ORIGINAL ARTICLE

Relationship between clopidogrel-related polymorphisms and variable platelet reactivity at 1 year: A cohort study from Han Chinese

Xiaodong Wang*, Yan Lai[1]*, Yu Luo, Xumin Zhang, Hua Zhou, Zi Ye[1], Jiani Tang[1], Xuebo Liu[1]

Department of Cardiology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai 200120, [1]Department of Cardiology, Shanghai Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China

*Xiaodong Wang and Yan Lai contribute to this paper equally.

Background: This study was designed to investigate the effect of clopidogrel-related gene polymorphisms on platelet reactivity and clinical outcome in Chinese Han patients. **Materials and Methods:** Three hundred and thirty-six percutaneous coronary intervention - treated patients were recruited and followed for 1 year. Blood samples were collected from all patients for DNA genotyping. The platelet reactivity unit was measured by the VerifyNow technique. The CYP2C19*2, CYP2C19*3, CYP2C19*17, ATP-binding cassette subfamily B member 1, ITGB3, CYP2C9*3, CYP2B6*9, and P2Y12 alleles were assessed. **Results:** The clinical endpoints were related to previous heart disease history (11.90% vs. 28.57%, $P = 0.017$), stroke (12.24% vs. 16.67%, $P = 0.039$), and diabetes (27.55% vs. 52.38%, $P = 0.047$). High on-treatment platelet reactivity (HTPR) was frequent in advanced age ($P = 0.019$), male gender ($P = 0.016$), hypertension ($P = 0.033$), and chronic renal failure ($P = 0.040$). There were more endpoints in the CYP2C19*2 and P2Y12 mutant carriers (76.19% vs. 43.20%, $P < 0.001$; 50.00% vs. 35.71%, $P = 0.001$, respectively), whereas fewer in the CYP2C19*17 mutant carriers (11.90% vs. 56.46%, $P = 0.001$). CYP2C19*2 and P2Y12 polymorphism manifested HTPR (194.25 ± 45.91 vs. 151.38 ± 58.14, $P < 0.001$; 180.33 ± 67.25 vs. 161.89 ± 56.49, $P = 0.008$, respectively), whereas CYP2C19*17 mutant improved platelet reactivity (97.17 ± 45.38 vs. 169.08 ± 57.15, $P = 0.003$). However, there were no further cardiovascular deaths in endpoint patients. **Conclusion:** In Han Chinese people of mainland China, clopidogrel-related gene polymorphisms are related to variable platelet reactivity after clopidogrel maintenance dosing, which influences major adverse cardiovascular events, without an effect on cardiac death.

Key words: Clopidogrel, polymorphism, VerifyNow

How to cite this article: Wang X, Lai Y, Luo Y, Zhang X, Zhou H, Ye Z, Tang J, Liu X. Relationship between clopidogrel-related polymorphisms and variable platelet reactivity at 1 year: A cohort study from Han Chinese. J Res Med Sci 2016;21:115.

INTRODUCTION

Dual antiplatelet therapy (DAPT) with aspirin and clopidogrel inhibits platelet activity and improves outcomes following acute coronary syndrome (ACS) and percutaneous coronary intervention (PCI).[1] However, there is remarkable inter-individual variability of clopidogrel response in some patients.[2] Clopidogrel is a predrug that necessitates biotransformation by hepatic cytochromes (CYPs) into its active metabolites. Data regarding *in vitro* metabolism and clinical outcomes suggest that the reduced-function CYP polymorphisms affect the conversion of clopidogrel to active metabolite and the variability of platelet activity.[3] These polymorphisms involve CYPs, ATP-binding cassette subfamily B member 1 (ABCB1), and purinergic receptor P2Y12.[4]

Metabolic activation by CYP2C19 has emerged as a crucial determinant of clopidogrel's pharmacodynamic response and clinical efficacy.[5] Carriers of this loss-of-function (LOF) polymorphism have reduced active metabolite generation and therefore a suboptimal platelet reaction index, which in turn results in a higher rate of post-PCI thrombotic events, such as stent thrombosis (ST).[6] Although several studies have

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/1735-1995.193502

Address for correspondence: Dr. Xuebo Liu, Department of Cardiology, Shanghai Tongji Hospital, Tongji University School of Medicine, No.389, Xincun Rd, Putuo Dist, Shanghai 200065, China. E-mail: lxb70@hotmail.com

Received: 29-01-2016; **Revised:** 04-04-2016; **Accepted:** 28-08-2016

shown that the CYP LOF polymorphism may be associated with poor outcomes,[7,8] the effect of the LOF allele and other mutations simultaneously on outcomes in a cohort of patients is unknown. In addition, the frequency of the CYP2C19*2 mutation is known to vary considerably according to ethnicity. Its frequency has been reported to be higher (30%) in Asians compared with Caucasians (15%).[4,9] It is unknown whether a similar association exists in Asians, especially in Han Chinese.

P2Y12 is the platelet receptor for adenosine diphosphate (ADP) targeted by the active form of clopidogrel. Primary studies led to the discovery of several P2Y12 polymorphisms (including intronic T744C polymorphism) forming two distinct haplotypes (H1 and H2).[10] However, subsequent studies using the same method could not find a correlation between P2Y12 haplotypes and high on-treatment platelet reactivity (HTPR).[11] Nevertheless, a mild impact on platelet function in patients homozygous for the H2 haplotype cannot be excluded.[12]

In the present study, we evaluated whether clopidogrel-related polymorphisms were associated with platelet activity and clinical outcomes.

MATERIALS AND METHODS

Patient population and study design

Between August 2012 and May 2013, 336 patients were enrolled in the prospective mono-center study. The study protocol is shown in Figure 1. It was conducted in accordance with the Declaration of Helsinki and approved by the Local Ethics Committee. We described probable side effects of the study to participants, and they were able to refuse participation in the study at any stage of the study for any reason. All participants had been informed about this right. They knew if they refused participation in the study, their therapeutic procedures would not be stopped or disturbed. We provided participants with information about human gene studies and the confidentiality that would be given to them, and their future and previous generations, before obtaining informed consent. All patients gave written informed consent before inclusion. Patients >18 years of age were enrolled in the study 1 month after undergoing PCI for one of three indications: Refractory angina pectoris in addition to optimal medical therapy, ischemia on thallium scintigraphy, or non-ST-elevation-ACS (NSTE-ACS). The exclusion criteria were a history of bleeding diathesis, persistent ST-segment elevation myocardial infarction, New York Heart Association functional Class III or IV, contraindications to antiplatelet therapy, platelet count <100 × 10^9/L, creatinine clearance <25 mL/min, use of a glycoprotein IIb/IIIa inhibitor and bivalirudin, sudden death, and concurrent severe illness with expected survival <1 year. In order to keep patients' and their relatives' data confidential, we locked the database after completion of statistical analysis.

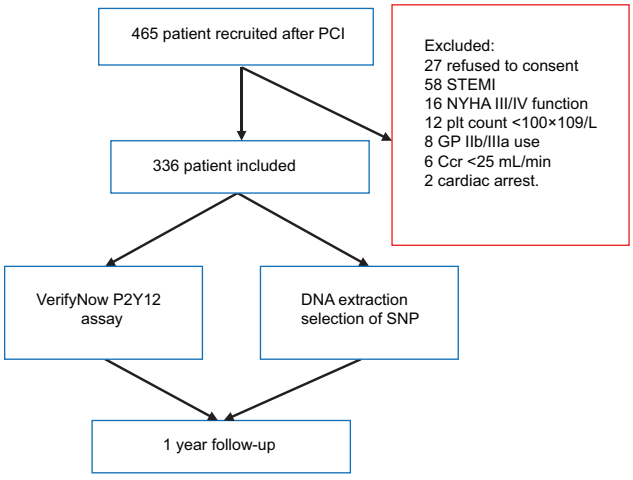

Figure 1: Study flow chart and follow-up

Coronary angiography and antithrombotic regimens

All patients received aspirin (300 mg) and clopidogrel (600 mg) at least 12 h before the start of the coronary procedure. A bolus of unfractionated heparin (100 U/kg) was administered immediately before PCI. The PCI was performed via a transradial or transfemoral approach using conventional methods with balloon predilation followed by drug-eluting stent (DES) deployment. Aspirin (100 mg) and clopidogrel (75 mg) were given for ≥12 months after PCI. Glycoprotein IIb/IIIa inhibitor use was excluded.

Blood sampling and VerifyNow P2Y12 assay

Blood samples were obtained 1 month after PCI. Each sample was placed in a tube containing 3.2% citrate, and the inhibitory effect of clopidogrel on platelet aggregation was measured with the VerifyNow P2Y12 test (ACCRIVA Diagnostics, Inc., California, USA). VerifyNow P2Y12 is a whole-blood, point-of-care turbidometric assay that measures the responsiveness to P2Y12 antagonists.[10,13-15] The cartridge consists of two channels: One channel contains fibrinogen-coated polystyrene beads, 20 μM ADP and 22 μM prostaglandin E1; the optical signal of this channel is reported as P2Y12 reaction units (platelet reactivity unit [PRU]; range, 0–550). The second channel contains fibrinogen-coated polystyrene beads, 3.4 μM iso-thrombin receptor-activating peptide. This channel was incorporated to estimate the maximal platelet function independent of P2Y12 receptor blockade.

DNA extraction, selection of single nucleotide polymorphism, and genotyping

One month after DES deployment, 4 mL of whole peripheral blood was obtained from patients. The genomic DNA was extracted from mononuclear cells with the DNA QIAamp

Midi kit (Qiagen Inc., California, USA), and the DNA was stored at − 20°C until used. The quantity and quality of the genomic DNA were verified with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) before assay with the TaqMan Open Array Genotyping System (Applied Biosystems, California, USA). The clopidogrel-metabolizing pathway single nucleotide polymorphisms (SNPs) used in the customized genotyping panel were: ABCB1 (rs1045642), CYP2B6*9 (rs3745274), CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893), CYP2C19*17 (rs12248560), and P2Y12 (rs2046934).[16] Sample processing was fully automated using the Freedom EVO150 Robotic Workstation (Tecan Group Ltd., Männedorf, Switzerland). Fluorescent signals were recorded by the OpenArray (Thermo Fisher Scientific, Inc., Waltham, USA) instrument, and specific cluster parameters were automatically obtained to precisely assign the relative genotypes. All samples were genotyped in duplicate to verify the results and avoid technical errors.

Outcomes and follow-up

The primary endpoint was major adverse cardiovascular and cerebral events (MACCE). MACCE was defined as the composite of cardiovascular death, nonfatal myocardial infarction, ST, repeat revascularization (including target lesion revascularizations [TLR] and target vessel revascularizations [TVR]), cerebral ischemia, transient ischemic attack (TIA), and acute heart failure (AHF). All deaths were considered to be cardiovascular deaths unless a clear noncardiovascular cause was identified. Myocardial infarction (MI) was defined as a recent ischemic symptom with electrocardiographic abnormalities in the ST-segment (depression or elevation of at least 0.1 mV) and a positive troponin concentration as defined locally. Repeat revascularization was defined as any repeat PCI or surgical bypass of any vessel. ST was defined as definite or probable according to the Academic Research Consortium criteria.[17]

Clinical safety outcomes were major and minor bleeding. Major bleeding was defined as intracranial bleeding or clinically overt bleeding associated with a decrease in hemoglobin of 50 g/L, or requiring blood transfusion according to the thrombolysis in MI (TIMI) criteria.[18] Minor bleeding was also defined according to TIMI criteria.

The cut-offs of HTPR were base d on previous definitions. HTPR was defined as ≥230 PRU. This cut-off was chosen because it was similar to those used in the receiver-operating characteristic curve analysis to identify HTPR in Chinese patients.

Statistics

The choice of sample size was based on the assumptions of: (1) an annual MACCE rate <10% and (2) HTPR occurrence in 30% of patients. Continuous variables were expressed as mean ± standard deviation, Categorical variables were expressed as frequency and percentage. Comparisons between groups were performed by Student's *t*-test, Chi-square test, or Fisher exact test. Hardy–Weinberg equilibrium was determined using the Chi-square goodness-of-fit test. Initially, to examine the effects of the selected SNP and of PRU ≥230, two-way analysis of variance was employed. As for the relationship between PRU and allele polymorphism, univariate and multivariate logistic regressions were taken, respectively. Kaplan–Meier curves were used to determine MACCE-free survival. Univariate and multivariate logistic regression was used to provide the relationships between PRU level and allele polymorphism with odds ratio (OR) and 95% confidence intervals (CIs). For all tests, $P < 0.05$ was considered statistically significant. Statistics was performed using SPSS version 17.0 software (SPSS Inc., Chicago, USA).

RESULTS

Patients' demographic, biological, and angiographic characteristics

A complete clinical follow-up was available for all 336 (100%) patients. MACCE at follow-up occurred in 31 (9.23%) patients. Specifically, cardiac death occurred in 1 (0.30%) patient; unstable angina pectoris was observed in 19 (5.65%) patients; nonfatal myocardial infarction in 1 (0.30%) patient; TLR in 3 (0.89%) patients; cerebral ischemia in 5 (1.49%) patients; TIA in 1 (0.30%) patient; and AHF occurred in 1 (0.30%) patient. Major TIMI bleeding was not observed, whereas minor TIMI bleeding was observed in 24 (7.14%) patients. Clinical, angiographic, and procedural features of the patients in the endpoints group and endpoints-free group are summarized in Table 1. The endpoints group had higher percentages of heart disease history (OR 4.05, 95% CI 1.28–12.77, $P = 0.017$), DM (OR 2.84, 95% CI 1.01–7.99, $P = 0.047$), and previous stoke (OR 3.64, 95% CI 1.07–12.37, $P = 0.039$) than did the endpoints-free group. The majority of patients in two groups (98.26% and 95.30%, respectively), received second-generation DES. The median duration of DAPT was 12.3 ± 2.8 and 12.5 ± 1.6 months, respectively.

Relationship between platelet reaction and clinical characteristics

Baseline characteristics according to HTPR are summarized in Table 2. Patients with HTPR were older (68.3 ± 9.1 vs. 64.0 ± 9.4, $P = 0.019$) and more likely to be Men (70.07% vs. 50.24%, $P = 0.016$). The rate of hypertension was higher in patients with HTPR (82.48% vs. 61.77%, $P = 0.033$). Heart disease history and chronic renal failure (CRF) were associated with HTPR (26.77% vs. 11.00%, $P = 0.037$ and 43.84% vs. 23.40%, $P = 0.040$, respectively).

Table 1: Clinical features at baseline of the global and major adverse cardiovascular and cerebral events-stratified populations

General characteristics	Endpoint-free group (*n*=294)	Endpoint group (*n*=42)	*P*
Age (years)	65.3±7.6	67.2±4.4	0.563
BMI	23.8±3.7	25.4±4.6	0.832
Male, *n* (%)	178 (60.54)	25 (59.52)	0.765
Current smokers, *n* (%)	132 (44.90)	16 (38.10)	0.684
Blood lipid			
Cholesterol	4.57±1.38	5.23±1.32	0.464
High-density lipoprotein	0.97±0.38	0.85±0.64	0.583
Low-density lipoprotein	3.16±0.24	3.55±0.70	0.387
Triglycerides	2.19±0.46	2.85±0.73	0.465
Medicine, *n* (%)			
Aspirin	290 (98.64)	40 (95.24)	0.967
Clopidogrel	294 (100)	42 (100)	1.000
Cilostazol	0	0	1.000
ACE-I	275 (93.54)	40 (95.24)	0.529
ARB	186 (63.27)	29 (69.05)	0.362
β-blocker	274 (93.20)	39 (92.86)	0.447
Statins	290 (98.64)	41 (97.62)	0.963
Proton-pump inhibitor	39 (13.27)	5 (11.90)	0.250
Calcium-channel blocker	49 (16.67)	7 (16.67)	1.000
Comorbidities, *n* (%)			
Heart disease history	35 (11.90)	12 (28.57)	0.017
LVEF <40%	42 (14.23)	8 (20.75)	0.184
CKD	80 (27.21)	6 (14.29)	0.082
Stroke	36 (12.24)	7 (16.67)	0.039
Diabetes	81 (27.55)	22 (52.38)	0.047
Hypertension	192 (65.31)	28 (66.67)	0.828
Stents			
Second-generation DES, *n* (%)	289 (98.26)	40 (95.30)	1.000
Stent length, mm	27.4±3.3	31.2±5.8	0.102
Stents per patient	2.2±0.82	2.5±0.74	0.266
PRU before PCI	170.3±65.6	162.9±53.7	0.382
DAPT (m)	12.3±2.8	12.5±1.6	0.405

ACE-I = Angiotensin-converting enzyme inhibitor; ARB = Angiotensin receptor blocker; BMI = Body mass index; CKD = Chronic kidney disease; DAPT = Dual anti-platelet therapy; DES = Drug-eluting stent; LVEF = Left ventricular ejection fraction; PCI = Percutaneous coronary intervention; PRU = Platelet reaction unit

Table 2: Baseline clinical characteristics of study patients on the basis of high on-treatment platelet reactivity

Clinical characteristics	PRU		*P*
	<230 (*n*=209)	≥230 (*n*=127)	
Age (year)	64.0±9.4	68.3±9.1	0.019
BMI	26.3±2.8	24.7±6.3	0.365
Male, *n* (%)	105 (50.24)	89 (70.07)	0.016
Hypertension, *n* (%)	129 (61.77)	104 (82.48)	0.033
Diabetes, *n* (%)	63 (30.14)	42 (33.07)	0.702
Dyslipidemia, *n* (%)	37 (17.70)	22 (17.32)	0.998
Heart disease history, *n* (%)	23 (11.00)	34 (26.77)	0.037
Stroke history, *n* (%)	23 (11.00)	15 (11.81)	0.836
CRF, *n* (%)	49 (23.40)	55 (43.84)	0.040

BMI = Body mass index; CRF = Chronic renal failure; PRU = Platelet reaction unit

Response and metabolizing status as major adverse cardiovascular and cerebral events predictors within the whole population

As assessed by the VerifyNow P2Y12 test, 127 of 336 patients (37.80%) had a PRU ≥230. HTPR occurred more in the endpoints group than in endpoints-free group (40.48% vs. 31.29%, $P = 0.030$). Genotyping was successful in all patients. CYP2C19*2 represented 43.20% of the endpoints-free group and 76.19% of the endpoints group, respectively ($P < 0.001$). CYP2C19*17 mutant alleles demonstrated a significant reverse association with clinical endpoints (11.90% vs. 56.46%, $P = 0.001$). There were more endpoints in P2Y12 mutant type than in wild-type (50.00% vs. 35.71%, $P = 0.001$). No significant differences were found between the endpoints group and the endpoints-free group when other analyzed SNPs were considered [Table 3].

Association of clinical characteristics with CYP2C19*2, CYP2C19*17, and P2Y12 polymorphisms

Because only CYP2C19*2, CYP2C19*17, and P2Y12 polymorphisms were related to clinical endpoints, we made the comparison between carriers and noncarriers of CYP2C19*2, CYP2C19*17, and P2Y12, respectively. The differences of clinical characteristics were not significant among these three polymorphisms [Table 4].

Distribution of major adverse cardiovascular and cerebral events and thrombolysis in myocardial infarction bleeding according to CYP2C19*2, CYP2C19*17, and P2Y12 polymorphisms

Based on the findings that only CYP2C19*2, CYP2C19*17, and P2Y12 polymorphisms were associated with clinical outcomes, we analyzed the MACCE and TIMI bleeding according to carriers and noncarriers of these polymorphisms. We found that CYP2C19*2 carriers, CYP2C19*17 noncarriers, and P2Y12 carriers had increased amounts of unstable angina pectoris and cerebral ischemia. However, TIMI bleeding was not different among the three allele polymorphisms, whether carriers or noncarriers [Table 5].

Relationship between high on-treatment platelet reactivity and polymorphisms

The results showed that a significantly higher PRU level had been detected in CYP2C19*2 carriers compared to noncarriers (194.25 ± 45.91 vs. 151.38 ± 58.14, $P < 0.001$). In contrast, CYP2C19*17 mutant carriers manifested lower PRU (97.17 ± 45.38 vs. 169.08 ± 57.15, $P = 0.003$). Whether in univariate or multivariate logistic regression, CYP2C19*2 carriers, P2Y12 carriers, and CYP2C19*17 noncarriers had significant relationships with high PRU, whereas there were no obvious relationships between CYP2C19*2 no-carriers, P2Y12 noncarriers, and CYP2C19*17 carriers [Table 6].

Distribution of cardiovascular death in CYP2C19*2, CYP2C19*17, and P2Y12 polymorphisms

During 1-year follow-up, only one cardiovascular death occurred [Table 5]. Neither CYP2C19*2, CYP2C19*17, nor P2Y12 polymorphisms affected cardiovascular death (data not shown).

Table 3: Summary of distributions of patients according to high on-treatment platelet reactivity and single nucleotide polymorphism, *n* (%)

Test	Comparison	Endpoint-free group (*n*=294)	Endpoint group (*n*=42)	*P*
HTPR	PRU □230	92 (31.29)	17 (40.48)	0.030
CYP2C19*2	rs4244285	127 (43.20)	32 (76.19)	<0.001
CYP2C19*3	rs4986893	75 (25.51)	12 (28.57)	0.565
CYP2C19*17	rs12248560	166 (56.46)	5 (11.90)	0.001
ABCB1	rs1045642	125 (42.52)	16 (38.10)	0.229
ITGB3	rs5918	213 (72.45)	26 (61.90)	0.532
P2Y12	rs2046934	105 (35.71)	21 (50.00)	0.001
CYP2C9*3	rs1799853	126 (42.86)	19 (45.24)	0.488
CYP2B6*9	rs3745274	57 (19.39)	10 (23.81)	0.058

ABCB1 = ATP-binding cassette subfamily B member 1; CYP = Cytochrome P450; ITGB3 = Integrin subunit beta 3; P2Y12 = Purinergic receptor P2Y12; PRU = Platelet reaction unit; HTPR = High on-treatment platelet reactivity

DISCUSSION

Three major findings of clopidogrel-related gene polymorphisms with platelet reactivity and clinical outcome in Chinese Han patients were drawn from the present study. First, advanced age, male gender, hypertension, and previous heart/renal disease are predictors of HTPR. Second, CYP2C19*2, CYP2C19*17, and P2Y12 gene polymorphisms are closely related to HTPR. Third, CYP2C19*2 and P2Y12 mutations result in adverse clinical outcomes, whereas CYP2C19*17 mutation improves outcomes, after 1-year follow-up.

It is well known that responses to clopidogrel are highly variable. Tidjane *et al*.[19] reported that age was a strong and independent predictor of HTPR. In that study, elderly patients had a higher rate of platelet reactivity at baseline than their younger counterparts. In the present study, elderly patients manifested higher PRU than younger patients, which was consistent with Tidjane's study. The other indicators of HTPR are renal disease and CRF. In our study, HTPR was related to CRF. Morel *et al*.[20] found that

Table 4: Clinical characteristics according to CYP2C19*2, CYP2C19*17, and P2Y12 polymorphisms

Clinical characteristics	CYP2C19*2			CYP2C19*17			P2Y12		
	Carrier	Noncarrier	*P*	Carrier	Noncarrier	*P*	Carrier	Noncarrier	*P*
	159 (47.4)	177 (52.6)		182 (54.2)	154 (45.8)		126 (37.7)	210 (62.3)	
Age (year)	66.4±5.3	65.3±2.8	0.327	65.9±4.4	67.2±5.8	0.159	66.8±3.5	67.4±4.2	0.657
BMI	24.7±4.1	23.9±2.8	0.445	25.2±3.7	24.8±5.3	0.409	24.9±1.8	25.3±3.0	0.398
Male, *n* (%)	95 (60.15)	103 (58.38)	0.572	111 (61.29)	93 (60.37)	0.828	73 (58.44)	129 (61.72)	0.552
Hypertension, *n* (%)	105 (66.27)	113 (63.85)	0.659	119 (65.77)	104 (67.87)	0.702	81 (64.58)	134 (63.74)	0.912
Diabetes, *n* (%)	50 (31.68)	57 (32.63)	0.581	62 (34.36)	52 (33.88)	0.656	41 (32.87)	72 (34.34)	0.663
Dyslipidemia, *n* (%)	29 (18.32)	31 (17.69)	0.535	30 (16.38)	27 (17.61)	0.865	24 (19.12)	35 (17.03)	0.437
Heart disease history, *n* (%)	18 (11.21)	24 (13.88)	0.456	23 (12.83)	16 (10.67)	0.422	15 (11.98)	28 (13.31)	0.625
Stroke history, *n* (%)	20 (12.79)	21 (11.96)	0.785	18 (10.3)	19 (12.67)	0.325	17 (13.40)	26 (12.49)	0.746
CRF, *n* (%)	42 (26.83)	49 (27.69)	0.850	51 (28.35)	42 (27.33)	0.604	32 (25.58)	56 (26.67)	0.835

BMI = Body mass index; CRF = Chronic renal failure

Table 5: Distribution of MACCE and TIMI bleeding during 1-year follow-up according to allele polymorphism, *n* (%)

	CYP2C19*2			CYP2C19*17			P2Y12		
	Carrier	Noncarrier	*P*	Carrier	Noncarrier	*P*	Carrier	Noncarrier	*P*
	159 (47.4)	177 (52.6)		182 (54.2)	154 (45.8)		126 (37.7)	210 (62.3)	
MACCE									
Cardiac death	1 (0.63)	0	0.26	0	1 (0.65)	0.55	1 (0.79)	0	0.18
Unstable angina pectoris	11 (6.92)	8 (4.52)	0.02	8 (4.40)	11 (7.14)	0.04	11 (8.73)	8 (3.81)	0.02
Nonfatal myocardial infarction	1 (0.63)	0	0.26	0	1 (0.65)	0.55	1 (0.79)	0	0.18
Target lesion revascularization	1 (0.63)	2 (1.13)	0.93	2 (1.10)	1 (0.65)	0.68	1 (0.79)	2 (0.95)	0.08
Cerebral ischemia	4 (2.52)	1 (0.56)	<0.01	1 (0.55)	4 (2.60)	0.01	3 (2.38)	2 (0.95)	0.02
Transient ischemic attack	1 (0.63)	0	0.26	0	1 (0.65)	0.55	1 (0.79)	0	0.18
Acute heart failure	1 (0.63)	0	0.26	1 (0.55)	0	0.49	1 (0.79)	0	0.18
Total	20 (12.58)	11 (6.21)	0.01	12 (6.60)	19 (12.34)	<0.01	19 (15.08)	12 (5.71)	<0.01
TIMI bleeding									
Major TIMI bleeding	0	0	-	0	0	-	0	0	-
Minor TIMI bleeding	11 (6.92)	13 (7.34)	0.92	14 (7.69)	10 (6.49)	0.36	10 (7.93)	14 (6.67)	0.41

CYP = Cytochrome P450; MACCE = Major adverse cardiovascular and cerebral events; P2Y12 = Purinergic receptor P2Y12; TIMI = Thrombolysis in myocardial infarction

Table 6: Univariate and multivariate logistic regression of the relationships between high platelet reaction unit level and allele polymorphism

Allele	PRU	Univariate			Multivariate		
		OR	95% CI	*P*	OR	95% CI	*P*
CYP2C19*2							
Carriers	194.25±45.91	2.26	1.28-3.37	<0.001	1.75	1.03-2.94	0.007
No-carriers	151.38±58.14	1.47	0.83-1.99	0.402	1.26	0.64-1.72	0.576
CYP2C19*17							
Carriers	97.17±45.38	1.97	1.16-3.45	0.003	1.58	1.26-3.28	0.004
No-carriers	169.08±57.15	0.73	0.38-1.66	0.865	1.10	0.84-1.59	0.664
P2Y12							
Carriers	180.33±67.25	2.95	1.87-3.01	0.008	2.48	1.64-3.53	0.002
No-carriers	161.89±56.49	0.92	0.74-2.62	0.468	1.21	0.55-2.65	0.397

CYP = Cytochrome P450; OR = Odds ratio; PRU = Platelet reaction unit; P2Y12 = Purinergic receptor P2Y12; CI = Confidence interval

the presence of low platelet response to clopidogrel was associated with increased mortality from all causes, ST, and MACE after PCI in patients with CRF. The mechanisms by which CRF affects platelet reactivity are multifactorial. A reduced bioavailability of nitric oxide secondary to increased oxidative stress has been proposed to play a major role in CRF-associated endothelial dysfunction, possibly leading to enhanced vasoconstriction and platelet adhesion/activation.[21]

The present study strengthens the theory that CYP2C19*2 mutation can be used to predict MACCE occurrence after stenting in stable coronary artery disease (CAD) patients.[5,8,9,22] CYP2C19*2 polymorphism is known to be associated with decreased clopidogrel active metabolites and thus reduced response to clopidogrel. Similar to Collet *et al*.'s study,[23] the present study supports the notion that the CYP2C19*2 allele increases the risk of MACCE (31/336, 9.23%). In that study, the primary endpoint in CYP2C19*2 carriers was 10.03%. The difference of MACCE between CYP2C19*2 carriers and noncarriers was explained by the fact that CYP2C19 is a highly polymorphic gene that is critical for two essential oxidative steps of clopidogrel bioactivation.[24] The clinical consequences of decreased clopidogrel activation due to deleterious effects of CYP2C19 variants have been extensively described.[4,6,7]

In contrast, the CYP2C19*17 mutation was associated with an increased activation of clopidogrel. A previous study indicated that the CYP2C19*17 allele was shown to be associated with increased function of the CYP2C19 enzyme, which plays a pivotal role in the activation process of clopidogrel.[25] In that study, whether in heterozygous (*wt/*17) or homozygous (*17/*17) allele carriers, lower platelet aggregation values were found compared with wild-type homozygotes (*wt/*wt). CYP2C19*17 allele carriage was significantly associated with an increased risk of bleeding. In the present study, the mutant CYP2C19*17 gene resulted in lower PRU values and fewer clinical endpoints, but not increased bleeding. The reason for this discrepancy was probably due to inter-ethnic difference. Nevertheless, both the increase in bleeding risk and the decrease in clinical endpoints highlighted the important role of CYP2C19*17 polymorphism in clopidogrel metabolism. Indeed, the CYP2C19*17 allele increases conversion of clopidogrel to an active compound in mutant carriers, leading to a higher degree of platelet inhibition assessed by aggregometry. Carriers of the CYP2C19*17 allele would have not only a higher overall percentage of clopidogrel activation but also a genetically determined faster activation of clopidogrel, and would therefore benefit more because of decreased thrombus burden before mechanical reperfusion.

The ADP receptor P2Y12 also plays a pivotal role in platelet aggregation. The importance of P2Y12 is emphasized by the fact that it is the target of the thienopyridine drug clopidogrel. In 2003, Fontana *et al*.[10] found that ADP-induced platelet aggregation was associated with P2Y12 gene variation in healthy subjects. The mutant genes, determined as haplotypes H1 and H2 phenotypes, were responsible for inter-individual variation. In addition, Rudez *et al*.[26] demonstrated that common variation in the P2Y12 gene was a significant determinant of the wide inter-individual variability in platelet reactivity. In that study, six common haplotypes were inferred from haplotype-tagging SNP (denoted A to F), and haplotype F was associated with significantly lower residual platelet reactivity compared with the haplotype A. Lee *et al*.[27] found that the P2Y12 haplotype had a significant association with ADP-induced platelet aggregation in a Korean population. In contrast, Angiolillo *et al*.[28] demonstrated a lack of association between P2Y12 polymorphism and platelet response in CAD patients. In another study, when a 600-mg loading dose of clopidogrel was prescribed in NSTE-ACS, there was no influence of the T744C polymorphism in the P2Y12 gene on clopidogrel response.[29] In the present study, the prevalence of P2Y12 mutation was associated with HTPR and adverse clinical outcomes. Three reasons may account for this discrepancy. First, the definition

of P2Y12 polymorphism varies according to different criteria (haplotypes H1/H2, denoted A to F, CC, CT, or TT). Second, the P2Y12 polymorphism was detected alone or coexisting with other alleles. In the latter study, the P2Y12 and CYP2C19 polymorphisms were detected at the same time. Third, some aforementioned studies were conducted in healthy volunteers, whereas others included ACS or CAD patients after PCI.

Studies using the VerifyNow P2Y12 assay have suggested that optimal cut-off of PRU for low responsiveness to clopidogrel was between 230 and 240.[30] Among many platelet function tests, the VerifyNow P2Y12 assay has proved to be particularly useful. Not only is it an effective tool for identifying high platelet reactivity in patients, but also knowing this information helps to predict which patients may experience an ischemic event. Consistent with these studies, we also found a very similar optimal cut-off value to predict ischemic events in Chinese Han patients undergoing elective PCI with a PRU value of ≥230.

CONCLUSION

An assessment of the CYP2C19*2, CYP2C19*17, and P2Y12 polymorphisms can be used to predict HTPR and adverse clinical endpoints in stable CAD patients receiving DES implantation, whereas assessment of HTPR with the VerifyNow P2Y12 test has a predictive value in older, male, and hypertensive CAD patients who have a history of heart disease and CRF.

Acknowledgments

We thank all the patients for their participation in the present study.

Financial support and sponsorship

This study was supported by a grant from the Shanghai Health Bureau (20124282) and Shanghai Pudong New Area Health and Family Planning Commission (PW2014A-16).

Conflicts of interest

There are no conflicts of interest.

AUTHORS' CONTRIBUTION

- XW contributed to the conception of the work, conducting the study, writing the draft, approving the final version of the manuscript, and agreement of all aspects of the work
- YL contributed to DNA extraction, selection of SNP, and genotyping
- YL contributed to blood sampling and VerifyNow P2Y12 assaying
- XZ contributed to statistical analysis
- HZ contributed to patient recruitment and follow-up
- ZY and JT contributed to patient registration and follow-up
- XL contributed to the conception and design of the work and program supervision.

REFERENCES

1. Antman EM, Hand M, Armstrong PW, Bates ER, Green LA, Halasyamani LK, *et al.* 2007 Focused Update of the ACC/AHA 2004 Guidelines for the Management of Patients with ST-Elevation Myocardial Infarction: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines: Developed in collaboration with the Canadian Cardiovascular Society Endorsed by the American Academy of Family Physicians: 2007 Writing Group to Review New Evidence and Update the ACC/AHA 2004 guidelines for the management of patients with ST-elevation myocardial infarction, writing on behalf of the 2004 writing committee. Circulation 2008;117:296-329.
2. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: Response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003;107:2908-13.
3. Bonello L, Tantry US, Marcucci R, Blindt R, Angiolillo DJ, Becker R, *et al.* Consensus and future directions on the definition of high on-treatment platelet reactivity to adenosine diphosphate. J Am Coll Cardiol 2010;56:919-33.
4. Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Méneveau N, *et al.* Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med 2009;360:363-75.
5. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, *et al.* Variability in individual responsiveness to clopidogrel: Clinical implications, management, and future perspectives. J Am Coll Cardiol 2007;49:1505-16.
6. Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, *et al.* Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. JAMA 2009;302:849-57.
7. Giusti B, Gori AM, Marcucci R, Saracini C, Sestini I, Paniccia R, *et al.* Relation of cytochrome P450 2C19 loss-of-function polymorphism to occurrence of drug-eluting coronary stent thrombosis. Am J Cardiol 2009;103:806-11.
8. Sibbing D, Stegherr J, Latz W, Koch W, Mehilli J, Dörrler K, *et al.* Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. Eur Heart J 2009;30:916-22.
9. Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. Clin Pharmacokinet 2002;41:913-58.
10. Fontana P, Dupont A, Gandrille S, Bachelot-Loza C, Reny JL, Aiach M, *et al.* Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. Circulation 2003;108:989-95.
11. von Beckerath N, von Beckerath O, Koch W, Eichinger M, Schömig A, Kastrati A. P2Y12 gene H2 haplotype is not associated with increased adenosine diphosphate-induced platelet aggregation after initiation of clopidogrel therapy with a high loading dose. Blood Coagul Fibrinolysis 2005;16:199-204.
12. Bura A, Bachelot-Loza C, Ali FD, Aiach M, Gaussem P. Role of the P2Y12 gene polymorphism in platelet responsiveness to clopidogrel in healthy subjects. J Thromb Haemost 2006;4:2096-7.
13. Malinin A, Pokov A, Spergling M, Defranco A, Schwartz K,

Schwartz D, *et al.* Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12(R) rapid analyzer: The VERIfy Thrombosis risk ASsessment (VERITAS) study. Thromb Res 2007;119:277-84.
14. von Beckerath N, Pogatsa-Murray G, Wieczorek A, Sibbing D, Schömig A, Kastrati A. Correlation of a new point-of-care test with conventional optical aggregometry for the assessment of clopidogrel responsiveness. Thromb Haemost 2006;95:910-1.
15. Jakubowski JA, Payne CD, Li YG, Brandt JT, Small DS, Farid NA, *et al.* The use of the VerifyNow P2Y12 point-of-care device to monitor platelet function across a range of P2Y12 inhibition levels following prasugrel and clopidogrel administration. Thromb Haemost 2008;99:409-15.
16. Sim SC, Ingelman-Sundberg M. The human Cytochrome P450 (CYP) Allele Nomenclature website: A peer-reviewed database of CYP variants and their associated effects. Hum Genomics 2010;4:278-81.
17. Kip KE, Hollabaugh K, Marroquin OC, Williams DO. The problem with composite end points in cardiovascular studies: The story of major adverse cardiac events and percutaneous coronary intervention. J Am Coll Cardiol 2008;51(7):701-7.
18. Braunwald E. Thrombolytic reperfusion of acute myocardial infarction: Resolved and unresolved issues. J Am Coll Cardiol 1988;12(6 Suppl A):85A-92A.
19. Tidjane MA, Voisin S, Lhermusier T, Bongard V, Sié P, Carrié D. More on: Adenosine diphosphate-inducible platelet reactivity shows a pronounced age dependency in the initial phase of antiplatelet therapy with clopidogrel. J Thromb Haemost 2011;9:614-6.
20. Morel O, El Ghannudi S, Jesel L, Radulescu B, Meyer N, Wiesel ML, *et al.* Cardiovascular mortality in chronic kidney disease patients undergoing percutaneous coronary intervention is mainly related to impaired P2Y12 inhibition by clopidogrel. J Am Coll Cardiol 2011;57:399-408.
21. Schiffrin EL, Lipman ML, Mann JF. Chronic kidney disease: Effects on the cardiovascular system. Circulation 2007;116:85-97.
22. Scott SA, Sangkuhl K, Gardner EE, Stein CM, Hulot JS, Johnson JA, *et al.* Clinical pharmacogenetics implementation consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther 2011;90:328-32.
23. Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, *et al.* Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: A cohort study. Lancet 2009;373:309-17.
24. Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, *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;38:92-9.
25. Sibbing D, Koch W, Gebhard D, Schuster T, Braun S, Stegherr J, *et al.* Cytochrome 2C19 * 17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. Circulation 2010;121:512-8.
26. Rudez G, Bouman HJ, van Werkum JW, Leebeek FW, Kruit A, Ruven HJ, *et al.* Common variation in the platelet receptor P2RY12 gene is associated with residual on-clopidogrel platelet reactivity in patients undergoing elective percutaneous coronary interventions. Circ Cardiovasc Genet 2009;2:515-21.
27. Lee SJ, Jung IS, Jung EJ, Choi JY, Yeo CW, Cho DY, *et al.* Identification of P2Y12 single-nucleotide polymorphisms and their influences on the variation in ADP-induced platelet aggregation. Thromb Res 2011;127:220-7.
28. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramírez C, Cavallari U, Trabetti E, *et al.* Lack of association between the P2Y12 receptor gene polymorphism and platelet response to clopidogrel in patients with coronary artery disease. Thromb Res 2005;116:491-7.
29. Cuisset T, Frere C, Quilici J, Morange PE, Saut N, Lambert M, *et al.* Role of the T744C polymorphism of the P2Y12 gene on platelet response to a 600-mg loading dose of clopidogrel in 597 patients with non-ST-segment elevation acute coronary syndrome. Thromb Res 2007;120:893-9.
30. Price MJ, Endemann S, Gollapudi RR, Valencia R, Stinis CT, Levisay JP, *et al.* Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. Eur Heart J 2008;29:992-1000.