



Cholesteryl ester transfer protein and atherosclerosis in Japanese subjects: a study based on coronary angiography

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Abstract

We undertook a cross-sectional analysis on CETP and atherosclerosis among Japanese subjects, by means of CETP mass assay, its gene polymorphism and coronary angiogram. The 110 consecutive patients who underwent coronary angiography were enrolled into the study except for those over 70 years and taking lipid-lowering drugs. Association was analyzed among plasma lipid and lipoproteins, CETP mass, its gene polymorphisms and the finding in coronary angiography. Four CETP-deficiency heterozygotes were identified and excluded from the analysis. CETP mass level showed neither significant correlation with the coronary score (CS) ($r = 0.06$, $P = 0.52$) nor the difference between the groups eventually diagnosed as coronary heart disease (CHD) positive and CHD negative (2.36 ± 0.57 vs. 2.24 ± 0.21 , $P = 0.24$). CETP mass correlated with the total and LDL cholesterol ($r = 0.43$, $P < 0.001$; $r = 0.36$, $P < 0.001$, respectively) but not with HDL cholesterol ($r = 0.08$, $P = 0.40$). While I405V polymorphism had no impact on CETP mass, HDL cholesterol or CS, CETP mass was low with *TaqIB* polymorphism (B1B1 > B2B2, $P < 0.05$) only in the low CS group (< 4). Among the lipid and lipoprotein, HDL cholesterol had a greater impact than LDL cholesterol on coronary atherosclerosis. We concluded that CETP mass in plasma does not correlate with coronary atherosclerosis as whole in the non-CETP-deficient. However, the B2B2 genotype in CETP *TaqIB* polymorphism, only when it decreases the CETP level, may act as a protective factor against atherosclerosis. It should also be noted that CETP mass in general correlates to total and LDL cholesterol, so that it would be an indirect atherogenic parameter. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: CETP; HDL; LDL; Coronary heart disease; Atherosclerosis; Gene polymorphism

1. Introduction

Plasma lipoprotein profile is one of the major factors to describe the risk of atherosclerotic cardiovascular disease. In many studies, the incidence of coronary heart disease (CHD) was shown to correlate positively with total cholesterol and low density lipoprotein

(LDL) cholesterol concentration in plasma [1–3] and negatively with high density lipoprotein (HDL) cholesterol [4–7]. Although the true mechanism by which HDL acts as an ‘anti-atherogenic’ factor is not fully understood, it is widely believed that HDL plays a central role in the pathway transporting cholesterol from peripheral cells to the liver where it is converted to bile acids and excreted. The regulation of the plasma LDL level has been well characterized, such as the mechanisms for its precursor secretion by the liver and its clearance from the plasma, and the clinical benefit of its reduction has also been well documented. On the

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other hand, many more factors may be involved in the regulation of the plasma HDL level, and it is premature to state whether its clinical manipulation is in general, beneficial for the patients.

Cholesteryl ester transfer protein (CETP) plays one of the major roles in regulation of plasma HDL by mediating the transfer of neutral lipids randomly between the lipoprotein cores such as cholesteryl ester and triglyceride. As cholesteryl ester is mainly generated by lecithin; cholesterol acyltransferase in HDL, the CETP reaction removes it from HDL in exchange for triglyceride [8] that is hydrolyzed by plasma lipases. Thus, low CETP results in high HDL, since cholesteryl ester tends to accumulate in HDL.

The hypothetical role of CETP in atherogenesis is controversial. CETP mediates the net cholesteryl ester transfer from HDL to other lipoproteins containing apolipoprotein B, which is eventually taken up by the liver to complete the pathway of cholesterol transport from the peripheral cells via HDL to the liver. Therefore, low CETP may cause retardation of this pathway and that is perhaps atherogenic. On the other hand, low CETP leads to the increase of HDL cholesterol, and high HDL may by all means be beneficial by increasing the negative risk factor for atherosclerosis. The results from animal experiments are indeed uncertain [9–15]. Clinical observation is also controversial with respect to the plasma CETP and atherosclerosis. The CETP-deficient patients seem at higher risk for atherosclerosis, either homozygotes or heterozygotes [16–18]. On the other hand, patients with CHD showed higher CETP activity than the normal control subjects [19], which may result from the positive correlation between CETP and LDL cholesterol among the non-CETP-deficient patients [20–22]. Various mutations in the CETP gene seem to cause changes in the HDL cholesterol levels. In polymorphism I405V, 405V is reportedly associated with low CETP, high cholesterol and elevation of the risk for CHD [23–25]. In contrast, low CETP and the consequent high HDL associated with the B2 allele in TaqIB polymorphism was shown to be protective against CHD [26–29].

It is of special interest to investigate the role of CETP in atherogenesis among the Japanese, because of extremely high prevalence of CETP deficiency [18,30] and low incidence of coronary heart disease despite the seemingly increasing plasma cholesterol level in the past few decades in Japan [31]. We have earlier demonstrated that there was no significant difference in CETP mass between the CHD and non-CHD groups of the Japanese patients who underwent coronary angiography [22]. In this study, we have analyzed yet another group of the Japanese patients who received coronary angiographic examination, for the CETP mass and its gene polymorphisms in relation to plasma lipid and lipoprotein concentration and coronary atherosclerosis, by excluding the CETP-deficient patients.

2. Methods

2.1. Subjects

The consecutive patients who were referred to Nagoya City University Hospital and its affiliated hospitals for coronary angiographic examination were enrolled for this study. Those of age above 70 years, of receiving lipid-lowering drugs or having a history of coronary bypass surgery and/or angioplasty were excluded from the study. All the eligible patients had symptoms suggesting CHD or cardiac dysfunction. Smokers and habitual alcohol consumers were identified by the information obtained in a patient questionnaire. The hypertensive and the diabetic patients were identified by their clinical history. An informed consent for the study was obtained in writing from all the patients.

2.2. Coronary angiography

All the patients underwent selective coronary angiography according to the Judkins technique, recorded on 35-mm films. The degree of coronary atherosclerosis were determined in 15 coronary artery segments according to definition by the Ad Hoc Committee on Grading of Coronary Artery Disease of the American Heart Association [32]. The coronary scoring system was based on the method described by Mabuchi et al [33]. Briefly, the stenosis in each segment was graded from 0 to 4 point (0, normal; 1, 1–25%; 2, 26–50%; 3, 51–75%; 4, more than 75%) and the coronary score was obtained as a sum of these points. The grading of the stenosis and calculation of the coronary score were based on the consensus opinion of the two cardiologists who were blinded for the history and lipid profile of the patients. The patient was classified into the clinical CHD group when the coronary angiography exhibited significant organized stenosis accompanied by clinical myocardial ischemia, and otherwise into the non-CHD group. The diagnosis was also made by the two cardiologists under the blind condition with respect to the lipid and CETP data.

2.3. Lipid and CETP measurement

Fasting venous blood was collected into an EDTA-containing tube. Plasma total cholesterol and triglyceride concentrations were determined by enzymatic methods. Plasma HDL-cholesterol concentration was measured after precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride. Plasma LDL-cholesterol was calculated according to the equation of Friedewald et al. [34]. CETP mass was measured by enzyme-linked immunosorbent assay as described previously [22] by using an

assay kit provided from Dai-ichi Pure Chemicals Ltd (Tokyo).

2.4. Analysis of CETP gene mutations

The two common mutations for the CETP deficiencies of intron 14 splicing defect and exon 15 missense, and the two common CETP polymorphisms of TaqIB and I405V were determined. DNA was extracted from peripheral white blood cells. The mutations were detected by polymerase chain reaction (PCR) amplification of the relevant sequence followed by digestion with the appropriate restriction endonuclease according to the manufacturers instructions. Detection of intron 14

splicing donor defect and exon 15 missense mutation were performed as described by Hirano [35] and Sakai [36], respectively. The TaqIB and I405V polymorphisms were analyzed as reported by Fumeron [37] and Gudnason [38], respectively. DNA restriction fragments were loaded onto 3 or 4% NuSieve 3:1 agarose gel for the electrophoretic analysis. Subsequently, the gel was stained with SYBR[®] Gold nucleic acid gel stain, and marked for the genotypes using UV translumination.

2.5. Statistical analysis

Results were given as mean \pm S.D. values. Two-tailed Student's *t*-tests or analysis of variance (ANOVA) followed by Bonferroni's test were used to determine group differences in continuous variables. Pearson's correlation coefficients were computed to assess the relation between continuous variables such as age, body mass index (BMI), CETP mass, coronary score and plasma lipids. All the tests were two-sided; *P* values below 0.05 were considered to indicate statistical significance.

3. Results

3.1. Patient characterization

One hundred and ten patients were enrolled into the study. The analysis of DNA identified four CETP deficient patients (all were heterozygotes of exon 15 missense mutation, mean CETP mass 1.65 ± 0.31 mg/l, mean HDL cholesterol 46.3 ± 7.1 mg/dl). These patients were excluded from further analysis, and the remaining 106 patients became eligible, characterized in Table 1. Twenty-eight patients were classified into the clinical CHD group (acute myocardial infarction, 3; old myocardial infarction, 8; and angina pectoris, 17), and 78 patients were into the non-CHD group (vasospastic angina pectoris, 21; valve disease, 9; dilated cardiomyopathy, 9; hypertensive heart, 5; peripheral artery disease, 2; arrhythmia, 1; and chest pain syndrome, 31). Coronary score showed significant difference between drinkers and non-drinkers, diabetics and non-diabetics and the CHD group and non-CHD group (Table 2).

3.2. CETP in categorical variables

The mean plasma CETP mass concentration was 2.27 ± 0.49 mg/l, and the distribution of CETP mass shown in Fig. 1 roughly demonstrated Gaussian curve fit. Differences of CETP in categorical variables are shown in Table 3. There was significant difference between male and female (2.17 ± 0.45 vs. 2.46 ± 0.51 mg/l, *P* < 0.01) and between drinker and non-drinker (2.14 ± 0.37 vs. 2.35 ± 0.54 mg/l, *P* < 0.05). Otherwise,

Table 1
Patient's characteristics^a

Age, year	59.1 \pm 8.4
Male gender, <i>n</i> (%)	69 (65.1)
Body mass index, kg/m ²	23.5 \pm 3.2
Smoker, <i>n</i> (%)	63 (59.4)
Alcohol intake, <i>n</i> (%)	40 (37.7)
Hypertension, <i>n</i> (%)	45 (42.5)
Diabetes, <i>n</i> (%)	25 (23.6)
CHD prevalence, <i>n</i> (%)	28 (26.4)
Total cholesterol, mg/dl	199.8 \pm 36.9
HDL cholesterol, mg/dl	46.2 \pm 12.8
LDL cholesterol, mg/dl	125.5 \pm 28.4
Triglyceride, mg/dl	139.5 \pm 88.5
CETP mass, mg/l	2.27 \pm 0.49

^a Values are mean \pm S.D. Body mass index is the weight in kg divided by the square of the height in m. Hypertension and diabetes were defined by clinical history.

Table 2
Differences of coronary score between categorical variables^a

Categorical variables	<i>n</i>	Coronary score	
Age	<65	71	7.8 \pm 8.8
	\geq 65	35	5.4 \pm 6.6
Male		69	7.1 \pm 8.1
	Female	37	4.5 \pm 5.7
Body mass index	\geq 23	51	5.5 \pm 6.1
	<23	55	6.8 \pm 8.5
Smoker		43	7.2 \pm 8.0
	Non-smoker	63	5.5 \pm 6.9
Drinker		40	4.3 \pm 5.6 ^b
	Non-drinker	66	7.4 \pm 8.2
Hypertension	(+)	45	7.6 \pm 9.0
	(-)	61	5.2 \pm 5.9
Diabetes	(+)	25	9.0 \pm 6.9 ^b
	(-)	81	5.3 \pm 7.4
CHD	(+)	28	16.1 \pm 6.7 ^c
	(-)	78	2.6 \pm 3.2

^a Values are mean \pm S.D. BMI is the weight in kg divided by the square of the height in m. Hypertension and diabetes were defined by clinical history.

^b *P* < 0.05.

^c *P* < 0.0001.

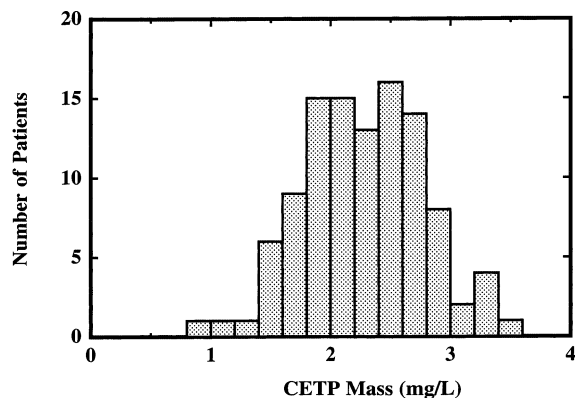


Fig. 1. Histogram for plasma CETP mass distribution of the 106 patients analyzed. Four CETP-deficient patients were excluded. Data width for each column is 0.2 mg/l.

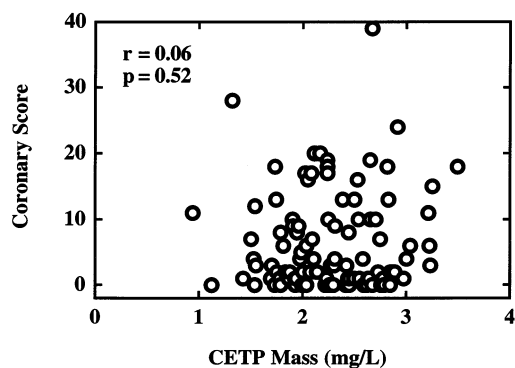


Fig. 2. Relationship between plasma CETP mass and coronary score.

Table 3
Differences of CETP mass between categorical variables^a

Categorical variables	N	CETP mass (mg/l)
Age		
<65	71	2.24 ± 0.50
≥65	35	2.33 ± 0.47
Male	69	2.17 ± 0.45 ^b
Female	37	2.46 ± 0.51
Body mass index		
≥23	51	2.36 ± 0.47
<23	55	2.18 ± 0.49
Smoker	43	2.16 ± 0.44
Non-smoker	63	2.34 ± 0.51
Drinker	40	2.14 ± 0.37 ^c
Non-drinker	66	2.35 ± 0.54
Hypertension		
(+)	45	2.31 ± 0.44
(-)	61	2.24 ± 0.52
Diabetes		
(+)	25	2.35 ± 0.49
(-)	81	2.25 ± 0.49
CHD		
(+)	28	2.36 ± 0.57
(-)	78	2.24 ± 0.21

^a Values are mean ± S.D. BMI is the weight in kg divided by the square of the height in meters. Hypertension and diabetes were defined by clinical history.

^b $P < 0.01$.

^c $P < 0.05$.

no significant difference was found between the groups examined.

3.3. CETP and coronary atherosclerosis

The impact of CETP mass on coronary atherosclerosis was examined in several aspects. Correlation between CETP and coronary score was not statistically significant ($r = 0.06$, $P = 0.52$) (Fig. 2). No significant difference in CETP mass was observed between the clinical CHD and non-CHD groups (Table 3) (2.36 ± 0.57 vs. 2.24 ± 0.21 mg/l, $P = 0.24$). In addition, neither the coronary score nor the prevalence of the clinical CHD was statistically different across the quintiles of the plasma CETP mass concentration, though the latter parameter seemed to increase with the CETP level (Fig. 3). When the patients were categorized into the groups with low, middle and high HDL (< 40 mg/dl, 40 mg/dl \leq and < 50 mg/dl, and 50 mg/dl \leq , respectively), the correlation coefficient showed an increase towards significance in the middle HDL group ($r = 0.17$ and $P = 0.12$ for the low HDL group, $r = 0.30$ and $P = 0.09$ for the middle HDL, and $r = -0.17$ and $P = 0.31$ for the high HDL). There was no significant difference in the level of CETP mass between the subgroups with lower (< 4) and higher ($4 \leq$) coronary score by the statistical analysis methods used. Interestingly, however, the distribution of CETP mass in these groups showed apparently different patterns (unimodal in the higher group and apparent bimodal in the lower group) (Fig. 4).

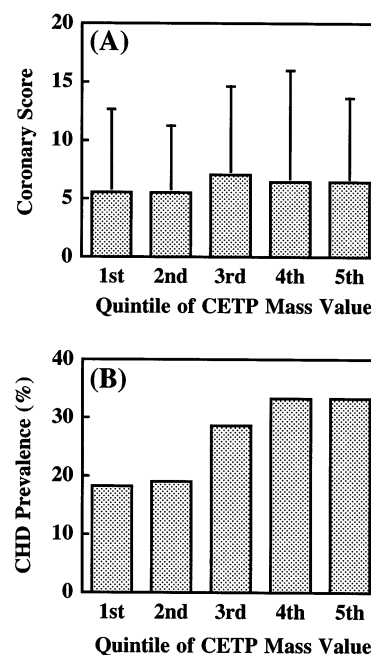


Fig. 3. Coronary score (A) and CHD prevalence (B) in the quintile distribution of CETP mass. The data of coronary score represent the mean ± S.D. The first quintile range was 1.87 mg/l or lower for 22 patients, and then 1.88–2.09, 2.11–2.42, 2.43–2.71 and 2.73 or higher for 21 patients each.

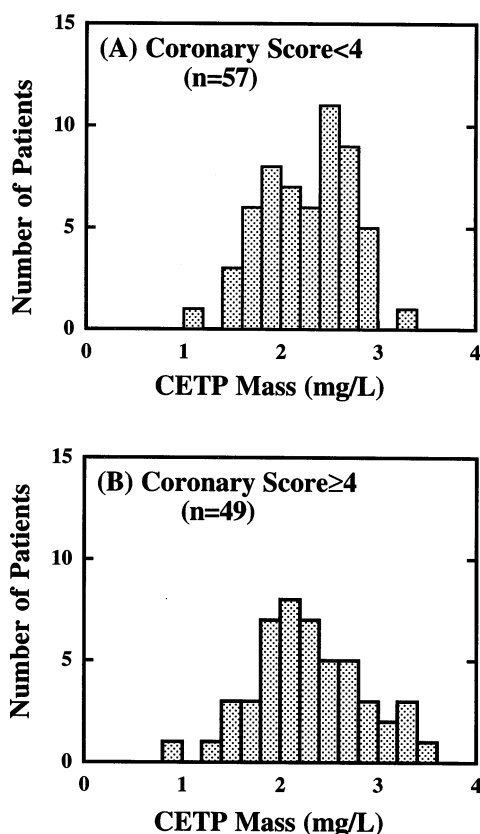


Fig. 4. CETP mass histogram of the patients with coronary score less than 4 (A) and with coronary score 4 or higher (B). Data width for each column is 0.2 mg/l.

3.4. Effect of CETP polymorphism

Influence of TaqIB and I405V polymorphisms on CETP mass, coronary score and HDL cholesterol were examined (Table 4). Neither polymorphism significantly influences these variables as whole. B2B2 homozygotes in TaqIB polymorphism showed lower CETP mass than B1B2 heterozygotes and B1B1 homozygotes but the differences were not statistically significant. HDL cholesterol was higher when B2 allele was present but again not significantly. When the patients were divided into the subgroups by coronary score, CETP mass was significantly lower in B2B2 homozygote than in B1B1

homozygote (2.36 ± 0.31 vs. 1.93 ± 0.40 mg/l, $P < 0.05$) in the group with lower coronary score (< 4) (Table 5). This difference reflected in the increased level of HDL cholesterol by B2B2 but not to the extent of the statistical significance. On the other hand, there was no such observation with I405V polymorphism.

3.5. Correlation between CETP and plasma lipids

Plasma CETP mass showed positive correlation with plasma total cholesterol ($r = 0.43$, $P < 0.0001$) and LDL cholesterol ($r = 0.36$, $P < 0.001$), but insignificant correlation with triglyceride ($r = 0.18$, $P = 0.06$) and with HDL cholesterol ($r = 0.08$, $P = 0.40$) (Fig. 5). CETP was not correlated with HDL even for the patients with TG > 150 mg/dl ($n = 32$, $r = -0.02$, $P = 0.93$).

3.6. Effects of plasma lipids on coronary atherosclerosis

Coronary score had an inverse correlation with HDL cholesterol ($r = -0.42$, $P < 0.0001$) and a mild correlation with triglyceride ($r = 0.24$, $P < 0.05$), but no correlation with total cholesterol ($r = 0.07$, $P = 0.46$) or LDL cholesterol ($r = 0.11$, $P = 0.27$). Coronary score and the prevalence of the CHD patients in each quintile of the lipid parameters are shown in Figs. 6 and 7. As the HDL cholesterol level increased, both coronary score and CHD prevalence decreased ($P < 0.0001$), and mild correlation was shown between triglyceride and coronary score ($P < 0.05$). Other lipid parameters did not show an apparent correlation with coronary score or CHD prevalence. When the relationship of coronary score with LDL cholesterol was analyzed for the subgroups stratified by plasma HDL cholesterol (low, < 40 mg/dl; middle $40 \text{ mg/dl} \leq$ and < 50 mg/dl; high, 50 mg/dl) (Fig. 8), significant correlation was observed only in the middle HDL group ($r = 0.43$, $P < 0.05$). In contrast, HDL cholesterol was inversely correlated with the coronary score in all subgroups stratified with LDL cholesterol (Fig. 8). The CHD prevalence showed the same tendency when analyzed in a similar manner (Fig. 9).

Table 4
CETP mass, HDL cholesterol and coronary score according to the genotypes of TaqIB and I405V polymorphisms^a

		<i>n</i>	CETP mass (mg/l)	HDL-cholesterol (mg/dl)	Coronary score
TaqIB	B1B1	37	2.33 ± 0.48	43.9 ± 10.8	7.2 ± 8.7
	B1B2	47	2.30 ± 0.46	47.4 ± 14.2	5.2 ± 6.1
	B2B2	22	2.11 ± 0.55	47.5 ± 12.6	6.5 ± 7.5
aa405	II	24	2.32 ± 0.49	42.5 ± 9.9	7.6 ± 9.9
	IV	43	2.28 ± 0.47	48.6 ± 14.1	5.0 ± 5.8
	VV	39	2.23 ± 0.52	45.8 ± 12.5	6.7 ± 7.3

^a Values are mean \pm S.D.

Table 5
CETP mass according to TaqIB and I405V RFLPs in the groups of high and low coronary score^a

		Coronary score <4			Coronary score ≥4		
		n	CETP mass (mg/l)	HDL-c (mg/dl)	n	CETP mass (mg/l)	HDL-c (mg/dl)
TaqIB	B1B1	18	2.36 ± 0.31	47.1 ± 11.4	19	2.31 ± 0.60	40.8 ± 9.5
	B1B2	29	2.30 ± 0.50	52.5 ± 15.0	18	2.29 ± 0.42	39.2 ± 7.5
	B2B2	10	1.93 ± 0.40 ^b	54.5 ± 9.3	12	2.25 ± 0.64	41.7 ± 12.3
I405V	II	13	2.27 ± 0.28	53.6 ± 9.7	11	2.37 ± 0.67	41.2 ± 10.5
	IV	25	2.27 ± 0.49	53.3 ± 16.2	18	2.29 ± 0.45	42.1 ± 6.5
	VV	19	2.23 ± 0.50	53.5 ± 8.9	20	2.24 ± 0.56	38.6 ± 11.1

^a Values are mean ± S.D.

^b $P < 0.05$, different from B1B1 genotype. The p values for the difference of HDL-c between B1B1 and B2B2 were, 0.16 for the low coronary score group and 0.81 for the high coronary score group. Differences among the three groups were tested ANOVA. Differences among the genotypes were tested by Bonferroni's test.

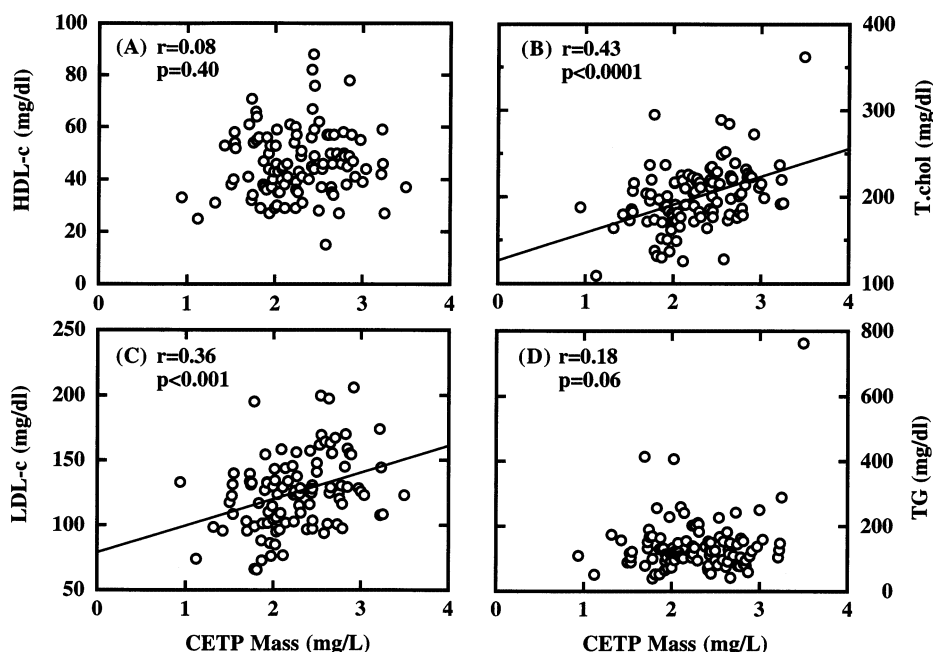


Fig. 5. Correlation of CETP mass with HDL cholesterol (A), total cholesterol (B), LDL cholesterol (C) and triglyceride (D). Solid lines indicate least linear regression lines when the relationship was statistically significant.

4. Discussion

Relationship of CETP with plasma lipoprotein and coronary atherosclerosis was investigated for Japanese subjects. The study was based on the measurement of plasma CETP mass concentration by enzyme-linked immunosorbent assay (ELISA) [22], the analysis of the CETP gene mutations, and on angiographic examination of the coronary arteries. Four CETP deficiency heterozygotes, frequently found among Japanese, were identified in the 110 subjects originally enrolled and excluded from further analysis. CETP mass did not correlate with any of plasma HDL cholesterol, coronary score, and clinical CHD, but with plasma total and LDL cholesterol. CETP phenotype of 405V did not

seem to relate with CETP mass change and coronary atherosclerosis. On the other hand, the B2 allele in TaqIB polymorphism was shown with a tendency of low CETP and high HDL. Since this was significant with B2B2 homozygotes only in the low coronary score group (<4), this allele would be protective against CHD only when it resulted in low CETP and high HDL. The results also demonstrated a strong negative correlation of HDL with coronary atherosclerosis among these patients, rather than total and LDL cholesterol.

A possible role of CETP in atherogenesis is still under debate, at the level of experimental animals and clinical observation [39]. Transgenic mice of CETP at a cholesterol-fed stage [9] or with LDL-receptor/apoE

knocked-out [13] exhibited enhanced atherogenesis. Suppression of CETP expression in cholesterol-fed rabbits by the antisense DNA treatment developed less atherosclerotic vascular lesion [11]. The same result was obtained by pharmacological and immunological inhi-

bition of the CETP activity [14,15]. On the other hand, expression of human CETP decreased atherogenesis in the mice with hypertriglyceridemia [10] or LCAT overexpression [12]. In humans, the results also seem controversial. Genetic CETP deficiency may have the

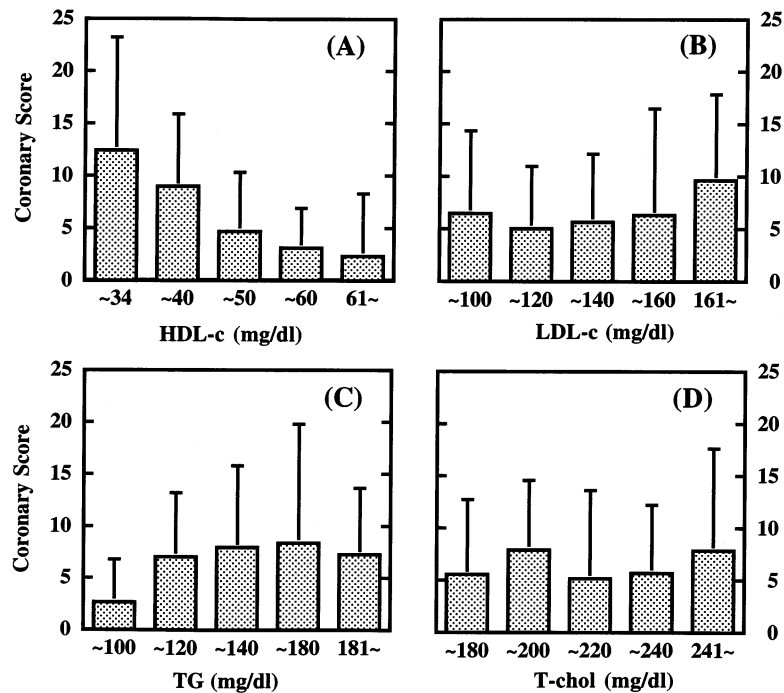


Fig. 6. Dependency of coronary score on HDL cholesterol (A), LDL cholesterol (B), triglyceride (C) and total cholesterol (D). The data represent the mean \pm S.D.

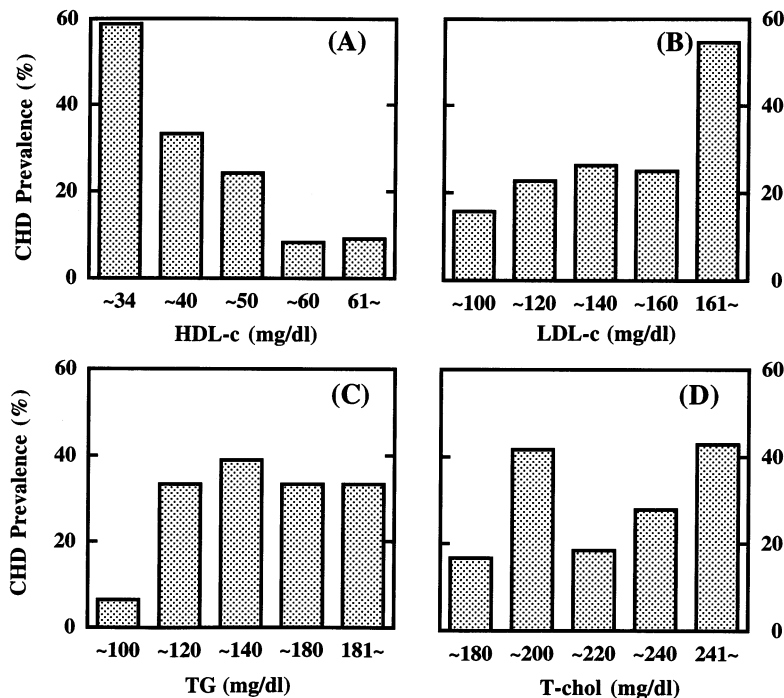


Fig. 7. The prevalence of the clinically defined CHD in each stratum group of HDL cholesterol (A), LDL cholesterol (B), triglyceride (C) and total cholesterol (D).

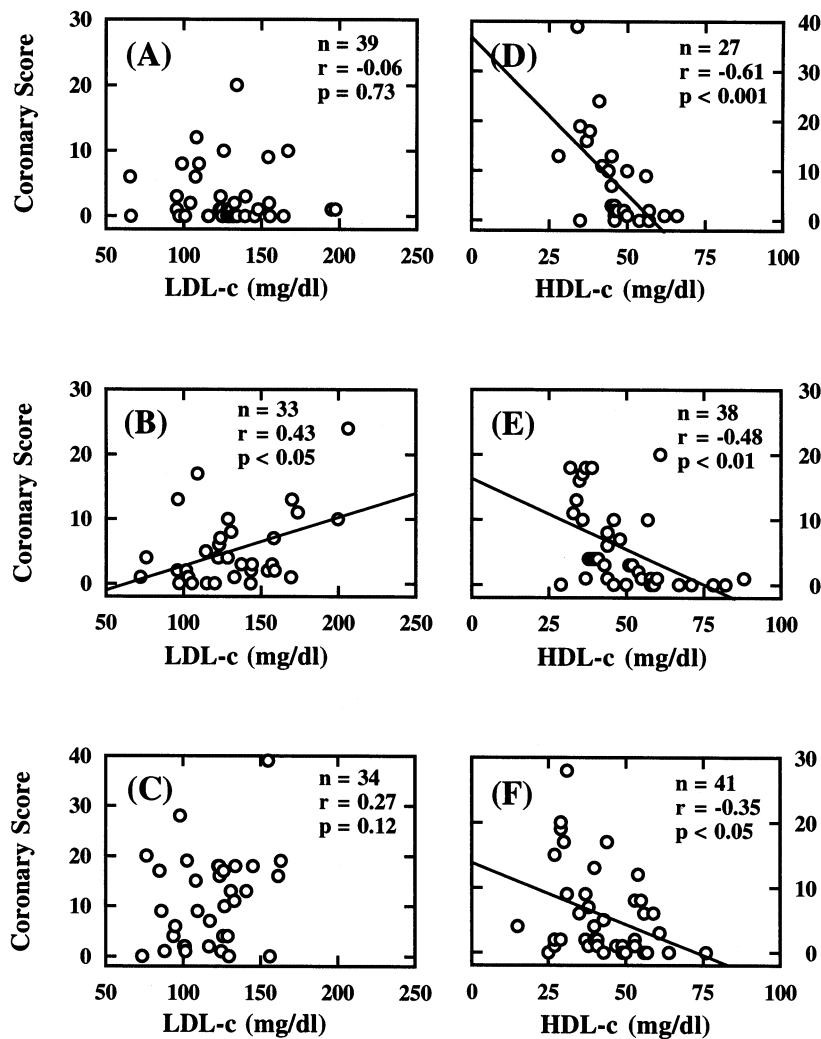


Fig. 8. Correlation of coronary score to LDL cholesterol in the patient groups having the HDL cholesterol high (50 mg/dl or higher) (A), middle (40 mg/dl or higher and lower than 50 mg/dl) (B) and low (lower than 40 mg/dl) (C), and to HDL cholesterol in the patient groups having LDL cholesterol high (140 mg/dl or higher) (D), middle (120 mg/dl or higher and lower than 140 mg/dl) (E) and low (lower than 120 mg/dl) (F).

disadvantage of atherogenesis either in homozygotes [17,18] or heterozygotes [16]. Mutation of CETP at the residue 405 from I to V may cause low CETP, high HDL and increase of CHD risk [23–25]. On the other hand, in another CETP polymorphism TaqIB, the B2 allele causes low CETP, high HDL and reduces the risk of CHD [27–29,40]. The reason for the discrepancy is not clear at this stage. Since CETP plays a complicated role in lipoprotein metabolism in plasma, other parameters in lipoprotein metabolism and magnitude of the change in CETP activity affect the outcome.

In the present study, patients who had ever taken lipid-lowering agents were excluded, since these drugs may influence the CETP mass in plasma [22,41–43]. CETP deficiency was also excluded because of the potential increase in atherogenesis both in HDL [44] and LDL [45]. In such a condition, CETP correlated with plasma LDL rather than HDL, and TaqIB muta-

tion of B2 showed antiatherogenic effect only when it lowers CETP and raises HDL. We failed to show a significant role of I405V variation of CETP either on lipoprotein metabolism or on atherogenesis in this study.

CETP may also be considered to be an indirect risk marker in human subjects [19], as well as in experimental animals [46] perhaps due to the positive correlation with atherogenic lipoproteins such as LDL [20,22,47,48]. This finding has been supported by experimental data such as that high cholesterol diet increased both plasma CETP and its mRNA in the liver in rabbits [49], and that cholesterol diet increased in mammalian plasma VLDL and LDL which positively correlated with the CETP mRNA in the adipose tissue and muscle [50]. The results imply that such conditions that induce the increase of LDL as high cholesterol diet induces the expression of the CETP gene. The plasma

HDL cholesterol level correlates with CETP only in hypertriglyceridemic humans [51] and monkeys [46], strongly suggesting that its regulation is mainly by hetero-exchange of cholesteryl ester in HDL with triglyceride in other lipoprotein fractions [8]. However, this tendency was not reproduced with our patient group of TG > 150 mg/dl.

In this study, we also analyzed the relationship between coronary atherosclerosis and plasma lipids. The results demonstrated that HDL cholesterol had higher impact on atherosclerosis than LDL cholesterol. While LDL tends to be correlated with coronary score only for the group with the middle HDL levels, HDL was a negative risk for all the high, middle and low LDL subgroups. This tendency was consistent with the earlier finding reported by Castelli et al. [52] except that the contribution of LDL to the risk seems even weaker in this study. A number of the intervention trials in Western countries demonstrated that lowering of plasma LDL cholesterol was beneficial for decreasing CHD incidence with respect to primary prevention [53,54]; or secondary prevention [55–58]. One of these studies, however, indicated that reduction of LDL cholesterol beyond 125 mg/dl may not contribute to the reduction of the risk [57]. It was also shown that the reduction of LDL did not reduce the CHD incidence for those with low HDL cholesterol [54]. Thus, HDL seems more important in terms of the CHD risk than LDL especially when the LDL cholesterol level is relatively low. CHD prevalence in Japan is considered to be a few times less than North America, and this might be partly because of a lower percentage of the population with high LDL [31]. Also, elimination of the patients who have ever been treated by lipid-lowering drugs presumably excluded some of the high LDL patients

from the analysis. These factors may have contributed to the low impact of LDL on the CHD risk in the results.

One of the limitations in the study is the use of coronary score as an index for the magnitude of coronary atherosclerosis. The region that appears normal in coronary angiography would often be considered atheromatous when examined by intravascular ultrasound [59]. Furthermore, acute myocardial infarction could frequently be inferred from the mild to moderate stenosis [60]. Thus, the extent of the stenosis does not always indicate the clinical severity of CHD. Nevertheless, it is still evident that a coronary scoring system is one of the good predictors for cardiac events [33].

The other bias in this study is that the subjects were those who required coronary angiographic examination. Therefore, prevalence of ‘clinical CHD’ may not be a highly reliable parameter since the population was so pre-selected. On the other hand, coronary score is still a fully valid and objective parameter to assess the contribution.

In conclusion, our study showed that CETP may modulate the risk of CHD by its TaqIB polymorphism only when B2 allele reduces CETP and perhaps subsequently increases HDL. CETP can also be an indirect risk for CHD as it increases in correlation with LDL.

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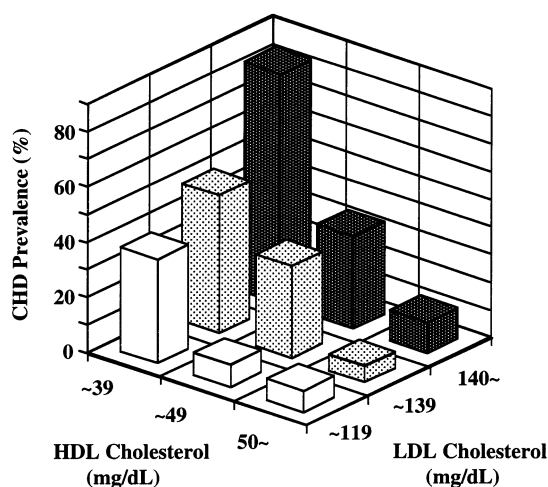


Fig. 9. Relationship of the CHD prevalence in the patient groups having high, middle and low HDL cholesterol and LDL cholesterol levels. Categories of the lipoprotein levels are defined in the legend of Fig. 8.

References

- [1] Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study. *Ann Int Med* 1971;74:1–12.
- [2] Kannel WB, Castelli WP, Gordon T. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. *Ann Int Med* 1979;90:85–91.
- [3] Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *J Am Med Assoc* 1986;256:2823–8.
- [4] Castelli WP, Doyle JT, Gordon T, et al. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. *Circulation* 1977;55:767–72.
- [5] Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am J Med* 1977;62:707–14.

- [6] Castelli WP, Garrison RJ, Wilson PW, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *J Am Med Assoc* 1986;256:2835–8.
- [7] Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 1989;79:8–15.
- [8] Ko KWS, Ohnishi T, Yokoyama S, et al. Triglyceride transfer is required for net cholesteryl ester transfer between lipoproteins in plasma by lipid transfer protein: evidence for a hetero-exchange transfer mechanism demonstrated by using novel monoclonal antibodies. *J Biol Chem* 1994;269:28206–13.
- [9] Marotti KR, Castle CK, Boyle TP, Lin AH, Murray RW, Melchior GW. Severe atherosclerosis in transgenic mice expressing simian cholesteryl ester transfer protein. *Nature* 1993;364:73–5.
- [10] Hayek T, Masucci-Magoulas L, Jiang X, Walsh A, Rubin E, Breslow JL, Tall AR. Decreased early atherosclerotic lesions in hypertriglyceridemic mice expressing cholesteryl ester transfer protein transgene. *J Clin Invest* 1995;96:2071–4.
- [11] Sugano M, Makino N, Sawada S, et al. Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. *J Biol Chem* 1998;273:5033–6.
- [12] Foger B, Chase M, Amar MJ, et al. Cholesteryl ester transfer protein corrects dysfunctional high density lipoproteins and reduces aortic atherosclerosis in lecithin cholesterol acyltransferase transgenic mice. *J Biol Chem* 1999;274:36912–20.
- [13] Plump AS, Masucci-Magoulas L, Bruce C, Bisgaier CL, Breslow JL, Tall AR. Increased atherosclerosis in ApoE and LDL receptor gene knock-out mice as a result of human cholesteryl ester transfer protein transgene expression. *Arterioscler Thromb Vasc Biol* 1999;19:1105–10.
- [14] Okamoto H, Yonemori F, Wakitani K, Minowa T, Maeda K, Shinkai H. A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature* 2000;406:203–7.
- [15] Rittershaus CW, Miller DP, Thomas LJ, et al. Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis. *Arterioscler Thromb Vasc Biol* 2000;20:2106–12.
- [16] Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, Tall AR. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J Clin Invest* 1996;97:2917–23.
- [17] Hirano K, Yamashita S, Kuga Y, et al. Atherosclerotic disease in marked hyperalphalipoproteinemia. Combined reduction of cholesteryl ester transfer protein and hepatic triglyceride lipase. *Arterioscler Thromb Vasc Biol* 1995;15:1849–56.
- [18] Hirano K, Yamashita S, Nakajima N, et al. Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. Marked hyperalphalipoproteinemia caused by CETP gene mutation is not associated with longevity. *Arterioscler Thromb Vasc Biol* 1997;17:1053–9.
- [19] Bhatnagar D, Durrington PN, Channon KM, Prais H, Mackness MI. Increased transfer of cholesteryl esters from high density lipoproteins to low density and very low density lipoproteins in patients with angiographic evidence of coronary artery disease. *Atherosclerosis* 1993;98:25–32.
- [20] Kinoshita M, Teramoto T, Shimazu N, et al. CETP is a determinant of serum LDL-cholesterol but not HDL-cholesterol in healthy Japanese. *Atherosclerosis* 1996;120:75–82.
- [21] Tato F, Vega GL, Tall AR, Grundy SM, et al. Relation between cholesterol ester transfer protein activities and lipoprotein cholesterol in patients with hypercholesterolemia and combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 1995;15:112–20.
- [22] Sasai K, Okumura-Noji K, Hibino T, Ikeuchi R, Sakuma N, Fujinami T, Yokoyama S. Human cholesteryl ester transfer protein measured by enzyme-linked immunosorbent assay with two monoclonal antibodies against rabbit cholesteryl ester transfer protein: plasma cholesteryl ester transfer protein and lipoproteins among Japanese hypercholesterolemic patients. *Clin Chem* 1998;44:1466–73.
- [23] Bruce C, Sharp DS, Tall AR, et al. Relationship of HDL and coronary heart disease to a common amino acid polymorphism in the cholesteryl ester transfer protein in men with and without hypertriglyceridemia. *J Lipid Res* 1998;39:1071–8.
- [24] Agerholm-Larsen B, Nordestgaard BG, Steffensen R, Jensen G, Hansen-Tybaerg A. Elevated HDL cholesterol is a risk factor for ischemic heart disease in white women when caused by a common mutation in the cholesteryl ester transfer protein gene. *Circulation* 2000;101:1907–12.
- [25] Kakko S, Tamminen M, Päivänsalo M, et al. Cholesteryl ester transfer protein gene polymorphisms are associated with carotid atherosclerosis in men. *Eur J Clin Invest* 2000;30:18–25.
- [26] Kuivenhoven JA, Jukema JW, Zwinderman AH, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. *New Engl J Med* 1998;338:86–93.
- [27] Ordovas JM, Cupples LA, Corella D, et al. Association of cholesteryl ester transfer protein -TaqIB polymorphism with variation in lipoprotein subclasses and coronary heart disease risk. *Arterioscler Thromb Vasc Biol* 2000;20:1323–9.
- [28] Radeau T, Vohl MC, Houde I, Lachance JG, Noël R, Després JP. HDL cholesterol and TaqIB cholesteryl ester transfer protein gene polymorphism in renal transplant recipients. *Nephron* 2000;84:333–41.
- [29] Corella D, Sáiz C, Guillén M, Portolés O, Mulet F, González J, Ordovas J. Association of TaqIB polymorphism in the cholesteryl ester transfer protein gene with plasma lipid levels in a healthy Spanish population. *Atherosclerosis* 2000;152:367–76.
- [30] Inazu A, Jiang XC, Haraki T, et al. Genetic cholesteryl ester transfer protein deficiency caused by two prevalent mutations as a major determinant of increased levels of high density lipoprotein cholesterol. *J Clin Invest* 1994;94:1872–82.
- [31] Anonymous. Current state of and recent trends in serum lipid levels in the general Japanese population. Research Committee on Serum Lipid Level Survey 1990 in Japan. *J. Atheroscler. Thromb.* 1996; 2: 122-32.
- [32] Austen WG, Edwards JE, Frye RL, et al. A reporting system on patients evaluated for coronary artery disease. Report of the Ad Hoc Committee for Grading of Coronary Artery Disease, Council on Cardiovascular Surgery, American Heart Association. *Circulation* 1975;51:5–40.
- [33] Mabuchi H, Koizumi J, Shimizu M, Takeda R. Development of coronary heart disease in familial hypercholesterolemia. *Circulation* 1989;79:225–32.
- [34] Friedewald WT, Levy RI, Fredrickson DS, et al. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
- [35] Hirano K, Yamashita S, Funahashi T, et al. Frequency of intron 14 splicing defect of cholesteryl ester transfer protein gene in the Japanese general population — relation between the mutation and hyperalphalipoproteinemia. *Atherosclerosis* 1993;100:85–90.
- [36] Sakai N, Yamashita S, Hirano K, et al. Frequency of exon 15 missense mutation (442D:G) in cholesteryl ester transfer protein gene in hyperalphalipoproteinemic Japanese subjects. *Atherosclerosis* 1995;114:139–45.
- [37] Fumeron F, Betoulle D, Luc G, et al. Alcohol intake modulates the effect of a polymorphism of the cholesteryl ester transfer protein gene on plasma high density lipoprotein and the risk of myocardial infarction. *J Clin Invest* 1995;96:1664–71.
- [38] Gudnason V, Kakko S, Nicaud V, Savolainen MJ, Kesaniemi YA, Tahvanainen E, Humphries S. Cholesteryl ester transfer

- protein gene effect on CETP activity and plasma high-density lipoprotein in European populations. The EARS Group. *Eur J Clin Invest* 1999;29:116–28.
- [39] Barter P. CETP and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2000;20:2029–31.
- [40] Kuivenhoven JA, de Knijff P, Boer JM, et al. Heterogeneity at the CETP gene locus. Influence on plasma CETP concentrations and HDL cholesterol levels. *Arterioscler Thromb Vasc Biol* 1997;17:560–8.
- [41] Carrilho AJ, Medina WL, Nakandakare ER, Quintao EC. Plasma cholesteryl ester transfer protein is lowered by treatment of hypercholesterolemia with cholestyramine. *Clin Pharmacol Therapeutics* 1997;62:82–8.
- [42] Contacos C, Barter PJ, Vrga L, Sullivan DR. Cholesteryl ester transfer in hypercholesterolaemia: fasting and postprandial studies with and without pravastatin. *Atherosclerosis* 1998;141:87–98.
- [43] McPherson R. Comparative effects of simvastatin and cholestyramine on plasma lipoproteins and CETP in humans. *Can J Clin Pharmacol* 1999;6:85–90.
- [44] Ishigami M, Yamashita S, Sakai N, et al. Large and cholesteryl ester-rich high-density lipoproteins in cholesteryl ester transfer protein (CETP) deficiency can not protect macrophages from cholesterol accumulation induced by acetylated low-density lipoproteins. *J Biochem* 1994;116:257–62.
- [45] Sakai N, Yamashita S, Hirano K, et al. Decreased affinity of low density lipoprotein (LDL) particles for LDL receptors in patients with cholesteryl ester transfer protein deficiency. *Eur J Clin Invest* 1995;25:332–9.
- [46] Quinet E, Tall A, Ramakrishnan R, Rudel L. Plasma lipid transfer protein as a determinant of the atherogenicity of monkey plasma lipoproteins. *J Clin Invest* 1991;87:1559–66.
- [47] Nakanishi T, Tahara D, Akazawa S, Miyake S, Nagataki S. Plasma lipid transfer activities in hyper-high-density lipoprotein cholesterolemic and healthy control subjects. *Metabol Clin Exp* 1990;39:225–30.
- [48] Tato F, Vega GL, Grundy SM, et al. Determinants of plasma HDL-cholesterol in hypertriglyceridemic patients. Role of cholesterol-ester transfer protein and lecithin cholesteryl acyl transferase. *Arterioscler Thromb Vasc Biol* 1997;17:56–63.
- [49] Quinet EM, Agellon LB, Kroon PA, Marcel YL, Lee YC, Whitlock ME, Tall AR. Atherogenic diet increases cholesteryl ester transfer protein messenger RNA levels in rabbit liver. *J Clin Invest* 1990;85:357–63.
- [50] Jiang XC, Moulin P, Quinet E, et al. Mammalian adipose tissue and muscle are major sources of lipid transfer protein mRNA. *J Biol Chem* 1991;266:4631–9.
- [51] Foger B, Ritsch A, Doblinger A, Wessels H, Patsch JR. Relationship of plasma cholesteryl ester transfer protein to HDL cholesterol. Studies in normotriglyceridemia and moderate hypertriglyceridemia. *Arterioscler Thromb Vasc Biol* 1996;16:1430–6.
- [52] Castelli WP. Cholesterol and lipids in the risk of coronary artery disease — the Framingham Heart Study. *Can J Cardiol* 1988;4 Suppl A:5A–10A.
- [53] Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *New Engl J Med* 1995;333:1301–7.
- [54] Downs JR, Clearfield M, Weis S, et al. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *J Am Med Assoc* 1998;279:1615–22.
- [55] Anonymous, Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S), *Lancet* 1994; 344: 1383-9.
- [56] Anonymous, Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group, *New Engl. J. Med.* 1998; 339: 1349-57.
- [57] Sacks FM, Pfeffer MA, Moye LA, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *New Engl J Med* 1996;335:1001–9.
- [58] Ericsson CG, Hamsten A, Nilsson J, Grip L, Svane B, de Faire U. Angiographic assessment of effects of bezafibrate on progression of coronary artery disease in young male postinfarction patients. *Lancet* 1996;347:849–53.
- [59] Mintz GS, Painter JA, Pichard AD, et al. Atherosclerosis in angiographically ‘normal’ coronary artery reference segments: an intravascular ultrasound study with clinical correlations. *J Am Coll Cardiol* 1995;25:1479–85.
- [60] Falk E, Shah PK, Fuster V, et al. Coronary plaque disruption. *Circulation* 1995;92:657–71.