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M.J. Mosher
Western Washington University, m.j.mosher@wwu.edu

L. J. Martin
University of Cincinnati

L. A. Cupples
Boston University

Q. Yang
Boston University

T. D. Dyer
Southwest Foundation for Biomedical Research

See next page for additional authors

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Authors
M.J. Mosher, L. J. Martin, L. A. Cupples, Q. Yang, T. D. Dyer, J. T. Williams, and K. E. North

Genotype-by-Sex Interaction in the Regulation of High-Density Lipoprotein: The Framingham Heart Study

M. J. MOSHER,[1] L. J. MARTIN,[2] L. A. CUPPLES,[3] Q. YANG,[3] T. D. DYER,[4] J. T. WILLIAMS,[4] AND K. E. NORTH[1]

Abstract Low levels of high-density lipoprotein (HDL) are widely documented as a risk factor for cardiovascular disease (CVD). Furthermore, there is marked sexual dimorphism in both HDL levels and the prevalence of CVD. However, the extent to which genetic factors contribute to such dimorphism has been largely unexplored. We examined the evidence for genotype-by-sex effects on HDL in a longitudinal sample of 1,562 participants from 330 families in the Framingham Heart Study at three times points corresponding approximately to 1971–1974, 1980–1983, and 1988–1991. Using a variance component method, we conducted a genome scan of HDL at each time point in males and females, separately and combined, and tested for genotype-by-sex interaction at a quantitative trait locus (QTL) at each time point. Consistent findings were noted only for females on chromosome 2 near marker D2S1328, with adjusted LOD scores of 2.6, 2.2, and 2.1 across the three time points, respectively. In males suggestive linkage was detected on chromosome 16 near marker D16S3396 at the second time point and on chromosome 18 near marker D18S851 at the third time point (adjusted LOD = 2.2 and 2.4, respectively). Although the heritability of HDL is similar in males and females, sex appears to exert a substantial effect on the QTL-specific variance of HDL. When genotype-by-sex interactions exist and are not modeled, the power to detect linkage is reduced; thus our results may explain in part the paucity of significant linkage findings for HDL.

Cardiovascular disease (CVD) is the leading cause of death in the United States (Arias et al. 2003). A major risk factor for CVD is high-density lipoprotein (HDL), identified nearly 30 years ago (Gordon et al. 1977). Since the original study, low plasma HDL levels have been associated with a greater prevalence of

[1]Department of Epidemiology, University of North Carolina at Chapel Hill, 137 E. Franklin Street, Suite 306 CB 8050, Chapel Hill, NC 27514.

[2]Department of Pediatrics, Cincinnati Children's Hospital Medical Center and the University of Cincinnati School of Medicine, Cincinnati, OH.

[3]Department of Biostatistics, School of Public Health, Boston University, Boston, MA.

[4]Southwest Foundation for Biomedical Research, San Antonio, TX.

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CVD throughout the world (de Backer et al. 1998). The association between HDL and CVD may be explained in part by HDL physiology. HDL, the primary constituent of the reverse cholesterol transport system, returns excess cholesterol from peripheral tissues to the liver for recycling or excretion. In addition, it mediates physiological traits connected to endothelial function, possesses antithrombotic properties, inhibits monocyte adhesion to vascular walls, and may act as an antioxidant (Barter et al. 2003). However, plasma HDL exhibits striking sexual dimorphism, which is expressed as higher HDL levels and lower CVD prevalence in females (Reilly et al. 1990; Barrett-Conner 1997). Factors underlying this dimorphism are not fully understood.

HDL is a complex trait influenced by both environment and genes (Wilson et al. 1994; Snieder et al. 1997). Environmental factors known to influence plasma HDL levels include smoking (Williams 1992), dietary patterns and alcohol intake (Perusse et al. 1997a; Friedlander et al. 2000), glucose metabolism (Tall 1990), adiposity (Stevens et al. 1993), sex hormones (Wu and von Eckardstein 2003), and age (Ordovas 2002). From a genetic standpoint a variety of family studies have reported that HDL has the highest heritability (h^2) of the lipid subfractions, with estimates ranging from 40% to 70% (Mitchell et al. 1996; Duggirala et al. 2000; Hokanson et al. 2003). Genome scans in several populations further identified regions of interest linked to HDL levels, although with relatively low LOD scores (Ordovas 2002), except for a region on chromosome 16q21 identified in Mexican Americans with a LOD = 4.3 (Mahaney et al. 2003).

One reason that few studies have identified significant linkage to HDL may be genotype-by-sex interaction, which, when present, reduces the power to localize quantitative trait loci (QTLs), although sex-specific QTLs that influence variation in HDL levels have been identified in animal studies (Kloting et al. 2001; Anunciado et al. 2003; Korstanje et al. 2004a). Recent studies have identified sex-specific HDL variation associated with several environmental factors: dietary patterns (Mosher et al. 2005; Vega-Lopez et al. 2001), alcohol (Gardner et al. 2000), glucose metabolism (Mittendorfer 2005), and patterns of adiposity and energy expenditure (Legato 1997; Gardner et al. 2000). Candidate gene studies, which focus on genes known to affect lipoprotein metabolism, have identified genes associated with plasma HDL levels (Kessling et al. 1992; Acton et al. 1999a; Pallaud et al. 2001; Ordovas 2002), with several studies reporting genotype-by-sex interaction affecting HDL associated with variants of the APOA1 (Kessling et al. 1992; Sigurdsson et al. 1992; Saha et al. 1994), APOB, and CETP (Kessling et al. 1992) genes. An extensive study of APOE variants now suggests that data combining males and females may lead to inferences about gene effects that do not accurately reflect true gene influence in either sex (Stengard et al. 2002).

We previously reported evidence for a QTL on chromosome 2q that influences HDL variation in a longitudinal sample of female Framingham Heart Study participants (North et al. 2003). Although the QTL was detected in the combined

sample of males and females at the first time point, linkage was not significant at subsequent time points. These previous analyses were conducted on data furnished to the Genetic Analysis Workshop 13 and lacked several relevant variables and possibly limited the outcome. Moreover, male-specific linkage results were not considered. In this paper we further examine the evidence for sex-specific linkage of HDL in Framingham Heart Study participants and include additional variables that may affect genotype-by-sex variation: menopausal status, hormone therapy, and lipid-lowering drugs.

Methods

Population. The Framingham Heart Study has remained an ongoing project since its inception in 1948. Data collection began with 5,209 subjects (ages 28–62 years) in 1948, and offspring of the original cohort were added in 1971 as well as spouses. Follow-up visits occurred every two or four years for the cohort and offspring groups, respectively. The longitudinal study design and methods have been previously documented (Dawber et al. 1951; Kannel et al. 1979). For the purpose of this study data from all participant generations were included from three time points, approximately 8 years apart. We assessed fasting HDL levels, choosing early observations to obtain maximum sample size. These time points, designated for this study as t_1, t_2, and t_3, refer to visits 11, 15, and 20 in the original cohort and visits 1, 2, and 4 in the offspring and spouses and correspond to 1971–1974, 1980–1983, and 1988–1991, respectively.

We selected individuals measured at all the exams of interest who had complete phenotypic data on age, sex, and cohort effects and included additional variables of smoking and drinking history, lipid-lowering drugs, menopause, estrogen use, and BMI. Fewer than 10 outliers (defined for this study as those observations beyond four standard deviations from the global mean) were removed, thus reducing the effects of nonnormality or kurtosis on linkage analysis. Kurtosis values in visits t_1, t_2, and t_3 were 0.3, 0.5, and 0.4, respectively, in the combined-sexes data. Kurtosis for males in these time periods was 0.52, 0.58, and 0.73 and in females, 0.43, 0.19, and 0.19, respectively. The resulting sample analyzed for this study included 1,562 individuals (766 males and 796 females), representing nearly 3,300 relative pairs in 330 pedigrees: 663 parent-offspring pairs, 1,273 sibling pairs, 445 avuncular pairs, and 717 pairs of first cousins.

Genotyping. DNA was obtained from 330 Framingham Heart Study families (ranging in size from 2 to 7) and was sent to the Mammalian Genotyping Service at the Marshfield Clinic (Marshfield, Wisconsin; http://research.marshfieldclinic.org/genetics). Using Weber marker set 9, we constructed a 10-cM density genome-wide map. The average heterozygosity of the sex-averaged markers for this population was 0.77 (Yuan et al. 1997). Genotype data cleaning was completed,

including verifications of family relationships and checks for Mendelian inconsistencies using "sibkin" in the Aspex program (Hinds 1996) and "gentest," a precursor of "infer" in PEDSYS (Dyke 1994).

To determine the physical location of significant markers and to search for candidate genes, we used three data banks: University of California, Santa Cruz (http://genome/ucsc.edu); Wellcome Trust, Sanger Institute (http://www.sanger.ac.uk/); and Online Mendelian Inheritance in Man (http://www3.ncbi.nlm.nih.gov/entrez/query.fcgi?db = OMIM).

Phenotyping. To determine HDL levels, we collected blood samples after a 12-hr fast in tubes containing 0.1% EDTA. Plasma was separated by centrifugation, and lipids were immediately analyzed. HDL was measured after precipitating out non-HDL lipoproteins (those containing apolipoprotein B) with dextran sulfate and magnesium sulfate (Ordovas et al. 2002).

Additional data were collected through standardized questionnaires. Menopause was defined as the cessation of menstrual periods for more than 1 year. Hormone replacement therapy was defined as the use of oral Premarin or patch estrogen. Information was also available on the use of lipid-lowering drugs. Drinking history was documented as the typical amount (in grams) of alcohol consumed per day during the year before the examination. Smoking history was defined as the typical number of cigarettes smoked per day during the 1 year before the date of examination (Gebara et al. 1995; Saccone et al. 2003).

Analysis

Covariate Adjustment. Descriptive statistics and stepwise multivariate regression were calculated using SPSS 10 to determine significant covariates of HDL to be considered in the genetic model. Subsequently, using SOLAR, version 2.1.2, we screened for statistically significant covariates from a list of covariates that have been shown to affect HDL variation: age, age by sex, cohort, BMI, drinking and smoking histories, blood pressure, lipid-lowering medications, menopause status, and estrogen therapy (Stevens et al. 1993; Perusse et al. 1997a; Friedlander et al. 2000; Wu and von Eckardstein 2003). We retained those covariates whose effects were significant at a $p \leqslant 0.10$ in the initial analysis, regardless of whether the significance levels decreased after inclusion of other covariates. We constructed two models for comparison, one using a minimum adjustment strategy (adjusting for age, age squared, age-by-sex, and cohort effects) and the second model adjusted for BMI, smoking and drinking history, lipid-lowering drugs, menopause, and estrogen therapy for women, or BMI, smoking and drinking history, and lipid-lowering drugs for men.

Genotype-by-Sex Interaction. To examine the evidence for genotype-by-sex interaction on HDL levels, we used a three-step approach. We first tested for

evidence of additive genotype-by-sex interaction in the polygenic model. Second, we compared the results from a linkage analysis of males and females (sex-stratified subsets) to the results of an analysis including both males and females (combined sample) to restrict the number of regions considered in the QTL-specific genotype-by-sex interaction analysis. Last, we examined the evidence for a QTL-specific genotype-by-sex interaction at regions identified in the linkage analysis. SOLAR version 2.1.2 was used for all quantitative genetic analyses (Almasy and Blangero 1998).

Additive Genotype-by-Sex Interaction. Univariate quantitative genetic analysis was done to partition the phenotypic variance of HDL into its additive sex-specific genetic and environmental components using maximum-likelihood variance decomposition methods (Robertson 1959; Eisen and Legates 1966; Comuzzie et al. 1993). The expected genetic covariance between a male and female relative pair i, j is defined as

$$\mathrm{Cov}(g_{i,M}, g_{j,F}) = 2\phi_{ij}\rho_{g(M,F)}\sigma_{g,M}\sigma_{g,F} \quad (1)$$

where ϕ is the coefficient of kinship between the two individuals, $\rho_{g(M,F)}$ is the additive genetic correlation between the expressions of the trait in the two sexes, and $\sigma_{g,M}$ and $\sigma_{g,F}$ are the respective genetic standard deviations for males and females, respectively. In the absence of additive genotype-by-sex interaction (i.e., the null hypothesis), the genetic correlation between male and female relative pairs should be $\rho_{g(M,F)} = 1.0$, and male and female genetic standard deviations will be identical ($\sigma_{g,M} = \sigma_{g,F}$). Conversely, if there is additive genotype-by-sex interaction, the genetic correlation between the sexes will be significantly less than 1.0 and/or the genetic standard deviations will not be equal between the sexes. The likelihood of the model including an additive genotype-by-sex interaction was compared to the likelihood of the restricted model in which such interaction was excluded using a likelihood-ratio test.

Variance Components Linkage Analysis. To identify regions of the genome of genotype-by-sex interaction, we completed a multipoint scan across the autosomes, in the combined-sexes sample, and also in samples stratified by sex. Regions of interest were identified as those with LOD scores greater than or equal to 1.77 (suggestive evidence for linkage; Rao and Gu 2001) and those regions that appeared to differ by sex. Genome scans for HDL were performed using both covariate adjustment strategy models at each of the three time points. To estimate multipoint identical-by-descent probabilities, a pairwise maximum-likelihood-base procedure was used, with an extension of Fulker and Cherny's (1996) technique, to permit the multipoint analysis for quantitative trait (QTL) mapping (Almasy and Blangero 1998). Using a variance component model (Blangero and Almasy 1997), we tested the null hypothesis that the additive genetic variance resulting from a QTL (σ^2_q) equals 0 (no linkage) by comparing the

likelihood of this restricted model with that of a model in which σ_q^2 is estimated. The difference between the two $\log_{10}$-likelihoods produces a LOD score that is the equivalent of the classical LOD score of linkage analysis. Twice the difference in log-likelihoods of these models yields a test statistic that is asymptotically distributed as a $\frac{1}{2}:\frac{1}{2}$ mixture of a chi-square variable and a point mass at 0 (Hopper and Mathews 1982).

To verify our major linkage findings, we calculated the adjusted distribution of the LOD scores under the assumption of multivariate normality, using 10,000 replicates and simulation methods incorporated into SOLAR (Almasy and Blangero 1998). We then used the adjusted distribution of the simulated LOD scores to assign percentiles to each replicate and calculated an expected test statistic on the basis of the percentile. SOLAR produces a correction constant by regressing the expected LOD scores on the observed simulated LOD scores, which we used to determine an adjusted LOD score [adjusted LOD score = observed LOD score × correction constant (Blangero et al. 2001)]. All correction constants were 0.88 or greater.

QTL-Specific Genotype-by-Sex Interaction. In the regions identified by comparison of genome scans of males, females, and the combined-sexes sample, we extended the additive genotype-by-sex interaction to include a QTL-specific component by including two more parameters: the QTL-specific standard deviation for males and for females (Towne et al. 1999). The expected genetic covariance between a male and female relative pair i, j is defined as

$$\mathrm{Cov}(g_{i,M}, g_{j,F}) = 2\phi_{ij}\rho_{g(M,F)}\sigma_{g,M}\sigma_{g,F} + \pi_{qij}\sigma_{q,M}\sigma_{q,F} \quad (2)$$

where π_{qij} is the probability that individuals i and j are identical by descent at a QTL that is linked to a genetic marker locus, and $\sigma_{q,M}$ and $\sigma_{q,F}$ are the marker-specific genetic standard deviations for males and females, respectively. To test for QTL-specific genotype-by-sex interaction, we compared the likelihood of the model in which the male and female marker-specific standard deviations are constrained to be equal ($\sigma_{q,M} = \sigma_{q,F}$) (i.e., the null hypothesis) to the likelihood of a general model in which all additive and QTL-specific parameters are estimated. Using a chi-square test with 1 degree of freedom, we compared the likelihood of this model including a QTL-specific genotype-by-sex interaction to the likelihood of the restricted model in which such interaction was excluded using a likelihood-ratio test (Self and Liang 1987; Williams et al. 1999a, 1999b).

Results

Descriptive Statistics. HDL levels were consistently lower in males than in females in both cohorts over all three time periods (Table 1). HDL levels and age

Table 1. Descriptive Statistics for Original Cohort and Offspring/Spouses in Three Time Periods: The Framingham Heart Study

Visit	*Sample*	*n*	*Age (Years), Mean (SD)*	*HDL Level (mg/dl), Mean (SD)*	*BMI (wt/ht^2), Mean (SD)*	*Menopausal Status, n (%)*	*Use of Estrogen, n (%)*	*Use of Lipid-Lowering Medications, n (%)*
Males								
t_1	Cohort	147	57 (5)	46 (12)	27 (3)			4 (0.5%)
	Offspring/spouses	619	33 (10)	44 (11)	26 (4)			
t_2	Cohort	147	65 (5)	45 (14)	27 (4)			9 (1.2%)
	Offspring/spouses	619	41 (10)	43 (10)	26 (3)			
t_3	Cohort	147	75 (5)	42 (14)	27 (4)			26 (3.4%)
	Offspring/spouses	619	48 (10)	43 (11)	27 (4)			
Females								
t_1	Cohort	201	57 (6)	58 (15)	26 (5)	262 (33%)	45 (6%)	7 (0.9%)
	Offspring/spouses	595	34 (10)	57 (14)	23 (4)			
t_2	Cohort	201	65 (6)	55 (14)	27 (5)	376 (47%)	19 (2%)	15 (1.9%)
	Offspring/Spouses	595	42 (10)	54 (12)	24 (5)			
t_3	Cohort	201	73 (6)	51 (14)	27 (5)	492 (62%)	41 (5%)	22 (2.8%)
	Offspring/spouses	595	49 (10)	55 (13)	26 (5)			

Table 2. Residual Heritability for HDL in Males, Females, and the Combined-Sexes Sample

Visit	*Sample*	*Heritability (± SE)*
t_1	Males[a]	0.62 ± 0.10
	Females[a]	0.48 ± 0.10
	Combined[b]	0.42 ± 0.05
t_2	Males	0.50 ± 0.10
	Females	0.41 ± 0.10
	Combined	0.42 ± 0.05
t_3	Males	0.51 ± 0.09
	Females	0.38 ± 0.09
	Combined	0.42 ± 0.05

a. Adjusted for age, age^2, and cohort.
b. Adjusted for age, age^2, age by sex, age^2 by sex, and cohort.

were not statistically associated in males; however, HDL was positively associated with age in females at t_1 ($p = 0.001$) and negatively associated with age in females at t_3 ($p = 0.003$). Males and females in the original cohort were marginally overweight, as illustrated by the mean BMI, whereas the offspring females in t_1 and t_2 were within the normal range (21–25). Although the number of females reporting menopause in t_3 was double that in t_1, the number of females reporting taking estrogen was consistently low, at approximately 5%. In addition, the percentage of both males and females taking lipid-lowering medications was low, less than 3.4% for all time periods in both males and females.

After accounting for covariates, there was strong residual heritability of HDL in the combined-sexes sample, in males, and in females (Table 2). Residual heritability was similar at all time points. Adjustment for BMI, smoking and drinking history, lipid-lowering drugs, menopause status, and estrogen therapy for women, and BMI, smoking and drinking history, and lipid-lowering drugs for men changed the heritabilities only slightly ($\pm < 0.05$) (data not shown).

Additive Genotype-by-Sex Interaction. We found evidence for genotype-by-sex interactions for HDL at all three time points, with genetic standard deviations larger in males than in females. The model in which the genetic correlation between males and females ($\rho_{g(M,F)} = 1.0$) was constrained to 1.0 was significantly different from the general model in which this correlation was estimated ($\rho_g = 0.003 \pm 0.10$ for t_1, $\rho_g = 0.11 \pm 0.11$ for t_2, and $\rho_g = 0.07 \pm 0.11$ for t_3; all $P < 0.001$). The implication is that there are some distinct additive genetic effects on HDL in males and females. Moreover, the fit of the model in which the male and female genetic standard deviations were constrained to be equal ($\sigma^2_{g,M} = \sigma^2_{g,F}$) was significantly different from the fit of the general model in which such interaction was allowed for t_1 ($\sigma^2_{g,F} = 11.0 \pm 0.95$, $\sigma^2_{g,M} = 14.89 \pm 1.13$, $P \leq 0.03$) and t_2 ($\sigma^2_{g,F} = 9.80 \pm 0.89$, $\sigma^2_{g,M} = 12.99 \pm 1.04$, $P \leq 0.04$) only. The

Table 3. Adjusted LOD Scores ≥ 1.7[a] for HDL Across Three Time Points in the Combined-Sexes Sample and Stratified by Sex

Visit	*Sample*	*Chromosome*	*Locus*	*Adjusted LOD Score*
t_1	Females[b]	2	133	2.6
	Females	12	170	1.8
	Combined[c]	2	150	3.1
	Combined	12	170	2.1
t_2	Males	16	79	2.2
	Combined	1	12	2.2
t_3	Males	18	75	2.4
	Females	2	132	2.1

a. Criteria for suggestive evidence of linkage according to Rao and Gu (2001).
b. Sex-stratified analysis adjusted for age, age^2, and cohort.
c. Analysis of combined data adjusted for age, age^2, age by sex, age^2 by sex, and cohort.

implication is that the magnitude of genetic effects on HDL is different in males and females.

Variance Components Linkage Analysis. Table 3 presents the adjusted LOD scores from our multipoint genome-wide scan using the minimum model and the location for all peaks greater than or equal to 1.77. Only the results using the minimum adjustment strategy are presented, because the adjusted LOD scores obtained using the Model 2 adjustment strategy were similar. Although the heritability of HDL is not significantly different in males and females, sex does appear to exert a substantial effect on the QTL-specific variance of HDL.

In the combined-sexes sample we obtained an adjusted LOD score of 3.1 on chromosome 2 at 150 cM for the t_1 observation. At t_2 and t_3 this region did not reach statistical significance, with adjusted LOD scores of 0.5 at 120 cM and 1.0 at 122 cM, respectively. Two other signals in the combined-sexes sample reached statistical significance: At t_1 the adjusted LOD score was 2.1 on chromosome 12 at 170 cM, and at t_2 the adjusted LOD score was 2.2 on chromosome 1 at 12 cM.

However, when stratifying the sample by sex, we documented a consistent finding of linkage in females on chromosome 2 near marker D2S1328 at 2q14.3 in all three time periods, with adjusted LOD scores of 2.6 at t_1, 1.7 at t_2, and 2.1 at t_3 (Figure 1). The one LOD unit support interval (SI) of the chromosome 2 signal in females spans 39 cM and 47 MB. A second location suggestive of linkage was identified on chromosome 12, near marker D12S392, with an adjusted LOD score of 1.8 at t_1, and the SI spanning 25 cM (Figure 2). In the male subset no linkage was detected in either of these regions (data not shown); however, at 170 cM an adjusted LOD score of 2.1 was detected on chromosome 12 in the combined-sexes sample.

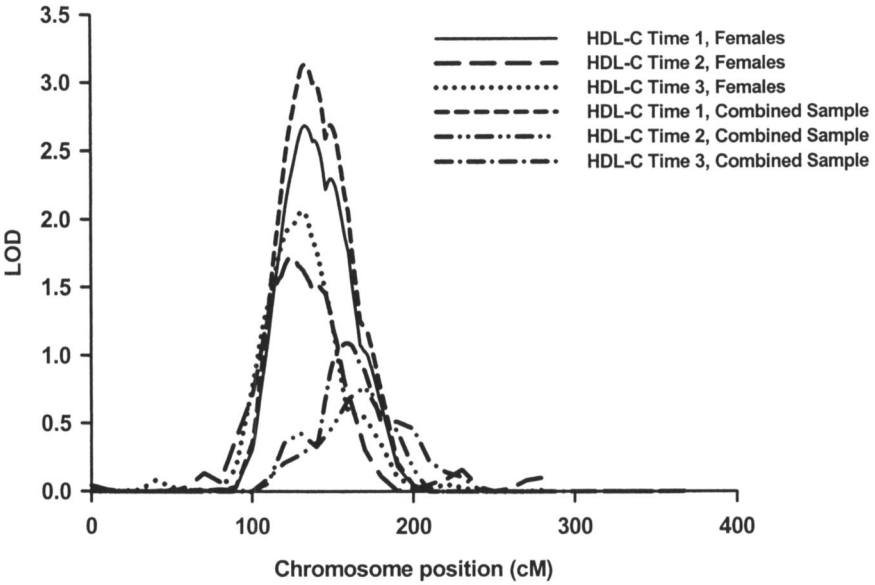

Figure 1. Adjusted LOD scores on chromosome 2 for HDL cholesterol in females and the combined-sexes sample at three time points. Data from the Framingham Heart Study.

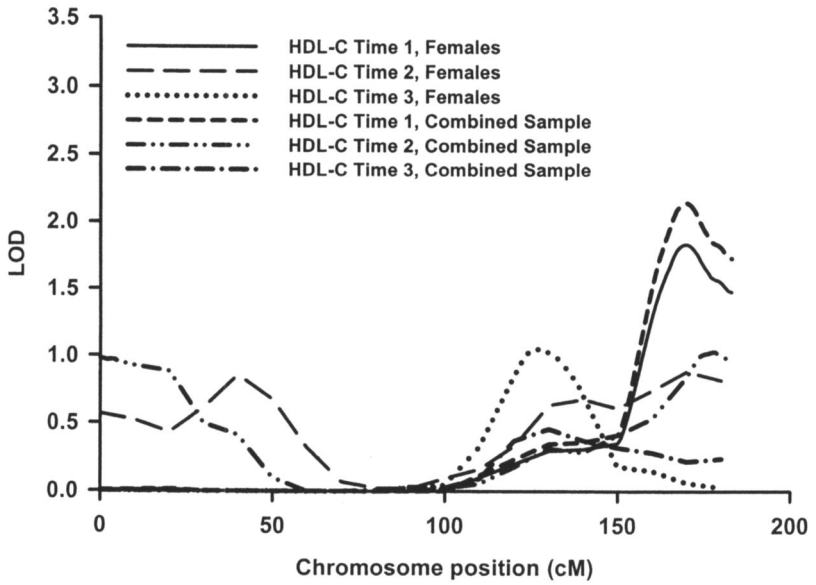

Figure 2. Adjusted LOD scores on chromosome 12 for HDL cholesterol in females and the combined-sexes sample at three time points. Data from the Framingham Heart Study.

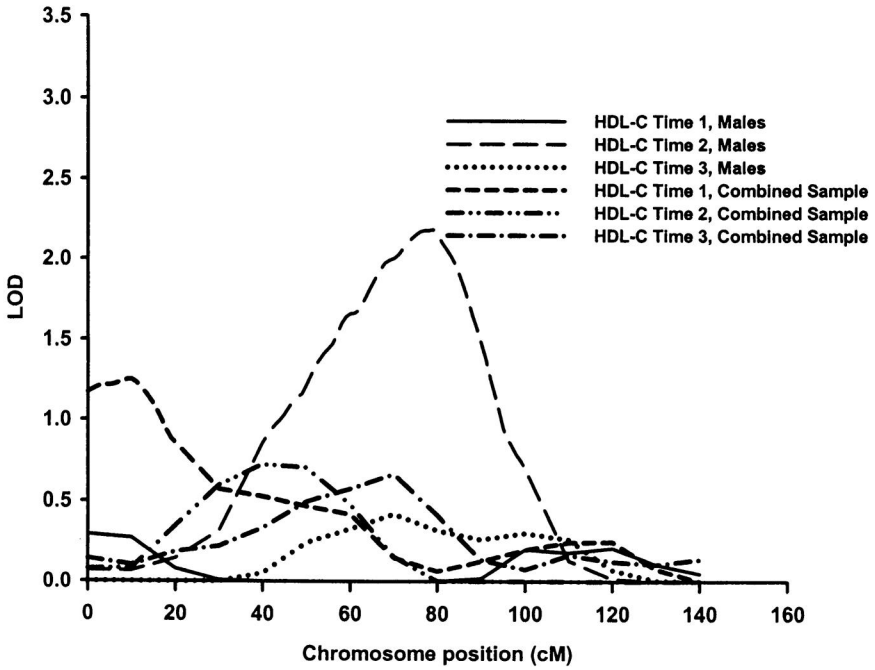

Figure 3. Adjusted LOD scores on chromosome 16 for HDL cholesterol in males and the combined-sexes sample at three time points. Data from the Framingham Heart Study.

In males the results are not consistent across observation time points. We detected an adjusted LOD score of 2.2 on chromosome 16 at 79 cM (nearest marker D16S3396, located at 16q12.1) at t_2 (Figure 3). The SI spans 36.5 MB. We also detected a suggestive linkage peak (adjusted LOD = 2.4) on chromosome 18 at 75 cM (nearest marker DS18S851, located at 18q21.1) at t_3 (Figure 4). The SI spans 36 MB. No adjusted LOD scores greater than 1.0 were detected in females in either of these regions (data not shown); however, at 106 cM an adjusted LOD score of 1.7 was detected in the combined sample.

QTL-Specific Genotype-by-Sex Interaction. QTL-specific genotype-by-sex interaction was noted at four chromosomal locations displaying increased linkage signals in one of the two sex subsets (chromosomes 2, 12, 16, and 18). On chromosome 2 QTL-specific interaction was noted for all three time periods and is reported in Table 3 (all $P < 0.03$). In contrast, on chromosome 12, 16, and 18 QTL-specific interaction was noted only at a single time point. QTL-specific interaction was identified on chromosome 12 for the first time point, on chromosome 16 for the second time point, and on chromosome 18 for the third time point (all $P < 0.03$).

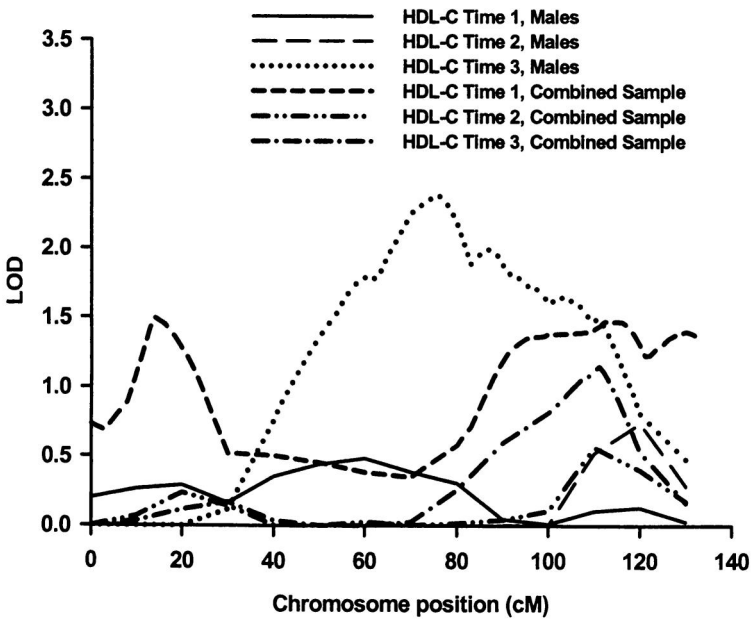

Figure 4. Adjusted LOD scores on chromosome 18 for HDL cholesterol in males and the combined-sexes sample at three time points. Data from the Framingham Heart Study.

Discussion

Sexual dimorphism in HDL levels is frequently reported in the literature (Reilly et al. 1990; Barrett-Conner 1997). However, most linkage studies of HDL levels have not explicitly explored genotype-by-sex interaction by testing whether the genes influencing HDL variability are differentially expressed in males and females. Therefore our objective was to examine the evidence for sex-specific linkage of HDL in a longitudinal sample of participants from the Framingham Heart Study. To approach this complex question, we assessed the evidence for additive genotype-by-sex interaction. In addition, we identified four regions on the genome that displayed differential evidence of linkage in males and females. By examining these regions further, we demonstrated sex-specific QTL effects at each of these loci.

We found strong evidence of additive genotype-by-sex interaction in HDL levels across all three time periods. The male and female genetic standard deviations were significantly different for time period t_1, suggesting a different magnitude of genetic effects on HDL in males and females. Moreover, the genetic correlation between males and females was significantly different from 1 across all three time periods ($p < 0.001$), suggesting distinct additive genetic effects on

HDL in males and females. Based on the estimates of ρ_g, the shared genetic component to the variance in HDL between the sexes in this population appears to be negligible. However, we must be cautious in interpreting this finding to specific loci because this is an estimate for additive effects, and thus the lack of a genetic correlation may be due to oligogenic effects that differ in the sexes (Carey 1988).

In the sex-specific linkage analysis we identified four regions, only one of which was identified in the full sample. Although these sex-specific linkages exhibited only suggestive evidence, these signals overlap previously reported QTLs associated with HDL and related phenotypes (Table 4). Moreover, the presence of biological candidate genes with sex-specific influence in several of these regions further supports our hypothesis of sex-specific loci affecting HDL variation.

We also found strong evidence of QTL-specific genotype-by-sex interaction, with suggestive QTLs identified in sex-stratified data on chromosomes 2q and 12q in females and chromosomes 16q and 18q in males. The inclusion of the QTL-specific genotype-by-sex component strengthens our findings from the linkage analysis that was performed in the sex-specific stratum, which was restricted to the relative pairs concordant for sex (826 and 808 male and female concordant relative pairs, respectively). When formally modeling QTL-specific interaction, we were able to use the information from the discordant relative pairs ($N = 1{,}612$), thereby increasing our power to detect genetic effects. The formal testing of QTL-specific genotype-by-sex interaction suggests that the underlying genetic structure of HDL may be sex specific. Table 5 documents our findings and the studies that they replicate.

In females the strongest evidence for linkage at chromosome 2q13.43 coincides with findings associated with lipid phenotypes in two different populations. This region contains several interleukin 1 (IL1) alpha and beta genes, which influence inflammation (Lord et al. 1991), as well as IL1 receptor antagonist, which inhibits IL1 action on endothelial cells (Dinarello and Wolff 1993). Sex hormones have been shown to influence the expression of IL1 (Morishita et al. 1999). The second finding for females, at chromosome 12q24, also coincides with a signal reported for triglycerides (Reed et al. 2001). Chromosome 12q24 harbors SCARB1, which is an HDL receptor that regulates cholesterol and triglycerides uptake (McCarthy et al. 2003; Trigatti et al. 2003; Osgood et al. 2003). Several studies have demonstrated sex-specific effects of this gene on lipoprotein variation (Acton et al. 1999b; McCarthy et al. 2003).

In males the marker identified at chromosome 16q21.1 coincides with a region linked to triglycerides in the Framingham Heart Study (Shearman et al. 2000) and several related phenotypes in Mauritian families (Francke et al. 2001). Our signal is also less than 20 MB from the signal reported for HDL on chromosome 16q22 in Mexican American families (Mahaney et al. 2003). Chromosome 16q12.1 includes the CETP and LCAT genes. Both have been shown to affect HDL variation (Brousseau et al. 2002; Ordovas et al. 2000). Genotype-by-sex

Table 4. QTL-Specific Standard Deviations ± Standard Errors for HDL Levels from the Analysis of Genotype-by-Sex Interaction on Chromosomes 2, 12, 16, and 18

	Males			*Females*		
σ_q^2 *for Chromosome and at Position*	t_1	t_2	t_3	t_1	t_2	t_3
2, 150 cM	3.71 ± 2.12[a]	0.0[b,c]	0.0[b,c]	10.82 ± 1.69	9.51 ± 1.71	10.51 ± 1.58
12, 170 cM	0.49 ± 2.04[b]	NA	NA	11.22 ± 1.70	NA	NA
16, 50 cM	NA	8.98 ± 1.07[b]	NA	NA	0.0[c]	NA
18, 100 cM	NA	NA	9.93 ± 1.30[a]	NA	NA	0.0[c]

NA, Not applicable.

a. Significantly different from females at $P < 0.03$.

b. Significantly different from females at $P < 0.007$.

c. Parameter estimated on boundary, no standard error computed.

Table 5. Studies of Lipid Profiles and Coronary Heart Disease That Corroborate Significant Locations Presented in This Paper

Chromosome	*Marker*	*LOD Score or P*	*Phenotype*	*Cytogenic Locale*	*cM*	*Citation*
2	D2S1328	3.3	HDL	2q13.43	133	Present study[b]
		$P = 0.003$	HDL (msx)[a]	2q24.2	156	Stein et al. (2003)[b]
	D2S410	$P = 0.005$	TG	2q14	125	Newman et al. (2003)
12	D12S392	2.3	HDL	12q24.33	179	Present study[b]
	D12SPAH	$P = 0.001$	TG	12q22–24	NA	Reed et al. (2001)
16	D16S3396	2.4	HDL	16q21.1	75	Present study[b]
	D16S262	4.3	HDL	16q22	95	Mahaney et al. (2003)
		1.5	TG/HDL		70–75	Shearman et al. (2000)[b]
		1.7	HDL	16q12	NA	Francke et al. (2001)
		2.8	CHD[c]		71	Francke et al. (2001)
18	D18S851	2.4	HDL	18q21.1	75	Present study[b]
		1.0	HDL		96	Coon et al. (2001)
		1.2	TG		86	Coon et al. (2001)
		1.1	LDL		78	Coon et al. (2001)

NA, data not available.

a. Metabolic syndrome.

b. Data for this study were derived from the Framingham Heart Study.

c. Coronary heart disease.

effects have been documented for CETP variants in Israeli (Kark et al. 2000), Finnish (Kauma et al. 1996), and American (Kastelein et al. 1999) populations, although some studies have identified female-specific effects and others male-specific effects. Our second finding for males, located at chromosome 18q21.1, supports the results noted on chromosome 18q21.31 for lipid phenotypes in families recruited for the HyperGEN Study (Coon et al. 2001). Chromosome 18q21.31 contains start-domain-containing protein 6 (STARD6), which binds cholesterol to the mitochondrial P450 cleavage enzymes in steroidogenic cells. STARD6 is identified as testis specific and is not reported in ovarian tissue. Because STARD6 is involved in cholesterol homeostasis and is testis specific, it is an interesting candidate for sex-specific linkage signals.

Differences in the genes contributing to the variation in HDL by sex or differences in the expression of HDL by sex are biologically plausible and are supported by several lines of evidence. First, sexual dimorphism in HDL levels is frequently reported in the literature (Reilly et al. 1990; Barrett-Conner 1997). Second, sex-specific QTLs that influence variation in HDL levels have been identified in animal models (Kloting et al. 2001; Korstanje et al. 2004b). Moreover, sex-specific QTLs for physiologically related phenotypes, such as serum insulin, triglyceride, total cholesterol, and phospholipid levels, have also been reported (Kloting et al. 2001; Anunciado et al. 2003).

This study has several limitations. First, there is a general lack of consistency in linkage results across time periods. One possible interpretation of these findings is age-dependent changes in the genetic control of HDL levels (Diego et al. 2003). Clearly, there is evidence of an age-related trend in the sexual dimorphism of circulating HDL levels (Williams 1992; Snieder et al. 1997). Such age-related genetic effects are supported by the sex-specific age trends in HDL variation. Sexual dimorphism in HDL is first noted during adolescence, when the concentration of HDL begins to decrease in males and increase in females. The differences remain throughout the life span, despite some decrease in HDL levels during the female menopause transition (Snieder et al. 1997; Barrett-Conner 1997). At this time, addressing both genotype-by-sex and genotype-by-age interaction together is not feasible; furthermore, this type of analysis would require substantially larger sample sizes to achieve appropriate statistical power. However, the lack of consistency may also be due to stochastic variation in HDL levels (Diego et al. 2003), environmental factors that change over time, and temporal changes in measurement techniques. A second limitation is the lack of available information on dietary intake of macronutrients (proteins, fat, and carbohydrate) and energy expenditure. More research to address the effects of diet, exercise, and age as well as an exploration of relevant candidate gene variants may further shed light on the underlying genetic architecture of HDL variation.

In conclusion, this study provides strong evidence of genotype-by-sex interaction on circulating HDL levels. In the additive genetic genotype-by-sex interaction analysis, both the genetic standard deviations in males and in females differed significantly and the genetic correlation was significantly different from

1. These results suggest that a different gene or suite of genes is contributing to the variance of HDL levels in this population. The inference of a suite of different genes influencing HDL levels in males and females is further supported by the variance components linkage analyses of the sample stratified by sex, where suggestive evidence for linkage to HDL was identified on two chromosomes in females (chromosomes 2 and 12) and on two chromosomes in males (chromosomes 16 and 18). Furthermore, we identified QTL-specific genotype-by-sex interaction at these four loci, suggesting a complex genetic architecture to HDL levels that varies by sex.

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