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Cholesteryl ester transfer protein B1B1 genotype as a predictor of coronary artery disease in Taiwanese with type 2 diabetes mellitus

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Abstract

Diabetes is known to be a high-risk factor for coronary artery disease (CAD), and lipid abnormalities have been found to possibly contribute to CAD in diabetic patients. Cholesteryl ester transfer protein (CETP) gene TaqIB polymorphism is associated with lipid profile variability, and this polymorphism may be a risk factor for CAD in diabetic patients. To clarify the relationship between CETP TaqIB gene polymorphism and CAD, we enrolled in our study 365 Taiwanese with type 2 diabetes mellitus (101 with CAD and 264 without CAD). The genotype of the subjects for TaqIB polymorphism of CETP in intron 1 was analyzed by using polymerase chain reaction-restriction fragment length polymorphism. The CETP B1B1 genotype (18.8% vs 8.5%, $P = .002$) and B1 allele (42.1% vs 29.7%, $P = .002$) were significantly more frequent in diabetic patients with CAD than those without CAD. Logistic regression analysis revealed that the CETP B1B1 genotype was associated with CAD in patients with type 2 diabetes mellitus (odds ratio, 3.18; 95% confidence interval, 1.54-6.54; $P = .002$). Interestingly, in diabetic patients, serum creatinine levels higher than 1.4 mg/dL were also associated with increased risk for CAD (odds ratio, 2.09; 95% confidence interval, 1.12-3.91; $P = .02$). Our results suggest that the CETP B1B1 genotype is a strong genetic predictor of CAD in Taiwanese with type 2 diabetes mellitus.

1. Introduction

Patients with type 2 diabetes mellitus are at high risk of developing coronary artery disease (CAD) [1]. This increased risk can be partly accounted for by the lipoprotein disorders linked to insulin resistance: elevated levels of very low-density lipoprotein and triglycerides (TGs), together with low levels of high-density lipoprotein cholesterol (HDL-C) [1,2]. In fact, decreased concentrations of HDL have been reported to be significantly related to CAD in patients with type 2 diabetes mellitus [3,4].

The cholesteryl ester transfer protein (CETP), a hydrophobic glycoprotein composed of 476 amino acids [5], mediates the transfer of cholesteryl ester from HDL to TG-rich lipoproteins [6]. It is involved in modulating concentrations of HDL concentration [7,8] and may, therefore, alter susceptibility to CAD. Several polymorphisms have been reported in the CETP gene locus [9-11]. The most studied polymorphism to date has been TaqIB polymorphism, which has been shown to be a silent base change affecting the 227th nucleotide in the first intron of the gene [9]. The B2 allele of this polymorphism has been associated with increased HDL-C levels [12,13]. Recently, Elosua et al [14] evaluated the association of 12 variants in 10 lipoprotein-related genes with carotid intimal medial thickness and found that only CETP TaqIB polymorphism was associated with carotid intimal medial thickness in men. Ordovas et al [15] reported that CETP TaqIB played a significant role in determining HDL-C variability and that this association translated into lower risk for coronary heart disease in men

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but not in women. However, the association with plasma HDL as well as with CAD may be population specific and influenced by environmental factors [16-19].

In general, CAD prevalence is lower in Asia than in Western countries. This Asian advantage is still identifiable when both groups live together. In a mixed-ancestry group living in Canada, those of Chinese heritage had a lower risk of CAD than their European counterparts [20]. In another study comparing Asians in similar living conditions, Chinese had a lower risk of myocardial infarction than Malays and Indians [21]. Chi et al [22] reported that the rate of large-vessel disease in Chinese diabetics was about half of that found in the combined centers of the World Health Organization Multinational Study of Vascular Disease in Diabetes. We hypothesized that a genetic factor might play some role in the Chinese's being at low risk for CAD. Although several studies on CETP TaqIB gene polymorphism and CAD have been performed in white and Japanese populations, the relationship between the polymorphism and heart disease has not been made explicit. Nor has there been a large-scale study of CETP TaqIB gene polymorphism in patients with type 2 diabetes mellitus in Taiwan. Therefore, our study was designed to further clarify the relationship between the CETP TaqIB gene and CAD and explore the relationship between the two in Taiwanese with type 2 diabetes mellitus.

2. Subjects and methods

2.1. Patient population

The study enrolled 365 Taiwanese patients with type 2 diabetes mellitus (101 with CAD and 264 without CAD) recruited from the diabetic clinic in the Metabolism Division at Kaohsiung Medical University Hospital, Kaohsiung, Taiwan. The diagnosis of diabetes was based on American Diabetes Association criteria [23]. The diabetic patients without CAD had a normal electrocardiogram and no history or clinical signs of CAD, based on a maximal negative exercise test. Coronary artery disease was diagnosed in patients having a clinical history of angina pectoris/myocardial infarction and was confirmed by coronary angiography. The hospital human research ethics committee approved the design, and informed consent was obtained from each patient. Individual interviews were held with patients about their disease and smoking history. Patients received a complete physical examination as well as an assessment for the presence and extent of macro- or microvascular complications. Routine blood and urine analyses were performed. Measurements were taken to calculate the body mass index (BMI).

2.2. Detection genotypes of CETP TaqIB

Cholesteryl ester transfer protein TaqIB genotypes were determined by polymerase chain reaction (PCR) amplification of genomic DNA, followed by restriction enzyme digestion. Genomic DNA was extracted from peripheral blood leukocytes by using either QIAamp mini kits (Qiagen, Hilden, Germany) or Generation Capture Column kits (Gentra Systems, Minneapolis, MN). A 535-base-pair (bp) fragment in intron 1 of the CETP gene was PCR-amplified with the following oligonucleotide primers: 5′-CAC-TAGCCCAGAGAGAGAGGAGTG CC-3′ (forward) and 5′-CTGAGCCCAGCCGCACACTAA C-3′ (reverse). Polymerase chain reaction cycling conditions were as follows: 1 cycle of denaturation at 94°C for 5 minutes followed by 35 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The PCR products were then digested with *Taq*IB restriction endonuclease (GIBCO-BRL, Rockville, MD) at 65°C for 2 hours, and the fragments were separated by electrophoresis in a 2% agarose gel. The resulting DNA fragments were 174 and 361 bp for the B1 allele and 535 bp for the undigested B2 allele.

2.3. Biochemical analyses

Total cholesterol and TG were measured by a Beckman Coulter biochemical analyzer (SYNCHRON CX-5CE, Beckman, Fullerton, CA). The total cholesterol and TG levels were analyzed by using the CHOD-POD method and the Lipase-GOD-POD method (Beckman reagent kit), respectively. The HDL-C and low-density lipoprotein cholesterol (LDL-C) fractions were measured by using an electrophoresis analyzer (Helena REP, Beaumont, TX). The Helena REP electrophoresis system separates very low-density lipoprotein, HDL-C, and LDL-C by agarose gel electrophoresis [24]. The specimen was applied to an agarose gel. Then, the lipoprotein fractions were separated by electrophoresis and stained with Fat Red 7B (CHROMA Division, Wadeck, Munich, Germany). The stained bands were quantified in a scanning densitometer (Rapid Electrophoresis Analyzer, Beaumont, TX) using a 525-nm filter. The control was used as a marker for locating the lipid bands and was measured to verify the accuracy of quantization.

2.4. Statistical analysis

Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained to compare differences between the patients with CAD and the distributions for allelic and genotype frequencies. The observed frequencies of the genotypes were compared with the frequencies under Hardy-Weinberg equilibrium by χ^2 tests [25]. The t test and 1-way analysis of variance were used to detect the mean differences in the biochemical data and in the CAD distribution or CEPT genotype. We used the logistic regression model to obtain the adjusted ORs for the factors associated with CAD of diabetic patients. All P values were calculated based on 2-sided tests, with statistical significance defined as a P value of less than .05.

3. Results

At baseline, the patients with type 2 diabetes mellitus with CAD and those without CAD had similar BMI,

Table 1
Clinical characteristics of patients with type 2 diabetes mellitus with and without CAD

	Type 2 diabetes mellitus with CAD (n = 101)	Type 2 diabetes mellitus without CAD (n = 264)
Sex (female/male)	57/44	148/116
Age (y)*	64.34 ± 10.5	58.15 ± 12.31
BMI (kg/m^2)	26.18 ± 3.30	25.51 ± 3.89
Cholesterol (mg/dL)*	180.05 ± 40.83	193.20 ± 45.61
TG (mg/dL)	140.63 ± 85.15	152.94 ± 137.48
HDL-C (mg/dL)	44.21 ± 10.75	44.95 ± 11.71
LDL-C (mg/dL)	109.88 ± 32.82	116.57 ± 34.93
Creatinine (mg/dL)	1.23 ± 0.89	1.10 ± 1.44
HbA_{1c} (%)	7.97 ± 2.13	7.81 ± 1.60
Duration of diabetes (y)	12.13 ± 3.46	11.46 ± 4.42

Data are presented as mean ± SD.
* $P < .05$.

durations of diabetes, and levels of hemoglobin A_{1c} (HbA_{1c}), serum TG, HDL-C, LDL-C, and creatinine (Table 1). Diabetic patients with CAD were significantly older compared with those without CAD. The serum cholesterol concentration was higher in diabetic patients without CAD than in those with CAD.

The CETP B1B1 genotype (18.8% vs 8.5%, $P = .002$) and B1 allele (42.1% vs 29.7%, $P = .002$) were significantly more frequent in diabetic patients with CAD than in those without CAD (Table 2). The CETP genotype distribution was measured to be in a Hardy-Weinberg equilibrium ($\chi^2 = 0.2$, $P > .05$). Taking sex into account, we found that the frequency of CETP B1B1 genotype was significantly higher in diabetic women with CAD than in those without CAD (21.1% vs 6.1%, $P = .002$), but this difference was not seen in men (15.9% vs. 12.1%, $P = .389$) (Table 3). However, there was a small number of CAD events and subjects with CETP B1B1 genotype in both women and men.

We summarized the characteristics of the patients with type 2 diabetes mellitus by CETP genotype (Table 4). There were no significant differences in age, BMI, durations of diabetes, HbA_{1c} level, and blood levels of cholesterol, TG, LDL-C, and creatinine. The serum HDL-C levels were significantly lower in diabetic patients with the B1 allele (B1B1 and B1B2) compared with those without the B1 allele (B2B2) (43.45 ± 10.93 vs 46.24 ± 11.48 mg/dL, $P = .02$). The frequencies of smoking and using statins and/or fibrates were not different among these 3 groups. However, CAD was more prevalent in patients with the CETP B1B1

Table 2
Distribution of CETP genotype and allele frequencies in patients with type 2 diabetes mellitus with and without CAD

Variable	Type 2 diabetes mellitus with CAD (n = 101), No. (%)	Type 2 diabetes mellitus without CAD (n = 264), No. (%)	OR (95% CI)	P
Genotypes				
B1B1	19 (18.8)	23 (8.5)	3.068 (1.054-6.26)	.002
B1B2	47 (46.5)	111 (40.8)	1.573 (0.949-2.608)	.078
B2B2	35 (34.6)	130 (47.7)	1.0	
Alleles				
B1	85 (42.1)	157 (29.7)	1.717 (1.227-2.402)	.002
B2	117 (57.9)	371 (70.2)	1.0	

Table 3
Distribution of CETP genotype and allele frequencies in patients with type 2 diabetes mellitus with and without CAD separated by sex

Variable	Type 2 diabetes mellitus with CAD, No. (%)	Type 2 diabetes mellitus without CAD, No. (%)	OR (95% CI)	P
Male	n = 44	n = 116		
Genotypes				
B1B1	7 (15.9)	14 (12.1)	1.530 (0.554-4.582)	.389
B1B2	19 (43.2)	45 (38.8)	1.337 (0.629-2.842)	.450
B2B2	18 (40.9)	57 (49.1)	1.0	
Alleles				
B1	33 (37.5)	73 (31.5)	1.307 (0.783)	.306
B2	55 (62.5)	159 (68.5)	1.0	
Female	n = 57	n = 148		
Genotypes				
B1B1	12 (21.1)	9 (16.1)	12.753	.002
B1B2	28 (49.1)	66 (44.6)	2.956	.085
B2B2	17 (29.8)	73 (49.3)	1.0	
Alleles				
B1	52 (45.6)	84 (28.4)	2.117 (1.354-3.309)	.001
B2	62 (54.4)	212 (71.6)	1.0	

Table 4
Clinical characteristics of patients with type 2 diabetes mellitus according to CETP genotype

	B1B1 (n = 42)	B1B2 (n = 158)	B2B2 (n = 165)
Sex (female/male)	22/20	94/64	89/76
Age (y)	59.05 ± 14.02	59.77 ± 12.78	59.84 ± 11.2
BMI (kg/m^2)	26.74 ± 3.69	25.69 ± 3.06	25.51 ± 4.26
Cholesterol (mg/dL)	188.95 ± 44.64	188.89 ± 43.53	189.45 ± 45.33
TG (mg/dL)	142.47 ± 72.94	155.03 ± 117.52	143.66 ± 138.55
HDL-C (mg/dL)*	43.31 ± 10.63	43.39 ± 11.09	46.24 ± 11.84
LDL-C (mg/dL)	114.98 ± 37.33	114.92 ± 35.58	114.21 ± 32.90
Creatinine (mg/dL)	1.17 ± 1.02	1.19 ± 1.65	1.06 ± 0.98
HbA_{1c} (%)	7.91 ± 1.70	7.77 ± 1.76	7.96 ± 1.79
Duration of diabetes (y)	12.19 ± 3.71	12.23 ± 3.61	11.96 ± 4.24
Smoker, No. (%)	7 (16.7)	27 (17.1)	29 (17.6)
Medications			
Statin, No. (%)	26 (61.9)	99 (62.7)	100 (60.6)
Fibrate, No. (%)	11 (26.2)	38 (24.1)	39 (23.6)
Type 2 diabetes mellitus with CAD, No. (%)†	19 (45.2)	47 (29.7)	35 (21.2)

Data are presented as mean ± SD unless otherwise indicated.
* $P = .02$, (B1B1 + B1B2) vs B2B2.
† $P = .02$, (B1B1+ B1B2) vs B2B2.

Table 5
Logistic regression analysis for CAD

	Adjusted OR (95% CI)	*P*
CETP		
B1B1 genotype	3.18 (1.54-6.54)	.002
B1B2 genotype	1.65 (0.99-2.76)	.055
Serum creatinine level ≥1.4 (mg/dL)	2.09 (1.12-3.91)	.02

genotype than in patients with the CETP B2B2 genotype (45.2% vs 21.2%, $P < .01$).

Multiple logistic regression analysis was also used to compare the data (Table 5). The CETP B1B1 genotype was associated with CAD in patients with type 2 diabetes mellitus independent of other risk factors, including age, sex, smoking status, cholesterol, TG, LDL-C, HDL-C, BMI, and HbA_{1c} (OR, 3.18; 95% CI, 1.54-6.54; $P = .002$). It was also interesting to note that in diabetic patients, serum creatinine levels higher than 1.4 mg/dL were also associated with increased risk for CAD (OR, 2.09; 95% CI, 1.12-3.91; $P = .02$).

4. Discussion

In the present study, we found an association of CETP TaqIB gene polymorphism with CAD in Taiwanese with type 2 diabetes mellitus. The relationship between the TaqIB genotype and the risk of CAD in diabetes has been investigated in some population-based studies, albeit without consistent results [26-28]. Two previous studies [26,27] revealed CETP TaqIB gene polymorphism to be associated with macrovascular complications of diabetes. However, Chaaba et al [28] reported that there was no association between CETP TaqIB gene polymorphism and CAD in type 2 diabetes mellitus. Recently, a meta-analysis study [29] reported that the CETP TaqIB variant was associated with the risk of CAD, and this association was substantially attenuated on adjustment for HDL-C levels. Although Han Chinese are generally accepted to be at low risk for CAD [20-22], our results revealed that CETP TaqIB gene polymorphism still exhibited a highly significant association with CAD after adjustment for HDL-C levels. Such a relation between CETP genotype and CAD independent of HDL-C levels has been reported in previous studies [30,31].

One previous study performed by Durlach et al [35] suggested that the effect of CETP polymorphism on CAD was sex dependent. Recently, Chaaba et al [28] found that CETP TaqIB polymorphism was associated with CAD only in men with type 2 diabetes mellitus. In our study, diabetic patients with the B1B1 genotype were found to be at risk for CAD, provided they were female. However, a meta-analysis conducted by Boekholdt et al [29] reported CETP TaqIB polymorphism to be associated with CAD regardless of sex. Because of the small number of patients with CAD in this study, we cannot draw any conclusions about the associations among CAD, CETP TaqIB polymorphism, and sex.

In this study, diabetic patients with the CETP B1 allele (B1B1 and B1B2) had significantly higher serum HDL-C levels compared with those without (B2B2). Hsu et al [32] reported that CETP gene polymorphism was associated with HDL-C levels in nondiabetic Taiwanese. The meta-analysis study conducted by Boekholdt et al [29] revealed that the CETP TaqIB variant was strongly associated with HDL-C levels in whites. However, there were inconsistent results from numerous studies about the relationship between CETP genotype and HDL-C levels in diabetic patients. Two studies reported no association between TaqIB polymorphism and HDL-C concentration in patients with type 2 diabetes mellitus [27,33]. Kauma et al [34], however, found an association between TaqIB polymorphism and HDL-C in women, whereas Durlach et al [35] found that TaqIB polymorphism seems to exert a modulating role in men only. Kawasaki et al [26] have discovered an association between TaqIB polymorphism and HDL-C in patients with type 2 diabetes mellitus. Many factors such as sex, smoking, and BMI have been reported to interact with this association [36,37]. However, the strong association demonstrated between CETP TaqIB polymorphism and HDL-C levels in diabetic patients is not influenced by sex, smoking, and BMI in our study. High frequency of lipid abnormalities and taking of lipid-lowering medications (statins and fibrates) were seen in patients with type 2 diabetes mellitus; thus, we hypothesize that the influence of CETP TaqIB polymorphism on lipid metabolism might be altered by diabetes and lipid-lowering medications. In the present study, 61.6% of diabetic patients took statins, and 24.1% of those took fibrates. The relationship between CETP gene variant and HDL-C levels were persistent after adjustment for lipid-lowering medications. The other possibility is that ethnic factors might play a role in their association in diabetic patients.

How CETP isoforms might influence the development of CAD in diabetes is unclear. CETP may mediate cholesterol redistribution by reducing the amount of cholesterol ester extraction from atherosclerotic lesions as a result of reduced HDL function. However, CETP regulated one of the steps in reverse cholesterol transport, an antiatherogenic process. CETP is involved in modulating concentration of HDL-C [7,8] and may therefore alter susceptibility to CAD. Because the CETP TaqIB polymorphism is located in an intron, it may not be a functional mutation. The results of the present study revealed CETP TaqIB polymorphism to be related to CAD independent of other cardiovascular risk factors including serum HDL-C level. Further studies are needed to determine if this polymorphism is a nonfunctional marker in linkage disequilibrium with functional variants of the CETP gene or other closely linked genes.

Our study also showed that serum creatinine levels higher than 1.4 mg/dL were found to be an independent risk factor for CAD in diabetic patients. The Framingham study [38] reported that minor renal dysfunction may be a predictor of cardiovascular risk. According to the study,

mortality rates in men with serum creatinine levels of 1.4 to 3.0 mg/dL were significantly higher than in controls. The association of mild renal failure with cardiovascular risk persisted after correction for established risks in the Hoorn study [39]. Wannamethee et al [40] reported that the risk of total mortality, cardiovascular mortality, and ischemic heart events was 20% higher in patients with serum creatinine levels higher than 1.6 mg/dL than in individuals with lower serum creatinine levels. These studies randomly selected individuals from the general population, but such information has recently become available for populations with high cardiovascular risk factors including diabetes from the HOPE study [41]. The HOPE randomized trial revealed that the cumulative prevalence of cardiovascular death, acute myocardial infarction, and stroke was higher in individuals with serum creatinine levels higher than 1.4 mg/dL than in those with lower values. Using observed and modeled data from 5097 subjects in the UK Prospective Diabetes Study, Adler et al [42] found that there was a trend for increasing risk of cardiovascular death with increasing nephropathy in patients with type 2 diabetes mellitus. The results of our study indicate that in Taiwanese diabetic patients, mild renal dysfunction (serum creatinine >1.4 mg/dL) is associated with CAD.

There are limitations inherent in the design of this study. This is a cross-sectional study. There is no basis in this study to determine the effect of CETP genotype on plasma CETP levels in diabetes. Because plasma samples that had not been freeze-thawed were unavailable for the present study, CETP levels were not measured. Ordovas et al [15] showed that CETP TaqIB genotype was a significant determinant of CETP and HDL-C levels. Another study on diabetic patients has reported a significant association between CETP genotype and both CETP and HDL-C concentration, but no correlation, however, between CETP mass and HDL-C concentrations [43]. However, other investigators have reported a lack of significant association between CETP activity and CETP TaqIB polymorphism [12,28]. These inconsistencies may derive from differences between study samples or from differences between the complex CETP activity assays that were used.

In conclusion, our data demonstrate that the CETP B1B1 genotype is a strong genetic predictor of CAD in Taiwanese with type 2 diabetes mellitus. We also confirm that diabetic patients with mild renal dysfunction are at independent risk for CAD.

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