Current Medical Science
DOI https://doi.org/10.1007/s11596-019-1998-2
39(1):44-51,2019

Association between CYP2C19*2/*3 Polymorphisms and Coronary Heart Disease*

Ying-ying ZHANG[1, 3†], Xin ZHOU[2†], Wen-jie JI[2], Ting LIU[1, 3], Jing MA[1, 3], Ying ZHANG[1, 3], Yu-ming LI[2#]
[1]*Department of Cardiology, Tianjin Chest Hospital, Tiangjin 300222, China*
[2]*Institute of Cardiovascular Disease and Heart Center, Pingjin Hospital, Logistics University of the Chinese People's Armed Police Forces, Tianjin 300102, China*
[3]*Tianjin Institute of Cardiovascular Disease, Tianjin 300222, China*

Summary: This study sought to explore the relationship between cytochrome P450 2C19 (CYP2C19) *2/*3 polymorphisms and the development of coronary heart disease (CHD), and to evaluate the influence of the single nucleotide polymorphisms (SNPs) on the occurrence of adverse clinical events in CHD patients. A total of 231 consecutive patients candidate for percutaneous coronary intervention genotyped for CYP2C19*2 (681G>A) and *3 (636G>A) polymorphisms were enrolled. The adverse clinical events were recorded during a follow-up period of 14 months. The incidence of CHD, according to coronary angiography, was significantly higher (P=0.025) in CYP2C19*2 carriers group. Stepwise binary logistic regression analysis revealed that among factors that potentially influenced the presence of CHD (age>60 years, gender, BMI, etc.), CYP2C19*2 carriers (OR 1.94, 95% CI: 1.08–3.50, P=0.028) and male gender (OR 2.74, 95% CI: 1.58–4.76, P=0.001) were independent predictors, which were associated with the presence of CHD. The follow-up results showed that the incidence of adverse cardiovascular events within 14 months of discharge was significantly higher in the CYP2C19*2 carriers than in the non-carriers (21.6% *vs.* 6.3%, P=0.019). The results of the multivariate Cox proportional hazards model showed that CYP2C19*2 loss-of-function was the only independent factor which predicted the coronary events during the follow-up period of 14 months (OR=3.65, 95% CI 1.09–12.25, P=0.036). The adverse impact of CYP2C19*2 polymorphisms was found not only in the risk of the presence of CHD, but also in the adverse cardiovascular events in CHD patients during the follow-up period of 14 months. However the same influence was not found in CYP2C19*3 mutation in Chinese Han population.
Key words: cytochrome P450 2C19; polymorphisms; coronary heart disease; clopidogrel

The pathogenesis of coronary heart disease (CHD) has not yet been clearly determined; nevertheless, it is known that a compound character is derived from a complex interaction of genetic and environment factors. First of all, atherosclerosis is believed to be a chronic inflammatory disease. Cytochrome P450 (CYP) epoxygenase participates in arachidonic acid metabolism[1]. Most CYP genes are primarily expressed in the liver, with significantly lower expression levels in extrahepatic tissues. However, some CYPs are predominantly detected in the heart, vasculature, gastrointestinal tract, kidney, and lung[2, 3] and even localized in vascular smooth muscle and endothelium contributing to regulation of vascular tone and homeostasis[4]. Due to the polymorphisms, there are large interindividuals as well as interethnic differences in the metabolism of different CYP substrates[5]. Ercan[6] found that smoker patients carrying CYP2C19*2 mutations have 3.7 fold risk of developing CHD in Turkey people. Yang reported that CYP2C19*3 genotype was significantly more prevalent in patients with CHD (6.25% *vs.* 2.96%; P=0.03) in Chinese Uighur population[7]. More evidence has tested that CYP family polymorphisms are associated with atherosclerosis[1, 8, 9].

The dual antiplatelet therapy of aspirin and clopidogrel is an important measurement to prevent the ischemic events that occur in CHD and can significantly

Ying-ying ZHANG, E-mail: lunwen.cardiolab@foxmail.com; Xin ZHOU, E-mail: xzhou@live.com
†The authors contributed equally to this work.
#Corresponding author, E-mail: liyuming.cardiolab@aliyun.com
*This work was supported by the National Natural Science Foundation of China (No. 81170238 and No. 81070121), Tianjin Municipal Science and Technology Committee (No. 09ZCZDSF04200 and No. 11JCYBJC12000) and Tianjin Chest Hospital Found 2018 (No. 2018XKZ01).

improve the clinical outcome of patients with acute coronary syndrome (ACS) and patients treated with percutaneous coronary intervention (PCI)[10, 11]. Clopidogrel is an inactive prodrug and requires metabolization and activation by the CYP2C19 to generate its active thiol metabolite, which can significantly inhibit platelet aggregation by binding to the P2Y12 receptor[12]. Initial studies have determined that carriers of the allelic variant (CYP2C19*2) have significantly lower levels of the active clopidogrel metabolite, which contributes to higher rate of cardiovascular events, including stent thrombosis[13]. Since these initial discoveries, multiple cohort studies have further linked the CYP2C19*2, CYP2C19*3 and other loss-of-function allelic variants of this gene to major adverse cardiovascular events in patients taking clopidogrel[14–16]. In March 2010, the US Food and Drug Administration approved a new label for clopidogrel with a "boxed warning" describing the diminished effectiveness of the standard drug dosing in individuals with impaired metabolic function (so-called poor metabolizers) based on their CYP2C19 genotype[11, 17]. The majority of researchers believe that CYP2C19*2 polymorphisms may interfere in major adverse cardiovascular events[18, 19]. However, a systematic review and meta-analysis by Holmes *et al* concluded that the genotype had no significant association with cardiovascular events[20].

To the best of our knowledge, the influence of genetic polymorphisms of CYP2C19 on the risk of the presence of CHD has not been studied in Chinese Han population. The main aim of the present study was to assess the relationship between CYP2C19 *2/*3 polymorphisms and the development of CHD. In patients treated with clopidogrel, the best genome-guided strategy remains to be determined. In light of the previous findings, we sought to investigate the influence of the genetic variant on CHD patients with the standard use of clopidogrel during a follow-up period of 14 months.

1 MATERIALS AND METHODS

1.1 Eligibility and Recruitment

We enrolled 231 consecutive patients suspected of CHD in Pingjin Hospital, Logistics University of Chinese People's Armed Police Forces from May 1st 2012 to Nov. 1st 2012. The enrolled patients had the following characteristics: (1) willing to provide informed consent and agreed to diagnostic coronary angiography; (2) had a normal routine troponin tested at least 12 h from symptom onset; (3) received a loading dose of 300 mg aspirin plus 300 mg clopidogrel for at least 24 h, or 100 mg aspirin plus 75 mg clopidogrel for at least 5 days; and (4) of Han nationality, the majority ethnic group in China. The exclusion criteria were as follows: (1) patients with congenital heart disease, valvular disease of the heart, congestive heart failure, renal failure, hepatic insufficiency, cerebrovascular disease, peripheral vascular disease, cancer or immune disorders; (2) patients who had undergone previous vascular surgery or percutaneous transluminal angioplasty; and (3) patients with a history of bleeding diathesis (platelet count $<150\times10^9$/L or $>450\times10^9$/L, hemoglobin <80 g/L). The research protocol was approved by the Ethical Committee of Pingjin Hospital, Logistics University of the Chinese People's Armed Police Forces, which was in accordance with the principles of the Declaration of Helsinki.

1.2 Coronary Angiography and Calculation of SYNTAX Score

Coronary angiography was applied via the femoral approach using the Standard Judkins Technique. SYNTAX score is a well-established coronary angiographic score based on complexity of coronary artery disease. SYNTAX score was retrospectively calculated by two experienced interventional cardiologists by visually assessing all coronary lesions with a diameter stenosis ≥50% in vessels >1.5 mm diameter, using the online algorithm (www.syntaxscore.com).

1.3 CYP2C19*2/*3 Genetic Polymorphisms

Genomic DNA was extracted from peripheral blood samples using Genomic DNA Purification Kit (Promega, USA). The PCR was performed in a 50-μL reaction volume containing 0.3 μmol/L of each of the following primers: CYP2C19*2 (F 5′-AATTACAACCAGAGCTTGGC-3′ and R 5′-TATCACTTTCCATAAAAGCAAG-3′, product length: 169 bp); CYP2C19*3 (F 5′-TGTGCTCCCTGCAATGTGAT-3′ and R 5′-TTTGGGGCTGTCACCAAAGT-3′, product length 419 bp). PCR was performed according to standard procedures and reaction mixtures were subjected to 35 cycles of amplification (94°C denaturation for 30 s, 60°C annealing for 30 s, and 72°C extension for 30 s). The PCR products were purified with a commercially available kit (Takara MiniBEST DNA Fragment Purification Kit Ver.3.0) and the genotypes of CYP2C19*2/*3 were identified using restriction fragment length polymorphisms with *Sma* Ⅰ (QuickCut Sma Ⅰ, Takara)/*Bam*H Ⅰ (QuickCut Sma Ⅰ, Takara). The products were then separated on a 3%/2% agarose gel, and the bands were visualized with ethidium bromide.

1.4 Follow-up and Study Endpoints

Clinical outcomes at 1, 2, 3, 6, 9, 12 and 14 months post-hospital discharge were assessed by telephone interviews. Clinical endpoints were cardiovascular death, an event of myocardial infarction, hospital readmission for ACS/stroke, and the major adverse cardiac events (MACE) consisting of stent thrombosis. Bleeding endpoints were based on the Thrombolysis

in Myocardial Infarction (TIMI) and Global Use of Strategies to Open Occlude Arteries (GUSTO) bleeding criteria.

1.5 Statistical Analysis

All analyses were carried out using SPSS version 18.0 (SPSS Inc., USA) and Empower (R). The Hardy-Weinberg equilibrium was assessed using chi-square analysis. Discrete variables, expressed as percentages, were compared by Chi-square or Fisher's exact test. Numerical variables were expressed as mean with standard deviation or median with interquartile range. Normally distributed continuous variables were compared by *t*-test, and non-normally distributed data were compared by nonparametric test. To determine clinical variables that independently predict CHD, stepwise binary logistic regression was used. The independent association between the presence of the CYP2C19*2 or *3 polymorphisms and CHD outcome was assessed by using multivariate Cox regression analysis and Cox proportional hazards model for event-free survival. In order to quantify the risk of CHD based on various risk factors, odds ratios (OR) with 95% confidence interval (CI) and *P* values were presented. A value of $P<0.05$ was considered as statistically significant.

2 RESULTS

2.1 Patient Characteristics

A total of 231 consecutive patients scheduled for coronary angiography (CAG) were enrolled in this study. The patients were grouped according to the CYP2C19*2 (non-carriers, $n=90$ and carriers, $n=141$) and *3 (non-carriers, $n=213$ and carriers, $n=18$) genotype. As shown in table 1, the demographic data of CYP2C19*2 and CYP2C19*3 determined were comparable. As shown in fig. 1A, the percentage of CHD people was significantly higher in CYP2C19*2 carriers group than that in non-carriers group (68.8% *vs*. 53.3%, $P=0.025$). No significant difference was

Table 1 Demographic data of 231 patients and subgroup characteristics

Items	Total patients (n=231)	CYP2C19*2		*P*	CYP2C19*3		*P*
		Non-carriers (n=90)	Carriers (n=141)		Non-carriers (n=213)	Carriers (n=18)	
CHD (%)	62.8	53.3	68.8	0.025*	62.9	61.1	1.000
Age (years)	58.36±8.40	58.03±7.31	58.57±9. 0	0.634	58.18±8.41	60.05±8.17	0.262
Gender (Male %)	51.5	50.0	52.2	0.787	52.1	44.4	0.626
Smoking (%)	44.2	38.9	47.5	0.223	44.6	38.9	0.806
BMI (kg/m^2)	24.53±4.46	24.50±4.56	24.55±4.41	0.929	24.68±4.40	22.77±4.90	0.082
Heart rate (beats/min)	70 (64, 77)	70 (63, 79)	70 (64, 76)	0.988	70.0 (64.0, 77.5)	67.0 (62.0, 77.0)	0.316
SBP (mmHg)	140 (121, 150)	140 (130, 150)	134 (120, 150)	0.153	135.0 (120.0, 150)	140.0 (130.0, 150.0)	0.345
DBP (mmHg)	80 (75, 90)	80 (79, 90)	80 (74, 90)	0.429	80.0 (75.0, 90.0)	80.0 (72.3, 95.5)	0.937
DM (%)	12.1	10.0	13.5	0.537	12.2	11.1	1.000
WBC counts (10^9/L)	6.59 (5.34, 7.84)	6.90 (5.49, 8.02)	6.34 (5.30, 7.84)	0.306	6.56 (5.33, 7.84)	6.79 (5.60, 8.11)	0.392
RBC counts (10^{12}/L)	4.46 (4.15, 4.83)	4.47 (4.21, 4.78)	4.44 (4.11, 4.86)	0.988	4.46 (4.16, 4.82)	4.45 (4.08, 5.05)	0.936
Neutrophil (%)	63.90±11.51	65.19±12.28	63.09±10.96	0.178	63.76±11.53	65.72±11.52	0.489
Lymphocyte (%)	26.66±9.23	25.93±9.02	27.12±9.37	0.342	26.71±9.22	26.04±9.67	0.768
Monocyte (%)	6.30 (4.90, 7.55)	6.35 (4.58, 7.60)	6.20 (5.10, 7.50)	0.677	6.30 (4.95, 7.50)	5.56 (4.88, 7.63)	0.649
Hemoglobin (g/L)	137.0 (127.0, 147.0)	137.0 (127.0, 145.0)	136.0 (128.0, 148.0)	0.698	137.0 (128.0, 147.0)	131.0 (124.3, 145.5)	0.214
Platelet counts (10^9/L)	218.84±52.43	222.08±52.47	218.06±52.58	0.776	217.58±53.04	233.83±43.10	0.207
ALT	16.0 (11.5, 27.00)	15.00 (10.75, 25.25)	18.00 (12.00, 29.00)	0.145	16.0 (12.0, 27.5)	13.5 (9.8, 23.5)	0.176
AST	17.0 (14.0, 23.0)	16.50 (13.00, 21.00)	17.00 (14.00, 23.50)	0.257	17.0 (14.0, 23.0)	15.5 (11.8, 18.0)	0.228
BUN	5.13±1.23	4.95±1.23	5.24±1.23	0.077	5.13±1.24	5.12±1.54	0.965
Creatinine (μmol/L)	63.86±13.46	61.73±14.06	65.21±12.93	0.055	64.00±13.57	62.22±12.30	0.593
Uric acid (μmol/L)	297.53±89.96	287.03±87.92	304.23±90.91	0.157	297.85±91.34	293.72±73.68	0.825
Total bilirubin (μmol/L)	10.40 (8.40, 14.25)	10.0 (8.38, 13.43)	10.90 (8.50, 14.45)	0.252	10.1 (8.4, 13.9)	12.4 (8.5, 16.5)	0.174
Total cholesterol (μmol/L)	4.35±0.86	4.37±0.96	4.33±0.79	0.771	4.32±0.85	4.72±0.93	0.055
TG	1.49 (1.06, 2.11)	1.50 (1.06, 2.11)	1.46 (1.07, 2.11)	0.328	1.46 (1.07, 2.09)	1.50 (0.95, 1.51)	0.920
Medications							
Nitrates (%)	51.9	51.1	52.5	0.893	46.0	66.7	0.149
β-blockers (%)	44.6	44.4	44.7	1.000	44.1	50.0	0.632
ACEI/ARB (%)	35.5	36.7	34.8	0.870	46.0	22.2	0.135
CCB (%)	32.9	31.1	35.5	0.479	31.5	50.0	0.122
Statins (%)	80.5	75.6	83.7	0.172	79.3	94.4	0.211
PPI (%)	13.4	10.0	15.6	0.243	13.6	11.1	1.000

ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blockers; BMI: body mass index; CCB: calcium channel blockers; DBP: diastolic blood pressure; PPI: proton pump inhibitors; RBC: red blood cells; SBP: systolic blood pressure; TG: triglycerides; WBC: white blood cells; DM: diabetes mellitus; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen

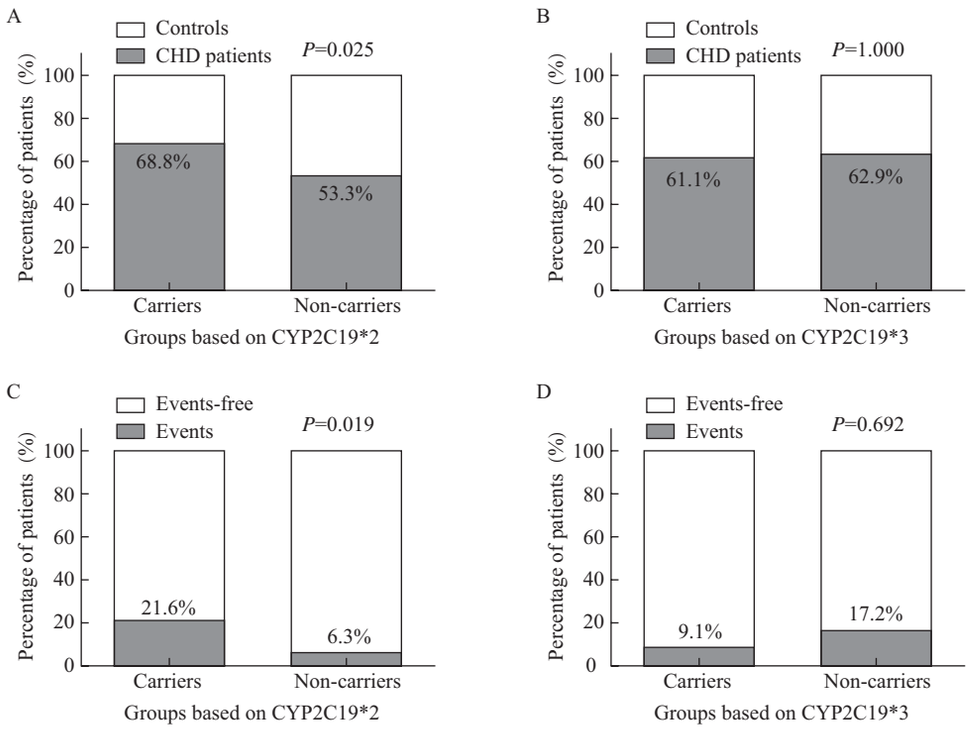

Fig. 1 The percentage of the patients in CYP2C19*2 or CYP2C19*3 determined groups
A: The percentage of CHD people was statistically higher in CYP2C19*2 carriers group than in non-carriers group. B: No significant difference was observed between CYP2C19*3-based groups among the 231 patients. C: The percentage of coronary events after 14-month follow-up was statistically higher in CYP2C19*2 carriers group than in non-carriers group. D: There was no significant difference between the subgroups based on CYP2C19*3 genotype.

observed between CYP2C19*3 based groups as shown in fig. 1B (61.1% *vs*. 62.9%, *P*=1.000). Among the 231 patients, 145 were diagnosed as CHD according to the result of CAG. Table 2 describes the baseline characteristics of the 145 CHD patients according to CYP2C19*2 phenotype. The percentage of coronary events after 14-month follow-up was statistically significantly higher in CYP2C19*2 carriers group than in non-carriers group (21.6% *vs*. 6.3%, *P*=0.019), as shown in fig. 1C, but there was no significant difference between the subgroups based on CYP2C19*3 genotype (9.1% *vs*. 17.2%, *P*=0.692, fig. 1D). All these baseline variables were well balanced among these groups (*P*>0.05). No patients experienced bleeding at all follow-up points. According to the power analysis, the unmatched power count was 0.896.

2.2 Logistic Regression and Cox Regression

To determine clinical variables that independently predict CHD, stepwise binary logistic regression was used. The results showed that in this model, CYP2C19*2 polymorphisms (OR 1.94, 95% CI 1.08–3.50, *P*=0.028) and male gender (OR 2.739, 95% CI 1.58–4.76, *P*=0.001) were statistically associated with CHD (table 3). In addition, the results of the multivariate Cox proportional hazards model showed that CYP2C19*2 loss-of-function was the only independent factor which predicted the coronary events during the follow-up period of 14 months (OR=2.70, 95% CI 1.15–6.34, *P*=0.023). There was a decreased adverse-event-free survival rate among patients carrying the CYP2C19*2 loss-of-function as compared with non-carriers (fig. 2).

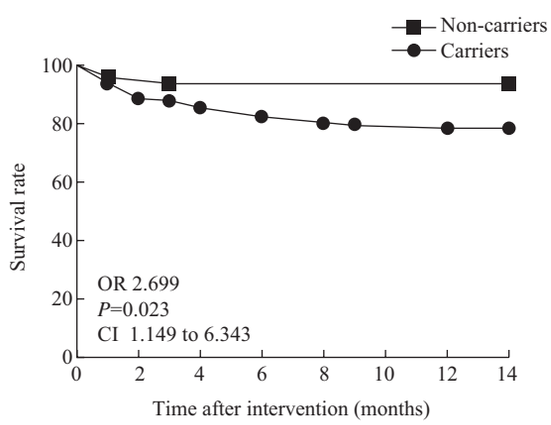

Fig. 2 Kapan-Meier curve for event-free-survival according to CYP2C19*2 loss-of-function polymorphisms carrier status among Chinese Han population with CHD.
There was a decreased adverse-event-free survival rate among patients carrying the CYP2C19*2 loss-of-function when compared with non-carriers.

3 DISCUSSION

This is the first study that not only connects a link

Table 2 Demographic data of 145 CHD patients and subgroup characteristics

Items	Total patients (*n*=145)	CYP2C19*2		*P*
		Non-carriers (*n*=48)	Carriers (*n*=97)	
Events (%)	16.6	6.3	21.6	0.019*
Age (years)	59.23±5.58	58.94±6.86	59.38±9.35	0.747
Gender (Male %)	60.7	58.3	61.9	0.720
Smoking (%)	51.7	47.9	53.6	0.597
SYNTAX Score	18.92±4.12	18.19±4.05	19.29±4.12	0.130
BMI (kg/m^2)	24.88±4.49	25.41±4.36	24.63±4.55	0.327
Heart rate (beats/min)	71.59±11.44	71.17±13.08	71.79±10.60	0.757
SBP (mmHg)	135.0 (120.5, 150.0)	140.0 (130.0, 150.0)	134.0 (120.0, 150.0)	0.369
DBP (mmHg)	80.0 (75.0, 90.0)	80.0 (75.25, 90.0)	80.0 (71.5, 90.0)	0.906
DM (%)	14.5	12.5	15.5	0.803
WBC counts (10^9/L)	6.61 (5.56,7.79)	6.91 (5.41, 7.77)	6.50 (5.56, 7.81)	0.575
RBC counts (10^{12}/L)	4.44 (4.09,4.82)	4.48 (4.05, 4.76)	4.43 (4.10, 4.82)	0.589
Neutrophil (%)	62.86±11.25	62.28±13.29	63.15±10.15	0.664
Lymphocyte (%)	27.26±9.41	27.44±9.28	27.18±9.51	0.876
Monocyte (%)	6.40 (5.10, 7.70)	6.90 (4.91, 7.88)	6.30 (5.15,7.60)	0.487
Hemoglobin (g/L)	136.12±18.21	135.77±15.95	136.29±19.31	0.872
Platelets counts (10^9/L)	212.92±52.99	207.21±48.29	215.75±55.18	0.363
ALT	16.0 (11.5, 29.0)	15.5 (11.0, 26.0)	17.0 (12.0,30.5)	0.308
AST	17.0 (14.0, 25.0)	17.0 (12.3, 21.8)	17.0 (14.0, 25.0)	0.325
BUN	5.26±1.17	5.08±1.20	5.35±1.15	0.184
Creatinine (μmol/L)	65.04±13.93	61.77±14.89	66.66±13.21	0.057
Uric acid (μmol/L)	301.29±93.21	286.20±81.73	308.75±97.94	0.277
Total bilirubin (μmol/L)	10.4 (8.25, 14.55)	10.0 (8.3, 13.73)	10.7 (8.1, 14.8)	0.688
Total cholesterol (mmol/L)	4.29±0.89	4.35±1.04	4.26±0.80	0.534
TG	1.43 (1.01, 2.03)	1.46 (0.98, 2.25)	1.38 (1.01, 1.96)	0.422
Medications				
Nitrates (%)	65.5	68.8	63.9	0.584
β-Blockers (%)	46.2	43.8	47.4	0.725
ACEI/ARB (%)	37.2	39.5	36.1	0.717
CCB (%)	31.0	29.2	32.0	0.849
Statins (%)	86.2	89.6	84.5	0.456
PPI (%)	12.4	10.4	13.4	0.790

ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blockers; BMI: body mass index; CCB: calcium channel blockers; DBP: diastolic blood pressure; PPI: proton pump inhibitors; RBC: red blood cells; SBP: systolic blood pressure; TG: triglycerides; WBC: white blood cells; DM: diabetes mellitus; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen

Table 3 Results of the binary logistic regression (*n*=231)

Variables	B	SE	*P* value	Odds ratio (95% CI)
Age >60 years old	0.518	0.292	0.077	1.68 (0.95, 3.00)
Male	1.008	0.282	0.001*	2.74 (1.58, 4.76)
Smoking (%)	0.539	0.349	0.123	1.72 (0.87, 3.40)
BMI (kg/m^2)	0.304	0.304	0.328	1.03 (0.97, 1.11)
DM	0.591	0.499	0.236	1.81 (0.68, 4.80)
HP	–0.004	0.301	0.988	1.00 (0.55, 1.80)
Hypertriglyceridemia	0.007	0.016	0.666	1.01 (1.08, 3.50)
Hypercholesterolemia	–0.197	0.175	0.261	0.82 (0.58, 1.16)
CYP2C19*2 carriers	0.663	0.301	0.023*	2.70 (1.15, 6.34)
CYP2C19*3 carriers	0.240	0.554	0.664	1.27 (0.43, 3.77)

BMI: body mass index; DM: diabetes mellitus; HP: hypertension

between CYP2C19*2 genotype and CHD, but also reports the association between CYP2C19*2 loss-of-function polymorphisms and the incidences of adverse outcomes of CHD within 14-month follow-up in Chinese Han population (among the CHD patients the PCI percentage was 71.1%).

According to previous reports, common polymorphisms in the CYP2C19 gene were approximately 30% in whites, 40% in blacks, and >55% in East Asians[21]. CYP2C19*2 polymorphisms

are responsible for 75%–85% poor metabolism (PM) phenotype in Caucasians and East Asians, which is significantly more frequent in East Asian populations. In the present study, the percentage of CYP2C19*2 in Chinese Han population was 61.0%. This study echoes the findings of previous studies that recognize the impact of ethnicity on the distribution of CYP2C19 polymorphisms[22]. CYP2C19*3 polymorphisms had been regarded as an Asian-specific variant polymorphisms that account for the PM phenotype in Asian populations, which occurs rarely in Caucasians. In the present study, the polymorphisms frequency of CYP2C19*3 in Chinese Han populations was 7.8%, and this frequency was similar to that of the previous reports (7.2%).

The role of CYP enzymes in atherosclerosis is controversial to date. It is known that CYP2C9 generates reactive oxygen species (ROS) that can cause the oxidation of low density lipoprotein (LDL), which leads to plaque formation in coronary arteries[23]. As the member of CYP2C subfamily, CYP2C19 can produce epoxyeicosatrienoic acids (EETs), i.e., the so-called endothelium-derived hyperpolarizing factors (EDHF), an important regulator of vascular tone under pathological condition. CYP2C19 gene is also expressed in endothelial beds, and it was shown that CYP2C19 mRNA was expressed in the right ventricle and aorta[6]. A link between CYP2C19*3 polymorphisms and CHD in Chinese Uighur population has been suggested by Yang *et al*[7], however, they did not investigate the CYP2C19*2 polymorphisms, the most common CYP2C19 polymorphisms in Asian[9]. In the present study, we found the percentage of CHD patients was statistically higher in CYP2C19*2 carriers than in non-carriers (68.8% *vs.* 53.3%, *P*=0.025), which is consistent with the previons finding that CYP2C19 enzyme participates in atherosclerosis, however the similar results were not found in CYP2C19*3 based groups in 231 Chinese Han population.

Clopidogrel, one of the most commonly prescribed cardiovascular medications in the word, is a prodrug that requires hepatic conversion to an active metabolite to exert its antiplatelet properties. The majority of absorbed clopidogrel (85% to 90%) is hydrolyzed by carboxylase to an inactive metabolite, whereas the remaining 10% to 15% is metabolized by hepatic CYP450 isoenzymes in a 2-step process[24]. Studies indicate that CYP2C19, CYP1A2, and CYP2B6 participate in the first metabolic step, whereas CYP2C19, CYP2C9, CYP2B6, and CYP3A are responsible for the second step[24]. Previous research strongly suggests that variable and insufficient active metabolite generation is the main explanation for clopidogrel response variability and high on-treatment platelet reactivity (HTPR)[25]. Importantly, CYP2C19 is involved in both steps of clopidogrel metabolism. Several studies have shown that CYP2C19*2 and *3 polymorphisms influence the platelet response to clopidogrel[26–31]. In the genetic sub-study of the TRITON-TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis In Myocardial Infarction 38) trial[13], carriers of these mutant polymorphisms receiving clopidogrel had a 5-fold increase in their risk of stent thrombosis and major adverse cardiovascular events compared with carriers of the wild-type genotype. In contrast to these findings, however, the recently published large-scale analysis of 2 randomized trials, CURE in ACS and ACTIVE A in atrial fibrillation, indicated that the primary outcome was similar in carriers of the CYP2C19*2 loss-of-function polymorphisms, as was seen in non-carriers[32, 33]. A reasonable explanation for this finding is the lower use of PCI with the stenting in the CURE trial (14.5%) and the relatively lower-risk population enrolled in the ACTIVEA study (non-ACS and non-PCI/stenting). Among patients treated with clopidogrel for PCI, carriage of even non-carriers increased risk of MACEs[18]. However, just because a large proportion of patients in the meta-analysis of Mega *et al*[18] underwent PCI with stenting in ACS (91.3% of whom underwent PCI and 54.5% of whom had an ACS) it remains to be determined whether the risks of CYP2C19*2 genetic testing can be generalized to non-ACS, or non-stented patients with chronic disease. So evaluating the influence of SNPs is perplexing, and in patients treated with clopidogrel, the best genome-guided strategy remains to be determined. In the present study, after follow-up for 14 months, the results of the multivariate Cox proportional hazards model showed that CYP2C19*2 loss-of-function was the only independent factor which predicted the coronary events (OR=2.70, 95% CI 1.15–6.34, *P*=0.023). However, the correlation between CYP2C19*3 polymorphisms and the adverse outcome was not found.

Several studies have provided evidence linking CYP2C19 genetic variation to reduced exposure to the active drug metabolite, lower platelet inhibition, and less protection from recurrent ischemic events in patients receiving clopidogrel[19]. In the present study, we reported the associations of CYP2C19*2 loss-of-function polymorphisms with the incidence of adverse outcomes of CHD patients (OR 1.94, 95% CI 1.08–3.50, *P*=0.028), among the CHD patients the PCI percentage was 71.1%. We found that patients with CYP2C19*2 loss-of-function polymorphisms have higher incidence of cardiovascular death, myocardial infarction and hospital readmission for ACS/stroke when compared with non-carries patients.

This study was a relatively small research, which did not assess other genetic polymorphisms that could be involved in the biological response. We did

not assess the platelet reactivity according to genetic polymorphisms. Therefore, further large-scale clinical studies are needed.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest or financial disclosures to report.

REFERENCES

1 Roman RJ. P-450 metabolites of arachidonic acid in the control of cardiovascular function. J Physiol Rev, 2002, 82(1):131-185

2 Oyekan AO, McAward K, Conetta J, *et al*. Endothelin-1 and CYP450 arachidonate metabolites interact to promote tissue injury in DOCA-salt hypertension. Am J Physiol, 1999,276(3):R766-R775

3 Wu S, Moomaw CR, Tomer KB, *et al*. Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. J Biol Chem, 1996,271(7):3460-3468

4 Lin JHC, Kobari Y, Zhu Y, *et al*. Human umbilical vein endothelial cells express P450 2C8 mRNA: cloning of endothelial P450 epoxygenase. Endothelium, 1996,4(3):219-229

5 Ingelman-Sundberg M, Sim SC, Gomez A, *et al*. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol Ther, 2007,116(3):496-526

6 Ercan B, Ayaz L, Cicek D, *et al*. Role of CYP2C9 and CYP2C19 polymorphisms in patients with atherosclerosis. Cell Biochem Funct, 2008,26(3):309-313

7 Yang YN, Wang XL, Ma YT, *et al*. Association of interaction between smoking and CYP 2C19*3 polymorphism with coronary artery disease in a Uighur population. Clin Appl Thromb Hemost, 2010,16(5):579-583

8 Fichtlscherer S, Dimmeler S, Breuer S, *et al*. Inhibition of cytochrome P450 2C9 improves endothelium-dependent, nitric oxide-mediated vasodilatation in patients with coronary artery disease. Circulation, 2004,109(2):178-183

9 Shin DJ, Kwon J, Park AR, *et al*. Association of CYP2C19*2 and *3 genetic variants with essential hypertension in Koreans. Yonsei Med J, 2012,53(6):1113-1119

10 Gasparyan AY, Watson T, Lip GY. The Role of Aspirin in Cardiovascular Prevention Implications of Aspirin Resistance. J Am Coll Cardiol, 2008,51(19):1829-1843

11 Holmes DR, Dehmer GJ, Kaul S, *et al*. ACCF/AHA Clopidogrel Clinical Alert: Approaches to the FDA "Boxed Warning": A Report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the American Heart Association Endorsed by the Society for Cardiovascular Angiography and Interventions and the Society of Thoracic Surgeons. J Am Coll Cardiol, 2010,56(4):321-341

12 Savi P, Pereillo JM, Uzabiaga MF, *et al*. Identification and biological activity of the active metabolite of clopidogrel. Thromb Haemost, 2000,84(5):891-896

13 Mega JL, Close SL, Wiviott SD, *et al*. Cytochrome p-450 polymorphisms and response to clopidogrel. N Engl J Med, 2009,360(4):354-362

14 Collet JP, Hulot JS, Pena A, *et al*. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. Lancet, 2009,373(9660):309-317

15 Shuldiner AR, O'Connell JR, Bliden KP, *et al*. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA, 2009,302(8):849-857

16 Simon T, Verstuyft C, Mary-Krause M, *et al*. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med, 2009,360(4):363-375

17 Food U, Drug Administration. FDA Drug Safety Communication: Reduced effectiveness of Plavix (clopidogrel) in patients who are poor metabolizers of the drug. Drug Safety and Availability. Food and Drug Administration (United States), 2010

18 Mega JL, Simon Y, Collet JP, *et al*. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. JAMA, 2010,304(16):1821-1830

19 Pare G, Mehta SR, Yusuf S, *et al*. Effects of CYP2C19 genotype on outcomes of clopidogrel treatment. N Engl J Med, 2010,363(18):1704-1714

20 Holmes MV, Perel P, Shah T, *et al*. CYP2C19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events. JAMA, 2011,306(24):2704-2714

21 Desta Z, Zhao X, Shin JG, *et al*. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. Clin Pharmacokinet, 2002,41(12):913-958

22 Man M, Farmen M, Dumaual C, *et al*. Genetic variation in metabolizing enzyme and transporter genes: comprehensive assessment in 3 major East Asian subpopulations with comparison to Caucasians and Africans. J Clin Pharmacol, 2010,50(8):929-940

23 Hunter AL, Cruz RP, Cheyne BM, *et al*. Cytochrome p450 enzymes and cardiovascular disease. Can J Physiol Pharmacol, 2004,82(12):1053-1060

24 Kazui M, Nishiya Y, Ishizuka T, *et al*. Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite. Drug Metab Dispos, 2010,38(1):92-99

25 Angiolillo DJ, Fernández-Ortiz A, Bernardo E, *et al*. High clopidogrel loading dose during coronary stenting: effects on drug response and interindividual variability. Eur Heart J, 2004,25(21):1903-1910

26 Brandt JT, Close SL, Iturria SJ, *et al*. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost, 2007,5(12):2429-2436

27 Umemura K, Furuta T, Kondo K. The common gene variants of CYP2C19 affect pharmacokinetics and pharmacodynamics in an active metabolite of clopidogrel in healthy subjects. J Thromb Haemostasis, 2008,6(8):1439-1441

28 Hulot JS, Bura A, Villard E, *et al*. Cytochrome P450

2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. Blood, 2006,108(7):2244-2247

29 Giusti B, Gori AM, Marcucci R, *et al*. Cytochrome P450 2C19 loss-of-function polymorphism, but not CYP3A4 IVS10+ 12G/A and P2Y12 T744C polymorphisms, is associated with response variability to dual antiplatelet treatment in high-risk vascular patients. Pharmacogenet Genomics, 2007,17(12):1057-1064

30 Trenk D, Hochholzer W, Fromm MF, *et al*. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. J Am Coll Cardiol, 2008,51(20):1925-1934

31 Frere C, Cuisset T, Morange PE, *et al*. Effect of Cytochrome P450 Polymorphisms on Platelet Reactivity After Treatment With Clopidogrel in Acute Coronary Syndrome. Am J Cardiol, 2008,101(8):1088-1093

32 Wallentin L, James S, Storey RF, *et al*. Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. Lancet, 2010,376(9749):1320-1328

33 Paré G, Mehta SR, Yusuf S, *et al*. Effects of CYP2C19 genotype on outcomes of clopidogrel treatment. New Engl J Med, 2010,363(18):1704-1714

(Received Jan. 25, 2018; revised Dec. 19, 2018)