Genetic Control of Coordinated Changes in HDL and LDL Size Phenotypes

David L. Rainwater, Lisa J. Martin, Anthony G. Comuzzie

Abstract—We investigated the correlation of high density lipoprotein (HDL) and low density lipoprotein (LDL) particle size distributions in samples from >1100 participants in the San Antonio Family Heart Study. By use of analyses of individual correlations of each HDL fraction with each LDL fraction, we devised new metrics for particle size phenotype, termed ΔHDL and ΔLDL, to optimally reflect the size correlations. Confirming previous studies, we found that the 2 size phenotype variables were positively correlated \((r=0.51)\). Quantitative genetic analysis indicated that nearly half (44%) of the variance in ΔHDL and in ΔLDL was explained by the additive effects of genes. Bivariate genetic analyses indicated that a positive genetic correlation \((r_g=0.56)\) exists between them and suggested that the pleiotropic effects of a gene or group of genes account for \(\approx 31\%\) \([i.e., r^2=0.56^2=0.31]\) of the genetic variance in the 2 traits. Triglyceride (TG) levels were negatively related to ΔHDL and ΔLDL, with phenotypic correlations of \(-0.48\) and \(-0.58\), respectively, and genetic correlations of \(-0.45\) and \(-0.76\), respectively, suggesting that genes exert significant pleiotropic effects on the covariation of TGs with each of the size variables. Finally, we evaluated a bivariate model for ΔHDL and ΔLDL in which TG level was included as a covariate. This analysis indicated that a small but significant genetic correlation remains for ΔHDL and ΔLDL, even after accounting for the effects of TGs. Thus, our study demonstrates that the phenotypic correlation of HDL and LDL sizes results in part from the pleiotropic actions of a set of genes, some of which also influence TG levels and some of which do not. (Arterioscler-Thromb-Vasc-Biol. 2001;21:1829-1833.)

Key Words: LDL size distributions ■ HDL size distributions ■ genetics ■ triglycerides ■ Mexican Americans

Lipoprotein particles of differing sizes play distinct roles in lipoprotein metabolism and the process of atherosclerosis. The predominance of small dense LDLs was identified as a risk factor for cardiovascular disease (CVD), and prospective studies now have confirmed this initial observation. Several mechanisms have been proposed to explain this association: (1) small dense LDLs are more readily converted to highly atherogenic oxidized LDLs; (2) they more readily bind to surface components in the vessel wall; and (3) their size permits a more efficient infiltration into the vessel wall.

There is growing evidence that HDL subpopulations differ in their abilities to protect against CVD. However, whether current methods of measuring HDL subpopulations provide a substantial increment in CVD-predictive power is more controversial. For example, 2 studies reported significant associations of small dense HDLs (ie, HDL₃, separated by electrophoresis and density fractionation) and CVD, but they differed as to whether HDL₃ improved the prediction. Using nuclear magnetic resonance methodology, Freedman et al found that lipoprotein subpopulation measures, for HDL and LDL, significantly increased the proportion of variation in the coronary artery occlusion score that could be predicted.

A number of studies have reported a positive correlation between LDL and HDL size phenotypes. Although triglyceride (TG) concentrations are associated with LDL and HDL size variation, recent results suggest that at least a portion of the correlation between LDL and HDL sizes is independent of TG levels. These observations suggest the existence of a metabolic mechanism(s) that governs the coordinated covariation in the relative sizes of several classes of lipoproteins. The purpose of the present study was to investigate the nature of the relationship between LDL and HDL size phenotypes. To facilitate this investigation, we have developed new metrics for lipoprotein size, ΔHDL and ΔLDL, which reflect the correlation of HDL and LDL size fractions, respectively. ΔHDL and ΔLDL are size distribution variables that are based on subtracting the absorbance in smaller particles from the absorbance in larger particles. With these new size variables, we have tested the hypothesis that there are “lipoprotein size” genes responsible for the coordinated covariation between LDL and HDL.

Methods

Subjects and Samples
The San Antonio Family Heart Study investigates risk factors for CVD and diabetes in families of Mexican Americans living in and
around San Antonio, Tex.16 Probands were selected at random, and all first-, second-, and third-degree relatives were invited to participate in the present study. At a clinic visit, participants provided information on lifestyle variables, including current medications, and they were subjected to a physical examination; subjects who were currently taking lipid-lowering medications (<2%) were excluded from the study. An oral glucose tolerance test was administered, and the presence of diabetes (yes/no) was determined by World Health Organization (WHO) criteria.17 In addition, a blood sample was obtained for lipoprotein analyses; plasma was isolated by low-speed centrifugation and stored at −80°C in single-use aliquots.18 Clinic procedures were reviewed by the Institutional Review Board for the University of Texas Health Science Center at San Antonio, and all subjects gave written informed consent.

### Measurement of Lipoprotein Phenotypes

Cholesterol and TG concentrations were measured enzymatically by using kits from Boehringer-Mannheim Diagnostics and Stanbio, respectively. HDL cholesterol (HDL-C) concentrations were determined19 in the supernatant after precipitating apoB-containing lipoproteins with dextran sulfate–Mg2+. Inters assay coefficients of variation for control products in these assays were 2.1% for total plasma cholesterol, 4.6% for HDL-C, and 3.7% for TGs. LDL cholesterol (in millimoles per liter) was estimated as follows: total plasma cholesterol–[HDL-C+(0.42TG)], which has been validated for TG values <8 mmol/L.20,21

Composite gradient gels, which enable the evaluation of LDL and HDL phenotypes in the same lane, were made in the laboratory.22 Samples were subjected to electrophoresis at 3000 V·h, and cholesterol was stained with Sudan black B.23 Lipoprotein size distributions of cholesterol stain were measured with an LKB-Ultroscan XL laser densitometer. Calibrators, run on each gel, were estimated as absorbance in large size particles minus absorbance of smaller size particles for a lyophilized plasma absorbance profile. The data indicated the existence of 2 size classes of LDL, which are strongly and positively correlated with 2 size classes of HDL: large LDLs (27.2 to 28.4 nm) with large HDLs (9.6 to 10.7 nm) and small LDLs (24.4 to 26.0 nm) with small HDLs (7.7 to 8.2 nm). As might be expected, large size lipoproteins were inversely correlated with small size lipoproteins. These results suggest that 2 relatively narrow size intervals in each lipoprotein class are largely responsible for the significant correlation of LDL and HDL size phenotype. The large and small size intervals tended to be on the leading and trailing edges of the main absorbance peak of the average LDL and HDL absorbance profiles (Figure 1). Thus, as LDL (or HDL) patterns trend toward larger particles, the large size interval increases relative to the small size interval (and vice versa).

Therefore, to generate a single variable representing coarsened size variation in each class of lipoproteins (ie, ΔHDL and ΔLDL), we subtracted absorbance in smaller particles from that in larger particles. When these variables were determined for all samples in the study, we found a strong positive phenotypic correlation between ΔHDL and ΔLDL.

### Quantitative Genetic Analyses

We used well-established statistical genetic methods26,27 to partition the phenotypic correlations among ΔHDL, ΔLDL, and TG levels into correlations due to shared genetic effects (ie, pleiotropy) and those due to random environmental effects (designated as ρa and ρe, respectively); total phenotypic correlations (ρt) were calculated as follows: ρt=[h2·ρa]+[√(1−h2)·ρe], where h2 and h2 are heritabilities of traits 1 and 2, respectively. We have described implementation of these methods in detail previously.28,29

### Results

#### Development of 2 Lipoprotein Size Variables, ΔHDL and ΔLDL

Initially, a subset of samples (n=482) was evaluated for correlations between HDLs and LDLs. Each absorbance profile was converted into units of particle size at increments of 0.1 nm, and Figure 1 shows the average absorbance profile for these subjects. A correlation coefficient was calculated for the relationship of each HDL fraction (n=58) with each LDL fraction (n=80), and Figure 2 shows a contour plot for these 4640 correlations. The data indicated the existence of 2 size classes of LDL, which are strongly and positively correlated with 2 size classes of HDL: large LDLs (27.2 to 28.4 nm) with large HDLs (9.6 to 10.7 nm) and small LDLs (24.4 to 26.0 nm) with small HDLs (7.7 to 8.2 nm). As might be expected, large size lipoproteins were inversely correlated with small size lipoproteins. These results suggest that 2 relatively narrow size intervals in each lipoprotein class are largely responsible for the significant correlation of LDL and HDL size phenotype. The large and small size intervals tended to be on the leading and trailing edges of the main absorbance peak of the average LDL and HDL absorbance profiles (Figure 1). Thus, as LDL (or HDL) patterns trend toward larger particles, the large size interval increases relative to the small size interval (and vice versa).
(Figure 3). The phenotypic correlation coefficient for ΔHDL and ΔLDL was stronger than the correlation coefficient for HDL and LDL median diameters ($r=0.54$ versus $r=0.46$, respectively; data not shown), an alternate particle distribution metric used previously.

**Quantitative Genetic Analyses of ΔHDL and ΔLDL**

ΔHDL and ΔLDL were subjected to quantitative genetic analyses to determine whether a genetic component contributed to their variation. Table 1 gives the results of the analyses of samples from 1157 individuals in the present study. Covariates that satisfied an entry criterion of $P<0.1$ in preliminary univariate analyses included sex, age, diabetes status, contraceptive use, and hypertension medications, and their coefficients are given in Table 1. Covariates accounted for 14% and 12% of total variation in ΔHDL and ΔLDL, respectively. Heritabilities of both traits were highly significant ($P<0.0000001$); the additive effects of genes accounted for 45% and 44% of residual variation (39% of total variation) for ΔHDL and ΔLDL, respectively. TG level, a trait associated with lipoprotein size, was also highly heritable ($h^2=0.47$), and covariates explained $\approx 16\%$ of total variation (Table 1).

**TABLE 1. Results of Quantitative Genetic Analyses of ΔHDL, ΔLDL, and Log, TG (millimolar), Including for Each Trait Mean, SD, $h^2$, $\beta$, and Proportion of Trait Variance Explained by Covariates**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ΔHDL</th>
<th>ΔLDL</th>
<th>Loge TGs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>($n=1157$)</td>
<td>($n=1157$)</td>
<td>($n=1135$)</td>
</tr>
<tr>
<td>Mean (male)</td>
<td>-91.6±7.5</td>
<td>-49.0±15.4</td>
<td>0.47±0.03</td>
</tr>
<tr>
<td>SD</td>
<td>122.9±2.8</td>
<td>251.2±5.7</td>
<td>0.44±0.01</td>
</tr>
<tr>
<td>$h^2$</td>
<td>0.45±0.07</td>
<td>0.44±0.06</td>
<td>0.47±0.06</td>
</tr>
<tr>
<td>$\beta$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (female)</td>
<td>93.2±7.3</td>
<td>115.2±14.9</td>
<td>-0.14±0.03</td>
</tr>
<tr>
<td>Age</td>
<td>-0.43±0.26</td>
<td>-2.6±0.5</td>
<td>0.009±0.001</td>
</tr>
<tr>
<td>Age$^2$</td>
<td>0.06±0.01</td>
<td>0.11±0.02</td>
<td>-0.0003±0.0004</td>
</tr>
<tr>
<td>Diabetes status</td>
<td>-20.9±10.7</td>
<td>-133.3±21.8</td>
<td>0.20±0.04</td>
</tr>
<tr>
<td>Contraceptive use</td>
<td>-3.9±14.4</td>
<td>-59.7±29.4</td>
<td>0.16±0.05</td>
</tr>
<tr>
<td>Hypertension medications</td>
<td>-23.9±12.8</td>
<td>-59.8±26.3</td>
<td>0.53±0.05</td>
</tr>
<tr>
<td>Variance explained</td>
<td>14</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>by covariates, % of total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\beta$ indicates covariate effects. Other covariates that were considered but did not satisfy requirement for entry into the model included several dietary variables (such as alcohol and fat intake), usage of medications (such as for diabetes and hypertension), smoking, and menopause status.

**Pleiotropic Effects on ΔHDL and ΔLDL**

Bivariate genetic analyses were used to evaluate the phenotypic correlations between ΔHDL and ΔLDL. The phenotypic correlation between the 2 traits was estimated to be 0.51 in the genetic analyses, which is similar to the simple correlation given in Figure 3, which did not account for the relatedness among individuals. At 0.56 (Table 2, model 1), the genetic correlation ($\rho_G$) between the 2 traits was highly significant, implying that $\approx 31\%$ of the genetic variance in the 2 traits is due to the pleiotropic actions of a gene or group of genes, ie, $\rho_G^2=(0.56)^2=0.31$. The remainder of covariation was due to factors not specified in the model, which could include unmeasured covariates as well as residual genetic effects.

**Effect of TG Concentrations on the Correlation of ΔHDL and ΔLDL**

Each of the lipoprotein size traits was strongly correlated with TG levels, with phenotypic correlations of $-0.48$ and $-0.58$ and genetic correlations of $-0.45$ and $-0.76$ for ΔHDL and ΔLDL, respectively (Table 2, models 2 and 3). Thus, shared genes accounted for 20% and 58%, respectively, of the genetic variance in each pair of traits, and this raised the possibility that the genetic correlation between ΔHDL and ΔLDL might be due exclusively to their common correlation with TG levels. After including TG level in the model as a covariate, we found that the phenotypic correlation decreased from 0.51 to 0.31 but that there remained a small, but significant, genetic correlation between ΔHDL and ΔLDL after the adjustment (Table 2, model 4).

**Discussion**

In the present study, we have devised new metrics for lipoprotein size distribution phenotype. The variables ΔHDL and ΔLDL were based on inspection of the pattern of correlations between HDL and LDL fractions. Potentially, the range of values can be $1000$ (all absorbance in the small size interval) to $1000$ (all absorbance in the large size interval). ΔLDL more closely approached that potential than...
did ΔHDL (−683 to 863 versus −424 to 356, respectively). Previously, we have used median diameter, the diameter, for which half the HDL (or LDL) absorbance is on larger and half is on smaller particles, to represent lipoprotein distribution.22

Our preliminary analyses indicated that HDL and LDL median diameters gave similar results to what we report in the present study but that their phenotypic correlations were not as strong as for ΔHDL and ΔLDL (r=0.457 versus r=0.540, respectively; data not shown).

Earlier investigations have noted that LDL and HDL sizes are significantly correlated,11–14 and the present study of >1100 subjects confirms that very strong correlation, accounting for ≈25% of the variance. These findings suggest a common pathway(s) that influences relative particle size distribution within the spectrum of possible sizes for LDLs and HDLs and for Lp(a) as well.13 Our data also indicate that particle size phenotypes are under substantial genetic control, with both traits having heritabilities (h²) of ≈45%, consistent with previous estimates of 30% to 50% for LDL30 and HDL29,31 size variables.

The novel finding of the present study is that genes account for a substantial proportion of the coordinated variation in lipoprotein size phenotypes. In fact, ≈31% of the genetic variance in the 2 traits is influenced by a set of genes with pleiotropic effects. This suggests that at least 1 of the pathways responsible for coordinated variation in lipoprotein sizes is under genetic control.

What sort of genes might these be? On the basis of known metabolic pathways, a number of proteins might be invoked as candidates for the coordinated changes that we have detected in the present study. For example, cholesteryl ester transfer protein (CETP) facilitates exchange of neutral lipids among lipoproteins. Studies have demonstrated that CETP induces the net transfer of TGs from VLDL to LDL and HDL, which, coupled with lipase activity, is associated with the reduction of HDL and LDL sizes.32–34 It is likely that this pathway would be influenced by TG levels, and recently, Pownall et al35 have shown that changes in TG levels and the attendant changes in CETP are determinants of HDL and LDL composition and structure. A number of polymorphisms in the CETP gene are associated with variation in CETP levels and with HDL measures.36 LIPC, which encodes hepatic lipase, is also a strong candidate for a lipoprotein size gene. Hepatic lipase activity is strongly associated with HDL size phenotype,37–40 and several studies have reported an association with LDL size as well.41–43 However, when it was investigated in the same study, HDL but not LDL size was associated with the C–514T polymorphism in the hepatic lipase gene previously reported to show differences in enzyme activities.44 Genetic studies have supported this association, with linkage of a locus affecting HDL size phenotype to the region on chromosome 15 containing the hepatic lipase gene45 and suggestive evidence for linkage of a gene for LDL peak diameter in the same region.46 Taken together, these data suggest hepatic lipase as a strong candidate for 1 of the genes globally affecting lipoprotein size distributions.

Our data help to delineate the nature of the relationship among TGs and LDL and HDL size variation. We found strong correlations of TGs with LDL and HDL size, as has been observed in numerous studies already cited, and we also noted that a substantial proportion of the correlation was due to pleiotropic effects of the genes. This suggested the possibility that the genetic correlation between LDL and HDL size might be due to genetic effects mediated through TGs. However, when TG level was included in the model as a covariate, we found that the genetic correlation between the LDL and HDL size remained significant, albeit small. Taken together, these observations suggest distinct sets of genes that influence lipoprotein size: those that influence TGs, a critical component of lipoprotein particles, and those that affect lipoprotein size independent of TGs (although we cannot exclude the possibility that a more direct measure of TG-rich lipoproteins might absorb the TG-independent correlation). This observation extends our earlier study, which suggested a significant TG-independent phenotypic correlation between HDL and LDL sizes.15 Thus, in addition to the factors uniquely influencing each trait, there appear to be genetic and nongenetic factors, TG-related and TG independent, that are responsible for covariation in lipoprotein size traits.

Acknowledgments

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References


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**TABLE 2. Results of Bivariate Quantitative Genetic Analyses of ΔHDL, ΔLDL, and Loge TG, Including Residual Heritabilities for Each Trait, Genetic and Environmental Correlations, and Overall Phenotypic Correlations Calculated as Given in the Text**

<table>
<thead>
<tr>
<th>Model</th>
<th>Traits</th>
<th>n</th>
<th>Trait 1 h²</th>
<th>Phenotypic Correlation</th>
<th>Genetic Correlation</th>
<th>Environmental Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ΔHDL, ΔLDL</td>
<td>1157</td>
<td>0.45</td>
<td>0.44</td>
<td>0.51</td>
<td>0.056 ± 0.08</td>
</tr>
<tr>
<td>2</td>
<td>ΔHDL, TG</td>
<td>1135</td>
<td>0.44</td>
<td>0.48</td>
<td>−0.48</td>
<td>0.056 ± 0.08</td>
</tr>
<tr>
<td>3</td>
<td>ΔLDL, TG</td>
<td>1135</td>
<td>0.43</td>
<td>0.48</td>
<td>−0.58</td>
<td>0.076 ± 0.06</td>
</tr>
<tr>
<td>4</td>
<td>ΔHDL, ΔLDL (+TG)†</td>
<td>1135</td>
<td>0.45</td>
<td>0.30</td>
<td>0.31</td>
<td>&lt;0.000001</td>
</tr>
</tbody>
</table>

*Covariates are same as in Table 1.

†Loge TG was included in the model as a covariate.


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