Brief Genetics Report

Relationship Between *Taq*IB Cholesteryl Ester Transfer Protein Gene Polymorphism and Macrovascular Complications in Japanese Patients With Type 2 Diabetes

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Cholesteryl ester transfer protein (CETP) is a key regulating factor of lipid metabolism, and the polymorphism of its gene may therefore be a candidate for modulating the lipid parameters, altering the susceptibility to atherosclerosis in type 2 diabetic subjects. In a group of 443 unrelated Japanese patients with type 2 diabetes, we studied the *B1B2* polymorphism at the *CETP* locus, which is detectable with the restriction enzyme *Taq*I. Patients were separated into three groups according to genotype and compared based on their clinical characteristics, lipid parameters, and macrovascular complications. The B2 allele was associated in a dose-dependent fashion with higher HDL cholesterol and apolipoprotein AI levels, together with lower CETP concentrations. Furthermore, the prevalence of macrovascular complications, such as coronary heart disease, arteriosclerosis obliterans, and cerebral vascular disease, was significantly higher in subjects with the *B1B1* genotype. Multiple logistic regression analysis also showed that the B1 allele of *CETP* genotype was associated with the incidence of these three complications independently of other risk factors. Thus, in type 2 diabetic patients, the *B1B2* polymorphism of *CETP* gene is likely to be a strong genetic predictor of macrovascular complications. *Diabetes* 51:871–874, 2002

Type 2 diabetic patients are at high risk of atherosclerotic diseases, such as coronary heart disease (CHD), cerebral vascular disease (CVD), or arteriosclerosis obliterans (ASO) (1). This increased risk can be partly accounted for by the lipoprotein disorders linked to insulin resistance: elevated VLDL, triglycerides, and cholesterol, together with low HDL cholesterol (2). The inverse relationship between the level of HDL cholesterol and the risk of cardiovascular disease is commonly explained by the crucial role of HDL in reverse cholesterol transport (3–5). By this process, cholesterol from peripheral cells is taken up by HDL and transported to the liver, where it is metabolized and excreted in the bile. Cholesteryl ester transfer protein (CETP) has a central role in the metabolism of HDL lipoprotein and may therefore alter the susceptibility to atherosclerotic vascular disease (3). In a normolipidemic population, the plasma CETP concentration varies mostly over a threefold range and is influenced by environmental factors (6). Plasma CETP has been shown to be elevated in smokers and to be decreased by heavy alcohol drinking and physical training (7–9).

Recently, the *B1B2* polymorphism of intron 1 of the *CETP* gene (presence or absence of a *Taq*IB restriction site) was shown to be a determinant of HDL cholesterol (10). It has been reported that both healthy and type 2 diabetic subjects with the *B2B2* genotype of *Taq*IB polymorphism have significantly higher HDL cholesterol levels than those with the *B1B1* or *B1B2* genotype (11,12). Furthermore, in subjects with the *B2B2* genotype, the progression of coronary atherosclerosis is more greatly suppressed by pravastatin than in those with the *B1B1* or *B1B2* genotype, and the incidence of CHD is lower in type 2 diabetic subjects with the *B2B2* genotype (11). In the present study, we tested the hypothesis that the *CETP* gene polymorphism is associated with an incidence of macrovascular diseases in 443 Japanese subjects with type 2 diabetes.

Analysis of *Taq*IB restriction fragment–length polymorphism showed that the frequencies of B1 (presence of cutting site) and B2 alleles were 58 and 42%, respectively. The genotype frequencies were in Hardy-Weinberg equilibrium (34.1, 47.9, and 18.0% for *B1B1*, *B1B2*, and *B2B2*, respectively). No statistically significant differences in sex distribution, age, diabetes duration, smoking index, BMI, HbA_{1c}, or homeostasis model assessment of insulin resistance ($HOMA_{IR}$) were observed among the three genetic groups (Table 1). The plasma concentrations in non-HDL cholesterol and triglycerides, as well as in apolipoproteins AII and B and lipoprotein(a) [Lp(a)] were similar in all genotypes. In contrast, *CETP* genotypes were significantly

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ASO, arteriosclerosis obliterans; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; CVD, cerebral vascular disease; $HOMA_{IR}$, homeostasis model assessment of insulin resistance; Lp(a), lipoprotein(a).

TABLE 1
Comparison of clinical characteristics in 443 type 2 diabetic patients according to CETP genotype

	B1B1	*B1B2*	*B2B2*	*P**
n	151	212	80	
Age (years)	56 (16–82)	59 (16–76)	56 (15–78)	0.2036
Diabetes duration (years)	7 (1–30)	10 (0–40)	10 (0–38)	0.6168
M/F	92/59	112/100	44/36	0.3035
BMI (kg/m^2)	23.8 (14.2–40.4)	22.4 (15.9–40.7)	22.3 (17.0–40.6)	0.0580
Smoking index (cigarette-years)	80 (0–3,200)	145 (0–2,940)	300 (0–1,500)	0.5683
Drinker/nondrinker	71/80	79/133	28/52	0.1230
HbA_{1c} (%)	8.4 (3.9–14.9)	9.0 (4.7–15.8)	8.6 (4.7–14.7)	0.0680
Non-HDL cholesterol (mmol/l)	3.79 (1.86–8.15)	3.83 (1.86–14.9)	3.72 (1.94–9.34)	0.5171
HDL cholesterol (mmol/l)	1.14 (0.08–2.46)	1.19 (0.52–2.46)	1.32 (0.67–2.77)	0.0012
Triglycerides (mmol/l)	1.28 (0.47–4.85)	1.29 (0.47–7.54)	1.17 (0.43–6.55)	0.7681
Apolipoprotein AI (mg/dl)	116.5 (68–287)	119.5 (75–197)	127.0 (77–248)	0.0127
Apolipoprotein AII (mg/dl)	28 (17–51)	29 (17–52)	28 (17–58)	0.4924
Apolipoprotein B (mg/dl)	100 (49–183)	102.5 (3.4–145)	101.5 (55–196)	0.8126
Lp(a) (mg/dl)	15 (1–110)	17 (1–160)	21 (1–160)	0.3815
$HOMA_{IR}$†	1.8 (0.37–10.5)	2.2 (0.34–34.6)	1.7 (0.20–50.7)	0.1347
CETP concentration (μg/ml)‡	1.98 (1.1–4.6)	1.70 (0.9–3.1)	1.20 (0.8–2.8)	0.0275

Data are *n* or median (range) **P* values were obtained by χ^2 test or Kruskal-Wallis test; †$HOMA_{IR}$ was calculated as the following formula: $HOMA_{IR}$ = fasting plasma insulin level (pmol/l) × fasting plasma glucose (mmol/l)/22.5; ‡CETP concentrations were obtained from 61 plasma samples (B1B1, $n = 21$; B1B2, $n = 23$; B2B2, $n = 17$).

associated with the plasma concentrations of HDL cholesterol ($P = 0.0012$, Kruskal-Wallis test). Moreover, although the number of plasma samples is limited, we identified a significant relationship between the *CETP* genotype and plasma concentrations of CETP ($P = 0.0275$). Specifically, our results showed that the B1 allele was associated in a dose-dependent fashion with higher CETP concentrations, which is in agreement with the previous findings in healthy subjects (13). *B1B1* had the highest CETP and the lowest HDL cholesterol concentrations, whereas *B2B2* had the lowest CETP and the highest HDL cholesterol concentrations. Moreover, serum apolipoprotein AI levels were higher in *B2B2* compared with other genotypes ($P = 0.0127$). Similar results were observed when data of male and female subjects were analyzed separately (data not shown).

The frequencies of macrovascular complications (CHD, ASO, and CVD) are shown according to the *CETP* genotype in Table 2. The association of genotype with macrovascular complications was tested by χ^2 test. Analysis of *CETP* genotype frequencies in the CHD, ASO, and CVD subgroups showed a greater percentage of patients with the *B1B1* genotype compared with the other genotypes (CHD: $\chi^2 = 8.90$, $P = 0.0117$; ASO: $\chi^2 = 6.83$, $P = 0.0329$; and CVD: $\chi^2 = 13.2$, $P = 0.0014$), thus indicating associations of *CETP* genotype with the prevalence of these complications.

The data were further examined by multiple logistic regression analysis, as shown in Table 3. The B1 allele of the *CETP* genotype was associated with the presence of CHD independent of other risk factors, including age, sex, smoking, HDL and non-HDL cholesterol, apolipoprotein Lp(a), BMI, HbA_{1c}, and $HOMA_{IR}$ (odds ratio 4.702 [95% CI 1.400–15.79], $P = 0.0122$). Interestingly, $HOMA_{IR}$ was also associated with increased risk of CHD (1.113 [1.022–1.211], $P = 0.0133$). In addition to CHD, the B1 allele of the *CETP* genotype was associated with the incidence of ASO and CVD independent of other risk factors (ASO: 8.551 [1.628–44.91], $P = 0.0112$; CVD: 3.903 [1.606–9.485], $P = 0.0026$). In regard to ASO, the BMI and apolipoprotein Lp(a) levels were also associated with increased risk of ASO, as follows: BMI 1.244 (1.000–1.540), $P = 0.0498$; Lp(a) 1.034 (1.001–1.060), $P = 0.0427$. Furthermore, HbA_{1c} was associated with the incidence of CVD independent of CETP genotype (1.358 [1.037–1.780], $P = 0.0263$).

The present study has demonstrated that the *B1B2* polymorphism of intron 1 of the *CETP* gene is closely associated with macrovascular complications, including CHD, ASO, and CVD in type 2 diabetic patients. It has furthermore confirmed the well-documented relationship between this polymorphism and HDL cholesterol (14,15). In this large population of type 2 diabetic patients, the distribution of *CETP* genotypes was similar to that previously reported in a healthy population (14–16), which does not suggest the occurrence of any linkage disequilibrium between the *CETP* gene and those that might cause type 2 diabetes.

In addition to the association of this polymorphism with

TABLE 2
Frequencies of clinical events in 443 type 2 diabetic patients according to CETP genotype

	B1B1	*B1B2*	*B2B2*	χ^2	*P**
n	151	212	80		
CHD (%)	15 (10.0)	8 (3.8)	1 (1.3)	8.898	0.0117
ASO (%)	12 (7.9)	7 (3.3)	1 (1.3)	6.828	0.0329
CVD (%)	23 (15.2)	12 (5.7)	3 (3.8)	13.204	0.0014

Data are *n* (%). **P* values were obtained by the χ^2 test.

TABLE 3
Multiple logistic regression analysis of determinants of CHD, ASO, and CVD in type 2 diabetic patients

Parameters	CHD		ASO		CVD	
	OR	95% CI	OR	95% CI	OR	95% CI
Age (years)	0.973	0.930–1.272	1.059	0.981–1.140	1.007	0.966–1.048
Sex (male)	0.911	0.175–4.735	2.582	0.183–36.50	0.520	0.520–8.212
BMI (kg/m^2)	1.099	0.950–1.272	1.244*	1.000–1.540	1.072	0.937–1.227
Smoking index (cigarette-years)	1.001	1.000–1.002	1.001	0.999–1.000	1.000	1.000–1.001
Alcohol (drinker)	0.745	0.154–3.614	0.285	0.029–2.830	0.602	0.180–2.017
HbA_{1c} (%)	1.081	0.770–1.517	1.506	0.955–2.370	1.358*	1.037–1.780
$HOMA_{IR}$	1.113*	1.022–1.211	0.613	0.319–1.180	1.010	0.879–1.161
HDL cholesterol (mmol/l)	1.024	0.970–1.081	1.029	0.959–1.100	1.023	0.984–1.063
Non-HDL cholesterol (mmol/l)	1.011	0.997–1.044	1.007	0.991–1.020	1.009	0.998–1.020
Lp(a) (mg/dl)	1.010	0.977–1.044	1.034*	1.001–1.060	1.005	0.982–1.029
Taq IB polymorphism (the number of B1 alleles)	4.702*	1.400–15.79	8.551*	1.628–44.91	3.903†	1.606–9.485

OR, odds ratio. *$P < 0.05$; †$P < 0.005$.

HDL cholesterol levels, we also identified a significant relationship between the B2 allele and higher levels of apolipoprotein AI, the major protein in HDL particles. After HDL particles accept cholesterol from nonliver cells, CETP facilitates the transfer of cholesteryl ester onto triglyceride-rich lipoproteins as part of the reverse cholesterol transport pathway, ultimately leading to cholesterol excretion by the liver (17). When CETP is dysfunctional, cholesterol accumulates in HDL, and the transfer of cholesterol from peripheral cells to the liver is blocked. In accordance with this, our data suggest the presence of an increased number of HDL particles (elevated HDL cholesterol and apolipoprotein AI) for subjects with the *B2B2* genotype. Because apolipoprotein AI is found only in HDL and chylomicrons, the effects we observed on apolipoprotein AI most likely reflect changes in levels of apolipoprotein AI in HDL cholesterol.

There was a significant relationship between variation at the *CETP* gene locus and the prevalence of macrovascular complications. This relationship was dose-dependent and independent of other risk factors, most notably sex and HDL cholesterol levels. Despite significant differences in mean HDL cholesterol levels between sexes (male subjects: 1.15 mmol/l; female subjects: 1.39 mmol/l; $P < 0.0001$, Mann-Whitney *U* test), there was no significant correlation between sex and the prevalence of macrovascular complications. In accordance with the present results, Tenkanen et al. (18) mentioned that there was no difference even when the sexes were analyzed separately. In contrast, Kauma et al. (19) showed that the association between the *Taq*IB polymorphism and HDL cholesterol was seen only in women, and Durlach et al. (12) reported that the *Taq*IB polymorphism seems to exert a modulating role in men only. Although the reason for the discrepancy between the previously reported data and our study is not known, one possible explanation may lie in the different lifestyles of the study subjects.

The role of CETP in atherogenesis is still under debate (20). CETP may play a proatherogenic role, in view of the fact that it mediates a redistribution of plasma cholesterol from lipoproteins, which is associated with protection against atherosclerosis, into the proatherogenic apolipoprotein B–containing lipoproteins. However, CETP mediates one of the steps in reverse cholesterol transport, an anti-atherogenic process. The results of the present study support the concept that increased HDL cholesterol levels resulting from lower CETP activity appear to be associated with a lower risk of macrovascular complications in Japanese diabetic subjects.

Transgenic mouse experiments have shown that environmental factors play an important role in the modulation of CETP gene expression (21). Some studies have analyzed the possible interaction between CETP genotype and some environmental factors, such as smoking and alcohol, on plasma HDL cholesterol levels. In this regard, Kondo et al. (14) showed that the association of the CETP gene with HDL cholesterol levels was present only in nonsmokers. Fumeron et al. (16) did not find an interaction with smoking, but they found an important interaction with alcohol consumption. In the present study, when gene-environmental terms were tested, no statistically significant interactions of the CETP genotype with drinking or smoking were found. These observations allow us to conclude that the effect of this polymorphism on plasma HDL cholesterol and incidence of macrovascular events seems statistically independent across several levels of these environmental factors.

In conclusion, our data demonstrate that the *Taq*IB polymorphism of the *CETP* gene is a strong genetic predictor of macrovascular complications in type 2 diabetes. The impact of these findings on the prevalence of these diseases will obviously require further prospective investigations.

RESEARCH DESIGN AND METHODS

A total of 443 patients with type 2 diabetes (248 men and 195 women) were selected randomly for the present study from among patients who were admitted to the diabetes center at Osaka City University Hospital from April 1997 to February 2000. The diagnosis of diabetes was based on a previous history of diabetes or on American Diabetes Association criteria (22). The mean duration of diabetes was 10.4 ± 8.3 years. Mean BMI was 23.0 ± 4.9 kg/m^2. The study was approved by the local ethics committee of Osaka City University Hospital, and appropriate informed consent was obtained from all the subjects.

Vascular pathologies. The diagnosis of CHD was assigned by a cardiologist, and the diagnosis was based on symptoms and clear ischemic changes in an electrocardiogram, either at rest or during an exercise test, or on findings from coronary angiography. CVD was diagnosed by a history of acute neurological deficits and evidence of cerebral infarction/hemorrhage on computed tomography and/or magnetic resonance imaging of the brain. ASO was defined

according to the La Fontaine classification (grade II or III) for leg arteriopathy and the ankle-brachial pressure ratio, and it was confirmed by peripheral angiography.

Determination of the *Taq*IB CETP polymorphism. Genomic DNA was extracted from peripheral-blood leukocytes by standard methods. The *Taq*IB polymorphism in intron 1 of the *CETP* gene was genotyped using a previously described PCR method (13). In brief, genomic DNA was amplified by PCR using a GeneAmp PCR System 9700 (Perkin-Elmer). Each amplification was performed using 100 ng of genomic DNA containing 20 pmol of each PCR primer and 1 unit of AmpliTaq Gold DNA polymerase (Perkin-Elmer) with 1.5 mmol/l MgCl2, 50 mmol/l KCl, 20 mmol/l Tris-HCl, pH 8.4, 200 μmol/l of each deoxynucleotide triphosphate, and 0.01 mg/ml BSA. The amplification consisted of 35 cycles of denaturation (1 min at 94°C), annealing (1 min at 60°C), and elongation (2 min at 72°C). To detect the *Taq*IB CETP polymorphism, 20-μl aliquots were digested with 5 units *Taq*I at 65°C for 3 h and then separated on a 2% agarose gel in 1× Tris acetate EDTA buffer. The gel was stained with ethidium bromide and visualized under ultraviolet light.

CETP concentrations. Concentrations of CETP protein were measured from frozen plasma samples stored at −80°C by enzyme-linked immunosorbent assay with two monoclonal antibodies (23). Because of a lack of sufficient aliquots of plasma, CETP concentrations were determined in 61 patients at baseline.

Statistical analyses. Statistical analyses were performed with the StatView 5 system (Abacus Concepts, Berkeley, CA) for the Apple Macintosh computer. All values were summarized as median and range, unless otherwise indicated. The differences for clinical parameters between the sexes were assessed by the Mann-Whitney nonparametric U test. Kruskal-Wallis and χ^2 tests were appropriately performed for comparison of genotyped groups. Predictive variables for risk were analyzed by the multiple logistic regression model. The assumption of Hardy-Weinberg equilibrium was tested by means of gene counting and χ^2 analysis. $P < 0.05$ was considered significant.

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