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Association of *CYP2C19*2* with hyperlipidemia in patients with the wild-type *CYP2C9*

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ABSTRACT. Arachidonic acids are metabolized by cytochromes P450 (*CYP450*) to epoxyeicosatrienoic acids (EETs). EETs have many cardiovascular protective effects such as vasodilation, anti-hypertension, anti-inflammation, lipid-lowering effects, and favorable effects on glucose homeostasis. This study aimed to investigate whether gene polymorphism of cytochrome P450 *CYP2C9* and *CYP2C19* are related to the risk of hypertension, hyperlipidemia, and diabetes mellitus. Four hundred and eighty patients were enrolled, and single nucleotide polymorphisms for *CYP2C9* and *CYP2C19* were genotyped. The percentages of patients with hypertension, hyperlipidemia, and diabetes mellitus with *CYP2C9* *1/*2, *1/*3, and wild-type (*CYP2C9 *1/*1*) were similar, and the chi-square (χ^2) analysis showed no statistically significant differences between each genotype. Further, no statistically significant differences were observed for the percentages of patients with hypertension, hyperlipidemia, and diabetes mellitus between *CYP2C19* genotypes. Subgroup analysis showed that among patients with the wild-type *CYP2C9* genotype (*CYP2C9*1/*1*, N = 297), patients with CYP2C19*1/*2 and *2/*2 genotypes have a significantly

higher percentage of hyperlipidemia than those with *CYP2C19* *1/*1, *1/*17, and *17/*17 genotypes. Binary logistic regression analysis also suggested that among patients with the wild-type *CYP2C9* genotype, the *CYP2C19*2* allele was associated with risk of hyperlipidemia (P = 0.027, odds ratio = 2.036; 95% confidence interval = 1.09-3.82). General linear model analysis showed that among our general research population, both *CYP2C9* and *CYP2C19* had no effect on hyperlipidemia independently, but the interaction between *CYP2C9* and *CYP2C19* and hyperlipidemia was statistically significant (F = 2.682, P = 0.031). Our study suggests that the association of *CYP2C19*2* with hyperlipidemia was specific among patients with the wild-type *CYP2C9*.

Key words: *CYP2C9*; *CYP2C19*; Diabetes mellitus; Dyslipidemia; Hypertension

INTRODUCTION

Arachidonic acid, a fatty acid in cell membranes, is metabolized by CYP450s to epoxyeicosatrienoic acid (EET). EETs serve as endothelial-derived hyperpolarizing factors (EDHF) and have numerous cardiovascular protective effects such as vasodilation, anti-hypertension, anti-atherosclerosis, and anti-inflammation effects (Fleming, 2001). EETs have also been shown to have a lipid-lowering effect. Zhang et al. (2009) showed that prevention of the degradation of EETs with a soluble epoxide hydrolase inhibitor caused reduction of serum lipid levels in a mouse model. Recently, a new study also suggested that EETs exert favorable effects on glucose homeostasis, either by enhancing pancreatic islet cell function or by increasing insulin sensitivity in peripheral tissues (Luther and Brown, 2016). *CYP2C9* is one of the predominant epoxygenases abundantly expressed in the endothelium (Imig, 2012). The role of the *CYP2C9* enzyme in the metabolism of warfarin and its gene polymorphism is well known and is highly associated with warfarin dose. The wild-type *CYP2C9*1* allele is associated with normal enzyme activity, and two common allelic variants, *CYP2C9*2* and *CYP2C9*3*, are associated with reduced enzyme activity. *CYP2C19* is another type of cytochrome P450 expressed in human endothelial cells and catalyzes the biosynthesis of EETs from arachidonic acid. *CYP2C19* gene variants, mainly *CYP2C19*2*, which is a loss-of-function allele, and *CYP2C19*17*, which is a gain-of-function allele, are well known to affect the efficacy of clopidogrel therapy (Hulot et al., 2006). Because EETs are metabolized by *CYP2C9* and *CYP2C19*, it is reasonable to suspect that patients with reduced enzyme activity variants of *CYP2C9* and *CYP2C19* are more likely to develop hypertension, hyperlipidemia, or diabetes mellitus due to less EET production. This study aimed to investigate the association between *CYP2C9* and *CYP2C19* polymorphisms and risk of hypertension, hyperlipidemia, and diabetes mellitus in our patient population.

MATERIAL AND METHODS

Subjects

A total of 480 patients were included in this study, and a consent form was signed by

every patient. This study was approved and monitored by the Copernicus Group Institutional Review Boards. This study was in compliance with the Declaration of Helsinki.

Genotyping

We used the magnetic bead-based method for concentrating DNA, which was obtained from buccal swab leukocytes by using QiangenQiaCube instrument and MagMAX™ DNA Multi-sample kits. *CYP2C9**1, *2, and *3 were genotyped in 480 patients. *CYP2C19**1, *2, and *17 were genotyped in 453 patients.

Study outcome definition

Hyperlipidemia is defined as fasting total cholesterol ≥200 mg/dL, and/or fasting low-density lipoprotein cholesterol ≥160 mg/dL and/or fasting triglycerides ≥150 mg/dL. Diabetes mellitus was diagnosed if the fasting plasma glucose concentration was ≥126 mg/dL at two different time points or hemoglobin A1c ≥ 6.5%. Hypertension was defined as systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg or treatment with antihypertensive medication.

Statistics

SPSS 16 was used for statistical analysis. Data are reported as means ± standard error and $P < 0.05$ was considered to be statistically significant. One-way analysis of variance and Crosstabs distribution with the chi-square (χ^2) analysis were performed to evaluate the differences between data. Binary logistic regression was used to evaluate the association of *CYP2C9* and *CYP2C19* gene polymorphism and diseases. A univariate general linear model was used to test for a potential interaction between *CYP2C9* and *CYP2C19* and hyperlipidemia.

RESULTS

CYP2C9 and *CYP2C19* allele frequency distribution

The allele frequency distribution of *CYP2C9* and *CYP2C19* in our patient population is presented in Table 1. The allele frequency of the wild-type allele is 79.5% for *CYP2C9*(*1) and 63.0% for *CYP2C19*(*1). One-way analysis of variance showed no significant age difference between patients with *CYP2C9**1/*1, *1/*2, and *1/*3. Further, no significant age difference in patients was noted among each genotype of *CYP2C19*.

CYP2C19 variants and diseases

For *CYP2C19*, because *CYP2C19*2* is a loss-of-function allele and *CYP2C19*17* is a gain-of-function allele, we divided patients into three groups: wild-type, patients with the *CYP2C19*2* allele, and patients with the *CYP2C19*17* allele. The percentages of patients with hypertension, hyperlipidemia, and diabetes mellitus in each group were similar, and the χ^2 analysis revealed no statistically significant difference between each group (Table 2). To further explore whether *CYP2C19* gene polymorphism played a role in the development of

hypertension, hyperlipidemia, and diabetes mellitus, binary logistic regression analysis was also used to detect the association between gene polymorphism and the three diseases. The analysis also suggested that there was no association of the *CYP2C19* gene polymorphism with risk of hypertension, hyperlipidemia, and diabetes mellitus (Table 3).

Table 1. *CYP2C9* and *CYP2C19* genotype distribution.

	Allele	N (%)	Genotype	N (%)	Age (years)
CYP2C9	1	763 (79.5)	*1/*1	297 (61.9)	70.6 ± 12.8
	2	129 (13.4)	*1/*2	111 (23.1)	70.1 ± 13.3
	3	68 (7.1)	*1/*3	58 (12.1)	74.5 ± 12.3
			*2/*2	6 (1.2)	82.0 ± 4.8
			*2/*3	6 (1.2)	83.3 ± 9.4
			*3/*3	2 (0.4)	73.0 ± 11.3
CYP2C19	1	571 (63.0)	*1/*1	182 (40.2)	72.1 ± 11.9
	2	152 (16.8)	*1/*2	87 (19.2)	71.3 ± 12.8
	17	183 (20.2)	*2/*2	15 (3.3)	71.9 ± 11.6
			*1/*17	120 (26.5)	70.7 ± 14.8
			*2/*17	35 (7.7)	68.9 ± 14.0
			*17/*17	14(3.1)	72.4 ± 12.7

Table 2. *CYP2C19* genotypes and diseases.

CYP2C19		*1/*1	*1/*2 and *2/*2	*1/*17 and *17/*17	χ^2	P
HTN	+	159 (87.4%)	91 (89.2%)	111 (82.8%)	2.275	0.32
	-	23 (12.6%)	11 (10.8%)	23 (17.2%)		
HLD	+	78 (42.9%)	53 (52.0%)	61 (45.5%)	2.195	0.33
	-	104 (57.1%)	49 (48.0%)	73 (54.5%)		
DM	+	48 (26.4%)	28 (27.5%)	39 (29.1%)	0.289	0.87
	-	134 (73.6%)	74 (72.5%)	95 (70.9%)		

HTN = hypertension; HLD = hyperlipidemia; DM = diabetes mellitus; χ^2 = chi-square test.

Table 3. Binary logistic regression analysis of *CYP2C9* and *CYP2C19* and diseases.

		HTN	HLD	DM
CYP2C9	P	0.811	0.787	0.346
	SE	0.196	0.139	0.152
	95%CI	0.65-1.40	0.73-1.26	0.86-1.56
CYP2C19	P	0.379	0.740	0.884
	SE	0.089	0.064	0.072
	95%CI	0.78-1.10	0.86-1.10	0.88-1.16

HTN = hypertension; HLD = hyperlipidemia; DM = diabetes mellitus; SE = standard error; 95%CI = 95% confidence interval.

Theoretically, both *CYP2C9* and *CYP2C19* can affect EET production, which will subsequently affect the risk of related diseases. To avoid interaction or confounding effect between gene polymorphism from these two genes, the relationship between the *CYP2C19* gene polymorphism and diseases was then analyzed only among patients with the *CYP2C9* wild-type (*CYP2C9 *1/*1*). In the wild-type *CYP2C9* patients, the χ^2 analysis showed that patients with the *CYP2C19*2* allele had a significantly higher percentage of hyperlipidemia than patients in the wild-type or *CYP2C19*17* allele group (χ^2 = 7.831, P = 0.02; Table 4). We also divided patients into two groups, those with *2 (*1/*2, *2/*2) genotypes and those without *2 (*1/*1, *17/*17, *1/*17) genotypes. Patients with the *CYP2C19*2* allele had a significantly higher percentage of hyperlipidemia than those without the *2 allele (χ^2 =

7.62, P = 0.0057). Binary logistic regression analysis also suggested that CYP2C19*2 was significantly associated with hyperlipidemia (P = 0.027; odds ratio = 2.036; 95% confidence interval = 1.09-3.82) in patients with the wild-type *CYP2C9*. No differences in the incidence of hypertension and diabetes mellitus were noted among these three groups in patients with the wild-type *CYP2C9*.

Table 4. *CYP2C19* genotypes and diseases in the wild-type *CYP2C9* patient.

CYP2C19		*1/*1	*1/*2 and *2/*2	*1/*17 and *17/*17	χ^2	P
HTN	+	71 (84.5%)	65 (89.0%)	76 (85.4%)	0.741	0.69
	-	13 (15.5%)	8 (11.0%)	13 (14.6%)		
HLD	+	33 (39.4%)	44 (60.3%)	38 (42.7%)	7.831	0.02
	-	51 (60.7%)	29 (39.7%)	51 (57.3%)		
DM	+	21 (25.0%)	19 (26.0%)	25 (28.1%)	0.221	0.90
	-	63 (75.0%)	54 (74.0%)	64 (71.9%)		

HTN = hypertension; HLD = hyperlipidemia; DM = diabetes mellitus; χ^2 = chi-square test.

CYP2C9 variants and diseases

For *CYP2C9*, there were not enough cases for patients with *2/*2, *2/*3, and *3/*3; hence, we compared differences only between *1/*1, *1/*2, and *1/*3. The percentages of patients with hypertension, hyperlipidemia, and diabetes mellitus in the wild-type, *1/*2, and *1/*3 *CYP2C9* groups were similar, and the χ^2 analysis showed no significant statistical differences between each genotype (Table 5). Binary logistic regression analysis also showed that the *CYP2C9* gene polymorphism was not associated with the risk of hypertension, hyperlipidemia, and diabetes mellitus (Table 3).

Table 5. *CYP2C9* genotypes and diseases.

		*CYP2C9**1/*1	*CYP2C9**1/*2	*CYP2C9**1/*3	χ^2	P
HTN	+	254 (85.5%)	96 (86.5%)	50 (86.2%)	0.069	0.966
	-	43 (14.5%)	15 (13.5%)	8 (13.7%)		
HLD	+	139 (46.8%)	48 (43.2%)	27 (46.6%)	0.422	0.810
	-	158 (53.2%)	63 (56.8%)	31 (53.4%)		
DM	+	78 (26.3%)	31 (27.9%)	18 (31.0%)	0.591	0.744
	-	219 (73.7%)	80 (72.1%)	40 (69.0%)		

HTN = hypertension; HLD = hyperlipidemia; DM = diabetes mellitus; χ^2 = chi-square test.

Subgroup analysis among patients in the wild-type CYP2C19 group showed a similar result, wherein no significant differences were found for the risk of hypertension, hyperlipidemia, and diabetes mellitus among patients in the wild-type, *1/*2, and *1/*3 *CYP2C9* groups.

Interaction between *CYP2C19* and *CYP2C9* and hyperlipidemia

A univariate general linear model was used to investigate the interaction between *CYP2C9* and *CYP2C19* and hyperlipidemia. In our general study population, both *CYP2C9* and *CYP2C19* had no effect on hyperlipidemia independently (F = 0.839, P = 0.523 for *CYP2C9* and F = 1.850, P = 0.102 for *CYP2C19*), but the interaction between *CYP2C9* and *CYP2C19* and hyperlipidemia was statistically significant (F = 2.682, P = 0.031).

DISCUSSION

In this study, we hypothesized that polymorphism in *CYP2C9* and *CYP2C19* affects enzyme activity, causes lower EET levels in endothelial cells, and then increases the risk of hypertension, hyperlipidemia, and diabetes mellitus in patients with the *CYP2C9* and *CYP2C19* variants. Our results suggest the association between *CYP2C19*2* allele and risk of hyperlipidemia among patients with the wild-type *CYP2C9* variants. Previous studies showed controversial results for the association between *CYP2C9* and *CYP2C19* gene polymorphism and the development of hypertension, hyperlipidemia, and diabetes mellitus. Regarding hypertension, Yu et al. (2004) showed that the allelic frequency of *CYP2C9*3* in patients with hypertension was significantly higher than in healthy controls in a Chinese population. Caucasian patients with secondary renal diseases had less favorable blood pressure with *CYP2C9* variant alleles (*2 and *3) (Joy et al., 2009). However, Dreisbach et al. (2005) showed that *CYP2C9*2* and *3 are not associated with increased risk of hypertension in the African American population. For the *CYP2C19* gene polymorphism, only one study on the association between *CYP2C19* and hypertension showed that *CYP2C19*2* variants did not have any association with hypertension in a Korean population (Shin et al., 2012). Concerning diabetes mellitus, *CYP2C9*2*, **3*, and *CYP2C19*2* have been shown to have no association with diabetes mellitus (Semiz et al., 2010; Weise et al., 2010), which is consistent with our results. One study even showed that individuals with the *CYP2C19*2* variant seem to protect against metabolic syndrome (Gaio et al., 2014). Regarding hyperlipidemia, the study by Luo et al. (2005) suggested an association of *CYP2C9*3* with hyperlipidemia in the female Chinese population. There are no reports on the relationship between *CYP2C19* variants and hyperlipidemia. To the best of our knowledge, our study is the first to show an association between *CYP2C19* variants and hyperlipidemia.

Several genes in the *CYP450* system are involved in EET metabolisms, such as *CYP2C9*, *CYP2C19*, *CYP2C8*, and *CYP2J2* (King et al., 2005). Thus, it is possible that gene polymorphism of a single gene, such as *CYP2C9* or *CYP2C19*, is not enough to determine the level of EET biosynthesis. It is more likely that the combined effect of genetic variants of several genes in the *CYP450* system determines the level of EET biosynthesis. Lundblad et al. (2005) showed that *CYP2C8*3/*3/CYP2C9*2/*2* exhibited a 34% ($P < 0.05$) decreased EET biosynthesis compared to other CYP2C8/CYP2C9 haplotypes, whereas there were no significant differences between the *CYP2C9* variants. In our study, patients with a combination of *CYP2C9*1/*1* and *CYP2C19*1/*2* or **2/*2* have significantly higher risk of hyperlipidemia than patients with other genotypes of *CYP2C19*. Individuals with *CYP2C19*17*, which is a gain-of-function allele, should theoretically have more EETs and lower risk of related diseases. However, there is no report to show that *CYP2C19*17* is a protective factor for risk of hypertension, hyperlipidemia, and diabetes mellitus and our results also did not support this theory. Thus, this is indirect evidence to support that one gene in the *CYP450* system is not enough to determine the level of EET biosynthesis. Our results used general linear model analysis to show that among our entire research population, both *CYP2C9* and *CYP2C19* have no effect on hyperlipidemia independently, but the interaction between *CYP2C9* and *CYP2C19* gene polymorphism and hyperlipidemia is statistically significant. Therefore, future studies should focus more on research regarding gene polymorphism combination effect or interactions for several related genes.

In summary, our study showed that the association of *CYP2C19*2* with hyperlipidemia is specific for patients with wild-type *CYP2C9* genotypes.

Conflicts of interest

The authors declare no conflict of interest.

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