Polymorphism in the CETP Gene Region, HDL Cholesterol, and Risk of Future Myocardial Infarction
Genomewide Analysis Among 18 245 Initially Healthy Women From the Women’s Genome Health Study

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Background—Recent trial data have challenged the hypothesis that cholesteryl ester transfer protein (CETP) and high-density lipoprotein cholesterol (HDL-C) have causal roles in atherothrombosis. One method to evaluate this issue is to examine whether polymorphisms in the CETP gene that impact on HDL-C levels also impact on the future development of myocardial infarction.

Methods and Results—In a prospective cohort of 18 245 initially healthy American women, we examined over 350 000 singe-nucleotide polymorphisms (SNPs) first to identify loci associated with HDL-C and then to evaluate whether significant SNPs within these loci also impact on rates of incident myocardial infarction during an average 10-year follow-up period. Nine loci on 9 chromosomes had 1 or more SNPs associated with HDL-C at genome-wide statistical significance ($P < 5 \times 10^{-8}$). However, only SNPs near or in the CETP gene at 16q13 were associated with both HDL-C and risk of incident myocardial infarction (198 events). For example, SNP rs708272 in the CETP gene was associated with a per-allele increase in HDL-C levels of 3.1 mg/dL and a concordant 24% lower risk of future myocardial infarction (age-adjusted hazard ratio, 0.76; 95% CI, 0.62 to 0.94), consistent with recent meta-analysis. Independent and again concordant effects on HDL-C and incident myocardial infarction were also observed at the CETP locus for rs4329913 and rs7202364. Adjustment for HDL-C attenuated but did not eliminate these effects.

Conclusion—In this prospective cohort of initially healthy women, SNPs at the CETP locus impact on future risk of myocardial infarction, supporting a causal role for CETP in atherothrombosis, possibly through an HDL-C mediated pathway.

Key Words: HDL-cholesterol ■ myocardial infarction ■ atherosclerosis ■ genetic association

Cholesteryl ester transfer protein (CETP) promotes the transfer of cholesteryl esters from high-density lipoprotein cholesterol (HDL-C) to other lipoprotein particles, and individuals genetically deficient for CETP often have extremely high HDL-C levels.1,2 In part on this basis, and because of consistent epidemiological evidence that elevated levels of HDL-C are protective against cardiovascular disease, inhibitors of CETP were developed with the hope that raising HDL-C through this mechanism would reduce vascular event rates. However, in the recently reported ILLUMINATE trial of torcetrapib conducted among more than 15 000 individuals at high risk for cardiovascular disease, mortality rates were increased in the active as compared with placebo treatment group3 leading to considerable debate as to whether CETP and/or HDL-C are in fact causal for heart disease and should remain viable pharmacological targets.4,5

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One approach to understanding causal pathways is to ascertain if genetic polymorphism known to impact on an intermediate phenotype (such as plasma lipid levels) also impacts on vascular risk.6 For example, recent work has found that those with polymorphism in PCSK9 not only have reduced levels of low-density lipoprotein cholesterol (LDL-C), but also reduced rates of vascular events.7 Similarly, recent reports have shown that polymorphism within several other LDL-related pathways determine both plasma LDL-C levels and vascular risk.8–11 By contrast, data demonstrating that polymorphism in genes known to affect plasma HDL-C...
levels also affect vascular event rates have been inconsistent, particularly with regard to CETP. However, such evidence, if available from a prospective cohort study free of selection bias, would support continued investigation into CETP and HDL-C as targets for therapy.

To address this issue, we evaluated more than 350,000 single-nucleotide polymorphisms (SNPs) across the human genome to first determine genetic loci associated with HDL-C and then evaluate whether any significant SNPs within these loci in turn impact on rates of incident myocardial infarction during an average 10-year follow-up period.

Methods

We evaluated the role of polymorphisms that impact on HDL-C as potential determinants of incident myocardial infarction among participants in the prospective Women’s Genome Health Study (WGHS). In brief, participants in the WGHS include initially healthy American women aged 45 and older with no previous history of cardiovascular disease, cancer, or other major chronic illness who provided a baseline blood sample during the enrollment phase of the Women’s Health Study between 1992 and 1995, and who gave consent for blood-based analyses related to risks of incident chronic diseases.

All study participants were followed up through March 2007 for incident myocardial infarction events that were adjudicated by an endpoints committee using standardized criteria and full medical record review. The end point of myocardial infarction was confirmed if symptoms met World Health Organization Criteria and if the event was associated with abnormal levels of cardiac enzymes or diagnostic electrocardiographic criteria. Only confirmed end points were included in this analysis.

All study participants had baseline blood samples assayed for total cholesterol, HDL-C, direct LDL-C, apolipoprotein A-1, and apolipoprotein B-100, and high-sensitivity C-reactive protein in a core laboratory certified by the national Heart Lung and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization Program; coefficients of variation were <5% for total cholesterol, HDL-C and LDL-C, and <5% for apolipoproteins A-1 and B-100.

As described elsewhere, DNA extracted from the baseline WGHS blood samples underwent SNP genotyping using the Illumina Infinium II assay to query a genome-wide set of 318,237 SNP markers (Human HAP300 panel) as well as an additional focused panel of 45,751 SNPs selected to enhance coverage of genomic regions without regard to allele frequency in which we had a strong a priori interest owing to presence of genes thought to be of relevance to metabolic, lipid, inflammatory, and other biological functions. To reduce the potential for population stratification, we evaluated only WGHS participants who were of European ancestry. As previously reported in the WGHS, a principal component analysis using 1443 ancestry-informative SNPs was used to confirm self-reported ancestry in 99.7% of the sample, leaving 18,245 participants with both self-reported and genetically inferred European ancestry for this study who also had both HDL-C and genotype data available. Further, in an additional principal component analysis performed for the exclusion of within European stratification based on 124,931 SNPs, 65% chosen to have pairwise disequilibrium r²<0.4, no correction for within European ancestry was required.

For the current analysis, we first ascertained those loci that contained 1 or more SNPs that associated with plasma HDL-C at a genome-wide level of statistical significance (P<5×10⁻⁸) and that had Hardy-Weinberg P=10⁻⁶, minor allele frequency greater than 1%, and genotyping call rates >90%. To evaluate for associations between any of these SNPs and plasma HDL-C, we assumed an additive model of inheritance and initially conducted univariate linear regression analysis to test the null hypothesis that HDL-C levels did not differ by the number of inherited copies of the SNP minor allele; in these initial analyses, we adjusted plasma HDL-C levels on an a priori basis for age, smoking, body mass index, hormone therapy, and menopausal status, and limited the evaluation to nondiabetic women who were not taking lipid-lowering agents.

For any locus containing at least 1 SNP that was associated with HDL-C at a genome-wide level of statistical significance, we next sought evidence of association between the significant SNPs in that loci and incident myocardial infarction. Association testing of these SNPs with incident myocardial infarction was performed with age-adjusted Cox proportional hazards models as well as models that additionally adjusted for HDL-C, and in fully adjusted models that further controlled for smoking status (current, not current), blood pressure (Framingham categories), diabetes (yes/no), parental history of myocardial infarction before age 60 years (yes/no), LDL-C, and log transformed triglycerides (milligram per deciliter).

Haplotype analysis was performed using the haplo.glm program from the haplo.stats analysis package in R within blocks of linkage disequilibrium as defined previously. Briefly, this program provided a method for logistic regression of myocardial infarction dependent on inferred haplotypes in a prespecified block of linkage disequilibrium. The key feature of the haplo.glm algorithm is its use of an expectation maximization procedure to optimize the likelihood of both the logistic model fit and the haplotype inference in an iterative fashion.

To replicate associations at the 2q24.3 locus, we used HDL-C measurements from PRINCE cohort for which genotype information was available through the PARC consortium. The genotype data derive from Illumina Human HAP300 genotyping among 670 PRINCE participants, of whom 168 (25.1%) were females.

The study protocol was approved by the Institutional Review Board of the Brigham and Women’s Hospital, Boston, Mass. All authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as it is written.

Results

Effects of Polymorphism on HDL-C

Nine loci on 9 chromosomes were identified that contained 1 or more SNPs that were associated with HDL-C at a genome-wide level of statistical significance (P<5×10⁻⁸) (Table 1). Eight of these 9 loci contain genes known to impact on HDL-C metabolism, whereas 1 locus near the genes COBLL1 and GRBI4 at 2q24.3 appears to be novel. The per-allele shifts in HDL-C for the 2 SNPs with genome-wide significance at 2q24.3, rs10490694 and rs7607980, were 1.35 mg/dL (P=3.9×10⁻⁹) and 1.29 mg/dL (P=1.5×10⁻⁸), respectively. These SNPs were in high linkage disequilibrium...
Table 2. SNPs in the CETP Gene With Genome-wide Effects on Plasma HDL Cholesterol

<table>
<thead>
<tr>
<th>SNP*</th>
<th>BP</th>
<th>MAF</th>
<th>M/m</th>
<th>HW-P</th>
<th>MM</th>
<th>Mm</th>
<th>Per Allele HDL Shift (mg/dL)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1800775</td>
<td>55552736</td>
<td>0.486</td>
<td>C/A</td>
<td>0.44</td>
<td>49 (41 to 59)</td>
<td>52 (43 to 62)</td>
<td>55 (46 to 67)</td>
<td>3.09</td>
</tr>
<tr>
<td>rs1532624</td>
<td>55562979</td>
<td>0.430</td>
<td>C/A</td>
<td>0.57</td>
<td>50 (42 to 60)</td>
<td>52 (44 to 63)</td>
<td>56 (46 to 68)</td>
<td>3.14</td>
</tr>
<tr>
<td>rs708272</td>
<td>55553788</td>
<td>0.428</td>
<td>G/A</td>
<td>0.28</td>
<td>50 (42 to 60)</td>
<td>52 (44 to 63)</td>
<td>56 (46 to 67)</td>
<td>3.08</td>
</tr>
<tr>
<td>rs1864163</td>
<td>55554733</td>
<td>0.255</td>
<td>G/A</td>
<td>0.27</td>
<td>54 (45 to 65)</td>
<td>50 (42 to 61)</td>
<td>48 (40 to 58)</td>
<td>−3.10</td>
</tr>
<tr>
<td>rs7499892</td>
<td>55564090</td>
<td>0.177</td>
<td>G/A</td>
<td>0.96</td>
<td>53 (44 to 64)</td>
<td>50 (41 to 60)</td>
<td>47 (40 to 57)</td>
<td>−3.50</td>
</tr>
<tr>
<td>rs9989419</td>
<td>55542639</td>
<td>0.395</td>
<td>G/A</td>
<td>0.35</td>
<td>54 (45 to 65)</td>
<td>51 (43 to 62)</td>
<td>50 (42 to 60)</td>
<td>−2.19</td>
</tr>
<tr>
<td>rs5880</td>
<td>55575951</td>
<td>0.053</td>
<td>G/C</td>
<td>0.90</td>
<td>52 (44 to 63)</td>
<td>49 (41 to 59)</td>
<td>45 (39 to 52)</td>
<td>−3.48</td>
</tr>
<tr>
<td>rs1800777</td>
<td>55574819</td>
<td>0.036</td>
<td>G/A</td>
<td>0.28</td>
<td>52 (44 to 63)</td>
<td>49 (40 to 58)</td>
<td>43 (37 to 47)</td>
<td>−3.91</td>
</tr>
<tr>
<td>rs12597002</td>
<td>55559904</td>
<td>0.300</td>
<td>G/A</td>
<td>0.96</td>
<td>53 (44 to 64)</td>
<td>51 (43 to 62)</td>
<td>50 (42 to 60)</td>
<td>−1.39</td>
</tr>
<tr>
<td>rs4784744</td>
<td>55568885</td>
<td>0.349</td>
<td>G/A</td>
<td>0.90</td>
<td>53 (44 to 64)</td>
<td>52 (43 to 62)</td>
<td>50 (41 to 61)</td>
<td>−1.32</td>
</tr>
<tr>
<td>rs289744</td>
<td>55575602</td>
<td>0.302</td>
<td>A/C</td>
<td>0.99</td>
<td>51 (43 to 62)</td>
<td>53 (44 to 63)</td>
<td>54 (45 to 64)</td>
<td>1.37</td>
</tr>
<tr>
<td>rs5882</td>
<td>55573592</td>
<td>0.318</td>
<td>A/G</td>
<td>0.42</td>
<td>51 (43 to 62)</td>
<td>53 (44 to 63)</td>
<td>53 (45 to 64)</td>
<td>1.30</td>
</tr>
<tr>
<td>rs7202364</td>
<td>55542890</td>
<td>0.267</td>
<td>A/G</td>
<td>0.40</td>
<td>52 (43 to 62)</td>
<td>53 (44 to 63)</td>
<td>53 (44 to 65)</td>
<td>1.10</td>
</tr>
<tr>
<td>rs8051691</td>
<td>55393212</td>
<td>0.267</td>
<td>C/A</td>
<td>0.44</td>
<td>52 (43 to 62)</td>
<td>52 (44 to 63)</td>
<td>53 (44 to 65)</td>
<td>1.09</td>
</tr>
<tr>
<td>rs5883</td>
<td>55564853</td>
<td>0.054</td>
<td>G/A</td>
<td>0.13</td>
<td>52 (43 to 62)</td>
<td>54 (45 to 64)</td>
<td>58 (49 to 67)</td>
<td>1.94</td>
</tr>
<tr>
<td>rs4329913</td>
<td>55462932</td>
<td>0.236</td>
<td>G/A</td>
<td>0.79</td>
<td>52 (43 to 62)</td>
<td>52 (43 to 62)</td>
<td>50 (42 to 60)</td>
<td>−1.03</td>
</tr>
<tr>
<td>rs1529929</td>
<td>55406996</td>
<td>0.411</td>
<td>G/A</td>
<td>0.09</td>
<td>51 (43 to 62)</td>
<td>52 (43 to 62)</td>
<td>53 (44 to 64)</td>
<td>0.89</td>
</tr>
<tr>
<td>rs2217332</td>
<td>55526648</td>
<td>0.145</td>
<td>G/A</td>
<td>0.74</td>
<td>52 (44 to 63)</td>
<td>51 (42 to 62)</td>
<td>51 (41 to 62)</td>
<td>−1.23</td>
</tr>
<tr>
<td>rs1306677</td>
<td>55483695</td>
<td>0.096</td>
<td>G/A</td>
<td>0.90</td>
<td>52 (43 to 62)</td>
<td>53 (44 to 63)</td>
<td>53 (45 to 62)</td>
<td>1.47</td>
</tr>
<tr>
<td>rs247615</td>
<td>55542263</td>
<td>0.232</td>
<td>A/G</td>
<td>0.76</td>
<td>52 (44 to 63)</td>
<td>52 (43 to 62)</td>
<td>51 (43 to 61)</td>
<td>−0.98</td>
</tr>
</tbody>
</table>

M indicates major allele; m, minor allele; MAF, minor allele frequency; HW-P, Hardy Weinberg P value; SNPs, single-nucleotide polymorphisms; HDL-C, high-density lipoprotein cholesterol.

*In order of decreasing significance for association with HDL-C.

($r^2=0.98$) with minor allele frequencies 12.3% and 12.5%, respectively. The minor allele frequencies of the 2 SNPs were comparable and their associations with HDL-C were significant with consistent direction of effects in separate genetic analysis in the PRINCE population. Specifically, the per-allele shifts were 2.9 mg/dL ($P=0.0008$) and 3.1 mg/dL ($P=0.0004$), respectively. The effects were larger among the 168 PRINCE women (both SNPs 7.3 mg/dL) than the 501 men (1.5 and 1.7 mg/dL, respectively), and an interaction with gender was observed ($P$ interaction = 0.002 and 0.003, respectively).

At the CETP locus, where genome-wide associations with HDL-C were both most significant and numerous, 20 SNPs were associated with plasma HDL-C at a genome-wide level of statistical significance (probability values ranging from $3.8 \times 10^{-8}$ to $3.7 \times 10^{-93}$) (Table 2). All of these were also highly associated with apolipoprotein A-1 levels (data not shown). These SNPs spanned about 242 kb of chromosome 16 with most clustered around the CETP gene but 3 mapping to the neighboring NUP93 gene encoding a nuclear pore protein, and another 6 in mapping to the neighboring SLC12A3 and HERPUD1 genes, respectively, encoding a sodium transporter and a protein of uncertain function localized to the endoplasmic reticulum (Figure 1).

As also shown in Table 2, several of the CETP SNPs were common (minor allele frequencies between 30% and 48%),
and several had substantive effects on plasma levels of HDL-C. For example, median HDL-C levels for homozygous major allele carriers, heterozygotes, and homozygous minor allele carriers at rs70872 (minor allele frequency 0.428) were 50, 52, and 56 mg/dL, respectively (P=1.7×10^{-90}), such that each minor allele increased HDL-C levels by an average 3.1 mg/dL or ≈6%. By contrast, other CETP SNPs that were associated with plasma HDL-C levels at a genome-wide level of significance were less common or had only marginal absolute effects on plasma HDL-C.

**Effects of Polymorphism on Incident Myocardial Infarction**

During follow-up, 198 incident myocardial infarction events were confirmed by the endpoints committee. Among the 9 loci in Table 1, only 1 at 1q63 (the site encompassing the CETP gene) contained SNPs that were both associated with HDL-C and also had significant impact on rates of incident myocardial infarction. For example, as shown in Table 3, polymorphism at rs708272 (minor allele frequency 0.428) were 50, 52, and 56 mg/dL, respectively (P=1.7×10^{-90}), such that each minor allele increased HDL-C levels by an average 3.1 mg/dL or ≈6%. By contrast, other CETP SNPs that were associated with plasma HDL-C levels at a genome-wide level of significance were less common or had only marginal absolute effects on plasma HDL-C.

### Table 3. Hazard Ratios (HRs) (95% CI) for the Risk of Incident Myocardial Infarction According to CETP Genotype

<table>
<thead>
<tr>
<th>SNP</th>
<th>N</th>
<th>Genotype</th>
<th>Per Allele (Age Adjusted)</th>
<th>Per Allele (HDL-C Adjusted)</th>
<th>Per Allele (Fully Adjusted*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MM</td>
<td>Mm</td>
<td>Mm</td>
<td>HR (Age Adjusted)</td>
</tr>
<tr>
<td>rs708272</td>
<td>18245</td>
<td>GG</td>
<td>GA</td>
<td>AA</td>
<td>0.76 (0.62–0.94)</td>
</tr>
<tr>
<td>rs4329913</td>
<td>18190</td>
<td>GG</td>
<td>GA</td>
<td>AA</td>
<td>1.34 (1.08–1.66)</td>
</tr>
<tr>
<td>rs1532624</td>
<td>17719</td>
<td>CC</td>
<td>CA</td>
<td>AA</td>
<td>0.78 (0.64–0.96)</td>
</tr>
<tr>
<td>rs1800775</td>
<td>18211</td>
<td>CC</td>
<td>CA</td>
<td>AA</td>
<td>0.82 (0.67–1)</td>
</tr>
<tr>
<td>rs7202364</td>
<td>18194</td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td>0.79 (0.62–0.99)</td>
</tr>
<tr>
<td>rs8051691</td>
<td>18237</td>
<td>CC</td>
<td>CA</td>
<td>AA</td>
<td>0.79 (0.62–1)</td>
</tr>
</tbody>
</table>

Data are provided on an individual genotype basis and on a per-allele basis, adjusted for age and adjusted for multiple risk factors.

HDL-C indicates high-density lipoprotein cholesterol.

*Fully adjusted model controlled for age (years), body mass index (kilogram per square meter), smoking status (current, not current), hormone replacement therapy use (yes/no), blood pressure (Framingham categories), diabetes (yes/no), parental history of myocardial infarction before age 60 yr (yes/no), LDL-C, HDL-C, and log transformed triglycerides (milligram per deciliter).

As also shown in Table 3, independent effects on both HDL-C and on incident myocardial infarction were also observed at rs4329913 (≈90 kb from the CETP gene; Figure 2B) and rs7202364 (≈210 kb from the CETP gene; Figure 2C). Three other SNPs showed similar concordant effects on both HDL-C and myocardial infarction risk, rs1800775 and rs1532624 (both of which were in high linkage disequilibrium with rs708272) and rs8051691 (which was in high linkage disequilibrium with rs7202364). For these SNPs, adjustment for HDL-C or for traditional risk factors again attenuated but did not eliminate effects on risk of incident myocardial infarction (Table 3).

Haplotype analysis was performed to evaluate whether combinations of SNPs at the CETP locus might be more strongly associated with myocardial infarction than individual SNPs. Haplotypes were inferred for neighboring SNPs in blocks with little evidence of historical recombination and tested for association with myocardial infarction. These analyses did not reveal stronger associations than were found in the single SNP analysis.

**Discussion**

Evaluating variation at 9 loci with genome-wide significance for HDL-C among 18 245 initially healthy American women followed prospectively over an average period of 10 years, we found that common SNPs exclusively in or near the CETP gene were also associated with risk of incident myocardial
infarction. Effects on HDL-C and myocardial infarction were concordant such that SNP alleles associated with increased plasma HDL-C (or ApoA1) levels were also associated with decreased vascular risk, and vice versa. SNP-based risk was attenuated but not eliminated by adjustment for either plasma HDL-C or traditional risk factors.

At the same time, our genome-wide design identified a novel candidate locus for HDL-C at 2q24.3. Analysis in the PRINCE population validated the 2q24.3 associations with HDL-C level and furthermore suggested potential gender specific effects. This region contains 2 genes, COBLL1 and GRB14, the latter of which may be the better candidate for a
functional role in determining HDL-C levels. **GRB14** encodes and adaptor protein known to form inhibitory interactions with the insulin receptor and other growth receptors and is expressed in the liver, skeletal muscle, and adipose tissue. Mice deficient of **GRB14** have altered patterns of glucose metabolism, a biological process that may also be linked to HDL-C levels.

As previous epidemiological work relating polymorphism in the **CETP** gene to vascular risk has been controversial and as 2 recent studies have not found significant relationships, external validation of our findings is important for interpretation. In this regard, in a recent comprehensive meta-analysis that included data from 38 previous studies employing a candidate gene approach, the same **rs708272** SNP uncovered in the WGHS using a genome-wide approach was associated with a 6% per allele reduction in coronary risk (odds ratio, 0.95; 95% CI, 0.92 to 0.99). For direct comparison, in our data, **rs708272** was associated with a 6% per allele increase in HDL-C and a 17% per allele concordant reduction in myocardial infarction after multivariate adjustment. Thus, for the primary observation in this large-scale genomiloids association study linking **CETP** genotype not only to HDL-C but also to incident vascular events, external validation is available for **rs708272** when previous studies are statistically pooled.

Although the direction of effect for **rs708272** in our data is consistent with that of the meta-analysis, the absolute magnitude of genetic impact on vascular events is somewhat larger. We believe there are several potential explanations for this effect. First, our data are limited to incident myocardial infarction whereas data in the recent meta-analysis includes many forms of coronary heart disease, often inclusive of angina and coronary revascularization, events that may have more to do with atherosclerotic progression than with acute plaque rupture.

Second, our data derive from a prospective cohort study of initially healthy women in which event status was determined solely by occurrence of disease rather than by any selection criteria imposed by the investigators or the patients that could result in inadvertent confounding or bias. In both theory and practice, inadvertent bias introduced in the conduct of even the best case-control studies may, in some situations, be greater in magnitude than the effect under study, particularly when that effect is modest to small in absolute magnitude.

Third, we limited our analysis to a white population, and thereby reduced the potential for ethnicity-based stratification to adversely impact the validity of our data. In this regard, it should be noted that several early candidate gene studies and previous meta-analyses of **CETP** polymorphism were unable to control for such effects and that substantial heterogeneity between studies has previously been observed.

We believe it of interest that one of the SNPs most significantly associated with myocardial infarction in our data was **rs708272**, the SNP that defines the B2 allele of the **CETP** TaqIB polymorphism (and is the core candidate **CETP** SNP defined in the recent comprehensive meta-analysis). Although using a different genotyping technology, the current data also extend to women early candidate gene work suggesting that the Taq1B polymorphism is associated with myocardial infarction in men with low HDL-C levels. In this regard, we note that some reports suggest that the Taq1B polymorphism is acting through linkage disequilibrium to a second SNP in the promoter of the **CETP** gene at position −629 from the transcription start site. This SNP is equivalent to rs1800775, which was the single most strongly associated SNP in our analysis with HDL-C (Table 1) and thus again is consistent with previous reports.

The second SNP within 16q13, **rs4329913**, which was independently associated with a decrease in HDL-C and an increase in the risk of myocardial infarction, is relatively far from the transcription start site for the **CETP** gene and within the transcribed region for a gene encoding a solute trans-
porter, SLC12A3. Neither SLC12A3 nor a second neighboring gene HERPUD1 are obvious candidates for regulation of HDL-C levels, perhaps suggesting the effect of rs4329913 is mediated by long range linkage disequilibrium to a causal variant nearer the CETP gene or long range effects on transcription at the entire locus. In our data, linkage disequilibrium between rs4329913 and at least one other SNP (rs1800777) nearer the CETP gene was as high as $D′=0.85$, reinforcing the potential for long range linkage effects. Finally, the third SNP in 1q13 independently associated with both HDL-C and incident myocardial infarction (rs7202364), is even further from the transcription site for the CETP gene and within the NUP93 gene, another unlikely candidate for effects on HDL-C and again suggesting effects mediated through linkage to the CETP gene.

In population-based epidemiological studies typically conducted among men of middle-age or older, it has been observed that the risk of coronary heart disease decreases $\approx 2\%$ for each $1\%$ increase in HDL-C. However, in our adjusted data, the reduction in risk associated with polymorphism in CETP was somewhat greater than this prediction. One possible explanation of this effect is that lifelong elevations of HDL-C may confer greater protection from vascular disease than would be anticipated from changes in HDL-C that might occur from pharmacological or dietary interventions begun at midlife. A second possibility is that gender specific differences exist in the relationships between CETP polymorphism, HDL-C, and vascular risk that were brought out by our study of women. Alternatively, it is possible that the biological effects of CETP polymorphism on myocardial infarction risk are not captured solely through the intermediate phenotype of HDL-C (or through the assessment of HDL-C at a single point in time). In this regard, adjustment for HDL-C only moderately attenuated the magnitude of the per allele relationships we observed between genotype and myocardial infarction risk. Further, as shown in Table 3, modest associations between rs708272 genotype and both LDL-C and triglycerides were also observed in our data. Nonetheless, even after additional adjustment for these lipid fractions (as well as for a large number of other major risk factors), the per allele HR for rs708272 was still 0.83. This lack of further attenuation suggests that CETP may well impact on atherothrombosis through additional intermediate pathways and/or intermediate phenotypes that go beyond its primary effects on HDL-C.

In sum, in these prospective data, we found specific polymorphisms in or near the CETP gene that impact on future risk of myocardial infarction. As such, these data support continued investigation of agents that target CETP as a potential method for vascular risk reduction.

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References
5. Tall AR, van Chernet L, Wang N. The failure of torcetrapib: was it the molecule or the mechanism? Arterioscler Thromb Vasc Biol. 2007;27: 257–260.
14. Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, Tall AR. Increased coronary heart disease in Japanese-Ame men with mutation in


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**CLINICAL PERSPECTIVE**

Understanding the pathophysiologic basis of the inverse correlation between plasma HDL-C levels and myocardial infarction risk is important for managing cardiovascular disease, especially in the context of potential therapeutic benefit of cholesteryl ester transfer protein (CETP) inhibitors, which raise plasma HDL-C. One approach to dissecting this relationship is to identify genetic associations with HDL-C and then to ask about their influence on myocardial infarction. In a prospective cohort including over 18,000 Caucasian women with 10 years of follow-up observation, we first surveyed the entire genome for common genetic variants associated with plasma HDL-C at baseline. We identified 9 genomic regions, with the strongest associations found at the gene encoding CETP. Among the 9 regions, only genetic variation at the CETP gene was also associated with the risk of future myocardial infarction. The nature of the association reinforces the critical role of CETP but also suggests that measurement of baseline HDL-C only partly accounts for the biological functions of CETP most relevant to myocardial infarction risk.
Polymorphism in the CETP Gene Region, HDL Cholesterol, and Risk of Future Myocardial Infarction: Genomewide Analysis Among 18 245 Initially Healthy Women From the Women's Genome Health Study
Paul M. Ridker, Guillaume Paré, Alex N. Parker, Robert Y.L. Zee, Joseph P. Miletich and Daniel I. Chasman

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