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Study on the effect of CYP2C19 genetic polymorphism and plasma concentration on clopidogrel resistance

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Abstract: At present, the mechanism for clopidogrel resistance (CR) is incompletely understood. Here, we aimed to analyze of the association of plasma concentration of clopidogrel active metabolites (CAM) and CYP2C19 genetic polymorphism with CR. We assigned 77 patients to receive CLP at a loading dose of 300mg on day 1, followed by 75mg per day from day 2 to day 6. Three peripheral venous blood samples were collected for analysis. Our results showed that plasma concentration of CAM in extensive metabolizers (EMs) group (2.48(1.31, 5.67) ng/mL) was higher than that in intermediate metabolizers (IMs) group (1.44(1.18,3.55) ng/mL) and that in poor metabolizers (PMs) (1.18(1.12,1.33) ng/mL) group was the lowest (H=14.58, P=0.001). Besides, the incidence of CR in EMs group(11.1%) was lower than that in IMs group (20.0%) and that in IMs group was lower than that in PMs group (45.5%) (χ^2=6.344, P=0.042). In addition, our findings confirmed that the incidence of chest tightness in IMs group (40.0%) and PMs group (50.0%) was higher than that in EMs group (9.1%) (P=0.015). Over the follow-up period, it was found that CYP2C19 and plasma concentration of CAM were related to the incidence of chest tightness. Our findings indicated that in addition to CYP2C19, plasma concentration of CAM may be an important factor in predicting CR.

Keywords: Clopidogrel, CYP2C19, clopidogrel resistance.

INTRODUCTION

Cardiovascular disease (CVD) is the most common cause of death among adults in the United States (Curry *et al*., 2018) and has become the first cause of death in China (Chin Circul, 2019). Clopidogrel (CLP) is an antiplatelet medication used to treat Acute Coronary Syndrome, recent myocardial infarction, recent stroke, or established peripheral arterial disease (Furlong *et al*., 2013). Because of significant interindividual variability in the CLP response, about 5-40% of patients do not achieve an adequate antiplatelet effect in the conventional dose. In severe cases, CLP lead to cardiovascular adverse events such as stent thrombosis, myocardial infarction, stroke and even death, which is called clopidogrel resistance (CR) (De Miguel *et al*., 2008; Guirgis *et al*., 2017). But the underlying mechanisms of CR remain unknown.

It is generally believed that CR can be attributed to the intrinsic factors and the extrinsic factors. The internal factors include genetic factors, activation of other platelet activation pathways and so on and the external factors include age, BMI, drug dosage, patient compliance, drug interaction, cigarette smoking and so on (Chen *et al*., 2018; Karazniewicz-Lada *et al*., 2019; Ramotowski *et al*., 2020).

Numerous studies show that the enzymes encoded by the CY2C19 genes influence CLP metabolism by affecting the absorption and activation of CLP, which will affect the clinical efficacy (Hulot *et al*., 2006; Mega *et al*., 2009; Bauer *et al*., 2011; Holmes *et al*., 2011; Osnabrugge *et al*., 2015; Dagmar *et al*., 2018; Pandey *et al*., 2019; Klein *et al*., 2019; Ferrari *et al*., 2019). However, CYP2C19 genetic polymorphism accounts for only 12% of clinical efficacy of CLP, which is not sufficient to predict clinical efficacy based on genetic polymorphism alone (De Miguel *et al*., 2008; Andrew *et al*., 2013; Mahdieh *et al*., 2018).

As a thienopyridine prodrug, CLP requires hepatic biotransformation to form an active metabolite, which could inhibit the purinergic P2RY12 receptor selectively and irreversibly (fig. 1). Previous research indicated that only 15% of the prodrug could transform to clopidogrel active metabolites (CAM), the remaining 85% was hydrolyzed by esterases to inactive forms (Scott *et al*., 2013). Wang *et al*. (2015) reported that the concentration of CAM may reflect CR more directly than platelet aggregation rate and was not affected by transporter and receptor genetic polymorphism. Thus, the concentration of CAM may also be an important factor affecting CR. Very limited data is available addressing the effect of CYP2C19 genetic polymorphism and plasma concentration on CR. We here aimed to study the association among the three, which is critically important to optimize treatment for patients with coronary heart disease (CHD).

MATERIALS AND METHODS

Patients

Our research was approved by the local Ethical Committee at The First Affiliated Hospital of Kunming Medical University ((2017) L No.33). All participants

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were written informed consent after an extensive description of the study aims and design. The following study included 77 patients from The First Affiliated Hospital of Kunming Medical University (Kunming, China) who were treated CLP prior to elective coronarography or percutaneous coronary intervention (PCI) procedure. All subjects were given a 300mg loading dose of CLP on day 1, followed by 75mg per day from day 2 to day 6. Subjects were excluded if they had a history or evidence of severe liver dysfunctions, severe renal dysfunctions, or hematologic abnormalities, or an allergy to any drugs.

Sample preparation

Patients were treated with CLP loading dose of 300 mg followed by 75 mg daily for 5 days. After the next dose of CLP was administered for 30-60min, three peripheral venous blood samples (each 2mL) were collected. One sample anticoagulated with 3.8% sodium citrate was collected and the platelet function was measured within 2 hours; the other one anticoagulated with EDTA was stored at -80℃ for CYP2C19 genotype analysis; the other one anticoagulated with EDTA was immediately added 20μl of 500mM 2-Bromo-3' -Methoxyacetophenone (MPB), gently mixed and reacted at room temperature for 10min. Finally, samples were centrifuged at 3500rpm for 10min, the supernatant was stored at -80℃ for LC-MS analysis.

Platelet function assay

Platelet function was measured by continuous platelet count (PL-11 platelet function analyzer, SINNOWA®). 0.5ml sodium citrate anticoagulant blood sample was added to PL-11 test tube and the instrument began to detect automatically. After two counts of basic platelet values in this sample, the instrument automatically added 40μl ADP with a concentration of 50mmol/L. The platelet count in the blood sample was calculated at continuous intervals, and the results were automatically converted when the minimum platelet count was obtained, expressed as the maximum platelet aggregation rate (MAR). That is {(initial platelet count-minimum platelet count)/initial platelet count}×100%. When MAR≥55, it was defined as clopidogrel resistance (CR) group and when MAR<55, it was defined as clopidogrel non-resistance (NCR) group.

Genotype analysis

CYP2C19 single nucleotide polymorphisms were genotyped in 77 patients and they were genotyped using CYP2C19 gene chip detection system (BaiO®). Operations were based on the instructions. The main steps include: DNA extraction, PCR amplification, chip hybridization and so on. The SNP information of CYP2C19 gene was given by scanning and automatic interpretation of the results of Array Doctor V2.0 gene chip image analysis software.

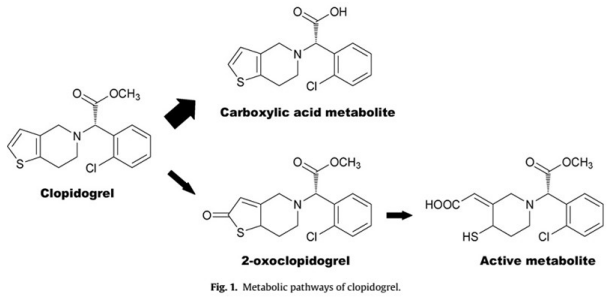

Fig. 1. Metabolic pathways of clopidogrel.

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LC-MS analysis

Chromatographic analysis was performed on a Waters ACQUITY UPLC system (Waters, USA). We use a Xevo TQD with Masslynx TM software (version 4.1) to detect target analytes. Chromatographic separations were performed on a Waters Acquity UPLC BEH C18 column (2.1mm×100 mm, 1.7μm) using a isocratic elution profile with acetonitrile at a flow rate of 0.2mL/min. The column temperature was maintained at 35℃. The injection volume was 8μL.The ion source temperature was 150℃, the capillary voltage was 2.90 kV, the cone voltage was 30V, the desolvation temperature was 500℃, the desolvation gas flow was 650L/h and the conegas flow was 50L/h. The protonated ions of analytes were detected in positive ionization in multiple reaction monitoring mode (MRM).

STATISTICS ANALYSIS

The statistical analysis was performed using SPSS Statistic 22.0 software. Continuous variables are presented as mean values ± standard deviation, while categorical variables are presented as percentages. The means for continuous variables in the two groups were compared using Student's t tests and the prevalence of categorical variables was compared using χ^2 tests. The means for continuous variables among the 3 groups were compared using analysis of variance tests and the significance of categorical variables was compared using χ^2 tests. Hardy-Weinberg equilibrium and allele frequency comparisons were compared using χ^2 tests. A signifcance level of $P<0.05$ was based on the two-sided probability test.

RESULTS

Patient characteristics

A total of 77 individuals who suffered from CHD were recruited, including 59 males (76.6%) and 18 females (23.4%). According to MAR, the patients were divided into CR and NCR group. There were no significant differences in the main baseline characteristics between the two groups. The results are shown in table 1.

Table 1: Baseline characteristics of the study populations [$\bar{x} \pm s$ 或 n(%)]

Characteristics	CR(n=15)	NCR(n=62)	χ^2/t 值	*P* 值
Age (years)	60.07±9.08	64.76±10.01	1.656	0.569
Gender (Female)	11(73.3)	48(77.4)	0.000	1.000
smoking	6(40.0）	24(38.7)	0.008	0.927
BSA	1.71±0.17	1.75±0.16	-0.830	0.983
BMI (18≤BMI＜24)	3(20.0)	23(37.1)	0.907	0.341
DM	6(40.0)	29(46.8)	0.224	0.636
SM	3(20.0)	20(32.3)	0.380	0.538
ACEI	5(33.3)	17(27.4)	0.019	0.891
ARB	7(46.7)	28(45.2)	0.011	0.916
CCB	5(33.3)	21(33.9)	0.002	0.968

BSA body surface area, BMI body mass index, DM diabetes mellitus, SM Traditional Chinese Medicine Preparation *Salvia miltiorrhiza,* ACEI Angiotensin converting enzyme inhibitor, ARB Angiotensin II receptor antagonist, CCB Calcium channel blocker.

Table 2: frequencies of CYP2C19 genotypes and alleles [n(%)]

Genotypes	Frequency (%)			Frequency (%)	
	G/G	G/A	A/A	G	A
CYP2C19*2	68(88.31)	9(11.69)	0(0)	145(94.16)	9(5.84)
CYP2C19*3	41(53.25)	29(37.66)	7(9.09)	111(72.08)	43(27.92)

Table 3: CYP2C19 genotype and CR[n(%)]

	EMs(n=36)	IMs(n=30)	PMs(n=11)	χ^2	*P*
CR	4(11.1)	6(20.0)	5(45.5)	6.344	0.042*
NCR	32(88.9)	24(80.0)	6(54.5)		

*P < 0.05 between CR and NCR.

Table 4: The plasma concentration of CAM in two groups [M (P25, P75)]

CR (n=15)	NCR (n=62)	*Z*	*P*
1.33 (1.18, 2.84)	1.63 (1.19, 4.26)	-0.842	0.400

Table 5: CYP2C19 genotype and plasma concentration of CAM [M (P25,P75)]

EMs(n=36)	IMs(n=30)	PMs(n=11)	*H*	*P*
2.48(1.31, 5.67)	1.44(1.18, 3.55)	1.18(1.12, 1.33)	14.58	0.001*

*P<0.05 between EMs, IMs and PMs.

Table 6: Association between CYP2C19 genetic polymorphism and follow-up results

Follow-up results	EMs(n=22)	IMs(n=25)	PMs(n=6)	Total(n=53)	χ^2	*P*
Rehospitalization	1(4.5%)	4(16.0%)	0	5(9.4%)	3.002	0.223
Chest tightness	2(9.1%)	10(40.0%)	3(50.0%)	15(28.3%)	7.781	0.020*
Bleeding	3(13.6%)	0	1(16.7%)	4(7.5%)	5.430	0.066
Revascularization	1(4.5%)	2(8.0%)	0	3(5.7%)	0.983	0.612
Adverse drug reaction (rash, gastrointestinal reaction , etc.)	3(13.6%)	5(20.0%)	0	8(15.1%)	2.435	0.296

*P<0.05 between EMs, IMs and PMs.

Table 7: Association between the plasma concentration of CAM and follow-up results [M(P25,P75)]

Follow-up results		The plasma concentration of CAM	*Z*	*P*
Rehospitalization	Yes	1.64(1.18, 3.79)	-0.380	0.704
	No	1.49(1.17, 2.84)		
Chest tightness	Yes	1.25(1.12, 1.80)	-1.984	0.047*
	No	1.66(1.18, 3.55)		
Bleeding	Yes	1.84(1.32, 2.59)	-0.404	0.686
	No	1.44(1.17, 3.06)		
Adverse drug reaction (rash, gastrointestinal reaction, etc.)	Yes	1.72(1.29, 3.38)	-0.770	0.441
	No	1.33(1.16, 2.84)		

*P<0.05 between patients with chest tightness and those without chest tightness.

Table 8: Association between CR and follow-up results [n(%)]

Follow-up results	NCR (n=41)	CR (n=12)	Total (n=53)	χ^2	*P*
Rehospitalization	4(9.8)	1(8.3)	5(9.4)	0.000	1.000
Chest tightness	11(26.8)	4(33.3)	15(28.3)	0.006	0.940
Bleeding	3(13.6)	1(8.3)	4(7.5)	0.000	1.000
Revascularization	2(4.9)	1(8.3)	3(5.7)	0.000	1.000
Adverse drug reaction (rash, gastrointestinal reaction, etc.)	7(17.1)	1(8.3)	8(15.1)	0.081	0.775

CYP2C19 genotype

Among the 77 patients, there were 68(88.31%) with wild homozygous, 9(11.69%) with heterozygous CYP2C19*2, 41(53.25%) with wild homozygous, 29(37.66%) with heterozygous, and 7(9.09%) with mutant homozygous CYP2C19*3. We divided these 77 patients into three phenotypes based on CYP2C19*2 and CYP2C19*3 genotypes. There were 36(42.51%) extensive metabolizers (EMs), 30(48.13%) intermediate metabolizers (IMs), and 11(9.36%) poor metabolizers (PMs) in the present study. Allele frequencies of all SNPs were in Hardy-Weinberg equilibrium (χ^2=0.30, 0.32, *P*=0.59, 0.57 respectively) (table 2).

CYP2C19 genotype and CR

The incidence of CR in EMs, IMs and PMs groups were 4 cases (11.1%), 6 cases (20.0%) and 5 cases (45.5%), respectively; whereas the incidence of NCR was 32 cases (88.9%), 24 cases (80.0%) and 6 cases (54.5%), respectively (χ^2=6.344, *P*<0.05). The results indicated that there was a statistics difference in the incidence of CR among the three groups (table 3). Further pairwise comparison, the incidence of CR in EMs group was lower than that in PMs group, but there was no significant difference between IMs group and other group.

The plasma concentration of CAM and CR

The plasma concentration M (P25, P75) of CAM in CR group was 1.33 (1.18, 2.84)ng/mL; whereas the plasma concentration M (P25, P75) in NCR group was 1.63 (1.194,4.26)ng/mL (*P*>0.05). Our results indicated that there was no significant difference in the plasma concentration of CAM between the two groups (table 4).

CYP2C19 genotype and plasma concentration of CAM.

The plasma concentration of CAM in EMs, IMs, PMs were 2.48(1.31,5.67)ng/mL, 1.44(1.18,3.55)ng/mL, 1.18 (1.12,1.33)ng/mL, respectively. There was significant difference in the plasma concentration of CAM among the three groups (H=14.58, P=0.001). Further pairwise comparison, there were differences between any two (table 5).

Association of CYP2C19 genetic polymorphism, plasma concentration of CAM and CR with follow-up results

Over the 3~12 months follow-up period, 24 cases were lost and 53 cases were followed up successfully. The follow-up rate was 68.83%. The follow-up results showed that there was statistic difference in the incidence of chest tightness among the three groups (*P*<0.05). The incidence of chest tightness in IMs (40.0%) and PMs (50.0%) was higher than that in EMs (9.1%), but there was no significant difference between IMs and PMs. There was no significant difference in the incidence of other clinical events among the three groups (table 6).

The association between the plasma concentration of CAM and follow-up results showed that the plasma concentration in patients with chest tightness was lower than that in patients without chest tightness (*P*<0.05). There was no significant difference in the incidence of other clinical events between the other two groups (table 7).

Finally, the association between CR and follow-up results showed that there were no significant differences between the two groups (table 8).

DISCUSSION

Coronary artery disease remains the major cause of mortality worldwide (Schilling *et al*., 2020). According to the American College of Cardiology recommendations, patients with acute coronary syndrome should continue to be treated with clopidogrel (CLP) combined with aspirin for at least 12 months, and patients with stable ischemic heart disease should continue treatment for 6 months (Levine *et al*., 2016). However, there are individual differences in the treatment of CLP, which may lead to poor clinical outcomes in some patients. At present, the mechanism is unclear. Previous studies focused on the effect of gene polymorphism on the clinical efficacy (Hulot *et al*., 2006; Mega *et al*., 2009; Bauer *et al*., 2011; Holmes *et al*., 2011; Osnabrugge *et al*., 2015; Dagmar *et al*., 2018; Pandey *et al*., 2019; Klein *et al*., 2019; Ferrari *et al*., 2019), but few studies included plasma concentration and gene polymorphism. Kim *et al*. (Kim *et al*., 2008) has done similar research before. However, only 24 healthy subjects were included in this study and the plasma levels of CLP was used as a pharmacokinetic parameter. CLP is a precursor drug that is converted into its active metabolites (CAM) form through 2 sequential oxidative steps after absorption into the bloodstream via the intestines (Kazui *et al*., 2010). Therefore, it is CAM rather than CLP that exerts clinical efficacy. In our study, the plasma levels of CAM and CYP2C19 genetic polymorphism were combined to comprehensively analyze the effects on CR. Our findings could provide a guidance for the development of clinical personalized antiplatelet therapy.

Our results showed that the plasma concentration of CAM in EMs group was higher than that in IMs group, and that in PMs group was the lowest. It indicated that CYP2C19 genetic polymorphism will affect the plasma concentrations of CAM in patients with CHD, follow by affected its clinical efficacy.

Besides, the incidence of CR in EMs group (11.1%) was lower than that in IMs group (20.0%) and that in IMs group was lower than that in PMs group (45.5%)(χ^2=6.344, P<0.05). These results indicated that CYP2C19 genetic polymorphism was associated with CR, which was in line with these previous findings. Furthermore, the pairwise comparison showed that only EMs group and PMs group (P=0.036) have significant difference, which indicated that CYP2C19 genetic polymorphism may not be the only factor predicting CR, and should be considered in combination with other factors.

In addition, our finding confirmed that CYP2C19 genetic polymorphism and the plasma concentration of CAM were associated with the incidence of clinical events (chest tightness). The incidence of chest tightness in IMs group (40.0%) and PMs group (50.0%) was higher than that in EMs group (9.1%). It is further verified that CYP2C19 genetic polymorphism is associated with the incidence of major adverse cardiovascular events (Hou *et al*., 2014; Jang *et al*., 2012; Karaźniewicz-Łada *et al*., 2014; Zhong *et al*., 2018).

Besides, it also found that the plasma concentration of CAM in patients with chest tightness was higher than that in patients without chest tightness (P<0.05), which indicated that the plasma concentration of CAM was related to the incidence of major adverse cardiovascular events. It is suggested that the plasma concentration of CAM may be an important factor in predicting CR.

It was also found that the plasma concentration M(P25, P75) of CAM was 1.33(1.18, 2.84)ng/mL in CR, whereas that was 1.63 (1.19,4.26)ng/mL in NCR. Although the statistical analysis showed no significant difference (P=0.400), the data suggested that the plasma concentration of CAM in NCR was higher than that in CR. Because of the relatively small sample size, we are not sure whether plasma concentration of CAM is associated with CR. Finally, the study also found that there was no significant difference in clinical events between CR and NCR, which suggested that there may be some differences between the experimental value of CR and clinical cardiovascular events. Therefore, it is recommended that the experimental value of CR can not be used as the only factor to evaluate the efficacy of CLP, which should be combined with CYP2C19 genetic polymorphism and the plasma concentration of CAM.

CONCLUSION

In conclusion, we studied the association between CYP2C19 genetic polymorphism and plasma concentration and CR in patients with CHD. It was found that CYP2C19 genetic polymorphism could affect the plasma concentration of CAM in patients with CHD, and CYP2C19 genetic polymorphism was associated with CR. Over the 3~12 months follow-up period, it was found that CYP2C19 genetic polymorphism and the plasma concentration were related to the incidence of clinical events (chest tightness). Our finding indicated that in addition to CYP2C19 genetic polymorphism, the plasma concentration of CAM may be an important factor in predicting CR. However, this is only a single-center study, so it may have some limitations. Further multi-center, large sample studies are needed to expand upon our findings.

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REFERENCES

Frelinger AL, Bhatt DL, Lee RD, Mulford DJ, Wu J, Nudurupati S, Nigam A, Lampa M, Brooks JK, Barnard MR and Michelson AD (2013). Clopidogrel pharmacokinetics and pharmacodynamics vary widely despite exclusion or control of polymorphisms (CYP2Y19, ABCB1, PON1), noncompliance, diet, smoking, co-medications (including proton pump inhibitors), and pre-existent variability in platelet function. *J. Am. Coll. Cardiol*., **61**(8): 872-879.

Bauer T, Bouman HJ, van Werkum JW, Ford NF, ten Berg JM and Taubert D (2011). Impact of CYP2C19 variant genotypes on clinical efficacy of antiplatelet treatment with clopidogrel: Systematic review and meta-analysis. *BMJ*., **343**: d4588.

Chen XL, Li XS, Ding YL, Deng YL and Wu H (2018). Research progress on non-genetic factors influenced the response to clopidogrel. *Chin. J. Clin. Pharmacol*., **34**(14): 1719-1722.

Curry SJ, Krist AH, Owens DK, Barry MJ and Caughey AB (2018). Risk assessment for cardiovascular disease with nontraditional risk factors: us preventive services task force recommendation statement. *JAMA*, **320**(3): 272-280.

Hernandez-Suarez DF, Tomassini-Fernandini JC, Cuevas A, Rosario-Berrios AN, Nuñez-Medina HJ, Padilla-Arroyo D, Rivera N, Liriano J, Vega-Roman RK, Renta JY, Melin K and Duconge J (2018). Clinical relevant polymorphisms affecting clopidogrel pharmacokinetics and pharmacodynamics: insights from the Puerto Rico newborn screening program. *Int. J. Environ. Res. Public Health,* **15**(6): 1115.

De Miguel A, Ibanez B and Badimón JJ (2008). Clinical implications of clopidogrel resistance. *Thromb. Haemost.,* **100**(2): 196-203.

Ferrari M, Tozzi M, Marino F, Tarallo A, Riva F, Mirabile M, Castelli P and Cosentino M (2019). Clopidogrel pharmacogenetics: Associations between genotype and stent thrombosis risk in patients with stent placement. *J. Cardiovasc. Med.* (Hagerstown), **20**(1): 46-48.

Furlong MT, Savant I, Yuan M, Scott L, Mylott W, Mariannino T, Kadiyala P, Roongta V and Arnold ME (2013). A validated HPLC-MS/MS assay for quantifying unstable pharmacologically active metabolites of clopidogrel in human plasma: Application to a clinical pharmacokinetic study. *J. Chromatogr. B*., **926**: 36-41.

Holmes MV, Perel P, Shah T, Hingorani AD and Casas JP (2011). CYP2C19 genotype, clopidogrel metabolism, platelet function, and cardiovascular events: A systematic review and meta-analysis. *JAMA*, **306**(24): 2704-2714.

Guirgis M, Thompson P and Jansen S (2017). Review of aspirin and clopidogrel resistance in peripheral arterial disease. *J. Vasc. Surg.,* **66**(5): 1576-1586.

Hou X, Shi J and Sun H (2014). Gene polymorphism of cytochrome P450 2C19*2 and clopidogrel resistance reflected by platelet function assays: A meta-analysis. *Eur. J. Clin. Pharmacol*., **70**(9): 1041-1047.

Hulot JS, Bura A, Villard E, Azizi M, Remones V, Goyenvalle C, Aiach M, Lechat P and Gaussem P (2006). Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood*, **108**(7): 2244-2247.

Itkonen MK, Tornio A, Lapatto-Reiniluoto O, Neuvonen M, Neuvonen PJ, Niemi M, Backman JT (2019). Clopidogrel increases dasabuvir exposure with or without ritonavir, and ritonavir inhibits the bioactivation of clopidogrel. *Clin. Pharmacol. Ther.,* **105**(1): 219-228.

Jang JS, Cho KI, Jin HY, Seo JS, Yang TH, Kim DK, Kim DS, Seol SH, Kim DI, Kim BH, Park YH, Je HG, Jeong YH and Lee SW (2012). Meta-analysis of cytochrome P450 2C19 polymorphism and risk of adverse clinical outcomes among coronary artery disease patients of different ethnic groups treated with clopidogrel. *Am. J. Cardiol*., **110**(4): 502-508.

Karaźniewicz-Łada M, Danielak D, Rubiś B, Burchardt P, Oszkinis G and Główka F (2014). The influence of genetic polymorphism of Cyp2c19 isoenzyme on the pharmacokinetics of clopidogrel and its metabolites in patients with cardiovascular diseases. *J. Clin. Pharmacol.,* **54**(8): 874-880.

Karaźniewicz-Łada M, Rzeźniczak J, Główka F, Gumienna A, Dolatowski F, Słomczyński M and Burchardt P (2019). Influence of statin treatment on pharmacokinetics and pharmacodynamics of clopidogrel and its metabolites in patients after coronary angiography/angioplasty. *Biomed. Pharmacother.*, **116**: 108991.

Kazui M, Nishiya Y, Ishizuka T, Hagihara K, Farid NA, Okazaki O, Ikeda T and Kurihara A (2010). 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.,* **38**(1): 92.

Kim KA, Park PW, Hong SJ and Park JY (2008). The effect of CYP2C19 polymorphism on the pharmacokinetics and pharmacodynamics of clopidogrel: A possible mechanism for clopidogrel resistance. *Clin. Pharmacol. Ther.*, **84**(2): 236-242.

Klein MD, Williams AK, Lee CR and Stouffer AG (2019). Clinical utility of CYP2C19 genotyping to guide antiplatelet therapy in patients with an acute coronary syndrome or undergoing percutaneous

coronary intervention. *Arterioscler. Thromb. Vasc. Biol.*, **39**(4): 647-652.

Levine GN, Bates ER, Blankenship JC, Bailey SR, Bittl JA, Cercek B, Chambers CE, Ellis SG, Guyton RA, Hollenberg SM, Khot UN, Lange RA, Mauri L, Mehran R, Moussa ID, Mukherjee D, Nallamothu BK and Ting HH (2016). ACC/AHA guideline focused update on duration of dual antiplatelet therapy in patients with coronary artery disease: A report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J. Thorac. Cardiovasc. Surg.,* **152**(5): 1243-1275.

Mahdieh N, Rabbani A, Firouzi A, Zahedmehr A, Hoseinimoghaddam M, Saedi S, Sanati H, Basiri H, Noohi F, Rabbani B and Maleki M (2018). Clopidogrel pharmacogenetics in iranian patients undergoing percutaneous coronary intervention. *Cardiovasc Toxicol,* **18**(5): 1-10.

Miao J, Rui L, Li Z(2009). Cytochrome p-450 polymorphisms and response to clopidogrel. *N. Engl. J. Med*., **360**(4): 354-362.

Osnabrugge RL, Head SJ, Zijlstra F, Ten Berg JM, Hunink MG, Kappetein AP and Janssens AC (2015). A systematic review and critical assessment of 11 discordant meta-analyses on reduced-function CYP2C19 genotype and risk of adverse clinical outcomes in clopidogrel users. *Genet. Med*., **17**(1): 3-11.

Pandey CP, Misra A, Negi MPS, Kanuri BN, Chhonker YS, Bhatta RS, Narain VS and Dikshit M (2019). Aspirin & clopidogrel non-responsiveness & its association with genetic polymorphisms in patients with myocardial infarction. *Indian J. Med. Res.*, **150**(1): 50-61.

Ramotowski B, Gurbel PA, Tantry U, Bracha JS, Karaźniewicz-Łada M, Lewandowski Z and Budaj A (2020). Effect of smoking cessation on the pharmacokinetics and pharmacodynamics of clopidogrel after pci: the smoking cessation paradox study. *Thromb. Haemost.*, **120**(3): 449-456.

Schilling U, Dingemanse J and Ufer M (2020). Pharmacokinetics and pharmacodynamics of approved and investigational P2Y12 Receptor Antagonists. *Clin Pharmacokinet*, **59**(5): 545-566.

Summary of China Cardiovascular Disease Report (2018). *Chin Circul. J*., **34**(03): 6-17.

Scott SA, Sangkuhl K, Stein CM, Hulot JS, Mega JL, Roden DM, Klein TE, Sabatine MS, Johnson JA, Shuldiner AR(2013). Clinical pharmacogenetics implementation consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 Update. *Clin. Pharmacol. Ther*., **94**(3): 317-323.

Wang L, Yuan LJ, Tang XY (2015). Study on correlation between the gene ploymorphisms of CYP2C19 and the curative effects of clopidogrel in patients with acute cerebral infarction. *J. Clin. Neurol*., **28**(2): 99-102.

Zhong ZX, Hou JY, Zhang QF, Li B, Li C, Liu ZD, Yang M, Zhong W, He XB, Wu HS, Zhong MC and Zhao PS (2018). Effect of cytochrome P450 2C19 polymorphism on adverse cardiovascular events after drug-eluting stent implantation in a large Hakka population with acute coronary syndrome receiving clopidogrel in southern China. *Eur. J. Clin. Pharmacol.,* **74**(4): 423-431.