G-50T Polymorphism of the Cytochrome P450 Epoxygenase CYP2J2 Gene is Not Associated with the Risk of Coronary Artery Disease among Chinese in Taiwan

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Background: Human cytochrome P450 (CYP) 2J2 is expressed in the vascular endothelium and metabolizes arachidonic acid to epoxyeicosatrienoic acids (EETs). The EETs are potent vasodilators and inhibitors of inflammation. Thus, the CYP2J2 gene variants may contribute to the risk of coronary artery disease (CAD).

Methods: In total, 209 patients with CAD (114 of them with myocardial infarction [MI]) and 209 age- and sex-matched control subjects were analyzed for the CYP2J2 G-50T polymorphism by polymerase chain reaction.

Results: The distribution of CYP2J2 genotype was similar in the CAD cases and controls; the GT genotype was present in 10.5% of CAD patients, as compared to 8.6% of CAD control subjects (odds ratio = 1.87, 95% C.I. = 0.81-4.32, *p* = 0.146). The frequency of the T allele was also similar in the CAD cases and controls (5.3% vs. 4.3%, *p* = 0.506). There was also no significant association between G-50T polymorphism carriers and the risk of myocardial infarction.

Conclusion: From our data, there is no evidence of an association between the CYP2J2 G-50T polymorphism and the risk of CAD and MI among Chinese in Taiwan.

Key Words: Polymorphism • Cytochromes P450 • Coronary disease

INTRODUCTION

Human cytochromes P450 (CYP) participate in the metabolism of arachidonic acid (AA). Human CYP2J2 is expressed at high levels in the heart, predominantly in cardiac myocytes and endothelial cells lining small and

large coronary arteries.[1,2] CYP2J2 is also expressed in other tissues, including the liver, kidney, lung, pancreas, and gastrointestinal tract.[1,3-5] The major products generated by CYP2J2-catalyzed AA metabolism are *cis*-epoxyeicosatrienoic acids (EETs) (5,6-, 8,9-, 11,12-, and 14,15-EETs) and 19-hydroxy metabolite (19-HETE).[1] The EETs are further metabolized by soluble epoxide hydrolase to the corresponding *vic*-dihydroxyeicosa-trienoic acids (DHET).[6,7] EETs have been shown to have numerous biological functions. In the heart, EETs dilate coronary arteries by activating Ca^{2+}-sensitive K^+ channels,[8,9] improve recovery of heart contractile function after prolonged global ischemia,[10] and affect the activity of cardiac L-type Ca^{2+} channels.[11,12] EETs are leading candidates for endothelium-derived hyperpolarizing factor, the nitric oxide/prostacyclin-independent component

of endothelium-dependent vasorelaxation.[13,14] In endothelial cells, CYP2J2-derived EETs have been shown to inhibit cell adhesion molecule expression and leukocyte adhesion to vascular wall, protect against hypoxia-reoxygenation injury, and induce tissue-plasminogen activator gene transcription.[2,15,16] Recently, King et al.[17] identified a G-50T polymorphism in the promoter region of the CYP2J2 gene. Furthermore, Spiecker et al.[18] demonstrated it was a functional polymorphism that abolished the binding of Sp1 transcription factor and reduced CYP2J2 promoter activity. Subjects with this polymorphism displayed decreased plasma stable EET metabolites levels and were independently associated with increased risk of coronary artery disease (CAD) in a Germany population.[18] These findings indicated that G-50T polymorphism might be an important genetic variant for CAD. However, no other studies have confirmed the findings. In this study, we assessed the influence of G-50T polymorphism of the CYP2J2 gene on the risk of CAD in a Chinese population in Taiwan.

METHODS

Study Population

Two hundred and eighteen patients with CAD and the same number of age- and sex-matched control subjects were enrolled for analysis. The demographic details of the CAD patients and their control subjects have been described previously.[19-21] Briefly, all the CAD patients were from Chang Gung Memorial Hospital (Taipei, Taiwan) and were recruited between August 1994 and September 1996. All CAD patients had > 50% stenosis in at least one major coronary artery, as documented by coronary angiography. The severity of CAD was determined by the number of significantly stenosed coronary arteries. Of the patients with CAD, 114 (52%) had experienced an myocardial infarction (MI) that was clinically verified by eletrocardiography and the development of left ventricular regional wall motion abnormality on left ventriculography. Control subjects were recruited during routine health examination, and were individually matched for age (within 2 years) and sex with CAD patients. Control subjects had no clinical evidence of CAD: (1) no history of typical angina pectoris, (2) no abnormal Q wave or ST-T changes on electrocardiography, and (3)

negative Master exercise test results. The presence of hypertension, diabetes mellitus, hypercholesterolemia or smoking was determined based on history-taking, previous medical records, current medication, or by examination during hospitalization. Obesity was defined as a body mass index (BMI) of 26 kg/m^2 or more. Since the DNA samples of nine CAD cases were used up during the several years of tests, the final study group included 209 cases and their controls. The study protocol was approved by the hospital's ethics committee, and oral informed consent was obtained from all subjects.

Genomic DNA Extraction and Genotyping of the CYP2J2 G-50T Polymorphism

Approximately 10 mL of blood was drawn into heparinized tubes, and white blood cells were separated by centrifugation. The genomic DNA was extracted from the peripheral blood leukocytes by the standard method with proteinase K digestion of nuclei. Phenol and chloroform extractions were followed by isopropanol precipitation of the DNA. Genotyping for the G-50T polymorphism of the CYP2J2 gene was carried out by polymerase chain reaction (PCR) and restriction enzyme digestion as described previously.[18] Briefly, 100 ng of forward (5′-TTTTCTGAGACCGGTGCGTG-3′) and 100 ng of reverse (5′-TAGGAGAGTCCGAGGATGGA-3′) primers designed to amplify the CYP2J2 promoter gene were used for amplification. The PCR yielded a 242-bp product. Incubation with *Alu*I resulted in 2 fragments (99 and 143 bp) in PCR products with the G-50T single nucleotide polymorphism but not in wild-type PCR products.

Statistical Analysis

The chi-square test and McNemar test whenever appropriate were used to examine differences in categorical variables. The clinical characteristics of continuous variables were expressed as mean ± SD, and were tested using a two-sample *t*-test or ANOVA. Conditional logistic regression analysis was used to evaluate the independent effect of investigated genotypes on the risk of CAD, adjusted for the presence of established risk factors including age, gender, hypertension, smoking, hypercholesterolemia, diabetes mellitus and obesity. Finally, all *p* values were calculated based on two-sided tests, with statistical significance being defined where the *p* value was less than 0.05.

RESULTS

Table 1 lists the characteristics of the cases and control subjects. The frequencies of classical risk factors for CAD, such as hypertension, hypercholesterolemia, diabetes mellitus, and smoking, were significantly higher in CAD patients than in the control subjects. The genotype and allele frequencies of the CYP2J2 gene G-50T polymorphism in CAD patients and controls are shown in Table 2. The distributions of genotypes in the two groups were in agreement with those predicted by the Hardy-Weinberg equilibrium. The genotype frequencies did not differ significantly between the two groups (McNemar $\chi^2 = 0.265$, $p = 0.607$). The T-allele also occurred at similar frequencies in the two groups ($\chi^2 = 0.442$, $p = 0.506$). The frequency of the GT genotype in CAD patients with MI (9.0%) was not significantly different from that of CAD patients without MI (12.2%), or from that of control subjects (8.6%) ($\chi^2 = 0.579$, $p = 0.447$, and $\chi^2 = 0.014$, $p = 0.905$, respectively). As shown in Table 3, there was no consistent relationship between the GT genotype and the number of significantly diseased vessels. Using conditional logistic regression analysis, subjects with the GT genotype did not have an increased probability of having CAD compared with those with a GG genotype (odds ratio = 1.87, 95% CI = 0.81-4.32, $p = 0.146$) after adjustment for common cardiovascular risk factors, including age, gender, hypertension, smoking, hypercholesterolemia, diabetes mellitus and obesity.

DISCUSSION

In the present case-control study, we found no evi-

Table 1. Clinical characteristics of the CAD patients and controls

	Control	CAD	p
Number	209	209	
Age, years	61.37 ± 8.79	61.50 ± 8.91	0.882
Sex (M/F)	159/50	159/50	1.000
Body mass index, kg/m^2	24.34 ± 3.83	25.20 ± 3.76	0.020
Hypercholesterolemia, %	16.3	29.7	0.001
Diabetes mellitus, %	4.8	30.6	0.000
Hypertension, %	34.4	45.0	0.028
Smoking, %	43.1	56.9	0.005

Table 2. CYP2J2 G-50T genotypes and the allele frequencies of CAD patients and controls

	Genotype			p	Allele		p
	GG	GT	TT		G	T	
Controls	191 (91.4)	18 (8.6)	0		95.7	4.3	
CAD	187 (89.5)	22 (10.5)	0	0.607	94.7	5.3	0.506

Figures in parenthesis represent percentages.

Table 3. Relationship between the CYP2J2 G-50T polymorphism and the severity of CAD, graded by number of significantly stenosed vessels

Genotype	Number of significantly (> 50%) stenosed vessels			
	1	2	3	LM
GG (n = 187)	79 (88.2)	49 (92.5)	42 (87.5)	17 (89.5)
GT (n = 22)	10 (11.2)	4 (7.5)	6 (12.5)	2 (10.5)

Figures in parenthesis represent percentages.

χ^2 (trend) = 0.340, $p = 0.560$, LM = left main coronary disease.

dence of an association between the CYP2J2 gene G-50T polymorphism and the risk of angiographically defined CAD/MI. There was no relationship between the CYP2J2 genotype and the severity of CAD. Thus, our results suggest that this polymorphism is not an important risk factor for CAD/MI among Chinese in Taiwan.

King et al.[17] first described the G-50T variant with variable frequency in different racial groups (13% for Asian, 8% for White, and 17% for African, respectively). However, the sample size of 24 subjects for each racial group was too small to apply to their represented populations. Reviewing the results from other reports with larger sample size, the T-allele frequency for our control population (4.3%) is similar to that reported for Chinese in China[22] (2.6%) and Korean[23] (4.23%), but lower than that reported for Caucasian populations[18,24] (5.49-7.7%) and African-American populations[24] (14.1%). Furthermore, Spiecker et al.[18] demonstrated that the G-50T polymorphism was functional and reduced transcriptional activity of CYP2J2 promoter in bovine aortic endothelial cells in vitro, by eliminating a Sp1 transcription factor binding site. Notably, this polymorphism was associated with reduced plasma 14,15-DHET levels, which was one of the major epoxidation products of AA by CYP2J2, in polymorphism carriers compared with non-carriers in vivo. In Spiecker's study,[18] the G-50T polymorphism was found in 10.6% of control subjects (0.4% for TT, and 10.2% for GT genotype) and 17.3% of CAD patients (2.4% for TT, and 14.9% for GT genotype). Spiecker et al.[18] concluded that the CYP2J2 G-50T polymorphism was significantly associated with an increased risk of CAD independent of other traditional risk factors, including hypertension (OR, 2.23; 95% CI, 1.04 to 4.79). In addition, they found that the frequency of acute coronary syndrome and cerebral ischemia was slightly higher in the T-allele carriers compared with the GG genotype subjects (without reaching statistical significance) among the CAD cases.[18] Our study did not confirm this association with CAD, nor for MI. It is possible that ethnic difference caused this discrepancy. Moreover, our patients, as a group, were slightly older (mean age 61 years) than patients in Spiecker's study[18] (mean age 51 years). Thus, we still could not exclude the possibility of an effect of this polymorphism in younger patient groups. On the other hand, to confirm a relative risk of at least 2.23 for the T-allele carriers being a risk factor for CAD as

proposed by Spiecker's study, the statistical power of our study, with this sample size (209 subjects in each group) and an incidence of the GT genotype of 8.6% in control population, could reach 0.77 at an alpha level of 0.05. Since CAD is a multifactorial disease, it is unlikely that a polymorphism in a single gene would have a profound effect on the risk of CAD. Although the statistical power of this study was not inadequate (acceptable requirement: 70-80%), our results still did not exclude the possibility of a smaller relative risk of CAD associated with this polymorphism.

The mechanism by which reduced CYP2J2 expression increases propensity to atherosclerosis development remains uncertain. EETs have also been extensively studied in the kidney, where they have been shown to affect renal vascular tone via inhibiting Na^+ reabsorption and K^+ secretion, affecting Na^+-K^+-ATPase activity, and modulating the actions of several renal hormones, including angiotensin II, arginine vasopressin, and renin.[25,26] These findings suggest that EETs may play a role in the pathogenesis of human hypertension.[25,26] However, the results of previous studies for the claim that CYP2J2 G-50T carriers bear increased risk of hypertension are inconsistent.[24] Similar to the results of Spiecker's study,[18] hypertension was not associated with G-50T polymorphism in our population (data not shown). On the other hand, Spiecker et al. did not demonstrate G-50T carriers with CAD exhibited lower plasma 14,15-DHET levels along with reduced vascular anti-inflammatory reserve, compared with GG genotype subjects with CAD in their study. Therefore, a larger, prospective and longitudinal study that stratifies patients according to inflammatory marker levels would be necessary to elucidate more clearly the underlying mechanisms by which CYP2J2 G-50T affects the risk of CAD.

In conclusion, using age- and sex-matched control groups for analysis, we found no evidence of an association between the CYP2J2 G-50T polymorphism and the risk of CAD and MI among Chinese in Taiwan.

ACKNOWLEDGEMENT

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細胞色素 P450 環氧化合酶 CYP2J2 基因上的 G-50T 多形性與台灣人發生冠狀動脈疾病的危險性沒有相關

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背景　人類細胞色素 P450 (CYP) 2J2 表現於血管內皮，能將花生四烯酸代謝成環氧二十碳三烯酸。環氧二十碳三烯酸是非常強的血管擴張劑和發炎抑制劑，所以 CYP2J2 基因上的多形性可能會影響發生冠狀動脈疾病的危險性

方法　利用聚合酶連鎖反應分析總共 209 個冠狀動脈疾病病人，(其中 114 個病人同時有心肌梗塞)，和 209 個與之年齡和性別配對的對照者的 CYP2J2 基因 G-50T 多形性。

結果　冠狀動脈疾病組和對照組兩組之 CYP2J2 多形性基因型的分佈是相似的，10.5% 之冠狀動脈疾病病人，和 8.6% 之對照者是 GT 基因型，(勝算比 = 1.87，95% 信賴區間 = 0.81-4.32，p = 0.146)。T 對偶基因的比率於冠狀動脈疾病組和對照組兩組之分佈也是相似的，(5.3% 相較於 4.3%，p = 0.506)。帶有 G-50T 多形性的人與發生心肌梗塞的危險性也沒有有意義的相關。

結論　由我們的資料中，沒有證據顯示 CYP2J2 基因上的多形性與臺灣人發生冠狀動脈疾病和心肌梗塞的危險性有相關。

關鍵詞：多形性、細胞色素 P450、冠狀動脈疾病。