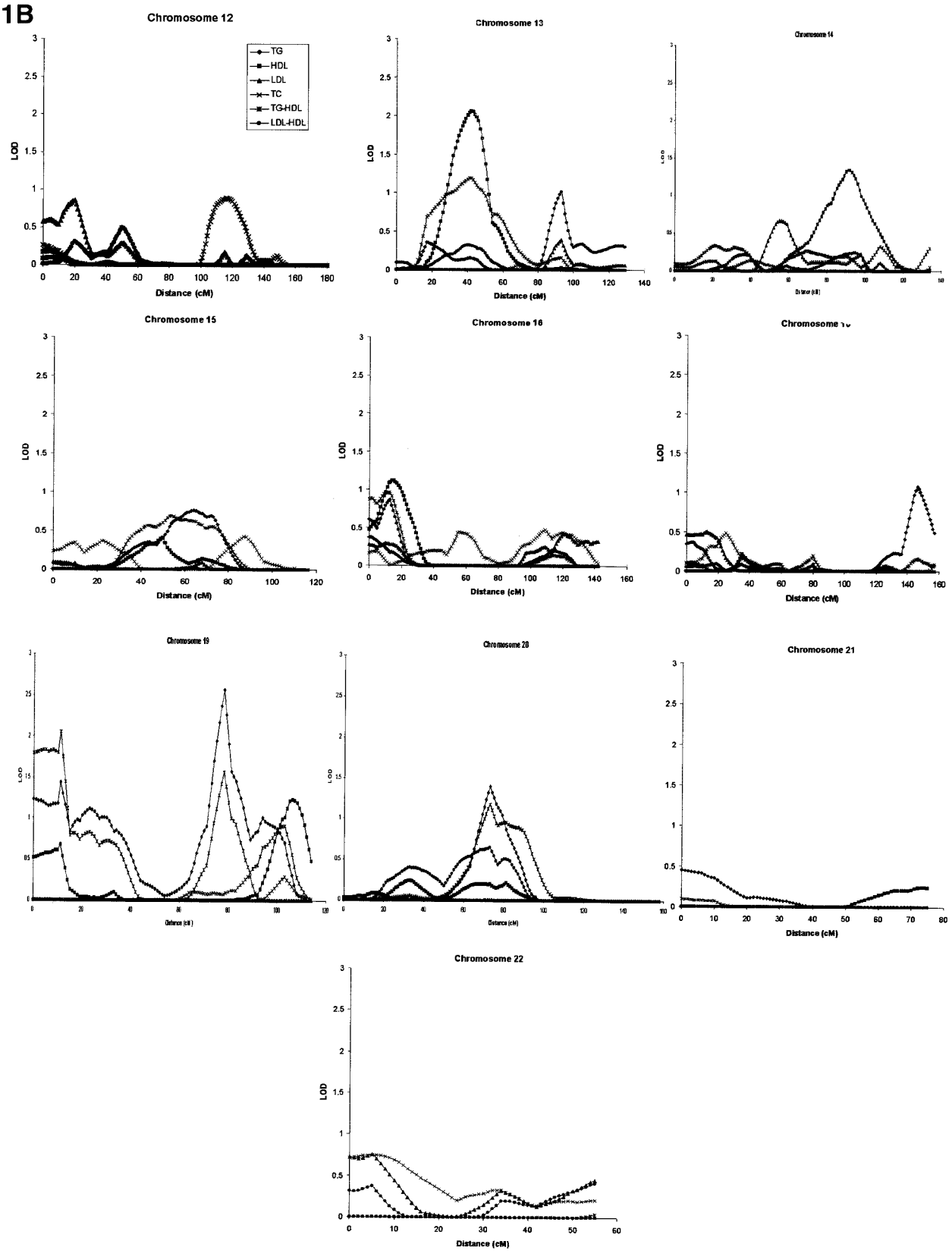

FIG. 1—*continued*

some 1q as a confirmed type 2 diabetes susceptibility gene (14–16). In contrast to the studies of FCHL as a dichotomous phenotype (affected versus unaffected), we examined lipids as quantitative (continuous) traits. We found no evidence for linkage of lipid-related traits to lipoprotein lipase (LPL), the ApoA1/ApoC3/ApoA4 cluster on 11q, the lecithin-cholesterol acyltransferase (LCAT) gene on 16q (7), or loci on chromosome 10 (12). We did find evidence

TABLE 3
LOD scores >1.5 for lipid-related traits in familial type 2 diabetes

Chromosome	Location (cM)	Trait	LOD, screening	LOD, extended	Marker
1	16–49	LDL	2.17 (28 cM)	2.43 (28 cM)	D1S214/D1S228
1	73–90	LDL:HDL	2.11 (82 cM)	1.89 (81 cM)*	D1S233/D1S193
1	190–210	HDL	1.49 (198 cM)	1.45 (198 cM)	D1S305/ApoA2
2	277–285	HDL	2.31 (285 cM)	1.14 (285 cM)	D2S338/D2S125
5	74–83	TC	1.92 (79 cM)	2.09 (79 cM)*	D5S427
5	191–201	LDL	1.96 (195 cM)	1.97 (195 cM)*	D5S211/D5S408
13	33–49	HDL	2.01 (42 cM)	1.93 (42 cM)*	D13S171/D13S263
19	0–14	TG:HDL	2.05 (11 cM)	1.92 (11 cM)	D19S247/D19S209
19	74–80	TG	2.56 (78 cM)	3.16 (78 cM)	D19S178/ApoC2

Trait, location (distance from the *p* telomere with LOD >1.5), and LOD scores for 10 regions that had LOD scores >1.5 for any lipid-related trait. TG, triglycerides (all family members); LDL:HDL, calculated ratio of LDL (calculated) to HDL; TG:HDL, triglyceride to HDL ratio. Maximum LOD score for each region is provided with the location of that maximum. The typed microsatellite marker closest to the maximum is provided for each trait. All distances are taken from the linkage map used in our analysis and may not correspond to other published linkage maps. Traits in the extended study included all traits available for all family members, including diabetic individuals. Regions marked with an asterisk (*) were not fully expanded to all individuals and all markers in expansion studies; thus, scores are likely to differ with full analysis and a dense map. TC, total cholesterol.

for linkage of HDL cholesterol in the region of chromosome 1q21–q23 that harbors the FCHL locus in Finnish families (10), but this modest evidence for linkage did not increase in the extended study. The ApoA2 gene is the strongest candidate in this gene-rich region. Transgenic mice overexpressing ApoA2 have elevated free fatty acid levels and increased triglycerides, altered HDL levels, and insulin resistance (44). We have screened the ApoA2 gene for variation and identified only noncoding changes in intronic and 3′ flanking regions (S.C.E., unpublished data), which show only modest association with diabetes. We have also failed to find coding region alterations of the retinoid X receptor γ gene in this region, which is known to form dimers with peroxisome proliferator–activator receptors α and γ. In preliminary analysis, we found little evidence for association of these variants with diabetes (S.C.E., unpublished data).

Our best evidence for linkage of any lipid trait was TG to 19q13.2. Because we have tested six different lipid-related phenotypes, and this value does not quite reach the levels proposed for genome-wide significance using allele-sharing methods (45), we would consider this linkage to fall between suggestive and significant. Two recent studies suggested that under some circumstances, variance-component analysis was subject to excessive error rates, particularly with extreme sampling distributions and skewed trait distributions (46,47). In the study of Hirschhorn et al. (47), empiric *P* values from simulation ranged from 0.02 to 0.24 for a LOD score of 3.1, albeit for smaller families and a trait with different characteristics. An LOD score of 3.1 may be compatible with failure to reach nominal genome-wide significance ($P < 0.05$). Thus, even our finding on chromosome 19q may represent false-positive linkage. However, several factors argue for the importance of this finding. In the present study, we normalized all data before analysis, and although we did not test the significance by simulation, extensive simulations by others have suggested that variance component analysis does not lead to inflated *P* values for normal data. Although similar artifacts (outliers or skewed data) might also alter LOD scores for nondiabetic family members, our finding of a suggestive LOD score for nondiabetic family members in this region of 2.54 close to the peak of linkage in all family members supports the biological significance of this region. This lower LOD score might be expected because diabetic subjects were excluded in this analysis. The close correspondence of these analyses suggests that the genetic predisposition to hypertriglyceridemia affects both diabetic and nondiabetic individuals similarly and is not likely to be due to hyperglycemia itself, nor to an interaction of this susceptibility locus with hyperglycemia, nor to diabetic individuals with very high triglyceride levels. Second, the TG-to-HDL ratio also maps to this location, although triglycerides and TG-to-HDL ratio show a strong correlation and this finding is expected. Third, this linkage is near a plausible cluster of candidate genes. These factors increase the confidence that a locus controlling TG levels is present in this region, despite some uncertainty regarding the statistical significance of the LOD scores derived from the variance-component linkage analysis. ApoE has been implicated in LDL cholesterol

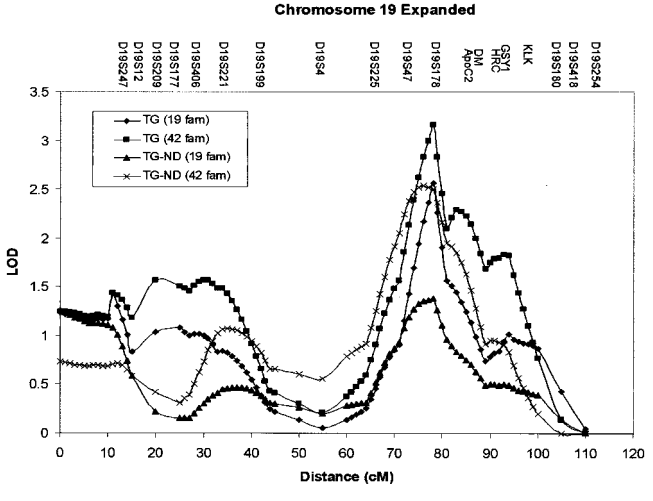

FIG. 2. LOD score tracings for chromosome 19 for triglycerides in both 19 and 42 family sets (screening and extended), and both including (TG) and excluding (TG-ND) individuals with diabetes. The 19 family triglyceride curves including individuals with diabetes are also shown in Fig. 1.

levels and in clearance of chylomicron remnants (7), but to our knowledge, no other study has reported linkage of TG to this region.

Despite strong suggestive evidence for linkage of TG to marker D19S178, the two-point LOD score for the ApoC2 microsatellite, which is within the cluster of genes that represent the obvious candidate genes in this region, is <0.5. For complex traits, LOD scores at any single locus depend on the amount of information that marker provides in the informative families in which susceptibility or protective alleles segregate (39). Thus, lack of linkage to ApoC2 does not exclude this gene or adjacent genes as explanations for this linkage. ApoC2 is a cofactor for lipoprotein lipase, and rare mutations cause familial hypertriglyceridemia (7). Unusual mutations in the promoter region, exons, and splice sites of the ApoC2 gene (48) cause hypertriglyceridemia by inactivating the gene, but paradoxically overexpression of ApoC2 in transgenic mice also causes hypertriglyceridemia (49). Among the common variants of ApoC2 are two amino acid changes (50) whose role in hypertriglyceridemia has not been evaluated, to our knowledge. Interaction of rare ApoC2 variants and the ApoE4 allele has also been described (51). Finally, the ApoE2 allele also may cause hypertriglyceridemia (52). The roles of the ApoC1 and ApoC4 genes are uncertain, but variation is a plausible cause of hypertriglyceridemia.

We also found linkage of TG-to-HDL ratio at the same location on chromosome 19 (LOD 2.727), with more modest evidence for linkage on chromosome 19p (LOD 1.9225) where total cholesterol was linked in Pima Indians (41). The TG-to-HDL ratio is an additional cardiovascular risk factor in type 2 diabetes, and an increased TG-to-HDL ratio commonly accompanies the insulin resistance-to-metabolic syndrome (20). Shearman et al. (20) reported heritability estimates for TG and TG-to-HDL ratio similar to ours (0.396 for TG, 0.488 for TG-to-HDL ratio). Interestingly, in their study both TG and TG-to-HDL ratio mapped to the same region of chromosome 7q, albeit with lower LOD scores than we report in the present study. Although both their study and ours might be interpreted to suggest that the same genes may control both TG levels and the TG-to-HDL ratio, these two phenotypes show a correlation of 0.94 in our complete sample. Thus, correspondence of the linkage may reflect the fact that TG and TG-to-HDL ratio in this sample in fact represents the same trait, as is reflected in the nearly invariant inverse relationship between TG and HDL cholesterol.

Replication of linkage findings is important in understanding the role of a locus in a complex disease, particularly when findings do not quite reach genome-wide significance. Such replication has been difficult to achieve and might require a study size at least three times larger than that of the current study (39). Given the strength of the ApoC2-to-ApoE-to-ApoC1-to-ApoC4 gene cluster as candidates for this linkage, and the higher power of association studies over linkage studies to detect variants in complex diseases (53), a direct search for association of common variants with TG and TG-to-HDL ratio appears justified. Such studies might lead to earlier intervention in the management of cardiovascular risk before the onset of hyperglycemia in these high-risk individuals.

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