Pleiotropic Genetic Effects on LDL Size, Plasma Triglyceride, and HDL Cholesterol in Families

Karen L. Edwards, Michael C. Mahaney, Arno G. Motulsky, Melissa A. Austin

Abstract—The interrelationships among low density lipoprotein (LDL) particle size, plasma triglyceride (TG), and high density lipoprotein cholesterol (HDL-C) are well established and may involve underlying genetic influences. This study evaluated common genetic effects on LDL size, TG, and HDL-C by using data from 85 kindreds participating in the Genetic Epidemiology of Hypertriglyceridemia (GET) Study. A multivariate, maximum likelihood–based approach to quantitative genetic analysis was used to estimate the additive effects of shared genes and shared, unmeasured nongenetic factors on variation in LDL size and in plasma levels of TG and HDL-C. A significant (P<0.001) proportion of the variance in each trait was attributable to the additive effects of genes. Maximum-likelihood estimates of heritability were 0.34 for LDL size, 0.41 for TG, and 0.54 for HDL-C. Significant (P<0.001) additive genetic correlations (ρg), indicative of the shared additive effects of genes on pairs of traits, were estimated between all 3 trait pairs: for LDL size and TG $\rho_g = -0.87$, for LDL size and HDL-C $\rho_g = 0.65$, and for HDL-C and TG $\rho_g = -0.54$. A similar pattern of significant environmental correlations between the 3 trait pairs was also observed. These results suggest that a large proportion of the well-documented correlations in LDL size, TG, and HDL-C are likely attributable to the influence of the same gene(s) in these families. That is, the gene(s) that may contribute to decreases in LDL size also contribute significantly to higher plasma levels of TG and lower plasma levels of HDL-C. These relationships may be useful in identifying genes responsible for the associations between these phenotypes and susceptibility to cardiovascular disease in these families. (Arterioscler Thromb Vasc Biol. 1999;19:2456-2464.)

Key Words: pleiotropy ■ LDL size ■ HDL cholesterol ■ triglycerides ■ families

Numerous studies have shown that plasma lipids and lipoproteins are associated with increased risk of cardiovascular disease (CVD), including smaller-size LDL particles, elevated plasma triglyceride (TG), and decreased levels of HDL cholesterol (HDL-C). However, whether these associations with CVD are independent of one another remains controversial.

Each of these cardiovascular risk factors also appears to be genetically influenced. For example, two of the most common forms of familial hyperlipidemia among families with documented coronary heart disease (CHD) have been classified as familial combined hyperlipidemia (FCHL) and familial “monogenic” hypertriglyceridemia (FHTG), both of which are characterized by variable and obligatory hypertriglyceridemia, respectively. Although the specific genetic bases of both FCHL and FHTG are poorly understood, studies indicate major gene effects on TG and apo B in FCHL families. In addition, there is also considerable evidence for genetic influences on LDL subclass phenotypes and HDL-C. Statistical and physiological relationships between measures of LDL size, plasma TG, and HDL-C are well established and may represent a high-CHD-risk lipid/lipoprotein phenotype with common underlying genetic influences. Sprecher et al first proposed that the combination of high TG and low HDL-C constituted an inherited “conjoint trait” associated with increased risk of CHD in families of hypertriglyceridemic or hypercholesterolemic probands participating in the Lipid Research Clinics Family Study. An atherogenic lipoprotein phenotype characterized by small dense LDL, low plasma levels of HDL-C, high plasma TG levels, and elevated apo B levels was previously described by Austin et al. Furthermore, a multivariate lipid factor characterized primarily by LDL size, plasma TG, and HDL-C has been identified and was shown to prospectively predict CVD. By comparing female monozygotic and dizygotic twins, this lipid factor was shown to be heritable and possibly linked to both the lipoprotein lipase gene and the hormone-sensitive lipase gene. Although the evidence supports common genetic influences on these cardiovascular risk factors, no study to date has simultaneously evaluated the genetic relationships among the 3 risk factors; LDL size, plasma TG, and HDL-C in high-risk families.

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Thus, the purpose of this investigation was to evaluate the evidence for shared genetic influences between phenotypic variation in LDL size, plasma levels of TG, and HDL-C in families at increased risk for CVD by using quantitative genetic analysis.

**Methods**

**Study Sample**

The families included in this cohort study are primarily white and are part of the Genetic Epidemiology of Hypertriglyceridemia (GET) Study, which is focused on understanding the risk of CVD in the common familial forms of elevated TGs, i.e., FCHL and FHTG, and on elucidating the underlying genetic influences of these disorders.

Family ascertainment for the GET Study was based on 2 family studies conducted at the University of Washington, Seattle, in the early 1970s. In brief, the first family study was published in 1973 and focused on families identified through a hyperlipidemic family member (proband) surviving a myocardial infarction. The second study was published in 1976 and identified families through a proband with hypertriglyceridemia but without coronary disease. In these baseline studies, FCHL was characterized by variable expression of hypercholesterolemia or hypertriglyceridemia in family members, while FHTG was characterized by families in which all affected relatives had an elevated plasma TG level but not an elevated cholesterol level. Thus, elevation in plasma TG levels in affected relatives was always seen in FHTG and commonly observed in FCHL at baseline.

As part of the GET Study, living individuals from a total of 85 families were recontacted between 1994 and 1997. Of the 85 families, 58 (68.2%) had been classified as FCHL and 27 (31.8%) as FHTG at baseline. In the 20 years since the baseline studies were initiated, many family members had died, including those with the highest TG levels, and could not be resampled. New blood samples were obtained from surviving family members, including probands and their spouses; siblings and spouses of siblings; and grandchildren, nieces, and nephews of the proband. For the current analysis, eligible family members were those who were over age 18 at follow-up, who were not pregnant, and who were not too ill to participate. Each eligible family member was asked to complete and return a self-administered medical history questionnaire by mail and to provide a fasting blood sample for lipid and lipoprotein determinations. Approximately 49% of participants lived in Washington state, with the remaining 51% residing in 28 other states. All information was kept confidential and was not shared with other family members. Each participant provided written, informed consent at the time they were enrolled in the follow-up study, and all methods used to contact family members were approved by the University of Washington Internal Review Board.

**Laboratory Measurements**

All subjects were instructed to fast overnight. Blood samples were centrifuged at 2500 rpm for 15 minutes using a standardized protocol. For participants living >100 miles from the Seattle area, plasma was separated and shipped on ice for overnight delivery to the University of Washington.

Lipid determinations including plasma TGs and total HDL-C were performed at the Core Laboratory, Northwest Lipid Research Laboratories in Seattle, a Centers for Disease Control and Prevention–certified lipid laboratory. HDL-C and plasma TG were measured by enzymatic techniques (Abbott Laboratories) according to the standardized procedures of the Lipid Research Clinics protocol.

Nondenaturing gradient gel electrophoresis was performed on isolated LDL using 2% to 14% polyacrylamide gradient gels, as previously described. The estimated diameters of LDL subclasses were calculated on the basis of a calibration curve constructed from high-molecular-weight standards run on the same gel. The size of the major LDL subclass, denoted LDL peak particle diameter (LDL size), was identified from the isolated LDL gel and used as a continuous variable in the quantitative genetic analysis as a measure of LDL size heterogeneity.

**Statistical Analysis**

Because TG and HDL-C distributions were positively skewed (2.16 and 0.96, respectively), the natural log transformations (ln) of these risk factors were used for all analyses. Transformation reduced the skewness in plasma TG and HDL-C to 0.12 and −0.01, respectively. Untransformed values are presented in descriptive tables and figures for ease of interpretation.

For the quantitative genetic analysis, pedigree and phenotypic data were prepared using the computer package PSEUDYS. Statistical genetic analysis was conducted using the modified version of the Pedigree Analysis Package, version 3.0, which is based on established genetic theory of partitioning the total phenotypic variance of a trait into genetic and environmental components. In this approach, the univariate heritability ($h^2$) of an individual trait represents the proportion of the total phenotypic variance due to additive genetic effects. The residual heritability is used here to reflect the proportion of variance attributable to additive genetic effects after accounting for age and sex effects.

Extension of univariate quantitative genetic analysis to the multivariate state has been described in detail. In this approach, the total phenotypic correlation between pairs of traits is partitioned into the additive genetic ($r_\text{G}$) and random environmental ($r_\text{E}$) components by using maximum-likelihood methods implemented in the modified version of the Pedigree Analysis Package. The 3 pairs of traits used in this trivariate quantitative genetic analysis were (1) LDL size and TG, (2) LDL size and HDL-C, and (3) TG and HDL-C. The additive genetic and environmental correlations between these pairs of traits were obtained from the genetic and environmental variance-covariance matrices, estimated by modeling the joint distributions of the traits as a function of their population means; their covariates and regression coefficients; the additive genetic values; random environmental deviations; and the degree of relationship among the individuals in this sample.

The genetic and environmental correlations represent the additive effects of shared genes (pleiotropy) and of shared, unmeasured environmental (nongenetic) factors on the phenotypic covariance for each pair of traits, respectively. An estimate of the total phenotypic correlation ($r_\text{T}$) between 2 traits was obtained using the following equation:

$$r_\text{T} = \sqrt{h^2_\text{G} + h^2_\text{E} + 2h^2_\text{G}h^2_\text{E}r_\text{G} + 2h^2_\text{E}r_\text{E} + h^2_\text{E}r_\text{E}} \times \sqrt{1 - h^2_\text{G} - h^2_\text{E} - 2h^2_\text{G}h^2_\text{E} + 2h^2_\text{E}r_\text{E} + h^2_\text{E}r_\text{E}}$$

where $h^2_\text{G}$ and $h^2_\text{E}$ represent the residual heritability of each trait in the pair.

The significance of each of the estimated parameters ($G\text{h}^2$, $E\text{h}^2$, and $G\text{r}_G$) was evaluated by likelihood-ratio tests by comparing the ln likelihood of a model in which the parameter is estimated to the ln likelihood of a more restricted model in which the same parameter is set to zero. The likelihood-ratio test yields a statistic that is asymptotically distributed approximately as a $\chi^2$ with degrees of freedom equal to the difference in the number of estimated parameters and is calculated as $-2\ln(\text{likelihood unrestricted model}) - \ln(\text{likelihood restricted model})$. Polygenic pleiotropy is indicated by $G\text{h}^2$ value that is significantly different from zero. An additional likelihood-ratio test was performed for each significant genetic correlation to test for complete pleiotropy ($r_\text{G} = 1.0$). In this test, the unrestricted model, wherein $r_\text{G}$ is estimated, is compared with a more restricted model wherein $r_\text{G}$ is fixed at either 1.0 or −1.0. When $r_\text{G}$ is significantly different from 1.0, pleiotropy is interpreted as incomplete.

Finally, to obtain an estimate of the shared additive effects of genes on each of the residual phenotypes in a pair, the squared additive genetic correlation for that pair is multiplied by the heritability estimate for each trait. Similarly, the proportion of residual phenotypic variance in an individual trait that is due to shared effects of unmeasured environmental factors is obtained by multiplying the squared environmental correlation by 1 minus the heritability estimate.

To better meet assumptions of normality, individuals with plasma TG >4.5 SDs above the mean (TG >905 mg/dL) were excluded from all analyses ($n = 5$). Thus, this analysis focuses on 780 individuals in 85 families who provided a fasting blood sample and completed a medical history questionnaire, including 78 spouse pairs, 501 parent-offspring pairs, and 518 sibling pairs. Quantitative genetic analysis was first performed in the whole data set and then separately in the FCHL and FHTG families. However, because there were no substantial differences when the quantitative genetic anal-
ysis was run separately in FCHL and FHTG families, only results based on the combined sample are presented.

**Results**

The average age of participants in this study was 49.0 years, and more than half of the participants were female (56.9%). As shown in Table 1, spouses tended to be older than relatives, although there were no differences in age between male and female relatives or between male and female spouses. Unadjusted measures of LDL size, plasma TG, and HDL-C for the individuals included in this analysis by relative type and sex are also shown in Table 1. Men tended to have smaller-size LDL particles and lower HDL-C levels than women, with similar patterns in relatives and spouses. The average level of plasma TG was higher in men than women and was higher in male relatives compared with male spouses. Plasma TG levels did not appear to be different in female relatives and spouses, and this may be due to increased mortality among relatives with high plasma TG levels relative to spouses.

Figures 1 through 3 present the frequency distributions for all individual study subjects (n = 780) for unadjusted measures of LDL size, plasma TG, and HDL-C, respectively. The mean LDL size was 265.7 Å (SD 9.13) in this sample (Figure 1). Figure 1 also shows a suggestive bimodal distribution of LDL size. The overall mean plasma TG level was 1.80 ± 1.27 mmol/L (159.5 mg/dL [SD 113.16]; median 1.46 mmol/L [129 mg/dL]) and was highly skewed (Figure 2). Mean HDL-C was 1.24 ± 0.38 mmol/L (47.8 mg/dL [SD 14.77]; median 1.16 mmol/L [45.0 mg/dL]) and was also positively skewed (Figure 3).

The maximum-likelihood estimates of the mean effects and variance components from the quantitative genetic analysis are presented in Table 2. Likelihood-ratio tests indicate significant (P < 0.001) residual heritabilities for each of the traits. Approximately one third of the residual variance in LDL size (h² = 0.34), slightly greater than one third of the residual variance in plasma TG (h² = 0.41), and more than half of the residual variance in HDL-C (h² = 0.54) were attributable to additive genetic effects. This table also includes maximum-likelihood parameter estimates for significant (P < 0.001) covariate effects. Based on the coefficients for sex (β-sex), mean values for females were 268.06 Å, 0.054 mmol/L (4.78 mg/dL), and 0.102 mmol/L (3.94 mg/dL) compared with 262.98 Å, 0.056 mmol/L (4.98 mg/dL), and 0.095 mmol/L (3.67 mg/dL) in males for LDL size, ln TG, and ln HDL-C, respectively. Significant age effects were also

### Table 1. Descriptive Summary (Mean±SD) for AGE, LDL Size, Plasma TG, and HDL-C in Relatives and Spouses by Sex

<table>
<thead>
<tr>
<th></th>
<th>Relatives</th>
<th></th>
<th>Spouses</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>n</td>
<td>(255)</td>
<td>(298)</td>
<td>(83)</td>
<td>(144)</td>
<td>(338)</td>
<td>(442)</td>
</tr>
</tbody>
</table>
| Age, y           | 46.3±15.1 | 45.9±14.9  | 54.1±16.3| 57.4±13.6  | 48.2±15.7| 49.7±15.5 | 2458 Arterioscler Thromb Vasc Biol. October 1999

Figure 1. Frequency distribution of LDL size in 780 family members. Mean LDL size was 265.7 Å (SD 9.13).
detected for each of the 3 traits. Overall, age and sex together accounted for 1.3%, 7.6%, and 17.6% of the total variance in LDL size, ln TG, and ln HDL-C, respectively.

In the multivariate analysis, all genetic correlations were significantly different from zero (P<0.001) based on likelihood-ratio tests (Table 3). The negative genetic correlation (r_G = -0.87) between LDL size and plasma TG was the strongest, suggesting that genes in common contribute to decreases in LDL size and increases in plasma TG. The positive genetic correlation (r_G = 0.65) between LDL size and HDL-C was modest and suggests that shared genes that increase LDL size also increase HDL-C. The negative genetic correlation between plasma TG and HDL-C (r_G = -0.54) was also significant. Based on likelihood-ratio tests, the hypothesis of complete pleiotropy (r_G = ±1.0) was rejected for all genetic correlations (P<0.001).

When squared, the additive genetic correlation between 2 traits in a pair provides an estimate of the additive genetic variance for each trait in the pair that is due to effects of the same gene(s). The squared additive genetic correlation between LDL size and plasma TG (r_G^2) was 0.757. Thus, almost 76% of the additive genetic variance in LDL size is shared with TG. The squared additive genetic correlation between LDL size and HDL-C (r_G^2) was 0.423, suggesting that nearly

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LDL Size (±SE)</th>
<th>In TG (±SE)</th>
<th>In HDL (±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>μ</td>
<td>262.98 ± 0.52</td>
<td>4.98 ± 0.04</td>
<td>3.67 ± 0.02</td>
</tr>
<tr>
<td>σ</td>
<td>8.77 ± 0.23</td>
<td>0.61 ± 0.02</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>h²</td>
<td>0.34 ± 0.07</td>
<td>0.41 ± 0.07</td>
<td>0.54 ± 0.07</td>
</tr>
<tr>
<td>β - Sex*</td>
<td>5.08 ± 0.61</td>
<td>-0.20 ± 0.04</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>β - Age*</td>
<td>-0.028 ± 0.029</td>
<td>0.004 ± 0.002</td>
<td>1.1 × 10⁻² ± 0.001</td>
</tr>
<tr>
<td>β - Agef*</td>
<td>-0.073 ± 0.026</td>
<td>0.013 ± 0.002</td>
<td>1.2 × 10⁻² ± 0.001</td>
</tr>
</tbody>
</table>

*All parameters are significant at P < 0.001.

50% of the additive genetic variance in each of these traits is due to shared genes. The squared additive genetic correlation between plasma TG and HDL-C (ρₐ²) was 0.292, suggesting that almost 30% of the additive genetic variance in each trait is due to genes shared between the pair.

Unmeasured environmental correlations were also significant (P < 0.001) between all pairs of traits. Consistent with the genetic correlations, the environmental correlations between LDL size and plasma TG and between plasma TG and HDL-C were negative, whereas the environmental correlation between LDL size and HDL-C was positive (Table 3). When squared, the environmental correlations provide an estimate of the proportion of the random environmental variance (i.e., that proportion of the shared residual phenotypic variance not attributable to additive genetic effects or covariates) for each trait that is due to the effects of the same unmeasured environmental factors. Phenotypic correlations were estimated by using the equation described in Methods and are shown in Table 3.

TABLE 3. Maximum-Likelihood Estimates of the Additive Genetic (ρₐ) and Environmental (ρₑ) Correlations From the Multivariate Quantitative Genetic Analysis of LDL Size, 1n TG, and 1n HDL-C in 85 Kindreds and the Phenotypic Correlations (ρₚ)*

<table>
<thead>
<tr>
<th>Phenotype Pairs</th>
<th>ρₐ ± SE</th>
<th>ρₑ ± SE</th>
<th>ρₚ</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL size–TG</td>
<td>-0.87 ± 0.06</td>
<td>-0.53 ± 0.05</td>
<td>-0.66</td>
</tr>
<tr>
<td>LDL size–HDL-C</td>
<td>0.65 ± 0.09</td>
<td>0.48 ± 0.06</td>
<td>0.54</td>
</tr>
<tr>
<td>TG–HDL-C</td>
<td>-0.54 ± 0.09</td>
<td>-0.53 ± 0.07</td>
<td>-0.53</td>
</tr>
</tbody>
</table>

*All genetic (ρₐ) and environmental (ρₑ) correlations are significant at P < 0.001.

to additive genetic effects shared with LDL size, and 17% was attributable to shared unmeasured environmental factors. However, the sum of the 2 proportions was less than 1 for each trait, suggesting that other nonshared factors contributed to the residual phenotypic variance in each trait of the pair: 55% for LDL size and 52% for TG (Figure 4A). Similar results were obtained for the other trait pairs; however, the proportion of nonshared residual variance was greater for traits in these pairs (Figures 4B and 4C).

Figure 4B indicates that 14% and 23% of the residual variance in LDL size and HDL-C, respectively, were due to shared additive genetic effects between the pair. Shared unmeasured environmental effects accounted for 15% and 11% of the residual variance in LDL size and HDL-C, respectively. Approximately 30% of the residual variance of each trait in this pair was shared; however, the majority of the residual variance was due to other nonshared factors, 71% and 66% for LDL size and HDL-C, respectively.

Similar results were observed for TG and HDL-C (Figure 4C). For both TG and HDL-C, 71% of the residual variance was attributable to other nonshared factors. Shared factors accounted for slightly less than 30% of the residual variance in each trait; additive genetic effects accounted for 12% and 16% of the residual variance, and shared unmeasured environmental factors accounted for 17% and 13% of the residual variance in TG and HDL-C, respectively.

Discussion

Although phenotypic associations between LDL size, TG, and HDL-C are well documented, this is the first demonstration that the observed phenotypic associations are largely genetic. That is, the results presented here demonstrate strong genetic correlations between each possible pair of these traits among families at increased risk for CVD.32 The negative genetic correlation between LDL size and plasma TG shows that genetic influences that decrease LDL size also result in increases in plasma TG levels. The positive genetic correlation between LDL size and HDL-C was also significant, as was the negative genetic correlation between HDL-C and plasma TG, indicating that genes in common explained a substantial proportion of the genetic covariance in each pair of traits. However, based on rejecting the hypotheses of complete pleiotropy, these results indicate that additional nonshared genes also contributed to the variation in each of the traits.

Environmental correlations between each pair of traits were also significant, although generally not as large as the corresponding genetic correlations. However, they still provide evidence of shared random environmental effects among this set of phenotypically related lipid and lipoprotein risk factors, indicating the existence of other important covariates that were not included in this analysis. At least 40% of the residual phenotypic variance in each of the 3 traits is attributable to nonshared effects that almost certainly include environmental factors and may even include some dominance genetic effects that were not modeled in this statistical genetic approach. Importantly, identification and addition of significant environmental factors to the genetic model would decrease the residual environmental contribution to the phenotypic variance and increase the relative importance of the genetic effects already detected.
Univariate residual heritability estimates for the lipids and lipoproteins in this sample of high-risk families are consistent with previous reports. For example, in a sample of Mexican-American families participating in the San Antonio Family Heart Study, Mahaney et al.\(^2\) reported heritability estimates of 0.53 and 0.55 for \(\ln\) TG and \(\ln\) HDL-C, respectively, compared with estimates from the current study of 0.41 and 0.54, respectively. Estimates of heritability for LDL size, plasma TG, and HDL-C based on female twins are also consistent with the estimates presented here, \(h^2 = 0.30,14\) \(h^2 = 0.50\) to 0.65, and \(h^2 = 0.73,4\) respectively. Based on data from male twins, the heritability estimate for HDL-C was 0.36.\(^4\)

The phenotypic correlations estimated in this study are also consistent with the well-documented patterns between these lipids and lipoproteins.\(^2\)–\(^5\) For example, in a recent report from the Physicians’ Health Study, the correlation for each possible pair of these variables was strong and highly statistically significant (\(P<0.01\)): \(-0.71\) for TG and LDL size, \(-0.57\) for TG and HDL-C, and 0.61 for LDL size and HDL-C\(^3\) compared with \(r_p = -0.66,\) \(r_p = -0.53,\) and \(r_p = 0.54,\) respectively, reported here.

The results presented in this study are also consistent with a previous quantitative genetic analysis of TG and HDL-C. Based on data from Mexican-American families participating in the San Antonio Family Heart Study, Mahaney et al.\(^2\) showed that shared genes contributed to the covariation of TG and HDL. The magnitude of the genetic correlation between TG and HDL-C reported in the current study of high-risk families is remarkably similar (\(r_p = -0.54\) versus \(-0.53,\) respectively).\(^2\) Heller et al.\(^6\) also found significant genetic correlations between serum lipids and apolipoproteins, including TG and HDL-C, in elderly Swedish twins. The results of the current study extend these previous reports...
by including a measure of LDL size and demonstrate common genetic influences between LDL size and both HDL-C and plasma TG.

The hypothesis of common genetic influences on pairs of lipids and lipoproteins was first suggested by Goldstein et al. Based on data from the original family study published in 1973, Goldstein et al suggested that the FCHL phenotype was due to variable expression of a single dominant gene and not segregation of 2 separate genes. The hypothesis of common underlying genetic influences is further supported by recent results from linkage studies of candidate genes involved in lipid and lipoprotein metabolism. Two recent reports, 1 based on families ascertained in Finland and the other based on a mouse model of FCHL, have strongly suggested the existence of a novel gene for FCHL on chromosome 1. In addition, Dallinga et al showed that DNA variations in the apo AI-CIII-AIV gene cluster modify plasma TGs, LDL-C, and apo CIII levels in families with FCHL. Recent sib-pair linkage analysis also provides evidence for common genetic influences on LDL size, TG, and HDL-C. Preliminary evidence for linkage between the apo B gene and a lipid factor identified by factor analysis and characterized by LDL size, plasma TG, and HDL-C is based on data from the Kaiser Women Twins Study. Furthermore, using data from the same women twins, Austin et al also provided evidence for linkage between the apo B gene and each of the following individual risk factors: LDL size, TG, HDL-C, and apo B levels. Several studies have also reported linkage between the apo B gene and individual measures of circulating lipids and lipoproteins, including measures of LDL heterogeneity and TG. Several other candidate genes, including the LDL receptor, lipoprotein lipase, and apo AI-CIII-AIV loci, have been shown to be associated with lipids and lipoproteins. Additionally, the lipoprotein lipase gene has recently been linked to LDL size, with simultaneous effects on TG and HDL-C in lipoprotein lipase–deficient families.

Together these findings suggest that each of the 3 lipid and lipoprotein measures reflect a common underlying process that is controlled, at least in part, by shared genes and provide strong support for the existence of genes with pleiotropic effects influencing covariation in plasma lipids and lipoproteins. Thus, it may be appropriate to consider the aggregate effects of these risk factors in predicting CHD, as has been recently reported in type 2 diabetes. The use of multivariate traits is an important alternative to traditional approaches of estimating independent effects when the traits have a common etiologic pathway. This may be particularly true when considering genetically related risk factors.

Furthermore, from the standpoint of genetic mapping, large-magnitude genetic correlations observed in the current study, particularly those between LDL size and plasma TG, can be used to delimit major-locus hypotheses, including major-locus pleiotropy and linkage, that can then be tested by multivariate segregation analysis and combined segregation and linkage analysis. For example, using a multivariate lipid phenotype characterized by LDL size, plasma TG, and HDL-C may be advantageous when pleiotropy is due primarily to major loci. Evidence for linkage with the multivariate lipid phenotype could represent a gene that is common to all 3 related traits, suggesting pleiotropic effects beyond those described in the current article.

In addition, when pleiotropy is due primarily to shared additive effects of polygenes, such traits with high genetic and environmental correlations can also be used as covariates in univariate segregation and linkage analyses. Specifically, by removing common genetic effects of other traits, the likelihood of detecting genes influencing the unique residual component of an individual trait may be increased. For example, on the basis of the results presented in this study, both HDL-C and plasma TG could be included as covariates in analyses of LDL size to account for shared additive genetic and random environmental contributions to the variance in LDL size. Evidence for linkage with the residual trait would represent a gene unique to that trait. Importantly, the pleiotropic effects identified in this study can thus be used to effectively increase the likelihood of detecting quantitative trait loci.

Finally, although these results were interpreted as representing polygenic pleiotropy, individual loci exerting substantial effects on 1 of these 3 phenotypes may be responsible for some or all of the pleiotropy detected in this analysis. However, it is important to emphasize that the analyses presented here do not explicitly distinguish between the pleiotropic effects of single, major loci and the pleiotropic effects of multiple loci. Thus, shared additive effects of individual loci may well be included in the genetic correlations. Additionally, the effects of closely linked loci exhibiting strong linkage disequilibrium could also contribute to the genetic correlations and the well-documented pattern of phenotypic associations between these lipids and lipoproteins. Linkage and other statistical genetic studies are currently underway to test and distinguish between these hypotheses in this population.

Overall, these results suggest that correlations between LDL size, plasma TG, and HDL-C are strongly influenced by shared genes and thus may be jointly involved in genetic susceptibility to CVD. Localizing and identifying these gene(s) may lead to a better understanding of the role of LDL size, plasma TG, and HDL-C in susceptibility to CVD in these high-risk families.

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