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Original Article

Correlation between CYP2C19 gene polymorphism and clinical efficacy of clopidogrel in patients with coronary artery heart disease

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Abstract: Objective: To investigate the correlation between CYP2C19 genotype polymorphisms in the CYP450 enzyme and the clinical efficacy of clopidogrel in patients with coronary artery heart disease (CHD). Methods: A total of 300 patients with CHD admitted to our hospital were divided into the clopidogrel normal response group (NCLR) (n = 152) and the clopidogrel basal response group (CLR) (n = 148), according to adenosine diphosphate (ADP)- and thromboelastography (TEG)-induced platelet inhibition ratio. The results of CYP2C19 genotype detection were classified into weak metabolism, middle and fast metabolism in order to find the relationship between poor clopidogrel to response with a CYP2C19 genotype. Results: There were significant differences in gender, smoking history, drinking history, aspartate aminotransferase (AST), platelet count (PLT), CYP2C19 genotype and triacylglycerol (TG) between the CLR group and NCLR group ($P < 0.05$). Little statistical significance was found with differences in hypertension, diabetes mellitus (DM), age, body mass index (BMI), low-density lipoprotein (LDL-C) and high-density lipoprotein (HDL-C) between the two groups ($P > 0.05$); the same was true between middle and weak metabolism in the CYP2C19 genotype detection ($P = 0.063$). The 3 types of metabolism showed notably different clopidogrel responses ($P < 0.05$). Multivariate logistic regression analysis found a relationship between CYP2C19 gene polymorphism, AST and PLT indicators with clopidogrel response ($P < 0.05$). ROC curves revealed the important value of CYP2C19 genotype detection in predicting clopidogrel responces. Conclusion: A poor clopidogrel response in CHD patients was correlated with CYP2C19 genotype polymorphisms. Recommended dosage of antiplatelet drugs may be changed in the light of the detection for a CYP2C19 genotype when applied in clinical practice.

Keywords: CYP2C19 genotypic polymorphism, coronary artery heart disease, clopidogrel, blood platelet

Introduction

In recent years, the increasing aging of the population in China has witnessed a significant annual elevation of the incidence of CHD. The cardiovascular mortality in China was 295.63/100,000 in 2014, while the mortality caused by heart disease was 143.72/100,000, which is still increasing every year [1]. Atherosclerotic lesions in coronary vessels leads to vascular obstruction or stenosis, causing hypoxia and myocardial infarction and therefore the occurrence of heart disease; which is collectively called "coronary artery heart disease". The related risk factors include: hypercholesterolemia, hypertension, diabetes, obesity, and smoking [2]. The occurrence of coronary artery heart disease is closely related to the severity and number of coronary atherosclerotic stenosis, and dyslipidemia is the principal factor causing atherosclerosis. Coronary artery heart disease is now involving luminal stenosis or occlusion due to inflammation, embolism and other causes. It is clinically classified into stable coronary artery heart disease and acute coronary syndrome. The differential diagnosis of coronary artery heart disease is therefore particularly important.

Coronary angiography serves as an accurate diagnostic technique and is one of the most commonly used methods for the diagnosis of coronary artery heart disease in clinical practice since the 1960s when it was first devel-

oped. Antiplatelets are considered to be the major regimen for the treatment of CHD. Dual antiplatelet therapy, clopidogrel + aspirin, is the referenced method for treatment and prevention of CHD ischemic events and acute coronary syndrome (ACS). Some patients, however, do not have good efficacy from the antiplatelets [3]. As a prodrug, Clopidogrel, has about 15% absorbance by the intestinal tract and is metabolically converted to antiplatelet metabolites by P450 enzymes in the liver [4]. Proteins encoded by CYP2C19 genes affects the metabolism of the clopidogrel. It has been found that CYP2C19 gene polymorphism plays an important role in clopidogrel activation [5]. The CYP2C19*2 genotype will lead to the production of nonfunctional proteins, while the CYP2C19*3 genotype will lead to the formation of immature codons, both of which can explain the 12% of attributional effect. The above two genotypes account for more than 99% of the weak metabolic phenotype in the Asian population. In this paper, 300 patients with CHD were enrolled to detect the correlation between platelet inhibition ratio and CYP2C19 genotype polymorphism in CHD patients given clopidogrel and the clinical efficacy.

Material and methods

Material

A total of 300 patients (172 males and 128 females, aged 38-77 years, mean age (45.2± 4.9) years) diagnosed with CHD and treated with clopidogrel in our hospital from October 2018 to April 2019 were enrolled. Inclusion criteria: The subjects were diagnosed with coronary artery heart disease and classified into stable angina pectoris (SAP), unstable angina pectoris (UAP) or acute myocardial infarction (AMI), have been taking oral clopidogrel for more than 1 d and were detected with the CYP2C19 gene and have signed an informed consent. This study was approved by the Ethics Committee of Zhangqiu District People's Hospital. Exclusion criteria: A patient who has a platelet count > 450×10^9/L or < 100×10^9/L, or has taken other antiplatelet drugs 2 weeks before surgery, or has contraindications to aspirin and clopidogrel, or was combined with dysfunction of the liver, kidney or coagulation issues, or any other infection, tumors or immune system diseases.

Methods

Before primary percutaneous coronary intervention (PCI), patients were given an oral loading dose of clopidogrel (300 mg) combined with aspirin (300 mg), while a maintenance dose of clopidogrel 75 mg/d combined with aspirin 100 mg/d on the next day, for more than 4 days. Coronary angiography was performed on these patients. The drug pressure was released to 8-16 atm. The eluting stent was provided by Johnson & Johnson (Cypher rapamycin) with adjusted length so as to cover the vascular lesion and sacculus injury. A successful stent implantation was indicated by less than 20% residual stenosis and restored blood supply without complications, cardiac death, myocardial infarction or ineffective revascularization. After surgery, clopidogrel 75 mg/d combined with aspirin 100 mg/d was administered for more than 1 year.

Platelet inhibition ratio by TEG

After 4 days of treatment, 2-3 ml of fasting venous blood was collected from all patients. The antiplatelet inhibition ratio of clopidogrel was measured using ADP, activator F, and kaolin TEG. Platelet ADP receptor inhibition < 30% refers to the CLR group, and ≥ 30% the NCLR group.

Determination of CYP2C19 gene polymorphism

Peripheral venous blood was collected in an ethylenediaminetetraacetate anticoagulant tube. The collected blood samples were tested. After DNA separation and purification using human blood DNA extraction kit, PCR was used for DNA amplification and specific hybridization to collect CYP2C19 gene chip. Six genotypes were determined on the basis of random assortment of CYP2C19*1, *2, and *3 (CYP2C19*1*1; CYP2C19*1*2, *1*3; CYP2C19*2*2, *2*3, *3*3). Fast, middle, and weak metabolism was decided according to the metabolic activity of translation products.

Statistics

SPSS 22.0 statistical software was used for data analysis. Measurement data following normal distribution were expressed as $\bar{x} \pm s$, and t-test was for comparisons between gr-

Table 1. Platelet inhibition ratio with different CYP2C19 genotypes [n (%)]

Group	CYP2C19	Case (n)	Total	CLR	ADP inhibition	F	P
Fast	*1*1	121 (40.33)	121 (40.33)	79 (26.33)	42.35±28.16	6.863	0.000
Middle	*1*2	98 (32.66)	120 (40.00)	86 (28.66)	37.42±28.48		
	*1*3	22 (7.33)					
Weak	*2*2	104 (34.66)	116 (38.66)	47 (15.00)	27.88±26.14		
	*2*3	8 (2.66)					
	*3*3	4 (1.33)					

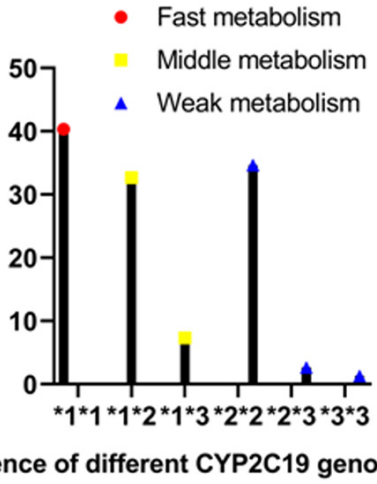

Figure 1. Incidence of different metabolic genotypes. There were remarkable differences between fast metabolism and middle metabolism (X^2 = 6.031, P = 0.008), and between fast metabolism and weak metabolism (X^2 = 11.478, P = 0.000), although the middle metabolism was not notably different from weak metabolism (X^2 = 2.334, P = 0.062).

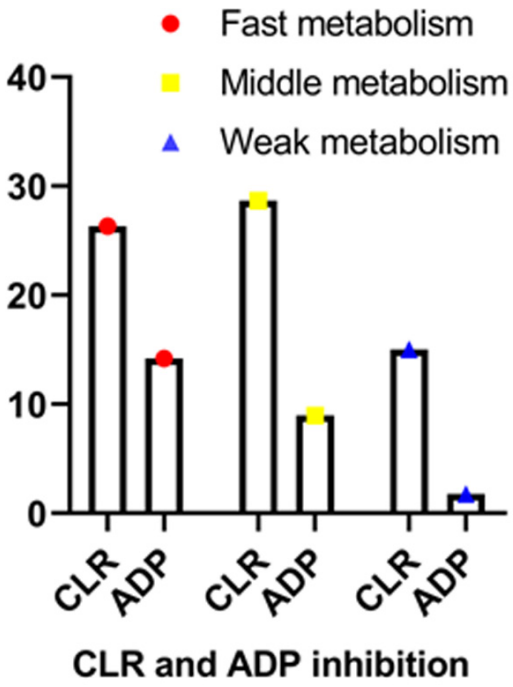

Figure 2. CLR and ADP inhibition by different genotypes. CLR and ADP inhibition by fast and middle metabolism were significantly higher than those by (P < 0.05).

oups. Enumeration data were expressed as percentage. Qualitative data were subject to chi-square test. Non-normally distributed measurement data were expressed as median (P25, P75) and subject to rank sum test. P < 0.05 indicated statistically significant differences.

Results

Platelet inhibition in patients of different CYP2C19 genotypes

Univariate analysis showed that there were significant differences in the metabolism among the three CYP2C19 genotypes (P < 0.05). Of the 300 patients, a fast metabolism was found in 121 patients (CYP2C19*1*1) while 79 were CLR, and middle metabolism included 98 patients (CYP2C19*1*2). There were 22 CYP2C19*1*3 patients, and 86 patients were CLR. Weak metabolism had 104 CYP2C19*2*2 patients, 4 CYP2C19*3*3 patients, 8 CYP2C19*2*3, and 47 CLR (**Table 1**). Clopidogrel reactivity among these patients showed a significant difference (X^2 = 13.841, P = 0.001) (**Figures 1** and **2**).

General information

There was no significant difference in age, BMI, DM, HDL-C, LDL-C and hypertension between the NCLR group and the CLR group (P > 0.05). Differences in gender, smoking history, drinking history, AST, PLT, TG and CYP2C19 genotype between the CLR group and the NCLR group suggested statistical significance (P < 0.05) (**Table 2**).

Table 2. General information of the two groups of patients [$\bar{x} \pm s$ (%)]

Item	NCLR (n = 152)	CLR (n = 148)	t/Z/X^2	P
Age (yr)	45.9±13.9	47.9±16.5	-1.856	0.159
Sex (M)	83 (54.60)	67 (45.27)	11.048	0.000
History of smoking	113 (74.34)	96 (64.86)	5.538	0.010
History of drinking	121 (79.60)	102 (68.91)	6.031	0.008
Dm	97 (63.81)	124 (83.78)	0.864	0.312
Hypertension	125 (82.23)	121 (81.75)	0.002	0.856
BMI (Kg/m^2)	25.41±3.84	25.17±3.56	0.705	0.304
PLT (×10^9)	220 (180, 265)	210 (170, 248)	-1.964	0.037
AST (IU/L)	45 (23, 120)	30 (21, 70.4)	-3.432	0.000
TG (mmol/L)	1.82±1.28	2.10±2.02	-2.006	0.034
LDL-C (mmol/L)	2.54±0.89	2.52±0.86	0.272	0.766
HDL-C (mmol/L)	1.06±0.29	1.04±0.28	0.728	0.391
CYP2C19 gene	86 (56.57)	67 (45.27)	13.849	0.001

Table 3. Prediction of different CYP2C19 genotypes on CLR reactivity [n (%)]

CYP2C19 genotypes	n	Platelet inhibition ratio	CLR poor response	X^2	P
*1*1	121	42.35±28.16	79 (26.33)	15.815	0.002
*1*2	98	37.42±28.48	78 (79.59)		
*1*3	22	39.10±28.79	20 (90.90)		
*2*2	104	28.51±27.58	41 (39.42)		
*2*3	8	24.73±20.52	6 (75.00)		

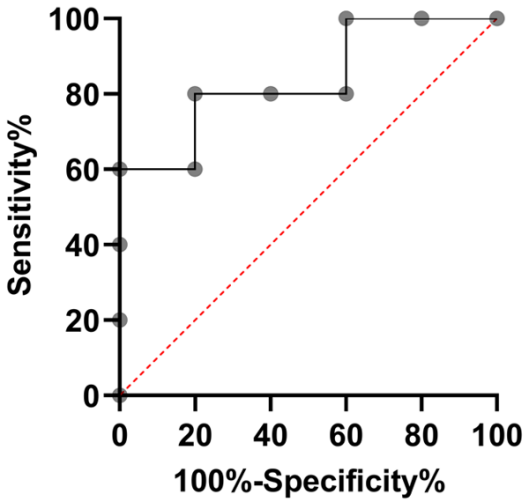

Figure 3. ROC curve. There is clear correlation between different genotypes and poor CLR response, and AUC > 0.5, suggesting that detection of the CYP2C19 gene may predict clopidogrel reactivity.

Different CYP2C19 genotypes with CLR

The platelet inhibition ratio of CYP2C19*1*1 was significantly higher than that of other genotypes (P < 0.05). Platelet inhibition ratio of *1*2 and CLR poor response were significantly different from those of *2*2 (P < 0.05), while comparisons among *1*2, *1*3 and *2*3 indicated little differences (P > 0.05) (**Table 3**). ROC curve showed that different CYP2C19 genotypes were notably different from CLR poor response (P < 0.05) (see **Figure 3**).

Logistic regression analysis

Multivariate logistic regression analysis was performed with CLR poor response as the dependent variable and independent variables included age, gender, smoking history, drinking history, AST, hypertension, DM, PLT, TG and CYP2C19 genotype. The results showed that PLT, AST and being male were negatively correlated with clopidogrel inhibition ratio. Age, PLT, AST and CYP2C19 genotype were the key factors responsible for the occurrence of CLR (**Table 4**).

Discussion

In recent years, the incidence of CHD patients has been significantly increased. When the coronary blood flow cannot meet the demands for healthy myocardial metabolism, it may lead to myocardial ischemia, angina pectoris and other complications in severe cases; while persistent myocardial ischemia may cause the occurrence of myocardial infarction [6-8]. Common complications of CHD are papillary muscle dysfunction or rupture, ventricular aneurysm, and the most dangerous cardiac rupture. In clinical practice the major concern is whether patients with mild CHD will develop an exacerbation of the condition [9, 10]. The study on risk factors for CHD severity is particularly important. An increasing age leads to slow metabolism and waste deposited in the vessel wall, narrowing of the vessel lumen and blood flow to the myocardium resulting in an increased incidence of complications [11, 12]. Hypertensive patients,

Table 4. Logistic regression analysis

Item	B	SE	Wald X^2	P	OR	OR (95% CI)
Sex	-0.699	0.242	7.562	0.006	0.484	0.300-0.803
Age	0.005	0.008	0.313	0.458	1.004	0.977-1.019
Smoking history	-0.061	0.208	0.016	0.633	0.829	0.605-1.429
Drinking history	-0.063	0.207	0.126	0.619	0.817	0.616-1.385
BMI	-0.036	0.022	2.499	0.103	0.853	0.919-1.008
DM	-0.039	0.167	0.042	0.808	0.859	0.666-1.359
Hypertension	-0.038	0.174	0.069	0.699	0.841	0.652-1.356
PLT ($\times 10^9$)	-0.005	0.001	11.046	0.001	0.884	0.881-0.887
AST	-0.003	0.001	7.056	0.005	0.886	0.884-0.888
LDL-C	-0.029	0.105	0.068	0.668	0.869	0.777-1.184
HDL-C	-0.379	0.305	1.439	0.204	0.573	0.364-1.235
TG	0.101	0.060	2.726	0.081	1.107	0.972-1.237
CYP2C19 genotype	-0.607	0.162	11.763	0.000	0.428	0.383-0.745

that is, patients with high systolic or diastolic blood pressure, are most likely to develop Coronary and cerebral arteries lesions. From 60% to 70% of hypertensive patients will develop atherosclerosis, and the incidence of CHD is 4 to 5 times that of normal people [13, 14]. Excessive low HDL will cause abnormal metabolism of lipids. Deposition of lipids in the arterial intima, plus accumulation of some atheroma-like lipids, form white plaques in the vascular wall leading to stenosis, blood flow obstruction, myocardial ischemia, or acute myocardial infarction if thrombi occurs and stops the blood flow completely [15, 16]. The mechanism of clopidogrel is to bind to platelets, reduce its inhibition of adenylate cyclase, and inhibit platelet aggregation.

Clopidogrel is able to reduce the expression of platelet selectin to regulate platelet activation [17, 18]. It may effectively prevent and treat thrombosis. With a relatively short half-life, it reaches an effective plasma concentration quickly and works fairly fast. It has been an effective drug for postoperative antithrombotic therapy in clinical practice [19, 20]. Although the mechanism of clopidogrel resistance has not been clarified, it has been shown that the loss of function of *2 and *3 in CYP2C19 genotypes which serves as a key enzyme in clopidogrel coding metabolism causes premature termination of protein synthesis and therefore clopidogrel hyporesponsiveness [21-23]. In this paper, 148 of 300 patients (49.33%) had poor CLR response, which was similar to the incidence of clopidogrel resistance (4%-44%) in Georgios J Vlachojannis et al. [24]. Little difference was found between poor metabolism and middle metabolism (P > 0.05). There were significant differences among fast metabolism, middle metabolism and weak metabolism (P < 0.05). CYP2C1911 was found to be superior to other genotypes (P < 0.05). CYP2C192 may be the factor directly affecting clopidogrel antiplatelets, which is supported by ELEVATE-TIMI56, etc. [25]. The small number of patients carrying CYP2C19*1*3 and *2*3 predicted the poor drug response, but the differences in platelet inhibition ratio were statistically significant (P < 0.05). The platelet inhibition ratio by weak and middle metabolism in CYP2C19 genotype was notably lower than that by fast metabolism, and all patients showing poor CLR response had CYP2C19*2 and *3.

In summary, there is a significant correlation between CYP2C19 gene polymorphisms and CLR poor response; especially for those carrying CYP2C19*2 and CYP2C19*3. Detection of CYP2C19 gene polymorphism helps to predict the response of clopidogrel.

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Disclosure of conflict of interest

None.

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