Associations of Genetic Variants in ATP-Binding Cassette A1 and Cholesteryl Ester Transfer Protein and Differences in Lipoprotein Subclasses in the Multi-Ethnic Study of Atherosclerosis

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BACKGROUND: ATP-binding cassette A1 (ABCA1) and cholesteryl ester transfer protein (CETP) play important roles in the reverse cholesterol transport pathway. The associations of ABCA1 and CETP polymorphisms with lipoprotein subclasses have not been extensively studied.

METHODS: We genotyped 2 ABCA1 and 5 CETP polymorphisms in 999 participants of the Multi-Ethnic Study of Atherosclerosis (MESA) and studied their associations with HDL and LDL subclass particle concentrations, measured by nuclear magnetic resonance spectroscopy.

RESULTS: ABCA1 and CETP polymorphisms were associated with different and distinct changes in lipoprotein subclass concentrations. The ABCA1 1051G/A AA genotype, previously found to be associated with cardioprotective effects in this cohort, was associated with a 5.5% higher concentration of small HDL particles ($P = 0.024$). The CETP TaqIB B2B2, $-2505C/A$ AA, and $-629C/A$ AA genotypes, previously demonstrated to lack cardioprotective effects, were associated with 15.2%, 15.4%, and 11.7% higher HDL cholesterol concentrations, respectively, and 36.5%, 40.7%, and 25.4% higher large HDL particle concentrations ($P < 0.0001$). The minor alleles of the A373P and R451Q polymorphisms were associated with lower large HDL particle concentrations.

CONCLUSIONS: Our study of the influence of ABCA1 and CETP genetic variants on lipoprotein subclasses demonstrates the importance of interpreting lipoprotein subclasses within the context of the biochemical processes involved in the alterations. In the case of HDL, the study of subclass particle numbers and sizes may not be sufficiently informative. Assays for HDL function may be needed to supplement quantification of HDL cholesterol and HDL particle numbers and sizes.

An independent and inverse association between HDL cholesterol (HDL-C)³ concentration and the risk of developing coronary artery disease (CAD) has now been unequivocally demonstrated (1, 2). In fact, a recent study demonstrated that HDL concentrations were predictive of cardiovascular events even among statin-treated individuals with LDL cholesterol (LDL-C) concentrations <70 mg/dL (1.813 mmol/L) (3). Although measurement of lipoprotein cholesterol such as HDL-C provides important information, measurement of lipoprotein subclasses may yield mechanistic insights and potentially further prognostic information beyond conventional lipoprotein measurements (4).

Lipoproteins such as VLDL, LDL, and HDL are each composed of a heterogeneous group of subclasses (5). Some studies have shown that larger HDL particle
subclasses are inversely associated with CAD incidence, whereas smaller HDL particle classes are positively correlated with CAD (5, 6). Studies have also demonstrated that measurement of LDL subclasses provides information beyond LDL-C concentration (5, 7).

Two proteins that play important roles in HDL metabolism are ATP-binding cassette transporter A1 (ABCA1) and cholesteryl ester transfer protein (CETP). ABCA1, a transmembrane protein present on peripheral tissue cells, participates in the first step of reverse cholesterol transport (RCT) by transporting free cholesterol out of the cell, where cholesterol binds with apoA1 to form pre-β HDL (8). The A allele of the ABCA1^1051G/A polymorphism has been associated with higher HDL-C concentrations (9-11), although its association with HDL or LDL subclass is not known.

CETP participates in HDL metabolism by facilitating the transfer of cholesteryl esters from HDL to apoB-containing lipoproteins in exchange for triglycerides being transferred to HDL (12). The association of CETP polymorphisms with HDL-C concentrations has been extensively studied (13-18). Previous studies have also shown that the B2B2 genotype of the TaqIB polymorphism is associated with higher concentrations of HDL2 (13) and HDL3 subclasses (13, 14).

Whereas polymorphisms in both the ABCA1 and CETP genes are associated with HDL-C concentrations, their influences on HDL-C are not reliable predictors of either CAD or subclinical markers of CAD (9, 19). Therefore, it is possible there are qualitative differences in HDL that are not detected by measuring HDL-C alone (20). In the current investigation, we examined the association of nuclear magnetic resonance (NMR)-determined lipoprotein subclasses with ABCA1 and CETP polymorphisms in a subsample from the Multi-Ethnic Study of Atherosclerosis (MESA) to determine if this approach explains the previously published differential clinical outcomes (19) in response to changes in HDL-C concentrations.

**Materials and Methods**

**SUBJECTS AND BLOOD SAMPLES**

The design of the MESA study was outlined by Bild et al. (21), and information about the MESA protocol is available at www.mesa-nhlbi.org. Briefly, 6814 men and women between the ages of 45 and 84 years without clinical evidence of cardiovascular disease were recruited from 6 communities in the US. Subjects who self-reported their race/ethnicity group as white or Caucasian, black or African-American, Chinese, or Spanish/Hispanic/Latino were potentially eligible. Institutional Review Board approval was obtained at all MESA sites, and all participants gave informed consent.

From the 5030 MESA participants enrolled before February 2002 (before completion of the overall recruitment), we randomly selected a sample of 999 participants for more extensive laboratory testing. All participants in this subsample consented to preparation and use of their DNA. The racial/ethnic breakdown of this group was 459 (45.9%) white, 99 (9.9%) Chinese-American, 210 (21.0%) African-American, and 231 (23.1%) Hispanic; demographic information on the entire MESA cohort and the participants included in this study are detailed in Benton et al. (22).

**DNA ANALYSES**

We extracted DNA from peripheral leukocytes isolated from packed cells of anticoagulated blood using commercially available reagents (Puregene; Gentra Systems). The ABCA1 1051G/A (rs#2230806) and −565C/T (previously designated as −477) (rs#2422493) polymorphisms were genotyped as described by Woll et al. (10) and CETP TaqIB (rs#708272), −2505C/A, −629C/A (rs#1800775), R451Q (rs#1800777), and A373P (rs#5880) polymorphisms as described (15-18).

All CETP polymorphisms were in Hardy–Weinberg equilibrium in each racial/ethnic group, as determined by gene counting and χ² tests. For ABCA1, both polymorphisms were in Hardy–Weinberg equilibrium in each racial/ethnic group except for the −565C/T polymorphism in whites (P = 0.02). For the CETP A373P and R451Q polymorphisms, the minor alleles were too rare to use for the Hardy–Weinberg test.

Minor allele frequencies of the ABCA1 polymorphisms were 1051, A allele, 39%, and −565, T allele, 49%. Minor allele frequencies of the CETP polymorphisms were TaqIB, B2 allele, 38%; −2505, A allele, 31%; −629, A allele, 49%; A373P, P allele, 5%; and R451Q, Q allele, 4%.

**BIOCHEMICAL ASSAYS**

We measured triglyceride, HDL-C, and LDL-C concentrations as described (19).

**LIPOPROTEIN SUBCLASS**

We used proton NMR spectroscopy to measure HDL and LDL subclass distribution (Liposcience) in μmol/L and nmol/L, respectively. The concentrations of 3 HDL and 2 LDL subclasses were determined: large HDL (defined as particles 8.8–13 nm), medium HDL (8.2–8.8 nm), small HDL (7.3–8.2 nm), large LDL (21.2–23

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10 Human genes: ABCA1, ATP-binding cassette, subfamily A, member 1; CETP, cholesteryl ester transfer protein, plasma.
Genetic Variants and Lipoprotein Subclasses

STATISTICAL ANALYSES
We performed statistical analysis using R (R Development Core Team, http://www.R-project.org). When each polymorphism was analyzed individually regarding its associations with lipoproteins and subclasses, multiple linear regression was used to adjust for age, race/ethnicity, sex, body mass index (BMI), waist circumference, diabetes, serum insulin level, smoking, and use of lipid-lowering medications. We used t-test to compare the adjusted means of 2 genotype groups and F-test to test the null hypothesis of no mean difference among 3 genotype groups. We also used F-test to determine if there were any interactions between race/ethnicity and ABCA1 1051G/A genotypes.

We conducted regression analysis with haplotypes of the 5 CETP polymorphisms in R, based on the method of Zaykin et al. (23). Haplotype frequencies were estimated using the EM algorithm implemented in the R package haplo.stats. Haplotypes with a frequency of <0.01 were pooled together. The same covariates used in single polymorphism analysis were also used in the haplotype analysis.

Results
Log-transformed triglyceride concentrations were associated with decreased large HDL subclass \( (R = -0.50) \) and moderately associated with increased medium and small HDL subclass concentrations, resulting in little change in total HDL particle concentration \( (R = -0.03) \), although they were associated with decreased HDL particle size \( (R = -0.52) \). With respect to LDL, triglyceride concentration was associated with decreased large LDL and increased small LDL \( (R = -0.38 \) and 0.56, respectively\), resulting in decreased average LDL size \( (R = -0.56) \) and increased LDL particle number \( (R = 0.49) \). As expected, increased HDL-C was highly correlated with increased large HDL \( (R = 0.90) \), and increased LDL-C was correlated with increased LDL particle number \( (R = 0.72) \) and small LDL \( (R = 0.45) \). HDL size was highly correlated with LDL size \( (R = 0.81) \).

Table 1 shows mean differences in triglyceride, HDL-C, and LDL-C concentrations as a function of ABCA1 and CETP genotypes. The ABCA1 1051 GA genotype was associated with an 8.1% increase in triglycerides, mg/dL

<table>
<thead>
<tr>
<th>Polymorphism (reference genotype)</th>
<th>Comparison genotype</th>
<th>Triglycerides, mg/dL</th>
<th>HDL-C, mg/dL</th>
<th>LDL-C, mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
</tr>
<tr>
<td><strong>ABCA1</strong></td>
<td></td>
<td><strong>P</strong></td>
<td><strong>P</strong></td>
<td><strong>P</strong></td>
</tr>
<tr>
<td>1051G/A (GG)</td>
<td>GA</td>
<td>0.078 (0.034)</td>
<td>-0.93 (0.89)</td>
<td>1.7 (2.2)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>-0.026 (0.047)</td>
<td>1.68 (1.24)</td>
<td>-5.8 (3.0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>0.02</strong></td>
<td><strong>0.03</strong></td>
<td><strong>0.056</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>0.02</strong></td>
<td><strong>0.03</strong></td>
<td><strong>0.056</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>0.02</strong></td>
<td><strong>0.03</strong></td>
<td><strong>0.056</strong></td>
</tr>
<tr>
<td>CETF</td>
<td>TaqIB (B1B1)</td>
<td>0.025 (0.033)</td>
<td>2.13 (0.86)</td>
<td>3.0 (2.2)</td>
</tr>
<tr>
<td></td>
<td>B2B2</td>
<td>-0.095 (0.047)</td>
<td>6.62 (1.22)</td>
<td>0.1 (3.0)</td>
</tr>
<tr>
<td></td>
<td>-2505C/A (CC)</td>
<td>-0.055 (0.032)</td>
<td>2.92 (0.84)</td>
<td>0.2 (2.1)</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>-0.111 (0.052)</td>
<td>6.72 (1.34)</td>
<td>4.7 (3.3)</td>
</tr>
<tr>
<td></td>
<td>-629C/A (CC)</td>
<td>-0.026 (0.038)</td>
<td>0.92 (0.98)</td>
<td>1.5 (2.4)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>-0.088 (0.043)</td>
<td>5.18 (1.12)</td>
<td>0.7 (2.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>0.03</strong></td>
<td><strong>0.02</strong></td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>0.02</strong></td>
<td><strong>0.03</strong></td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>0.02</strong></td>
<td><strong>0.03</strong></td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td></td>
<td>A373P (AA)</td>
<td>0.115 (0.051)</td>
<td>-3.03 (1.34)</td>
<td>4.2 (3.3)</td>
</tr>
<tr>
<td></td>
<td>PP</td>
<td>0.530 (0.338)</td>
<td>-18.78 (8.87)</td>
<td>-1.0 (2.1)</td>
</tr>
<tr>
<td></td>
<td>R451Q (RR)</td>
<td>0.114 (0.058)</td>
<td>-3.39 (1.52)</td>
<td>5.7 (3.7)</td>
</tr>
<tr>
<td></td>
<td>QQ</td>
<td>0.528 (0.338)</td>
<td>-18.75 (8.87)</td>
<td>-0.9 (2.1)</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, race/ethnicity, BMI, waist circumference, diabetes, serum insulin level, smoking, and use of lipid-lowering medications.

** To convert triglyceride concentrations to mmol/L, multiply by 0.0113; to convert HDL-C and LDL-C concentrations to mmol/L, multiply by 0.0259. Triglyceride values are log-transformed.

* P values are based on t-test comparing mean of comparison genotype to the indicated reference genotype for each polymorphism using a codominant model.
Table 2. Differences in HDL subclass particle concentrations between **ABCA1** and **CETP** polymorphisms compared with reference genotype, in a subsample of the MESA population.

<table>
<thead>
<tr>
<th>Polymorphism (reference genotype)</th>
<th>Comparison genotype</th>
<th>Large HDL, μmol/L</th>
<th>Medium HDL, μmol/L</th>
<th>Small HDL, μmol/L</th>
<th>HDL size, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>P</td>
</tr>
<tr>
<td><strong>ABCA1</strong></td>
<td></td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td>Mean (SE)</td>
<td></td>
</tr>
<tr>
<td>1051G/A (GG)</td>
<td>GA</td>
<td>−0.25 (0.24)</td>
<td>−0.51 (0.30)</td>
<td>0.086</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>0.33 (0.33)</td>
<td>1.11 (0.41)</td>
<td>0.99 (0.44)</td>
<td>0.024</td>
</tr>
<tr>
<td><strong>CETP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqIB (B1B1)</td>
<td>B1B2</td>
<td>1.77 (0.32)</td>
<td>−0.20 (0.28)</td>
<td>−0.86 (0.30)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>B2B2</td>
<td>0.32 (0.41)</td>
<td>0.49</td>
<td>0.044</td>
<td>0.104</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>1.95 (0.36)</td>
<td>−0.83 (0.45)</td>
<td>0.79 (0.48)</td>
<td>0.102</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>1.52 (0.26)</td>
<td>0.01 (0.33)</td>
<td>−0.48 (0.35)</td>
<td>0.414</td>
</tr>
<tr>
<td></td>
<td>A373P (AA)</td>
<td>1.28 (0.30)</td>
<td>0.04 (0.38)</td>
<td>−1.07 (0.40)</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>PP</td>
<td>−0.95 (0.36)</td>
<td>0.39 (0.45)</td>
<td>0.74 (0.48)</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>R451Q (RR)</td>
<td>−5.29 (2.36)</td>
<td>1.79 (2.96)</td>
<td>−1.83 (3.15)</td>
<td>0.237</td>
</tr>
<tr>
<td></td>
<td>QQ</td>
<td>−0.81 (0.40)</td>
<td>0.30 (0.51)</td>
<td>0.76 (0.54)</td>
<td>0.513</td>
</tr>
</tbody>
</table>

* Values are adjusted for age, sex, race/ethnicity, BMI, waist circumference, diabetes, serum insulin level, smoking, and use of lipid-lowering medications.

**P** values are based on t-test comparing mean of comparison genotype to the indicated reference genotype for each polymorphism using a codominant model.

Using linear regression, we calculated estimated mean differences between minor allele–containing heterozygotes and homozygotes compared with wild-type homozygotes for all **ABCA1** and **CETP** polymorphisms. Table 2 shows that the GA and AA genotypes of the **ABCA1** 1051G/A polymorphism were associated with 3.5% and 5.5% higher small HDL subclass particle concentrations, respectively, compared with the GG genotype (P = 0.045 and 0.024, respectively; 2-degree-of-freedom t-test, P = 0.04). The AA genotype was also associated with 21.4% lower medium HDL particle concentrations compared with the GG genotype (P = 0.007; 2-degree-of-freedom t-test, P = 0.02). The −565C/T polymorphism was not associated with any HDL subclass particle concentrations (data not shown).

Table 2 also shows that the **CETP** TaqIB B2B2, −2505 CA, −2505 AA, and −629 AA genotypes were associated with 36.5%, 18.3%, 40.7%, and 25.4% higher large HDL subclass particle concentrations (P < 0.0001 for all) compared with the TaqIB B1B1, −2505 CC, and −629 CC genotypes. The A373P AP and PP genotypes and R451Q RQ and QQ genotypes were associated with 7% (AP and RQ genotypes) and 41% (PP and QQ) lower HDL-C (P = 0.024–0.035 for all).

Using linear regression, we calculated estimated mean differences between minor allele–containing heterozygotes and homozygotes compared with wild-type homozygotes for all **ABCA1** and **CETP** polymorphisms. Table 2 shows that the GA and AA genotypes of the **ABCA1** 1051G/A polymorphism were associated with 3.5% and 5.5% higher small HDL subclass particle concentrations, respectively, compared with the GG genotype (P = 0.045 and 0.024, respectively; 2-degree-of-freedom t-test, P = 0.04). The AA genotype was also associated with 21.4% lower medium HDL particle concentrations compared with the GG genotype (P = 0.007; 2-degree-of-freedom t-test, P = 0.02). The −565C/T polymorphism was not associated with any HDL subclass particle concentrations (data not shown).

Table 3 shows the estimated genotypic effect of **ABCA1** and **CETP** polymorphisms on LDL subclass particle concentrations. Although the **ABCA1** 1051G/A polymorphism was not, genotypes of the **CETP** polymorphisms were associated with significant differences in LDL subclass particle concentrations. The **CETP** TaqIB B2B2, −2505 CA, −2505 AA, and −629AA ge-
notypes were associated with significantly higher (13%–26%) large LDL subclass particle concentrations ($P < 0.0002$ for all genotypes) and significantly lower (8%–17%) concentrations of small LDL subclass particles ($P < 0.0002$ for TaqIB B2B2, $P < 0.0005$ for $−2505$ CA, $P = 0.002$ for $−2505$ AA, and $P = 0.001$ for $−629$ AA). In contrast, the A373P and R451Q polymorphisms had the opposite effect on LDL subclass particle concentrations. PP genotype of A373P and QQ genotype of R451Q were associated with 81% lower particle concentrations. PP genotype of A373P and QQ

### Table 3. Differences in LDL subclass particle concentrations between $ABCA1$ and $CETP$ polymorphisms compared with reference genotype, in a subsample of the MESA population.$^a$

<table>
<thead>
<tr>
<th>Polymorphism (reference genotype)</th>
<th>Comparison genotype</th>
<th>Large LDL, nmol/L</th>
<th>Small LDL, nmol/L</th>
<th>LDL size, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (SE) $^b$</td>
<td>Mean (SE) $^b$</td>
<td>Mean (SE)</td>
</tr>
<tr>
<td>$ABCA1$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1051G/A (GG)</td>
<td>GA</td>
<td>$−19.5$ (12.9)</td>
<td>$28.0$ (29.5)</td>
<td>$0.1$ (0.1)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>$−23.6$ (17.9)</td>
<td>$−0.8$ (40.8)</td>
<td>$0.0$ (0.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$CETP$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TaqIB (B1B1)</td>
<td>B1B2</td>
<td>$42.5$ (12.6)</td>
<td>$−80.8$ (28.6)</td>
<td>$0.2$ (0.1)</td>
</tr>
<tr>
<td></td>
<td>B2B2</td>
<td>$81.0$ (17.7)</td>
<td>$−173.0$ (40.3)</td>
<td>$0.3$ (0.1)</td>
</tr>
<tr>
<td></td>
<td>$−2505$C/A (CC)</td>
<td>$55.1$ (12.2)</td>
<td>$−114.0$ (27.8)</td>
<td>$0.2$ (0.1)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>$87.4$ (19.4)</td>
<td>$−137.4$ (44.2)</td>
<td>$0.3$ (0.1)</td>
</tr>
<tr>
<td></td>
<td>$−629$C/A (CC)</td>
<td>$25.7$ (14.3)</td>
<td>$−47.2$ (32.5)</td>
<td>$0.1$ (0.1)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>$61.0$ (16.4)</td>
<td>$−128.6$ (37.2)</td>
<td>$0.3$ (0.1)</td>
</tr>
<tr>
<td></td>
<td>A373P (AA)</td>
<td>$34.1$ (19.4)</td>
<td>$135.6$ (44.0)</td>
<td>$0.2$ (0.1)</td>
</tr>
<tr>
<td></td>
<td>PP</td>
<td>$−300.5$ (128.5)</td>
<td>$786.7$ (291.1)</td>
<td>$−1.2$ (0.5)</td>
</tr>
<tr>
<td></td>
<td>R451Q (RR)</td>
<td>$−16.4$ (22.0)</td>
<td>$118.3$ (49.9)</td>
<td>$−0.2$ (0.1)</td>
</tr>
<tr>
<td></td>
<td>QQ</td>
<td>$−297.7$ (128.7)</td>
<td>$781.5$ (291.6)</td>
<td>$−1.2$ (0.5)</td>
</tr>
</tbody>
</table>

$^a$ Values are adjusted for age, sex, race/ethnicity, BMI, waist circumference, diabetes, serum insulin level, smoking, and use of lipid-lowering medications.

There were 7 $CETP$ haplotypes with a frequency $>1\%$. The 2 most common haplotypes were B1-C-C-A-R (frequency 43%) and B2-A-A-A-R (frequency 26%) (alleles listed in the order TaqIB, $−2505$C/A, $−629$C/A, A373P, R451Q). For many of the subclass particle concentrations, the B2-A-A-A-R haplotype showed a significant difference compared with the wild-type B1-C-C-A-R haplotype. In particular, the B2-A-A-A-R haplotype showed a significant difference compared with the wild-type B1-C-C-A-R haplotype. In particular, the B2-A-A-A-R haplotype was associated with higher large HDL subclass concentration [mean (SE) 1.36 (0.29) $\mu$mol/L; $P = 4 \times 10^{-6}$] and with lower small HDL subclass concentration [1.16 (0.39) $\mu$mol/L; $P = 0.003$]. It was also associated with higher HDL-C [5.25 (1.10) mg/dL, 0.136 (0.0285) mmol/L; $P = 2 \times 10^{-6}$] and HDL particle size [0.16 (0.03) nm; $P = 2 \times 10^{-7}$]. Interestingly, while the B2-A-A-A-R haplotype was not associated with LDL-C, it was associated with lower concentrations of small LDL subclass particles [118.0 (37.0) nmol/L; $P = 0.0015$] and higher large LDL [54.4 (15.8) nmol/L; $P = 0.0006$], as well as LDL particle size [0.26 (0.059) nm; $P = 1 \times 10^{-5}$].

### Discussion

$ABCA1$ and $CETP$ are proteins that play important roles in the RCT pathway of HDL metabolism. It has been well documented that polymorphisms in these genes are associated with changes in HDL-C concen-
tations (9–11, 13, 14, 19, 22). The differences in HDL-C concentrations associated with these polymorphisms, however, are not reliable predictors of their potential cardiovascular benefit (9, 10, 19, 24). In the current study, we measured lipoprotein subclasses by NMR to determine whether additional information gained from such measurement yields insights not provided by conventional lipoprotein measurements.

Whereas ABCA1 and CETP play important roles in the RCT pathway, changes in HDL subclasses associated with polymorphisms of each gene were distinct. We demonstrated that the ABCA1 1051AA genotype was associated with a significantly higher concentration of small HDL particles and a lower concentration of medium HDL particles than the 1051GG genotype. With respect to the CETP gene variants, the TaqIB B2, −2505A, and −629A alleles of the noncoding region polymorphisms were associated with higher concentrations of large HDL particles and lower concentrations of small HDL particles. The minor alleles of the A373P and R451Q polymorphisms (P and Q, respectively) were associated with lower concentrations of large HDL particles.

Large HDL particles (HDL2) are thought to be more atheroprotective than small HDL (HDL3) particles (5, 6). Increased serum triglycerides decrease total HDL-C and result in a decreased HDL2/HDL3 ratio. Thus, individuals with relatively high serum triglycerides (>250 mg/dL (>2.825 mmol/L]) often have low HDL2/HDL3 ratios, and this pattern of HDL subclasses has been shown to be associated with increased risk of CAD (25). In the entire MESA cohort, studies of lipoprotein particles by NMR also confirmed that large HDL particles were inversely correlated with intima-media thickness (IMT) (7). In contrast, increases in small HDL particle concentrations have also been demonstrated to be associated with reduced risk of CAD when the increase of small HDL occurs in the absence of decreased total or large HDL particle concentrations. For example, significantly higher small HDL particle concentrations have been reported in the Veterans Affairs HDL Cholesterol Intervention Trial (VA-HIT) (26) and Bezafibrate Coronary Atherosclerosis Intervention Trial (BECATI) (27), where participants were treated with gemfibrozil and bezafibrate, respectively. The increase in small HDL particle concentration was independent of HDL-C concentration and was shown to be a strong and clinically significant predictor of CAD event reduction (26). The increased small HDL particle concentration is thought to be the result of the binding of fibrates to the nuclear receptor PPAR-α (peroxisome proliferator–activated receptor α) which increases LXR-α (liver X-receptor α) and ABCA1 expression (28), which in turn increases cholesterol efflux and leads to increased formation of HDL particles.

In a previous publication (22), we demonstrated in this same cohort that the ABCA1 1051A allele is atheroprotective. Our finding in the current study that the 1051A allele is associated with a significantly higher small HDL particle concentration is consistent with the concept that the atheroprotective role of the 1051A allele may be due to increased ABCA1 activity, resulting in increased conversion of either the discoidal pre-β-migrating HDL or the larger α-nascent apoAI-containing particles to HDL3-like particles (29, 30). The NMR spectroscopy used in the current study does not detect pre-β HDL. However, increased pre-β HDL as detected by 2-dimensional gel electrophoresis has been demonstrated to be inversely correlated with CAD, most likely due to deficiencies in lecithin:cholesterol acyltransferase required to convert pre-β HDL to mature spherical HDL particles (31, 32). We speculate that our finding of increased small HDL particles is due to increased pre-β HDL particles that were rapidly converted to HDL3-like particles. Direct measurement of pre-β HDL particles is needed to confirm this hypothesis.

We have previously shown that the minor allele genotypes of the noncoding region CETP variants (TaqIB B2B2, −2505AA, −629AA) are associated with lower CETP activity and higher HDL-C concentration (19). In the current study, these genotypes were also associated with higher large HDL subclass particle concentrations, higher large LDL particle concentrations, and lower small LDL particle concentrations, patterns typically interpreted as protective against atherosclerosis (6, 25, 33, 34). However, our previous study (19) showed that these genotypes were not associated with a decrease in subclinical cardiovascular disease, assessed as degree of carotid IMT or the presence of either carotid stenosis or coronary artery calcium, a finding in agreement with Kakko et al. (35). We speculate that although decreased CETP results in increased quantity and size of HDL particles, such benefit may be offset either by a potential decrease of RCT and/or feedback inhibition on cholesterol efflux from peripheral cells (36). Most recently, clinical trials of torcetrapib, a CETP inhibitor that significantly raises serum HDL-C concentrations and HDL particle sizes, was terminated because torcetrapib did not provide cardioprotective effects as measured by the progression of carotid IMT (37, 38), despite a 50% increase in HDL-C. This may be partly due to reduced RCT, particularly when CETP inhibitors are administered along with statins (36).

In contrast to the noncoding region CETP polymorphisms, which were not associated with subclinical markers of CAD, our previous study demonstrated that the 373P and 451Q alleles, which were associated with
higher activity and concentration of the CETP protein and lower HDL-C, were also associated with significantly increased presence of coronary artery calcium and carotid stenosis (19). In the current study, we found that these 2 coding region polymorphisms were associated with lower concentrations of large HDL particles, lower large LDL particle concentrations and higher small LDL particle concentrations, changes that are usually interpreted as proatherogenic.

Thus, we have demonstrated that ABCA1 and CETP polymorphisms are associated with different, and distinct, changes in HDL and LDL subclass particle concentrations. We showed that the ABCA1 1051A allele was not associated with a statistically higher HDL-C concentration, yet it was associated with a significantly higher small HDL particle concentration. This is in agreement with the finding that higher small HDL particle concentrations in individuals treated with fibrates may be mediated through activation of ABCA1 gene expression (26, 27), and higher small HDL particle concentration in the absence of lower large HDL particle concentration may be viewed as a marker of increased cholesterol efflux.

With regard to the noncoding region CETP polymorphisms, decreased CETP activity may have both anti- and proatherogenic properties. This may account for the lack of significant cardioprotective effects of the noncoding region CETP polymorphisms in our previous study (19) as well as the contradictory results on the association of CETP polymorphisms with cardioprotective effects (35, 39, 40). It is likely that the degree of changes in CETP may result either in a net increase or decrease in RCT (36).

A limitation of this study is that in the analysis of CETP haplotypes, the large number of rare haplotypes greatly diminished the analytical power to determine risk of haplotypes. Also, only 1 ABCA1 polymorphism was analyzed for this study, and therefore confirmatory studies using other polymorphisms are needed.

In conclusion, our study of the influence of ABCA1 and CETP genetic variants on lipoprotein subclasses demonstrates the importance of interpreting lipoprotein subclasses within the context of the biochemical processes involved in the alterations. Thus, the association of higher large HDL subclass particle concentration with lower CETP activity, caused either by a genetic variant or a CETP-inhibiting drug, may not be protective, as there may be a loss of function of these large HDL particles. In our previous study on the association of CETP polymorphisms with subclinical markers of cardiovascular disease (19), we speculated that there is a threshold effect of CETP activity and that CETP inhibitors may be most useful in individuals with high CETP activity, such as those with hypertriglyceridemia. The current study demonstrates that whereas study of the lipoprotein subclasses is thought to provide information beyond that of measuring LDL-C and HDL-C concentrations alone, at least with regard to HDL, the study of subclasses based on size alone may not be sufficient. Thus, reliable assays of HDL function are needed, both for the assessment of the usefulness of drugs that raise HDL-C concentration and for use in specialized clinical laboratories to improve cardiovascular risk assessment.

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