Sex-Specific Effects of the Glutamine/Histidine Polymorphism in Apo A-IV on HDL Metabolism

Arnold von Eckardstein, Harald Funke, Ali Chirazi, Changting Chen-Haudenschild, Helmut Schulte, Rainer Schonfeld, Ekkehart Köhler, Sigrid Schwarz, Armin Steinmetz, Gerd Assmann

Abstract In Caucasians, a histidine for glutamine substitution (Gln->His) at residue 360 in apolipoprotein (apo) A-IV leads to an electrophoretically detectable polymorphism whose contribution to lipid metabolism regulation is controversial. In this study of 426 male and 188 female coronary heart disease patients, we analyzed the impact of this polymorphism on lipid metabolism, particularly high-density lipoprotein (HDL). The frequency of the rarer apo A-IV (360: His) allele was .069. This polymorphism exerted opposite effects in men and women in terms of serum concentrations of total cholesterol; triglycerides; HDL cholesterol; LDL cholesterol; lipoprotein (Lp) A-I; and apo A-I, A-II, and B. Only the difference in Lp A-I levels between male apo A-IV (360: Gin/Gln) homozygotes and apo A-IV (360: Gin/His) heterozygotes was significant (P<0.05). In randomly selected subgroups of 38 male and 15 female apo A-IV (360: Gin/Gln) heterozygotes and 104 male and 15 female apo A-IV (360: Gin/His) heterozygotes, heterozygosity for apo A-IV (360: Gin/His) in both sexes was associated with lower plasma cholesterol transfer protein (CETP) activity (P<0.05) and higher serum apo A-IV concentrations (P<0.01) in men. Moreover, only men had significantly higher mean plasma activity levels of lecithin:cholesterol acyltransferase (LCAT) (P<0.01). Multivariate analysis of the effects of age, diabetes mellitus, intake of lipid-lowering drugs, body mass index, smoking, and apo A-IV polymorphism revealed that the apo A-IV polymorphism contributed 1%, 3% to 4%, 6% to 7%, and 5% to 18% of the variation in serum concentrations and plasma activities of Lp A-I, apo A-IV, LCAT, and CETP, respectively. In men, univariate and multivariate regression analyses revealed significant interrelationships between concentrations and activities of HDL cholesterol, Lp A-I, apo A-IV, LCAT, and CETP. We conclude that the 360:Gln/Gln apo A-IV polymorphism influences HDL metabolism via apo A-IV concentration and/or plasma activities of LCAT and CETP. However, these effects appear to be strongly modulated by other factors, including gender, and do not contribute importantly to cardiovascular risk. (Arterioscler Thromb. 1994;14:1114-1120.)

Key Words • lecithin:cholesterol acyltransferase • cholesteryl ester transfer protein • HDL subfractions • apolipoprotein polymorphisms • coronary heart disease

Several epidemiological and clinical studies have revealed an inverse correlation between low plasma concentrations of high-density lipoprotein (HDL) cholesterol and the risk of myocardial infarction (reviewed in Reference 1). The reverse cholesterol transport model is the one most widely used to explain the protective role of HDL in atherogenesis. In this model, HDL mediates efflux of excess cholesterol from peripheral cells into plasma as well as the esterification of cholesterol in plasma by lecithin:cholesterol acyltransferase (LCAT). From HDL, cholesterol esters are subsequently delivered to the liver by several mechanisms. In humans the most important pathway apparently involves the exchange of cholesterol esters from triglycerides from low-density lipoprotein (LDL) and very low-density lipoprotein by cholesteryl ester transfer protein (CETP) (reviewed in References 2 through 4). Family and twin studies suggest that low HDL cholesterol levels are partially hereditary and have estimated the influence of genes to be 35% to 50%. It has therefore been hypothesized that genetic defects that interfere with either the regular structure of HDL or the processes that generate and remove HDL are disadvantageous to those who carry such defects. To date, however, only a few structural defects in apolipoprotein (apo) A-I, LCAT, and CETP have been shown to regulate plasma HDL cholesterol concentrations. Taken together, these defects contribute neither to the high prevalence of low HDL cholesterol levels in the population nor to the associated coronary risk. Among those proteins that either constitute or process HDL, only apo A-IV and E are known to exhibit structural polymorphisms with frequent isomers in Caucasian populations. The impact of these polymorphisms on the regulation of HDL cholesterol levels is controversial.8-10

Apo A-IV, as a component of chylomicrons, is secreted by enterocytes into lymphatic fluid. In fasting plasma, most of apo A-IV is associated with HDL. In vitro, apo A-IV activates LCAT,15,16 which in turn modulates lipoprotein lipase activation through apo C-II,17 and specifically interacts with HDL-binding sites on various cells, thereby promoting cholesterol efflux.21,26

The primary structure of apo A-IV is encoded by three exons of the apo A-I/C-III/A-IV gene cluster on three exons of the apo A-I/C-III/A-IV gene cluster.
chromosome 11.27-29 In Caucasian populations the apo A-IV gene exhibits four nonsynonymous polymorphisms in codons −8, 127, 347, and 360 and additional variation with rare mutant alleles.5,10,30-35 The histidine for glutamine replacement (Gln→His) at position 360 of the mature protein can be detected by isoelectric focusing (IEF).26,28 Reported allele frequencies for the two isoforms, apo A-IV-1 (apo A-IV360Gln) and apo A-IV-2 (apo A-IV360His), are 91 to 93% and 0.7 to 0.9%, respectively.36-43 In some population studies, apo A-IV-1/2 heterozygotes exhibit significantly higher levels of HDL cholesterol37,38 and lower levels of triglycerides38,39 compared with apo A-IV-1/1 homozygotes. Other studies have not shown these relations.34,35,40-44 In two groups of probands we recently observed that apo A-IV (360: His) decreased lipoprotein(a) levels.

To obtain more detailed information of possible effects of the Gln→His polymorphism in apo A-IV on HDL metabolism, we analyzed the impact of this substitution on the concentrations of HDL apolipoproteins and HDL subfractions as well as plasma activities of LCAT and CETP in coronary heart disease patients.

Methods

Subjects

The electrophoretically detectable apo A-IV polymorphism was analyzed in 426 male and 188 female patients from a cardiac rehabilitation center who underwent coronary angiography because of symptoms of coronary heart disease.44 Venous blood samples were drawn as early as 4 weeks after myocardial infarction or cardiac surgery and after overnight fasting. Serum was used for quantification of lipids, lipoproteins, and apolipoproteins and for determination of the apo A-IV polymorphism at codon 360 by IEF. EDTA-blood samples were immediately placed on ice to yield plasma for measurements of plasma lipid transfer activities. After centrifugation at 4°C for 15 minutes at 200g, sera and plasma samples were divided into aliquots and immediately frozen at −70°C.

Measurements of Lipids, Lipoproteins, and Apolipoproteins

Serum concentrations of triglycerides and cholesterol were quantified with an autoanalyzer (Hitachi/Boehringer). HDL cholesterol concentrations were measured after precipitation of apo B-containing lipoproteins with phosphotungstic acid/Mg2+. Briefly, 0.3 μCi [3H]cholesterol in a 3 μL ethanol was dried on 5-mm filter paper plates that were subsequently incubated overnight at 4°C with 200 μL NaCl. After 30 minutes' preincubation at 37°C, 50 μL assay buffer with 80 g/L bovine serum albumin and 10 μL β-mercaptoethanol was added. Esterification of [3H]cholesterol was started by addition of 15 μL plasma and was terminated after 30 minutes by the addition of 4 mL chloroform/methanol (2:1, vol/vol). Phases were separated by addition of 1.5 mL of 150 mmol/L NaCl and subsequent centrifugation at 4°C. 2000g Unesterified cholesterol and cholesterol esters in the lower phase were separated by thin-layer chromatography and counted for radioactivity. LCAT activity was calculated by multiplying the percentage of radiolabeled cholesterol esters produced per time by the concentration of unesterified cholesterol in the substrate. Every sample was determined in duplicate in a series of no more than 10 samples. The intra-assay coefficient of variation (CV) was 5% and the interassay CV 10%.

Determination of LCAT Activity in Exogenous Substrates

The activity of plasma to esterify radiolabeled cholesterol was determined by using either exogenous50 or endogenous51 substrates. LCAT activity on exogenous substrates has been shown to correlate with LCAT mass.52 By contrast, esterification of radiolabeled cholesterol on endogenous substrates appears to reflect interaction between LCAT and various endogenous lipoproteins.51 For the exogenous substrate assay (ie, LCAT activity), 10 μg apo A-I was incorporated into liposomes that were prepared by the method of Batzri and Korn53 and that contained 1 μCi [3H]cholesterol (New England Nuclear), 93 μg egg yolk lecithin, and 11 μg unesterified cholesterol (molar ratio, 4:1) in 140 μL assay buffer with 10 mmol/L Tris-HCl, pH 7.4, 5 mmol/L EDTA, and 150 mmol/L NaCl. After 30 minutes' preincubation at 37°C, 50 μL assay buffer with 80 g/L bovine serum albumin and 10 μL β-mercaptoethanol was added. Esterification of [3H]cholesterol was started by addition of 15 μL plasma and was terminated after 30 minutes by the addition of 4 mL chloroform/methanol (2:1, vol/vol). Phases were separated by addition of 1.5 mL of 150 mmol/L NaCl and subsequently centrifugation at 4°C. 2000g Unesterified cholesterol and cholesterol esters in the lower phase were separated by thin-layer chromatography and counted for radioactivity. LCAT activity was calculated by multiplying the percentage of radiolabeled cholesterol esters produced per time by the concentration of unesterified cholesterol in the substrate. Every sample was determined in duplicate in a series of no more than 10 samples. The intra-assay coefficient of variation (CV) was 5% and the interassay CV 10%.

Determination of CETP Activity

Endogenous substrate assay (ie, fractional esterification rate [FER]) measured the activity of plasma (P-FER)51 or apo B-free plasma (H-FER),54 to esterify [3H]cholesterol at 37°C after initial equilibration with endogenous plasma lipoproteins at 4°C. Apo B-free plasma was obtained by precipitation with phosphotungstic acid/Mg2+. Briefly, 0.3 μCi [3H]cholesterol in 3 mL ethanol was dried on 5-mm filter paper plates that were subsequently incubated overnight at 4°C with 200 μL plasma (for P-FER) or apo B-free plasma (for H-FER). Before and after subsequent incubation at 37°C, 50 μL plasma was removed for incubation with 1 mL of 99% ethanol for lipid extraction. Unesterified cholesterol and cholesterol esters were separated by thin-layer chromatography so that their radioactivities could be counted separately. FER was calculated as the difference between the percentage of radiolabeled cholesterol esters before and after incubation at 37°C. All samples were measured in duplicate in series of no more than 10 samples. The intra-assay and interassay CVs for both assays ranged from 6% to 8% and from 11% to 14%, respectively.

Determination of CETP Activity

CETP activity was determined as described by Kato et al55 and Nakanishi et al.56 In this assay, apo A-I-containing proteoliposomes were used as donors and LDL as acceptors of radiolabeled cholesterol esters. Proteoliposomes containing 3 mg apo A-I, 7 mg egg yolk lecithin, 1.16 mg unesterified cholesterol, 77.5 μg cholesteryl oleate, and 10 μCi [3H]cholesteryl oleate (New England Nuclear) (molar ratio of phosphatidylcholine to unesterified cholesterol to cholesteryl ester, 75:25:1) were prepared by the cholate dialysis method.51 Aliquots with 25 μg apo A-I were mixed at a final volume of 300 μL with 100 μL LDL in a buffer with 39 mmol/L sodium phosphate, pH 7.4, 60 mmol/L NaCl, 5 mmol/L EDTA, and 1.4 mmol/L dithiothreitol to achieve an acidic condition. [3H]cholesterol was added to the reaction mixture. The assay was started by addition of 5 μL plasma and was terminated after 20 minutes' incubation at 37°C by placing the
TABLE 1. Impact of the Apo A-IV (360:Gln/His) Polymorphism on Lipid Metabolism

<table>
<thead>
<tr>
<th>Age, y</th>
<th>360:Gln/Gln, 1/1 (n=367)</th>
<th>360:Gln/His, 1/2 (n=57)</th>
<th>360:Gln/Gln, 1/1 (n=167)</th>
<th>360:Gln/His, 1/2 (n=19)</th>
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<tr>
<td>155±1.5</td>
<td>54.1±1.3</td>
<td>54.1±1.3</td>
<td>60.5±1.3</td>
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<td>156±1.5</td>
<td>54.2±1.0</td>
<td>54.9±1.0</td>
<td>57.9±1.0</td>
<td>58.9±1.0</td>
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<tr>
<td>156±1.5</td>
<td>226.5±39.5</td>
<td>220.5±27.9</td>
<td>242.5±34.6</td>
<td>242.5±35.6</td>
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<tr>
<td>155±1.5</td>
<td>79.7±125.4</td>
<td>73.7±120.6</td>
<td>82.9±125.4</td>
<td>93.7±125.4</td>
</tr>
<tr>
<td>156±1.5</td>
<td>35.8±8.4</td>
<td>57.8±8.4</td>
<td>41.7±11.0</td>
<td>37.8±11.0</td>
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<tr>
<td>156±1.5</td>
<td>162.0±54.7</td>
<td>157.0±27.9</td>
<td>172.4±37.7</td>
<td>177.0±50.8</td>
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<td>155±1.5</td>
<td>4.7±13.9</td>
<td>5.1±11.4</td>
<td>6.3±16.2</td>
<td>4.4±10.5</td>
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<td>156±1.5</td>
<td>39.7±10.1</td>
<td>43.8±11.0</td>
<td>50.3±12.7</td>
<td>46.4±6.0</td>
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<td>156±1.5</td>
<td>113.7±17.7</td>
<td>116.6±14.5</td>
<td>124.1±21.0</td>
<td>117.6±11.8</td>
</tr>
<tr>
<td>156±1.5</td>
<td>30.8±6.2</td>
<td>30.6±5.1</td>
<td>30.3±5.3</td>
<td>30.0±4.5</td>
</tr>
<tr>
<td>156±1.5</td>
<td>89.4±17.0</td>
<td>86.7±14.0</td>
<td>90.7±18.1</td>
<td>95.8±22.0</td>
</tr>
</tbody>
</table>

**Note:** Apo indicates apolipoprotein; Ml, myocardial infarction; HDL, high-density lipoprotein; chol, cholesterol; LDL, low-density lipoprotein; and Lp, lipoprotein. Concentrations of triglycerides and Lp(a) are presented as geometric means with ±1S confidence intervals as calculated by natural logarithm transformation. Data were corrected for age, intake of lipid-lowering drugs, diabetes mellitus, and smoking status except that age at first Ml was corrected only for medication use, diabetes mellitus, and smoking status.

Results

Table 1 summarizes the data on lipid metabolism of 367 male and 167 female apo A-IV (360:Gln/Gln) homozygotes and 57 male and 19 female apo A-IV (360:Gln/His) heterozygotes. Only two male and two female homozygotes for apo A-IV (360:His/His) were identified, and because of this low number, were not considered in the statistical analysis (Table 2). The apo A-IV (360:Gln/His) polymorphism had opposite effects in men compared with women in terms of all parameters of lipid metabolism except Lp(a), which was lower in apo A-IV-1/2 heterozygotes of both sexes compared with apo A-IV-1/1 homozygotes (Table 1). Apo A-IV-1/2 was associated with lower mean levels of cholesterol, triglycerides, LDL cholesterol, and apo B in men but...
Table 3. Impact of the Apo A-IV (360: Gln/His) Polymorphism on Lipids, Lipoproteins, Apo lipoproteins, and Lipid Transfer Activities in Male and Female Coronary Heart Disease Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men</th>
<th>Women</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>360: Gln/Gln, 1/1 (n=104)*</td>
<td></td>
<td></td>
<td>360: Gln/His, 1/2 (n=38)*</td>
<td></td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>228.7±40.9</td>
<td>218.2±30.7</td>
<td>240.6±45.8</td>
<td>247.1±62.3</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>73.4±117.4&lt;187.9</td>
<td>70.5±110.3&lt;172.4</td>
<td>97.7&lt;123.1±155.1</td>
<td>102.0&lt;145.9±208.6</td>
</tr>
<tr>
<td>HDL chol, mg/dL</td>
<td>36.1±8.7</td>
<td>37.2±7.9</td>
<td>40.4±4.9</td>
<td>37.4±5.1</td>
</tr>
<tr>
<td>LDL chol, mg/dL</td>
<td>163.0±38.5</td>
<td>154.9±30.1</td>
<td>174.6±42.6</td>
<td>179.1±54.5</td>
</tr>
<tr>
<td>Lp A-I, mg/dL</td>
<td>39.7±10.0</td>
<td>44.4±11.7‡</td>
<td>50.9±8.9</td>
<td>46.8±6.4</td>
</tr>
<tr>
<td>Apo A-I, mg/dL</td>
<td>113.8±16.2</td>
<td>116.2±15.1</td>
<td>121.6±8.4</td>
<td>117.3±13.5</td>
</tr>
<tr>
<td>Apo A-II, mg/dL</td>
<td>30.8±5.2</td>
<td>30.6±5.2</td>
<td>29.0±5.2</td>
<td>30.0±2.9</td>
</tr>
<tr>
<td>Apo A-IV, mg/dL</td>
<td>13.4±3.8</td>
<td>15.1±3.5‡§</td>
<td>11.9±3.6</td>
<td>14.3±3.7</td>
</tr>
<tr>
<td>Apo B, mg/dL</td>
<td>68.8±18.1</td>
<td>85.5±15.5</td>
<td>91.2±18.4</td>
<td>98.3±22.3</td>
</tr>
<tr>
<td>LCAT, (nmol/h)/mL</td>
<td>20.5±4.2</td>
<td>24.4±9.4†§</td>
<td>15.4±1.7</td>
<td>14.3±1.5</td>
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<tr>
<td>FER, (%)/h/mL</td>
<td>9.6±2.2</td>
<td>9.8±2.2</td>
<td>9.3±2.7</td>
<td>10.9±4.0</td>
</tr>
<tr>
<td>H-FER, (%)/h/mL</td>
<td>17.2±6.3</td>
<td>16.6±5.0</td>
<td>15.3±6.1</td>
<td>15.6±3.0</td>
</tr>
<tr>
<td>CETP, (nmol/h)/mL</td>
<td>113.3±26.0</td>
<td>101.3±27.9†</td>
<td>107.7±154.2&lt;220.8</td>
<td>47.2&lt;98.6&lt;206.8†</td>
</tr>
</tbody>
</table>

Ratio LCAT to CETP: 0.131<0.183<0.255 0.130<0.245<0.46

See the footnote to Table 1 for an explanation of same abbreviations. LCAT indicates lecithin:cholesterol acyltransferase; P-FER, fractional esterification rate in plasma; H-FER, fractional esterification rate in HDL; and CETP, cholesteryl ester transfer protein. Concentrations of triglycerides and the ratio of LCAT to CETP in both men and women as well as CETP activity in women are presented as geometric means with ±1S confidence intervals as calculated by natural logarithm transformation. Data are presented from a subgroup of patients presented in Table 1. Data were corrected for age, intake of lipid-lowering drugs, diabetes mellitus, and smoking.

*Activities of LCAT, FER, H-FER, and CETP were determined in 77 apo A-IV (360:Gln/Gln) homozygotes and 39 apo A-IV (360:Gln/His) heterozygotes.

†P<.05, ‡P<.001, Mann-Whitney U test.

With higher levels in women. In contrast, concentrations of HDL cholesterol, Lp A-I, and apo A-I were higher in male heterozygotes for apo A-IV-1/2 but lower in female heterozygotes. After adjustment for age, smoking status, diabetes mellitus, and intake of lipid-lowering drugs, only the differences in Lp A-I values between male apo A-IV-1/2 heterozygotes and apo A-IV-1/1 homozygotes remained significant (P<.05, Student's t test). In a multiple regression analysis we calculated the effect of apo A-IV polymorphism on Lp A-I serum concentrations after accounting for age, body mass index, diabetes mellitus, use of lipid-lowering drugs, and smoking status. The apo A-IV polymorphism contributed 1% of the variation in Lp A-I levels in this population.

To analyze in more detail the effect of the Gln→His polymorphism on apo A-IV in HDL metabolism in men, we randomly selected 38 male and 15 female apo A-IV-1/2 heterozygotes and 104 male and 15 female apo A-IV-1/1 homozygotes who were matched by age and measured the concentrations of apo A-IV and the activities of plasma lipid transfer enzymes (Table 3). Also in these subgroups, the apo A-IV polymorphism exerted opposite effects on men and women in terms of serum concentrations of triglycerides, HDL cholesterol, LDL cholesterol, Lp A-I, apo A-I, and apo B. In men the differences for Lp A-I were significant (P<.05). Mean serum concentrations of apo A-IV were higher in apo A-IV (360:Gln/His) heterozygotes of both sexes but only in men was this difference significant (P<.01, Student's t test). Furthermore, apo A-IV-1/2 heterozygotes of both sexes had significantly lower CETP activities (P<.05, Student's t test). LCAT activity was oppositely affected by the apo A-IV polymorphism in men versus women, but the differences were significant only for men (P<.05, Student's t test). In the aforementioned multiple regression model, the apo A-IV polymorphism explained 3% to 4% of the variation in apo A-IV levels and 5% (men) to 18% (women) of the variation in CETP activity. Although opposite effects were seen in men and women, 6% to 7% of the variation in plasma LCAT activity was determined by the apo A-IV polymorphism.

In univariate regression analysis, we tested the relations of serum concentrations of apo A-IV and Lp A-I as well as plasma activities of LCAT and CETP to other variables of lipid metabolism. Table 4 summarizes the results of univariate Pearson regression analyses obtained in men and Table 5 the results obtained in women. In both sexes, as expected, Lp A-I had strongly positive correlations with HDL cholesterol and apo A-I and negative correlations with triglycerides, which remained after multivariate regression analysis (not shown). In both sexes apo A-IV correlated positively with Lp A-I. In men this relation was highly significant and remained so after multivariate regression analysis. Furthermore, apo A-IV concentration correlated positively with HDL cholesterol, apo A-I, and apo A-II in men but not in women. In men, LCAT activity correlated positively with HDL cholesterol, Lp A-I, and apo A-IV and negatively with triglycerides and CETP activity. The correlations of LCAT activity with Lp A-I and triglycerides remained stable after multivariate regres-
### TABLE 4. Pearson Correlation Coefficients Between Lipid Transfer Activities and Lipid, Lipoprotein, and Apolipoprotein Variables in Men

<table>
<thead>
<tr>
<th></th>
<th>LCAT</th>
<th>CETP</th>
<th>Apo A-IV</th>
<th>Lp A-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>.2441</td>
<td>.026</td>
<td>.184*</td>
<td>.141t</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-.103</td>
<td>.167</td>
<td>.230†</td>
<td>.035</td>
</tr>
<tr>
<td>In triglycerides</td>
<td>-.284†</td>
<td>.080</td>
<td>.038</td>
<td>-.113t</td>
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<tr>
<td>HDL chol</td>
<td>.333‡</td>
<td>-.094</td>
<td>.296‡</td>
<td>.590‡</td>
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<tr>
<td>LDL chol</td>
<td>-.077</td>
<td>.183</td>
<td>.165§</td>
<td>-.086t</td>
</tr>
<tr>
<td>In Lp(a)</td>
<td>-.194</td>
<td>.332‡</td>
<td>-.077</td>
<td>-.014</td>
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<tr>
<td>Lp A-I</td>
<td>.359†</td>
<td>-.087</td>
<td>.445‡</td>
<td>1.0</td>
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<td>Apo A-I</td>
<td>.234*</td>
<td>-.030</td>
<td>.373‡</td>
<td>.540‡</td>
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<tr>
<td>Apo A-II</td>
<td>.169</td>
<td>-.050</td>
<td>.155</td>
<td>-.066*</td>
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<tr>
<td>Apo A-IV</td>
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<td>Apo B</td>
<td>-.154</td>
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<tr>
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<td>CETP</td>
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<td>-.002</td>
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See the footnote to Tables 1 and 3 for an explanation of abbreviations.

n=113 for LCAT, CETP, H-FER, and fractional esterification rate in plasma (P-FER); n=152 for apo A-IV; n=1025 for Lp A-I.

*P<.05, †P<.01, ‡P<.001. No significant correlation coefficient was found for P-FER.

### TABLE 5. Pearson Correlation Coefficients Between Lipid Transfer Activities and Lipid, Lipoprotein, and Apolipoprotein Variables in Women

<table>
<thead>
<tr>
<th></th>
<th>LCAT</th>
<th>In CETP</th>
<th>Apo A-IV</th>
<th>Lp A-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-.011</td>
<td>.368*</td>
<td>.259</td>
<td>.057</td>
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<tr>
<td>Cholesterol</td>
<td>.157</td>
<td>.292</td>
<td>.131</td>
<td>.130</td>
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<tr>
<td>In triglycerides</td>
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<td>-.235</td>
<td>.070</td>
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<tr>
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<td>Lp A-I</td>
<td>.183</td>
<td>-.257</td>
<td>.217</td>
<td>1.0</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>.090</td>
<td>-.336</td>
<td>.035</td>
<td>.749†</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>.055</td>
<td>-.233</td>
<td>-.070</td>
<td>.223*</td>
</tr>
<tr>
<td>Apo A-IV</td>
<td>-.150</td>
<td>.062</td>
<td>1.0</td>
<td>.217</td>
</tr>
<tr>
<td>Apo B</td>
<td>.007</td>
<td>-.056</td>
<td>.054</td>
<td>-.087</td>
</tr>
<tr>
<td>LCAT</td>
<td>1.0</td>
<td>-.012</td>
<td>-.150</td>
<td>.184</td>
</tr>
<tr>
<td>H-FER</td>
<td>-.203</td>
<td>-.080</td>
<td>.121</td>
<td>-.180</td>
</tr>
<tr>
<td>CETP</td>
<td>-.012</td>
<td>1.0</td>
<td>.062</td>
<td>-.257</td>
</tr>
</tbody>
</table>

See the footnote to Tables 1 and 3 for an explanation of abbreviations.

n=30 for LCAT, CETP, FER-HDL, and fractional esterification rate in plasma (P-FER); n=22 for apo A-IV; n=186 for Lp A-I.

*P<.05, †P<.001.
His) heterozygotes may also stabilize binding of apo A-IV to HDL, since in vitro the inhibition of LCAT prevents the association of apo A-IV with HDL.51,62

Whereas it is well known that CETP contributes to the distribution of apo A-IV among various lipoproteins,60 it is not clear how the apo A-IV polymorphism may influence CETP activity in plasma. One possibility is that the apo A-IV polymorphism influences the residence time of CETP-containing particles in plasma. Alternatively, as a component of chylomicrons and a modulator of lipoprotein lipase activity, apo A-IV may influence postprandial hyperlipidemia and thereby CETP gene expression.53 Also, the different CETP activities in plasma of apo A-IV (360:Gln/Gln) homozygotes and apo A-IV (360:Gln/His) heterozygotes are difficult to explain. Because of the opposite associations in men and women, it is unlikely that differences in CETP activity by apo A-IV isoforms are responsible.33,58 Moreover, apo A-I activates CETP more efficiently than does apo A-IV,6,64 and is present in plasma at concentrations that exceed those of apo A-IV by several fold. Because CETP activity as measured in exogenous substrates reflects LCAT mass52 and because CETP is associated with distinct HDL subclasses,65,66 it is more likely that the apo A-IV polymorphism determines the serum concentration of CETP-containing particles whose identity has yet to be defined.

In conclusion, the association of apo A-IV-1/1 and apo A-IV-1/2 with significantly different serum concentrations of apo A-IV, plasma activities of lipid transfer enzymes, and serum levels of HDL subfractions indicates that the Gln—His polymorphism at residue 360 of apo A-IV influences HDL metabolism. The opposite effects on parameters of lipid metabolism in men and women as well as the small variation exerted in women on this polymorphism on lipids and lipoproteins compared with the higher variation exerted on apo A-IV levels and lipid transfer enzyme activities point to the importance of other modulating factors, including gender. In men, the association of the apo A-IV (360:His) allele with higher CETP activity, lower CETP activity, and higher concentrations of Lp(a) indicates a reverse role of this polymorphism for reverse cholesterol transport and coronary risk. Some reduced coronary risk is also expected in view of the lower serum concentration of Lp(a) associated with apo A-IV-1/2 heterozygosity (References 34 and 45 and Table 1). The allele frequency of apo A-IV-2 observed in this study of coronary heart disease patients, however, did not differ from that observed in a recent study of healthy students from the same geographic region (.069 versus .070).64 Although insignificant, the mean age of male patients at their first myocardial infarction differed by approximately 2 years. This difference may indirectly indicate some antithrombotic effects of the apo A-IV-2 allele. However, this effect does not appear to be large or to affect life expectancy, since in a recent study of octogenarians we did not observe an increased frequency of apo A-IV-2 alleles in the elder generation.

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