Multiple QTLs influencing triglyceride and HDL and total cholesterol levels identified in families with atherogenic dyslipidemia[S]

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Abstract We conducted a genome-wide scan using variance components linkage analysis to localize quantitative-trait loci (QTLs) influencing triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol, and total cholesterol (TC) levels in 3,071 subjects from 459 families with atherogenic dyslipidemia. The most significant evidence for linkage to TG levels was found in a subset of Turkish families at 11q22 [logarithm of the odds ratio (LOD) = 3.34] and at 17q12 (LOD = 3.44). We performed sequential oligogenic linkage analysis to examine whether multiple QTLs jointly influence TG levels in the Turkish families. These analyses revealed loci at 20q13 that showed strong epistatic effects with 11q22 (conditional LOD = 3.15) and at 7q36 that showed strong epistatic effects with 17q12 (conditional LOD = 3.21). We also found linkage on the 8p21 region for TG in the entire group of families (LOD = 3.08). For HDL-C levels, evidence of linkage was identified on chromosome 15 in the Turkish families (LOD = 3.05) and on chromosome 5 in the entire group of families (LOD = 2.83). Linkage to QTLs for TC was found at 8p23 in the entire group of families (LOD = 4.05) and at 5q13 in a subset of Turkish and Mediterranean families (LOD = 3.72). These QTLs provide important clues for the further investigation of genes responsible for these complex lipid phenotypes. These data also indicate that a large proportion of the variance of TG levels in the Turkish population is explained by the interaction of multiple genetic loci.—Yu, Y., D. F. Wyszynski, D. M. Waterworth, S. D. Wilton, P. J. Barter, Y. A. Kesäniemi, R. W. Mahley, R. McPherson, G. Waeber, T. P. Bersot, Q. Ma, S. S. Sharma, D. S. Montgomery, L. T. Middleton, S. S. Sundseth, V. Mooser, S. M. Grundy, and L. A. Farrer. **Multiple QTLs influencing triglyceride and HDL and total cholesterol levels identified in families with atherogenic dyslipidemia. *J. Lipid Res.* 2005.** 46: **2202–2213.**

Supplementary key words cardiovascular disease • epistatic effects • genetic linkage analysis • metabolic syndrome • variance components analysis

Atherogenic dyslipidemia is a combination of high triglyceride (TG) and low high density lipoprotein-cholesterol (HDL-C) concentrations often concomitant with high low density lipoprotein-cholesterol (LDL-C) and high total cholesterol (TC) levels (1, 2). Hypertriglyceridemia (defined as TG levels ⩾150 mg/dl) and decreased HDL-C levels (⩽40 mg/dl) are two important components of the metabolic syndrome, a condition associated with increased risk for atherosclerotic cardiovascular disease (CVD) (3–6). According to the National Health and Nutrition Examination Survey III, the age-adjusted prevalence of high TG

Manuscript received 7 April 2005 and in revised form 18 May 2005 and in re-revised form 1 July 2005.

Published, JLR Papers in Press, August 1, 2005.
DOI 10.1194/jlr.M500137-JLR200

Abbreviations: cM, centimorgan; CVD, cardiovascular disease; FCHL, familial combined hyperlipidemia; GEMS, Genetic Epidemiology of Metabolic Syndrome; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; LOD, logarithm of the odds ratio; QTL, quantitative trait locus; TC, total cholesterol; TG, triglyceride.

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[S] The online version of this article (available at http://www.jlr.org) contains an additional four figures.

(≥150 mg/dl) is ~30% of the adult population in the United States. High plasma TG levels and low HDL-C levels are commonly associated with other constituent phenotypes of the metabolic syndrome, including hyperinsulinemia, central obesity, hypertension, and impaired glucose tolerance (7). Low serum HDL-C is a characteristic component of familial combined hyperlipidemia (FCHL) (8, 9). Subjects affected with FCHL have a 2- to 10-fold greater risk for premature coronary artery disease (10–12). A meta-analysis of 17 prospective population-based studies showed that increased plasma TG level is significantly associated with CVD risk independent of HDL-C (13). High LDL-C and TC levels also contribute to the progression of atherosclerosis and are strong predictors of CVD risk (14, 15).

Numerous studies have shown that TG levels are at least partially affected by genetic factors (16–18). Duggirala et al. (19) reported significant linkage of TG levels to chromosome 15q11-q13 in Mexican American families. More recently, TG levels have been linked to chromosome 7q35-q36 (20). A gene for FCHL was localized to chromosome 1q21-q23, and the USF1 gene in this region has been associated with TG levels (21, 22). Other studies found suggestive linkage signals on chromosomes 2q14, 6q15-q16, 8p21-p23, 9p21, 10p11, 11p14-q12, and 19q13 in genome-wide scans using TG concentrations as quantitative traits (23–27). Loci for HDL-C levels have also been mapped. A study of Mexican Americans reported linkage of HDL-C concentrations to chromosome 9p21-p22 (28) and linkage of the unesterified HDL_{2a}-C component of HDL-C to chromosomes 8q24 and 15q22 (29). More than 20 loci for LDL and related lipid phenotypes have been reported (30), not including the recent finding of a quantitative trait locus (QTL) for TC and LDL-C on chromosome 2q21.2-q24.1 (31). Overall, the results of these studies were not definitive, because few minor susceptibility loci have been confirmed and no major putative locus affecting plasma TG and HDL-C levels has been identified.

In this study, we sought to map loci for plasma TG, HDL-C, LDL-C, and TC concentrations by linkage analysis of genetic marker data obtained from a genome-wide scan in the Genetic Epidemiology of Metabolic Syndrome (GEMS) Project (32). Identification of genetic loci that contribute to variation in these phenotypes may enhance our understanding of the pathways and mechanisms of lipid metabolism and eventually provide insight into the development of effective strategies for the prevention and treatment of hypertriglyceridemia, diabetes, metabolic syndrome, FCHL, and CVD.

SUBJECTS AND METHODS

Sample population

The GEMS Project is a large, international, and family-based study designed to explore the genetic basis of the metabolic syndrome and its constituent phenotypes. A description of this study was reported previously (32). Families containing at least one individual showing evidence of atherogenic dyslipidemia (plasma TG ≥ 75th percentile and serum HDL-C ≤ 25th percentile, both adjusted for age, gender, and population of origin) were ascertained from six centers in Adelaide (Australia), Ottawa (Canada), Oulu (Finland), Lausanne (Switzerland), Istanbul (Turkey), and Dallas (United States). Members of the nuclear or extended families were contacted and invited to participate in the study. A total of 3,071 relatives of 459 probands provided detailed medical history and epidemiological information and donated blood samples for biochemical and genetic analysis. The proband was the only member in 145 families meeting the criteria for atherogenic dyslipidemia. One hundred thirty-seven families had two affected members, and 177 families had three or more affected members. A total of 1,222 subjects including probands were affected. To calculate and assign proper marker allele frequencies in the linkage analysis, families were classified on the basis of their ethnic background as European (presumed European ancestry for individuals living in Switzerland, Canada, Australia, and the United States), Finns (those living in Finland), and Turk/Mediterranean. The Turk/Mediterranean group included 39 families residing in Turkey and 21 other families recruited in Ottawa and Adelaide. Eighty percent of the genotyped Turk/Mediterranean group subjects were Turks. Of the 21 families residing in Canada and Australia, 17 self-characterized their ethnicity as Mediterranean and 4 self-characterized as Middle Eastern. The efficacy of treating Finns as a discrete population for gene-mapping studies of complex traits was reviewed recently (33). Whereas most of the families were small sibships, several families, particularly those from Turkey, were larger and included more distant relatives. One large inbred Turkish family contributed 170 individuals to the analysis. One family self-identified as Hispanic and containing three phenotyped subjects was excluded from further analysis. The study protocol was approved on a yearly basis by the institutional review board at each participating institution.

Questionnaire information and phenotyping

Demographic, lifestyle, and medical history information, including medication use, risk factors, and family history, was obtained using standardized questionnaire instruments that were formatted for optical scanning. These data were transferred electronically to a centralized database at Boston University. Subjects had a thorough physical examination, and height, weight, waist circumference, and blood pressure measurements were obtained in a standardized manner. Body mass index was computed as weight (kg) divided by the square of height (m). Blood pressure was measured three times after a 5 min seated rest. The average of the second and third measurements was used in the analysis. Blood samples were collected after a 12 h fasting period. Clinical chemistry measurements, including TG, HDL-C, LDL-C, TC, and glucose levels, were obtained by standard procedures defined by the Clinical Laboratory Improvement Amendments (http://www.phppo.cdc.gov/clia/). Subject profiles are shown in **Table 1**.

Genotyping

DNA was extracted using the Puregene system (Gentra Systems) at the Center for Human Genetics at Duke University Medical Center. The DNA samples from the study subjects were organized into genotyping lists, producing a standardized order of samples; quality control samples were incorporated at specified slots in the list to act as interplate and intraplate controls to ensure consistency and accuracy of allele calling. The laboratory technicians were blinded to the identity of the quality control samples and to the affection status and family composition of all samples. A set of 448 microsatellite markers at an average density of <10 centimorgan (cM) was genotyped at the Australian Neuromuscular Research Institute in Perth. The average marker heterozygosity was 0.76. Marker locations and intermarker distances

TABLE 1. Subject characteristics

Variable	Probands (n = 459)	All Subjects (n = 2,850)	European (n = 1,744)	Turk/Mediterranean (n = 770)	Finn (n = 336)
Age (years)	46.1 ± 10.1	44.8 ± 14.8	45.1 ± 14.2	42.0 ± 16.4	49.1 ± 12.9
Gender (% male)	68.0	49.8	51.4	46.0	50.3
Body mass index (kg/m^2)	28.7 ± 3.6	27.5 ± 5.0	27.8 ± 5.0	27.0 ± 5.0	27.1 ± 4.4
TG (mg/dl)	330.0 ± 234.6	209.3 ± 196.0	230.9 ± 213.2	174.1 ± 175.6	177.8 ± 114.6
HDL-C (mg/dl)	34.2 ± 6.8	43.5 ± 14.2	43.4 ± 14.1	41.2 ± 12.3	49.3 ± 16.7
LDL-C (mg/dl)	122.0 ± 40.9	119.8 ± 39.7	112.7 ± 39.7	111.0 ± 37.2	126.7 ± 41.9
TC (mg/dl)	216.8 ± 50.0	203.1 ± 49.2	209.7 ± 49.9	186.1 ± 46.9	208.3 ± 41.6
Waist girth (cm)	98.6 ± 10.7	93.5 ± 14.6	95.5 ± 14.9	89.8 ± 13.5	91.5 ± 13.4
Glucose (mg/dl)	95.9 ± 16.6	95.8 ± 25.1	94.8 ± 23.5	95.9 ± 29.3	101.0 ± 21.9
Diastolic BP (mmHg)	83.6 ± 11.6	82.7 ± 12.1	82.5 ± 11.6	82.6 ± 13.4	83.8 ± 11.2
Systolic BP (mmHg)	135.1 ± 19.0	134.5 ± 21.6	133.6 ± 19.6	134.4 ± 24.9	139.2 ± 22.2

BP, blood pressure; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride. Values are means ± SD.

were obtained from the Marshfield sex-average genetic map (http://research.marshfieldclinic.org/genetics/) (34). The fluorescently labeled amplification products were fractionated on 6% sequencing slab gels under denaturing conditions and detected using a FMBIO II scanner (Hitachi). Alleles were identified and compared with known Human Genome Diversity Project (HGDP) Centre d'Etude du Polymorphisme Humain (CEPH) diversity panel standards (http://www.cephb.fr/HGDP-CEPH-Panel/) and submitted for pedigree analysis at Boston University once the internal quality control checks (i.e., zero mismatches called within the interplate and intraplate controls) were passed.

Statistical analysis

Pedigree and genotyping error detection and handling of inbreeding loops. Genotyping errors (1.4% of all genotypes) were detected using Pedcheck software (35) and reported to the genotyping laboratory for reanalysis. After a single reread of the alleles, the overall accuracy of allele calling was estimated to be 99.5%, and the remaining inconsistent genotypes were converted to missing values. Pedigrees were examined for potentially misspecified relationships based on analysis of the genotype data for 438 autosomal markers using the program PREST (36, 37). When the data showed a high likelihood of a misspecified relationship (e.g., a half-sib wrongly classified as a full-sib), the family relationships were adjusted based on the suggestion by PREST or problematic individuals were deleted from the pedigree. Of the 51 pedigrees revealed by this analysis to have a problem in pedigree structure, reassignment of a full-sib to a half-sib relationship resolved the problem in 18 pedigrees, and one individual in each of 33 pedigrees determined to be unrelated to any other family members was deleted. Inbreeding loops were broken into individual family structures with MEGA2 software (38). In these instances, one individual was duplicated and his/her genotypic data were set to missing values. Large families were trimmed using a computer routine written in PERL that we developed. All individuals uninformative for linkage analysis were removed from the pedigrees.

Data adjustment and normalization. Because blood lipid profiles change dramatically in puberty as a consequence of changes in sex steroid hormone levels (39) and this effect may not be accounted for by simple age adjustment, the phenotype data of 218 subjects younger than 16 years were excluded from the linkage analysis. A total of 2,850 individuals from 458 families (324 European, 60 Turk/Mediterranean, 74 Finn) were included in the genetic analyses (see Table 1 for subject characteristics at baseline). Plasma TG and HDL-C concentrations, waist girth, and fasting glucose (in the European group) were significantly different ($P < 0.0001$) between men and women. TG, HDL-C, LDL-C, and TC levels were log-transformed because they were not normally distributed and the variance components analysis method is known to produce spurious results when the assumption of normality is violated. We included age, age^2, gender, interaction terms for age-by-gender and age^2-by-gender, number of cigarettes smoked per day, amount of alcohol consumed per day, body mass index, and ethnicity as covariates in the models analyzed for linkage. Historical lipid values were used for 530 subjects who were taking lipid-lowering medication at the time that blood samples were drawn for phenotyping. However, use of these medications, which most prominently influence LDL-C concentrations (40–42), was not factored into the linkage analysis for TG and HDL-C.

Variance components analysis. We performed single-locus variance component linkage analysis for TG and HDL-C using the computer software SOLAR (version 2.13) (43). The autosomal marker data were evaluated by multipoint linkage analysis using the Marshfield genetic map, whereas the X chromosome data were evaluated by two-point linkage analysis only because of the limitations of SOLAR. To minimize the potential for bias attributable to nonrandom sampling, we corrected for ascertainment by conditioning the likelihood of each family on the phenotype of the proband (44). We also performed multipoint oligogenic linkage analyses to test for the effects of multiple loci on the same trait (45). Using this iterative approach, the genome scan data from the single-locus analysis were reanalyzed after conditioning the linkage model on the putative locus position with the highest logarithm of the odds ratio (LOD) score in the initial genome scan. If a LOD score for a second locus, conditioned on the first locus, exceeded the recommended LOD score cutoff of 2.2 for suggestive linkage (46), a three-locus model was evaluated by conditioning the linkage model on the first two loci. This procedure was repeated until no new multilocus model yielded a total LOD score greater than two LOD units obtained with the previous model. In addition to testing for additive genetic effects of a multilocus system, SOLAR allows a one-pass epistasis scan based on the oligogenic model (47). For example, the covariance matrix for a simple two-locus epistasis model can be written as:

$$\Omega = \Pi_1\sigma^2_{q1} + \Pi_2\sigma^2_{q2} + \Pi_1 \otimes \Pi_2\sigma^2_{q1q2} + 2\Phi\sigma^2_g + I\sigma^2_e$$

where σ^2_{q1q2} represents the variance components corresponding to the additive interaction effects between the two loci, Π_1 and Π_2 are the identical-by-descent matrices of the two loci, respectively, and $\otimes$ denotes the Hadamard matrix product operator. Oligogenic analyses have been demonstrated to yield more accurate estimates of the relative effect of each locus and increase the statistical power to detect linkages (45). The proportion of the variance accounted for by each component in the above model

TABLE 2. Heritability (h^2) estimates for the serum lipid traits

Trait	All Subjects (n = 2,850)	European (n = 1,744)	Turk/Mediterranean (n = 770)	Finn (n = 336)
ln (TG)	0.50 ± 0.04	0.46 ± 0.06	0.52 ± 0.06	0.58 ± 0.11
ln (HDL-C)	0.62 ± 0.04	0.52 ± 0.05	0.80 ± 0.05	0.69 ± 0.09
ln (LDL-C)	0.50 ± 0.04	0.46 ± 0.05	0.65 ± 0.07	0.19 ± 0.11
ln (TC)	0.43 ± 0.04	0.38 ± 0.05	0.53 ± 0.07	0.33 ± 0.11

(i.e., σ_i^2, where i refers to the terms in the model) was estimated by SOLAR as described previously (45). Hypothesis testing was conducted by comparing the maximum likelihoods for a restricted model in which σ_i^2 was constrained to zero and a more general model in which σ_i^2 was estimated. The $\log_{10}$ of the likelihood ratio between the oligogenic model and the polygenic model yields a sequential LOD score for the oligogenic model. The $\log_{10}$ of the likelihood ratio between the oligogenic model and the model on which it was conditioned yields a conditional LOD score for the new locus tested. SOLAR was also used to compute estimates of heritability for log-transformed values of TG and HDL-C, which were compared between groups using *Z* statistics.

Empirical P value estimation. Type I error rates for the LOD scores were determined by simulating 100,000 fully informative markers with SOLAR using the pedigree data under the null hypothesis of no linkage. For each simulated marker, identical-by-descent information was calculated and linkage analysis was then conducted. This simulation allowed for the random assignment of phenotypic data only to those subjects who were actually studied.

RESULTS

Heritability analysis revealed that serum lipids are under significant genetic influence (**Table 2**). The heritability estimates of HDL-C and LDL-C levels for the European families were substantially lower than those for the Turkish/Mediterranean families ($P = 0.00004$ and $P = 0.0006$, respectively), probably reflecting the comparatively greater genetic and cultural homogeneity of the latter groups.

Chromosomal regions suggesting possible linkage (LOD > 2.4) are listed in **Table 3**. The European families revealed suggestive evidence of linkage (LOD = 2.80) for TG level to a locus on chromosome 8p21 at 54 cM from

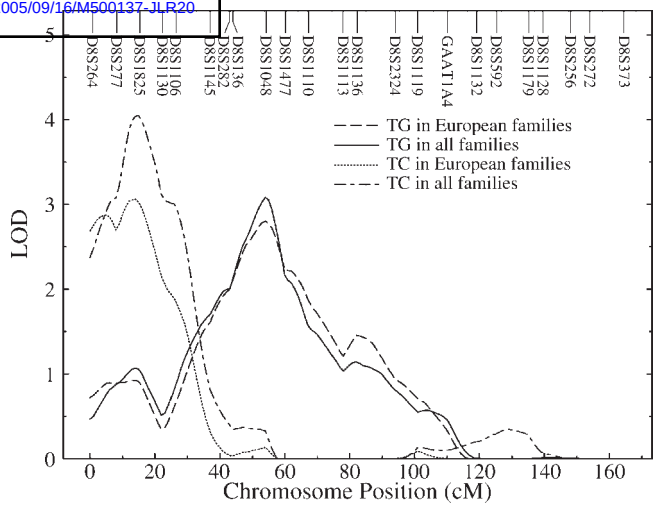

Fig. 1. Linkage of triglyceride (TG) and total cholesterol (TC) levels to chromosome 8. The maximum logarithm of the odds ratio (LOD) score of 3.08 for TG was obtained from analysis of all families in the study (solid line), although most of the evidence was derived from the European families (dashed line). Similarly, the maximum LOD score of 4.05 for TC was obtained from the entire group of families (dot-dashed line), with most of the evidence contributed by the European families (dotted line). cM, centimorgan.

pter near D8S1048. After including the Turkish/Mediterranean and Finnish families and adjusting for ethnicity, the LOD score increased to 3.08. Based on the 1-LOD unit support interval (corresponding to the 95% confidence limits), the linkage peak encompassed ~20 cM (**Fig. 1**). We detected significant evidence of linkage (LOD = 3.41) for TG level at 100 cM on chromosome 11q22 between D11S4159 and D11S1986 in the Turkish/Mediterranean families. The 1-LOD support interval for this linkage peak was ~12 cM (**Fig. 2**). We also found suggestive linkage for TG level in the Turkish/Mediterranean families at 82 cM on chromosome 5q13 (LOD = 2.90) close to D5S1501 (**Fig. 3**).

We repeated the genome-wide linkage analysis in the Turkish families to determine the extent to which the linkage peaks in the Turkish/Mediterranean families were accounted for by the Turkish families alone and, possibly, to

TABLE 3. Multipoint linkage analysis peaks with LOD score >2.4

Trait	Chromosome Location	Distance	Closest Markers	Sample Population	LOD	Empirical *P* Value
		cM				
TG	5q13	82	D5S1501	Turkish/Mediterranean	2.90	0.00039
	8p21	54	D8S1048	European	2.80	0.00014
				All families	3.08	0.00011
	11q22	100	D11S4159/D11S1986	Turkish/Mediterranean	3.41	0.00012
		99	D11S4159	Turkish	3.34	0.00011
	17q12	57	D17S1293	Turkish	3.44	0.00008
HDL-C	5p15	7	D5S2849	All families	2.83	0.00021
	15q22	58	D15S643/D15S153	Turkish/Mediterranean	3.02	0.00010
		66	D15S983	Turkish	3.05	0.00009
TC	5q13	83	D5S1501	Turkish/Mediterranean	3.72	0.00020
	8p23	15	D8S1825	European	3.06	0.0011
				All families	4.05	0.00038

cM, centimorgan; LOD, logarithm of the odds ratio.

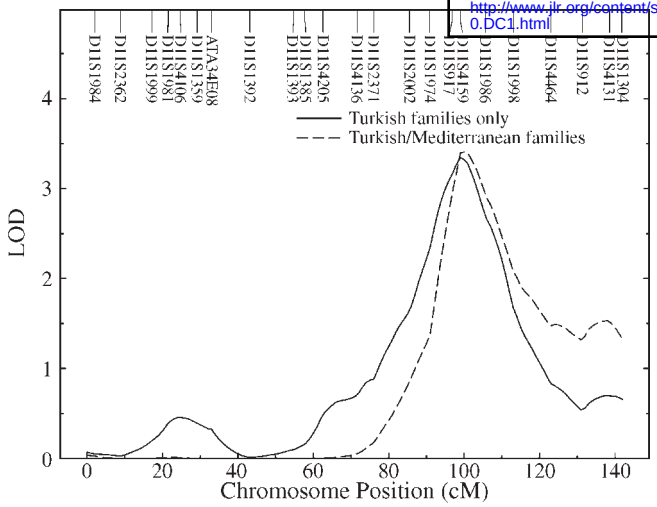

Fig. 2. Linkage of TG level to chromosome 11q22 in Turkish/Mediterranean families (dashed line). All of the evidence for linkage (LOD = 3.34) was derived from Turkish families (solid line).

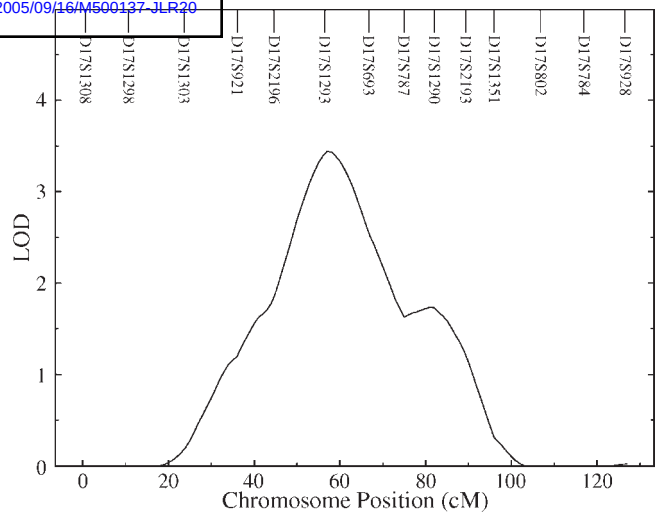

Fig. 4. Linkage of TG level to chromosome 17q12 in Turkish families.

identify QTLs unique to this population. The maximum LOD score for TG level on chromosome 11q22 was nearly identical (3.34), suggesting that Turkish families account for all of the evidence for linkage at this location (Fig. 2). Significant evidence of linkage (LOD = 3.44) for TG level was also detected at 57 cM on chromosome 17q12, with a 1-LOD support interval of 21 cM (**Fig. 4**). This QTL did not reach the threshold for suggestive linkage in the sample including the Mediterranean families (LOD = 2.01). The secondary linkage peak on chromosomes 5q13 was not evident in the Turkish families alone (LOD = 1.0).

We extended the variance component linkage analysis to examine simultaneously linkage to multiple QTLs for TG levels in the Turkish families. Analysis of the genome scan data conditioned on the putative locus with the highest LOD score suggested by the single-locus analysis (chromosome 17q12) yielded a LOD score of 6.13 for the two-locus model, with a second QTL on chromosome 11q22 between D11S4159 and D11S1986 (**Table 4**). Further iteration did not reveal a third locus that had a conditional LOD score >2.0. To search for loci influencing TG level through epistatic interaction with the major loci at 17q12 and 11q22, we tested two models, each including one of the 17q12 and 11q22 loci and an interaction term for a second locus. Conditioned on linkage to the 17q12 QTL, we detected a LOD score of 3.21 on chromosome 7q36 near D7S559 at 182 cM (**Fig. 5**). A model conditioned on linkage to the 11q22 QTL revealed a LOD score of 2.60 on chromosome 20q13 near D20S171 at 96 cM (**Fig. 6**). A three-locus epistasis model conditioned on linkage to QTLs at 17q12 and 11q22 and interaction between the 17q12 and 7q36 loci showed a sequential LOD score of 9.0. These three loci accounted for 87% of the variance of TG levels in the Turkish families, with the interaction term accounting for most of this proportion. An even higher sequential LOD score (9.3) was obtained from a three-locus epistasis model predicated on interaction between the 11q22 and 20q13 loci (conditional LOD score = 3.15 for the 20q13 locus). The loci in this model accounted for 91% of the variance of TG levels, with the interaction term accounting for 65% of the variance. A model including all four loci could not be evaluated by SOLAR, which allows for only one epistatic locus.

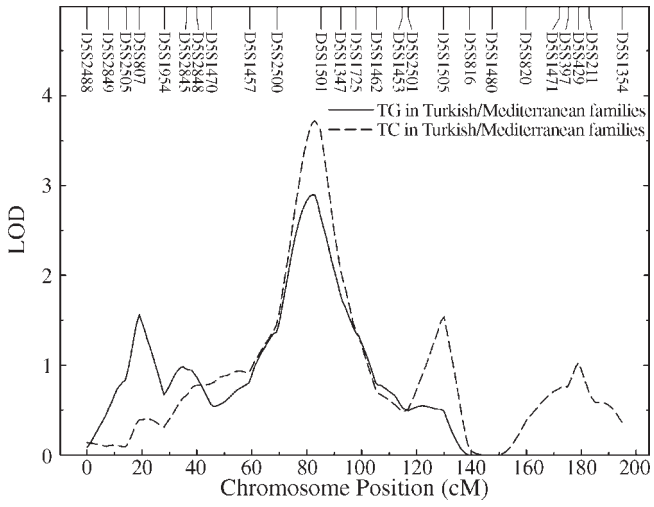

Fig. 3. Linkage of TG (solid line) and TC (dashed line) levels to chromosome 5q13 in Turkish/Mediterranean families.

We detected linkage (LOD = 3.02) for HDL-C level between D15S643 and D15S153 at 58 cM on chromosome 15q22 in the Turkish/Mediterranean families; however, the linkage peak was >20 cM wide (**Fig. 7**). This result remained unchanged in the subset of Turkish families, although the peak was now centered at 66 cM near D15S983. The most significant result from analysis of the entire group of families (LOD = 2.83) was obtained near D5S2849, which is located ~7 cM from 5pter (**Fig. 8**).

For TC level, we found significant linkage near D8S1825 on chromosome 8p23 in the entire group of families (LOD = 4.05), with most evidence contributed by the European families (LOD = 3.06). The 1-LOD support interval for this linkage peak of ~17 cM does not encompass

TABLE 4. Results of oligogenic linkage analysis of TGs in Turkish families

Model	New Quantitative Trait Locus Region Detected	Distance	Conditional LOD	Sequential LOD	Percentage of Variation Explained by					
					17q12	11q22	7q36	20q13	17q12×7q36	11q22×20q13
		cM								
One-locus	17q12	57	3.44	3.44	27	—	—	—	—	—
Two-locus (conditioned on 17q12)	11q22	101	2.69	6.13	25	29	—	—	—	—
Two-locus epistasis (conditioned on 17q12)	7q36	182	3.21	6.65	14	—	5	—	52	—
Two-locus epistasis (conditioned on 11q22)	20q13	96	2.60	5.94	—	15	—	0	—	56
Three-locus epistasis (conditioned on 17q12, 11q22)	7q36	182	2.85	8.98	10	23	0	—	54	—
Three-locus epistasis (conditioned on 17q12, 11q22)	20q13	96	3.15	9.28	25	1	—	0	—	65

the QTL for TG at 8p21 identified in the same ethnic subgroup (Fig. 1). In the Turkish/Mediterranean families, we obtained a LOD score of 3.72 at 83 cM on chromosome 5q13, the precise location showing linkage to TG in the same group of families (Fig. 3).

We did not find evidence for linkage with LDL-C level. The highest LOD score of 2.03 was obtained at 177 cM on chromosome 5 between markers D5S397 and D5S429 in the Turkish/Mediterranean families.

DISCUSSION

We conducted multipoint linkage analyses of genome scan data to identify loci influencing serum lipid levels in families participating in the GEMS Project. Analyses of log-transformed TG and HDL-C levels showed that these serum lipid traits are substantially heritable, especially HDL-C levels in the Turkish/Mediterranean families ($h^2 = 0.8$). Previously, it has been shown that the Turkish population has a very high prevalence of low HDL-C. The mean HDL-C levels in Turkish men and women are 36 and 42 mg/dl, respectively (48). The low HDL-C appeared to be genetic in origin, because the HDL-C levels were similar in Turks living in Germany and the United States (49, 50). A substantial proportion of the Turkish subjects in the present study lived in a rural area of central Turkey. Collectively, the Turkish/Mediterranean families provided more statistical power than the European or Finnish families. Of note, three large families containing 234 genotyped individuals contributed disproportionately to several of the linkage findings in the Turkish sample. We did

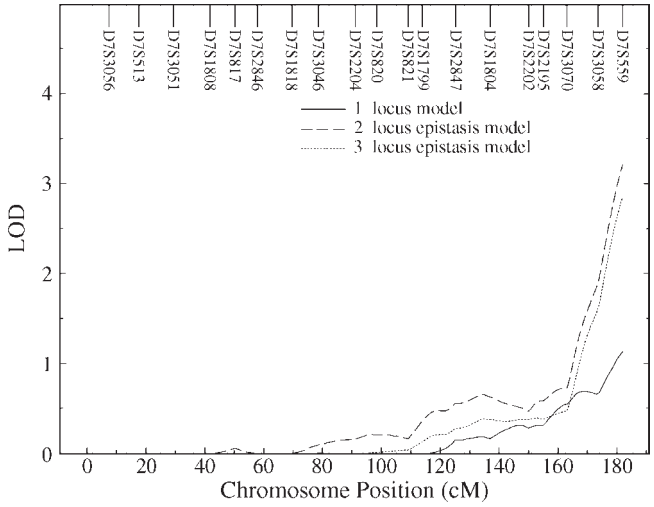

Fig. 5. Linkage of TG level to chromosome 7q36 in Turkish families. The maximum LOD score of 1.2 at 182 cM (near D7S559) obtained from the single-locus model (solid line) was much less remarkable than the LOD score of 3.2 at the same location obtained from the two-locus epistasis model (dashed line) conditioned on the linkage to a quantitative trait locus (QTL) at 17q12 or the LOD score of 2.8 obtained from the three-locus epistasis model (dotted line) conditioned on the linkage to the 17q12 QTL and including the 11q22 QTL.

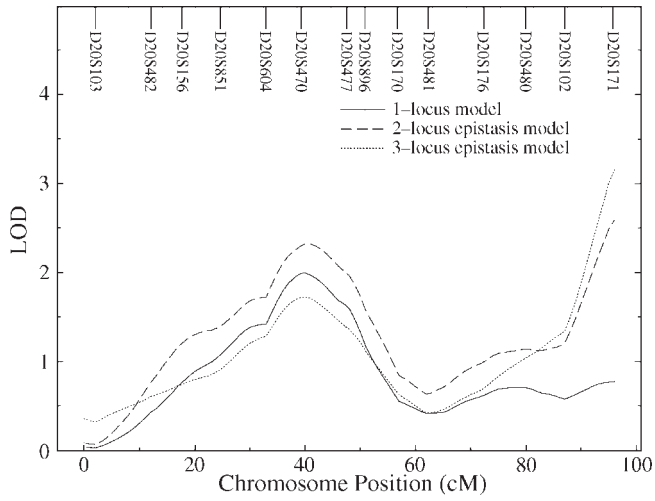

Fig. 6. Linkage of TG level to chromosome 20q13 in Turkish families. The maximum LOD score of 2.0 at 40 cM (near D20S470) obtained from the single-locus model (solid line) was much less remarkable than the LOD score of 2.6 at 96 cM (near D20S171) obtained from the two-locus epistasis model (dashed line) conditioned on the linkage to a QTL at 11q22. The highest LOD score of 3.2 at 96 cM was obtained from the three-locus epistasis model (dotted line) conditioned on linkage to the 11q22 QTL and including the 17q12 QTL.

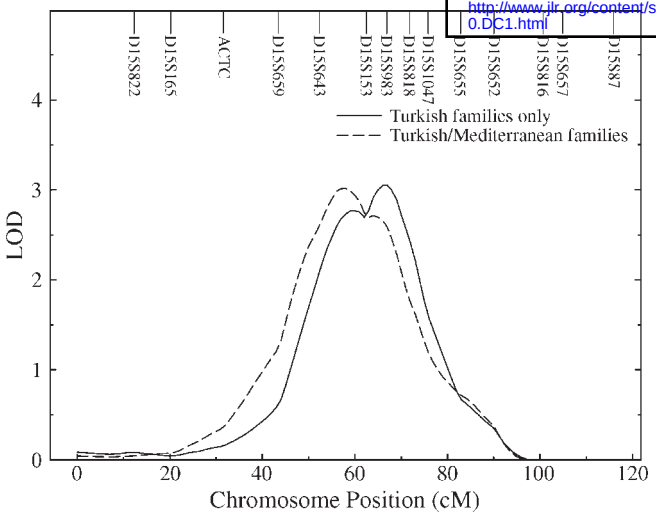

Fig. 7. Linkage of high density lipoprotein-cholesterol (HDL-C) level to chromosome 15q22. The maximum LOD score of 3.02 at 58 cM (near D15S643) in the Turkish/Mediterranean families (dashed line) was nearly identical to the maximum LOD score of 3.05 at 66 cM in the subset of Turkish families (solid line).

not find evidence of linkage for any of the traits in the Finn group alone, most likely because of the small sample size, which afforded much lower statistical power than the other groups, or perhaps because of heterogeneity.

We identified four loci contributing to TG level using univariate multipoint linkage analysis (Table 3). Evidence for all but the 8p21 locus was exclusive to the Turkish families. Evaluation of oligogenic models allowing epistatic effects involving the primary QTLs at 11q22 and 17q13 in the Turkish families revealed loci at 7q36 and 20q13 that showed no evidence for linkage independently but yielded conditional LOD scores of 3.2, a value approaching genome-wide significance. This may be attributable to the poor performance of the single-locus model in detecting loci that influence TG through interaction with other genes (51). Although complex traits such as TG levels are

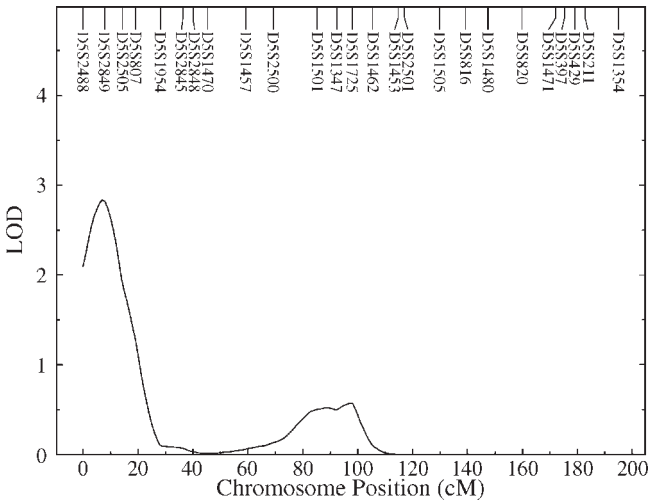

Fig. 8. Linkage of HDL-C level to chromosome 5p15 in all families.

shown to have large genetic components, it is unlikely that any locus accounts for the majority of the genetic contribution. The net effect of the oligogenic analyses was the identification of new linkage peaks but not the narrowing of intervals around QTLs identified in the univariate analyses. This suggests that part of the additive effects may be shared between these major loci. In spite of our success in identifying four loci in a single population isolate, the real panorama of genes determining TG levels in outbred populations is likely to be more extensive.

Arnett and colleagues (23) recently reported a QTL for fasting plasma TG level on chromosome 8 at a location ~25 cM from our peak in all families combined (**Table 5**). Other studies indicate that this genetic region is linked to HDL (29, 52) and to FCHL and type II diabetes (26, 53–55). LPL and leptin receptor-overlapping transcript-like 1 are among the genes in the vicinity of this linkage peak that may influence TG levels (**Table 6**). LPL plays a major role in plasma TG metabolism, and a number of genetic variants have been linked to hypertriglyceridemia and familial combined dyslipidemia (56).

The TG locus we identified on chromosome 11q22 is 6 cM from a linkage peak for LDL-C (30) and ~20 cM from a locus for hypoalphalipoproteinemia (low HDL-C) (57) and near the APOAI/CIII/AIV/AV gene cluster. APOAV has been shown to be an important determinant of plasma TG level (58, 59). Our 11q22 QTL appears distinct from the one at 11p14.1-q12.1 identified in studies of FCHL for log-transformed TG level (26, 60).

A QTL affecting LDL peak particle diameter was mapped to 17q21.33 near marker D17S1301 in a French-Canadian family sample (61). This locus is near the apolipoprotein H gene (APOH), in which a polymorphism was found to influence TG levels (62). However, our linkage peak at 17q12 is >35 cM from both D17S1301 and APOH. The monocyte chemotactic protein 1 (MCP1) gene is located within 1 cM of our linkage peak at 17q12. Increased MCP1 may alter the expression of several adipogenic genes, such as lipoprotein lipase and adipsin (63).

Our finding for linkage between TG levels and a QTL at 7q36 is consistent with a previous report of a QTL at 7q35-q36 that affects both plasma TG and LDL-C variation in U.S. families of predominantly northern European (but presumably not Finnish) ancestry (20). A recent genome scan conducted in families ascertained for obesity also found linkage between TG levels and markers in the 7q36-7qtel region (64). Suggestive evidence for linkage to TG levels at this location was identified in populations of Mexican Americans and U.S. Caucasians (19). It is unclear whether the locus implicated in these other studies is the same as the 7q36 QTL we identified in the Turkish families, whose influence on TG levels is mediated through interaction with the 17q12 QTL. The insulin-induced gene 1 (INSIG1) is a very attractive candidate for the 7q36 QTL influencing TG levels. INSIG1 plays an essential role in cholesterol homeostasis through binding the sterol-sensing domain of sterol-regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) and facilitating the retention of the SCAP/SREBP complex in the en-

TABLE 5. Linkage of lipid traits in other studies near linkage peaks identified in this study

Chromosome	Map Position[a]	Sample Population	Trait	LOD Score	References
	cM				
	2	Caucasian	LDL-C	1.9	(79)
	7	European/Finn/Turk	HDL-C	2.8	This study
5pter-p13	40	Caucasian	HDL-C	3.6	(80)
	48	Caucasian	HDL-C	2.7	(81)
	79	U.S. (ethnicity unspecified)	Cholesterol	2.1	(27)
	81	Caucasian	Apolipoprotein A-II	2.1	(82)
5q13-q14	82	Turk/Mediterranean	TG	2.9	This study
	83	Turk/Mediterranean	TC	3.7	This study
	85	French Canadian	LDL-PPD[b]	2.4	(61)
	155	Caucasian	TG	1.8	(83)
	155	Caucasian	Ratio TG/HDL-C	2.5	(83)
	158	Mexican American	TG	1.9	(19)
7q35-qter	174	Caucasian	TG	3.7	(20)
	174	Caucasian	LDL	2.2	(20)
	174	European/African American	TG	3.5	(64)
	182	Turkish	TG	3.2	This study
	8	British	TC	2.2	(26)
	15	European/Finn/Turk	TC	4.1	This study
	54	European/Finn/Turk	TG	3.1	This study
8p12-p23	60	Mexican American	Unesterified HDL_{2b}-C	2.1	(29)
	61	Mexican American	HDL-C	2.0	(53)
	79	Caucasian	TG	2.8	(23)
	99	Turk	TG	3.3	This study
	105	French Canadian	LDL-C	3.2	(84)
11q22-q24	118	Northern European descent	Low HDL	3.5	(52)
	123	French Canadian	TG	1.9	(84)
	44	Dutch Canadian	LDL-PPD	2.2	(85)
	52	Caucasian	TG	2.6	(86)
	57	Mexican American	Unesterified HDL_{2a}-C	3.3	(29)
15q21-q25	66	Turk	HDL-C	3.1	This study
	76	Caucasian	TG	2.4	(87)
	79	Mexican American	Unesterified HDL_{2b}-C	2.5	(29)
	57	Turk	TG	3.4	This study
	86	U.S. (ethnicity unspecified)	TG	2.2[c]	(87)
17q12-q25	89	Western European	LDL-C	2.3	(41)
	101	French Canadian	LDL-PPD	6.7	(61)
	87	Finn	High TG	2.4	(67)
	96	Finn	HDL-C	1.9	(88)
20q13	96	Turk	TG	3.2	This study
	101	European/African American	TG	2.3	(64)

[a] The distance of loci from pter of the chromosome was calibrated to the Marshfield map.
[b] LDL peak particle diameter.
[c] LOD score calculated from the *Z* score reported in the reference.

doplasmic reticulum (65) and in the degradation regulation of HMG-CoA reductase (66), which catalyzes the committed step in cholesterol biosynthesis.

Suggestive evidence of linkage to a locus in 20q13 influencing TG levels has been observed in two other studies (Table 5), including a Finnish sample (67). There are no obvious candidate genes in this region having a direct influence on TG levels. Hepatic nuclear factor 4α (HNF4A) regulates the expression of several hepatic genes, including microsomal transfer protein (68), which plays a critical role in the assembly of TG-rich lipoproteins. The phosphoenolpyruvate carboxykinase-1 (PCK1) gene regulates glyceroneogenesis, a pathway critical for adipocyte TG synthesis (69). PCK1 could thus have effects on the availability of free fatty acids for hepatic TG synthesis.

In our multipoint genome-wide scan, none of the linkage peaks for HDL-C were found overlapping with linkage peaks for TG. Our most significant linkage finding for HDL-C was in the interval between D15S643 and D15S153 on 15q22 (LOD = 3.05) in the subset of Turkish families. The LIPC gene encoding hepatic lipase, an important enzyme in high density lipoprotein metabolism, is located in 15q21-15q23 and may account for our linkage peak in this region (70). Increased hepatic lipase activity and mass have been observed in Turks with low HDL-C levels. Compared with individuals of Western European descent,

TABLE 6. Candidate genes located under the linkage peaks identified in this study

Chromosome Location	Trait	Candidate Genes
5p15	HDL-C	HMGCS
5q13	TG	HMGCR
7q36	TG	INSIG1, NOS3 (eNOS)
8p21-p23	TG	LPL, LEPROTL1, FDFT1
11q22	TG	APOAI/CIII/AIV/AV, APOAV
15q22	HDL-C	LIPC
17q12	TG	APOH, MCP1
20q13	TG	PCK1, HNF4A

Turkish men and women have 25–30% greater hepatic lipase activity (49, 71). Almasy et al. (29) reported linkage of the unesterified HDL_{2a}-C component of HDL-C to this region on chromosome 15 in Mexican Americans (Table 5), but their study failed to show significant linkage to HDL-C concentration. We also found suggestive evidence of linkage near marker D5S2849 on chromosome 5p15 in the total sample of families, but none of the individual ethnic groups yielded a LOD score >2. The 5p15 locus has previously been linked to diabetes (72) and age of onset of diabetes in carriers of HNF1A mutations in MODY-3 (73). HMG-CoA synthase (HMGCS), a transcriptionally sterol-regulated enzyme of cholesterologenesis, is located in 5p14-5p13 (74) and is thus a positional candidate gene. However, the function of HMGCS in human lipid metabolism remains poorly understood.

In the Turkish/Mediterranean families, the most significant linkage peak for TC level was coincident with a linkage peak for TG on 5q13. Suggestive evidence of linkage for cholesterol levels has been reported at this location in type 2 diabetes families (27) (Table 5). HMG-CoA reductase (HMGCR), a rate-limiting enzyme in cholesterol biosynthesis, is located under our linkage peak (Table 6). Polymorphisms in HMGCR may be associated with plasma lipid levels (75). Further studies will be needed to determine whether the QTL for TC that we found on 8p23 in the total family sample is distinct from our QTL for TG located ~30 cM away. Naoumova et al. (26) reported suggestive evidence for linkage for both TC (LOD = 2.2) and TG (LOD = 1.4) at 8p22-p23.3. Farnesyldiphosphate farnesyltransferase 1 (FDFT1), an enzyme in the cholesterol biosynthetic pathway, is a candidate gene for the 8p23 QTL.

We did not find linkage to LDL-C in our genome scan. One potential explanation for this result is the use of lipid-lowering medication, primarily statins, by 530 subjects at the time blood samples were collected. Although we adjusted for the use of these medications in the linkage analyses for LDL-C, the tendency of these medications to decrease extremely high LDL-C values may have compromised our ability to detect linkage attributable to reduced intrafamilial trait variability.

One potential weakness of the results presented here is that they are based on a family sample ascertained through probands with atherogenic dyslipidemia and, thus, enriched for subjects with extreme lipid values. We conditioned the likelihood of a family on the phenotype of the proband to minimize the effects of this ascertainment scheme (38). However, there is no proven method for correcting this skewed sample. Second, comparison of the LOD scores in Table 3 with the empiric *P* values estimated by simulation suggests that in some instances the evidence for linkage might be overstated, perhaps indicating that the phenotype distributions were not perfectly normal even after adjustment for covariates and log transformation. In addition, we recognize that the estimate of the percentage of the phenotypic variance attributed to a particular QTL may be inflated (76), although this bias is minimized when jointly modeling effects from two or more loci (77), as was implemented in our oligogenic analyses.

Several strengths of this study should also be mentioned. First, the probands and their relatives were evaluated extensively using a fixed, detailed, and strict protocol, ensuring diagnostic uniformity. Second, the large sample size (2,850 subjects from 458 families with questionnaire, anthropometric, biochemical, and genetic marker data) makes this one of the most statistically powerful whole genome family studies for QTLs influencing lipid levels conducted to date, providing a solid basis for future candidate gene analysis. Third, three large consanguineous families yielded the frequently unattainable statistical power needed to detect linkage between TG level and multiple loci simultaneously, including loci with epistatic effects. Epistasis that should be accounted for in the complex trait studies are too often neglected or undetectable because of the lack of statistical power (78). To the best of our knowledge, this is the first study of human linkage in a complex lipid phenotype that demonstrates the interaction of two loci.

The genetic regions identified in our study provide important clues for the investigation of genes determining the variation in TG, HDL-C, and TC levels as well as metabolic diseases, including hypertriglyceridemia, diabetes, metabolic syndrome, FCHL, and CVD. The QTLs identified encompass relatively broad intervals, and these genomic regions should be scrutinized in independent samples and with panels of higher density markers. If the linkage peaks are confirmed, association study approaches would be the next step to identify the gene sequence polymorphisms that modulate the serum concentrations of TG, HDL-C, and TC in humans.

The authors thank all of the families whose participation made this project possible. The authors gratefully acknowledge the contributions of the study personnel at each of the collaborating sites: John Farrell, Vikki Nolan, Nicholas Nikolopoulos, and Maureen Sutton (Boston); Judy Walshe, Monica Prentice, Anne Whitehouse, Julie Butters, and Tori Nicholls (Adelaide); Anthony Akkari, Rachael Duff, Tina Ly, and Kate Hollingsworth (Perth); Heather Doelle, Lynn Lewis, and Anna Toma (Ottawa); Kari Kervinen, Seppo Poykko, Liisa Mannermaa, and Sari Paavola (Oulu); Claire Hurrel, Diane Morin, Alice Mermod, Myriam Genoud, and Roger Darioli (Lausanne); Guy Pepin, Sibel Tanir, Erhan Palaoglu, Kerem Ozer, Linda Mahley, and Aysen Agacdiken (Istanbul); and Deborah A. Widmer, Rhonda Harris, and Selena Dixon (Dallas). The authors also thank Dr. Allen Roses, Shyama Brewster, Anita Nelsen, and Julia Perry for their support and Dennis Sprecher, Annie McNeill, and Sara Ephross for their useful discussions.

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