Elevated HDL Cholesterol Is a Risk Factor for Ischemic Heart Disease in White Women When Caused by a Common Mutation in the Cholesteryl Ester Transfer Protein Gene

Birgit Agerholm-Larsen, MSc, PhD; Børge G. Nordestgaard, MD, DMSc; Rolf Steffensen, MD; Gorm Jensen, MD, DMSc; Anne Tybjærg-Hansen, MD, DMSc

Background—The level of HDL cholesterol is inversely related to the risk of ischemic heart disease.

Methods and Results—In 9168 women and men from a general population and 946 women and men with ischemic heart disease (all white), we tested the hypothesis that the Ile405Val mutation in the cholesteryl ester transfer protein gene (CETP) affects HDL cholesterol levels and the risk of ischemic heart disease. The relative frequencies of Ile/Ile, Ile/Val, and Val/Val carriers were 0.46, 0.43, and 0.11 for both women and men. Women with these 3 genotypes had mean HDL cholesterol levels of 1.68, 1.75, and 1.82 mmol/L, respectively ($P=0.001$, ANOVA), as well as a significant decrease in the ratio of total to HDL cholesterol ($P<0.002$, ANOVA). On multiple logistic regression analysis, women not treated with hormone replacement therapy who were heterozygous or homozygous for Val405 had a 1.4-fold (95% CI 1.0 to 1.9) to 2.1-fold (95% CI 1.3 to 3.4) increase in the risk of ischemic heart disease. No significant associations were found in men.

Conclusions—Increased HDL cholesterol levels caused by mutations in CETP are associated with an increased risk of ischemic heart disease in white women. (Circulation. 2000;101:1907-1912.)

Key Words: transfer proteins | genetics | lipoproteins | heart diseases | apolipoproteins

There is a strong inverse relationship between plasma HDL cholesterol levels and the risk of ischemic heart disease risk (IHD).1,2 Cholesteryl ester transfer protein (CETP) mediates the transfer of cholesteryl esters from HDL to triglyceride-rich lipoproteins.3,4 This stimulates reverse cholesterol transport (ie, the transport of cholesterol from peripheral cells to the liver for excretion).2,3 A genetic deficiency in CETP is associated with marked increases in HDL cholesterol levels in homozygotes and with moderate increases in heterozygotes.4–7 Although CETP deficiency might be an antiatherogenic state, due to HDL cholesterol elevation, the role of CETP in reverse cholesterol transport suggests the opposite.4,5 The common Ile405Val mutation in CETP is associated with a stepwise decrease in CETP activity in whites.8 We studied the effect of this mutation on lipid, lipoprotein, and apolipoprotein levels and on the risk of IHD in 5069 women and 4099 men from a general white population sample and in 946 patients with IHD.

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lines of the European Society of Cardiology, including location, character, and duration of pain and the relation of pain to exercise plus at least 1 of the following: severe stenosis on coronary angiography (70% stenosis of at least 1 coronary artery or 50% stenosis of the left main coronary artery), a previous myocardial infarction, or a positive exercise ECG test.

More than 99% of participants in this study were white and of Danish descent. The study was approved by Danish ethical committees 100.2039/91 and KA 93125.

DNA Analysis
The substitution of valine for isoleucine is caused by an A→G mutation at codon 405 in exon 14 of the CETP gene on chromosome 16. Exon 14 was amplified by PCR with primers located in intron 13 (5'-AATGCTTGTCCGAGCTTGCACT-3') and in intron 14 (5'-CAGTTTCCTCCAGCC-CACACTTA-3'). The PCR product was digested with FnuI, followed by 2% agarose gel electrophoresis. This resulted in bands of 35 and 85 bp for the A allele, a 120-bp band for the G allele, and a common band of 55 bp.

Other Analyses
Colorimetric and turbidimetric assays were used to measure plasma levels of total cholesterol, HDL cholesterol, triglycerides, apoA-I, apoB (all from Boehringer-Mannheim), and Lp(a) (DAKO). LDL cholesterol levels were calculated as total cholesterol−HDL cholesterol−(triglycerides/2.2) (all in mmol/L).

Statistical Analysis
Statistical analyses were separately performed for each sex with SPSS software. A P value on a 2-sided test of <0.05 for group comparisons and <0.10 for interaction tests was considered statistically significant. \( \chi^2 \) tests were used to compare genotype frequencies in different groups. ANOVA or Kruskal-Wallis ANOVA (for unequal variances) was used to evaluate the heterogeneity of levels of lipids, lipoproteins, and apolipoproteins across CETP genotypes. Student’s \( t \) test or Mann-Whitney \( U \) test was used as a post-hoc test for 2-genotype comparisons. We used Levene’s test to examine the homogeneity of variance. The interaction between CETP genotype and cholesterol, apoB, HDL cholesterol, apoA-I, triglycerides, Lp(a), body mass index, waist/hip ratio, glucose, wine consumption, 10-year age groups, hypertension, diuretic use, diabetes mellitus, smoking, physical activity, menopausal status (women), and hormone replacement therapy (HRT; women) in the prediction of levels of lipids, lipoproteins, and apolipoproteins was tested through the introduction, 1 at a time, for all possible 2-factor interaction terms in an ANCOVA model that already included genotype and the covariate in question. Evidence of interaction was further explored through division of the interacting covariate into categorical groups, tertiles, or quintiles, followed by tests of homogeneity of mean values across the 3 genotypes. In case of heterogeneity of variance between groups when testing for interaction, the dependent variable was weighted by the inverse variance.

Logistic regression analysis, with an allowance for age only, age and HDL cholesterol quintiles, or a group of known cardiovascular risk factors (age, cholesterol, body mass index, lipid-lowering medication, hypertension, diabetes mellitus, and smoking) plus HDL quintiles, was used to explore the impact of CETP genotype on the risk of IHD. Interaction between CETP genotype and the above-mentioned covariates was explored in logistic regression models that included CETP genotype, the risk factor in question, and an interaction term of these 2 factors; the likelihood ratio test between complete and reduced models was used to determine statistical significance.

Results
The basic characteristics of the subjects have been reported previously. The relative CETP genotype frequencies in this white Danish general population sample were 0.46 for Ile/Ile, 0.43 for Ile/Val, and 0.11 for Val/Val. These frequencies did not differ significantly from those predicted with the Hardy-Weinberg equilibrium (\( P>0.70, \chi^2 \)) and did not differ between women and men (\( P=1.00, \chi^2 \)).

CETP Genotype Frequencies
The relative CETP genotype frequencies in this white Danish general population sample were 0.46 for Ile/Ile, 0.43 for Ile/Val, and 0.11 for Val/Val. These frequencies did not differ significantly from those predicted with the Hardy-Weinberg equilibrium (\( P>0.70, \chi^2 \)) and did not differ between women and men (\( P=1.00, \chi^2 \)).

Lipids, Lipoproteins, and Apolipoproteins as a Function of CETP Genotype
In the general population sample, there was a stepwise increase in HDL cholesterol levels, apoA-I, and HDL cholesterol/apoA-I ratio from Ile/Ile to Ile/Val to Val/Val in women (\( P<0.001 \), all ANOVA) but not in men (Table 1). For HDL cholesterol, this pattern was confirmed in the same individuals with the use of levels measured 10 years earlier. With post-hoc tests, both Ile/Val and Val/Val carriers had higher levels of HDL cholesterol, apoA-I, and HDL cholesterol/apoA-I ratio than did female Ile/Ile carriers. Plasma levels of triglycerides, cholesterol, apoB, and Lp(a) were unaffected by Ile405Val genotype.

Furthermore, the relative frequency of subjects heterozygous or homozygous for Val405 increased significantly as a function of HDL cholesterol level in quintiles in women (\( P<0.001, \chi^2 \)) but not in men (\( P=0.55, \chi^2 \) (data not shown)).

In women, the total cholesterol/HDL cholesterol ratio was highest in noncarriers for Val405, intermediate for heterozygous carriers of Val405, and lowest for homozygous carriers of Val405 (\( P=0.002, \) ANOVA), whereas this ratio was unaffected by genotype in men (\( P=0.56, \) ANOVA) (Table 1).

Interaction With Other Cardiovascular Risk Factors
In women, the interaction of CETP genotype with HRT had an affect on HDL cholesterol levels (\( P=0.08 \)); the genotype affected HDL cholesterol levels of premenopausal women (\( P=0.005, \) ANOVA) and of postmenopausal women who were not treated with HRT (\( P<0.001, \) ANOVA) but not of postmenopausal women who were treated with HRT (\( P=0.80, \) ANOVA) (Figure 1). The use of HRT also reduced levels of LDL cholesterol, apoB, and Lp(a) in postmenopausal women (Table 2).

In men, there was an interaction between CETP genotype and plasma triglycerides on HDL cholesterol (\( P=0.09 \)). As suggested in an earlier study of Japanese men, the interaction was due to a borderline significantly higher HDL cholesterol level in homozygous VV men compared with IV and II men among those with triglyceride levels of >1.86 mmol/L but not in those with triglyceride levels of <1.86 mmol/L (Figure 1).

CETP Genotype and Risk of IHD
CETP genotype interacted with HRT on IHD risk (\( P<0.05 \)). In premenopausal women and postmenopausal women without HRT, when age was adjusted for, the odds ratio (OR) for the risk of IHD for Ile/Val and Val/Val versus Ile/Ile was 1.21 (95% CI 0.90 to 1.62), and 1.65 (95% CI 1.06 to 2.58), respectively (Figure 2). This was even more pronounced when in addition to age, the analyses allowed for HDL
of genotype on risk of IHD only in women): (1) patients with IHD versus the total general population sample (case-referent design) or (2) subjects in the general population sample with IHD versus those without (cross-sectional design).

In women, the association between carrying the valine allele and an increased risk of IHD was most pronounced in the upper tertiles of LDL, apoB, and Lp(a), whereas the middle and lower tertiles, which included relatively fewer patients, showed similar, although not statistically significant, trends (Figure 3). These results were supported by a lack of interaction between genotype and LDL cholesterol, apoB, or Lp(a) on risk of IHD in women. In men, there was no association between genotype and risk of IHD in tertiles of either LDL, apoB, or Lp(a) (Figure 3), with the exception of an apparently increased risk in heterozygote men in the middle tertile of apoB, suggesting a chance effect.

**Discussion**

We report that the common Ile405Val mutation in the CETP gene, despite being associated with increased levels of HDL cholesterol, is an independent risk factor for IHD in white women who are not treated with HRT. The evidence is based on genotyping of 10,114 women and men, of whom 1446 had IHD and the control subjects were from a general population sample.

**Effect on HDL Cholesterol**

Previous studies found that homozygosity for Val405 was associated with increased HDL cholesterol levels in 234 Dutch men, in 145 Icelandic men, and in 102 Japanese men with hypertriglyceridemia. We extended these findings in a large general population sample to demonstrate that HDL cholesterol level increases in both homozygotes and heterozygotes of Val405 in premenopausal women and in postmenopausal women not treated with HRT, whereas in

**TABLE 1. Effect of CETP Ile405Val Genotype in Subjects in the General Population Sample**

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ile/Ile</td>
<td>Ile/Val</td>
<td>Val/Val</td>
</tr>
<tr>
<td>No. of individuals</td>
<td>2300</td>
<td>2155</td>
<td>528</td>
</tr>
<tr>
<td>Age, y</td>
<td>58±0.3</td>
<td>59±0.3</td>
<td>58±0.7</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.68±0.01</td>
<td>1.75±0.01</td>
<td>1.82±0.02</td>
</tr>
<tr>
<td>HLD cholesterol, mmol/L*</td>
<td>1.23±0.01</td>
<td>1.27±0.01</td>
<td>1.31±0.02</td>
</tr>
<tr>
<td>apoA1, mg/dL</td>
<td>149±6</td>
<td>153±6</td>
<td>155±12</td>
</tr>
<tr>
<td>HLD cholesterol/apoA1, ×10^-2</td>
<td>43.3±0.1</td>
<td>43.9±0.1</td>
<td>44.7±0.3</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.25±0.03</td>
<td>6.32±0.03</td>
<td>6.30±0.06</td>
</tr>
<tr>
<td>Total cholesterol/HDL cholesterol</td>
<td>4.02±0.03</td>
<td>3.91±0.04</td>
<td>3.76±0.06</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.70±0.02</td>
<td>1.68±0.02</td>
<td>1.65±0.04</td>
</tr>
<tr>
<td>apoB, mg/dL</td>
<td>86.4±0.5</td>
<td>86.3±0.5</td>
<td>85.3±1.1</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>32.2±0.6</td>
<td>32.6±0.8</td>
<td>30.8±1.7</td>
</tr>
</tbody>
</table>

ANOVA indicates 1-way ANOVA for unequal variances. Values are mean±SEM. Individuals receiving cholesterol-lowering treatment (39 women and 43 men) were excluded from all analyses. To approach normal distribution, triglycerides and Lp(a) were transformed logarithmically before statistical tests, but untransformed values are shown.

*Values were obtained at the second examination of the Copenhagen City Heart Study in 1981 through 1983; all other values were obtained at the third examination in 1991 through 1994.

†P<0.001, †P<0.05 compared with Ile/Ile, post hoc test.

‡P<0.05 compared with Ile/Ile, post hoc test.

cholesterol levels in quintiles (OR 1.36, 95% CI 1.00 to 1.83; OR 1.89, 95% CI 1.20 to 2.98) or for HDL cholesterol in quintiles plus a group of known cardiovascular risk factors (OR 1.38, 95% CI 1.01 to 1.90; OR 2.07, 95% CI 1.27 to 3.37). In contrast, in postmenopausal women treated with HRT, there was no effect of genotype on risk of IHD (Figure 2). In men, there was no effect of genotype on risk of IHD (Figure 2).

The effects shown in Figure 2 are based on all subjects with IHD versus all subjects without IHD (case-control design). However, in other study designs that we have previously used, the results or trends were similar (ie, showing an effect...
hypertriglyceridemic men, only Val/Val homozygosity is associated with increased HDL cholesterol. In accordance with a previous study, we also observed an apoA-I–raising effect of the Val405 allele in women.

Mechanistically, it seems plausible that the Ile405Val mutation in CETP will affect levels of HDL cholesterol and apoA-I, the major protein in HDL particles. Complete CETP deficiency as seen in the Japanese leads to massively elevated levels of HDL cholesterol and apoA-I, and previous studies have demonstrated that the Ile405Val mutation leads to reduced CETP mass and activity in plasma. After HDL particles accept cholesterol from nonliver cells, CETP facilitates the transfer of cholesteryl ester onto triglyceride-rich lipoproteins as part of the reverse cholesterol transport pathway, ultimately leading to cholesterol excretion by the liver. When CETP is dysfunctional, cholesterol accumulates in HDL, and the transfer of cholesterol from peripheral cells to the liver is blocked. In accordance with this, our data suggest the presence of both an increased number of HDL particles (elevated HDL cholesterol and apoA-I) and cholesterol enrichment of HDL particles (elevated HDL cholesterol/apoA-I ratio) for both heterozygous and homozygous female carriers of the Ile405Val mutation. Because apoA-I is found only in HDL and chylomicrons, the effects we observed on apoA-I most likely reflect changes in levels of apoA-I in HDL.

In men, an interaction between CETP genotype and plasma triglyceride levels on HDL cholesterol was previously observed. CETP exchanges cholesterol in HDL for triglycerides in triglyceride-rich lipoproteins, making it plausible that the effect of CETP genotype on HDL cholesterol is seen only in men with high triglyceride levels, a situation in which the rate-limiting factor in cholesteryl ester transfer is in fact CETP.

**Effect on IHD Risk**

The most important novel observation in the present study is the clear codominant pattern of increased risk of IHD from Ile/Ile to Ile/Val to Val/Val in untreated white women. This is supported by similar, but less clear and less significant, results of an earlier study of hypertriglyceridemic men of Japanese descent and of men of Japanese descent with the Asp442Gly mutation in CETP.

It is quite likely that a genetic deficiency of CETP caused by mutations like Ile405Val affects IHD risk. CETP is essential in the reverse cholesterol transport pathway, the

### Table 2. Age-Adjusted Levels of LDL Cholesterol, apoB, and Lp(a) as a Function of Menopausal Status and HRT in Women From the Copenhagen City Heart Study

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal Women (n=1493)</th>
<th>Postmenopausal Women Without HRT (n=3150)</th>
<th>Postmenopausal Women With HRT (n=714)</th>
<th>ANOVA P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.03±0.03†</td>
<td>4.21±0.02*</td>
<td>3.65±0.04†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>apoB, mg/dL</td>
<td>70.7±0.5†</td>
<td>93.9±0.4*</td>
<td>86.1±0.8†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>26.2±0.8†</td>
<td>36.8±0.8*</td>
<td>28.7±1.3†</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ANOVA indicates 1-way ANOVA for equal variances or Kruskal-Wallis ANOVA for unequal variances. Values are mean±SEM.

Individuals receiving cholesterol-lowering treatment (39 women) were excluded from all analyses. To approach normal distribution, Lp(a) was transformed logarithmically before statistical tests, but untransformed values are shown.

*P<0.001 compared with premenopausal women, post hoc test.
†P<0.001 compared with postmenopausal women without HRT, post hoc test.
main route by which the body can eliminate excess cholesterol. Dysfunctional CETP that causes reduced reverse cholesterol transport is reflected as an increase in HDL cholesterol levels, suggesting that cholesterol may also accumulate in the arterial intima, ultimately leading to increased risk of atherosclerosis and IHD.

The effect of the interaction between CETP genotype and HRT on IHD risk may reflect the corresponding effect of the interaction between CETP genotype and HRT on HDL cholesterol levels. If HRT overrides the effect of CETP genotype on HDL cholesterol, it is equally possible that HRT may override the effect of CETP genotype on IHD risk. This apparent cardiovascular protective effect of estrogens in postmenopausal women treated with HRT may also reflect the known effects of HRT to reduce LDL cholesterol, apoB, and Lp(a) concentrations, effects that were also found in the Copenhagen City Heart Study (Table 2).

Sex-Specific Effects

Sex-specific effects of the CETP Ile405Val polymorphism on plasma levels of HDL cholesterol and risk of IHD are interesting but not easy to explain biologically. It is well known, however, that men develop IHD at an earlier age than women and that HDL cholesterol levels are lower in men than in women. A priori, we stratified the data analyses by sex and observed that the effects of the Ile405Val mutation on HDL cholesterol, apoA-I, and IHD risk differed between women and men. In accordance with this, CETP genotype and sex interacted on HDL cholesterol (P = 0.001) and apoA-I (P < 0.001) but not on IHD risk (P = 0.35). Because CETP levels appear to be higher in women than in men and because CETP levels are raised in late pregnancy in parallel with estrogen elevation, although testosterone administration to both women and men does not appear to affect CETP levels, it is not unlikely that the effects of mutations in CETP will influence lipoprotein metabolism and IHD risk differently in women and men.

Although the risk of IHD was increased only modestly at the level of the individual, the Ile405Val mutation may be important for the total risk of IHD in untreated women in the population at large. It can be calculated based on our data that 24% of female IHD risk can be attributed to this mutation in CETP, an attributable risk similar in magnitude to that of a conventional cardiovascular risk factor such as hypertension in our sample. This must be confirmed in other independent studies but nevertheless suggests that genetic variability in CETP may explain a large proportion of the genetic component of IHD risk in the population at large.

In conclusion, our data suggest that increased HDL cholesterol levels caused by mutations in CETP may be associated with an increased risk of IHD in white women and that the clinical use of the ratio of total to HDL cholesterol as a risk indicator may be misleading in persons with CETP mutations.

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