Association of Cholesteryl Ester Transfer Protein Activity and TaqIB Polymorphism With Lipoprotein Variations in Japanese Subjects

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Cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl ester from high-density lipoprotein (HDL) to apolipoprotein (apo)B-containing lipoproteins, whereby it potentially regulates steady-state concentrations of HDL-cholesterol (HDL-C), as well as low-density lipoprotein-cholesterol (LDL-C). We performed a multicenter trial to assess the association of CETP activity with plasma lipoprotein levels in 591 Japanese subjects. Women had significantly higher CETP activity (15%) and mass (24%) compared to men. For both genders CETP activity was negatively correlated with HDL-C and HDL_2-C, but positively correlated with LDL-C. B2 allele frequency in TaqIB polymorphism was 40%, with no gender difference. TaqIB genotypes were significantly associated with CETP activity and HDL-C level (both $P < .001$). B1B1 had the highest CETP activity and the lowest HDL-C concentrations, whereas B2B2 had the lowest CETP activity and the highest HDL-C concentrations. However, no statistically significant differences in triglycerides (TG) or LDL-C were observed across TaqIB genotypes. Multivariate analysis revealed that determinants of HDL-C were age, gender, body mass index (BMI), smoking, alcohol intake, exercise, CETP activity, and TG, and for LDL-C were BMI, age, and CETP. These data demonstrate that CETP activity is a significant determinant of HDL-C and LDL-C levels and that TaqIB CETP gene polymorphism affects CETP activity and HDL-C level in Japanese population examined.

HIGH-DENSITY lipoprotein-cholesterol (HDL-C) levels have been shown to be inversely and independently correlated with the risk of coronary artery disease (CAD). Although the antiatherogenic properties of HDL have not been fully elucidated, collective data led to a hypothesis that HDL exerts its cardioprotective function through a process called reverse cholesterol transport (RCT), in addition to anti-inflammatory and antioxidative effects. RCT describes a metabolic pathway initiated by HDL-mediated efflux from peripheral tissues and subsequent delivery to the liver.[1] Indeed, the latest guideline from the Adult Treatment Panel III designates high HDL-C equal to or greater than 60 mg/dL as a negative risk factor.[2]

Cholesteryl ester transfer protein (CETP) facilitates redistribution of cholesteryl ester (CE) and triglyceride (TG) among lipoproteins.[3] There is a net transfer of CE from HDL to apolipoprotein (apo)B-containing lipoproteins via an exchange of TG. Transferred CE is eventually converted to low-density lipoprotein (LDL), then delivered to the liver via a LDL receptor. Thus, in humans, RCT may be dependent on the CETP-mediated pathway relative to direct removal of CE on HDL by scavenger receptor B1 or apoE-mediated holo-particle uptake.[4] The essential role of CETP on human lipoprotein metabolism is evident based on a markedly altered lipoprotein profile in patients with genetic CETP deficiency, mostly reported from Japan.[5,6] Humans homozygous for CETP deficiency have much higher (3- to 6-fold increase) and larger HDL and lower and polydispersed LDL.[7-9] Apart from these extreme cases, however, the inverse relationship between CETP and HDL-C is only apparent in a handful of studies,[10-12] whereas other studies have found nonsignificant[13-18] and even positive relationships.[19] In contrast to the inconsistent association of CETP with HDL, to which CETP primarily functions, it is surprising to find a positive relationship with LDL-C in most studies.

CETP gene polymorphism in intron 1 by TaqIB, originally reported by Kondo et al,[20] together with other studies,[11,16,21,22] showed that the B2 allele was associated with decreased CETP activity (or concentration) and increased HDL-C level, and further associated with a lower CAD risk in some studies.[21,22] In contrast, recent studies from Japan reported that frequency of the B2 allele was somewhat lower (38%) than in Caucasian populations (44%) and the B2 allele has not been associated with higher levels of HDL-C.[18,23,24]

In Japan, HDL-C levels are generally higher when compared to Western countries, at least in part, due to 2 major *CETP* gene mutations, intron14G to A and D442G missense mutation, which are present in 5% to 10% of the general Japanese population.[5] Previously, 2 large-scale observational studies[25,26] reported conflicting associations between *CETP* gene mutations (CETP deficiency) and CAD, as found in experimental animal and clinical studies.[27] Although the exact reason for the discrepancy remains unclear, environmental factors may be partially responsible, since both investigations were performed in relatively restricted areas of Japan.

In this study, we performed a multicenter trial to assess the association between CETP and lipid, plus apolipoprotein concentrations in population samples extracted from various areas throughout Japan, in order to minimize environmental factors, as well as to determine the allele frequency of TaqIB polymorphism and its effect on CETP and HDL-C levels.

MATERIALS AND METHODS

Subjects

The initial study population consisted of 862 unrelated Japanese patients (524 men and 338 women) attending hospitals registered for this project. A total of 234 patients (133 men and 101 women) were

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Table 1. Baseline Demographic and Lipid Parameters of Study Subjects

	Men	Women	Total	*P* Value
n	372	219 (76/143)*	591	—
Age (yr)	53.2 ± 13.4	53.9 ± 15.7	53.4 ± 14.3	.540
BMI (kg/m^2)	24.0 ± 3.0	22.2 ± 3.4	23.3 ± 3.3	<.001
CETP activity (nmol/h/mL)	353.2 ± 75.8	405.1 ± 90.7	372.4 ± 85.3	<.001
CETP mass (μg/mL)	2.28 ± 0.72	2.83 ± 0.88	2.48 ± 0.83	<.001
Total cholesterol (mg/dL)	207.5 ± 38.4	225.7 ± 59.0	214.3 ± 47.8	<.001
HDL-C (mg/dL)	48.9 ± 17.0	64.2 ± 22.9	54.6 ± 20.7	<.001
HDL_2-C (mg/dL)	30.1 ± 12.2	41.7 ± 17.2	34.4 ± 15.3	<.001
HDL_3-C (mg/dL)	16.0 ± 3.3	16.7 ± 3.6	16.3 ± 3.4	.016
LDL-C (mg/dL)	132.5 ± 35.3	136.6 ± 50.8	134.0 ± 41.7	.249
TG (mg/dL)	147.1 ± 95.9	114.1 ± 79.0	134.9 ± 91.3	<.001
ApoA-I (mg/dL)	132.7 ± 32.5	156.7 ± 36.9	141.6 ± 36.1	<.001
ApoA-II (mg/dL)	31.7 ± 7.3	32.2 ± 6.8	31.9 ± 7.1	.459
ApoB (mg/dL)	106.8 ± 26.8	104.8 ± 38.1	106.0 ± 31.4	.453
ApoC-II (mg/dL)	4.8 ± 1.7	4.5 ± 1.5	4.7 ± 1.6	.021
ApoC-III (mg/dL)	10.6 ± 4.3	10.2 ± 3.7	10.4 ± 4.1	.246
ApoE (mg/dL)	4.9 ± 1.3	5.5 ± 2.0	5.1 ± 1.6	<.001
LCAT (μmol/mL/h)	420.6 ± 96.1	446.2 ± 103.4	430.1 ± 99.6	.003
HDL_2-C/HDL_3-C	1.87 ± 0.61	2.52 ± 1.02	2.11 ± 0.85	<.001
ApoA-I/HDL-C	2.80 ± 0.34	2.56 ± 0.39	2.71 ± 0.38	<.001
Atherogenic index†	3.00 ± 1.17	2.43 ± 1.31	2.79 ± 1.25	<.001
TaqIB B1B1	134	83	217	
TaqIB B1B2	179	100	279	.845
TaqIB B2B2	59	36	95	
Gene frequency (%)				
B1 allele	60.1	60.7	60.3	.825
B2 allele	39.9	39.3	39.7	
Smoker (%)	62.1	12.8	43.8	.001
Daily drinker (%)	25.3	5.9	18.1	.001
Daily exercise (%)	1.3	4.1	2.4	.195
On diet (%)	20.4	25.6	22.3	.267
CAD (%)	30.6	11.0	23.4	.001
DM (%)	14.0	14.0	13.9	.924
HT (%)	33.1	23.3	29.4	.012

NOTE. Values are mean ± SD.

Abbreviations: DM, diabetes mellitus; HT, hypertension.

*Pre/postmenopause.

†(Total cholesterol − HDL-C)/HDL-C.

excluded due to the use of drugs affecting lipid metabolism, uncontrolled diabetes mellitus ($HbA_{1C} > 8\%$), or liver, renal, or thyroid disease. Sixteen subjects with homozygous CETP deficiency, 18 patients with familial hypercholesterolemia, and 3 patients with chylomicronemia were excluded. After these exclusions, 591 subjects were judged eligible for this study. Among the participants, 138 subjects had CAD and 82 subjects were type II diabetic, as shown in Table 1. Criteria for classifying CAD include myocardial infarction and angina pectoris verified by electrocardiogram, cardio-specific enzyme and coronary angiography, or history of coronary angioplasty or coronary bypass surgery. Information on smoking, alcohol intake, and exercise were obtained by interview. In addition, nutrition status was evaluated, in particular, whether the subjects executed nutritional intervention provided by nutrition professionals. The protocol was approved by the ethics committee at each hospital. All subjects gave informed consent.

Plasma Lipid, Lipoprotein, and Apolipoprotein

After an overnight fast, blood samples were obtained by venipuncture into vacuum tubes containing EDTA. Plasma was immediately centrifuged at 3,000 rpm for 30 minutes at 4°C. Both blood and plasma samples were delivered immediately to the core laboratory (BML, Saitama, Japan) for lipid measurement and genotyping. Plasma total cholesterol (TC) and TG levels were measured by an enzymatic method. HDL-C was directly measured using a homogenous method.[28] HDL_2 and HDL_3 (d = 1.063 to 1.125 g/mL for HDL_2, d = 1.125 to 1.210 g/mL for HDL_3) were separated sequentially in a table-top ultracentrifuge (CS100, Hitachi Koki., Tokyo, Japan), then cholesterol was quantified enzymatically. Plasma apoA-I, apoA-II, apoB, apoC-II, apoC-III, and apoE concentrations were determined using immunoturbidometric assays.[29] LDL-C was directly measured using homogeneous enzymatic assay[30] from Daiichi Pure Chemicals (Tokyo, Japan), which yielded values highly comparable to those of the Friedewald formula, as evidenced by the result that "direct" LDL-C = 0.991 × "Friedewald" LDL-C + 0.35 mg/dL, r = 0.969 by regression analysis. Atherogenic index is defined as (TC − HDL-C)/HDL-C. Lecithin: cholesterol acyltransferase (LCAT) activity in plasma was determined by a method using dimyristoyl phosphatidilcholine-cholesterol liposome as substrate.[31]

CETP Activity and Mass

Plasma CETP activity was measured using a method reported by Nagano et al.[32] In brief, diluted (1:40) plasma sample was incubated

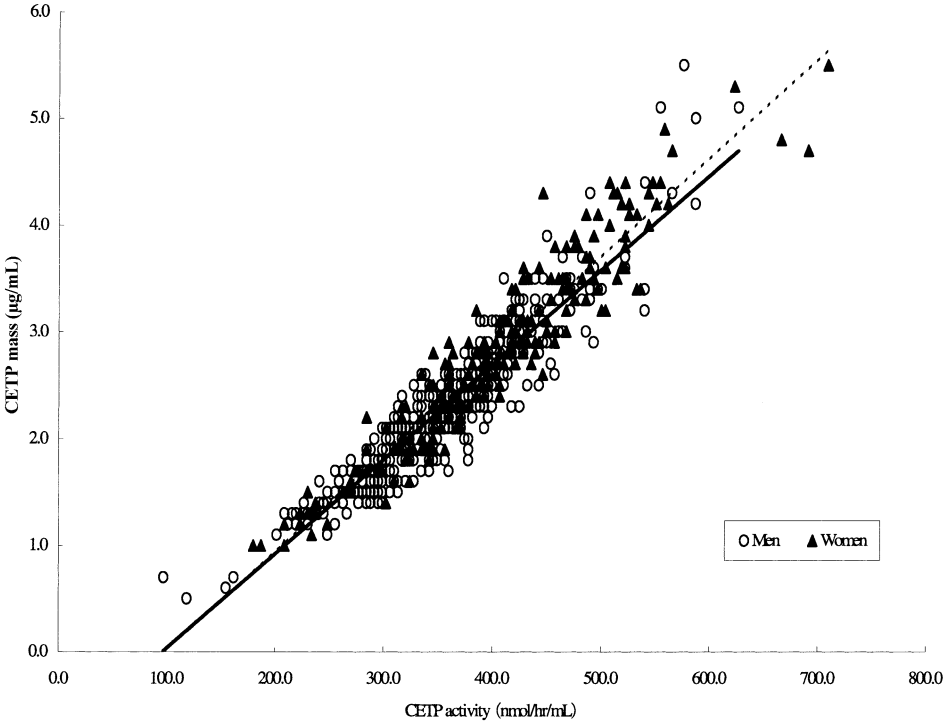

Fig 1. Pearson correlation of plasma CETP activity with CETP mass in study subjects (○, men; ▲, women). Solid and broken lines represent linear regression lines for men and women, respectively.

with LDL and reconstituted HDL at 37°C for 30 minutes. LDL was precipitated with 0.05% dextran sulfate and 30 mmol/L $MgCl_2$, then the radioactivity in the precipitates was measured in a scintillation counter. CETP mass was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using 2 monoclonal antibodies, JHC1 and JHC2, as reported previously.[32]

TaqIB Polymorphism of CETP Gene

Genomic DNA was extracted from blood leukocytes by phenol extraction. A fragment of 535 base pairs in intron 1 of the *CETP* gene was amplified by polymerase chain reaction (PCR) using allele-specific oligonucleotides (U: CACTAGCCCAGAGAGAGAGGAGTGCC, L: CTGAGCCCAGCCGCACACTAAC), according to the method of Fumeron et al.[33] The PCR products were then digested in the presence of TaqIB.

Statistical Analysis

Univariate comparisons of demographic, clinical, and laboratory variables between men and women were done using Student's *t* test for continuous variables and Fisher's exact test for categorical data. Pearson's correlation coefficients were calculated to examine the relationship of CETP activity to CETP mass and to serum lipid parameters. The variation in CETP activity, CETP mass, and serum lipid parameters, according to CETP TaqIB genotype, were evaluated by 1-way analysis of variance (ANOVA). Finally, multivariate regression analysis of serum HDL-C and LDL-C was performed in relation to CETP activity and other covariates including age, sex, smoking, alcohol intake, body mass index (BMI), exercise, and TG. In this analysis, categorical terms were translated into dummy variable as follows: gender, 1 men, 2 women; smoking, 1 never-smoker, 2 exsmoker and current smoker; alcohol, 1 nondrinker, 2 infrequent (~2 d/wk) drinker, 3 frequent (~5 d/wk) drinker, 4 daily drinker; exercise, 1 no exercise, 2 irregular-basis, 3 regular-basis, 4 daily basis. All statistical analyses were done using SAS version 6.12 (SAS Institute, Cary, NC).

RESULTS

Baseline demographic and lipid parameters are summarized in Table 1. Age was well matched and average BMI was normal in both groups, but men had a significantly higher average BMI than women. Prevalence of smokers, including exsmokers, daily drinkers, CAD, and hypertension was significantly higher in men than in women. For CETP, both activity (+15%) and mass (+24%) were significantly greater in women than in men ($P < .001$). Despite the elevated CETP activity, women had, on average, 31% higher HDL-C levels than men, and this difference was exclusively due to higher HDL_2-C levels. LDL-C level, however, was similar between genders, thus resulting in a significantly decreased atherogenic index in women. Plasma TG levels were significantly higher in men compared to women, likely reflecting the fact there were more subjects with type IIb and IV hyperlipidemia (37% in men *v* 18% in women) and drinkers among the men. CETP TaqIB genotype showed a 40% prevalence of the B2 allele with no significant differences between men and women. The distribution of alleles of the gene was consistent with Hardy-Weinberg equilibrium. Chemical composition of HDL showed a gender difference: women had a significantly lower apoA-I/HDL-C than men, a finding consistent with larger HDL particle sizes.

A correlation between CETP activity and mass is shown in Fig 1. Strong positive correlations were observed in both genders (men $r = 0.931$, women $r = 0.949$, both $P < .001$). Therefore, CETP activity was used to investigate associations with lipid and apolipoprotein concentrations (Table 2). In men, CETP activity was inversely correlated with HDL-C, HDL_2-C, and HDL_3-C. Similar associations, except for HDL_3-C, were observed in women. ApoA-I and apoA-II, major protein con-

Table 2. Partial Correlation Coefficients Between CETP Activity and Lipid and Apolipoprotein Concentrations

	Men (n = 372)		Women (n = 219)	
	r	*P*	*r*	*P*
Total cholesterol	0.143	.006	0.369	<.001
TG	−0.055	.289	0.161	.017
LDL-C	0.230	<.001	0.398	<.001
HDL-C	−0.173	<.001	−0.196	.004
HDL_2-C	−0.164	.002	−0.205	.002
HDL_3-C	−0.122	.018	0.083	.224
ApoA-I	−0.140	.007	−0.099	.146
ApoA-II	−0.223	<.001	0.039	.570
ApoB	0.206	<.001	0.402	<.001
ApoC-II	−0.100	.054	0.117	.084
ApoC-III	−0.193	<.001	0.076	.262
ApoE	0.043	.405	0.269	<.001
CETP mass	0.931	<.001	0.949	<.001
LCAT	0.027	.605	0.178	.008
HDL_2-C/HDL_3-C ratio	−0.075	.148	−0.243	<.001
ApoA-I/HDL-C ratio	0.150	.004	0.246	<.001
Atherogenic index	0.218	<.001	0.354	<.001

stituents of HDL, were negatively correlated with CETP in men, but this correlation was not evident in women. Consequently, stronger associations of CETP with HDL_2-C/HDL_3-C ratio and apoA-I/HDL-C ratio were observed in women, indicating that decreased CETP was associated with larger and more cholesterol-rich HDL. LCAT activity was positively correlated with CETP in women. LDL-C and apoB were positively correlated with CETP in both genders, but women had a significantly stronger positive correlation compared to men. ApoC-III was inversely correlated with CETP in men, while TG and apoE were positively correlated in women. Overall, atherogenic index, representing cholesterol balance between apoB-containing lipoprotein and HDL, was positively correlated with CETP in both groups, demonstrating that CETP indeed modulates redistribution of cholesterol among lipoproteins.

Table 3 provides a summary of the association of TaqIB polymorphism with CETP activity, mass, plasma lipids, lipoprotein, and apolipoprotein levels. Age and gender distributions are equivalent among genotypes. TaqIB genotypes were significantly associated with the plasma concentrations and activities of CETP and HDL-C (all $P < .001$). B1B1 subjects had higher CETP activity and mass (396.4 ± 89.1 nmol/h/mL for CETP activity, 2.68 ± 0.88 μg/mL for CETP mass) than did B1B2 (365.7 ± 80.2 nmol/h/mL, 2.41 ± 0.78 μg/mL) and B2B2 (337.4 ± 75.6 nmol/h/mL, 2.22 ± 0.74 μg/mL) subjects. HDL-C level was lowest in B1B1, highest in B2B2, and intermediate in B1B2 subjects. Similar associations were observed for HDL_2-C, apoA-I, and LCAT activity. No statistically significant differences in TG, LDL-C, HDL_3-C, and other apolipoproteins were observed across TaqIB genotypes. TaqIB genotype modulated HDL particle size as indicated by apoA-I/HDL-C ratio; B2 allele carrier had larger particles relative to B1 allele carrier. Higher HDL_2-C level in B2 allele carriers also supports this observation.

Finally, we performed a multivariate analysis to assess how HDL-C and LDL-C concentrations were determined by factors such as sex, BMI, smoking, alcohol, exercise, TG, and CETP activity. Each model is summarized in Table 4. In the HDL-C model, all parameters were statistically significant. Age, alcohol, and exercise increased, but male gender, obesity, smoking, TG, and CETP decreased HDL-C levels. Overall, these factors accounted for 36% of HDL-C variation. In contrast, some, but not all, parameters were statistically significant for modulation of LDL-C level: CETP, obesity, and, to a lesser extent, aging increased LDL-C level.

Table 3. Clinical and Lipid Characteristics According to CETP TaqIB Genotype

	B1B1	B1B2	B2B2	*P* Value
N	217	279	95	—
Age (yr)	53.6 ± 14.5	53.2 ± 14.5	53.7 ± 13.1	.920
Sex (M/F)	134/83	179/100	59/36	.845
CETP activity (nmol/h/mL)	396.4 ± 89.1	365.7 ± 80.2	337.4 ± 75.6	<.001
CETP mass (μg/mL)	2.68 ± 0.88	2.41 ± 0.78	2.22 ± 0.74	<.001
Total cholesterol (mg/dL)	211.7 ± 48.4	213.7 ± 48.4	221.9 ± 44.4	.219
HDL-C (mg/dL)	50.9 ± 17.7	55.1 ± 21.1	61.4 ± 24.0	<.001
HDL_2-C (mg/dL)	31.6 ± 12.8	34.8 ± 15.6	39.7 ± 18.2	<.001
HDL_3-C (mg/dL)	16.0 ± 3.3	16.4 ± 3.5	16.6 ± 3.3	.308
LDL-C (mg/dL)	134.3 ± 43.5	133.3 ± 41.1	135.4 ± 40.0	.910
TG (mg/dL)	141.6 ± 92.3	131.0 ± 93.1	130.8 ± 83.4	.393
ApoA-I (mg/dL)	136.0 ± 33.1	142.5 ± 36.7	151.8 ± 38.6	.001
ApoA-II (mg/dL)	31.7 ± 6.9	31.9 ± 7.3	32.4 ± 6.9	.667
ApoB (mg/dL)	107.0 ± 32.2	105.3 ± 31.1	106.2 ± 31.0	.833
ApoC-II (mg/dL)	4.8 ± 1.6	4.6 ± 1.7	4.7 ± 1.6	.330
ApoC-III (mg/dL)	10.4 ± 4.0	10.3 ± 4.2	10.7 ± 3.9	.677
ApoE (mg/dL)	5.1 ± 1.5	5.1 ± 1.8	5.3 ± 1.5	.396
LCAT (μ mol/mL/hr)	417.5 ± 92.0	433.9 ± 106.0	447.4 ± 93.9	.034
HDL_2-C/HDL_3-C ratio	1.97 ± 0.71	2.13 ± 0.88	2.38 ± 0.97	<.001
ApoA-I/HDL-C ratio	2.77 ± 0.37	2.70 ± 0.36	2.61 ± 0.40	<.001
Atherogenic index	2.94 ± 1.27	2.74 ± 1.22	2.57 ± 1.26	.039

Table 4. Multivariate Analysis for Parameters Regulating HDL-C and LDL-C

Parameters	Estimate	*F*	*P*
Model 1	HDL-C		
(intercept)	104.264		
Age	−0.128	6.93	.0087
Sex	−14.399	73.91	<.0001
BMI	−0.896	15.47	<.0001
Smoking	−0.200	9.83	.0018
Alcohol	2.974	21.51	<.0001
Exercise	3.257	11.58	<.0007
CETP activity	−0.035	16.87	<.0001
TG	−0.071	75.65	<.0001
R^2		0.365	
Model 2	LDL-C		
(intercept)	16.834		
Age	0.234	4.13	.0427
Sex	1.340	0.11	.7357
BMI	1.753	11.57	.0007
Smoking	0.126	0.73	.3943
Alcohol	−0.995	0.43	.5130
Exercise	4.187	3.43	.0646
CETP activity	0.155	59.54	<.0001
R^2		0.119	

DISCUSSION

In the present study, we found an inverse correlation between CETP and HDL-C, in particular for HDL_2-C, and a positive correlation between CETP and LDL-C, both in men and women. In men, apoA-I and apoA-II, major protein constituents of HDL, were also negatively correlated with CETP. The positive correlation between CETP and LDL-C agreed with most previous studies.[11,12,15,18] However, the negative correlation between HDL-C is a controversial finding. Both Kark et al[11] and Gudnason et al[12] found a significant negative correlation, but 2 reports from Japan,[15,18] Bernard et al,[16] and Dachet et al[17] did not find this relationship. Although it is not entirely clear what the reason is for this discrepancy, all positive data are based on gender-separated analysis. Indeed, when all data in the current study subjects were analyzed together, we could not find a significant correlation between CETP and HDL-C ($r = -0.059$, $P = .153$). Thus, we speculate that, since mean HDL-C level differs significantly between men (48.9 mg/dL) and women (64.2 mg/dL), pooled analysis may decrease the sensitivity of detecting potential correlations. We also employed a multiple regression analysis of HDL-C and LDL-C. CETP was found to be an independent factor for decreasing HDL-C and increasing LDL-C levels, along with other factors including age, sex, BMI, smoking, alcohol, and exercise for HDL-C, plus age and BMI for LDL-C. We previously performed HDL[8] and LDL[9] apolipoprotein metabolic studies using stable isotopically labeled tracer in homozygous CETP-deficient patients, in order to assess the effect of CETP deficiency on lipoprotein metabolism. We found that, for CETP deficiency, rates of catabolism of apoA-I were markedly slower, thereby causing elevated apoA-I levels. Low LDL apoB level was found to be due to an increased rate of catabolism in CETP deficiency, likely due to upregulation of LDL receptor activity. Findings from the present study, together with the aforementioned kinetic evidence, thus support the concept that CETP activity is a relevant factor regulating steady-state levels of HDL-C as well as LDL-C, in a range of subjects whose lipid levels vary widely.

We also found a positive correlation between CETP activity and LCAT activity in women. Although we are not certain about this gender-specific effect, a positive relationship was reported previously, in a healthy Japanese population.[15] Our correlation analysis also revealed that CETP activity affects the chemical composition of HDL particles, particularly in women. CETP activity was correlated negatively with HDL_2-C/HDL_3-C and positively with apoA-I/HDL-C ratios, indicating that low CETP activity is associated with large, cholesterol-rich HDL particles. This is consistent with the observation that homozygous subjects with genetic CETP deficiency have very large HDL particles.[8,34] CETP activity was positively correlated with LDL-C and apoB concentrations, a finding consistent with previous studies.[11,12,15,18,35] Of note, women showed a stronger positive association than men. Although the actual reason is not clear, the observed gender difference may be attributed to higher HDL-C relative to LDL-C concentrations in women, as HDL is the primary substrate of CETP, leading to more CE transfer into LDL. In this regard, CETP may be considered to be proatherogenic, by increasing LDL level, when removal through the LDL receptor pathway cannot fully compensate for the increased LDL input. However, it should also be kept in mind that correlation analysis itself does not imply any causal relationship between CETP and LDL as evidenced by increased CETP due likely to elevated LDL concentration in familial hypercholesteromia.

A notable finding in the present study was the significant effect of TaqIB polymorphism on CETP activity/mass, HDL-C (HDL_2-C in particular) and apoA-I levels which have not previously been observed in Japanese.[18,23,24] B2 allele was associated with a lower CETP activity/mass and higher HDL-C compared to B1 allele, whereas TaqIB polymorphism did not affect LDL-C level, despite the fact that CETP activity is positively associated with LDL-C level, a similar observation to previous studies.[16,18,21,22] This is an important finding based on the fact that there are 2 common functional CETP gene mutations (D442G and intron 14 splicing defect) in the general Japanese population, which could confound the association of TaqIB polymorphism with HDL-C. Although we did not genotype the 2 CETP mutations in this study, we hypothesized that if the 2 mutations are present more in B2 carriers than B1 carriers, one could expect the effect of TaqIB polymorphism to not be significant in subjects whose HDL-C levels are in a low or normal range. However, the effect of TaqIB polymorphism on CETP activity and HDL-C was still found to be significant when subjects with HDL-C $\geq$ 80 mg/dL were excluded (CETP activity: B1B1 [n = 197] 396 $\pm$ 86 nmol/h/mL, B1B2 [n = 246] 367 $\pm$ 79 nmol/h/mL, B2B2 [n = 76] 342 $\pm$ 72 nmol/h/mL, $P < .001$; HDL-C: B1B1 46.9 $\pm$ 13.0 mg/dL, B1B2 49.2 $\pm$ 13.2 mg/dL, B2B2 51.8 $\pm$ 14.7 mg/dL, $P = .02$). Thus, it is unlikely that there is an interaction between the mutations and TaqIB polymorphism, rather we believe that this polymorphism affects CETP and HDL-C through a mechanism independent from the one mediated by the 2 mutations. Nonethe-

less, future studies monitoring both CETP gene variations and TaqIB polymorphism are needed to clarify this issue.

The allele frequency of B2 in the present study was 40% and did not significantly differ between genders (Table1). This allele frequency is highly consistent with Caucasian populations[16,21,22] and a recent report in Japanese diabetic patients,[18] supporting findings that B2 allele frequency is independent of ethnicity. The TaqIB polymorphism site is located in intron1, and thus this is unlikely to represent a functional mutation per se. Rather, it is plausible to speculate that the polymorphism is in linkage disequilibrium with unknown functional variations in the *CETP* gene. Two studies have presented evidence supporting this hypothesis. Dachet et al[17] recently discovered a new polymorphic site at −629 A/C in the *CETP* gene. The −629A allele was associated with lower CETP mass plus higher HDL-C and was highly concordant with the TaqIB B2 allele. Transient transfection assays revealed that the −629A allele modulated the transcription rate and plasma CETP. Talmud et al[36] also identified a tetranucleotide repeat within the CETP promoter, "short" alleles strongly associated with the B2 allele at the TaqIB polymorphic site. Although not proven yet, these hypervariable regions within promoters are likely to be functional as demonstrated by the (tttta)n repeat polymorphism in the 5′ flanking region of the lipoprotein(a) gene 37.

There were several limitations in this study. First, we did not genotype the 2 CETP mutations (intron14G to A mutation and D442G missense mutation). Therefore, we could not exclude the possibility that interaction of the CETP mutations with TaqIB polymorphism underlies the observed effect of the latter on CETP and HDL-C levels. Second, lipoprotein and clinical profiles, as well as menopausal status, vary among study subjects. The majority of subjects were hyperlipidemic, but 29% were normolipidemic. Further, our study subjects included patients with CAD (23%) or diabetes (14%), who may have different metabolic backgrounds to a normal, healthy population. Although subgroup analysis revealed significant differences in CETP and some lipid parameters, depending on the presence or absence of hyperlipidemia, CAD, or diabetes mellitus or between premenopausal and postmenopausal women, no clear difference was observed in the association of CETP activity with lipoprotein levels, except that in male patients with CAD (n = 100), CETP activities did not show significant association with HDL-C or LDL-C (data not shown). Thus, in our opinion, these factors did not detract from our main findings.

In summary, the present study demonstrated that (1) CETP activity modulated both HDL and LDL-C levels, and (2) TaqIB polymorphism plays an important role in determining CETP activity and HDL-C level, as previously observed in Caucasian populations. However, human clinical studies will be necessary in the future to assess the potential benefit of therapies based on high HDL-C/low LDL-C by CETP inhibition.

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APPENDIX

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