A genome scan for serum triglyceride in obese nuclear families

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Abstract Serum triglyceride (TG) levels are increased in extremely obese individuals, indicating abnormalities in lipid metabolism and insulin resistance. We carried out a genome scan for serum TG in 320 nuclear families segregating extreme obesity and normal weight. Three hundred eighty-two Marshfield microsatellite markers (Screening Set 11) were genotyped. Quantitative linkage analyses were performed using family regression and variance components methods. We found linkage on the 7q36 region [D7S3058, 174 centimorgan (cM), Logarithm of Odds (LOD) = 2.98] for log-transformed TG. We also found suggestive linkages on chromosomes 20 (D20S164, 101 cM, LOD = 2.34), 13 (111 cM, LOD = 2.00), and 9 (104 cM, LOD = 1.90) as well as some weaker trends for chromosomes 1, 3, 5, 10, 12, and 22. In 58 African American families, LOD scores of 3.66 and 2.62 were observed on two loci on chromosome 16: D16S3369 (64 cM) and MFD466 (100 cM). To verify the 7q36 linkage, we added 60 nuclear families, and the LOD score increased to 3.52 (empirical *P* < 0.002) on marker D7S3058.—Li, W-D., C. Dong, D. Li, C. Garrigan, and R. A. Price. **A genome scan for serum triglyceride in obese nuclear families.** ***J. Lipid Res.*** **2005. 46: 432–438.**

Supplementary key words quantitative trait linkage • chromosome 7 • hypertriglyceridemia

Serum triglyceride (TG) level is usually increased in individuals who are obese or have type 2 diabetes (1–4). Although the causes of cardiovascular disease, obesity, metabolic syndrome, and type 2 diabetes are complicated, increased serum TG level is common to both obese and type 2 diabetic individuals (1–4). Epidemiologic studies have indicated that TG is an independent risk factor of cardiovascular disease (5–11) and stroke (12). Hypertriglyceridemia reflects abnormalities of lipid metabolism and insulin sensitivity. In a case/control study from the National Heart, Lung, and Blood Institute Family Heart Study, Hopkins et al. (13) found that increased TG levels in familial combined hyperlipidemia and hypertriglyceridemia were strongly related to metabolic syndrome and associated with the risk of coronary artery diseases.

Serum TG levels are largely controlled by genetic factors. More than 60 studies during the past 30 years have shown the heritability of plasma TG to range from 20% to 75%, with most studies indicating heritabilities of 30–40% (14–19). However, hypertriglyceridemia is not only a result of obesity. It may also increase the risk for obesity by inducing leptin resistance at the blood-brain barrier (20).

Some specific genes that influence hypertriglyceridemia have been identified. For example, mutations of the ABC1 gene cause Tangier disease, an autosomal recessive disease characterized by hypertriglyceridemia, hypocholesterolemia, and absence of normal HDL in plasma [Mendelian Inheritance in Man (MIM) 205400] (21). Association studies have found that apolipoprotein A-V gene (APOA5) variations were associated with plasma TG levels (22). Also, APOA4 gene polymorphisms were found to be related to quantitative plasma lipid risk factors of coronary heart disease (23). Mutations in the LCAT gene cause fish eye syndrome (24), including corneal opacities, HDL-cholesterol < 10 mg/dl, normal plasma cholesteryl esters, and increased TG. However, the genetic factors that influence common forms of hypertriglyceridemia remain unclear.

There are also nongenetic factors that play a role in hypertriglyceridemia. Certain diseases such as severe diabetes, hypothyroidism, and Gaucher's disease can cause secondary hypertriglyceridemia. In addition, environmental factors such as a high-fat diet increase serum TG levels.

To date, there are more than 20 published genome scans for TG and related lipid phenotypes; 1q, 7q, and 16q are among the most replicated regions (see **Table 1** and Discussion). To find plausible loci affecting serum TG, we carried out a genome scan in 320 nuclear families segregating extreme obesity and normal weight.

SUBJECTS AND METHODS

Subjects

Three hundred twenty families (1,514 subjects) were chosen from an ongoing study, as previously described (25). Briefly, all

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TABLE 1. Summary of linkages found in the 7q36-7qtel region for TG-related phenotypes

References	Subjects	Location/Markers	LOD	Phenotypes
Duggirala et al. (32)	Mexican Americans, 418 subjects	D7S1824 (139.4 Mb)	1.86	Log (TG)
Shearman et al. (33)	1,702 subjects from the Framingham Heart Study	D7S3070 (151.0 Mb, 163 cM)	1.8, 2.5	Log (TG), log (TG/HDL)
Lin (36)	1,702 subjects from the Framingham Heart Study	D7S3058 (154.0 Mb, 174 cM)	1.7	Log (TG)
Reed et al. (52)	75 obese nuclear families	D7S530	2.44 (*Z* score)	TG
Hsueh et al. (54)	Old Order Amish, 672 subjects	~20 cM downstream of leptin (164 cM)	1.77	BMI-adjusted leptin
Sonnenberg et al. (37)	507 Caucasian families, 2,209 subjects	D7S3058 (154.0 Mb, 174 cM)	3.7	Log (TG)

BMI, body mass index; cM, centimorgan; LOD, Logarithm of Odds; Log (TG), log-transformed triglyceride; TG, triglyceride.

family probands [body mass index (BMI) ⩾ 40 kg/m^2] had at least one obese sibling (BMI ⩾ 30 kg/m^2) and at least one parent and one sibling who were of normal weight (BMI < 27 kg/m^2). Most of these families were European American (260 families, 1,258 individuals) or African American (58 families, 247 individuals); only two families (9 individuals) reported other ethnic origins. Phenotypes were available for most parents. Genome scan results for body weight-related phenotypes (BMI, percentage fat, and waist circumferences) in 260 European American families were reported elsewhere (26). All subjects gave informed consent, and the protocol was approved by the Committee on Studies Involving Human Beings at the University of Pennsylvania.

After the genome scan, 53 European American families (237 individuals) and 7 African American families (37 individuals) were added to verify the most significant results.

Phenotypes

All blood samples were collected after fasting for >6 h. Fasting serum TG was measured by Quest Diagnostics (Philadelphia, PA). Log-transformed TG levels were adjusted for linear effects of age within generation, sex, and race using SPSS 11.0 (**Table 2**), after which higher order age effects were not significant. Specifically, we coded the original proband and his/her siblings as generation 1; their parents were coded as generation 0. Linear regressions were carried out between serum TG levels (log transformed) and age within each sex by race by generation category. Standardized residuals were calculated and saved (SPSS) to yield an overall mean = 0 and standard deviation = 1 for the combined sample. The heritability of log-transformed, adjusted fasting glucose in our sample was 0.40 [as calculated by the variance components computer program in the SOLAR package (27)].

DNA preparation and genotyping

DNA was extracted using a high-salt method (28) and diluted to 10 ng/μl for genotyping. Three hundred eighty-two polymorphic Marshfield microsatellite markers from Marshfield Screening Set 11 were genotyped by the Marshfield Center for Medical Genetics. Map distances were taken from the Marshfield database (http://research.marshfieldclinic.org/genetics/). Sex-averaged recombination rates were used in this study. One family was duplicated (coded as a different family) and used as an inner control for genotyping. Also, sex-specific PCR markers were amplified to verify gender. Mendel checks were performed by the computer program MERLIN, and all errors were corrected or dropped.

Statistical analysis

Log-transformed, adjusted serum TG levels were analyzed using the family regression test [multipoint linkage using MERLIN_regress (29)]. This program regresses identity by descent onto squared differences and sums of phenotype values for relative pairs. The family regression method is particularly appropriate for the TG data because it is insensitive to trait distribution (30). Variance components analyses were also conducted using the MERLIN computer program (multipoint linkage using MERLIN_vc). Only multipoint linkage analyses are reported.

Allele frequencies were based on allele counting for the European American, African American, and combined samples. More than 98% of families had one or more parental genotypes. DNA

TABLE 2. Descriptive statistics of TG-related phenotypes in 380 obese nuclear families

Phenotypes	Valid N	Minimum	Maximum	Mean	SD	Skewness	Kurtosis
			mg/dl				
Combined samples							
TG	1,732	28.00	1,482.0	178.3	129.4	3.51	21.42
Log (TG)	1,732	1.45	3.17	2.17	0.25	0.37	0.37
TG_LRES	1,710	−2.75	4.29			0.33	0.31
TG_LRES_BMI	1,689	−2.61	4.51			0.44	0.45
European Americans							
TG	1,447	28.00	1,482.0	185.5	136.1	3.45	20.78
Log (TG)	1,447	1.45	3.17	2.19	0.25	0.35	0.39
TG_LRES	1,442	−2.75	4.29			0.33	0.43
African Americans							
TG	276	38.0	520.0	140.9	78.9	1.88	4.77
Log (TG)	276	1.58	2.72	2.09	0.21	0.33	−0.08
TG_LRES	268	−2.20	2.75			0.34	−0.31

Log (TG), log-transformed TG; TG_LRES, age-adjusted, log-transformed TG; TG_LRES_BMI, BMI- and age-adjusted, log-transformed TG.

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was available for both parents in 194 families, for one parent in 120 families, and for neither parent in only 6 families.

Additional genotyping on 7q36

After detecting linkage on 7q36, 53 European American families (237 individuals) and 7 African American families (37 individuals) were added. We then genotyped markers D7S3058 and D7S3070 for those 60 additional families. Both family regression and variance components analyses (MERLIN) were conducted after new genotyping data were added. Genotyping of microsatellite markers was performed as previously described (31).

Simulations

To verify our linkage result on 7q36, simulations (500 replicates) were carried out for both family regression and variance components studies using the "simulate" function of MERLIN. Empirical *P* values were obtained by dividing the number of replicates that exceeded the observed Logarithm of Odds (LOD) score by the number of replicates (500).

RESULTS

Linkages on chromosome region 7qtel for plasma TG

Based on our genome scan data (382 microsatellite markers and 320 families: 260 European American and 58 African American), we carried out quantitative linkage analyses for serum TG. We analyzed log-transformed TG after adjusting for linear effects by age within sex, race, and generation. Distributions of original serum TG, log-transformed TG, and age-adjusted TG are shown in Table 2. Both family regression (MERLIN_regress) and variance components (MERLIN_vc) gave positive linkage on 7q36 for log-transformed adjusted TG [LOD = 2.98, D7S3058, 174 centimorgan (cM)] (**Table 3**).

To verify the linkage for TG on 7q36-7qtel, additional genotyping data for 53 European American and 7 African American families were added to marker D7S3070 (163 cM) and D7S3058 (174 cM). There were 380 total families: 313 European American and 65 African American. As shown in Table 3, the LOD score at 174 cM (D7S3058) increased from 2.98 to 3.52 (family regression method) for log-transformed adjusted TG (**Fig. 1**). For variance components analyses, the LOD score increased from 2.98 to 3.10.

Race-specific analyses showed linkage in the same region in European Americans as in the combined samples (Fig. 1). The LOD scores were 3.41 and 2.98 by family regression and variance components methods, respectively.

Because our study has limited information on HDL (we have HDL data for only 470 individuals), we could not perform analyses on TG/HDL ratio. The linkage result for serum cholesterol at 7q36-7qtel was not supportive of linkage (LOD < 0.2).

Because we also found linkages for BMI in the 7q36-7qtel region, we adjusted log-transformed TG by BMI and age within sex, race, and generation. After the adjustment, the linkage signals for TG remained significant: the family regression analyses found a LOD of 3.46 for all samples, and the variance components analyses found a LOD of 3.25.

Empirical *P* values were obtained by 500 replicates of simulations in both family regression and variance components analyses; only once did we obtain a LOD > 2.98 (empirical $P = 0.002$).

Suggestive or weak linkages in other chromosome regions of the human genome

Aside from the quantitative trait loci on 7q36, we also found suggestive linkages on chromosomes 20 (D20S164, 101 cM, LOD = 2.34), 13 (111 cM, LOD = 2.00), and 9 (104 cM, LOD = 1.90) as well as weak linkage signals on chromosomes 1, 3, 5, 10, 12, 19, and 22 (**Table 4**, **Fig. 2**).

Linkages on 16q and 13q in African American families

Within 58 African American families, we found two linkage peaks on 16q (Fig. 2): one on D16S3396 (64 cM, LOD = 3.66) and another on the marker MFD466 (100 cM, LOD = 2.62). We also obtained a LOD of 2.68 on 13qtel (AGAT113, 111 cM).

DISCUSSION

Fasting serum TG levels were linked to chromosome region 7q36 in a sample of nuclear families segregating extreme obesity and normal weight. The linkage was detected in the context of a whole genome scan and was strengthened by additional families and markers. The linkage finding was consistent across regression and variance components analyses. Analyses of the augmented sample localized the putative gene to a small interval of less than 5 Mb, a region small enough to be suitable for fine mapping analysis.

Although it is well known that there are high false-positive rates near telomeres, the peak linkage in chromosome region 7q36 is not at the terminal marker, at which there is a downturn in the LOD score. Moreover, there are multiple replications, as detailed below.

At least five published genome scans found linkage for TG in the 7q36-7qtel region (Table 1), but the linkage sig-

TABLE 3. Linkages of log-transformed, adjusted TG on 7q36-7qtel after adding 60 additional families

Analysis	Marker	Location	Samples	LOD	Empirical *P* Value
		cM			
Family regress	D7S3058	174	All	3.52 (2.98)[a]	<0.002
	D7S3058	174	European Americans	3.41	<0.002
Variance components	D7S3058	174	All	3.10 (2.98)[a]	<0.002
	D7S3058	174	European Americans	2.98	0.002

A total of 380 families (313 European American and 65 African American) were included in this study.
[a] Original genome scan results are shown in parentheses.

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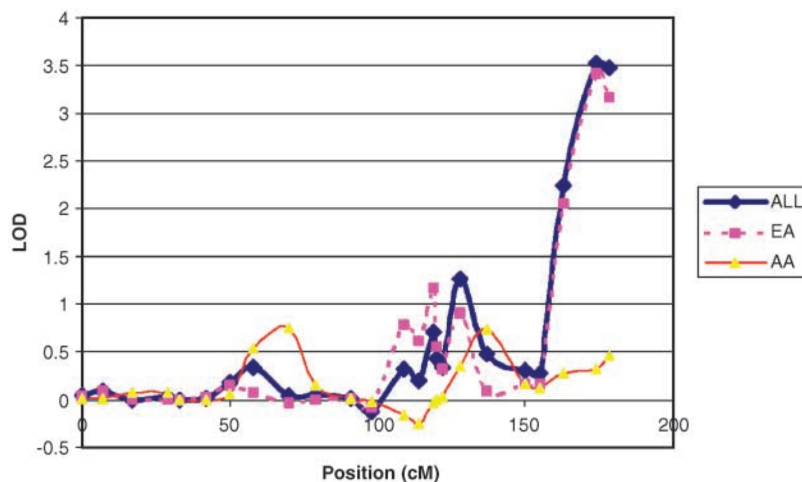

Fig. 1. Family regression quantitative linkage (MERLIN_regress) for log-transformed adjusted serum triglyceride (TG) on chromosome 7 in combined samples (ALL), European Americans (EA), and African Americans (AA), after an additional 60 families were added. cM, centimorgan.

nal has been poorly localized. Duggirala et al. (32) found linkage on D7S1824 for log-transformed TG in their Mexican American genome scan. Their linkage peak on D7S1824 was ~10 Mb upstream of our major linkage region. We, along with Shearman et al. (33), found linkages on markers D7S3058 to D7S3070. Horne, Malhotra, and Camp (34) identified a linkage of the TG/HDL ratio at 163 cM on chromosome 7 by variance components analysis (LOD = 2.67) in Framingham Heart Study subjects. Likewise using Framingham Heart Study samples, Zhang and Wang (35) and Lin (36) also found suggestive linkages of TG in the 7q36 region. Recently, Sonnenberg et al. (37) reported linkage on marker D7S3058 (LOD = 3.7) for TG in 507 Caucasian families. Interestingly, their population (Caucasians), markers (Marshfield), and phenotype (log-transformed TG) were similar to ours, and the similarity in the results is striking.

In our study, we obtained comparable results from family regression and variance components analyses. This result was not surprising because the distribution of log-transformed TG was approximately normal (Table 2).

TABLE 4. Linkages or suggestive linkages (LOD > 1.9) for log-transformed, adjusted TG in combined samples, European Americans, and African Americans found in genome scans by family regression analyses (MERLIN_regress, multipoint)

Sample	Chromosomes	Location	Marker	LOD
		cM		
Combined	7	174	D7S3058	3.52[a]
	9	104	D9S910	1.90
	13	111	AGAT113z	2.00
	20	101	D20S164	2.34
European Americans	7	174	D7S3058	3.41[a]
	9	104	D9S910	2.29
African Americans	13	111	AGAT113z	2.68
	16	64	D16S3396	3.66
	16	100	MFD466	2.62
	20	101	D20S164	2.48

[a] After 60 new families were added.

The 1 LOD confidence interval of the 7q36 linkage was narrow (<15 cM). According to the Human Genome Working Draft (http://genome.ucsc.edu/), the physical distance of the 15 cM region is only ~5 Mb.

There are 66 genes located in the 150–158 Mb (7q36-7qtel) interval, including 30 known genes. From the linkage mapping results, it is impossible at present to determine which of the genes with either known or unknown function may be related to TG levels. However, several are plausible candidates: INSIG1 (insulin-induced gene 1; 154.48–154.49 Mb; MIM 602055) is a key element for cholesterol processing, binding to the SREBP Cleavage-Activating Protein (SCAP)/sterol-regulatory element binding protein complex as a "brake" for cholesterol release to the Golgi body (38–40). ABCF2 (ATP binding cassette subfamily F, member 2; 150.30 Mb) is a homolog of ABCA1 (MIM 600046), the gene responsible for Tangier disease (high density lipoprotein deficiency) (41). Other genes, including PTPRN2 (protein tyrosine phosphatase receptor type N, polypeptide 2; MIM 601968), NOS3 (nitric oxide synthase 3; MIM 163729), PRKAG2 (protein kinase, AMP-activated, γ2 noncatalytic subunit; MIM 602743), and FABP5L3 (fatty acid binding protein 5-like 3), are plausible candidate genes based on functional significance.

The ABCA1 gene (MIM 600046) could be a candidate gene for the peak on D9S910 (104 cM). Mutations of the ABCA1 gene were found in Tangier disease and are characterized by low HDL and hypertriglyceridemia (41).

Several studies have reported linkage of 20q markers with obesity- and diabetes-related phenotypes. We too found suggestive linkage on 20q at the marker D20S149. PCK1 (phosphoenolpyruvate carboxykinase 1; MIM 261680), VAPB (vesicle-associated membrane protein B; MIM 605704), GNAS1 (guanine nucleotide binding protein α-stimulating activity polypeptide 1; MIM 139320), and NTSR1 (neurotensin receptor 1; MIM 162651) are among the candidate genes in this region. After adjustment by BMI,

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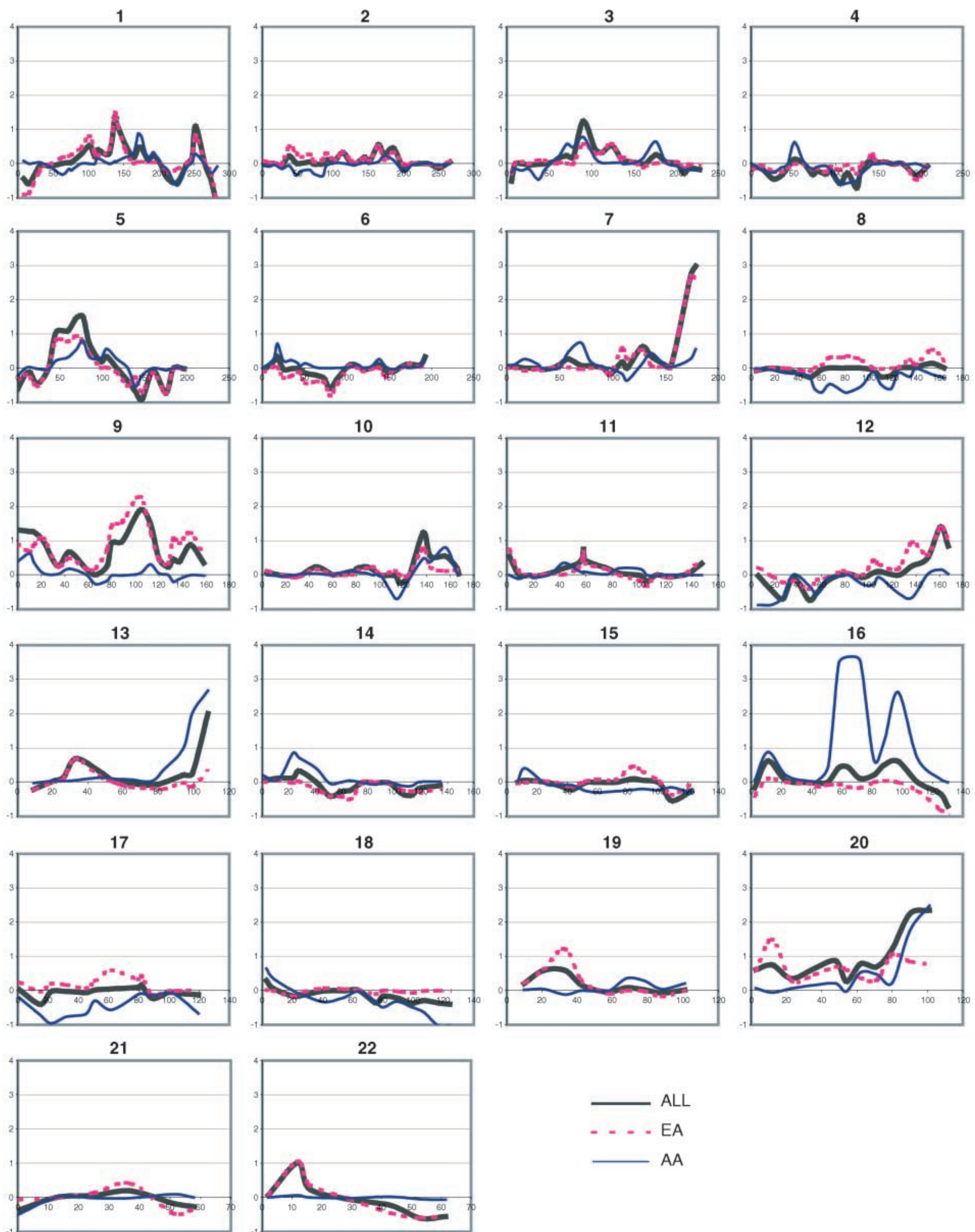

Fig. 2. Genome scan results [Logarithm of Odds (LOD) scores obtained by family regression analyses] for serum TG in combined samples (ALL), European Americans (EA), and African Americans (AA). The *x* axes represent map distances (centimorgan).

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the 20q peak decreased from LOD = 2.34 to LOD = 1.23, suggesting an overlap between TG and obesity quantitative trait loci.

The linkage on chromosome 13 was close to IRS2 (insulin receptor substrate 2; MIM 600797). IRS2 knockout mice develop type 2 diabetes (42), and human studies also yielded a positive association between IRS2 sequence variances and insulin resistance (43, 44).

The suggestive linkage on chromosome 5 (77 cM) was close to the linkage found by Bosse et al. (45). CART (co-

caine- and amphetamine-regulated transcript protein precursor; MIM 602606) could be a candidate gene. Also, the weak linkage on 3p overlapped with the linkage found by Imperatore et al. (46).

Although only 58 African American families were included in the genome scan, we obtained significant linkage on 16q (LOD = 3.66). As the locus on 7q36, the 16q linkage was replicated by several studies: Zhang and Wang (35) and Shearman et al. (33) found linkage on marker D16S3396 in the Framingham Heart Study. Mahaney et al. (47) also detected significant linkage (LOD = 3.73) on marker D16S518 for HDL-cholesterol levels in Mexican Americans. Several candidate genes, including CETP (cholesteryl ester transfer protein; MIM 118470) (48) and LCAT (MIM 606967) (49), locate in the 16q12-22 region. Recently, Badzioch et al. (50) also found linkage (LOD = 3.0) for LDL size near CETP in familial combined hyperlipidemia.

African Americans on average have lower TG and higher HDL levels than Caucasians (51). In our samples, African Americans have significantly lower TG levels (137.3 ± 78.0 mg/ml in African Americans vs. 181.9 ± 135.8 mg/ml in European Americans; $P < 0.001$). It is possible that different genes account for the serum TG variation in these two ethnic groups. On the other hand, the relatively small sample size of African Americans could have exaggerated the true difference.

Compared with our first genome scan in 75 families (52), our sample size has increased more than 4-fold (320 families, not including the additional 60 families used to verify the 7q36 results). Suggestive linkages found by our previous genome scan on chromosomes 1, 12, and 20 were verified in this study but remained weak. The linkage on D7S530 (found by our previous genome scan) was ~25 Mb upstream of the current TG peak on 7q36. Sample size, power, analytic methods, and genetic heterogeneity could have contributed to differences between the previous and current genome scans. In addition, marker selection was totally different between the current and previous genome scans. We used Marshfield Screening Set 11 markers in this genome scan, and most markers were tetranucleotide repeats, whereas our previous scan used mostly dinucleotide repeats.

Like other complex traits, serum TG levels are controlled by multiple genes. Using genome-wide RNA interference analysis, Ashrafi et al. (53) found more than 400 genes that can reduce or increase body fat storage in *Caenorhabditis elegans* (~2.5% of all *C. elegans* genes). In humans, the total number of genes influencing fat storage could be more.

In summary, we carried out a genome scan for serum TG levels and found significant linkage in the 7q36-7qtel region in nuclear families segregating obesity and normal weight. Our study localized the putative gene influencing TG to a small region of less than 5 Mb. Suggestive linkages were also found on chromosomes 20, 13, and 9. In African Americans, we detected linkage in chromosome region 16q12-22. These regions could harbor genes that regulate serum TG levels. Further analyses, including fine linkage and linkage disequilibrium mapping, are needed to identify those genes.

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