

Pleiotropic Genetic Effects Contribute to the Correlation between HDL Cholesterol, Triglycerides, and LDL Particle Size in Hypertensive Sibships

Iftikhar J. Kullo, Mariza de Andrade, Eric Boerwinkle, Joseph P. McConnell, Sharon L.R. Kardia, and Stephen T. Turner

Objective: Abnormalities of HDL cholesterol (HDL-C), triglycerides, and LDL particle size are present in familial dyslipidemic hypertension. We investigated heritability of these three lipid traits and the extent to which shared effects of genes (pleiotropy) contribute to the additive genetic variation in each trait in hypertensive sibships.

Methods: Subjects included 788 individuals (60% women) ascertained through sibships with ≥ 2 members diagnosed with hypertension before age 60 years. The LDL particle size was measured by polyacrylamide gel electrophoresis. Triglycerides were log transformed to reduce skewness, and age- and sex-adjusted lipid traits were used in the analyses. Heritability and pairwise genetic correlations were computed using a variance components approach. The genetic correlation between a pair of traits was squared to yield genetic covariance, a measure of pleiotropic effects of genes influencing both traits concomitantly.

Results: Heritability estimates indicated significant genetic effects on HDL-C (0.58), log triglycerides (0.47), and LDL particle size (0.71). Genetic correlation was strongest between HDL-C and log triglycerides (-0.642), followed by log triglycerides and LDL particle size (-0.493), and HDL-C and LDL particle size (0.334). HDL-C and log triglycerides showed the strongest genetic covariance (41%), followed by LDL particle size and log triglycerides (24%), and HDL-C and LDL particle size (11%).

Conclusions: Multivariate quantitative genetic analyses in hypertensive sibships reveal that pleiotropy contributes to the additive genetic variation in HDL-C, triglycerides, and LDL particle size. These findings provide the rationale for multivariate linkage analyses to identify novel genetic loci with pleiotropic effects on the traits. Am J Hypertens 2005;18:99–103 © 2005 American Journal of Hypertension, Ltd.

Key Words: Multivariate analysis, genetic correlation, HDL cholesterol, LDL particle size, triglycerides.

Dyslipidemia and hypertension are common medical problems and up to 27 million American adults have both hypertension and dyslipidemia.^{1,2} The co-existence of the two disorders may be due to a shared genetic background.³ Abnormalities in HDL cholesterol (HDL-C) and triglycerides were found to be present in nearly half of the members of hypertensive families.³ More recently, abnormalities in LDL particle size have been found in patients with high triglyceride–

low HDL-C dyslipidemia, and the three lipid traits appear to be correlated.⁴ The genetic basis of the correlations between these lipid traits is not well understood. In families ascertained on the basis of hyperlipidemia, Edwards et al⁵ demonstrated that the correlations between these lipid traits were in part due to effects of shared genes.

Elucidating the genetic architecture of these lipid traits in the context of hypertension may provide insight into the increased coronary heart disease (CHD) risk of hyperten-

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From the Divisions of Cardiovascular Diseases (IJK), Biostatistics (MA), and Nephrology & Hypertension (STT), Department of Laboratory Medicine and Pathology (JPM), Mayo Clinic and Foundation, Rochester, Minnesota; Human Genetics Center and Institute of Molecular Medicine, University of Texas-Houston Health Science Center (EB), Houston, Texas; and Department of Epidemiology (SLRK), University of Michigan, Ann Arbor, Michigan.

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Address correspondence and reprint requests to Dr. Iftikhar J. Kullo, Division of Cardiovascular Diseases, Mayo Clinic, 200 First Street Southwest, Rochester, MN 55905; e-mail: kullo.iftikhar@mayo.edu

sive individuals,⁶ as well as a better understanding of the genetic basis of alterations of metabolic pathways common to blood pressure (BP) control and lipoprotein metabolism. A step in this direction is to determine to what extent shared genes (or pleiotropy) are responsible for the genetic correlation between HDL-C, triglycerides, and LDL particle size in hypertensive families. We therefore investigated heritability of HDL-C, triglycerides, and LDL particle size and the extent to which pleiotropy contributes to the phenotypic and genetic correlation between these traits in a cohort of hypertensive sibships.

Methods

Study Participants

The study was approved by the Institutional Review Board of the Mayo Clinic and written informed consent was obtained from participants in the Genetic Epidemiology Network of Arteriopathy (GENOA) study, a multicenter community-based study of hypertensive sibships that aims to identify genes influencing BP.⁷ In the initial phase of the GENOA study (9/1995 to 6/2001), sibships containing at least two individuals with essential hypertension diagnosed before age 60 years (non-Hispanic white subjects) were enrolled in Rochester, MN. In phase II of GENOA, participants underwent measurement of novel cardiovascular risk factors including LDL particle size, and assessment of target organ damage due to hypertension. Through October 2002, 815 of the original 1583 GENOA participants had completed the phase II study protocol. We excluded 27 subjects with missing data.

Resting systolic BP and diastolic BP levels were measured in the right arm with a random-zero sphygmomanometer (Hawksley and Sons, West Sussex, UK). Three measures at least 2 min apart were taken and the average of the second and third measurements was used in the analysis. The diagnosis of hypertension was confirmed based on BP levels measured at the study visit (systolic BP ≥ 140 mm Hg or diastolic BP ≥ 90 mm Hg) or report of a prior diagnosis of hypertension and current treatment with antihypertensive medications. Information about the use of lipid-lowering medications was obtained from a questionnaire administered to the participants.

Measurement of Lipid Variables

Blood samples were obtained by venipuncture after an overnight fast. Standard enzymatic methods were used to measure total cholesterol, HDL-C, and triglycerides.⁸ The LDL particle size was measured by polyacrylamide gel electrophoresis as follows.⁹ Plasma (25 μ L) was mixed with 200 μ L of loading gel (containing Sudan black-B dye and riboflavin) and added to the top of a precast 3% polyacrylamide gel tube (Quantimetrix Corporation, Redondo Beach, CA). After photopolymerization for 30 min, specimens were electrophoresed for 1 h. The dye preferentially binds to lipoprotein particles and remains

with them during electrophoresis. Separation is based primarily on particle size due to the sieving action of the polyacrylamide gel. The separated lipoprotein particles were scanned with an ArtixScan 1100 (Microtek, Carson, CA). The electrophoretograms were quantitatively analyzed using the public domain National Institutes of Health Image program (<http://rsb.info.nih.gov/nih-image/>). The program divides the electrophoretogram at designated electrophoretic mobility values and calculates the area under the curve for each mobility fraction. Particle diameter for each fraction was calculated as previously described¹⁰ and weighted based on the percent area under the curve for each fraction in relation to the area occupied by the entire LDL band. The mean LDL particle size was then determined by averaging weighted diameters for each fraction. Two controls (mean particle diameter 264 Å and 254 Å) were analyzed with every 10 study samples. Inter-assay coefficients of variation were 0.77% (standard deviation = 2.0 Å), and 1.46% (standard deviation = 3.7 Å), respectively.

Statistical Analyses

Triglyceride values were log transformed to reduce skewness. Age- and sex-adjusted HDL-C, log triglycerides, and LDL particle size were used in the analyses. Multivariate quantitative genetic analyses using a variance components approach was performed with the Expectation Maximization Variance Components (EMVC) software.¹¹ An extension of the variance components approach to multivariate quantitative genetic analyses has been described by Lange and Boehnke.¹² The variance-covariance matrix of m traits for a particular sibship s (V_s) was partitioned into additive genetic effects and random environmental effects. The additive genetic effects were represented by the direct product of the matrix \mathbf{A} , whose off-diagonal elements $\sigma_{g,lj}$ represent the genetic covariance of traits l and j , and the diagonal elements $\sigma_{g,l}^2$ represent the genetic variance of trait l , for $l = 1, 2, \dots, m$, and the matrix of the coefficients of relationship for the sibship \mathbf{G}_s . The environmental effects were represented by the direct product of the matrix \mathbf{B} , whose off-diagonal elements $\sigma_{e,lj}$ represent the environmental covariance of traits l and j , and the diagonal elements $\sigma_{e,l}^2$ represent the environmental variance of trait l , for $l = 1, 2, \dots, m$, and the identity matrix \mathbf{I}_s , such that $V_s = \mathbf{A} \otimes \mathbf{G}_s + \mathbf{B} \otimes \mathbf{I}_s$. The symbol \otimes represents the direct product of two matrices, which consists of all possible products of an element of first matrix multiplied by an element of the second matrix.

For each trait, the heritability (h_l^2) was calculated as the proportion of the total phenotypic variance due to additive genetic effects. The following three pairwise combination of traits were included in the multivariate genetic analyses: 1) HDL-C and log triglycerides, 2) HDL-C and LDL particle size, and 3) log triglycerides and LDL particle size. Pairwise genetic and environmental correlations were

Table 1. Subject characteristics (*n* = 788)

Characteristic	
Age (y)	62.0 ± 8.5
Women (%)	60.0
Hypertension (%)	76.4
HDL-C (mg/dL)	51.9 ± 15.4
Triglycerides (mg/dL)	158.9 ± 94.3
LDL particle size (Å)	270.9 ± 4.9
Total cholesterol (mg/dL)	201.4 ± 33.7
Lipid-lowering medication (%)	31.4

Table entries are means ± standard deviations for quantitative traits or percentages for categorical traits.

calculated as $\rho_{g,lj} = \frac{\sigma_{g,lm}}{\sqrt{\sigma_{g,l}^2 \times \sigma_{g,j}^2}}$, and $\rho_{e,lj} =$

$$\frac{\sigma_{e,lm}}{\sqrt{\sigma_{e,l}^2 \times \sigma_{e,j}^2}}$$

Phenotypic correlations between two traits were calculated based on the pairwise genetic and environmental correlations. An estimate of the phenotypic correlation (ρ_p) between two traits was obtained using the following equation: $\rho_{p,lj} = \rho_{g,lm} \sqrt{h_l^2 h_j^2} + \rho_{e,lj} \sqrt{(1-h_l^2)(1-h_j^2)}$, where h_l^2 and h_j^2 represent the heritability of each trait in the pair (*l* and *j*).¹³ This method provides an estimate similar to Pearson’s correlation estimate, but it has the advantages of yielding an unbiased estimate of phenotypic correlation. The squared genetic correlation between two traits provides an estimate of the additive genetic variance in the traits that is due to effects of shared genes and can be interpreted as a measure of pleiotropic effects of genes influencing both traits simultaneously.¹⁴ The significance of each of the estimated parameters (eg, h_l^2 , $\rho_{g,lj}$, and $\rho_{e,lj}$) was evaluated by likelihood-ratio tests by comparing the log likelihood of a model in which the parameter was estimated to the log likelihood of a more restricted model in which the same parameter was set to zero. The likelihood-ratio test yields a statistic that is asymptotically distributed approximately as a χ^2 with degrees of freedom equal to the difference in the number of parameters estimated in the two models and is calculated as $-2(\log \text{likelihood}_{\text{restricted model}} - \log \text{likelihood}_{\text{general model}})$.

Pleiotropy due to shared genes is indicated by a ρ_G value that is significantly different from zero.

Finally, to estimate the percentage of residual phenotypic variation in a particular trait due to genes shared with another trait, we multiplied the heritability of the trait by the square of the genetic correlation between the two traits.

Results

Subjects (*n* = 788) belonged to 324 sibships, mean age was 62 years, 60% were women, 77% were hypertensive, and 30% were on statins (Table 1). The heritability estimates for the age- and sex-adjusted measures of HDL-C, log triglycerides, and LDL particle size were 0.58, 0.47, and 0.71, respectively. The standard error of mean was 0.08 for all three estimates and the estimates were significantly different from zero ($P < .0001$).

In the multivariate analysis, all genetic correlations were significantly different from zero ($P < .001$) based on likelihood-ratio tests (Table 2). There were relatively strong negative genetic correlations between HDL-C and log triglycerides and between log triglycerides and LDL particle size, suggesting that shared genes that increase triglycerides, decrease HDL-C and LDL particle size. A modest positive genetic correlation between HDL-C and LDL particle size suggests that shared genes increase HDL-C and LDL particle size together (Table 2).

The strongest genetic covariance was noted between HDL-C and log triglycerides, indicating that almost 41% of the additive genetic variation in each trait was due to genes shared between the two traits (Table 2). Eleven percent of the additive genetic variation in LDL particle size was attributable to genes shared with HDL-C and 24% of the additive genetic variation in LDL particle size could be attributed to genes shared with log triglycerides (Table 2).

The percentages of (age and sex adjusted) phenotypic variation in each trait due to shared additive genetic effects are shown in Table 3. Pleiotropy made the greatest contribution to the phenotypic variances in the trait pair of HDL-C and log triglycerides. Genes shared between HDL-C and log triglycerides accounted for 24% and 19% of the phenotypic variation in HDL-C and log triglycer-

Table 2. Maximum likelihood estimates of the additive genetic, environmental, and phenotypic correlations of HDL-cholesterol, log triglycerides, and LDL particle size

	Bivariate Phenotype Pairs		
	HDL-C & log triglycerides	HDL-C & LDL size	Log triglycerides & LDL size
Genetic correlation (ρ_G)	-0.64	0.33	-0.49
Environmental correlation (ρ_E)	-0.29	0.25	-0.59
Phenotypic correlation (ρ_P)	-0.47	0.30	-0.52
Genetic correlation squared (ρ_G^2)	0.41	0.11	0.24

All genetic and environmental correlations are statistically significant at $P < .0001$.

Table 3. Percentage of phenotypic variation in a trait due to additive genetic effects shared with the other trait of a trait pair

	Percentage of Phenotypic Variation due to Pleiotropy
HDL-C	23.8
Log triglycerides	19.3
HDL-C	6.4
LDL size	7.8
Log triglycerides	11.3
LDL size	17.0

ides, respectively. The trait pair for which pleiotropy had the least influence on phenotypic variances was the HDL-C and LDL particle size combination.

Discussion

Using multivariate quantitative genetic analysis in hypertensive sibships, we confirm significant genetic correlation between HDL-C, triglycerides, and LDL particle size, abnormalities of which are prevalent in familial dyslipidemic hypertension. In addition, a significant genetic covariance was noted between each possible pair of these traits, indicating that shared genes explain varying proportions (0.11 to 0.41) of the additive genetic variation in each trait. A previous study by Edwards et al⁵ demonstrated pleiotropic genetic effects on these lipid measures in families with hyperlipidemia. However, to our knowledge, the present study is the first to report on multivariate quantitative genetic analyses of HDL-C, triglycerides, and LDL particle size in hypertensive sibships and is a step toward identifying genetic loci/genes with pleiotropic effects on lipid traits that contribute to the increased CHD risk in these sibships.

Univariate residual heritability estimates for HDL-C have varied from 0.38¹³ to 0.55¹⁵ and for triglycerides from 0.35¹⁶ to 0.54,¹³ values similar to the heritabilities reported in the present study for these two lipid phenotypes. However, the heritability for LDL particle size in our study was higher than previously reported by Edwards et al⁵ (0.34) or by Bosse et al¹⁷ (0.59). It is possible that in hypertensive sibships the genetic influence on LDL particle size is greater than in these other populations. Alternatively, the differences in the heritability estimates may be due to differences in study design (sibships in the present study versus pedigrees in the studies of Edwards et al⁵ and Bosse et al¹⁷), different methods of measuring LDL particle size (polyacrylamide gel electrophoresis in the present study versus gradient gel electrophoresis in the other two studies), as well as the compact distribution of LDL particle size with less phenotypic variation in the present study (standard deviation of 4.19 Å versus 9.13 Å in the study by Edwards et al⁵). Genetic correlation between HDL-C and triglycerides have been reported in

several studies, whereas correlations between the HDL-C, triglycerides, and LDL particle size have been reported only in the family-based study of Edwards et al,⁵ in which subjects were ascertained based on the presence of hyperlipidemia. The results of the current study extend this previous report by demonstrating common genetic influences between HDL-C and both triglycerides and LDL size particle size in hypertensive sibships.

Pleiotropic effects contributed in varying degrees to the phenotypic variation in each trait. Shared genetic effects accounted for a significant proportion of the phenotypic variation in the trait pair of HDL-C and log triglycerides, suggesting the possibility of one or more loci with pleiotropic effects on both traits. Shared genetic effects accounted for only a modest proportion of the phenotypic variation of the HDL-C and LDL particle size trait pair. Therefore, a large proportion of the phenotypic variation in this trait pair can be attributed to nonshared genes and environmental effects.

Lipid abnormalities in hypertensive subjects are associated with other features of the insulin resistance syndrome.¹⁸ The pathophysiologic mechanisms that link insulin resistance and lipid abnormalities are unclear. It is likely that there are common metabolic pathways that influence components of the atherogenic lipoprotein profile. Thus, pathways that are known to lower plasma HDL-C concentrations are also involved in triglyceride metabolism and determination of LDL particle size. For example, concurrent changes in HDL-C and triglycerides may occur due to the exchange of cholesterol between HDL-C and triglyceride-rich particles mediated by lipid transfer proteins such as phospholipid transfer protein and cholesterol ester transfer protein (CETP)^{19,20} or due to hepatic lipase activity.²¹

Thirty percent of subjects in the present study were on lipid-lowering medications (statins constituted >90% of the lipid-lowering medications). Statins do not have a significant effect on LDL particle size²² and the effect on HDL-C is relatively modest (ranging from 5% to 15% increase in levels).²³ The effect of statins on triglycerides depends on the baseline triglyceride level and a decrease ranging from 7% to 30% can occur.²³ Inclusion of statin use in the statistical models did not significantly alter estimates of heritability (analyses not shown).

Detecting pleiotropic genetic effects is an important step toward understanding the phenotypic variation in the lipid traits associated with familial dyslipidemic hypertension. The results of our multivariate analyses suggest that a proportion of the additive genetic variation in HDL-C, log triglycerides, and LDL particle size in hypertensive sibships may be due to the pleiotropic effects of shared genes. Elucidating the underlying genetic architecture of these traits will facilitate mapping of genes contributing to familial dyslipidemic hypertension and CHD susceptibility. The present multivariate approach, by providing insights into the patterns of genetic relationships between

HDL-C, log triglycerides, and LDL particle size, also provides the rationale for multivariate linkage analyses to identify novel genetic loci with pleiotropic effects on the traits.

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