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Cholesteryl Ester Transfer Protein Genetic Polymorphisms, HDL Cholesterol, and Subclinical Cardiovascular Disease in the Multi-Ethnic Study of Atherosclerosis

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Abstract

The cholesteryl ester transport protein (CETP) plays a key role in high-density lipoprotein (HDL) metabolism. Genetic variants that alter CETP activity and concentration may cause significant alterations in HDL-cholesterol (HDL-C) concentration; however, controversies remain about whether these genetic variants are associated with atherosclerosis. We genotyped the *CETP* R451Q, A373P, -629C/A, Taq1B, and -2505C/A polymorphisms in a cohort of Caucasian, Chinese, African-American, and Hispanic individuals within the Multi-Ethnic Study of Atherosclerosis. Genotypes were examined in relationship to HDL-C, CETP activity, CETP concentration, and three measures of subclinical cardiovascular disease (CVD): coronary artery calcium (CAC) measured by fast CT scanning, and carotid intimal-medial thickness (IMT) and carotid artery plaque, measured by ultrasonography. Carriers of the 451Q and 373P alleles have significantly higher CETP concentration (22.4% and 19.5%, respectively; $p<0.001$) and activity (13.1% and 9.4%, respectively; $p<0.01$) and lower HDL-C (5.6% and 6.0%, respectively; $p<0.05$). The minor alleles of the R451Q and A373P polymorphisms are associated with the presence of CAC, even after adjusting for CVD risk factors and HDL-C ($p=0.006$ and $p=0.01$, respectively). The R451Q polymorphism is also associated with presence of carotid artery plaque ($p=0.036$). Neither polymorphism is associated with common or internal carotid IMT. We confirmed that the -629A, Taq1B B2, and -2505A alleles are significantly associated with lower CETP concentration

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(20.8%, 25.0%, and 23.7%, respectively; p<0.001) and activity (14.8%, 19.8%, and 18.4%, respectively; p<0.001) and higher HDL-C concentration (9.7%, 11.5%, and 10.4%, respectively; p<0.01). However, we did not find any associations between these non-coding polymorphisms and subclinical CVD.

Keywords

CETP; CVD; HDL; MESA

1. Introduction

High-density lipoprotein cholesterol (HDL-C) has long been recognized as being atheroprotective [1]. In recent years, epidemiological studies have shown that low HDL-C concentrations are the most common lipid abnormality in patients with premature cardiovascular disease (CVD) [2–4]; thus, there has been increased interest in the regulation of HDL-C by proteins such as cholesteryl ester transfer protein (CETP) that are involved in HDL-C metabolism.

CETP facilitates the exchange of cholesteryl esters and triglycerides between high-density lipoprotein (HDL) and Apo-B containing lipoproteins [5]. Thus, CETP plays an important role in the reverse cholesterol transport pathway, as changes in CETP activity are inversely correlated with plasma HDL-C concentrations [6]. CETP inhibitors significantly raise plasma HDL-C concentration; however, clinical trials of one CETP inhibitor, torcetrapib, have been discontinued due in part to the lack of atheroprotective effects. Studying CETP genetic variants that also alter CETP activity may potentially provide insight into why torcetrapib failed to provide atheroprotective effects.

Many common *CETP* polymorphisms have been shown to be associated with CETP activity and/or concentration and with HDL-C concentration. Two *CETP* polymorphisms (R451Q and A373P) located in the coding region of the gene have been studied. The minor alleles of these two polymorphisms, Q and P, respectively, appear at a low frequency in the general population, each having a minor allele frequency of 2–7% in Western European cohorts [7, 8]. The minor alleles of these polymorphisms have been associated with lower HDL-C concentrations [7] and higher CETP activity [9].

Two other polymorphisms (-629C/A and Taq1B) are relatively prevalent in Caucasians [10–12]. The -629C/A promoter polymorphism, unlike Taq1B, is thought to be a functional polymorphism [7, 9, 11], and the A allele is associated with higher HDL-C concentrations [11, 13–15]. The Taq1B polymorphism, located in intron 1 of the *CETP* gene, has been extensively studied; the B2 allele of this polymorphism has been shown to be associated with higher HDL-C concentrations in several studies [7, 10, 16]. Decreased CETP activity [10, 16] and CETP concentration [11, 15] have also been associated with the Taq1B B2 and -629A alleles. Allele frequencies of the Taq1B polymorphism have been previously reported to differ by racial/ethnic group [17, 18].

Recently, a report described another promoter region polymorphism, -2505C/A. Lu et al. [19] studied this polymorphism in 357 elderly Japanese men from the general population and found the A allele at a frequency of 20%. The AA genotype of -2505C/A was associated with significantly lower CETP concentration and higher HDL-C.

Although CETP is important for the metabolism of HDL, and *CETP* polymorphisms are known to be associated with differences in HDL-C concentrations, reports on the association of *CETP* polymorphisms with risk of CVD have been inconsistent. The Taq1B B1 allele has

been marginally associated with risk of developing CVD [10, 12]. The Q allele of R451Q and the P allele of A373P were found to be associated with lower HDL-C, while risk of ischemic heart disease was paradoxically reported to be decreased 36% in women with the P allele, when adjusted for HDL concentrations [8]. The association of *CETP* polymorphisms with subclinical CVD is even less well characterized. Carotid intimal-medial wall thickness (IMT) was found not to differ by Taq1B or -629C/A polymorphism, while men with AP genotype of the A373P polymorphism had thinner IMT than those with the AA genotype. The reverse was found in women: those with the AP genotype had thicker IMT compared to those with the AA genotype [13]. Similarly, the RQ genotype of the R451Q polymorphism was associated with thinner IMT in men compared to the RR genotype, but this association was not significant in women [9]. To our knowledge, no studies have explored the relationship between *CETP* polymorphisms and coronary artery calcification (CAC).

The purpose of this study is to investigate the associations between five common *CETP* polymorphisms (R451Q, A373P, -629C/A, Taq1B, and -2505C/A) with HDL-C, CETP activity, CETP concentration, and three measures of subclinical CVD (CAC, carotid artery plaque, and carotid IMT) in a cohort of apparently healthy men and women from four racial/ethnic groups included in the Multi-Ethnic Study of Atherosclerosis (MESA).

2. Materials and methods

2.1. Subjects and blood samples

The primary aim of MESA is to investigate characteristics related to subclinical CVD development and progression including racial/ethnic and genetic factors that may influence the disease process. Background information concerning objectives and design of the MESA study are outlined by Bild et al. [20], and information about the MESA protocol is available at www.mesa-nhlbi.org. Briefly, 6,814 men and women between the ages of 45 and 84 years without clinical evidence of CVD were recruited from six communities in the United States (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; New York, NY; and St. Paul, MN). During recruitment, questions on race/ethnicity were asked based on the U.S. 2000 census questionnaire. Subjects who self-reported their race/ethnicity group as White or Caucasian, Black or African-American, Chinese, or Spanish/Hispanic/Latino were potentially eligible. Recruitment and baseline examinations began in July 2000 and were conducted over a 24-month period. Institutional Review Board approval was obtained at all MESA sites, and all participants gave informed consent.

A sample of 999 participants was randomly selected from the 5,030 MESA participants enrolled prior to February 2002, before completion of the overall recruitment, for more extensive laboratory testing. All participants in this subgroup consented to preparation and use of their DNA. Individuals were excluded if they were taking lipid-lowering medication or did not have complete *CETP* genotype data (N=144), thus leaving 855 individuals for the present analysis.

Serum and EDTA-anticoagulant tubes were collected from participants in the fasting state and processed using a standardized protocol [21]. The serum and plasma samples were aliquoted and stored at −70°C until time of use.

2.2. DNA analysis

DNA was extracted from peripheral leukocytes isolated from packed cells of anticoagulated blood using commercially available reagents (Puregene, Gentra Systems, Minneapolis, MN). The DNA was stored at −70°C until time of use.

The R451Q and A373P polymorphisms were genotyped following Agerholm-Larsen et al. [8]; -629C/A polymorphism was genotyped as described by Eiriksdottir et al. [14], and the Taq1B polymorphism was genotyped as described by Fumeron et al. [22]. To detect the -2505C/A polymorphism, the method of Lu et al. [19] was followed, and the resulting restriction enzyme DNA fragments were detected by electrophoresis on a 3.5% agarose gel stained with Sybr Gold (Molecular Probes, Eugene, OR). All genotyping methods were verified by sequencing, and known samples of each genotype were assayed within each batch.

2.3. Biochemical assays

Total cholesterol and HDL-C were measured in EDTA plasma on the Roche/Hitachi 911 Automatic Analyzer (Roche Diagnostics Corporation, Indianapolis, IN) using a cholesterol esterase, cholesterol oxidase reaction (Chol R1, Roche Diagnostics Corporation). Before measurement of HDL-C, the non-HDL-C fractions were precipitated with magnetic 50,000 MW dextran sulfate and magnesium chloride. Triglycerides were measured using a glycerol blanked enzymatic method (Trig/GB, Roche Diagnostics Corporation). LDL-C was calculated in specimens having a triglyceride value <400 mg/dL using the Friedewald equation.

2.4. CETP activity and concentration

CETP activity was measured in EDTA-anticoagulated plasma using the fluorescence-based CETP Activity Kit from Roar Biomedical, Inc. (New York, NY).

CETP concentration was measured in serum using the CETP Test ELISA from Wako Chemicals USA (Richmond, VA).

2.5. Subclinical disease measures

CAC was determined using computed tomography of the chest, as described by Bild et al. [20], (Imatron C-150, Imatron, San Francisco, CA; Lightspeed, General Electric Medical Systems, Waukesha, WI; or Volume Zoom, Siemens, Erlanger, Germany) and was an average of two scores adjusted for phantoms of known physical calcium concentration [23, 24]. Analyses were performed for presence of CAC (Agatston score >0) and also for the degree of CAC in those with a CAC score >0.

Carotid IMT was measured using high-resolution B-mode ultrasonography (Logiq 700, General Electric Medical Systems) as the distance between lumen-intima and media-adventitia interfaces of the near and far walls of the common carotid artery and the internal carotid artery (including the bifurcation and 1 cm distal to the bifurcation). A maximum IMT for each of these two segments was calculated. The current analyses included separate measures of internal and common carotid IMT [25].

Carotid artery plaque was defined using both visual and Doppler measurement (Signa CV/i, General Electric Medical Systems; Signa LX, General Electric; Vision, Siemens; or Symphony, Siemens), as described by Bild et al. [20]. For statistical analyses, carotid plaque was analyzed as a dichotomous variable defined as “present” when focal intima-media thickening was at least twice the width of the adjacent smooth wall and/or a Doppler peak systolic velocity of 150 cm/second or greater in either carotid artery. Carotid plaque was defined as “absent” when carotid plaque was not observed for either carotid artery.

2.6. Statistical analysis

Deviation from Hardy Weinberg proportions were assessed using the Chi-square goodness of fit test, stratified by race/ethnicity and in the combined cohort. D′ linkage disequilibrium

estimates were calculated. Differences in gene allele frequencies between racial/ethnic groups were determined using gene-counting and Chi-square tests. Additive models were constructed for comparison of mean HDL-C, CETP activity, and CETP concentration between genotypes of each polymorphism. Probability of CAC and probability of carotid plaque were modeled using relative risk regression methodology with log link and Gaussian error structure specified so that direct estimates of relative risk could be obtained. Agatston score was used as the measure of CAC amount and was restricted to participants with positive scores in the analyses. Agatston scores were natural log transformed due to their skewed distribution and modeled using ordinary least squares regression. Common carotid artery IMT and internal carotid artery IMT were modeled using ordinary least squares regression. Analyses were initially stratified by self-reported race/ethnicity. Since no significant interactions between each polymorphism, outcome, and race/ethnicity were detected, pooled analyses, adjusted for race/ethnicity, are presented. Three regression models were constructed (Table 5). The first model (model 1) used age, race/ethnicity, and gender-adjusted values to determine relative risk or coefficients. Body mass index, smoking, diabetes, hypertension, and LDL-C were added to the first model covariates to compose model 2, and model 3 included all these covariates plus HDL-C to determine whether the associations between polymorphisms and subclinical markers of CVD were independent of HDL-C. Reported p-values from the models described above are based on robust standard errors. Significance is defined as $p<0.05$ without adjustment for multiple comparisons. Statistical analyses were performed using STATA 9.1 (College Station, TX).

3. Results

3.1 Frequencies of each polymorphism differed by race/ethnicity groups

Demographic information on the 855 MESA participants included in this study is shown in Table 1. There were significant differences in all factors listed in the table between racial/ethnic groups, except for percentage of males and LDL-C concentrations.

Minor allele frequencies of the R451Q, A373P, -629C/A, Taq1B, and -2505C/A *CETP* polymorphisms in each of the four racial/ethnic groups are shown in Table 2. All polymorphisms were in Hardy-Weinberg equilibrium, both in the MESA cohort selected for this study and within each race/ethnicity stratum of the cohort. The *CETP* R451Q and A373P polymorphisms were not analyzed for Hardy-Weinberg equilibrium, as the minor alleles were too rare, limiting the power to test for equilibrium. There were significant differences in the minor allele frequencies ($p<0.001$) between the four race/ethnicity groups for all the polymorphisms. Both the 451Q and 373P alleles are rare in all four races/ethnicities: the alleles are absent in the Chinese and appear at a frequency of 1% in the African-American cohort. On the other hand, the -629A and Taq1B B2 alleles were common in all four groups. Frequency of the -629A allele was highest in African-Americans, while frequency of the Taq1B B2 allele was lowest in this group. The -2505A allele frequency was similar in all racial/ethnic groups except the Chinese, where the A allele was present at a frequency of 17% compared to about 33% in the other groups.

Pairwise linkage disequilibrium (LD) coefficients (D′) for all *CETP* polymorphisms are shown in Table 3. There was strong LD between the two coding-region polymorphisms, R451Q and A373P, especially in Caucasian and Hispanic subjects. There was also strong LD between the three non-coding polymorphisms, -629C/A, Taq1B, and -2505 C/A.

3.2 *CETP* polymorphisms are associated with HDL-C, CETP activity, and CETP concentration

Table 4 shows the associations of *CETP* polymorphisms with HDL-C, CETP activity and concentration, adjusted for race/ethnicity, using additive models for all polymorphisms.

There were no significant interactions between race/ethnicity and the polymorphisms or the outcome variables. All five polymorphisms were associated with HDL-C, CETP activity, and CETP concentration. Marginal means adjusted for age, gender, and race/ethncity are reported. The extreme sparseness (n=1) of the minor allele homozygous genotypes suggests caution in interpretation of the data for the R451Q and A373P polymorphisms. When the R451Q and A373P polymorphisms were analyzed using dominant models where the heterozygous and minor allele homozygous genotypes were combined for comparison to the major allele homozygous genotype, significance was maintained, similar to that of the additive models. HDL-C concentration was lower in the presence of the 451Q and the 373P alleles (p=0.035 and p=0.018, respectively), but CETP activity and concentration were significantly higher in the presence of the 451Q and 373P alleles (p<0.01 for both polymorphisms). In contrast, the -629A, Taq1B B2, and -2505A alleles were associated with higher HDL-C concentration (p<0.01 for each polymorphism), lower CETP activity (p<0.001 for each polymorphism) and lower CETP concentration (p<0.001 for each polymorphism), in a dose-dependent fashion. Overall, CETP concentration and activity are inversely associated with HDL-C concentration in all five polymorphisms. On average, there is a 2% change in CETP activity and 3% change in CETP concentration for every 1% change in HDL-C concentration

3.3 *CETP* R451Q and A373P polymorphisms are associated with CAC, and R451Q shows association with carotid artery plaque but not carotid IMT

There is an inverse association between HDL-C and common carotid IMT ($r = -0.102$, $p = 0.003$, $n = 847$) and between HDL-C and internal carotid IMT ($r = -0.079$, $p = 0.022$, $n = 840$). There is also a significant difference in HDL-C concentration in individuals without CAC present versus those with CAC > 0 (53.1 vs. 48.4 mg/dL, respectively; $p < 0.001$). However, there is no significant difference in HDL-C concentration in those with or without carotid plaque present (50.6 vs. 51.5 mg/dL, respectively; $p = 0.347$). Therefore, considering the association of *CETP* polymorphisms with differences in HDL-C concentrations, we studied the association of the *CETP* polymorphisms with subclinical CVD measures.

The association of subclinical CVD measures and *CETP* polymorphisms are shown in Table 5. The Q allele of the R451Q and P allele of the A373P polymorphisms were associated with the presence of CAC (relative risk=1.27, p=0.009 and relative risk=1.22, p=0.018, respectively) in model 1, and this association persisted for both polymorphisms after adjustment for other CVD risk factors (model 2). The effect is independent of HDL-C as this association remained significant after adjusting for HDL-C (model 3) (relative risk=1.26, p=0.006 for R451Q; relative risk=1.21, p=0.01 for A373P). There were also modest associations between these polymorphisms and the degree of CAC among those with CAC present (p=0.048 for R451Q and p=0.085 for A373P, n=352) in model 1. After adjustment for other CVD risk factors (model 2), the associations with degree of CAC were attenuated (p=0.060 for R451Q and p=0.078 for A373P), and the association was no longer present when adjusted for HDL-C (model 3).

The R451Q polymorphism was also associated with the presence of carotid artery plaque, in all three models (relative risk = 1.32, p=0.009, model 1; relative risk = 1.26, p=0.037, model 2; relative risk = 1.26, p=0.036, model 3). The A373P polymorphism exhibited marginal association with the presence of carotid plaque in model 1 (p=0.056), but after adjusting for CVD risk factors and HDL-C (model 3), the association was no longer significant (p=0.235).

None of the polymorphisms examined were associated with common or internal carotid IMT, except for a borderline association between R451Q and thicker internal carotid IMT in

model 1 (p=0.058). This association was no longer significant after adjustment in model 3 (p=0.139).

The Taq1B, -629C/A, and -2505C/A polymorphisms were not associated with CAC presence or degree, carotid artery plaque, or common or internal IMT.

4. Discussion

Controversies exist about the role of CETP in atherosclerosis, especially with regard to the effect of genetic variants that alter the activity and concentration of this protein and its regulation of HDL-C. Studies of these genetic variants may be important, particularly since CETP inhibitors were and are still being tested as potential therapeutic agents for raising plasma HDL concentrations. The discontinuation of clinical trials of the CETP inhibitor torcetrapib raised the question of whether the failure of torcetrapib is unique for this molecule or whether inhibition of CETP in general may not provide protection against atherosclerosis [26, 27].

In the current study, we examined *CETP* R451Q and A373P, polymorphisms associated with increased CETP activity and concentration, as well as *CETP* -629C/A, Taq1B and -2505C/A, polymorphisms associated with decreased CETP activity and concentration, and their association with three measures of subclinical CVD: CAC, carotid IMT, and carotid artery plaque.

We found significant differences between racial/ethnic groups with regard to allele frequencies of the five *CETP* polymorphisms in agreement with previous publications [17, 18, 28, 29]. However, despite these differences in allele frequencies between the racial/ethnic groups, we found no significant interactions between HDL-C concentration, CETP activity or concentration and race/ethnicity. Also, there were no interactions between common or internal carotid IMT, CAC, or carotid artery plaque with race/ethnicity. Therefore, pooled analyses adjusted for race/ethnicity are presented.

Our study shows a dose-dependent effect of the three non-coding region polymorphisms on HDL-C concentration. We confirmed findings of others that the A allele of the promoter –629C/A polymorphism and the B2 allele of the intronic Taq1B variant are associated with higher concentrations of HDL-C [7, 10, 11, 13–16]. In addition, higher HDL-C concentration found in carriers of these polymorphisms was associated with both lower activity and lower concentration of the CETP protein, as previously reported [10, 11, 15, 16]. The study of Lu et al. performed in a Japanese population has been the only report concerning the -2505C/A promoter polymorphism [19]. The current study confirms findings of Lu et al. that the -2505A allele is associated with higher HDL-C and lower CETP activity and concentration. With regard to the two CETP coding-region polymorphisms, R451Q and A373P, the minor alleles of these two polymorphisms are relatively rare [7, 13]. In this MESA cohort, the prevalence of the minor allele is 4.0% for 373P and 3.3% for 451Q. In agreement with others [7, 9] we report that carriers of these rare alleles have lower HDL-C concentrations and higher concentration and activity of the CETP protein.

In the current study, we confirmed that HDL-C concentrations are inversely associated with carotid IMT [30, 31]. Despite these findings, and the fact that the five *CETP* polymorphisms investigated were associated with differences in HDL-C concentrations, variants in the CETP gene were not associated with either common or internal carotid IMT. Our results are in agreement with a previous study showing that the Taq1B and -629C/A polymorphisms were not associated with carotid IMT [13]. Although the five *CETP* polymorphisms were not associated with either common or internal carotid IMT, we found that the 451Q allele was associated with carotid artery plaque.

In addition to carotid IMT, we also investigated the association of CETP polymorphisms with a second measure of subclinical atherosclerosis, coronary artery calcification (CAC) as measured by computed tomography. The only previous study that showed an association between CAC and CETP was in a group of diabetic and non-diabetic subjects [32]. In that study, there was an inverse association between CAC and CETP activity measured using endogenous substrates in plasma. We show that the 373P and 451Q alleles, which are associated with higher CETP activity and concentration and lower HDL-C concentration, are also associated with atherogenic effects as manifested by a greater presence of CAC. The finding that the coding-region polymorphisms A373P and R451Q, but not the promoter and intronic polymorphisms Taq1B, -629C/A and -2505C/A, are associated with CAC is intriguing. The exact mechanism for the differential influence of these polymorphisms on CAC is not known. One possibility is that there may be a threshold effect, since the 373P and 451Q alleles are associated with higher CETP activity and lower HDL-C concentrations than are the -629C, Taq1B B1, and -2505C alleles. Our finding that the 451Q allele is also associated with carotid artery plaque strengthens the case that higher CETP activity above a threshold level may predispose individuals to subclinical atherosclerosis. Moreover, it is possible that the effects of the 373P and 451Q alleles may be more pronounced than is reflected by the in vitro activity assays. Our finding is in contrast to that of Agerholm-Larsen et al. [8] in that the atherogenic effects of the 451Q allele persisted when adjusted for HDL-C. Thus measurements downstream of HDL-C concentration may be needed to understand the mechanism.

In a previous study of the same MESA population, we demonstrated that the G1051A (R219K) polymorphism in the *ABCA1* gene, which is associated with slightly higher HDL-C concentrations, is also associated with a significantly lower prevalence of CAC [33]. In the case of the *ABCA1* gene, an increased activity of this protein is postulated to significantly increase the export of cholesterol from peripheral tissues. While the increased ABCA1 activity results in only slightly- to moderately-elevated plasma HDL-C, increased efflux of cholesterol from macrophages (known as macrophage reverse cholesterol transport) is thought to play a particularly important role in protection from atherosclerosis [34]. In contrast, higher HDL-C concentration resulting from lower CETP activity caused by the promoter-region polymorphisms in this study did not offer protection against atherosclerosis. This could be due to the fact that the benefit of slightly elevated HDL-C may be offset by a possible decrease in reverse cholesterol transport under certain circumstances when CETP concentration is decreased. Alternatively, as recently demonstrated by Tanigawa et al. [35], decreased CETP causes the formation of large but functionally inactive HDL molecules. Along with previous findings, our results demonstrate that the genetic influence of CETP, which results in increased HDL-C concentration, does not always lead to diminished atherosclerosis. Thus, while there is a global inverse relationship between HDL-C and risk of CVD and atherosclerosis, the relationship may be modified by specific genetic and epigenetic factors. Further studies on the qualitative aspects of HDL, such as determination of lipoprotein subspecies and the use of functional assays of HDL, are needed for a better understanding of this important phenomenon.

The results of the current genetic study may be relevant to the ongoing clinical trials of other CETP inhibitors such as Roche's JTT-705. Our findings that the genetic variants that were associated with higher CETP activity and lower HDL-C were associated with an increased prevalence of CAC, but that the genetic variants associated with lower CETP activity and higher HDL-C were not associated with less CAC suggests that modulating CETP activity may be beneficial in a select subset of individuals. Further studies are clearly needed to decipher the intricacies of the role CETP plays in atherosclerosis.

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Abbreviations

ABCA1	ATP-binding cassette transporter A1
CAC	coronary artery calcium
CETP	cholesteryl ester transfer protein
CVD	cardiovascular disease
HDL	high-density lipoprotein
HDL-C	HDL-cholesterol
IMT	intimal-medial thickness
MESA	Multi-Ethnic Study of Atherosclerosis

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Table 1

Demographic characteristics of study sample (mean ± S.D or percent).

Variable	Caucasian	Chinese	African-American	Hispanic	Total	p-value
Number of subjects	387 (45 %)	91 (11 %)	176 (21 %)	201 (24 %)	855 (100%)	
Age (years)	60.3 ± 10.6	56.2 ± 8.9	59.4 ± 8.7	56.9 ± 9.0	58.8 ± 9.8	<0.0001
Male	43.9 %	41.8 %	35.8 %	45.8 %	42.5 %	0.216
Diabetes	5.7 %	14.5 %	16.5 %	15.0 %	11.0 %	0.001
BMI (kg/m^2)	27.7 ± 5.3	23.7 ± 3.4	30.9 ± 6.3	29.3 ± 5.0	28.3 ± 5.6	<0.0001
HDL-C (mg/dl)	52.8 ± 16.0	48.4 ± 10.8	52.1 ± 14.0	48.5 ± 12.6	51.1 ± 14.5	<0.0001
LDL-C (mg/dl)	121.2 ± 30.9	111.6 ± 24.5	118.0 ± 32.3	118.6 ± 32.4	118.9 ± 31.0	0.063
Triglycerides (mg/dl)	130.3 ± 70.6	128.4 ± 55.6	103.3 ± 63.3	161.8 ± 141.3	132.0 ± 92.0	<0.0001
Systolic Blood Pressure (mmHg)	123.2 ± 22.0	121.6 ± 21.1	128.3 ± 20.2	120.8 ± 20.1	123.5 ± 21.2	0.004
Diastolic Blood Pressure (mmHg)	69.9 ± 10.0	71.5 ± 10.5	74.0 ± 10.0	70.8 ± 9.8	71.1 ± 10.1	<0.0001
Hypertension medications	28.2 %	17.6 %	45.1 %	26.4 %	30.1 %	<0.0001
Current Smokers	16.0 %	3.3 %	20.1 %	19.4 %	16.3 %	<0.0001
Former Smokers	41.3 %	12.1 %	35.1 %	26.9 %	33.5 %	<0.0001
Prevalence of Coronary Calcification	48.8 %	30.8 %	40.3 %	34.3 %	41.8 %	0.001
Maximum Internal Carotid IMT (mm)	1.064 ± 0.547	0.782 ± 0.324	1.035 ± 0.562	0.936 ± 0.468	0.998 ± 0.520	<0.0001
Maximum Common Carotid IMT (mm)	0.862 ± 0.198	0.789 ± 0.136	0.899 ± 0.174	0.818 ± 0.162	0.851 ± 0.182	<0.0001

The p-value is for the difference in mean or percent between race/ethnicity.

Table 2

Minor allele frequencies of five *CETP* polymorphisms.

		Allele frequency				
Race/ethnicity	n	451 Q[a]	373 P[a]	-629 A[a]	TaqIB B2[a]	-2505 A[a]
Caucasian	387	0.04	0.07	0.47	0.42	0.34
Chinese	91	0.00	0.00	0.51	0.42	0.17
African-American	176	0.01	0.01	0.64	0.27	0.33
Hispanic	201	0.08	0.08	0.48	0.39	0.31

[a] $p<0.001$ for the difference between race/ethnicity.

Table 3

Pairwise linkage disequilibrium coefficients between *CETP* gene polymorphisms.

	D′			
Position (location)	**373 (amino acid)**	**-629 (promoter)**	**Taq1B (intron 1)**	**-2505 (promoter)**
451 (amino acid)	0.999[a] (W)	0.850[b]	0.752[c]	0.589[d]
	---(C)	---	---	---
	0.747[a] (AA)	0.403	0.984	0.981
	0.999[a] (H)	0.647[c]	0.492[d]	0.706[d]
373 (amino acid)		0.648[b]	0.387[d]	0.277
		---	---	---
		0.993[d]	0.984	0.987
		0.649[c]	0.482[d]	0.729[d]
-629 (promoter)			0.982[a]	0.910[a]
			0.941[a]	0.714[e]
			0.999[a]	0.906[a]
			0.945[a]	0.903[a]
Taq1B (intron 1)				0.880[a]
				0.849[c]
				0.767[a]
				0.815[a]

For each pair of *CETP* gene polymorphisms, four coefficients are given, representing the four race/ethnicities, in the order: (W) white or Caucasian; (C) Chinese; (AA) African-American; and (H) Hispanic. A dashed line (---) indicates the rare allele of that polymorphism was not present in the indicated race/ethnicity, therefore no comparison is possible.

[a] $p<10^{-15}$;

[b] $p<10^{-5}$;

[c] $p<0.001$;

[d] $p<0.05$;

[e] $p<10^{-9}$.

Table 4

Associations of *CETP* polymorphisms with mean values of HDL cholesterol, CETP activity, and CETP concentration.

Polymorphism	Genotype	N	HDL-C, mg/dL (SE)	% change	P-value	CETP activity, nmol/mL/hr (SE)	% change	P- value	CETP concentration, ug/mL (SE)	% change	P-value
R451Q	RR	787	49.7 (0.5)		0.035	40.6 (0.5)		<0.001	1.74 (0.02)		<0.001
	RQ	67	46.9 (1.7)	−5.6		45.9 (1.5)	+13.1		2.13 (0.06)	+22.4	
	QQ	1	26.0 (--)	--		55.9 (--)	--		2.00 (--)	--	
A373P	AA	765	49.7 (0.5)		0.018	40.6 (0.5)		0.003	1.74 (0.02)		<0.001
	AP	89	46.7 (1.5)	−6.0		44.4 (1.4)	+9.4		2.08 (0.06)	+19.5	
	PP	1	26.0 (--)	--		55.8 (--)	--		1.99 (--)	--	
-629C/A	CC	199	47.5 (1.0)		<0.001	43.9 (0.9)		<0.001	1.97 (0.04)		<0.001
	CA	432	49.0 (0.7)	+3.2		41.4 (0.7)	−5.7		1.80 (0.03)	−8.6	
	AA	224	52.1 (0.9)	+9.7		37.5 (0.9)	−14.8		1.56 (0.04)	−20.8	
Taq1B	B1B1	325	47.8 (0.8)		<0.001	44.0 (0.7)		<0.001	1.92 (0.03)		<0.001
	B1B2	404	49.8 (0.7)	+4.2		40.0 (0.7)	−9.1		1.75 (0.03)	−8.9	
	B2B2	126	53.3 (1.2)	+11.5		35.3 (1.1)	−19.8		1.44 (0.05)	−25.0	
-2505C/A	CC	408	48.2 (0.7)		0.001	43.0 (0.6)		<0.001	1.90 (0.03)		<0.001
	CA	352	50.5 (0.8)	+4.8		39.6 (0.7)	−7.9		1.68 (0.03)	−11.6	
	AA	93	53.2 (1.4)	+10.4		35.1 (1.3)	−18.4		1.45 (0.05)	−23.7	

Values are mean (standard error). No standard error was calculated (--) when 1 individual represented the group. The percentage change represents the increase (+) or decrease (−) over the baseline genotype; baseline genotypes were designated as: 451 RR, 373 AA, -629 CC, Taq1 B1B1, and -2505 CC. No percentage change was calculated (--) when 1 individual represented the group.

Table 5

Additive genetic model associations of *CETP* polymorphisms with CAC, carotid plaque, and IMT.

Polymorphism	Model[a]		CAC presence	CAC degree	Carotid plaque presence	Common carotid IMT degree	Internal carotid IMT degree
R451Q	1	relative risk	1.27	---	1.32	---	---
		coefficient	---	0.53	---	0.01	0.11
		p-value	0.009	0.048	0.009	0.706	0.058
	2	relative risk	1.29	---	1.26	---	---
		coefficient	---	0.53	---	0.01	0.08
		p-value	0.002	0.060	0.037	0.656	0.136
	3	relative risk	1.26	---	1.26	---	---
		coefficient	---	0.51	---	0.01	0.08
		p-value	0.006	0.074	0.036	0.762	0.139
A373P	1	relative risk	1.22	---	1.23	---	---
		coefficient	---	0.42	---	0.01	0.07
		p-value	0.018	0.085	0.056	0.567	0.218
	2	relative risk	1.23	---	1.15	---	---
		coefficient	---	0.45	---	0.01	0.06
		p-value	0.007	0.078	0.238	0.642	0.285
	3	relative risk	1.21	---	1.15	---	---
		coefficient	---	0.43	---	0.01	0.05
		p-value	0.010	0.096	0.235	0.769	0.294
-629C/A	1	relative risk	0.97	---	0.95	---	---
		coefficient	---	0.02	---	−0.002	−0.02
		p-value	0.582	0.856	0.360	0.846	0.473
	2	relative risk	0.98	---	1.00	---	---
		coefficient	---	0.01	---	0.0002	0.003
		p-value	0.592	0.915	0.945	0.979	0.866
	3	relative risk	1.00	---	0.99	---	---
		coefficient	---	0.02	---	0.002	0.004
		p-value	0.951	0.875	0.928	0.767	0.824
Taq1B	1	relative risk	0.99	---	0.94	---	---

Polymorphism	Model[a]		CAC presence	CAC degree	Carotid plaque presence	Common carotid IMT degree	Internal carotid IMT degree
		coefficient	---	0.05	---	0.003	−0.01
		p-value	0.809	0.35	0.301	0.745	0.714
	2	relative risk	0.99	---	0.97	---	---
		coefficient	---	0.06	---	0.003	0.01
		p-value	0.800	0.630	0.589	0.724	0.643
	3	relative risk	1.02	---	0.97	---	---
		coefficient	---	0.08	---	0.01	0.01
		p-value	0.746	0.556	0.570	0.505	0.590
-2505C/A	1	relative risk	1.02	---	1.01	---	---
		coefficient	---	−0.003	---	0.002	0.02
		p-value	0.667	0.983	0.859	0.808	0.292
	2	relative risk	1.01	---	1.02	---	---
		coefficient	---	0.0004	---	0.001	0.03
		p-value	0.802	0.998	0.730	0.873	0.137
	3	relative risk	1.04	---	1.02	---	---
		coefficient	---	0.008	---	0.003	0.04
		p-value	0.425	0.954	0.737	0.665	0.114

[a]Model 1: adjusted for age, gender, and race/ethnicity.

[a]Model 2: adjusted for age, gender, race/ethnicity, body mass index, smoking, diabetes, hypertension, and LDL-C.

[a]Model 3: adjusted for age, gender, race/ethnicity, body mass index, smoking, diabetes, hypertension, LDL-C, and HDL-C.