

## No association of platelet aggregation function after administration of clopidogrel with *CYP2C19* gene polymorphisms in coronary heart disease patients.

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### Abstract

**Objective:** The present study aimed to investigate the relation between *CYP2C19* gene polymorphism and ADP-induced platelet aggregation ratio (MAR) in patients with coronary heart disease (CHD) administrated with clopidogrel.

**Methods:** 1251 CHD patients were admitted to the Heart Center of the First Affiliated Hospital of Xinjiang Medical University from January 2011 to June 2017 were included the present study. *CYP2C19* genotype and ADP-induced platelet aggregation rate were detected after given loading dose of 300 mg clopidogrel for 24 hours. We observed the relation between *CYP2C19* genotype and platelet aggregation rate after loading clopidogrel.

**Results:** *CYP2C19* genotyping results showed that 608 (48.6%), 515 (41.1%) and 128 (10.2%) patients were extensive metabolism (EM, *CYP2C19*\*1/\*1), intermediate metabolism (IM, *CYP2C19*\*1/\*2, \*1/\*3) and poor metabolism (PM, *CYP2C19*\*2/\*2, \*2/\*3, \*3/\*3) carriers, respectively. Platelet function test results showed that there were 60.5% of patients with on-treated high platelet response (MAR  $\geq$  55%). Nevertheless, we did not find any association of MAR with *CYP2C19* in the present study.

**Conclusion:** The present study indicated that both on-treated high platelet response patients and *CYP2C19* PM carriers are frequent in CHD patients in China. However, there was no association of MAR after 300 mg of loading clopidogrel with *CYP2C19* genotypes.

**Keywords:** Coronary heart disease, *CYP2C19*, Platelet aggregation function.

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### Introduction

Clopidogrel plays an important role in the antiplatelet therapy of coronary heart disease (CHD). However, 4% to 30% of CHD patients experience clopidogrel resistance in clinical application [1]. Clopidogrel is a prodrug metabolized by the *CYP2C19* enzyme in the liver and is transformed into a pharmacologically active substance that acts an antiplatelet agent [2]. Previous studies have shown that there are a variety of *CYP2C19* mutant alleles, such as *CYP2C19*\*1, \*2, \*3 and \*17, that affect the antiplatelet activity of clopidogrel [3-5]. In Chinese populations, two mutant alleles, *CYP2C19*\*2 and \*3, cause *CYP2C19* enzyme activity to be decreased or completely lost, therefore affecting clopidogrel metabolism in the liver [6,7]. Additionally, previous studies have shown that the *CYP2C19* gene polymorphism is an important factor causing clopidogrel resistance [8,9]. Our previous study also suggested that personalized anti-platelet therapy guided by *CYP2C19* gene testing could significantly improve the clinical outcome

of CHD patients after PCI [10,11]. Therefore, the idea of precision antiplatelet therapy based on the *CYP2C19* genotype has been widely accepted by many scholars [12,13].

Recently, both *CYP2C19* and platelet function testing were recommended in high-risk CHD populations by the AHA/ACC guidelines [14]. Additionally, important progress has been made in guiding anti-platelet therapy of CHD based on platelet function tests [15,16]. There are various methods to detect platelet function, including PL-11, LTA, VerifyNow, and TEG [15-18]. PL-11 is a novel method (platelet count method) to detect platelet aggregation function, with a cut-off value of 55%, for which the sensitivity and specificity are 100% [18]. Theoretically, platelet aggregation rate should be significantly reduced after clopidogrel administration in patients with CHD. *CYP2C19* is a key enzyme in clopidogrel metabolism, so platelet aggregation rate will be affected in the carriers with loss-of-function alleles of *CYP2C19*. However, it is still unclear whether the *CYP2C19* genotype will affect platelet

aggregation function. In this study, we aimed to analyse the correlation between *CYP2C19* genotype and platelet aggregation function in CHD patients to whom clopidogrel had been administered.

## Subject and Methods

### Subjects

This study included 1251 CHD patients (996 males and 255 females) aged from 21 to 83 years ( $58.4 \pm 12.4$ ) admitted to the First Affiliated Hospital of Xinjiang Medical University from Jan. 2011 to June 2017. All patients were administered secondary prevention of CHD. Prior to enrolment in the study, 300 mg of aspirin and 300 mg of clopidogrel were administered.

**Inclusion criteria:** All the patients were diagnosed with CHD according to the coronary angiography, with stenosis  $\geq 50\%$  of at least one of the main coronary arteries.

**Exclusion criteria:** (1) Platelet count  $>450 \times 10^9/L$  or  $<100 \times 10^9/L$ ; (2) the presence of aspirin or clopidogrel contraindications; (3) coagulation dysfunction or severe liver disease; or (4) severe anemia, infection or hyperthyroidism or other diseases. All subjects signed informed consent forms, and the present study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University.

### Blood sampling

Antecubital vein blood samples were collected for monitoring platelet function in all subjects after administration with 300 mg of clopidogrel for 24 hours. A total of 2 ml of blood was collected in a 3.2% sodium citrated tube (Becton-Dickinson, Franklin Lakes, NJ) for PL-11 tests. Additionally, 2 ml of blood was collected in a sodium citrate anticoagulant tube for *CYP2C19* genotyping. Additionally, we collected 2 ml of non-anticoagulant blood for detection of biochemical indicators. Blood samples for PL-11 and biochemical indicators should be stored at room temperature before being tested. The whole procedure was performed within 2 hours of sampling. Blood samples for *CYP2C19* genotyping were stored at  $-4^\circ\text{C}$  before being detection.

### Platelet aggregation function testing

In the present study, we utilized a PL-11 platelet function analyzer (SINNOWA Medical Science & Technology Co., Nanjing, China), which is a novel point-of-care apparatus for platelet function analysis *via* an automated impedance technique [18]. The whole procedure was completed after transferring 500 ml of citrated blood sample into a polycarbonate tube and inserting it into the detection position. The blood sample in the polycarbonate tube was mixed gently throughout the testing process. Platelet count was detected in duplicate at the start, and the mean value of platelet count was set as the baseline. A total of 40 l of adenosine diphosphate (ADP, 50  $\mu\text{mol/L}$ ) was trickled into the blood sample after the

second detection time. The single platelet count dropped when aggregates became too large to be counted as single platelets. PL-11 counted platelets several times until it detected the lowest level. The whole process was finished within 15 minutes (six detection times). The system calculated the maximal platelet aggregation ratio according to the following formula:

MAR%

$$= \frac{(1\text{st platelet count} + 2\text{nd platelet count})/2 - \text{lowest platelet count}}{(1\text{st platelet count} + 2\text{nd platelet count})/2}$$

If ADP-induced platelet aggregation (MAR) was  $\geq 55\%$ , it was defined as clopidogrel resistance.

### Statistical analysis

SPSS version 17.0 (SPSS Inc., Chicago, Illinois) was used for all statistical analyses. Comparison of continuous variables was performed using Student's t-test (normally distributed continuous variables) or, in the case of non-normal distribution, the Mann-Whitney U test. Categorical variables were presented as numbers or percentages and compared using chi-square or Fisher's exact tests.  $P < 0.05$  (2-sided) was considered statistically significant.

## Results

### Characteristics of participants

The demographic parameters, biochemical parameters, and the physical examination results of the 1251 CHD patients are shown in Table 1.

**Table 1.** Characteristics of participants.

Characteristics	Mean $\pm$ SD or N (Frequency)
Age	58.4 $\pm$ 12.4
Weight	78.8 $\pm$ 53.8
SBP	133.24 $\pm$ 45.69
PLT	229.41 $\pm$ 127.04
BUN	5.76 $\pm$ 4.50
Cr	75.97 $\pm$ 30.90
UA	323.15 $\pm$ 92.23
TG	2.13 $\pm$ 1.81
TC	7.04 $\pm$ 3.41
HDL-C	1.02 $\pm$ 0.41
LDL-C	2.43 $\pm$ 0.93
ApoAI	1.16 $\pm$ 0.45
ApoB	0.78 $\pm$ 0.33
EF	62.11 $\pm$ 28.2
MAR	55.34 $\pm$ 19.37

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Male	996 (0.80)
Smoking	1087 (0.87)
Drinking	1100 (0.88)
Family history	804 (0.64)
Diabetes	875 (0.70)
Hypertension	1113 (0.89)
Hyperlipidemia	1000 (0.80)

**CYP2C19 genotype test results**

Among the 1251 patients, there were 608 (48.6%) cases with *CYP2C19* \*1/\*1, 452 (36.1%) cases with *CYP2C19* \*1/\*2, 63 (5.0%) cases with *CYP2C19* \*1/\*3, 93 (7.4%) cases with *CYP2C19* \*2/\*2, 33 (2.6%) cases with *CYP2C19* \*2/\*3, and 2 (0.2%) cases with *CYP2C19* \*3/\*3. Therefore, 608 (48.6%) patients were EMs, 515 (41.1%) patients were IMs, and 128 (10.2%) patients were PMs, which suggests that 52.4% of patients in this study carry a *CYP2C19* loss-of-function allele (*CYP*\*2 or \*3) (Table 2).

**Table 2.** Genotypes and phenotypes of *CYP2C19* in coronary heart disease.

Genotype	N	%	Phenotype	N	%
*1/*1	608	48.6	EM	608	48.6
*1/*2	452	36.1	IM	515	41.2
*1/*3	63	5	PM	128	10.2
*2/*2	93	7.4			
*3/*3	2	0.2			
*2/*3	33	2.6			

**Results of ADP-induced platelet aggregation rate test**

As shown in the Table 3, among the 1251 CHD patients, there were 757 patients with MAR ≥ 55%, suggesting that 60.5% of patients still had a high platelet aggregation response after taking 300 mg of clopidogrel.

**Table 3.** Comparison of MAR among three groups.

Genotype	Phenotype	N	MAR (%)	F value	P value
*1/*1	EM	608	55.13 ± 19.84	0.027	0.973
*1/*2 and *1/*3	IM	515	54.88 ± 19.46		
*2/*2,*3/*3, *2/*3	PM	128	55.04 ± 19.73		

**Relation between CYP2C19 genotype and MAR**

As shown in Table 4, there were 372 (61.18%) patients with high platelet aggregation response (MAR ≥ 55%) in the EM group, 312 (60.58%) patients with high platelet aggregation response (MAR ≥ 55%) in the IM group, and 73 (57.03)

patients with high platelet aggregation response (MAR ≥ 55%) in the PM group. The prevalence of high platelet aggregation response was not significantly different between the three groups (P =0.682).

**Table 4.** Comparison of frequency of high on-treat platelet response among three groups.

Genotype	Phenotype	N	MAR ≥ 55%	MAR<55%	P value
*1/*1	EM	608	372 (61.18)	236 (38.82)	0.682
*1/*2 and *1/*3	IM	515	312 (60.58)	203 (39.42)	
*2/*2,*3/*3, *2/*3	PM	128	73 (57.03)	55 (42.97)	

**Discussion**

The present study suggests that both the frequency of *CYP2C19* loss-of-function allele carriers and the number of high platelet aggregation response subjects were high after the administration of 300 mg of clopidogrel to CHD patients (51.4% and 60.45%, respectively). Although both *CYP2C19* genotype detection and platelet function testing have provided the basis for clinical personalized antiplatelet therapy, no association of the *CYP2C19* genotype with high platelet aggregation response was observed.

The current evidence shows that *CYP2C19* gene polymorphisms have a significant impact on the metabolism of clopidogrel drug, especially in carriers with loss-of-function alleles [19-21]. Additionally, our previous study demonstrated that personalized antiplatelet therapy guided by the *CYP2C19* genotype can significantly reduce MACEs in patients with CHD after PCI [10]. In the present study, we found that 51.4% of patients carry *CYP2C19* loss-of-function alleles, which is consistent with previous reports [19-21]. Patients with *CYP2C19* loss-of-function alleles have an increased risk of MACEs after PCI. Theoretically, *CYP2C19* loss-of-function allele carriers should show increased platelet responses after taking 300 mg of clopidogrel compared with individuals with normal metabolisms. However, we did not find any correlation between *CYP2C19* genotype and MAR in this study.

MAR reflects the function of platelet aggregation, which can be used to monitor the efficacy of anti-platelet drugs. In this study, MAR results detected using the PL-11 platelet aggregation analyser are reliable. PL-11 analyser using the platelet count difference method, for which both the sensitivity and the specificity are very high, has been widely used clinically. Zhang et al. [17] detected MAR using a PL-11 platelet aggregation analyser in CHD patients after PCI; the authors found that the incidence of MACEs was significantly increased in patients with elevated MAR.

Since both the MAR and *CYP2C19* genotype test can predict and evaluate the antiplatelet effect and clinical outcome in patients with CHD, it is worth discussing why there is no correlation between the two indicators. On the one hand, the identification of *CYP2C19* genotypes has been thoroughly verified, and the genotyping accuracy is very high. We also

verified the genotyping results using direct sequencing and demonstrated that the *CYP2C19* genotype detection method in this study is reliable. On the other hand, the PL-11 analyser used in this study passes the quality inspection, and the testing staff was formally trained before detection. The quality of the testing method is reliable, and the repeatability is good. Therefore, we conclude that the results were not due to mistakes in either genotyping of *CYP2C19* or MAR detection.

In conclusion, our findings revealed that *CYP2C19* genotype had no effect on platelet aggregation after clopidogrel administration in patients with CHD. However, our results still need verification in the future study.

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### Declaration of Interest

The authors report no conflicts of interest.

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