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ORIGINAL RESEARCH

CYP2C19 *2/*2 Genotype is a Risk Factor for Multi-Site Arteriosclerosis: A Hospital-Based Cohort Study

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Background: Vascular diseases such as atherosclerosis usually affect multiple organs. Genetic factors have a certain proportion in the risk factors of atherosclerosis. The purpose was to investigate the relationship of cytochrome P450 2C19 (*CYP2C19*) polymorphisms with multi-site atherosclerosis.

Methods: The study included 410 patients with single-site atherosclerosis and 529 patients with multi-site atherosclerosis. The relationship between *CYP2C19* rs4244285 and rs4986893 polymorphisms and single-site atherosclerosis and multi-site atherosclerosis was analyzed.

Results: The proportion of *CYP2C19* rs4244285 A allele (35.9% vs 29.9%, *P*=0.007) and rs4986893 G allele (97.7% vs 94.8%, *P*=0.001) in multi-site atherosclerosis group was significantly higher than that in single-site atherosclerosis group. The distribution of *CYP2C19* genotypes was significantly different between the two groups (*P*=0.002). The results of univariate logistic regression indicated that *CYP2C19* *1/*3 genotype (*1/*3 vs *1/*1: odds ratio (OR) 0.456, 95% confidence interval (CI): 0.231–0.902, *P*=0.024) may decrease risk of multi-site atherosclerosis, while *2/*2 genotype (*2/*2 vs *1/*1: OR 1.780, 95% CI: 1.100–2.880, *P*=0.019) may increase risk of multi-site atherosclerosis. Multivariate logistic regression (adjusted for gender, age, smoking, drinking, hypertension, and diabetes) indicated that *CYP2C19* *1/*3 genotype (*1/*3 vs *1/*1: OR 0.459, 95% CI: 0.231–0.909, *P*=0.026) may be an independent protective factor for multi-site atherosclerosis, while *2/*2 genotype (*2/*2 vs *1/*1: OR 1.767, 95% CI: 1.091–2.864, *P*=0.021) may be an independent risk factor for multi-site atherosclerosis.

Conclusion: *CYP2C19* *1/*3 genotype may be an independent protective factor for multi-site atherosclerosis, while *2/*2 genotype may be an independent risk factor for multi-site atherosclerosis.

Keywords: CYP2C19, genotype, multi-site atherosclerosis, polyvascular disease

Introduction

The incidence of cardiovascular and cerebrovascular diseases is on the rise at present.[1,2] Atherosclerosis is the main pathological basis of many cardiovascular and cerebrovascular diseases.[3] Atherosclerosis is a chronic disease of arterial wall and is caused by the damage of the intima of the artery by risk factors, the deposition of lipids in the intima of the artery, the chronic inflammatory reaction of the artery wall, and the gradual formation of atherosclerotic plaque.[4–6] The formation of atherosclerotic plaque and plaque rupture cause platelet activation and aggregation, plaque surface and cavity thrombosis, and eventually leads to vascular stenosis and occlusion, resulting in a variety of diseases.[7] With the local immune response and lipid infiltration of the intima, the proliferation of fibrous tissue and the formation of calcium deposits can lead to plaque rupture and thrombosis.[8] Atherosclerosis can sometimes involve multiple vessels or multiple sites of a vessel. Polyvascular disease refers to clinically obvious atherosclerosis in multiple arterial sites (coexisting disease in ≥2 arterial beds), which is regarded as a disease prone to cardiovascular and cerebrovascular adverse events, and has been widely concerned.[9,10]

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The occurrence of atherosclerosis is influenced by a number of factors, especially alcohol abuse and chronic hyperlipidemia, and the other risk factors include smoking, hypertension, diabetes, ambient air pollution and noise.[11,12] Atherosclerosis is also related to genetic factors.[13] Cytochrome P450 (CYP450) superfamily is involved in the metabolism of endogenous and exogenous substances.[14] The occurrence and progression of many diseases may be related to the CYP450 family.[15] Cytochrome P450 2C19 (CYP2C19) is a member of the CYP450 family.[16] Arachidonic acid (AA) can be metabolized by CYP2C19 into endodermal hyperpolarized factor (EDHF),[17] while EDHF is beneficial to vascular dilation and inhibits vascular calcification.[18] Reactive oxygen species (ROS) produced by coronary endothelial cells in the process of CYP2C19-catalyzed reaction[19] is a risk factor for atherosclerosis.[20,21]

CYP2C19 is encoded by the *CYP2C19* gene. The single-nucleotide polymorphisms (SNPs) rs4244285 and rs4986893 are the most common polymorphisms of *CYP2C19* gene. *CYP2C19**2 (rs4244285, 681G>A) and *CYP2C19**3 (rs4986893, 636G>A) are defined as loss-of-function (LOF) alleles related to decreased platelet response to clopidogrel or increased incidence of clopidogrel resistance.[22] According to its ability to metabolize drugs, CYP2C19 enzyme can be divided into three phenotypes: extensive metabolizer (EM) type, intermediate metabolizer (IM) type, and poor metabolizer (PM) type. The metabolic phenotype of wild-type genotype (*CYP2C19**1/*1) was EM type. The CYP2C19 activity of the mutant heterozygote (*CYP2C19**1/*2, or *1/*3) was weakened, and its metabolic phenotype is IM type. The metabolic activity of CYP2C19 encoded by the mutant homozygote (CYP2C19*2/*2, *2/*3, or *3/*3) is completely lost, and its metabolic phenotype is PM type.[23] At present, studies on CYP2C19 mainly focus on the relationship between *CYP2C19* polymorphism and treatment effect and response to clopidogrel in patients with arterial disease.[24,25] However, there are relatively few studies on the relationship between *CYP2C19* polymorphism and atherosclerosis susceptibility. The *CYP2C19**2 frequency in Han patients with coronary artery disease (CAD) was significantly higher than that in Han healthy groups, while the *CYP2C19**3 frequency in Uygur patients with coronary artery disease was significantly lower than that in Uygur healthy groups, in Northwestern Xinjiang, China.[26] The *CYP2C19**3 was an independent risk factor for CAD in a Uighur population.[27] *CYP2C19* PM might be associated with the susceptibility of CAD in women from a Japanese population.[28] *CYP2C19* loss-of-function allele was a risk factor for ischemic stroke event in an American population.[29] *CYP2C19* loss-of-function allele was a risk factor for stroke composite events in a Caucasian population.[25]

Differences in ethnicity, lifestyle and genetic background can affect the onset and progression of atherosclerosis.[15,30,31] However, the study in the relationship of *CYP2C19* gene polymorphisms and multi-site atherosclerosis is still at a blank stage. In the current study, we evaluated the association between *CYP2C19* gene polymorphisms and single- or multi-site atherosclerosis.

Materials and Methods

Study Population

This study retrospectively analyzed the clinical data of 410 patients with single-site atherosclerosis and 529 patients with multi-site atherosclerosis who were admitted to Meizhou People's Hospital in China from January 2016 to July 2019. This study was approved by the Ethics Committee of Meizhou People's Hospital.

Arteriosclerosis is determined by tests such as angiography, magnetic resonance imaging (MRI), computed tomography (CT) or color Doppler flow imaging (CDFI). Plaque was defined as a focal structure that invaded the lumen of the artery by at least 0.5 mm, or by 50% of the surrounding endo-media thickness, or by a thickness greater than 1.5 mm measured from the outer membrane to the endo-lumen interface.[32] The diagnosis of atherosclerosis was determined by two senior radiologists evaluating the results of imaging examinations in a double-blind evaluation. In the presence of atherosclerotic plaque, thickening of the vessel wall is seen with or without lumen stenosis.[33,34] In this study, atherosclerosis was observed in coronary artery, carotid artery, cerebral artery, and limb artery. The inclusion criteria were as follows: (1) Clinically diagnosed as atherosclerosis; (2) The clinical data of the patients were complete; (3) Adults. The exclusion criteria were as follows: (1) Incomplete clinical data of the patients; (2) Atherosclerosis patients with severe infectious diseases, autoimmune diseases, organ insufficiency and other diseases.

The demographic characteristics, personal history and disease history (vascular risk factors) of patients were collected from the Hospital Information System (HIS). The diagnostic criteria for hypertension are systolic blood pressure of

≥140 mm Hg, diastolic blood pressure of ≥90 mm Hg.[35] The diagnostic criteria for diabetes were fasting blood glucose ≥126.1 mg/dL, blood glucose ≥200 mg/dL 2 hours after loading.[36]

Genetic Analysis

Genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, North Rhine-Westphalia, Germany). *CYP2C19* variants were detected by *CYP2C19* genotyping kit (BaiO Technology Co., Ltd, Shanghai, China) with PCR-gene chip method.[37] The specific detection steps are carried out by referring to the method published by our colleagues before.[37]

Statistical Analysis

Data analysis was performed using SPSS statistical software version 21.0 (IBM Inc., USA). Categorical variables are expressed as percentages. The differences of genotype composition ratios and allele frequencies among patients with single- and multi-site atherosclerosis were analyzed by the χ^2 test. The significance of the Hardy-Weinberg equilibrium (HWE) of the *CYP2C19* polymorphisms in the patients was analyzed by the χ^2 test. The level of significance adopted was $P<0.05$ in single- and multi-site atherosclerosis patients. To measure the relative risk of *CYP2C19* genotype, multiple regression analysis was performed after adjusting for the factors of demographic characteristics, personal history and disease history. $P<0.05$ was used as the level of statistical significance for all statistical analyses in this study.

Results

Characteristics of Subjects

This study included 939 individuals, including 645 (68.7%) men and 294 (31.3%) women. There were 303 (32.3%) patients <65 years old and 636 (67.7%) patients ≥65 years old. There were 410 patients with single-site atherosclerosis and 529 patients with multi-site atherosclerosis in this study. There were no significant differences in age distribution, gender distribution, proportion of patients with a history of smoking and alcohol consumption, and proportion of patients with hypertension and diabetes between the single- and multi-site atherosclerosis groups (all $P>0.05$) (Table 1).

Frequencies of *CYP2C19* rs4244285 and rs4986893 Genotypes in Patients with Single-Site Atherosclerosis and Multi-Site Atherosclerosis

The χ^2 test was used to test the significance of the Hardy-Weinberg equilibrium of the polymorphism of the *CYP2C19* gene in the patients with single-site atherosclerosis and patients with multi-site atherosclerosis. The genotype distributions of *CYP2C19* rs4244285 (*CYP2C19* *2) in patients with single-site arteriosclerosis ($\chi^2=2.421$, $P=0.120$) and patients with multi-site arteriosclerosis ($\chi^2=0.981$, $P=0.322$) were consistent with Hardy-Weinberg equilibrium, respectively. The genotype distributions of *CYP2C19* rs4986893 (*CYP2C19* *3) in patients with single-site atherosclerosis ($\chi^2=0.752$, $P=0.386$) and patients with multi-

Table 1 Clinical Characteristics of Patients with Single-Site Arteriosclerosis and Patients with Multi-Site Arteriosclerosis

	Total (n=939)	Patients with Single-Site Arteriosclerosis (n=410)	Patients with Multi-Site Arteriosclerosis (n=529)	*P* values
Age, years				
<65, n(%)	303 (32.3)	138 (33.7)	165 (31.2)	0.439
≥65, n(%)	636 (67.7)	272 (66.3)	364 (68.8)	
Gender				
Male, n(%)	645 (68.7)	281 (68.5)	364 (68.8)	0.944
Female, n(%)	294 (31.3)	129 (31.5)	165 (31.2)	
History of smoking, n(%)	195 (20.8)	86 (21.0)	109 (20.6)	0.935
History of alcohol consumption, n(%)	33 (3.5)	14 (3.4)	19 (3.6)	1.000
Hypertension, n(%)	628 (66.9)	263 (64.1)	365 (69.0)	0.124
Diabetes, n(%)	300 (31.9)	133 (32.4)	167 (31.6)	0.778

site atherosclerosis (χ^2=2.037, *P*=0.153) were consistent with Hardy-Weinberg equilibrium, respectively. The frequencies of *CYP2C19* rs4244285 and rs4986893 genotypes and alleles were compared between the single and multi-site atherosclerosis groups. The proportion of *CYP2C19* rs4244285 A allele in multi-site atherosclerosis group was significantly higher than that in single-site atherosclerosis group (35.9% vs 29.9%, *P*=0.007). The proportion of *CYP2C19* rs4986893 G allele in multi-site atherosclerosis group was significantly higher than that in single-site atherosclerosis group (97.7% vs 94.8%, *P*=0.001) (Table 2).

The percentages of *CYP2C19**1, *CYP2C19**2, and *CYP2C19**3 alleles were 63.2%, 33.3%, and 3.6%, respectively. Of the 939 individuals included in this study, 575 (61.2%) were carriers of *CYP2C19**2 or *3 LOF allele. The distribution of *CYP2C19* genotypes was significantly different between the single- and multi-site atherosclerosis groups (*P*=0.002). The distribution of *CYP2C19**1, *2, and *3 alleles was significantly different between the two groups (*P*<0.001) (Table 3).

Table 2 Frequencies of *CYP2C19* Genotypes in Patients with Single-Site Arteriosclerosis and Patients with Multi-Site Arteriosclerosis

Genotype/Allele	Total (n=939)	Patients with Single-Site Arteriosclerosis (n=410)	Patients with Multi-Site Arteriosclerosis (n=529)	*P* value
*CYP2C19**2 (rs4244285)				
G/G	407(43.3%)	195(47.6%)	212(40.1%)	0.016
G/A	439(46.8%)	185(45.1%)	254(48.0%)	
A/A	93(9.9%)	30(7.3%)	63(11.9%)	
G	1253(66.7%)	575(70.1%)	678(64.1%)	0.007
A	625(33.3%)	245(29.9%)	380(35.9%)	
HWE (χ^2, *P*)	χ^2=2.614, *P*=0.106	χ^2=2.421, *P*=0.120	χ^2=0.981, *P*=0.322	
*CYP2C19**3 (rs4986893)				
G/G	875(93.2%)	369(90.0%)	506(95.7%)	0.001
G/A	61(6.5%)	39(9.5%)	22(4.2%)	
A/A	3(0.3%)	2(0.5%)	1(0.2%)	
G	1811(96.4%)	777(94.8%)	1034(97.7%)	0.001
A	67(3.6%)	43(5.2%)	24(2.3%)	
HWE (χ^2, *P*)	χ^2=2.931, *P*=0.087	χ^2=0.752, *P*=0.386	χ^2=2.037, *P*=0.153	

Abbreviation: HWE, Hardy Weinberg Equilibrium.

Table 3 Distribution of *CYP2C19* Genotypes, *CYP2C19**2 and *CYP2C19**3 Loss-of-Function Alleles in the Study Population

Genotype	Total (n=939)	Patients with Single-Site Arteriosclerosis (n=410)	Patients with Multi-Site Arteriosclerosis (n=529)	*P* value
Genotypes				
*1/*1	364(38.8%)	167(40.7%)	197(37.2%)	0.002
*1/*2	418(44.5%)	172(42.0%)	246(46.5%)	
*1/*3	40(4.3%)	26(6.3%)	14(2.6%)	
*2/*2	93(9.9%)	30(7.3%)	63(11.9%)	
*2/*3	21(2.2%)	13(3.2%)	8(1.5%)	
*3/*3	3(0.3%)	2(0.5%)	1(0.2%)	
Alleles				
*1	1186(63.2%)	532(64.9%)	654(61.8%)	<0.001
*2	625(33.3%)	245(29.9%)	380(35.9%)	
*3	67(3.6%)	43(5.2%)	24(2.3%)	

Table 4 Association of the Risk Factors with Multi-Site Arteriosclerosis

Variables		Univariate OR (95% CI)	*P* values	Multivariate OR (95% CI)	*P* values
Age (≥65/<65)		1.119(0.850–1.474)	0.423	1.093(0.820–1.459)	0.543
Gender (Male/ Female)		1.013(0.767–1.337)	0.929	0.998(0.739–1.349)	0.992
History of smoking (Yes/No)		0.978(0.712–1.343)	0.890	0.992(0.696–1.414)	0.964
History of alcohol consumption (Yes/No)		1.054(0.522–2.128)	0.884	1.060(0.513–2.191)	0.874
Hypertension (Yes/No)		1.244(0.947–1.635)	0.117	1.269(0.959–1.678)	0.095
Diabetes (Yes/No)		0.961(0.729–1.267)	0.777	0.926(0.698–1.228)	0.593
CYP2C19	*1/*1	1.000(reference)		1.000(reference)	
	*1/*2	1.212(0.913–1.610)	0.183	1.209(0.908–1.609)	0.194
	*1/*3	0.456(0.231–0.902)	0.024	0.459(0.231–0.909)	0.026
	*2/*2	1.780(1.100–2.880)	0.019	1.767(1.091–2.864)	0.021
	*2/*3	0.522(0.211–1.289)	0.159	0.498(0.200–1.236)	0.133
	*3/*3	0.424(0.038–4.716)	0.485	0.435(0.039–4.915)	0.501

Association of the Risk Factors with Multi-Site Atherosclerosis

To gain insight into the independent risk factors on multi-site atherosclerosis, logistic regression analysis was performed. The results of univariate logistic regression showed that *CYP2C19* *1/*3 genotype (*1/*3 vs *1/*1: odds ratio (OR) 0.456, 95% confidence interval (CI): 0.231–0.902, *P*=0.024) may decrease risk of multi-site atherosclerosis, while *2/*2 genotype (*2/*2 vs *1/*1: OR 1.780, 95% CI: 1.100–2.880, *P*=0.019) may increase risk of multi-site atherosclerosis.

The results of multivariate logistic regression (adjusted for gender, age, smoking, drinking, hypertension, and diabetes) indicated that *CYP2C19* *1/*3 genotype (*1/*3 vs *1/*1: OR 0.459, 95% CI: 0.231–0.909, *P*=0.026) may be an independent protective factor for multi-site atherosclerosis, while *2/*2 genotype (*2/*2 vs *1/*1: OR 1.767, 95% CI: 1.091–2.864, *P*=0.021) may be an independent risk factor for multi-site atherosclerosis (Table 4).

Discussion

The result of the relationship of *CYP2C19* gene polymorphisms and multi-site atherosclerosis is still unclear. In the current study, the relationship between *CYP2C19* rs4244285 and rs4986893 polymorphisms and single-site atherosclerosis and multi-site atherosclerosis was analyzed.

In this study, the proportion of *CYP2C19* rs4244285 A allele in multi-site atherosclerosis group was significantly higher than that in single-site atherosclerosis group, while the proportion of *CYP2C19* rs4986893 G allele in multi-site atherosclerosis group was significantly higher than that in single-site atherosclerosis group. The distribution of *CYP2C19* genotypes was significantly different between the single- and multi-site atherosclerosis groups. Multivariate logistic regression showed that *CYP2C19* *1/*3 genotype may be an independent protective factor for multi-site atherosclerosis, while *2/*2 genotype may be an independent risk factor for multi-site atherosclerosis. In endothelial cells, CYP2C-mediated arachidonic acid endothelium-derived hyperpolarizing factor (EDHF) metabolite is the most important cause of endothelial relaxation.[17] Reactive oxygen species (ROS) produced by CYP2C19 catalyzed reactions, excessive ROS can have harmful effects on arterial endothelial cells.[15] Generally speaking, the mechanism by which CYP2C19 is involved in the process of atherosclerosis may be related to its role in the metabolism of vascular endothelial cells and vascular endothelial biology.[17–21] There may be differences in the levels of metabolic substrates at different arterial sites. Different *CYP2C19* genotypes express different CYP2C19 enzymes with different conformations, affecting their ability to bind to substrates at different arterial sites.[38] Several studies have reported the relationship between *CYP2C19* polymorphism and susceptibility to atherosclerosis.[26–29] To our knowledge, this study is the first report of the relationship of *CYP2C19* genotypes and multi-site atherosclerosis.

In this study, the percentages of *CYP2C19* *2 and *3 alleles were 33.3% and 3.6%, respectively. It is consistent with the results of a study based on the Chinese Han population.[39] The prevalence of the *CYP2C9**2 and *3 alleles was 53.1% and 10.2% in a Taiwanese population,[40] 20.5% and 2.5% in a Vietnamese population,[41] 25.6% and 2.5% in a Thai population.[42] In the European populations, the prevalence of the *CYP2C9**2 and *3 alleles was 13.6% and 7.4% in the population from the Republic of Srpska in Bosnia and Herzegovina,[43] 13.1% and 0% in a Greek population.[44] The

prevalence levels of *2 and *3 alleles in the Caucasian population were 13.3% and 5.3%, respectively.[45] On the African continent, the percentages of *CYP2C19* *2 and *3 alleles were 11.38% and 0% in a Moroccan population,[46] 6% and 0% in a Ghanaian population,[47] 12.6% and 0.3% in an Egyptian population.[48] The *CYP2C19*2 allele is present in only about 13% of the Middle Eastern population.[49,50] In the American population, the proportion of the CYP2C19*2 allele is about 8%.[51,52] In general, the frequencies of *CYP2C19* LOF alleles are relatively high in Southeast Asian populations.

Studies on the relationship between traditional influencing factors and atherosclerosis risk have also had some inconsistent results. In this study, multivariate logistic regression showed no significant relationship between gender, age, smoking, alcohol consumption, hypertension, and diabetes and the risk of multi-site atherosclerosis. Study has shown that smoking, alcohol abuse, a history of hypertension and diabetes are risk factors that increase the likelihood of developing cardiovascular disease.[53] A study has found that the incidence of arterial disease may be different between men and women.[54] It can be seen that the occurrence of atherosclerosis is the result of genetic factors, environmental factors, living habits and other comprehensive effects. The inconsistent results between different studies may be related to the number of patients included and the influencing factors analyzed.

Our study found that *CYP2C19* *1/*3 genotype may be an independent protective factor for multi-site atherosclerosis, while *2/*2 genotype may be an independent risk factor for multi-site atherosclerosis. To our knowledge, this study is the first report of the relationship of *CYP2C19* genotypes and multi-site atherosclerosis. The strengths of our study include that we analyzed atherosclerosis at multiple vascular sites, and the number of included cases was not small. However, there are some limitations in this study. First, the association between these polymorphisms and the degree of atherosclerosis (atherosclerosis index or grade I-IV) was not investigated in this study because this study is a retrospective study. Second, it is a study conducted among single- and multi-site atherosclerosis patients in a medical institution, there was inevitably selection bias as the population is not completely representative. Third, this study only studied the common polymorphisms of *CYP2C19* gene, and did not investigate the relationship between the full-length variation of *CYP2C19* gene and the risk of multi-site atherosclerosis. Future studies that include larger sample sizes, the degree of atherosclerosis, and the analysis of the full-length variation of *CYP2C19* gene are needed.

Conclusion

In the cohort of this study, we found that *CYP2C19* *2/*2 genotype is an independent risk factor for multi-site atherosclerosis after adjusting for the factors of demographic characteristics, personal history and disease history.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval

All participants were informed on the study procedures and goals and the study obtained written informed consent from all the participants. We confirm that all methods were performed in accordance with relevant guidelines and regulations. The study was performed under the guidance of the Declaration of Helsinki and approved by the Ethics Committee of Medicine, Meizhou People's Hospital.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests in this work.

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