Genotype Frequencies of CYP2C19, P2Y₁₂ and GPIIIa Polymorphisms in Coronary Heart Disease Patients of Han Ethnicity, and Their Impact on Clopidogrel Responsiveness

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SUMMARY

To investigate the genotype frequencies of cytochrome P450, family2, subfamily C, polypeptide19 (CYP2C19); P2Y₁₂ receptor; and glycoprotein IIIa polymorphisms in patients with coronary heart disease and their impact on clopidogrel responsiveness and major adverse cardiac events (MACEs).

A total of 146 coronary heart disease patients of Han ethnicity, on a clopidogrel regimen, were enrolled. Polymerase chain reaction and DNA sequencing were used to detect the genotype and allelic frequencies of CYP2C19 (*2,*3,*17), P2Y₁₂ (C34T, G52T, T744C) and GPIIIa (T1565C) polymorphisms. Clinical and laboratory data were compared between the high on-treatment platelet reactivity (HTPR) versus normal groups.

HTPR was identified in 35 (24%) patients. CYP2C19*2 (G681A) polymorphism was found to be significantly associated with HTPR ($P < 0.05$). Allele frequencies were significantly higher in the HTPR group versus the normal group ($P < 0.05$). On logistic regression analysis, CYP2C19*2 (G681A) polymorphism was found to be an independent risk factor associated with HTPR. No link could be established between genetic polymorphisms and recurrence of MACEs, or between HTPR and recurrence of MACEs.

The genetic polymorphisms in CYP2C19*2 were closely associated with HTPR. The frequency of the A allele of CYP2C19*2 was significantly associated with HTPR, with A allele carriers being more likely to develop HTPR. (Int Heart J 2016; 57: 586-592)

Key words: Genetic polymorphism, CYP2C19*2 A allele frequency, P2Y₁₂ receptor, GPIIIa receptor gene, Clopidogrel efficacy, HTPR, MACEs recurrence

The most extensively used therapy for significantly reducing the risk of acute myocardial infarction, coronary stent thrombosis, and the incidence of major adverse cardiovascular events (MACEs) includes the combination of clopidogrel and aspirin, which is referred to as a dual antiplatelet therapy.¹⁻⁴⁾ In recent years, research findings have shown that the antiplatelet effect and dose of clopidogrel varies between individuals, and even when patients receive a regular loading dose, they may still develop recurrent clinical thrombotic events. The phenomenon of poor- or non-responsiveness to clopidogrel is called high on-treatment platelet reactivity (HTPR).⁵⁾ Some reports have shown that approximately 4-30% of patients exhibit HTPR after coronary stent implantation;^{6,7)} however, an important factor driving this phenomenon includes the presence of inherited polymorphisms in human genes that impact the metabolism of cardiovascular drugs.⁸⁾ Significant racial/ethnic differences in pharmacodynamic and pharmacokinetic characteristics have been noted for a majority of cardiovascular drugs, including clopidogrel.⁹⁾ Therefore, inherited

genetic polymorphisms in ethnic groups may have a major impact on the efficacy of clopidogrel, yet there are limited studies that address this fundamental gap in the field.

We aimed to investigate the frequency of polymorphisms in genes, which impact the activation of clopidogrel, such as CYP2C19 (CYP2C19*2, CYP2C19*3, CYP2C19*17), which affect its biological activity, such as P2Y₁₂ receptor (P2Y₁₂ C34T, P2Y₁₂ G52T, P2Y₁₂ T744C), and which affect its receptor-mediated platelet activity, such as GPIIIa (GPIIIa T1565C), in coronary heart disease patients of Han ethnicity. In addition, we also studied the impact of these gene polymorphisms on clopidogrel responsiveness and recurrence of MACEs. Genetic variants associated with CYP2C19, P2Y₁₂ receptor, and GPIIIa are associated with reduced formation of the clopidogrel active metabolite, reduced pharmacodynamic response, and reduced platelet function.¹⁰⁻¹⁴⁾ A thorough assessment of the impact of these genetic variants on coronary heart disease and MACE in all major ethnic populations, including those within China, is likely to provide important insights into the risks and possible

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benefits of platelet-directed pharmacotherapy in relation with specific genotypes.

METHODS

Study population: We recruited 146 patients from January 2014 to August 2014 at the Second Affiliated Hospital of Guangzhou Medical University. Inclusion criteria included: 1) male or female patients of Han ethnicity, aged 18 to 85 years, who had no blood relation with each other, and residing in Guangzhou city; 2) patients diagnosed with coronary heart disease by coronary angiography, which included ST segment elevation myocardial infarction (STEMI), non-ST segment elevation myocardial infarction (NSTEMI), and unstable angina pectoris (UA). 3) patients on a standard clopidogrel maintenance dose (75 mg daily) for more than 5 days (some patients did also receive aspirin or glycoprotein IIb/IIIa inhibitor treatment, dependent on the progressive state of their disease). The following criteria were used to exclude patients from the study: 1) platelet counts of $\leq 150 \times 10^9/L$ or $\geq 350 \times 10^9/L$, 2) severe liver disease and/or coagulation abnormalities, 3) contraindications to anti-platelet therapy, 4) recent surgery, 5) history of hemorrhagic stroke, autoimmune disease, or other severe disease within the last 6 months, 6) history of valvular heart disease, and 7) allergy or intolerance to clopidogrel. The Ethics Committee of the Second Affiliated Hospital of Guangzhou Medical University approved this study and written informed consent was obtained from all the patients before enrollment in the study.

Platelet aggregation assay: Peripheral blood samples were obtained from patients for a platelet aggregation assay. Four milliliters (mL) of blood was collected from fasting patients (in the morning after they had been administered 75 mg clopidogrel for more than 5 days) in tubes containing 3.8% sodium citrate. The platelet aggregation assay was performed within 3 hours of sampling to minimize environmental effects.

The platelet aggregation assay was performed by light transmittance aggregometry (LTA) using an LBY-NJ4 automatic platelet aggregation instrument (PuLisheng Corporation, Beijing, China). Blood-citrate mixtures were centrifuged at

950 rpm for 10 minutes to collect platelet-rich plasma (PRP). Samples were further subjected to centrifugation at 3000 rpm for 15 minutes to collect platelet-poor plasma (PPP). A fully automated blood cell counting instrument (Sysmex XE-2100, Japan) was used to adjust platelet counts to between $210\text{--}270 \times 10^9/L$. Platelets in the PRP group were stimulated with a final concentration of $20 \mu\text{mol/L}$ adenosine diphosphate (ADP) (Sigma Corporation, USA).¹⁵⁾ Platelet aggregation was expressed as the maximum aggregation rate (MPA) in light transmittance compared to baseline using PPP as a reference. All laboratory procedures were performed at room temperature. According to the standards of MPA, when the MPA was $\geq 50\%$, it was regarded as HTPR; however, when the MPA was $< 50\%$, it was regarded as normal.¹⁶⁾

Genetic analysis: Fasting peripheral venous blood samples were obtained from all 146 patients for genetic analysis. Genomic DNA was extracted from 2 mL of EDTA-treated anticoagulated whole blood using the TIANamp Blood DNA Kit (Tiagen Biotech, Beijing, China). Each DNA sample was assessed for concentration and quality by the Bio Tek Epoch spectrophotometer to ensure that the OD260/OD280 ratio was between 1.7 and 2.0.

Primers were designed for detecting 7 polymorphic loci in 3 genes using Primer 5.0 software, which included the CYP2C19*2 (681G>A), CYP2C19*3 (636G>A), CYP2C19*17 (808C>T), P2Y₁₂ (34C>T), P2Y₁₂ (52G>T), P2Y₁₂ (744T>C) and GPIIIa (1565T>C) polymorphisms (Table I). The polymorphic loci were amplified by polymerase chain reaction (PCR), using the following protocol: denaturing at 95°C for 3 minutes followed by 35 cycles at 95°C for 10 seconds, 63°C for 30 seconds, 68°C for 30 seconds, and a final extension at 68°C for 3 minutes with storage at 12°C. The PCR reaction mix contained genomic DNA 2.0 μL , 2 $\times$ Taq Master Mix 25.0 μL , 1.2 μL of primers, and 21.8 μL ddH₂O, making a total reaction volume of 50 μL . Samples were sent to GenScript Inc (Nanjing, China) for direct DNA sequencing. To verify correct sample handling, approximately 10% of the patients were randomly selected for repeat analysis, which revealed identical results.

Follow-up: The follow-up of patients was performed either by office visits or by telephone at a median time interval of 7

Table I. Primer Sequences Utilized to Amplify Seven Polymorphic Loci

Polymorphic locus	Primer sequences
CYP2C19*2 (G681A, rs4244285)	Forward: 5'-GCAGGTATAAGTCTAGGAAATGA-3' Reverse: 5'-TAAAGTCCCAGGGTTGTT-3'
CYP2C19*3 (G636A, rs4986893)	Forward: 5'-CTGTGATCCCACCTTTCATCC-3' Reverse: 5'-CAAACACCTCTGCCCTATTC-3'
CYP2C19*17 (C808T, rs12248560)	Forward: 5'-GCTGTGGGTCCATTAGTGA-3' Reverse: 5'-CGTGAAGGCAGGAATTGTTAT-3'
P2Y ₁₂ receptor (T744C, rs2046934)	Forward: 5'-GGGATGAACCTAAGACCACAC-3' Reverse: 5'-TTATTACCACAATAGGCAGC-3'
P2Y ₁₂ receptor (C34T, rs6785930 G52T, rs6809699)	Forward: 5'-TGACGGAGGTAACCTTGACAC-3' Reverse: 5'-GCTTTAAGAGGCAACATTC-3'
GPIIIa subreceptor (T1565C, rs5918)	Forward: 5'-CCCAGGAAAGACCACAACA-3' Reverse: 5'-TCTATGCCACCTGCTTCA-3'

months (range, from 3-12 months). This time interval was chosen as it was considered to be appropriate for obtaining information about the recurrence of major adverse cardiovascular events in patients, which included recurrent angina, recurrent myocardial infarction, stent thrombosis, stent restenosis, and sudden cardiac death.

Statistical analysis: Continuous data are expressed as the mean $\pm$ standard deviation (SD). Data are presented as numbers and frequencies for categorical variables. For comparison between groups, the chi-square test (or Fisher's exact test when any expected cell count was < 5 for a 2×2 table) was used for categorical variables and the independent-sample t test was applied for continuous data. The chi-square test for goodness of fit was used to verify agreement with Hardy-Weinberg equilibrium using Fisher's exact test. Non-conditional logistic regression analysis was performed to determine independent risk factors related to genotype and HTPR. Two-sided P values < 0.05 were considered statistically significant. Statistical tests were performed using SPSS version 19.0 software (SPSS Inc, Chicago, USA).

RESULTS

Characteristics of the study population: Out of a total of 146 patients enrolled in the study, 35 (24%) were found to have HTPR. The proportion of patients with AMI in the HTPR and normal groups was 34% and 46%, respectively. Table II presents the demographic, clinical characteristics, angiographic findings, and laboratory data of the study population according to clopidogrel responsiveness. No significant differences in patient characteristics were found between the HTPR and normal group, except for MPA.

Genotype analyses: The genotype distribution and allele frequencies of the CYP2C19*2 (681G>A), CYP2C19*3 (636G>A), CYP2C19*17 (808C>T), P2Y₁₂ (34C>T), P2Y₁₂ (52G>T), P2Y₁₂ (744T>C), and GPIIIa (1565T>C) among 146 patients

are presented in Table 3. All of the alleles conformed to the Hardy-Weinberg equilibrium.

Relationship of genetic polymorphisms with HTPR: In the present study, the genotype frequencies of the CYP2C19*2 (681G>A) polymorphism were significantly different between the two groups ($P < 0.05$). The frequency of the A allele was significantly higher in the HTPR group than in the normal group (41.4% versus 27.0%, $P < 0.05$; Table III). In addition, the A allele carriers were more likely to develop HTPR (OR = 1.91, 95% CI: 1.091-3.344, $P < 0.05$). Logistic regression analysis adjusted for the demographic characteristics suggested that the CYP2C19*2 (681G>A) polymorphism was an independent risk factor for HTPR (OR = 2.837, 95% CI: 1.246-6.458, $P < 0.05$).

Since no patient was wild type for CYP2C19*3 and heterozygous for CYP2C19*3 (636G>A), no statistical analysis could be performed for these polymorphisms, hence, the relation between this genetic polymorphism and clopidogrel responsiveness could not be investigated (Table III).

There was no significant difference in the genotype and allele frequencies of CYP2C19*17 (808C>T), P2Y₁₂ (34C>T, 52G>T, 744T>C), and GPIIIa (1565T>C) between the two groups ($P > 0.05$). To eliminate the effect of CYP2C19*2 on HTPR, we selected patients with wild type CYP2C19*2 and analyzed the distribution of genotype frequencies of the above-mentioned gene-loci and still found that there were no significant differences between the two groups ($P > 0.05$; Table III).

Relationship of genetic polymorphisms with MACEs: Out of 146 patients, 94 were successfully followed up. We could not find any association between the various polymorphisms of the above-mentioned gene-loci and the recurrence of MACEs ($P > 0.05$; Table IV).

Relationship of HTPR with MACEs: We could not find any association between HTPR and the recurrence of MACEs ($P > 0.05$; Table V).

Table II. Clinical Characteristics of Patients and Angiographic Findings in the HTPR and Normal Groups

	HTPR ($n = 35$)	Normal ($n = 111$)	P
MPA, %	57.21 $\pm$ 5.08	30.36 $\pm$ 13.58	< 0.01
Age	70.26 $\pm$ 9.15	68.53 $\pm$ 11.85	0.831
Sex (male/female), n	24/11	72/39	0.687
Smoking, n	14	36	0.411
Hypertension, n	24	78	0.849
Diabetes mellitus, n	10	27	0.614
BMI (kg/m ²)	23.55 $\pm$ 2.76	23.43 $\pm$ 3.13	0.837
TG (mmol/L)	1.57 $\pm$ 1.09	1.35 $\pm$ 0.73	0.480
TCHO (mmol/L)	4.25 $\pm$ 1.02	4.26 $\pm$ 1.21	0.658
LDL (mmol/L)	2.23 $\pm$ 1.04	2.02 $\pm$ 1.15	0.236
HDL (mmol/L)	1.44 $\pm$ 0.82	1.73 $\pm$ 1.06	0.294
Number of diseased vessels			
One	15	48	0.968
Two	11	40	0.618
Three	7	20	0.792
Left main disease	1	3	0.961
Number of vessels treated per patient	1.2 $\pm$ 0.4	1.2 $\pm$ 0.4	0.112
Number of stents per patient	1.5 $\pm$ 0.8	1.5 $\pm$ 0.8	0.681
Minimal stent diameter (mm)	3.9 $\pm$ 18.6	3.6 $\pm$ 14.7	0.764
Maximal length of stent (mm)	22.0 $\pm$ 14.9	21.5 $\pm$ 0.7	0.613
At least one drug-eluting stent patient	18	66	0.402

Table III. Genotype Distributions and Allele Frequencies of All Investigated Genetic Variants

	HTPR (<i>n</i> = 35)	Normal (<i>n</i> = 111)	χ^2	<i>P</i>
CYP2C19*2 (681G>A)				
GG	10 (28.6%)	59 (53.2%)		
GA	21 (60.0%)	44 (39.6%)		
AA	4 (11.4%)	8 (7.2%)	6.457	0.040*
G allele	41 (58.6%)	162 (73.0%)		
A allele	29 (41.4%)	60 (27.0%)	5.209	0.022*
CYP2C19*3 (636G>A)				
GG	35 (100%)	111 (100%)		
GA	0 (0%)	0 (0%)		
AA	0 (0%)	0 (0%)	---	--- Δ
G allele	70 (100%)	222 (100%)		
A allele	0 (0%)	0 (0%)	---	--- Δ
CYP2C19*17 (808C>T)				
CC	35 (100%)	110 (99.1%)		
CT	0 (0%)	1 (0.9%)		
TT	0 (0%)	0 (0%)	0.550	0.458
G allele	70 (100%)	221 (99.5%)		
A allele	0 (0%)	1 (0.5%)	0.549	0.459
P2Y ₁₂ (34C>T)				
CC	22 (62.9%)	71 (64.0%)		
TC	11 (31.4%)	33 (29.7%)		
TT	2 (5.7%)	7 (6.3%)	0.046	0.977
T allele	55 (78.6%)	175 (78.8%)		
C allele	15 (21.4%)	47 (21.2%)	0.002	0.963
P2Y ₁₂ (52G>T)				
GG	27 (77.1%)	81 (73.0%)		
GT	8 (22.9%)	26 (23.4%)		
TT	0 (0%)	4 (3.6%)	2.258	0.323
T allele	62 (88.6%)	188 (84.7%)		
C allele	8 (11.4%)	34 (15.3%)	0.653	0.419
P2Y ₁₂ (744T>C)				
TT	21 (60.0%)	76 (68.5%)		
TC	12 (34.3%)	30 (27.0%)		
CC	2 (5.7%)	5 (4.5%)	0.856	0.652
T allele	54 (77.1%)	182 (82.0%)		
C allele	16 (22.9%)	40 (18.0%)	0.804	0.370
GPIIIa (1565T>C)				
TT	34 (97.1%)	108 (97.3%)		
TC	1 (2.9%)	3 (2.7%)		
CC	0	0	0.002	0.961
T allele	69 (98.6%)	219 (98.6%)		
C allele	1 (1.4%)	3 (1.4%)	0.002	0.961

**P* < 0.05, Δ indicates that statistical analysis on this population was impossible.

DISCUSSION

In patients undergoing percutaneous coronary intervention (PCI) with stent implantation, clopidogrel in combination with aspirin is recommended for reducing the risk of MACEs and to prevent stent thrombosis.¹⁷⁾ However, some patients receiving regular or even loading doses of clopidogrel develop HTPR, yet not many studies have been carried out to explore the underlying mechanisms of this phenomenon.

In this study, we screened for the presence of HTPR by light transmittance aggregometry (LTA) in patients of Han ethnicity presenting with coronary heart disease. We found that 24% of the study subjects had HTPR, which was similar to the findings reported from Fujian province (36%) and northern areas (24%) of China.^{18,19)} In other Asian populations, the incidence rate of HTPR is reported to be 21% in Japan²⁰⁾ and 25.9% in Iran.²¹⁾ Similarly, the incidence rate of HTPR is reported to range from 23% to 28% in western countries.²²⁻²⁵⁾ In-

terestingly, Jeong, *et al*²⁶⁾ reported the incidence rate of HTPR as 50% in Korea, while Geisler, *et al*²⁷⁾ reported the HTPR rate as 6% in Germany. The reasons for the differences in these results may be due to the lack of uniformity in laboratory tests used for detection of HTPR, lack of a standard definition of HTPR, lack of standardization in sample selection and sample sizes in each cohort, and differing study designs.

In recent years, extensive research has been performed on HTPR and CYP450, as CYP2C19*2 is a locus with the highest mutation rate. The CYP2C19*2 A allele frequency was found to be 30.5% in coronary heart disease patients of Han ethnicity residing in Guangzhou city, which was similar to the patients of Han ethnicity in other areas of China.²⁸⁾ Also, we identified that A allele carriers were more likely to develop HTPR, while the CYP2C19*2 genetic polymorphism was an independent risk factor for HTPR. Our results are in agreement with those reported by earlier studies.²⁹⁻³¹⁾ Interestingly, no patient was wild type and heterozygous for CYP2C19*3. In addition, the

Table IV. Relationship of Genetic Polymorphisms With MACEs

	Incidence of major adverse cardiovascular events		χ^2	<i>P</i>
	Yes (<i>n</i> = 40)	No (<i>n</i> = 54)		
CYP2C19*2 (681G>A)				
GG	18 (45.0%)	25 (46.3%)		
GA	18 (45.0%)	23 (42.6%)		
AA	4 (10.0%)	6 (11.1%)	0.066	0.968
G allele	54 (67.5%)	73 (67.6%)		
A allele	26 (32.5%)	35 (32.4%)	0.0001	0.989
CYP2C19*3 (636G>A)				
GG	40 (100%)	54 (100%)		
GA	0 (0%)	0 (0%)		
AA	0 (0%)	0 (0%)	---	--- Δ
G allele	80 (100%)	108 (100%)		
A allele	0 (0%)	0 (0%)	---	--- Δ
CYP2C19*17 (808C>T)				
CC	40 (100%)	54 (100%)		
CT	0 (0%)	0 (0%)		
TT	0 (0%)	0 (0%)	---	--- Δ
G allele	80 (100%)	108 (100%)		
A allele	0 (0%)	0 (0%)	---	--- Δ
P2Y ₁₂ (34C>T)				
CC	26 (65.0%)	37 (68.5%)		
TC	10 (25.0%)	16 (29.6%)		
TT	4 (10.0%)	1 (1.9%)	3.162	0.206
T allele	62 (77.5%)	90 (83.3%)		
C allele	18 (22.5%)	18 (16.7%)	1.010	0.315
P2Y ₁₂ (52G>T)				
GG	31 (77.5%)	37 (68.5%)		
GT	7 (17.5%)	17 (31.5%)		
TT	2 (5.0%)	0 (0%)	5.506	0.064
T allele	69 (86.3%)	91 (84.3%)		
C allele	11 (13.8%)	17 (15.7%)	0.144	0.705
P2Y ₁₂ (744T>C)				
TT	29 (72.5%)	34 (63.0%)		
TC	8 (20.0%)	19 (35.2%)		
CC	3 (7.5%)	1 (1.9%)	3.965	0.138
T allele	66 (82.5%)	87 (80.6%)		
C allele	14 (17.5%)	21 (19.4%)	0.115	0.735
GPIIIa (1565T>C)				
TT	37 (92.5%)	53 (98.1%)		
TC	3 (7.5%)	1 (1.9%)		
CC	0 (0%)	0 (0%)	1.813	0.178
T allele	77 (96.3%)	107 (99.1%)		
C allele	3 (3.8%)	1 (0.9%)	1.760	0.185

Δ indicates that statistical analysis on this population was not possible.

Table V. Relationship Between HTPR and MACEs

	Incidence of major adverse cardiovascular events		χ^2	<i>P</i>
	Yes (<i>n</i> = 40)	No (<i>n</i> = 54)		
HTPR	9 (22.5%)	14 (25.9%)		
Normal	31 (77.5%)	40 (74.1%)	0.146	0.702

number of heterozygous patients for CYP2C19*17 was also rare. Thus, the above two polymorphisms appeared to have no impact on clopidogrel responsiveness.

Clopidogrel acts through the P2Y₁₂ receptor, which is a central receptor that triggers antiplatelet activity. Thus, we also examined P2Y₁₂ receptor gene (34C>T, 52G>T, 744T>C) polymorphisms, which might have an impact on drug pharmacodynamics. We could identify patients that were wild type as well as those who were heterozygous for the P2Y₁₂ receptor

gene; however, they were not found to be associated with a response to clopidogrel. These results were consistent with several other reports from China and western countries.³¹⁻³⁵⁾

The last genetic polymorphism that was evaluated in this study was glycoprotein IIIa (GPIIIa;1565T>C), which is part of the integrin complex present on platelets, and acts as a receptor for fibrinogen and von Willebrand factor that aids in platelet activation. Since genetic polymorphisms in the P2Y₁₂ receptor were not associated with HTPR, we sought to deter-

mine whether downstream pathways from the P2Y₁₂ receptor, such as polymorphisms in the GPIIIa receptor gene, may account for the development of HTPR. We demonstrated that the GPIIIa polymorphism was not associated with poor responsiveness to clopidogrel. These results are in agreement with those reported by Cooke³⁶⁾ and Isordia-Salas.³⁷⁾ Hence, genetic screening for GPIIIa polymorphisms may not be warranted, as they are less likely to be associated with HTPR.

We found no significant association between HTPR and the recurrence of MACEs. On the contrary, some research findings³⁸⁾ have suggested that HTPR is associated with significantly increased adverse cardiovascular events. The incidence of MACE in our study is considered relatively high (42.6%). Reasons for the high incidence of MACEs may be due to the small sample size of patients at follow-up and the follow-up time period being too short. We also specifically analyzed the relationship between genetic polymorphisms with HTPR and MACEs, but could not demonstrate any association between the two. Our results are in disagreement with those reported by Viviani Anselmi, *et al*³⁹⁾ which suggested that polymorphisms in the CYP2C19 gene were an independent predictor of MACEs after drug eluting stent (DES) implantation and can be used as a prognostic tool to determine the stability of CAD patients. Several possible reasons could explain the lack of association between HTPR and MACEs in our study. One reason could relate to population-specific effects, as Asians have been shown to exhibit a favorable endogenous thrombolytic profile, while Westerners exhibit a more prothrombotic profile.⁴⁰⁾ Thus, modest platelet inhibition may be enough to avoid MACEs in Han patients. Another reason could be that aspirin may have an important role in MACEs. Genetic and environmental factors, including angiographic and procedural characteristics, that were not accounted for in our study, may play an important role in recurrent ischemic events in patients. In addition, the low rate of follow-up may also impact our results on MACEs.

Though the present study has generated some important findings, there are certain limitations. A single method was used to evaluate clopidogrel responsiveness, which was not confirmed by another assay. Clopidogrel responsiveness was assessed only in patients who were already on clopidogrel treatment without having assessed the baseline reactivity data. The sample size of the study was relatively small, which may have impacted the reliability of the results. Finally, the patient follow-up time period was also limited in our study.

In conclusion, the incidence of HTPR in coronary heart disease patients of Han ethnicity in Guangzhou city was found to be 24%. The genetic polymorphisms in CYP2C19*2 were closely associated with HTPR. The frequency of the A allele of CYP2C19*2 was significantly associated with HTPR, with A allele carriers being more likely to develop HTPR.

DISCLOSURE

Conflicts of interest: none declared

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