Pleiotropic QTL on Chromosome 12q23–q24 Influences Triglyceride and High-Density Lipoprotein Cholesterol Levels: The HERITAGE Family Study

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Abstract To determine whether a common quantitative trait locus (QTL) influences the variation of fasting triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) levels, we used a bivariate multipoint linkage analysis with 654 polymorphic markers in 99 white and 101 black families. The phenotypes were investigated under two conditions: at baseline and after a 20-week exercise training intervention. A maximum genome-wide bivariate LOD score of 3.0 ($p = 0.00010$) was found on chromosome 12q23–q24, located within the *IGF1* gene (insulin-like growth factor 1, at 107 cM) for TG and HDL-C at baseline in whites. This bivariate linkage peak is considerably higher than the univariate linkage results at the same chromosome location for either trait (for TG, LOD = 2.07, $p = 0.00108$; for HDL-C, LOD = 2.04, $p = 0.00101$). The genetic correlations between baseline TG and HDL-C levels were -0.14 for the residual and -0.33 for the QTL components. Moreover, association analysis showed that TG, HDL-C, and *IGF1* are significantly associated ($p = 0.04$). In conclusion, these results suggest that a QTL on chromosome 12q23–q24 influences the variation of plasma TG and HDL-C levels. Further investigation should confirm whether *IGF1* or another nearby gene is responsible for the concomitant variation in TG and HDL-C levels.

High-density lipoprotein cholesterol (HDL-C) and plasma triglyceride (TG) levels are inversely correlated, and both are involved in important metabolic

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pathways. Each trait is also likely to be influenced by genetic and environmental factors, some of which are probably unique to each trait and some of which may be common to both. Consequently, in the current investigation we explore the nature of the genetic factors underlying the covariation in fasting HDL-C and TG levels.

With regard to the correlated metabolic pathway, elevated TG levels increase the risk of acute coronary events and are an independent risk factor, whereas elevated HDL-C levels are anti-atherogenic (Carmena et al. 2004). Low HDL-C level is related to several disorders, including hypertriglyceridemia, accumulation of small and dense low-density lipoprotein cholesterol (LDL-C), central obesity, and insulin resistance—that is, the cluster of disorders seen in metabolic syndrome (Martinez et al. 2004). In addition, the intima media thickness of the common carotid artery, a well-established marker of early atherosclerosis, is associated with hypertriglyceridemia and low HDL-C (Temelkova-Kurktschiev and Hanefeld 2004). Also, HDL relates to reverse cholesterol transport, a process by which cholesterol is removed from the periphery and transported to the liver for catabolism and excretion in the bile. Complex interactions are involved in the determination of plasma levels of HDL-C, including those proteins contributing to HDL formation [e.g., lipoprotein lipase or ATP-binding cassette A1 protein (ABCA1)] and to HDL catabolism [e.g., hepatic lipase (LIPC), cholesteryl ester transfer protein (CETP), scavenger receptor-class B type I (SR-BI) and membrane-bound ATP synthase/hydrolase] (Eckardstein et al. 2005). TG and other lipoproteins are also involved in HDL formation. HDL-C cholesteryl ester is exchanged for TG in apoB-rich particles, LDL-C, and very low density lipoprotein (VLDL) cholesterol through CETP, whereas LIPC hydrolyzes TG to small HDL-C particles and pre-β HDL-C (cf. Ashen and Blumenthal 2005).

Concerning possible genetic factors underlying each trait, it is known that several genetic and environmental factors as well as their interactions influence lipid and lipoprotein levels. Maximal heritability estimates of the fasting TG and HDL-C levels accounted for 48% and 58% of phenotypic variance at baseline and 22% and 26% of the responses to exercise training, respectively, in normolipidemic individuals from the HERITAGE Family Study (Feitosa et al. 2005a). In addition, significant genetic ($\cong$0.40) and environmental ($\cong$0.40) pleiotropic correlations between HDL-C and TG levels have been reported in the HERITAGE Family Study (Feitosa et al. 2005a) and in the IRAS Family Study (Hokanson et al. 2003). The moderate magnitude of these correlations confirms that some of the correlations between HDL-C and TG are due to common causes and that some are unique to each trait.

Strong evidence of linkage for the HDL-TG and LDL-TG subfractions was detected on chromosomes 13q12–q14, 14q31, and 10p14 at baseline levels and on chromosomes 13q12–q14 and 10p14 for their responses to regular exercise in whites in the HERITAGE study (Feitosa et al. 2005b). The linkage signals for HDL-C were only moderate in the HERITAGE study (LODs of 2.0 and 2.6 at

baseline in whites and blacks, respectively). However, Cohen et al. (2004) demonstrated that rare alleles with major phenotypic effects contributed significantly to low HDL-C levels in the general population.

Moreover, evidence of linkage for HDL-C (Almasy et al. 1999; Ng et al. 2004) and TG levels (Reed et al. 2001), analyzed separately, have been localized to the same or to the nearby genomic region 12q23–q24, where we found a pleiotropic QTL that influences HDL-C and TG levels.

Despite the evidence of genetic effects on plasma HDL-C and TG levels, knowledge about genes that commonly affect both lipoprotein levels is scarce. The bivariate linkage approach has been reported to improve the power for localizing genes that map to the same chromosome location for correlated quantitative traits (Almasy et al. 1997). For example, Lin (2003), using bivariate linkage analysis, reported evidence of a common quantitative trait locus (QTL) on chromosome 6 that influences the variation of HDL-C and TG levels in the Framingham population. Recently, we found evidence of a common QTL for the covariation of plasma levels of TG and adiposity traits in white HERITAGE families on chromosome 19q12–q13, where the genes *APOE* and *LIPE* are located (Feitosa et al. 2006). Mendelian disorders are caused by a spectrum of different mutations in a gene (or genes) (Reich and Lander 2001); however, the contribution of rare alleles to more common quantitative traits has not usually been investigated. In the current study we seek to identify QTLs underlying the covariation of fasting TG and HDL-C levels at baseline and in response to exercise training in white and black families from the HERITAGE study using bivariate multipoint linkage analysis.

Materials and Methods

Study Design and Data. The specific aims, study design, exclusion criteria, and standardized training exercise protocol of the four clinical centers of the HERITAGE Family Study have been described in detail elsewhere (Bouchard et al. 1995). In summary, the subjects were recruited in family units consisting of two parents and at least three offspring, although this structure was relaxed in the black families. The age range was 17–65 years old. All subjects for the HERITAGE Family Study were recruited primarily if they were in good health and had not engaged in regular vigorous physical activity over the previous 6 months. Subjects were excluded if they were on hypertensive, hyperglycemic, or hypercholesterolemic medication. In the exercise training program, participants trained three times per week for 20 weeks on a cycle ergometer under supervision. The intensity and duration of exercise were adjusted every 2 weeks, so that subjects were working at a heart rate associated with 75% of their maximum oxygen uptake volume for 50 minutes during the last 6 weeks of training. The power output was adjusted automatically by means of built-in computers in the cycle's ergometer in order to maintain the prescribed exercise heart rate. Blood samples

were collected from an antecubital vein into vacutainer tubes containing EDTA in the morning after a 12-hr fast with participants in a semirecumbent position. The study was approved by the Institutional Review Board at each center, and written informed consent was obtained from each subject.

Measures and Data Adjustment. Blood was drawn twice at baseline at least 24 hours apart and twice at 24 and 72 hours after the last training session. The post-training measures were corrected for plasma volume changes associated with exercise, and the responses to training were computed as the difference between corrected post-training values and baseline values. TG concentrations were determined in total plasma by enzymatic methods using a Technicon RA-500 Analyzer (Bayer Corporation Inc., Tarrytown, New York). The HDL-C fraction was obtained after precipitation of LDL-C in the infranatant by the heparin manganese chloride method (Burstein and Samaille 1960). Extensive quality control procedures ensured the validity and reproducibility of the lipid-lipoprotein measurements (Després et al. 1999). Post-training lipid values were corrected for plasma volume changes resulting from exercise (Leon et al. 2000). Body mass index (BMI, kg/m^2), fasting insulin level (measured by radioimmunoassay after polyethylene glycol separation) (Desbuquois and Aurbach 1971), and hormone use (contraceptives and hormone replacement therapy; 0 = no, 1 = yes, and 2 = unknown) were included as covariates. Both baseline and training response TG levels were transformed using natural logarithms to improve distributional properties. Stepwise regression analysis was used to remove the effects of age (age, age^2, and age^3), hormone use, fasting insulin levels, and BMI within sex and ethnic groups for each phenotype. Similar adjustment procedures were used for the training response, except that the corresponding baseline value was also included.

Marker Data. Genotyping methods and quality control of molecular data have been previously described by Chagnon et al. (2001). Map locations were taken from the Genetic Location Database. *IGF1* (insulin-like growth factor 1) polymorphisms were typed using the polymerase chain reaction (PCR). Genotyping analysis has been previously outlined (Sun et al. 1999). In summary, 27 different genotypes were found. The frequency of 189/189-bp homozygotes reached 48.5%, and the frequency of 189-bp heterozygotes was 36.9%, whereas the frequency of all other 25 genotypes (non-189-bp carriers) was 14.6%. There were not enough subjects for effective analysis when non-189-bp carriers were divided among several genotypes. Thus, for comparisons between genotypes, we grouped the individuals into 189/189-bp homozygotes, 189-bp heterozygotes, and non-189-bp carriers.

Association Analysis. Associations between *IGF1* and TG and HDL-C levels were analyzed using the Mixed procedure in the SAS software package (version 9; SAS Institute, Cary, North Carolina). We adjusted for nonindependence

among family members using a sandwich estimator, which asymptotically yields the same parameter estimates as ordinary least squares or regression methods, but the standard errors and consequently the hypothesis tests are adjusted for the dependences. This method assumes the same degree of dependency among all members of a family.

Possible gene-by-covariate interaction (e.g., *IGF1* and HDL-C level) were tested using the Mixed model procedure by including main effects (TG levels) and interaction terms in the same model.

Linkage Analysis. Linkage analyses using a variance component approach were implemented in the computer program SOLAR, version 2.1.2 (Almasy and Blangero 1998). The multipoint identity-by-descent probabilities were estimated using GeneHunter software and were imported into SOLAR. In brief, the parameters include trait-specific means, standard deviations, variance components resulting from additive genetic contributors of a QTL (h_q^2), residual additive genetic effects (h^2), and environmental effects (e^2). The bivariate multipoint linkage approach is an extension of the univariate analysis and tests whether or not the correlation pattern between two quantitative traits in families is due to pleiotropic genetic effects (Williams et al. 1999). The pleiotropy is modeled as cross-trait correlations caused by a QTL (ρ_q), a residual additive genetic effect (ρ_g), and a random environmental effect (ρ_6). All parameters were estimated using maximum-likelihood methods, and the linkage hypothesis was tested using a likelihood-ratio comparison. The significance of the genetic contribution of the QTL was tested by comparing the log-likelihood of the full model with that of a reduced model in which the additive genetic variance caused by the QTL is fixed to 0 for both traits.

In the current model bivariate LODs are converted from effective LODs with 2 degrees of freedom to ones with 1 degree of freedom (LOD_{eq}), which are comparable to univariate LODs (Amos et al. 2001). That is, the difference in likelihoods is asymptotically distributed as a 50:50 mixture of a chi-square with 1 degree of freedom and a point mass at 0.

The hypotheses of pleiotropy and coincident linkage were tested against the complete model using likelihood-ratio tests (Almasy et al. 1997). Pleiotropic effects ($\rho_q \neq 0$) occur when two traits are influenced by the same QTL, whereas for coincident linkage ($\rho_q = 0$) no shared QTL affects both traits. To test complete pleiotropy, the likelihood of a restricted model in which ρ_q is constrained to 1 (or -1) is compared to that of a model in which ρ_q is estimated.

Ninety-nine white families and 101 black families were studied using 654 polymorphic markers. For the variance components linkage analysis, 843 baseline and 739 training response subjects had phenotype data. The data yielded a maximum of 382 white and 138 black sib pairs at baseline, and a maximum of 300 white and 73 black sib pairs for the training responses.

A false discovery rate (Benjamini and Hochberg 1995), used as a measure of global error for multiple tests, was estimated using the SAS package. The

Table 1. Descriptive Characteristics for Unadjusted Traits and Covariates

	Whites			*Blacks*		
Covariate and Trait	*N*	*Mean ± SD*	*Range*	*N*	*Mean ± SD*	*Range*
Age (years)	529	35.4 ± 14.5	17.0–65.2	326	32.9 ± 11.6	17.0–6 5.9
BMI (kg/m^2)	522	25.9 ± 5.0	17.0–47.5	321	28.0 ± 6.2	17.4–50.9
Insulin level (pmol/L)	492	64.8 ± 46.2	1.0–378.6	259	81.5 ± 68.2	1.0–5 19.6
Hormone use[a]	267	39%		209	22%	
Baseline						
TG level (mmol/L)	520	1.37 ± 0.78	0.38–6.33	315	1.03 ± 0.60	0.36–4.62
HDL-C level (mmol/L)	520	1.04 ± 0.26	0.49–2.02	315	1.08 ± 0.30	0.56–3.55
Training response						
TG level (mmol/L)	468	−0.02 ± 0.42	−1.83–2.31	222	−0.04 ± 0.35	−1.68–1.41
HDL-C level (mmol/L)	468	0.04 ± 0.11	−0.33–0.54	222	0.03 ± 0.12	−0.46–0.43

To convert mmol/L to mg/dL, multiply HDL-C level by 38.7 and TG level by 88.54.

a. *N* and % denote the number of women.

genome-wide p values were adjusted for false discovery rate, denoted here by the q value.

Results

Table 1 presents the descriptive characteristics for the covariates and the unadjusted plasma TG and HDL-C levels in the white and black samples. Age, fasting insulin level, and BMI were predictors for the baseline phenotypes and accounted for about 25% of the phenotypic variance. For the responses to training the baseline level was the most consistent predictor and accounted for about 15% of the phenotypic variance. Consequently, the effects of age, sex, hormone use, fasting insulin levels, and BMI were removed from the lipid values, and the standardized residuals (adjusted lipids) were used in the SOLAR program. The overall additive genetic heritability for baseline adjusted TG levels was higher in whites (48%) than in blacks (21%), whereas for adjusted TG responses to training the heritability was higher in blacks (32%) than in whites (22%). The heritabilities for adjusted HDL-C level at baseline (≅ 60%) and for training responses (≅ 0.27%) were similar in both ethnic groups (Feitosa et al. 2005a).

Genome-wide univariate linkage scans for TG levels have been previously reported in the HERITAGE study (Feitosa et al. 2005b). In the current study we performed bivariate analysis to explore a pleiotropic hypothesis for TG and HDL-C levels. Evidence of a pleiotropic QTL was found only on chromosome 12q23–q24 (at 107 cM, *IGF1* marker) for TG and HDL-C at baseline in white families. The maximum genome-wide bivariate LOD_{eq} score (which is equivalent to a LOD score with 1 degree of freedom) was 3.0 (at 107 cM), whereas at the same chromosome location the univariate LOD scores were 2.07 (at 109 cM) and

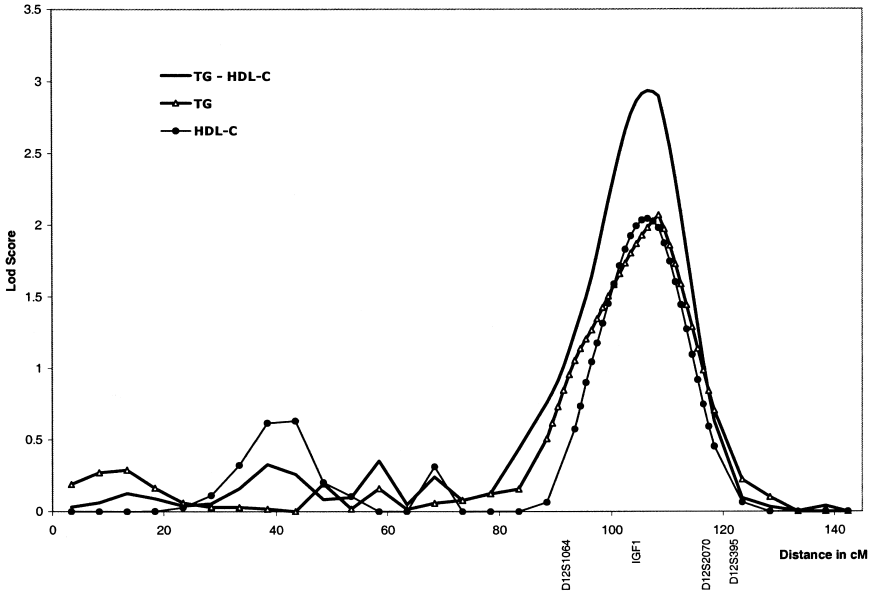

Figure 1. Distributions of univariate and bivariate LOD scores for TG and HDL-C levels on chromosome 12. LOD scores are along the *y*-axis and map locations (cM) are on the *x*-axis. The marker names are listed only for the range of linkage.

2.04 (at 107 cM) for TG and HDL-C levels, respectively (Figure 1). The 1-LOD support interval surrounding the peak extended from 99 cM to 115 cM from p-ter. The correlation between baseline TG and HDL-C levels resulting from QTL effects ($\rho_q \pm$ SE) was -0.33 ± 0.23, and the correlation resulting from residual genetic effects was -0.14. Both the complete pleiotropy hypothesis (i.e., locus-specific correlation between TG and HDL-C levels is $\rho_q = -1$) and the coincident linkage hypothesis (i.e., locus-specific correlation pair TG–HDL-C is $\rho_q = 0$) were rejected ($p < 0.001$).

Because our maximum LOD peak coincided with the marker *IGF1* in the HERITAGE data, we used an association analysis of the *IGF1* gene and TG and HDL-C levels. There was no significant association of *IGF1* with TG and HDL-C levels when the phenotypes were considered separately. However, significant association ($p = 0.04$) was found when TG level (dependent variable), *IGF1*, and HDL-C level were modeled simultaneously. The mean TG levels were higher in 189/189-bp homozygotes than in non-189-bp homozygous carriers.

The evidence for a common gene influencing the covariation between TG and HDL-C levels was not replicated in black families (univariate and bivariate LODs < 0.5) on chromosome 12q23–q24 (or other chromosome locations), which may simply reflect reduced power because of the much smaller sample

size of blacks, differences in allele frequencies, or interactions between QTLs and other unmeasured environmental factors. For training responses, weak signals of linkage on chromosome 12q23–q24 were found for HDL-C level in white families (LOD = 0.6, 113 cM) and black families (LOD = 0.8, 125 cM) but not for TG levels. Also, there were no pleiotropic linkage signals for the responses to exercise training of TG and HDL-C levels ($LOD_{eq} < 0.5$).

Discussion

The bivariate linkage approach improved the evidence for linkage to a QTL on chromosome 12q23–q24 having a pleiotropic effect on baseline TG and HDL-C levels in whites. A significant increase in the LOD score was observed for the bivariate analysis, where the maximum LOD scores coincided with the *IGF1* marker in the HERITAGE genotype data. Further tests suggest incomplete pleiotropic linkage of the QTL on chromosome 12q23–q24 that jointly influences the plasma levels of TG and HDL-C. The observation of incomplete pleiotropy may suggest that some of the functional variation within the QTL is phenotype specific, or it may be indicative of trait-specific epistatic or gene-by-environment interactions (Almasy et al. 1997).

Linkage signals on chromosome 12q23–q24 for HDL-C level (LOD = 2.1, 122–130 cM) were reported in Chinese families from Hong Kong (Ng et al. 2004) and for unesterified HDL2a-C level (LOD = 2.1, 108 cM) (Almasy et al. 1999), TG level ($Z = 3.0$, 104–123 cM) (Reed et al. 2001), and apoA2 level (LOD = 2.2, 122–128 cM) (Klos et al. 2001) in US families. These findings suggest that a QTL located on chromosome 12q23–q24 plays an important role in lipid profile variation, at least for TG, HDL-C, and apoA1. However, the hypothesis of a QTL influencing lipid-lipoprotein levels on chromosome 12q23–q24 had not been previously tested.

Further association analysis showed significant ($p = 0.04$) association of TG with HDL-C level and *IGF1* (on chromosome 12q23, at 107 cM) in which the mean TG level was higher in 189/189-bp homozygotes than in non-189-bp homozygous carriers. Also, we tested *IGF1* in the bivariate TG–HDL-C linkage analysis using SOLAR. However, the linkage result did not reach the level of significance, which could be due to lack of power in the analysis.

Interestingly, another study has reported evidence of a wild-type allele of the *IGF1* gene associated with increased levels of TG in glucose-tolerant white subjects (Nielsen et al. 2004). IGF1 plays an important role in the regulation of glucose homeostasis (Nielsen et al. 2004), and IGF1 levels have been shown to be correlated with the atherosclerotic profile (Colao et al. 2005). These results suggest that *IGF1* may be involved in variation of TG and HDL-C levels. However, further studies should investigate the relation of lipid-lipoprotein levels and the *IGF1* gene.

The QTL chromosome location at 12q23–q24 is rich with candidate genes that are related to lipid-related metabolism, among them *PLA2G1B* (phospholipase A2, group IB; chromosome 12q23–q24, 119 cM), *SRBI* (chromosome 12q24, 124 cM), *TCF1* (transcription factor 1; chromosome 12q24, 124 cM), and NIDDM2 (non-insulin-dependent diabetes mellitus, type 2; chromosome 12q24, 124 cM). In particular, *SRBI* is a prominent gene for lipid lipoproteins because it has high affinity binding for HDL-C and mediates the transport of cholesterol and cholesteryl ester from HDL particles. Extensive reviews have suggested that the *SRBI* function is not only crucial for cholesterol delivery to the liver but also important in many pathways of HDL metabolism, with variation in uptake depending on the cell type (Martinez et al. 2004; Eckardstein et al. 2005; Connelly and Williams 2004). However, this strong candidate gene lies outside the LOD interval with 1 degree of freedom for our linkage peak.

In summary, the evidence from the bivariate linkage approach used here points to a QTL located on chromosome 12q23–q24 that influences both TG and HDL-C levels in the HERITAGE Family Study data. These findings can guide subsequent efforts to identify the particular gene and test its role in clinical syndromes affiliated with HDL deficiency.

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