Inheritance of High Density Lipoprotein and Lipoprotein Lipase and Hepatic Lipase Activity

Timo Kuusi, Y. Antero Kesäniemi, Matti Vuoristo, Tatu A. Miettinen, and Markku Koskenvuo

The role of genetic and environmental factors in the regulation of plasma high density lipoprotein (HDL) was estimated in 17 monozygotic (MZ) and 18 dizygotic (DZ) male twins randomly selected from the Finnish Twin Cohort Study. In addition to HDL cholesterol, we determined the HDL subfractions, HDL2 and HDL3, and the major HDL apoproteins (apo) A-I and A-II. The activities of lipoprotein lipase (LPL) and hepatic lipase (HL) were also assayed from postheparin plasma to get information on their possible contribution to the heritability of HDL. Evidence for the genetic component in the regulation of plasma HDL received support from the heritability estimate of 0.34. The different heritability estimates of HDL2 and HDL3 (h2 of 0.56 and <0, respectively) support the idea that the HDL subfraction distribution might be important in the genetic regulation of plasma HDL level. This also received support from the heritability of apo A-I (h2 = 0.66), mainly varying in HDL2, and the lack of it in apo A-II, found mainly in HDL3. These conclusions were strengthened by standardizing the data with relative ponderosity. Postheparin plasma HL activity had a high pairwise correlation coefficient in the MZ twins (r=0.80, p<0.001), whereas LPL displayed no within-pair correlation. Neither of the lipolytic enzymes, LPL or HL, showed any correlation in the DZ twins. Therefore, it is suggested that part of the genetic regulation of the HDL and its subfraction distribution might be mediated through the activity of HL.

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A familial clustering of coronary heart disease (CHD) is evident from several studies.1-3 However, it is not known whether genetic or shared environmental factors play a dominating role in the aggregation of the disease in some families. Recently, it has been shown in a large study on siblings that the clustering of CHD might not be due largely to the major classical risk factors of CHD such as serum cholesterol or blood pressure. This suggests that other lipoprotein abnormalities (as well as nonlipoprotein factors) warrant exploration as possible factors that might be involved in the familial aggregation of CHD.4

A low level of serum high density lipoprotein (HDL) is a well-established risk factor of CHD.5 Accordingly, much interest has recently been focused on environmental and genetic factors regulating its serum level. In fact, HDL cholesterol shows a correlation between siblings ranging from 0.16 to 0.46.6,7 In twin studies, some genetic influence has been demonstrated in the variance of both major HDL apoproteins, apo A-I6 and apo A-II.8,9 However, studies on HDL in a large pedigree have supported a polygenic mode of inheritance.10 Studies on hypoalphalipoproteinemia have suggested a major gene effect in the familial aggregation of low HDL cholesterol in addition to the multifactorial background of the disorder.11-13 Thus, the regulation of plasma HDL by genetic factors appears to be quite complex in nature.

Plasma HDL is actually a mixture of different lipoproteins. At least two major subfractions, HDL2 and HDL3, can be identified in the HDL density range.14 The metabolism of the subfractions is interrelated, and it appears to be closely related to the function of two endothelial lipolytic enzymes, lipoprotein lipase (LPL) and hepatic endothelial lipase (HL).15 Thus, HDL2 is formed during catabolism of plasma triglyceride-rich lipoproteins by LPL,16,17 whereas its degradation is associated with the function of HL.18 Information about the genetic aspects of interrelations between HDL and the two lipolytic enzymes are lacking. Therefore, we have calculated heritability estimates for plasma HDL, apo A-I and A-II, and the subfractions, HDL2 and HDL3, in twins. In addition, the postheparin plasma activities of LPL and HL were measured to get information on the possible role of the lipolytic enzymes in the genetic regulation of plasma HDL concentration.

Methods

Subjects

A group of 17 monozygotic (MZ) and 18 dizygotic (DZ) male twins living in the Helsinki metropolitan area were
randomly selected from the Finnish Twin Cohort Study\(^\text{19}\) that included about 15,000 twin pairs. The zygosity diagnosis in the registry is based on a mailed questionnaire which is validated by using blood markers as described in detail earlier.\(^\text{19}\) The twins in the present study were aged between 48 and 63 years and their weight ranged from 56 to 103 kg with a mean of 78 kg ± 9.5 kg (SD). The subjects were studied as outpatients at the Central University Hospital, and the protocol of the study was approved by the Ethical Committee. The subjects gave their written informed consent.

**Lipids and Lipoproteins**

Blood was obtained after an overnight fast at 8:00 A.M. Serum was separated after clotting at 4°C, and the lipoprotein analysis was started the same day. Very low density lipoprotein (VLDL) was separated by ultracentrifugation in a Beckman L-70 ultracentrifuge (Beckman Instruments Incorporated, Palo Alto, California) in a Kontron T74 45.6 rotor (Kontron AG, Basel, Switzerland) operated at 4°C for 18 hours at a density of 1.006 g/ml and at 38,000 rpm. LDL and HDL\(_2\) were then precipitated sequentially from the bottom fraction using heparin-MnCl\(_2\) and dextran sulphate by the method of Gidez et al.\(^\text{20}\) Cholesterol concentration was determined using an enzymatic kit (No 187313, Boehringer Mannheim, FRG) in a Kone Olli-D fully automated analyzer (Kone Limited, Espoo, Finland).

**Apolipoprotein A-I and A-II**

These concentrations were determined by immunoturbidimetry\(^\text{21}\) using monospecific antisera against apo A-I and A-II (Catalogue Nos 726 428 and 726 486, respectively, Boehringer GmbH, Mannheim, FRG).

**Postheparin Plasma Lipase Activities**

After taking samples for lipoprotein analysis, 100 IU/kg of heparin was injected intravenously. Blood was taken after 5 and 15 minutes into precooled heparinized tubes, and plasma was separated at 4°C and stored frozen at −20°C until assayed. Postheparin plasma LPL and HL activities were determined by a selective immunological method using gum arabic stabilized tritiated (1-\(^14\)C-oleyl) glycerol as a substrate.\(^\text{22}\) The activity of LPL was determined after inactivation of HL by a specific antiserum to human serum. The HL activity was determined at 1.0 M NaCl and in the absence of serum to inactivate LPL.

22 The activity of LPL was determined after inactivation of HL by a specific antiserum to human serum. The HL activity was determined at 1.0 M NaCl and in the absence of serum to inactivate LPL. After a 90-minute incubation at 28°C, the released free fatty acids were separated and counted for radioactivity in a Wallac Rack-Beta liquid scintillation spectrometer.\(^\text{22}\) The activities are expressed as μmoles of free fatty acids released per milliliters of postheparin plasma in an hour (μmol/hr/ml).

**Statistical Analysis**

Statistical treatment of the data was performed using a Honeywell DPS computer using BMDP statistical software (Biomedical Data Processing System, Department of Biostatistics, University of California, California). The MZ and DZ pairs were compared using the heritability estimate of Falconer\(^\text{23}\) calculated by the formula:

\[
h^2 = 2 \times (r_{MZ} - r_{DZ})
\]

where \(r_{MZ}\) and \(r_{DZ}\) are the pairwise intraclass correlations of the MZ and DZ twins calculated using double entry of the data. In addition, we calculated the intrapair variances by the formula:

\[
\Sigma (A - B)^2 / 2N
\]

where \(A\) and \(B\) are the values of the twin pair and \(N\) is the number of twin pairs.\(^\text{24}\) We calculated the F-ratios by dividing the intrapair variances for DZ by those for MZ twins. Heritability was also estimated using the formula:

\[
h^2 = (\text{intrapair variance}_{DZ} - \text{intrapair variance}_{MZ}) / \text{intrapair variance}_{DZ}
\]

\(^\text{3}\) Results

The two groups of twins were relatively similar with respect to their plasma HDL cholesterol and apo A-I and A-II concentrations (Table 1). The cholesterol concentrations of the HDL subfractions, HDL\(_2\) and HDL\(_3\), and the postheparin plasma lipolytic enzymes, LPL and HL, were also similar in the MZ and DZ twins.

The plasma HDL cholesterol concentration was positively correlated with postheparin plasma LPL activity in all subjects (\(r = 0.33, p<0.01\)) and in the MZ group (Figure 1A). The respective correlation of plasma HDL cholesterol with postheparin plasma HL activity was negative in the MZ twins (Figure 1B), whereas no significant relationships existed between HDL cholesterol concentration and postheparin plasma lipase activities in DZ twins.

The pairwise correlation coefficients for plasma HDL cholesterol and postheparin plasma lipase activities are shown in Table 2. This correlation was significant for HDL cholesterol in both MZ and DZ twins. However, the correlations in the MZ pairs were higher in comparison with the DZ pairs. Thus, a heritability estimate of 0.34 could be obtained for HDL cholesterol. Calculation of the estimate for HDL subfractions revealed that this was mainly due to the

**Table 1. Plasma Cholesterol and Apolipoprotein Concentrations and Postheparin Plasma Lipase Activities in Monozygotic and Dizygotic Twins**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MZ twins</th>
<th>DZ twins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 34)</td>
<td>(n = 36)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>44.1 ± 9.3</td>
<td>50.7 ± 12.0</td>
</tr>
<tr>
<td>HDL(_2)*</td>
<td>13.5 ± 4.6</td>
<td>16.6 ± 9.3</td>
</tr>
<tr>
<td>HDL(_3)*</td>
<td>34.8 ± 7.0</td>
<td>37.2 ± 8.1</td>
</tr>
<tr>
<td>Apolipoprotein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo A-I</td>
<td>132.5 ± 14.0</td>
<td>142.7 ± 21.0</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>37.3 ± 4.2</td>
<td>36.0 ± 4.2</td>
</tr>
<tr>
<td>Lipoprotein lipo</td>
<td>31.1 ± 8.2</td>
<td>36.1 ± 15.8</td>
</tr>
<tr>
<td>Hepatic lipase</td>
<td>29.4 ± 12.7</td>
<td>26.8 ± 11.6</td>
</tr>
</tbody>
</table>

Values for cholesterol and apolipoproteins are given as mg/dl. Values for lipoprotein lipoase and hepatic lipase are given as μmol/hr/ml. All values are means ± SEM. MZ = monozygous; DZ = dizygous.

*HDL subfractionation was performed in 20 MZ and 24 DZ men.
high heritability for HDL₂ cholesterol. In addition, genetic regulation could be observed for plasma apo A-I but not for apo A-II concentration (Table 2). Since there appeared to be considerable sharing of relative ponderosity between both sets of twins (r of body mass index (BMI) 0.58 and 0.40 for MZ and DZ twins, respectively) we also calculated the pairwise correlations after adjustment with BMI (shown in parenthesis in Table 2). Thus, it is evident that the heritability estimates for HDL and its subfractions were only slightly changed by the adjustment with BMI. However, the situation was opposite with apo A-I and A-II, as it appeared that the $h^2$ for both apoproteins were significantly improved by standardizing the data with BMI.

Postheparin plasma HL activity displayed a highly significant pairwise correlation in MZ twins, whereas no significant correlation could be observed in their LPL activities. The postheparin plasma lipases, LPL and HL, showed low pairwise correlation in the DZ group (Table 2). Accordingly, a high heritability estimate ($h^2 = 1.18$) could be calculated for HL activity in our twin material, and this appeared to be rather independent of the effect of ponderosity as judged from the correlations obtained after adjusting with BMI.

The heritability data on HDL and the factors that influence its concentrations were also evaluated by calculating the intrapair variances. The F-values thus obtained were most significant for HDL₂, HL, and apo A-I (Table 3). This suggests that HDL subtraction distribution and its regulators might have a marked role in the mediation of genetic influence on plasma HDL concentration.

Discussion

Several twin studies have been performed to evaluate the role of nature vs. nurture as a determinant of plasma HDL cholesterol. This research was largely stimulated by the finding of low HDL cholesterol concentrations in male relatives of patients with coronary heart disease. CHD is common in families with hypoalphalipoproteinemia and Tangier disease but is rare in pedigrees with hyperalphalipoproteinemia, a familial disorder with grossly elevated plasma HDL cholesterol. The common risk factors such as serum cholesterol and hypertension have failed to explain much of the genetic/familial transmission of CHD. Thus familial aggregation of low HDL cholesterol might have a role in the familial aggregation of atherosclerotic disease. In the present study the genetic influence on HDL cholesterol concentration was evaluated by comparing the pairwise correlation of HDL in both MZ and DZ twins. The heritability estimate thus obtained ($h^2 = 0.34$) is very close to that calculated in a previous twin study ($h^2 = 0.35$) and in a large pedigree using path analysis ($h^2 = 0.59$). In the present study, we also performed HDL subfractionation, which was not reported in earlier twin studies, to evaluate the contribution of the HDL subfractions to the inheritance of HDL cholesterol level. It is well known that most variation in plasma HDL cholesterol is due to the HDL₂ subfraction. Therefore it was not surprising to find high $h^2$ for HDL₂ but not for HDL₃ cholesterol (Table 2). This was confirmed by calculating the heritability estimates by the intrapair variance method (Table 3). These results suggest that factors regulating the HDL subfraction distribution might also participate in a genetic regulation of plasma HDL.
Earlier studies\(^8\)\(^{-10}\) have resulted in conflicting data on the heritability of the major HDL apoproteins, apo A-I and A-II. Thus, high heritability has been reported for apo A-I but not for apo A-II, a finding contrasted in other studies.\(^8\)\(^{-10}\) In the present study we could demonstrate a genetic influence on serum apo A-I but not on apo A-II by both the Falconer and the Intrapair variance methods. The ad
determined than apo A-II. This is compatible with the pre-
other hand, apo A-II is mainly found in the HDL3 subfrac-
tions.\(^8\)\(^{-10}\) In the present study we could demonstrate a genet-
ic influence on serum apo A-I but not on apo A-II by both
apoproteins, apo A-I being again more strongly genetically
determined than apo A-II. This is compatible with the pre-
view that the variation of serum apo A-I is largely due
to the apo A-I of the HDL2 subfraction, which contains
particles with apo A-I as the main apoprotein.\(^3\)\(^1\) On the
other hand, apo A-II is mainly found in the HDL3 subfrac-
tion,\(^3\)\(^1\) and this subfraction seems to have a minor role in
the variation of serum HDL level.

The HDL subfractions are metabolically interrelated,
since they are transformed to each other. Thus, HDL2 is
the variable constituent whereas HDL3 forms a metabolic
reservoir for the events leading to the formation of HDL2.\(^1\)\(^5\)
This occurs at least partly during catabolism of triglyceride-
rich lipoproteins by LPL.\(^1\)\(^8\)\(^{-17}\) Degradation of HDL2 and its
conversion back to HDL3 is initiated by another lipolytic
enzyme, HL.\(^1\)\(^5\)\(^{-13}\) Familially increased HDL cholesterol is
associated with either increased LPL or decreased HL
activities.\(^3\)\(^3\)\(^{-34}\) In normal subjects, plasma HDL (HDL\(_3\)) cho-
lesterol concentration is positively correlated with LPL and
negatively with HL activity.\(^1\)\(^6\) This was also the case in all
the subjects and in the MZ twins of the present study
(Figure 1).

Postheparin plasma HL activity displayed remarkable
pairwise correlation in the MZ twins, and therefore a high
heritability estimate was obtained (Tables 2 and 3). This
appeared to be independent of the effect of relative pon-
derosity. The activity of the other lipolytic enzyme, LPL,
showed no significant pairwise correlation in either kind of
twins. Thus it is plausible to suggest that if lipolytic en-
zymes are involved, HL might have a more important role
in the genetic regulation of plasma HDL cholesterol than
LPL. This is consistent with the fact that LPL activity can be
largely affected by factors such as nutritional status, insulin
and glucose infusion, and fat infusion\(^1\)\(^7\) whereas HL is
fairly resistant to these interventions. In addition, the re-
sponse of HL activity to various factors seems to depend
on the initial activity of the enzyme. Thus, subjects with low
levels of HL have only a marginal increase of HL upon
induction of the enzyme, for example with levonorgestrel,
a progestin with androgenic characteristics.\(^3\)\(^5\) The present

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### Table 2. Within-Pair Correlation Coefficients for Cholesterol Concentrations and Lipase Activities In Monozygotic and Dizygotic Twins

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MZ twins</th>
<th>DZ twins</th>
<th>Heritability estimates*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>0.70</td>
<td>&lt;0.01</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>(0.73)</td>
<td></td>
<td>(0.56)</td>
</tr>
<tr>
<td>HDL(_2)</td>
<td>0.74</td>
<td>&lt;0.05</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>(0.79)</td>
<td></td>
<td>(0.58)</td>
</tr>
<tr>
<td>HDL(_3)</td>
<td>0.14</td>
<td>NS</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>(0.37)</td>
<td></td>
<td>(0.42)</td>
</tr>
</tbody>
</table>

### Table 3. Intrapair Variances for High Density Lipoproteins, Apolipoproteins A, and Lipase Activities In Monozygotic and Dizygotic Twins

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intrapair variance</th>
<th>Heritability index</th>
<th>F ratio†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MZ</td>
<td>DZ</td>
<td></td>
</tr>
<tr>
<td>HDL</td>
<td>24.4</td>
<td>64.4</td>
<td>0.62</td>
</tr>
<tr>
<td>HDL(_2)</td>
<td>5.9</td>
<td>45.0</td>
<td>0.87</td>
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<tr>
<td>HDL(_3)</td>
<td>39.4</td>
<td>24.1</td>
<td>-0.64</td>
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<tr>
<td>Apo A-I</td>
<td>145.4</td>
<td>403.2</td>
<td>0.64</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>20.7</td>
<td>13.0</td>
<td>-0.60</td>
</tr>
<tr>
<td>LPL</td>
<td>67.8</td>
<td>150.9</td>
<td>0.55</td>
</tr>
<tr>
<td>HL</td>
<td>31.7</td>
<td>100.2</td>
<td>0.68</td>
</tr>
</tbody>
</table>

LPL = lipoprotein lipase, HL = hepatic lipase, NS = not significant, MZ = monozygous; DZ = dizygous.

*The heritability index was calculated using the formula \(h^2 = \frac{(\text{intrapair variance}_{MZ} - \text{intrapair variance}_{DZ})}{\text{intrapair variance}_{DZ}}\).
†The F ratios were calculated by dividing the intrapair variance for DZ with that for MZ.
‡p<0.05; $p<0.01.
References


Index Terms: HDL • apolipoprotein A-I • apolipoprotein A-II • hepatic lipase • lipoprotein lipase • twin studies • heritability
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