© 2000 Oxford University Press Human Molecular Genetics, 2000, Vol. 9, No. 9 **1315–1320**

Evidence for a gene influencing the TG/HDL-C ratio on chromosome 7q32.3–qter: a genome-wide scan in the Framingham Study

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Received 22 December 1999; Revised and Accepted 17 March 2000

Some studies show that plasma triglyceride (TG) levels are a significant independent risk factor for cardiovascular disease (CVD). TG levels are inversely correlated with high density lipoprotein cholesterol (HDL-C) levels, and their metabolism may be closely interrelated. Therefore, the TG/HDL-C ratio may be a relevant CVD risk factor. Our analysis of families in the Framingham Heart Study gave a genetic heritability estimate for log(TG) of 0.40 and for log(TG/HDL-C) of 0.49, demonstrating an important genetic component for both. A 10 cM genome-wide scan for log(TG) level and log(TG/HDL-C) was carried out for the largest 332 extended families of the Framingham Heart Study (1702 genotyped individuals). The highest multipoint variance component LOD scores obtained for both log(TG) and log(TG/HDL-C) were on chromosome 7 (at 155 cM), where the results for the two phenotypes were 1.8 and 2.5, respectively. The 7q32.3–qter region contains several candidate genes. Four other regions with multipoint LOD scores greater than one were identified on chromosome 3 [LOD score for log(TG/HDL-C) = 1.8 at 140 cM], chromosome 11 [LOD score for log(TG/HDL-C) = 1.1 at 125 cM], chromosome 16 [LOD score for log(TG) = 1.5 at 70 cM, LOD score for log(TG/HDL-C) = 1.1 at 75 cM] and chromosome 20 [LOD score for log(TG/HDL-C) = 1.7 at 35 cM, LOD score for log(TG) = 1.3 at 40 cM]. These results identify loci worthy of further study.

INTRODUCTION

The genetic and environmental factors that determine plasma lipid levels and their impact on cardiovascular disease (CVD) risk have been the focus of much investigation. Genetic factors play a significant role in determining serum lipid levels, but identification of specific quantitative trait loci (QTL) for lipid phenotypes in humans has been a challenge. CVD risk is positively associated with increased plasma triglyceride (TG) and decreased levels of plasma high density lipoprotein cholesterol (HDL-C) (1–6). Until recently, the balance of evidence suggested that the association between CVD and TG was often removed in statistical analyses when HDL-C levels were taken into account (4,6). TG and HDL-C levels are inversely correlated, their metabolism may be closely interrelated and combined information on these two variables may be a more precise CVD risk factor (4). Concurrent hypertriglyceridemia and low HDL-C are characteristic of insulin-resistant subjects, may represent a single inherited phenotype (7) and is emerging as a significant risk factor for CVD (8).

Genetic heritabilities of the commonly measured fasting lipids, including HDL-C and TG, have been estimated to range from 0.40 to 0.65, whilst cultural heritabilities of the phenotypes range from 0.02 to 0.10 (9,10). The clinical relevance and substantial evidence for a genetic component in TG and TG/HDL-C prompted our investigation of linkage for these traits in a genome scan of the Framingham Heart Study families.

RESULTS

Mean values of the measures used in this study and heritability estimates from SOLAR of the standardized residual lipid variables are shown in Tables 1 and 2. For log(TG) the covariates [age, body mass index (BMI), smoking, alcohol consumption, physical activity and estrogen therapy for women] accounted for 6.0–8.5% of the variance in the original cohort and for 15.0–17.5% of the variance in the offspring. For TG/HDL-C the covariates accounted for 3.3–7.0% of the variance in the original cohort and 12.5–13.0% of the variance in the offspring.

Both log(TG) and log(TG/HDL-C) were approximately normally distributed (Table 3). Using the pedigrees in this study we have simulated 10 replicates of phenotypic data mimicking log(TG/HDL-C) under the assumption of no

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Table 1. Mean values for subjects in the genome scan of Framingham Heart Study families

Variable	Framingham Original Cohort members			Framingham Offspring Study members		
	n[a]	Mean ± SD	Range	*n*	Mean ± SD	Range
Sex[b]	767	1.52 ± 0.50	1–2	1678	1.51 ± 0.50	1–2
Age (years)	767	59.7 ± 7.4	49–81	1665	32.6 ± 10.5	5–64
Alcohol (ounces/week)	767	3.7 ± 5.6	0–51	1647	3.5 ± 4.8	0–43
Cigarettes (per day)	758	6.5 ± 11.6	0–70	1657	13.0 ± 15.5	0–90
Physical activity index	738	33.5 ± 5.2	25–63	1387	34.7 ± 5.9	26–65
Body mass index (kg/m^2)	761	26.8 ± 4.0	13.7–46.9	1664	25.0 ± 4.4	13.5–52.2
Estrogen[c]	746	0.09 ± 0.28	0–1	1661	0.08 ± 0.28	0–1
HDL-C (mg/dl)	767	51.4 ± 15.3	18–129	1632	50.4 ± 14.1	16–118
TG (mg/dl)	767	128.0 ± 75.8	33–633	1642	91.3 ± 68.6	12–795
log(TG)	767	4.73 ± 0.48	3.5–6.5	1642	4.32 ± 0.61	2.5–6.6
log(TG/HDL-C)	767	0.83± 0.68	-0.90-3.4	1632	0.44± 0.77	1.7–3.9

[a]*n* is the sample size.
[b]Sex was coded 1 if male and 2 if female.
[c]Estrogen status was coded 1 for estrogen therapy and 0 for none.

Table 2. Heritability estimates for lipid measures

Measure	Heritability	*n*	Families
Residual log(TG)	0.396 ± 0.033	4527	1366
Residual TG/HDL-C	0.332 ± 0.031	4527	1366
Residual log(TG/HDL-C)	0.488 ± 0.044	2052	332

Table 3. Moments for the normal distribution and studied phenotypes

	Mean	Variance	Skewness	Kurtosis
Normal distribution	0	1	0	0
Residual log(TG)	0.068	0.896	0.220	0.412
Residual TG/HDL-C	0.021	0.903	3.110	17.145
Residual log(TG/HDL-C)	0.048	0.904	0.339	0.671

linkage and performed multipoint variance component analyses using the actual data. Due to computational constraints, examination of a large number of replicates (e.g. 1000) was not feasible. The simulated trait had a mean heritability of 0.48, mean skewness of 0.06 and mean kurtosis of 0.14. In the absence of linkage the mean number of maximum multipoint LOD scores >1 observed per genome scan was 2.6 and LOD scores >2 was 0.2. Our genome scan with Framingham phenotypic data gave five peaks with maximum LOD scores >1 and one peak with a maximum LOD score >2.

The highest multipoint variance component LOD scores (Fig. 1) obtained for both log(TG) and log(TG/HDL-C) were on chromosome 7q (at 155 cM), where the results for the two phenotypes were 1.8 and 2.5, respectively. This locus is within 7q32.3–qter and accounts for an estimated 25% of the variability in log(TG) and an estimated 26% of the variability in log(TG/HDL-C). The next highest LOD scores for log(TG/HDL-C) were 1.8 on chromosome 3 [at 140 cM, where log(TG) gave a LOD score of 0.92] and 1.7 on chromosome 20 [at 35 cM, LOD score for log(TG) = 1.3 at 40 cM]. Two other regions with multipoint LOD scores >1 were identified on chromosome 11 [LOD score for log(TG/HDL-C) = 1.1 at 125 cM] and chromosome 16 [LOD for log(TG) = 1.5 at 70 cM, LOD score for log(TG/HDL-C) = 1.5 at 75 cM].

DISCUSSION

In a genome-wide scan for log(TG) and log(TG/HDL-C) in the 332 largest extended families in the Framingham Heart Study we have identified five regions producing multipoint LOD scores of 1 or greater. The strongest evidence for linkage was found on chromosome 7q32.3–qter. While some of them may be false positives we consider the loci with LOD scores of 1 or greater to be worthy of further examination of log(TG), log(TG/HDL-C), HDL-C or other interrelated lipid phenotypes in other populations. Nevertheless, other regions where we obtained relatively modest LOD scores in this randomly selected population may represent loci with very significant contributions in families selected for presence of lipid disorders.

The highest multipoint LOD scores that we obtained were 2.5 for Log(TG/HDL-C) and 1.8 for log(TG), both at the same locus within chromosome 7q32.3–qter. This locus contains a number of candidate genes. *ABC28* (*ABCF2*, ATP-binding cassette subfamily F, member 2) at 7q35–q36 may be an interesting candidate due to similarity to *ABC1*, which is responsible for Tangier disease (11–13). Farnesyl pyrophosphate synthetase-like 2 (*FPSL2*) has been tentatively identified and mapped to 7q (14) and is similar to *FPS* (also known as cholesterol repressible protein *CHR39A*), which is coordinately regulated with HMG-CoA reductase and involved in cholesterol biosynthesis. Smith–Lemli–Opitz syndrome (*SLOS*), which is characterized by abnormal cholesterol metabolism and recurrent translocations involving 7q32, maps between *D7S3061* (128 cM) and *D7S1804* (137 cM), within our region of interest (15–17).

A number of genes that affect lipid levels have been identified through investigation of rare genetic dyslipidemia, population studies or functional studies (5,7,18,19). These genes encode apolipoproteins, lipases, lipoprotein receptors, lipid transfer proteins, enzymes that function in the cholesterol and bile acid synthetic pathways and proteins involved in insulin metabolism. Many of the genes that could conceivably be involved in control of the traits analyzed in this study lie in regions of the genome where we found no evidence of linkage to these traits (LOD scores of 0) in our analyses. Our linkage analysis in a random sample may not have sufficient power to detect genes that have detectable effects only in populations selected for a particular phenotype or specific genetic origin or genes that have a modest influence on TG or TG/HDL-C. So, absence of linkage does not rule out the presence of lipid-modifying genes within a region.

In conclusion, linkage analysis was carried out using variance component methods to analyze genome-wide scan data from 332 families for two quantitative phenotypes: log(TG) and log(TG/HDL-C). Of the five regions with multipoint LOD scores >1 the most significant was on chromosome 7q32.3–qter. Identification of genes associated with blood lipid levels and characterization of the contributory physiological and metabolic processes may reveal new targets for therapeutic intervention in CVD. In particular, treatments might be generated to compensate for a gene-associated metabolic profile that results in a high risk of CVD. Such treatments could be tailored specifically for a genetically predisposed subset of the population to control or prevent CVD.

MATERIALS AND METHODS

Subjects

In 1948 a random sample of households in Framingham, MA, was selected to participate in the Framingham Heart Study. The aim of this prospective study was to evaluate the multivariable components associated with CVD development. The original 1948 cohort consisted of 5209 people (2336 men and 2873 women) aged 28–62 years at examination 1 (mean age 44.1 years). The Original Cohort included 1644 spouse pairs and other related individuals. Subjects in the Original Cohort are now aged between 80 and 100 years and have undergone biennial examinations since the study began.

The Framingham Offspring Study was started in 1971 in part to evaluate the genetic components of CVD etiology. The Offspring Study consists of 5124 subjects aged 5–70 at entry to the study (mean 36.3 years), of whom 2616 are offspring of the original spouse pairs and 34 are stepchildren. A total of 898 Offspring are children of Cohort members where only one parent was a study participant and 1576 are spouses of the offspring. The Offspring Cohort have been followed every 4 years (except between examinations 1 and 2 with an intervening 8 years) using protocols similar to those used for study of the Original Cohort. The study design, Cohort composition and clinical and laboratory methods have been described in detail (20,21).

The genome-wide scan was carried out in the 332 largest extended Framingham Heart Study families, which were not selected for any trait value. Lipid levels and other phenotypes described here (Table 1) were measured at offspring examination 1 and original cohort examinations 10–12 (in the early to mid 1970s). The average ages of the Offspring and Original Cohort members that made up the 332 families in this study were 32.6 and 59.7 years, respectively. All participants provided information on gender, age, cigarette smoking, alcohol consumption, physical activity and BMI. Approximately 2% of the subjects were treated with cholesterol-lowering agents and were excluded from our analyses. The 332 families included a total of 1702 genotyped individuals and ranged in size from two to 29 genotyped individuals. The genotyped sample (77% offspring and 23% Original Cohort Members) included 87 spouse pairs, 933 parent–offspring pairs, 1545 sibling pairs, 742 cousin pairs and 468 avuncular pairs.

Blood samples and measurement of lipids

Clinical data and blood samples were obtained with informed consent as approved by the Boston University Human Subjects Committee. After a 12–14 h overnight fast, venous blood was drawn and mixed with EDTA (final concentration 0.1%). Plasma triglyceride and HDL-C levels were measured by Lipid Research Clinic Methods. Lipid analyses were performed at the Framingham Heart Study Laboratory, which participates in the Standardization Program of the Center for Disease Control (22).

Measurement of covariates

BMI [weight (kg)/height squared (m^2)] was determined for all participants. Subjects were weighed in light clothing, without shoes, on a calibrated spring balance scale. Height was measured with subjects standing erect with their heads in the Frankfort plane. The average number of cigarettes smoked per day over the year prior to each examination was based on self-report by the subjects. Alcohol consumption was recorded by subject report as their usual number of drinks (of comparable ethanol content) per day. Physical activity was determined by survey of the number of hours a day spent in various activities. Statistical weights were applied to the number of hours spent in five different categories (sleep, 1.0; sedentary, 1.1; slight, 1.5; moderate, 2.4; heavy, 5.0) to account for the variable energy expenditure required. The sum of the weighted hours was recorded as the physical activity index. Estrogen consumption was recorded as yes, if women were using oral contraceptives, or hormone replacement therapy and no, if they were not.

Genotyping

Genomic DNA was extracted from peripheral lymphocytes using a Qiagen Blood and Cell Culture DNA Maxi Kit. A genome-wide scan was carried out by the Marshfield Mammalian Genotyping Service. The set of 399 microsatellite markers (23) covers the genome at an average density of one marker every 10 cM and has an average heterozygosity of 0.77 (Screening Set v.8; ref. 24). The screening set and genotyping protocols are available at the website of the Center for Medical Genetics, Marshfield Medical Research Foundation. Map distances were taken from Screening Set v.9 and the Marshfield 'build your own map' facility.

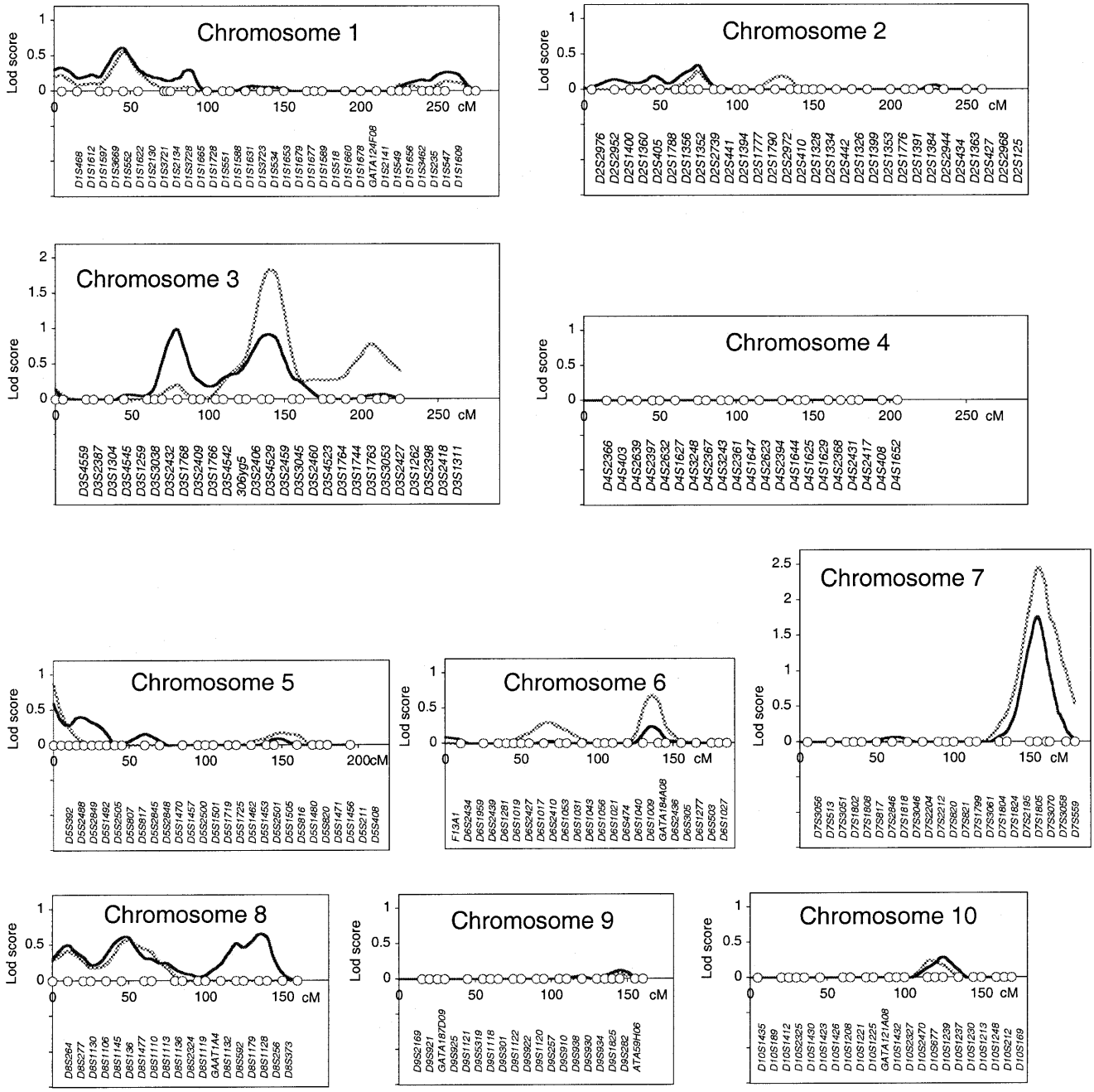

Figure 1. Multipoint LOD scores, plotted against genetic distance along each chromosome, for log(TG) (solid line) and log(TG/HDLC) (dotted line) in the genome-wide scan on 332 families. Genetic marker locations are indicated with a circle.

Statistical analysis

To enhance our ability to use linkage analysis to detect genetically determined variation, variation in the traits due to known factors was first removed by multiple linear regression. In particular, log(TG) and log(TG/HDL-C) were adjusted for the effects of age (including squared and cubic terms to allow for non-linearity), BMI, smoking, alcohol consumption, physical activity and estrogen therapy for women. Separate regression models were used for male Offspring, female Offspring, male Cohort and female Cohort subjects. These adjustments for known covariates yielded standardized residuals that were then used in the heritability and linkage analyses reported here.

Variance component linkage analysis was carried out using SOLAR (25). This approach makes use of all the information present in pedigrees of any size or complexity. One assumption of variance component analysis is that there is multivariate normality, that both log(TG) and log(TG/HDL-C) approximate to a normal distribution (Table 3), otherwise an increased false positive rate may result (26), as would be the case with the TG/HDL-C ratio prior to log transformation. Although variance component models require relatively few assumptions regarding the mode of inheritance, they do assume that the genetic effect is additive. SOLAR evaluates linkage by comparing a variance component model that permits a partic-

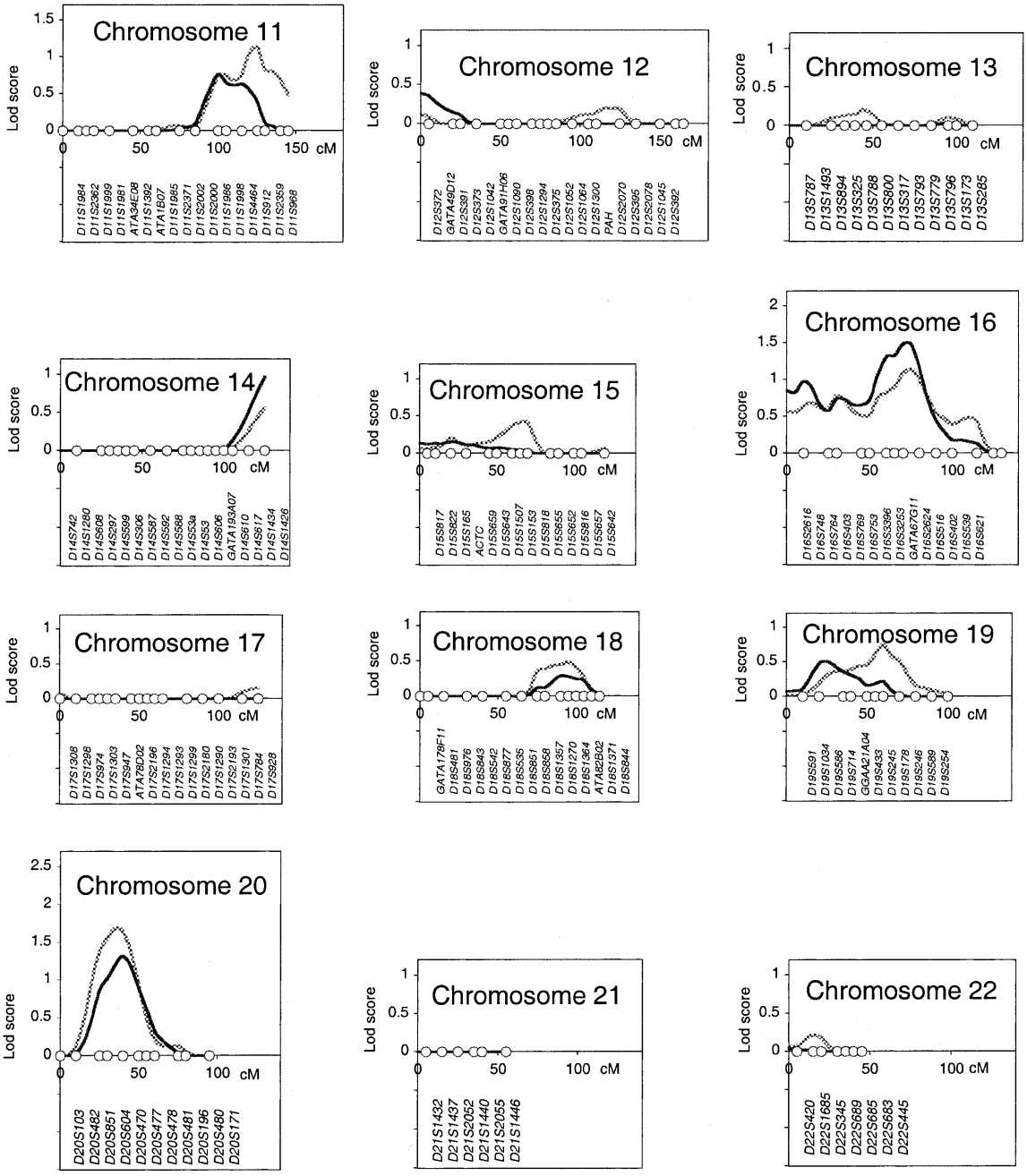

Figure 1. *Continued.*

ular locus (possible quantitative trait locus) to account for some of the additive genetic variance (along with a residual polygenic component) to a purely polygenic model, using likelihood ratio tests. Multipoint analyses are based on an extension of a regression approach that at each cM obtains a weighted average of the identity by descent probabilities over the nearby two-point probabilities as proposed by Fulker *et al.* (27). Allele frequencies used in the identity by descent calculations were calculated from the study participants. Initially we performed maximum likelihood estimates of allele frequencies for several chromosomes and found that these were very close to the estimates obtained from simple allele counting, which were then used in this study.

For the pedigrees used in this study we have ~80% power to detect a LOD score of 2.0 or higher for a quantitative trait locus that accounts for ~20% of the variation in the phenotype. This estimate of power is based upon simulations with the pedigrees, calculating the probability that a QTL with specified heritability is identified by linkage analysis at a specific LOD score threshold. Heritability estimates were obtained from the variance component analysis. LOD scores were derived in the usual fashion by calculating the $\log_{10}$ of the likelihood ratio.

Electronic database information

URLs for data in this article are as follows: Center for Medical Genetics, Marshfield Medical Research Foundation: http://www.marshmed.org/genetics/order and distances of markers ; Genetic Location Database, http://cedar.genetics.soton.ac.uk/public_html ; Genome Database, http://www.gdb.org ; Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim

ACKNOWLEDGEMENTS

We are grateful to all those who participated in the Framingham Heart Study. We thank John Blangero, Laura Almasy and Tom Dyer for their many hours of assistance in the use of SOLAR, the development that is supported by NIH grant MH59490. The work is from the National Heart, Lung and Blood Institute's Framingham Heart Study, National Institutes of Health (NIH/NHLBI contracts N01-HC-38038 and HL-54776). The Framingham Heart Study is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with Boston University. This manuscript has been reviewed by Boston University and NHLBI for scientific content and consistency of data interpretation with previous Framingham publications and significant comments have been incorporated prior to submission for publication. The genome-wide scan was carried out by the NHLBI Mammalian Genotyping Service. The work was supported in part by NIH/NHLBI POE PO1-HL41484.

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