Common Cholesteryl Ester Transfer Protein Mutations, Decreased HDL Cholesterol, and Possible Decreased Risk of Ischemic Heart Disease

The Copenhagen City Heart Study

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

Background—Cholesteryl ester transfer protein (CETP) mediates the transfer of cholesteryl ester from HDL in exchange for triglycerides in apolipoprotein B–containing lipoproteins.

Methods and Results—We studied 2 common mutations in CETP, A373P and R451Q, in 8467 healthy women and men from the Danish general population and in 1636 Danish women and men with ischemic heart disease. The prevalence of 373P and 451Q was 0.10 and 0.07, respectively, for heterozygous carriers and 0.003 and 0.002, respectively, for homozygous carriers. All carriers of the 451Q allele also carried the 373P allele. HDL cholesterol in female noncarriers, heterozygotes, and homozygotes of 373P was 1.74 ± 0.01 (mean ± SE), 1.62 ± 0.02, and 1.38 ± 0.09 mmol/L, respectively (ANOVA, P < 0.001). In men, equivalent values were 1.40 ± 0.01, 1.26 ± 0.02, and 1.19 ± 0.09 mmol/L, respectively (ANOVA, P < 0.001). HDL cholesterol decreased similarly as a function of 451Q genotypes and all 373P/451Q genotype combinations. Furthermore, apolipoprotein AI and the HDL cholesterol/apolipoprotein AI ratio was also lower in carriers of either of these mutations for both sexes. Finally, the CETP genotype was not associated with risk of ischemic heart disease unless we adjusted for HDL cholesterol: female heterozygous and homozygous carriers versus noncarriers had 36% lower risk of ischemic heart disease (95% CI 4% to 57%); in male carriers, we observed a similar trend.

Conclusions—The A373P/R451Q polymorphism in CETP is associated with decreases in HDL cholesterol of 0.12 to 0.36 mmol/L in women and 0.14 to 0.21 mmol/L in men and possibly with a paradoxical 36% decrease in the risk of ischemic heart disease in women. (Circulation. 2000;102:2197-2203.)

Key Words: epidemiology ■ lipids ■ heart diseases ■ ischemia

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was no difference in genotype frequencies between subjects receiving cholesterol-lowering therapy and untreated subjects.

DNA Analyses
A G→C mutation at codon 373 in exon 12 of CETP leads to a proline for arginine (R451Q).\(^4,5\) Exon 12 was amplified by use of a primer overlapping the intron 11/exon 12 splice site (5'-GTTTCTCTCAGGAGATATCGTGAC 3') and a primer at the 3' end of exon 12 (5'-GTCAGTTGGAACACTTTTGGTG 3'); exon 15 was amplified by use of a primer overlapping the intron 14/exon 15 splice site (5'-TCGCCAGGCTGAGTATTGTGGT 3') and a primer ∼150 bp upstream from exon 16 (5'-ACCCAACTTCACACGCGTG 3'). For the A373P mutation, the restriction enzyme digestion by Scal resulted in a common band (on 3% agarose) of 206 bp plus bands of 36 and 281 bp for the alanine allele and a band of 317 bp for the proline allele. For the R451Q mutation, the result of digestion with the restriction enzyme Xhol was a common band of 11 bp plus bands of 62 and 362 bp for the arginine allele and a band of 424 bp for the glutamine allele.

Inconsistency was found between observed and predicted bands for exon and intron 12 (GenBank accession Nos. AC:M32997 and ID:HSCETP6); therefore, we sequenced exon and intron 12. Compared with the GenBank HSCETP6 sequence, a 13-bp DNA deletion from position 643 to 655 in intron 12 and an insertion of 97 bp were found at this position by us; the following sequence was as follows:

5'-AGGGCCTGGGAGGAGGAGAGCGCTGCCCG
3'

Continuous variables are shown as mean±SD. Triglycerides, lipoprotein(a), and body mass index were logarithmically transformed before statistical tests to approach normal distribution. Student t test (or Mann-Whitney U test if variances were unequal) and likelihood ratio test compared women and men in the 2 samples.

### Table 1. Characteristics of Subjects Genotyped for CETP A373P and R451Q Mutations

<table>
<thead>
<tr>
<th></th>
<th>General Population*</th>
<th>Patients With IHD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women</strong></td>
<td><strong>Men</strong></td>
<td><strong>Women</strong></td>
</tr>
<tr>
<td>No. of individuals</td>
<td>4793</td>
<td>3674</td>
</tr>
<tr>
<td>Age, y</td>
<td>57.7±0.2</td>
<td>55.5±0.3</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.73±0.01</td>
<td>1.39±0.01</td>
</tr>
<tr>
<td>Apolipoprotein AI, mg/dL</td>
<td>151.2±0.4</td>
<td>130.1±0.4</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.67±0.02</td>
<td>2.09±0.03</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.27±0.02</td>
<td>5.92±0.02</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>85.8±0.4</td>
<td>85.4±0.4</td>
</tr>
<tr>
<td>Lipoprotein(a), mg/dL</td>
<td>33.3±0.6</td>
<td>30.1±0.6</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.2±0.1</td>
<td>26.0±0.1</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>18.1</td>
<td>19.4</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>2.1</td>
<td>3.9</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>46.0</td>
<td>52.7</td>
</tr>
<tr>
<td>Former smokers, %</td>
<td>22.6</td>
<td>28.2</td>
</tr>
<tr>
<td>**CETP 451Q allele, %</td>
<td><strong>5.2</strong></td>
<td><strong>5.4</strong></td>
</tr>
<tr>
<td>**CETP 373P allele, %</td>
<td><strong>3.6</strong></td>
<td><strong>3.8</strong></td>
</tr>
</tbody>
</table>

### Table 2. Subjects With CETP A373P and R451Q Genotypes in General Population

<table>
<thead>
<tr>
<th>CETP R451Q</th>
<th>Noncarriers (RR)</th>
<th>Heterozygous Carriers (RQ)</th>
<th>Homozygous Carriers (QQ)</th>
<th>All Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CETP A373P, n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncarriers (AA)</td>
<td>8226</td>
<td>1</td>
<td>0</td>
<td>8227</td>
</tr>
<tr>
<td>Heterozygous carriers (AP)</td>
<td>280</td>
<td>632</td>
<td>0</td>
<td>912</td>
</tr>
<tr>
<td>Homozygous carriers (PP)</td>
<td>2</td>
<td>10</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td>All genotypes, n</td>
<td>8508</td>
<td>643</td>
<td>15</td>
<td>9166</td>
</tr>
</tbody>
</table>

Other Analyses
Plasma levels of total cholesterol, HDL cholesterol, apolipoprotein AI, apolipoprotein B, lipoprotein(a), and triglycerides were measured by enzymatic and turbidimetric assays.\(^6\)

Statistical Analyses
SPSS release 8.5 was used. A value of \(P<0.05\) was considered significant. We used the Student t test, Mann-Whitney U test, ANOVA, Kruskal-Wallis ANOVA, ANCOVA (to test for interaction between genotype and cardiovascular risk factors on lipoprotein levels), likelihood ratio test, and \(\chi^2\) test.

Multifactorial logistic regression analysis examined the association between CETP genotype and the risk of IHD. Models were
adjusted for (1) age, (2) age, body mass index, total cholesterol, triglycerides, cholesterol-lowering treatment, smoking habits, diabetes mellitus, and hypertension, or (3) all above-mentioned variables plus HDL cholesterol. We also tested for interaction between genotype and cardiovascular risk factors on IHD.

Results
HDL cholesterol was lower, and age, triglycerides, cholesterol, apolipoprotein B, lipoprotein(a), body mass index, and the relative frequencies of hypertension, diabetes mellitus, and former smoking status were higher in female and male patients with IHD than in women and men from the general population without IHD (Table 1). Female patients had slightly lower apolipoprotein AI than did healthy women from the general population, and fewer male patients were active smokers than were healthy men from the general population (patients often become exsmokers).

Genotype Frequencies
The numbers of subjects with A373P and R451Q CETP genotypes in the general population are shown in Table 2. Observed frequencies of A373P genotypes were consistent with those predicted by the Hardy-Weinberg equilibrium ($\chi^2$, $P>0.60$), as were observed frequencies of R451Q genotypes ($\chi^2$, $P>0.30$). There was deviation between observed and expected numbers of the 9 possible combinations of the 2 mutations (Pearson $\chi^2$, $P<0.001$). Whenever a subject carried the 451Q allele, the 373P allele was also present, except for one subject, whereas the 373P mutation was observed in 282 subjects who did not carry the 451Q variant (Table 2). The one subject with the 451Q variant without the 373P variant was confirmed by sequencing.

HDL Cholesterol
Plasma levels of HDL cholesterol differed as a function of A373P genotypes in both women and men (both ANOVAs, $P<0.001$; Figure 1); there was no interaction between A373P genotype and sex for HDL cholesterol (ANCOVA, $P=0.40$). On post hoc tests, heterozygous carriers of the 373P allele had lower levels of HDL cholesterol than did noncarriers for both sexes ($P<0.001$). HDL cholesterol also differed as a function of R451Q genotypes in both women and men (both ANOVAs, $P<0.001$; Figure 1). On post hoc tests, heterozygous carriers of the 451Q allele had lower levels than did noncarriers for both sexes ($P<0.001$).

HDL cholesterol levels also differed as a function of genotype in both women and men when both A373P and R451Q genotypes were considered together (both ANOVAs, $P<0.001$; Figure 1). Post hoc tests showed that double heterozygous carriers of the 451Q and 373P alleles (AP and RQ) had even lower HDL cholesterol than did single heterozygous carriers of the 373P allele (AP and RR) for women ($P<0.05$) but not for men.

The association between genotype and HDL cholesterol in women was also observed in premenopausal women and in postmenopausal women not treated with hormonal replacement therapy (HRT) but not in postmenopausal women treated with HRT (Figure 2). However, the test of interaction between HRT and A373P genotype was not statistically significant (ANCOVA, $P=0.77$).

Other Lipids, Lipoproteins, and Apolipoproteins
Plasma levels of apolipoprotein AI and the ratio of HDL cholesterol to apolipoprotein AI differed as a function of A373P and R451Q genotypes in both women and men (all ANOVAs, $P<0.001$; Table 3). Post hoc tests showed that heterozygous carriers of 373P or 451Q versus noncarriers had lower levels of apolipoprotein AI and HDL cholesterol/apolipoprotein AI ratios in both women and men ($P<0.001$). Levels of plasma triglycerides, cholesterol, apolipoprotein B, and lipoprotein(a) were statistically unaffected; however, there was a trend toward a genotype-dependent decrease in cholesterol in men for both mutations and in women for the A373P mutation (Table 3). Although the CETP genotype was associated with different levels of HDL cholesterol and not with different levels of triglycerides, HDL cholesterol and
plasma triglycerides were, as expected, inversely correlated in the cohort (data not shown).

We also performed 504 tests of interaction on total cholesterol, triglycerides, HDL cholesterol, and apolipoprotein AI for each sex separately between genotypes (A373P, R451Q, or combination) and age, total cholesterol, apolipoprotein B, lipoprotein(a), triglycerides, HDL cholesterol, apolipoprotein AI, fibrinogen, body mass index, waist/hip ratio, glucose, alcohol consumption, smoking, physical activity, blood pressure, hypertension, diabetes mellitus, menopausal status (women), HRT (women), diuretic medication, heart medication, and medication against high blood pressure. The vast majority of these tests were not statistical significant, and among variables with a value of $P<0.05$, plots of stratified data did not reveal monotonic and consistent associations but revealed different irregular patterns suggesting chance findings rather than plausible interactions. Furthermore, when correction for multiple comparison was done, none of the tests of interaction were significant.

### Risk of IHD

Because stratified analysis suggested that risk of IHD was associated with CETP genotype in untreated women but not in treated women (in accordance with associations with HDL cholesterol, Figure 2), results are shown separately for untreated premenopausal and postmenopausal women and postmenopausal women treated with HRT (Figure 3). However, the test of interaction between HRT and A373P genotype on IHD risk was not statistically significant (likelihood ratio test, $P=0.17$).

### TABLE 3. Association Between CETP A373P and R451Q Genotypes and Lipoprotein Levels in Subjects Sampled From General Population

<table>
<thead>
<tr>
<th></th>
<th>CETP A373P</th>
<th>CETP 451</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AP</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of individuals</td>
<td>4482</td>
<td>489</td>
</tr>
<tr>
<td>Age, y</td>
<td>58±0.2</td>
<td>58±0.7</td>
</tr>
<tr>
<td>Apolipoprotein AI, mg/dL</td>
<td>152±0.4</td>
<td>147±1.3*</td>
</tr>
<tr>
<td>HDL cholesterol/apolipoprotein AI ($\times 10^{-3}$)</td>
<td>43.8±0.1</td>
<td>42.2±0.3*</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.69±0.02</td>
<td>1.70±0.05</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.30±0.02</td>
<td>6.19±0.06</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>86.3±0.4</td>
<td>86.0±1.0</td>
</tr>
<tr>
<td>Lipoprotein(a), mg/dL</td>
<td>33.6±0.6</td>
<td>33.3±1.9</td>
</tr>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of individuals</td>
<td>3600</td>
<td>399</td>
</tr>
<tr>
<td>Age, y</td>
<td>57±0.3</td>
<td>57±0.8</td>
</tr>
<tr>
<td>Apolipoprotein AI, mg/dL</td>
<td>130±0.4</td>
<td>125±1.2*</td>
</tr>
<tr>
<td>HDL cholesterol/apolipoprotein AI ($\times 10^{-3}$)</td>
<td>40.8±0.1</td>
<td>38.6±0.3*</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.11±0.03</td>
<td>2.17±0.08</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.97±0.02</td>
<td>5.87±0.06</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>86.4±0.4</td>
<td>86.5±1.1</td>
</tr>
<tr>
<td>Lipoprotein(a), mg/dL</td>
<td>30.9±0.6</td>
<td>30.1±1.8</td>
</tr>
</tbody>
</table>

Values are mean±SE. Individuals on cholesterol-lowering treatment (39 women and 44 men) were excluded from all analyses. CETP 451 RR group includes only individuals without 373P allele. Number of individuals varies slightly for different covariates according to availability of data. To approach a normal distribution, triglycerides and lipoprotein(a) were transformed logarithmically before statistical tests, but untransformed values are shown. One-way ANOVA was used in case of equal variances, Kruskal-Wallis ANOVA was used in case of unequal variances. Post hoc tests were by Student $t$ test or Mann-Whitney $U$ test.

* $P<0.001$, † $P<0.05$, and ‡ $P<0.01$ compared with subjects without mutations.
When we adjusted for age alone or a group of risk factors, CETP genotype was not significantly associated with the risk of IHD (Figure 3). When we adjusted for a group of risk factors plus HDL cholesterol, untreated women carrying CETP 451Q and/or 373P had a 36% lower risk of IHD compared with noncarriers (odds ratio 0.64, 95% CI 0.43 to 0.96; Figure 3). There was a similar trend in male carriers compared with noncarriers (odds ratio 0.86, 95% CI 0.67 to 1.12) but not in female carriers on HRT (odds ratio 1.51, 95% CI 0.67 to 3.62). Sex did not interact with genotype for the risk of IHD (likelihood ratio test, P=0.28).

In addition, we tested for interaction between genotype and age, total cholesterol, triglycerides, body mass index, smoking, physical activity, hypertension, diabetes mellitus, menopausal status (women), and cholesterol-lowering medication on the risk of IHD for each sex separately. None of these tests reached statistical significance. This means that the association between CETP genotype and IHD risk does not depend on the presence or absence of these risk factors.

Discussion
In this sample from the Danish general population, the relative allele frequency of CETP 373P was 5.6%, and that of CETP 451Q was 3.8%. The present data suggest linkage of the 451Q allele with the 373P allele, not documented previously. This linkage is most likely because the 451Q mutation occurred on an existent 373P allele. One subject was heterozygous for the 451Q allele without carrying the 373P allele; ie, there was a haplotype frequency of 1/(2×9166) = 5.5×10⁻⁵ in the population at large.

HDL Cholesterol
Our data suggest that the 373P mutation alone is associated with lower HDL cholesterol levels. Furthermore, it is possible that the 373P and 451Q mutations combined versus 373P alone are associated with even lower HDL cholesterol levels, particularly in women. We cannot exclude that the lower HDL cholesterol associated with the 451Q mutation is due to linkage disequilibrium with 373P. Homozygous subjects with either or both mutations may have lower HDL cholesterol levels than heterozygous subjects, although in most instances, this was not statistically significant, most likely because of a lack of power due to the relatively few homozygous subjects (4 to 13 homozygous subjects per group, Figure 1).

The fact that carriers of mutant alleles versus noncarriers had lower HDL cholesterol, apolipoprotein A1, and HDL cholesterol/apolipoprotein A1 ratios indicates that carriers compared with noncarriers have fewer HDL particles, with each carrying less cholesterol. A biologically plausible explanation for such an association could be that the mutation at position 373 (and/or 451) induces higher CETP activity, transferring more cholesteryl esters out of HDL particles in carriers than in noncarriers. We were not able to test this hypothesis in the Copenhagen City Heart Study; however, a previous Finnish study of 21 noncarriers and 7 heterozygous carriers of the R451Q variant suggested that plasma CETP activity was higher by 26% in male carriers compared with noncarriers (P<0.01). This would reduce the amount of cholesteryl ester within each HDL particle and perhaps also stimulate downregulation of the total number of HDL particles. This hypothesis also implies that fewer HDL particles would be available for reverse cholesterol transport, a potentially deleterious effect, which could be counteracted by a faster turnover of cholesterol in HDL particles due to the increased CETP activity, and would thereby induce the decrease in risk of IHD that we have observed in untreated women. This hypothesis is supported by in vitro studies showing that interaction of HDL with CETP promotes the generation of small pre-β migrating HDL particles, which are the preferred acceptors of cell cholesterol promoting reverse cholesterol transport.

The A373P and R451Q variants are situated ≈2000 bp apart in the CETP gene and 78 amino acids apart in the terminal region of the 476 amino acid CETP protein. Structure-function studies of CETP have shown that insertion mutagenesis at codon 373-379 in exon 12 resulted in impaired CETP activity, suggesting that an amino acid substitution at position 373 may in fact affect CETP activity. Codon 451 in exon 15 is located in the putative lipid-binding region. An amino acid substitution such as the present (resulting in loss of positive charge at this position) could therefore influence the binding of CETP to HDL cholesteryl ester and thereby indirectly affect the efficiency of the protein transferring cholesteryl ester out of HDL particles. Therefore, it seems plausible that these 2 substitutions may affect the activity of CETP and, through this effect, decrease the levels of HDL cholesterol. Further studies of structure and function

Figure 3. Risk of IHD in heterozygous + homozygous carriers of 373P and 451Q vs noncarriers by logistic regression analysis. Models allowed either for (1) age, (2) a group of known cardiovascular risk factors (age, cholesterol, body mass index, triglycerides, lipid-lowering medication, hypertension, diabetes mellitus, and smoking), or (3) the group of known cardiovascular risk factors mentioned above plus HDL cholesterol in quintiles.
of CETP in carriers of these genetic variants may help explain the apparent paradoxical association of the CETP genotypes with HDL cholesterol and IHD risk.

**Risk of IHD**

This is the first study to present data suggesting that CETP mutations increasing CETP activity lead to decreased HDL cholesterol and possibly a lower risk of IHD in carriers. However, the association with a lower risk of IHD was found only in untreated women and not in those treated with HRT, whereas in men, there was only a similar trend. Furthermore, this association was seen only after we adjusted for a group of risk factors and HDL cholesterol. Therefore, our findings on the risk of IHD should be interpreted with caution until confirmed by another independent group.

Nevertheless, our results are indirectly supported by the opposite observation: low plasma CETP activity is associated with high HDL cholesterol and possibly with high risk of IHD.2,9,15–18 The finding that the B1 versus B2 CETP allele was associated with higher CETP concentrations, lower HDL cholesterol, and the progression of coronary atherosclerosis may seem to contradict this idea;20 however, this intron variant probably is not the causative mutation, and the end points studied in this20 and other studies differ. Taken together, CETP mutations leading to increased HDL cholesterol levels may increase the risk of IHD, whereas the present study suggests that CETP mutations associated with low HDL cholesterol possibly may lead to the opposite situation, namely, a low risk of IHD.

Mutations that increase CETP activity may increase reverse cholesterol transport and thus slow the progression of atherosclerosis, ultimately leading to a reduced risk of IHD.2 Altered CETP activity could also lead to altered hepatic production of apolipoprotein B,20 which is likewise of importance in the development of atherosclerosis; however, in our sample, CETP genotype was not associated with a variation in plasma apolipoprotein B levels (Table 3). Furthermore, the association between CETP genotype and reduced risk of IHD was not influenced by differences in apolipoprotein B levels (data not shown).

**Interaction With HRT and Sex**

An interaction between CETP genotype and HRT in predicting HDL cholesterol and IHD risk seems plausible, because as reported in other studies, HRT raises HDL cholesterol in women (Copenhagen City Heart Study) and thereby may override the effect of CETP genotype. However, it should be emphasized that statistical tests could not document the interaction between CETP genotype and HRT on either HDL cholesterol levels or risk of IHD, most likely because of the relatively few women in the postmenopausal group (see Figure 2).

The association between CETP genotype and HDL cholesterol levels was similar for the 2 sexes, but it was largest in women, whereas IHD risk was lower in untreated female carriers versus noncarriers but was statistically unaffected in male carriers. Nevertheless, we cannot exclude that the association with IHD risk may indeed be similar in men and women, because (1) when HDL cholesterol was adjusted for, we observed a trend toward lower risk in male carriers (see Figure 3), and (2) genotype and sex did not interact in predicting IHD risk with statistical significance. On the other hand, because CETP levels appear higher in women than in men,21,22 it is not unlikely that mutations in CETP could influence IHD risk differently in women and men.

**Interaction With Other Covariates**

CETP mutations may be particularly important in mediating lipoprotein-related risk of IHD in patients with diabetes mellitus. However, we had only 122 women and 184 men with diabetes mellitus in the Copenhagen City Heart Study. Therefore, although we were not able to show interaction between genotype and diabetes mellitus on HDL cholesterol or risk of IHD, we cannot fully exclude such interactions.

Alcohol intake may also influence associations between CETP genotype and lipoproteins and risk of IHD.23 We did not observe an interaction between CETP genotype and alcohol intake on HDL cholesterol in the present study, and we were not able to test for the interaction on IHD risk because we lacked information on alcohol intake among patients with IHD.

**Conclusions**

We demonstrate that CETP 373P/451Q alleles are associated with decreases in HDL cholesterol of 0.12 to 0.36 mmol/L (7% to 21%) in women and 0.14 to 0.21 mmol/L (10% to 15%) in men and possibly with a paradoxical 36% lower risk of IHD in women. These data support the hypothesis that it may not be the low HDL cholesterol per se that explains the strong inverse association between HDL cholesterol levels and IHD risk observed in prospective epidemiological population studies.1 This also implies that some individuals with low HDL cholesterol may have a reduced rather than an increased risk of IHD and vice versa for some individuals with high HDL cholesterol. Finally, the data suggest that genotyping for CETP mutations may improve cardiovascular risk assessment in the future.

**Acknowledgments**

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