Familial Correlations of HDL Subclasses Based on Gradient Gel Electrophoresis

Paul T. Williams, Karen M. Vranizan, Melissa A. Austin, and Ronald M. Krauss

We used nondenaturing polyacrylamide gradient gel electrophoresis to examine the familial correlations of high density lipoprotein (HDL) subclasses for 150 offspring in 47 nuclear families. The absorbance of protein stain was used as an index of mass concentrations at intervals of 0.01 nm within five HDL subclasses: HDL_{x} (7.2-7.8 nm), HDL_{y} (7.8-8.2 nm), HDL_{z} (8.2-8.8 nm), HDL_{a} (8.8-9.7 nm), and HDL_{b} (9.7-12 nm). Parent–offspring correlations were computed for two different characterizations of the parents: 1) by sex (i.e., mother versus father) and 2) by their relative values (highest versus lowest HDL). Sibling resemblance was assessed by using the intraclass correlations coefficient. Family members were significantly related for the following subclasses: HDL_{x} (sibling and father–offspring), HDL_{y} (sibling), HDL_{z} (sibling and mother–offspring), HDL_{a} (mother–offspring), and HDL_{b} (sibling, father–offspring, and mother–offspring). The offspring's HDL_{x} and HDL_{y} values were more strongly related to their fathers' than to their mothers' values, whereas their HDL_{z} levels were more strongly related to their mothers' values. In addition, fathers' HDL_{a} values were inversely correlated with the offspring's HDL_{b}. The parents' HDL subclass levels were more strongly related to subclass levels of their younger (<20 years) than their older offspring. Among all subclasses, HDL_{b} showed the strongest parent–offspring relation, with the parents' HDL values accounting for over 30% of the variance in offspring's HDL_{b}. Correlating the offspring with the highest- and lowest-valued parent revealed possible differences in the inheritance of apolipoprotein (apo) A-I with apo A-II–containing HDL subclasses (HDL_{a} and HDL_{b}) and apo A-I without A-II–containing subclasses (HDL_{x}, HDL_{y}, and HDL_{z}). Specifically, the offspring's HDL_{a} and HDL_{b} values were correlated with the low-valued parent only, whereas the offspring's HDL_{x} and HDL_{b} levels correlated equally with the high-valued and low-valued parent. The familial correlations for HDL subclasses remained significant when adjusted for the parents' HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. We conclude that gradient gel electrophoresis reveals correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I values. 

KEY WORDS • gradient gel electrophoresis • high density lipoproteins • genetics • family studies • apolipoprotein A-I

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Plasma high density lipoprotein (HDL) cholesterol and apolipoprotein (apo) A-I are carried on multiple distinct subclasses of particles that have different electrophoretic mobilities on nondenaturing polyacrylamide gradient gels. Electrophoresis has been used to identify particle-size intervals that approximate the location of five HDL subclasses: HDL_{x} (7.2–7.8 nm in diameter), HDL_{y} (7.8–8.2 nm), HDL_{z} (8.2–8.8 nm), HDL_{a} (8.8–9.7 nm), and HDL_{b} (9.7–12 nm). The relations of HDL subclasses to age, adiposity, exercise, weight loss, and plasma concentrations of other lipoproteins have been reported.

Case-control and angiographic studies suggest that coronary heart disease risk may be increased when HDL_{b} is reduced relative to HDL_{a} and HDL_{x}.

Plasma HDL cholesterol and apo A-I levels are correlated between parents and offspring and among siblings. A variety of genetic transmission schemes have been proposed for HDL inheritance: polygenic transmission for HDL cholesterol and apo A-I and A-II; recessive, dominant, and additive major gene transmission for HDL cholesterol; and additive major gene transmission for apo A-I. There are also reports of the absence of genetic transmission for HDL cholesterol and apo A-I. No report to date has used gradient gel electrophoresis to investigate the familial relations of HDL subclasses.

The apparently contradictory results from previous family studies of HDL cholesterol and apo A-I may be due to heretofore unstudied heterogeneity within these lipoprotein measurements. Correlations among family members provide initial assessment of familial effects, which may be due to genetics, cultural transmission, or shared environment. This report assesses the relations of HDL subclasses among family members. The absorbance value of protein-stained polyacrylamide gradient...
gels was used as an index of mass concentrations of HDL by particle diameter. After adjustment for the effects of sex, age, menstrual status, alcohol consumption, and exogenous hormones, protein-stained HDL levels in parents and offspring were used to (1) assess familial correlations at corresponding HDL subclasses with diameter values between 7.2 and 12.0 nm, (2) test whether the parent–offspring correlations for HDL subclasses depend on the parent's gender (mother versus father) or their relative value (i.e., highest versus lowest), (3) test whether parents' and offspring's HDLs are correlated at noncorresponding diameters, and (4) test whether adjustment of HDL subclass values for HDL cholesterol or apo A-I eliminates the familial correlations among HDL subclasses.

Methods

Family Set

The recruitment of families took place primarily among the Mormon community in the San Francisco Bay Area. Additional families were recruited after the first report from this study. Families were not selected for lipid disorders or family history of cardiovascular disease, but sequential sampling of kindreds for a low density lipoprotein (LDL) subclass phenotype expression was used. Persons were excluded if they reported taking lipid-lowering drugs or other drugs known to affect plasma HDL. Women were included if they used oral contraceptives and exogenous estrogen. This left 453 individuals belonging to 51 kindreds.

Clinical Measurements and Questionnaires

Participants completed questionnaires during their clinic visits, which included questions on date of birth, drug and medication use, current and recent pregnancy, lactation, medication and hormone use, cigarette use, drug and medication use, current and recent pregnancy, oral contraceptives and exogenous estrogen. This left 453 individuals belonging to 51 kindreds.

Laboratory Measurements

All participants provided blood samples after an overnight fast. Plasma total cholesterol and triglyceride concentrations were measured by enzymatic techniques by using the Gilford 3500 autoanalyzer. HDL cholesterol was measured after precipitation with heparin-MnCl₂. Plasma was ultracentrifuged at d<1.006 g/ml, cholesterol determined in the infranatant, and LDL cholesterol calculated from the formula by Friedewald et al. Plasma apo A-I concentrations were determined by radial immunodiffusion.

Electrophoresis of HDL in the ultracentrifuged d<1.20 g/ml fraction was performed on Pharmacia electrophoresis apparatus (GE 4-II) by using slab gradient gels (PAA 4/30, Pharmacia, Piscataway, N.J.) as described by Blanche et al. The protein-stained gradient gels were scanned with a model RFT densitometer (Transidyne Corp., Ann Arbor, Mich.) at a wavelength of 603 nm. A mixture of four globular proteins (High Molecular Weight Calibration Kit, Pharmacia) was run on the central lane to calibrate for particle size. The HDL migration distances (Rf) were measured relative to the migration distance of the peak of bovine serum albumin, one of the protein standards. The HDL distributions were converted from the migration distance scale to the particle diameter scale with a method previously described.

Statistical Analysis

The parents' and offspring's total plasma HDL cholesterol and apo A-I concentrations and absorbance values of the protein-stained HDL at each diameter value were adjusted for the effects of age, alcohol intake, and hormone use. Specifically, multiple linear regression was used to obtain the residual values from females' HDL measurements when adjusted for alcohol intake (ounces per week), oral contraceptive use, postmenopause estrogen use, and menstrual status (i.e., whether a woman was premenstrual, premenopausal, or postmenopausal). The males' HDL levels were adjusted for alcohol intake and whether he was ≤11 years old, 12–17 years old, or >18 years old. Values for both men and women were adjusted to have the same mean value. Simple and partial correlations and multiple linear regression were used to compare parent and offspring HDL measurements. Sibling HDL levels were compared by using the intraclass correlation coefficients. The correlations were computed at each 0.01 nm between 7.2 and 12 nm. Contour plots were used to examine the correlations of HDL over the two-dimensional plane of parents' and offspring's HDL diameter values. Regression analyses were used to test whether the effects of the parents' HDL levels on the offspring's HDL levels were: 1) different in sons and daughters; 2) different for the fathers and mothers; and 3) different for the high-valued parent (maximum [HDL₂₀, HDL₁₀]) and low-valued parent (minimum [HDL₂₀, HDL₁₀]) to assess potential dominance effects. Parent–offspring correlation and regression coefficients were computed with all families receiving equal weight. The familial correlations are expected to underestimate the true correlation because of the ascertainment bias. However, the bias is probably small because families were not directly selected for their HDL levels, and few probands are included in the sample. All significance levels are two-tailed.

Conversion from absorbance value to plasma concentration is not necessary for analyzing protein-stained HDL by particle diameter because the significance levels used in this report (i.e., for t tests, Pearson's correlation coefficients, partial correlation, and regression analyses) are invariant to translations of scale or location. This means that the significance levels for absorbance values will be identical to those based on unknown plasma concentrations when the conversion involves the addition and/or multiplication of numerical constants. In fact, different constants may be used at each diameter, so that variation in chromogenicity across the HDL particle size spectrum will not affect the results. The correlation coefficient is also invariant to translations of scale or location. Variations in chromogenicity will, however, affect the magnitude of the regression coefficients.

Within-Gel Correlations

Each polyacrylamide gel contained up to seven plasma samples. Each sample included a known con-
centration of thyroglobulin (0.35 mg/ml), which served as an internal standard. The areas of thyroglobulin standards on the same gel were found to be highly correlated with each other (intraclass correlation of 0.60 for 50 samples on 10 gradient gels) presumably because they shared the same staining procedure. Thus, correlations among family members will be inflated whenever related individuals are analyzed on the same gel. The 453 family members were measured on 158 separate gels (the other lanes were used for samples from other studies). Seventeen percent of the children shared their parents’ gradient gel, and 25% shared their mothers’. Thirty-seven percent of the siblings and 68% of the mothers and fathers were measured together.

In the analyses to follow, we eliminated the correlation within gels from the parent–offspring correlations by excluding children whose samples were measured on the same gel as either of their parents. In doing this we adopted the principle that it was preferable to increase type II rather than type I errors. This procedure reduced the sample to 83 male and 67 female offspring in 47 families. The intraclass correlation for siblings required exclusion of siblings whose HDLs were measured on the same gel and also exclusion of all families with only one child. Thus, the intraclass correlation coefficients are based on data for 83 siblings in 29 families. Too few mothers’ and fathers’ HDLs were measured on separate gradient gels to provide meaningful estimates of between-spouse correlations. The correlations presented in this report probably underestimate the true correlations. By excluding family members whose HDLs were measured on the same gradient gel, we have diluted the parent–offspring correlations by adding gel-to-gel variation to the unexplained variance. The correlations are therefore attenuated by the intergel measurement error.

**Results**

Table 1 summarizes the characteristics of the parents and offspring. The total and LDL cholesterol levels of the fathers tend to be low relative to the Lipid Research Clinic prevalence study but are otherwise normal.

**Familial Correlations for HDL Cholesterol and Apo A-I**

Table 2 shows that the offspring’s adjusted plasma HDL cholesterol and apo A-I concentrations are significantly correlated with those of their father and the minimum parent. Mother–offspring and maximum parent–offspring correlations were significant for apo A-I but not for HDL cholesterol. Plasma apo A-I was more strongly correlated between parent and offspring than was HDL cholesterol. Parent–offspring correlations for HDL cholesterol were not significantly different between sons and daughters or between mothers and fathers. The intraclass correlations for siblings were not significant for either HDL cholesterol or apo A-I (Table 2).

**Sibling Correlation of HDL Subclasses**

Figure 1 displays the sibling intraclass correlation coefficient by HDL particle diameter. The correlation coefficient is represented along the vertical axis, and HDL particle diameter is represented along the horizontal axis. For example, the intraclass correlation is 0.44 for HDL particles that are 8.0 nm in diameter and 0.32 for 10.5-nm particles. The correlations that were

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**Table 1. Characteristics of the Families**

<table>
<thead>
<tr>
<th></th>
<th>Fathers</th>
<th>Mothers</th>
<th>Sons</th>
<th>Daughters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>47*</td>
<td>47*</td>
<td>83*</td>
<td>67*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.0±13.8</td>
<td>53.9±13.3</td>
<td>27.7±12.7</td>
<td>26.9±12.6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.4±2.9</td>
<td>27.2±5.3</td>
<td>22.9±4.0</td>
<td>22.1±5.7</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>177.7±41.5</td>
<td>210.3±50.0</td>
<td>170.1±41.6</td>
<td>162.8±37.9</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>125.0±66.9</td>
<td>132.7±79.5</td>
<td>102.6±67.4</td>
<td>84.9±58.6</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>40.7±8.9</td>
<td>51.3±13.5</td>
<td>45.0±10.0</td>
<td>48.5±10.2</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>112.5±37.5</td>
<td>132.5±44.5</td>
<td>104.8±40.6</td>
<td>97.4±35.0</td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/dl)</td>
<td>128.0±21.6</td>
<td>149.2±23.8</td>
<td>127.3±17.5</td>
<td>136.6±15.8</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; LDL, low density lipoprotein. Values are mean±SD.

*Plasma apolipoprotein A-I concentrations were measured in 44 fathers and mothers, 71 sons, and 56 daughters.

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**Table 2. Spouse, Parent–Offspring, and Sibling Correlations for HDL Cholesterol and Apolipoprotein A-I Concentrations**

<table>
<thead>
<tr>
<th></th>
<th>Spouse</th>
<th>Father</th>
<th>Mother</th>
<th>Maximum parent</th>
<th>Minimum parent</th>
<th>Sibling</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL cholesterol</td>
<td>0.04</td>
<td>0.27*</td>
<td>0.11</td>
<td>0.18</td>
<td>0.26*</td>
<td>0.09</td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>0.23</td>
<td>0.41*</td>
<td>0.37*</td>
<td>0.44*</td>
<td>0.47*</td>
<td>0.12</td>
</tr>
<tr>
<td>7.2–7.8 nm (approx HDL₃)</td>
<td>...</td>
<td>0.25*</td>
<td>0.06</td>
<td>0.17</td>
<td>0.17</td>
<td>0.27</td>
</tr>
<tr>
<td>7.6–8.2 nm (approx HDL₄)</td>
<td>...</td>
<td>0.14</td>
<td>0.14</td>
<td>0.11</td>
<td>0.22</td>
<td>0.44*</td>
</tr>
<tr>
<td>8.2–8.8 nm (approx HDL₅)</td>
<td>...</td>
<td>0.06</td>
<td>0.23*</td>
<td>0.13</td>
<td>0.19</td>
<td>0.29*</td>
</tr>
<tr>
<td>8.8–9.7 nm (approx HDL₆)</td>
<td>...</td>
<td>0.11</td>
<td>0.33*</td>
<td>0.20</td>
<td>0.30*</td>
<td>0.05</td>
</tr>
<tr>
<td>9.7–12.0 nm (approx HDL₇)</td>
<td>...</td>
<td>0.47*</td>
<td>0.32*</td>
<td>0.46*</td>
<td>0.48*</td>
<td>0.26</td>
</tr>
</tbody>
</table>

HDL, high density lipoprotein; approx, approximately. Correlation coefficients were computed for 83 siblings in 29 families (sibling), for 150 offspring in 47 nuclear families (parent–offspring), and for 47 spouse pairs. Data were adjusted for age, sex, alcohol intake, menstrual status, and hormone use before analyses.

*Significant at p<0.05.
significant at \( p < 0.05 \) are identified by the solid portions of the bar at the bottom of the graph. The analyses show that sibling HDL levels were significantly correlated for HDL\(_{3b}\), HDL\(_{3a}\), and HDL\(_{1b}\) subcategories and for larger HDL\(_{3x}\) (specifically significant between 7.5 and 8.86 nm and between 10.11 and 11.82 nm).

**Parent–Offspring Correlation of HDL Subclasses**

Figure 2 displays the parent–offspring correlation coefficients for HDL by particle diameter (solid curve). The analyses show that HDL\(_{3b}\) levels are significantly correlated between fathers and sons (specifically between 9.69 and 11.27 nm) and between fathers and daughters (9.73–11.91 nm). In contrast, plasma HDL\(_{3a}\) levels showed the strongest correlation between mothers and sons (between 8.96 and 10.97 nm) and between mothers and daughters (8.59 and 9.50 nm). Plasma levels of smaller-diameter HDL, including HDL\(_{3x}\) and HDL\(_{2b}\), were significantly correlated only between fathers and sons (i.e., 7.28–7.99 nm).

A second set of curves in Figure 2 displays the partial correlation with adjustment for the fathers’ and the mothers’ BMI (dashed curve). This was done because of the strong association between BMI and plasma levels of HDL\(_{3a}\) and HDL\(_{3b}\). Adjustment for the parent’s BMI had little effect on the correlation coefficients for HDL\(_{3a}\) and HDL\(_{3b}\). However, the adjustment eliminated the correlation for small HDL in fathers and sons while accentuating the correlation for small HDL in fathers and daughters. Thus, the parent–offspring correlations for HDL subclasses do not appear to be secondary to familial aggregation of BMI.

The parent–offspring correlations of Figure 2 are generally similar for sons and daughters but appear different for fathers and mothers. Multiple regression analyses were used to formally test whether the effects of the parents’ HDL values on the offspring’s HDL values were different for sons and daughters or for mothers and fathers. There was no difference in the coefficients for sons and daughters except for a minor portion of HDL\(_{2b}\) (11–12 nm; analyses not displayed), in which the effect of the father was greater on daughters than on sons. Therefore, sons and daughters are usually combined in the analyses to follow.

Figure 3 (uppermost panel) displays the fathers’ and mothers’ regression coefficients for predicting the offspring’s HDL values. For example, the coefficients were

\[
\beta_{\text{father}} = 0.23 \quad \beta_{\text{mother}} = -0.10 \quad \beta_{\text{son}} = -0.04 \quad \beta_{\text{daughter}} = 0.41
\]

The significance levels for the differences between the fathers’ and mothers’ coefficients are displayed at the bottom of the panel. The graph shows that the offspring HDL value was more strongly influenced by the father than by the mother for the HDL\(_{3x}\) and HDL\(_{3b}\) subclasses and more strongly influenced by the mother than by the father for the HDL\(_{3a}\) subclass.

We also tested whether the relation of the offspring’s HDL values to those of their parents might depend on the relative magnitude of the parental HDLs (Figure 3, middle panel). At each diameter value, the offspring’s HDL was predicted by the HDL of the highest-valued (maximum) and lowest-valued (minimum) parent. The parent with the lowest HDL value was the strongest predictor of the offspring’s HDL value within the HDL\(_{3x}\) and HDL\(_{3b}\) intervals. The maximum parent was unrelated to the offspring’s HDL\(_{3a}\) and HDL\(_{3b}\) values. Comparison of the uppermost and middle panels suggests that the relation of the offspring’s HDL\(_{2b}\) and HDL\(_{3x}\) values to those of their parents appeared to have more to do with the parent’s gender than their relative HDL values.

The lowermost panel displays the percentage of the offspring’s variance explained by the fathers’ and mothers’
HDL values and the highest-valued and lowest-valued parents' HDL (i.e., $100R^2$ for the uppermost and middle panels, where $R^2$ is the multiple correlation coefficient). Both models show that the parents accounted for the greatest percentage of the offspring's variance within the HDL-2b region (e.g., the father and mother explain 34% of the variance at 10.5 nm). The explained variance suggests that the offspring's HDL-2b and HDL-3c values are best described when the parents are characterized by their sex, whereas the offspring's HDL-2a and HDL-3b values are best described when the parents are characterized by their relative values.

The influence of age on parent–offspring resemblance was examined by dividing the offspring into two groups, those ≤20 years and >20 years. Offspring ≤20 years were presumably more likely to reside with their parents than those >20 years. Figure 4 shows that the parent–offspring relation was stronger in the younger offspring, particularly within HDL-3b. The younger and older offspring exhibited similar asymmetry with respect to the father’s and mother’s contribution to the offspring’s HDL levels (i.e., significant father’s effect for HDL-3c and HDL-3a and significant mother’s effect within HDL-2b). The lowermost panel of Figure 4 shows that the parent–offspring similarity for HDL levels was greater for offspring ≤20 years old than older offspring. The younger offspring were also more strongly related to their parents than the older offspring when the parents were characterized by their minimum and maximum values (analyses not displayed).

Partial correlations were then used to assess whether the parent–offspring correlations for the HDL subclasses were simply the consequence of the parent–offspring relations for HDL cholesterol and apo A-I.

Figure 5 shows that the parent–offspring correlations for HDL subclasses remained significant when adjusted for the parents’ and offspring’s HDL cholesterol and apo A-I values. Thus, Figures 2 and 3 display relations between parents and offspring that are not simply the consequence of the apo A-I and HDL cholesterol correlations of Table 2 and the known relation of apo A-I and HDL cholesterol to the HDL subclasses.4

Parent–Offspring Correlations: Contour Plots Across All Diameters

Figure 6 examines the parent–offspring correlations over the two-dimensional plane of parent and offspring HDL diameter values. (The correlations of Figure 2 are the special case of the parent–offspring correlation represented along the diagonal.) The contour lines join points of equal correlation. Each contour line represents an increment in the correlation of 0.1 unit. The shading designates the sets of parents’ and offspring’s diameters where their HDL levels are significantly correlated. Father–offspring HDL levels are positively correlated throughout the HDL-2a range. The fathers' HDL-2b levels are also inversely correlated with the offspring’s HDL-3b. The mother–son correlation is significant throughout the HDL-2b and HDL-3b regions.

Discussion

Our analyses suggest that specific types of HDL particles are influenced by genetic or cultural transmission between parents and offspring.Sibling HDL levels were correlated within the HDL-2b, HDL-3a, HDL-3b, and larger...
HDL subclasses but not HDL-2b (Figure 1). The fathers’ and offspring’s HDL values were correlated for HDL-3a and HDL-3b, and the mothers’ and offspring’s HDL values were correlated for HDL-2a and HDL-2b (Figure 2). Parent–offspring resemblance was stronger for younger offspring (Figure 4), possibly reflecting the influence of the shared household environment for offspring who reside at home, a dilution of genetic effects by environmental influences occurring after age 20, or physiological changes modifying the expression of a trait.

The data in Figure 3 show that HDL-3a and HDL-3b levels in offspring were more strongly associated with those of their fathers than their mothers and that their HDL-2a levels were more strongly associated with those of their mothers than their fathers. The basis for a possible difference in the paternal and maternal influence on the offspring’s HDL subclass distributions is not known. None of the genes for HDL apolipoproteins, lecithin:cholesterol acyltransferase, or cholesteryl ester transfer protein are located on the sex chromosomes. Various multifactorial and cultural transmission models have been proposed in which the contributions of the father and mother are different. Alternatively, equal genetic contributions by both the mother and father may only appear to affect the offspring differently because the phenotypic expression of HDL is different in fathers and mothers. Adult women have higher levels of HDL-2a, HDL-2b, and HDL-3b and lower levels of HDL-3b than do men. The offspring may show different relations to the mother and father because of sexual differences in penetrance of the genes regulating HDL subclasses.

Parent–offspring relations were assessed for two different characterizations of the parents: 1) by sex (i.e., mother versus father) and 2) by their relative values (highest versus lowest HDL). Fitting separate coefficients to the fathers’ and mothers’ HDL-2a and HDL-3b levels gave the best prediction of offspring HDL-2a and HDL-3b values. Characterizing the parents by their minimum and maximum HDL values, not by sex, gave better predictions.

**Figure 5.** Graphs showing partial correlation coefficient for father–offspring and mother–offspring correlations adjusted for high density lipoprotein (HDL) cholesterol (top panel) and apolipoprotein A-I (bottom panel). Solid portions of the bars at the bottom of the graphs designate the range of diameter values that correlate significantly when adjusted at p<0.05.

**Figure 6.** Contour plots of the Pearson correlation between high density lipoprotein (HDL) protein levels of parents and offspring for protein-stained HDL between 7.2 and 12 nm. Contour lines connect points of equivalent correlation and are plotted every 0.1 unit. Solid and dashed lines represent positive and negative correlations, respectively. Shading designates significance at p<0.05. The diagonal represents the special case of correlating parents and offspring at the same HDL diameter value.
Diameter value explained a greater proportion of the offspring HDL_A and HDL_B variance than characterizing the parents by sex. The minimum parent was significantly related to the offspring HDL_B and HDL_A subclasses. The different results may be due to the fact that the HDL_A and HDL_B subclasses and the HDL_A and HDL_B subclasses belong to two separate particle class distributions. HDL_A and HDL_B contain both apo A-I and apo A-II and therefore belong to the HDL(A-I with A-II) particle distribution.\textsuperscript{38,39} HDL_A and HDL_B contain apo A-I but no apo A-II and therefore belong to the HDL(A-I without A-II) particle distribution.\textsuperscript{38,39} Simulation studies suggest that these results could arise from the inheritance of low HDL levels through a nonrecessive uncommon major gene (P.T. Williams et al, unpublished results). The results of Figure 3 may point to a difference in the mode of inheritance of the HDL(A-I with A-II) and HDL(A-I without A-II) distributions.

Adiposity clusters among families.\textsuperscript{40,41} The association between adiposity and HDL_B is known from intervention studies.\textsuperscript{5} However, the parent–offspring correlations within the HDL_B region remained largely unchanged when adjusted for the parents' BMIs. Thus, metabolic factors associated with adiposity may not be major determinants of the parent–offspring HDL_B relationship. This concurs with the observation that familial correlations for HDL cholesterol are unaffected by adjustment for adiposity.\textsuperscript{12} Terry et al\textsuperscript{42} showed that measurements of upper body obesity are more strongly related to plasma HDL subfractions than to BMI. The influence of upper body obesity on familial subclass correlations cannot be assessed in our study because regional adiposity was not measured.

Our analyses have largely focused on the HDL levels at corresponding HDL diameters. However, genetic and environmental factors that influence HDL metabolism are likely to promote changes throughout HDL particle distribution.\textsuperscript{8} We may, therefore, expect parent and offspring HDL levels to be related at noncorresponding diameter values as well. The contour plots of Figure 6 show that the fathers' HDL_B levels correlate inversely with the offspring's HDL_B. The inverse association between HDL_B and HDL_A has been observed cross sectionally in population-based studies.\textsuperscript{4} Metabolic interconversion between HDL subclasses appears to occur predominantly within the HDL(A-I with A-II) and HDL(A-I without A-II) distributions.\textsuperscript{29} Thus, the inverse correlations between HDL_A and HDL_B could be due in part to inherited metabolic factors (e.g., hepatic\textsuperscript{43,44} and lipoprotein lipase\textsuperscript{44} activities and plasma triglyceride concentrations\textsuperscript{25,26}) that promote parallel changes in the HDL(A-I with A-II) and HDL(A-I without A-II) distributions.

Estimates of genetic heritability have been presented from twin studies and pedigrees for HDL cholesterol (0.35,\textsuperscript{20} 0.34,\textsuperscript{43} 0.59,\textsuperscript{15} 0.66,\textsuperscript{45} and 0.74\textsuperscript{46}; HDL_A cholesterol (0.37\textsuperscript{47} and 0.50\textsuperscript{9}); HDL_A cholesterol (<0.04\textsuperscript{3} and 0.28\textsuperscript{48}); apo A-I (0.50–0.58\textsuperscript{9} and 0.66\textsuperscript{49}); and apo A-II (<0.43\textsuperscript{9} 0.26,\textsuperscript{10} 0.30, and 0.35\textsuperscript{29}). The higher heritability estimates for HDL_B vis-à-vis HDL_A, cholesterol\textsuperscript{47} and apo A-I vis-à-vis apo A-II\textsuperscript{43,48} are consistent with our finding that parents and offspring have the highest correlation for HDL_B (Figure 3). Although estimates from path-analysis models suggest that the genetic heritability of plasma HDL cholesterol is greater than its cultural heritability,\textsuperscript{10,49–52} these models will overestimate the genetic component if the environmental index variables are measured incompletely or imprecisely and if gene–environment interactions are ignored.\textsuperscript{53} Recent studies suggest that the HDL responses to exercise\textsuperscript{44} and short-term caloric excess\textsuperscript{55} are genotype dependent, thereby violating the path-analysis restriction on gene–environment interactions.

Our analyses have shown that HDLs exhibit familial relations that are subclass specific and that remain significant when adjusted for HDL cholesterol and apo A-I. The parent–offspring and sibling correlations were substantially greater for HDL_B than for either HDL cholesterol or apo A-I. The HDL_B correlations were also generally greater than those reported by others for HDL cholesterol between parent and offspring (r=0.20,\textsuperscript{13} 0.24,\textsuperscript{11} 0.26,\textsuperscript{12} 0.27,\textsuperscript{10,13} 0.29,\textsuperscript{11,13} and 0.30,\textsuperscript{10,13}) and among siblings (0.20,\textsuperscript{10} 0.29,\textsuperscript{10,13} 0.32,\textsuperscript{11} and 0.41\textsuperscript{12}). Caution is warranted in generalizing these results to other populations, since the families were principally selected from among the Mormon community. Our analyses suggest that measurements of HDL particle distributions by gradient gel electrophoresis may be better suited for genetic studies than are either HDL cholesterol or apo A-I measurements.

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References


Familial correlations of HDL subclasses based on gradient gel electrophoresis.

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