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Prevalence and types of genetic polymorphisms of CYP2C19 and their effects on platelet aggregation inhibition by clopidogrel

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Abstract. – OBJECTIVE: The current study was conducted to determine the distribution of genetic polymorphisms in CYP2C19 in Iraqi patients and their role in inter-individual variability of clopidogrel efficacy.

PATIENTS AND METHODS: A prospective controlled study was done on 100 patients under high risk of cardiovascular diseases who started clopidogrel prophylactic therapy. Polymerase chain reaction-restriction fragment length polymorphism method was used to determine the existence of the CYP2C19 gene mutation. Vasodilator-stimulated phosphoprotein (VASP) index baseline besides one-month post-therapy was analyzed by dual-color flow cytometry analysis.

RESULTS: Eight gene mutations of CYP2C19 were found (*1/*1), (*1/*2), (*1/*3), (*1/*8), (*1/*17), (*2/*2), (*2/*4), and (*3/*3) with higher prevalent CYP2C19*1 gene. Homozygous CYP2C19*1 allele was shown to be the rapid metabolizer comparing to the heterozygous CYP2C19*1 allele, whereas, CYP2C19*2 and CYP2C19*3 were resistant alleles and were present in 28% of patients. The analysis of VASP phosphorylation produces accurate inter-individual response variability in platelets inhibition by antiplatelet drugs.

CONCLUSIONS: *In vitro* gene analysis and VASP index improve the clinical outcome of a patient candidate to clopidogrel as prophylaxis in cardiovascular events.

Key Words:
Genetic polymorphisms, CYP2C19, Clopidogrel, VASP index, Cardiovascular diseases.

Introduction

Antiplatelet therapy has a central role in the prevention of coronary artery diseases. Clopidogrel is one of many antiplatelet drugs frequently considered for unrestrained use in patients with peripheral artery diseases[1]. It can decrease mortality and improve cardiovascular outcomes without increases bleeding tendency in patients with acute coronary syndrome[2]. Cannon et al[3] indicated that clopidogrel in a dosage of 75 mg daily has a significant reduction in cardiovascular endpoints by approximately 25%[3].

Current approaches in patients undergoing optional percutaneous coronary intervention (PCI) include administration of aspirin, a P2Y12 antagonist (clopidogrel, prasugrel, or ticagrelor), an antithrombin agent, and occasionally a glycoprotein (GP) IIb/IIIa receptor antagonist. Clopidogrel inhibits adenosine diphosphate (ADP) binding to the P2Y12 platelet receptor; thus, prevents platelet activation and, consequently, the process of aggregation[4].

There are many well-known factors associated with pharmacokinetics and pharmacodynamics variability of clopidogrel, including patient compliance, diet, smoking, alcohol, demographic factors, drug-drug interaction (enzyme inducer or enzyme inhibitor), and platelet hyperactivity. Moreover, the genetic polymorphisms of

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cytochrome P4502C19 (CYP2C19), which is the main hepatic enzyme activator of clopidogrel, can lead to significant inter-individual variation in the antiplatelet activities[5].

Clopidogrel is a prodrug, which needs process of enzymatic activation in two steps within hepatocyte by a CYP-dependent pathway. Less than 15% of clopidogrel dose transformed into thiol metabolite (active metabolite), while the remaining (>85%) converted into two inactive metabolites (1-oxo-clopidogrel-carboxylic acid and thiol metabolite carboxylic acid)[6]. Inter-individual variability in pharmacokinetics and pharmacodynamics, which may affect drug efficacy, is mainly related to the genetic polymorphism in the CYP oxidative enzyme family[7]. CYP2C19 consists of nine coding exons spanning 90,209 bases with a coding region of 1473 bases (Figure 1); it could hold a variety of genetic alleles; some of them can powerfully affect their metabolic activity[8]. Types of these alleles were vigorously studied and documented more than seventeen variant alleles of single nucleotide polymorphisms (SNPs). The most prevalent alleles of CYP2C19 are designated as CYP2C19*1, CYP2C19*2, CYP2C19*3, and CYP2C19*17[9]. Genetic allele CYP2C19*1/*1 has regular activity, whereas CYP2C19*2 and CYP2C19*3 have no metabolic activity for clopidogrel. Moreover, the genetic allele of CYP2C19*17 has been associated with the higher antiplatelet activity of clopidogrel[10]. The genetic allele is commonly present as two SNPs, which lead to unexpected activity. Other less frequently alleles, which are characterized by reduced or loss of enzymatic activity, include CYP2C19*4, *5, *6, *7, and *8[11]. The objective of this study was to determine the prevalence of genetic CYP2C19 polymorphisms in Iraqi patients and their effects on clopidogrel responsiveness using the principle of the vasodilator-stimulated phosphoprotein (VASP) index.

Patients and Methods

Patients

A prospective controlled study included 100 patients admitted to the Baghdad Teaching Hospital, Medical City, Baghdad, Iraq from June 2019 to January 2020, aged between 40 to 65 years old, not on clopidogrel, antiplatelet or anticoagulant therapy. Those patients are candidates to use clopidogrel as prophylaxis against cardiovascular diseases. All patients are informed of the research steps and objectives, and they are given written informed consent to sign before inclusion.

The patients excluded from the present study are those contraindicated for antiplatelet therapy, smokers, pregnant women, patients concomitantly using drugs causing enzyme inducers or inhibitors, patients with a history of pathological bleeding, and patients who have VASP index below 55%. The patient's demography was documented, including age, sex, blood pressure, diabetes, heart failure, and myocardial infarction (Table I).

Research Method

Before the initiation of clopidogrel therapy, all patients had their blood samples from the antecubital vein by non-traumatic venipuncture, and their VASP index baseline was analyzed. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to determine the existence of the CYP2C19 gene mutation. All patients started with clopidogrel 75 mg daily and re-evaluation was established after 30 days.

Efficacy of Clopidogrel by VASP Index

Clopidogrel efficacy was evaluated for one-month and the efficacy was tested within 48 h after collection of blood. Blood samples were stored

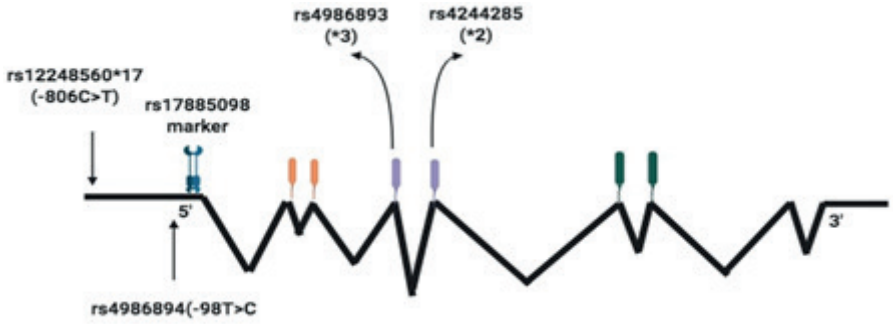

Figure 1. Cytochrome P4502C19.

Table I. Demographic distribution of patients.

Disease	Female (n=30) Age (59.47±5.56)	Male (n=70) Age (56.20±6.86)	Total frequency (n=100) Age (57.18±6.64)
Hypertension	6 (20.00%)	18 (25.71%)	24 (24.00%)
Hypertension + Diabetes mellitus	14 (46.67%)	26 (37.14%)	40 (40.00%)
Hypertension + Heart failure	6 (20.00%)	16 (22.86%)	22 (22.00%)
Hypertension + Myocardial infarction	4 (13.33%)	10 (14.29%)	14 (14.00%)

at room temperature (18-25°C) in trisodium citrate (0.129 M) in the ratio of 9:1 (blood to citrate) and were analyzed using PLT VASP/P2Y12 kit (Biocytex, Marseille, France) by following company guidelines. Initially, blood samples were incubated with prostaglandin E1 (PGE1) alone or PGE1 with ADP. All samples were labeled with clone 16C2 by indirect no-wash immunofluorescence. Dual-color flow cytometry analysis (Coulter EPICS XL cytometer; Beckman Coulter Inc., Fullerton, CA, USA) was used to compare the results of two samples to inhibit VASP phosphorylation by ADP. The platelet reactivity index was measured by the ratio, 100 × [(Mean fluorescence intensity PGE1− Mean fluorescence intensity ADP+PGE1)/ Mean fluorescence intensity PGE1]. The cut-off value of the VASP index for detecting clopidogrel activity was 55%[12].

Polymorphism in CYP2C19 Gene

SNPs of interest were detected using primer extension technique, as described by Adithan et al[13]. Patients were grouped according to the CYP2C19 genotypes to evaluate their effect on the VASP index.

Ethics Statement

The Research Ethics Committee at Baghdad Teaching Hospital, Baghdad, Iraq approved this study protocol (Ethical approval No: 1448 on May-2019). Further, an informed verbal consent was obtained from all the study participants to include in the study.

Statistical Analysis

Statistical analysis was performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Statistically significant values are considered at $p < 0.05$. The mean-standard deviation is used to express variables. Frequency and percentage are used to represent the categorical variables. A Chi-square test was used to assess independent variability. The normal platelet reactivity without clopidogrel is above 60-70%[14].

Results

Genotype Distribution

The genotype results are shown in Figure 2. For total 100 patients, eight gene mutations of

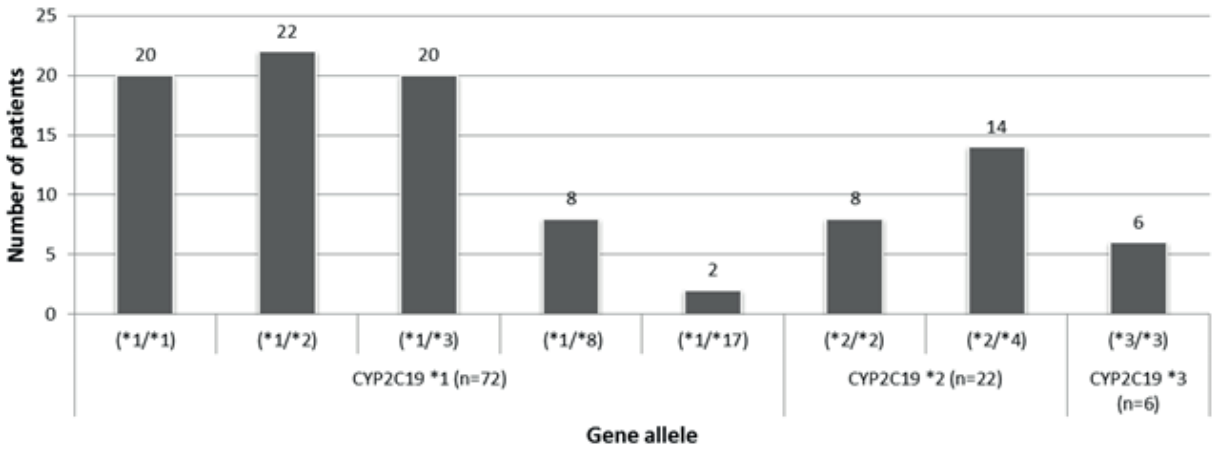

Figure 2. Genotype distribution.

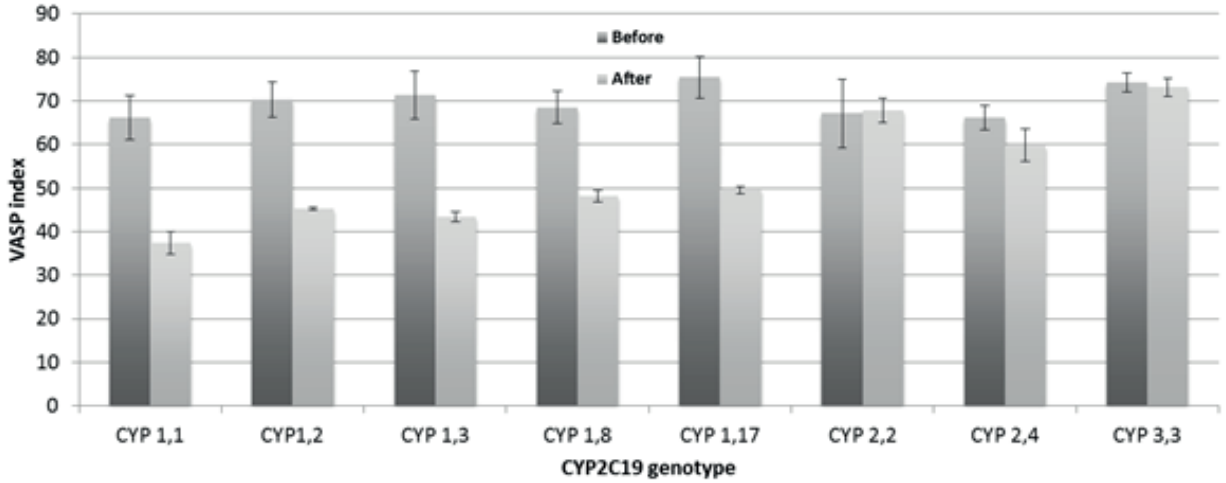

Figure 3. CYP2C19 gene mutation in relation to the VASP index.

CYP2C19 were found (*1/*1), (*1/*2), (*1/*3), (*1/*8), (*1/*17), (*2/*2), (*2/*4), and (*3/*3). The distribution of these mutations was: 72 patients found to have CYP2C19 *1 gene and 28 patients were detected to have CYP2C19 *2 and CYP2C19 *3 gene mutation.

VASP phosphorylation

A total of 100 patients had a baseline VASP index of 69.13 ± 5.47% before starting clopidogrel therapy. Thirty days after clopidogrel therapy in a dose of 75 mg/day resulted in a significant reduction in the VASP index to 49.58 ± 11.68 (p = 0.001). The result indicated a small variation in the VASP index between the patients before therapy, ranging from 50.37 to 80.42; conversely, higher variation seen after therapy with a range of 35.11 to 78.17 (Table II).

The distribution of the CYP2C19 gene mutation related to the VASP index was illustrated in Table III and Figure 3. One month after clopidogrel therapy, the VASP index significantly decreased in patients with CYP2C19*1 allele. Homozygous CYP2C19*1 allele was shown to be the rapid metabolizer comparing to the heterozygous CYP2C9*1 allele. In contrast, patients with homozygous CYP2C19*2 alleles show no significant effect on the VASP index (p = 0.84), while heterozygous CYP2C19*2 alleles significantly reduced VASP index. For patients with homozygous CYP2C19*3 alleles, the effect on the VASP index was non-significant.

Discussion

Genetic polymorphism of CYP2C19 is widely distributed and results in wide variability in clopidogrel therapeutic effect[15]. The most common polymorphism of CYP2C19 is the genotype of CYP2C19*1, CYP2C19*2, CYP2C19*3, and rare type of CYP2C19*4, CYP2C19*5, CYP2C19*8[16]. The activity of CYP2C19 was expressed as fast, intermediate, and slow metabolizer. Homozygous type of CYP2C19*1 allele (CYP2C19*1/*1) is a rapid metabolizer, whereas heterozygous types of CYP2C19*1/*2, CYP2C19*1/*3, CYP2C19*1/*4, and CYP2C19*1/*8 alleles are described as intermediate metabolizer. The homozygous CYP2C19*2/*2 and CYP2C19*3/*3, as well as the heterozygous CYP2C19*2/*3 and CYP2C19*2/*4 alleles exhibit slow metabolizing activity[5,17]. The high risk

Table II. VASP index before and after clopidogrel therapy.

VASP index (total patients)				
	Mean ± SD	Range	Difference	*p*-value
Before treatment	69.13 ±5.47	50.37 - 80.42	30.05	.001
After treatment	49.58 ± 11.68	35.11 - 78.17	43.06	

Table III. CYP2C19 gene mutation in relation to the VASP index.

Genotype		VASP index		Difference of mean	*p*-value
		Before clopidogrel treatment	One month after clopidogrel treatment		
CYP2C19 *1 (N=72)	CYP2C19 (*1/*1) (N=20)	66.08 ± 5.09	37.37 ± 2.52	28.71	.00
	CYP2C19 (*1/*2) (N=22)	70.35 ± 4.07	45.32 ± 0.34	25.03	.00
	CYP2C19 (*1/*3) (N=20)	71.36 ± 5.43	43.37 ± 1.17	27.99	.00
	CYP2C19 (*1/*8) (N=8)	68.52 ± 3.69	48.09 ± 1.36	20.43	.00
	CYP2C19 (*1/*17) (N=2)	75.42	49.53	25.89	-
	Total (N=72)	69.41 ± 5.12	42.94 ± 4.13	26.47	.00
CYP2C19 *2 (N=22)	CYP2C19 (*2/*2) (N=8)	67.15 ± 7.84	67.96 ± 2.77	0.81	.84
	CYP2C19 (*2/*4) (N=14)	66.21 ± 2.82	59.81 ± 3.70	6.4	.03
	Total= 22	66.81 ± 6.28	64.87 ± 4.98	1.94	.43
CYP2C19 *3 (N=6)	CYP2C19 (*3/*3)	74.28 ± 2.21	73.15 ± 2.12	1.13	.55

of major adverse cardiovascular events (MACE) associated with clopidogrel use in *CYP2C19* loss of function allele carriers suggests that the use of genotype-guided dual antiplatelet therapy (DAPT) in practice may improve clinical outcomes[18]. Friedman et al[19] consistently demonstrate that a genotype-guided DAPT reduces the risk of MACE in ≈30% of the US population, with less risk of bleeding tendency. Moreover, the use of genotype-guided antiplatelet therapy improves patient outcomes following PCI[20]. Similar results were found in patients with cerebrovascular diseases using clopidogrel[21]. The relationship between CYP2C19 metabolizer status and clinical outcomes was extensively studied. CYP2C19 rapid and ultrarapid metabolizers treated with clopidogrel show high plasma concentration of clopidogrel active metabolite and increased inhibition of platelet aggregation, as well as higher bleeding risk and lower MACE risk among clopidogrel-treated patients[22]. Therefore, assessment of patients' clinical risk is required to determine if a patient is a poor metabolizer before starting clopidogrel therapy. Genetic testing is essential for patients believed to be at moderate or high risk for poor outcomes, like patients undergoing elective high-risk PCI procedures[23].

In the current study, the CYP2C19 gene alleles were three types (CYP2C19*1, CYP2C19*2, and CYP2C19*3). The higher frequency was CYP2C19*1 (72%), whereas CYP2C19*2 and CYP2C19*3 were less frequent (22% and 6%, respectively). Different results have been reported in other studies (Table IV). The frequency of CYP2C19*1 alleles found in the present study was close to Iranian and Saudi Arabian populations but lower than the frequencies in the Jordanian, Egyptian, and Turkish people (84.0%, 87.8%, and 88.0%, respectively). CYP2C19*2 alleles frequencies in Iranian, Saudi Arabian, and Turkish people were 12.0%, 11.2%, and 11.6%, respectively, are closed to our finding, but less than Jordanian (16.0%) and more than Egyptian (10.9%) population[24-28]. CYP2C19*3 alleles were absent in Saudi Arabian and Jordanian populations, as well as the Iranian and Turkish populations[24-28]. These individualized differences could be due to variability in the studied populations and the small size of our study (one of its limitations).

Platelet function testing is a promising alternative option for patients with suboptimal efficacy of clopidogrel[29]. The latest American Heart Association (AHA) guideline recommends that patients under high risk of thrombosis, such as bifurcating left main, unprotected left main, or last patent coronary vessel, higher dose of clopidogrel (150 mg daily) is considered if the inhibition of platelet aggregation was less than 50%[30].

VASP is a widely distributed vertebrate intracellular protein activated by phosphorylation that controls all vasodilator-stimulated phosphor-pro-

Table IV. Alleles and genotype frequencies of CYP2C19 in different countries.

Country	No.	Reference	CYP2C19 allele frequency (%)			CYP2C19 genotype frequency (%)							
			*1	*2	*3	*1/*1	*1/*2	*1/*3	*1/*8	*1/*17	*2/*2	*2/*4	*3/*3
Iraq	100	Current study	66.3	12.8	1	46.9	13.7	10	4	1	4	7	3
Iran	84	24	71	12	1	71	0	0	-	-	12	-	1
Saudi Arabia	201	25	62.9	11.2	0	40.3	14.5	0	-	20.4	0.4	-	0
Jordan	78	26	84.0	16	0	74.4	19.2	0	-	-	6.4	-	0
Egypt	120	27	87.8	10.9	12.8	78.5	20	0.4	-	-	0.8	-	-
Turkish	404	28	88	11.6	0.4	37	35	-	-	-	6	6	0

tein function[31]. Platelet activation begins after adhesion to the damaged vascular wall, mainly regulated by endothelial factors, nitric oxide (NO), and prostacyclin. These factors activate soluble guanylyl cyclase that subsequently increases platelet cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP). The NO/cGMP and prostacyclin/cAMP signaling are mainly targeting the cGMP-dependent protein kinase I and cAMP-dependent protein kinase[32]. The common substrate of these kinases is the VASP, which is distributed in a variety of cells and tissues in addition to the platelets. In platelets, VASP is strategically located at the intersection of two major inhibitory pathways[33].

Studies showed the direct correlation of VASP phosphorylation with the inhibition of platelet activation and the expression of GP IIb/IIIa receptors[34]. Moreover, in VASP-deficient mice, platelets show more expression of P-selectin and GP IIb/IIIa receptors[32]. Meanwhile, the antagonism of platelet ADP receptor P2Y12 by clopidogrel was found to suppress VASP phosphorylation and prevent platelet aggregation. Therefore, VASP is associated with the regulation of platelet stimulation both *in vitro* and *in vivo*[34]. In accordance with these observations, the inhibition of platelet aggregation by antiplatelet drugs can impair VASP knockout. Consequently, VASP phosphorylation could be used as a marker for antiplatelet activities[14].

One of the most common principles to describe inter-individual variability in patients treated with P2Y12 receptor antagonists is via the VASP index as explained by Bal Dit Sollier et al[35]. As clopidogrel inhibits platelet aggregation by blocking ADP-dependent platelets receptor activation, the present study's result allows for two main conclusions: firstly, the measurement of VASP phosphorylation by cytometry was useful for determination of reduction in platelets' activity by clopidogrel therapy. Secondly, the analysis of VASP phosphorylation produces accurate inter-individual response variability in platelets' inhibition by antiplatelet drugs.

Our finding illustrates three types of genotype distributed in the studied patients. The most prevalent was the CYP2C19*1 allele that demonstrates an excellent response to clopidogrel therapy with a significant reduction in the VASP phosphorylation index (<55%). This result supported by Samoš et al[36] found a good activity of P2Y12 receptor antagonist measured as the VASP phosphorylation index. Meanwhile, the clopidogrel loading dose adjustment significantly improves the clinical outcome after PCI in patients using the VASP index[37]. The homozygous alleles of the CYP2C19*1 allele found to be rapid metabolizers comparing to the heterozygous alleles. This was illustrated by a more significant reduction in the VASP index for homozygous *vs.* heterozygous alleles. Our result was consistent with many global studies[38-40].

Two groups of patients were found to be resistant to clopidogrel therapy carrying CYP2C19*2 and CYP2C19*3 alleles that have a VASP phosphorylation index >55%. This result supported by Nasyuhana et al[41] concluded that the CYP2C19*2 allele was related to a decrease in platelet responsiveness to clopidogrel. Despite the higher prevalence of CYP2C19*1 allele, the occurrence of total resistant alleles was 28% of the separately studied patients; CYP2C19*2 and CYP2C19*3 were founded in 22% and 6% of patients, respectively. For patients with CYP2C19*2 alleles, both homozygous and heterozygous alleles seemed re-

sistant to clopidogrel therapy. Mega et al[42] studied the effect of different clopidogrel dosing and found that a subset of CYP2C19*2 homozygous patients, even with a dose of 300 mg of clopidogrel was unable to accomplish on-treatment platelet reactivity. Conversely, the high clopidogrel-loading dose of 900 mg was able to adequately lower on-treatment platelet reactivity.

Other potential mechanisms of genetic variability of clopidogrel activity, which should be considered, are polymorphisms in the ABCB1 genes encoding P-glycoprotein (an efflux transporter)[43] and paraoxonase-1 (PON1) (the rate-limiting enzyme in clopidogrel metabolic activation). Momary et al[44] studied the effect of polymorphisms in ABCB1 genes and found a significant association with variability in oral bioavailability of clopidogrel. Polymorphism in PON1-192Q and R isoforms found to cause different rates of clopidogrel activation[45]. Furthermore, Polasek et al[46] found lower plasma concentrations of the active metabolite of clopidogrel and lower platelet inhibition in association with a lower PON1 plasma activity.

The polymorphism in the P2Y12 receptor for clopidogrel and its effector, glycoprotein IIb/IIIa, could be another cause for variability in clopidogrel effect. A study conducted on 375 patients with ischemic stroke did not show any association between polymorphisms of P2Y12 or GPIIIa and clinical outcomes[47]. Besides, Lev et al[48] and Floyd et al[49] did not find significant associations between polymorphisms in P2Y12, P2Y1, and GPIIb/IIIa with responsiveness clopidogrel in patients with cardiovascular diseases. In contrast, recent studies[50,51] have shown that SNPs in P2Y12 and GPIIIa genes may affect antiplatelet drug responsiveness. These mechanisms need further investigation to confirm their role in clopidogrel efficacy.

Conclusions

An *in vitro* genotype analysis can predict the antiplatelet activity of clopidogrel and improves clinical outcomes in the treatment setting with PCI or patients with cardiovascular diseases. The CYP2C19*1 has rapid metabolic activity and results in the good antiplatelet activity of clopidogrel, whereas the CYP2C19*2 and CYP2C19*3 are characterized by poor platelet responsiveness to clopidogrel and may, therefore, be an essential genetic indicator to therapeutic failure of clopidogrel in the clinical settings. Long-term trials with large-scale data are necessary to determine the prevalence of other CYP2C19 alleles and their precise role in clopidogrel efficacy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Franchi F, Angiolillo DJ. Novel antiplatelet agents in acute coronary syndrome. Nat Rev Cardiol 2015; 12: 30-47.
2) Aradi D, Komócsi A, Vorobcsuk A, Serebruany VL. Impact of clopidogrel and potent P2Y 12-inhibitors on mortality and stroke in patients with acute coronary syndrome or undergoing percutaneous coronary intervention: a systematic review and meta-analysis. Thromb Haemost 2013; 109: 93-101.
3) Cannon CP, Harrington RA, James S, Ardissino D, Becker RC, Emanuelsson H, Husted S, Katus H, Keltai M, Khurmi NS, Kontny F. Comparison of ticagrelor with clopidogrel in patients with a planned invasive strategy for acute coronary syndromes (PLATO): a randomised double-blind study. Lancet 2010; 375: 283-293.
4) Wijeyeratne YD, Heptinstall S. Anti-platelet therapy: ADP receptor antagonists. Br J Clin Pharmacol 2011; 72: 647-657.
5) Brown SA, Pereira N. Pharmacogenomic impact of CYP2C19 variation on clopidogrel therapy in precision cardiovascular medicine. J Pers Med 2018; 8: 8.
6) Jiang XL, Samant S, Lesko LJ, Schmidt S. Clinical pharmacokinetics and pharmacodynamics of clopidogrel. Clin Pharmacokinet 2015; 54: 147-166.
7) Hirota T, Eguchi S, Ieiri I. Impact of genetic polymorphisms in CYP2C9 and CYP2C19 on the pharmacokinetics of clinically used drugs. Drug Metab Pharmacokinet 2013; 28: 28-37.
8) Sanford JC, Guo Y, Sadee W, Wang D. Regulatory polymorphisms in CYP2C19 affecting hepatic expression. Drug Metabol Drug Interact 2013; 28: 23-30.
9) Vu NP, Nguyen HT, Tran NT, Nguyen TD, Huynh HT, Nguyen XT, Nguyen DT, Nong HV, Nguyen HH. CYP2C19 genetic polymorphism in the Vietnamese population. Ann Hum Biol 2019; 46: 491-497.
10) Klein MD, Williams AK, Lee CR, Stouffer GA. 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 2019; 39: 647-652.
11) Mirabbasi SA, Khalighi K, Wu Y, Walker S, Khalighi B, Fan W, Kodali A, Cheng G. CYP2C19 genetic variation and individualized clopidogrel prescription in a cardiology clinic. J Community Hosp Intern Med Perspect 2017; 7: 151-156.
12) Pettersen AA, Arnesen H, Opstad TB, Seljeflot I. The influence of CYP 2C19*2 polymorphism on plate-

let function testing during single antiplatelet treatment with clopidogrel. Thromb J 2011; 9: 4.
13) Adithan C, Gerard N, Vasu S, Rosemary J, Shashindran CH, Krishnamoorthy R. Allele and genotype frequency of CYP2C19 in a Tamilian population. Br J Clin Pharmacol 2003; 56: 331-333.
14) Schwarz UR, Geiger J, Walter U, Eigenthaler M. Flow cytometry analysis of intracellular VASP phosphorylation for the assessment of activating and inhibitory signal transduction pathways in human platelets--definition and detection of ticlopidine/clopidogrel effects. Thromb Haemost 1999; 82: 1145-1152.
15) Qiu LN, Sun Y, Wang L, Han RF, Xia XS, Liu J, Li X. Influence of CYP2C19 polymorphisms on platelet reactivity and clinical outcomes in ischemic stroke patients treated with clopidogrel. Eur J Pharmacol 2015; 747: 29-35.
16) Lee SJ. Clinical Application of CYP2C19 Pharmacogenetics toward more personalized medicine. Front Genet 2013; 3: 318.
17) Dean L. Clopidogrel Therapy and CYP2C19 Genotype. In: Pratt VM, McLeod HL, Rubinstein WS, et al, eds. Medical Genetics Summaries. Bethesda (MD): National Center for Biotechnology Information (US), 2012.
18) Ma TK, Lam YY, Tan VP, Yan BP. Variability in response to clopidogrel: how important are pharmacogenetics and drug interactions? Br J Clin Pharmacol 2011; 72: 697-706.
19) Friedman KG, Gauvreau K, Hamaoka-Okamoto A, Tang A, Berry E, Tremoulet AH, Mahavadi VS, Baker A, deFerranti SD, Fulton DR, Burns JC, Newburger JW. Coronary artery aneurysms in Kawasaki disease: risk factors for progressive disease and adverse cardiac events in the US population. J Am Heart Assoc 2016; 5: e003289.
20) Klein MD, Lee CR, Stouffer GA. Clinical outcomes of CYP2C19 genotype-guided antiplatelet therapy: existing evidence and future directions. Pharmacogenomics 2018; 19: 1039-1046.
21) Lee CR, Sriramoju VB, Cervantes A, Howell LA, Varunok N, Madan S, Hamrick K, Polasek MJ, Lee JA, Clarke M, Cicci JD, Weck KE, Stouffer GA. Clinical outcomes and sustainability of using CYP2C19 genotype-guided antiplatelet therapy after percutaneous coronary intervention. Circ Genom Precis Med 2018; 11: e002069.
22) Subraja K, Dkhar SA, Priyadharsini R, Ravindra BK, Shewade DG, Satheesh S, Sridhar MG, Narayan SK, Adithan C. Genetic polymorphisms of CYP2C19 influences the response to clopidogrel in ischemic heart disease patients in the South Indian Tamilian population. Eur J Clin Pharmacol 2013; 69: 415-422.
23) Sibbing D, Aradi D, Alexopoulos D, Ten Berg J, Bhatt DL, Bonello L, Collet JP, Cuisset T, Franchi F, Gross L, Gurbel P, Jeong YH, Mehran R, Moliterno DJ, Neumann FJ, Pereira NL, Price MJ, Sabatine MS, So DYF, Stone GW, Storey RF, Tantry U, Trenk D, Valgimigli M, Waksman R, Angiolillo DJ. Updated expert consensus statement on platelet function and genetic testing for guiding P2Y12 receptor inhibitor treatment in percutaneous coronary intervention. JACC Cardiovasc Interv 2019; 12: 1521-1537.
24) Zahedi M, Shabestari M, Ayatollahi H, Gholoobi A. CYP2C19 genetic polymorphism in the East of Iran: its association with the severity and pattern of coronary artery disease. J Cardio-Thorac Med 2019; 7: 417-421.
25) Saeed LH, Mayet AY. Genotype-phenotype analysis of CYP2C19 in healthy saudi individuals and its potential clinical implication in drug therapy. Int J Med Sci 2013; 10: 1497-1502.
26) Zalloum I, Hakooz N, Arafat T. Genetic polymorphism of CYP2C19 in a Jordanian population: influence of allele frequencies of CYP2C19*1 and CYP2C19*2 on the pharmacokinetic profile of lansoprazole. Mol Biol Rep 2012; 39: 4195-4200.
27) Hamdy SI, Hiratsuka M, Narahara K, El Enany M, Moursi N, Ahmed MS, Mizugaki M. Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. Br J Clin Pharmacol 2002; 53: 596-603.
28) Nassar S, Amro O, Abu-Rmaileh H, Alshaer I, Korachi M, Ayesh S. ABCB1 C3435T and CYP2C19*2 polymorphisms in a Palestinian and Turkish population: A pharmacogenetic perspective to clopidogrel. Meta Gene 2014; 2: 314-319.
29) Lordkipanidzé M, So D, Tanguay J-F. Platelet function testing as a biomarker for efficacy of antiplatelet drugs. Biomark Med 2016; 10: 903-918.
30) von Beckerath N, Kastrati A, Wieczorek A, Pogatsa-Murray G, Sibbing D, Graf I, Schömig A. A double-blind, randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days. Eur Heart J 2007; 28: 1814-1819.
31) Tsai EJ, Kass DA. Cyclic GMP signaling in cardiovascular pathophysiology and therapeutics. Pharmacol Ther 2009; 122: 216-238.
32) Massberg S, Grüner S, Konrad I, Konrad I, Garcia Arguinzonis MI, Eigenthaler M, Hemler K, Kersting J, Schulz C, Müller I, Besta F, Nieswandt B. Enhanced in vivo platelet adhesion in vasodilator-stimulated phosphoprotein (VASP)-deficient mice. Blood 2004; 103: 136-142.
33) Tantry US, Bliden KP, Gurbel PA. Resistance to antiplatelet drugs: current status and future research. Expert Opin Pharmacother 2005; 6: 2027-2045.
34) Benz PM, Laban H, Zink J, Günther L, Walter U, Gambaryan S, Dib K. Vasodilator-Stimulated Phosphoprotein (VASP)-dependent and -independent pathways regulate thrombin-induced activation of Rap1b in platelets. Cell Commun Signal 2016; 14: 21.
35) Bal Dit Sollier C, Berge N, Boval B, Dubar M, Drouet L. Differential sensitivity and kinetics of response of different ex vivo tests monitoring functional variability of platelet response to clopidogrel. Thromb Haemost 2010; 104: 571-581.
36) Samoš M, Šimonová R, KováĐ F, Duraj L, Fedorová J, Galajda P, Staško J, Fedor M, Kubisz P, MokáĐ M. Clopidogrel resistance in diabetic patient with acute myocardial infarction due to stent thrombosis. Am J Emerg Med 2014; 32: 461-465.
37) Bonello L, Camoin-Jau L, Arques S, Boyer C, Panagides D, Wittenberg O, Simeoni MC, Barragan P, Dignat-George F, Paganelli F. Adjusted clopidogrel

loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance: a multicenter randomized prospective study. J Am Coll Cardiol 2008; 51: 1404-1411.
38) Chaudhry AS, Kochhar R, Kohli KK. Genetic polymorphism of CYP2C19 & therapeutic response to proton pump inhibitors. Indian J Med Res 2008; 127: 521-530.
39) Ragia G, Arvanitidis KI, Tavridou A, Manolopoulos VG. Need for reassessment of reported CYP2C19 allele frequencies in various populations in view of CYP2C19*17 discovery: the case of Greece. Pharmacogenomics 2009; 10: 43-49.
40) Santos PC, Soares RA, Santos DB, Nascimento RM, Coelho GL, Nicolau JC, Mill JG, Krieger JE, Pereira AC. CYP2C19 and ABCB1 gene polymorphisms are differently distributed according to ethnicity in the Brazilian general population. BMC Med Genet 2011; 12: 13.
41) Nasyuhana Sani Y, Sheau Chin L, Luen Hui L, Mohd Redhuan Shah Edwin NE, Teck Hwa G, Serebruany VL, Kah Hay Y. The CYP2C19(*)1/(*)2 genotype does not adequately predict clopidogrel response in healthy malaysian volunteers. Cardiol Res Pract 2013; 2013: 128795.
42) Mega JL, Hochholzer W, Frelinger AL, Kluk MJ, Angiolillo DJ, Kereiakes DJ, Isserman S, Rogers WJ, Ruff CT, Contant C, Pencina MJ. Dosing clopidogrel based on CYP2C19 genotype and the effect on platelet reactivity in patients with stable cardiovascular disease. JAMA 2011; 306: 2221-2228.
43) Schumacher T, Benndorf RA. ABC transport proteins in cardiovascular disease–A brief summary. Molecules 2017; 22: 589.
44) Momary KM, Dorsch MP, Bates ER. Genetic causes of clopidogrel nonresponsiveness: which ones really count? Pharmacotherapy 2010; 30: 265-274.
45) Mackness M, Mackness B. Human paraoxonase-1 (PON1): gene structure and expression, promiscuous activities and multiple physiological roles. Gene 2015; 567: 12-21.
46) Polasek TM, Doogue MP, Miners JO. Metabolic activation of clopidogrel: in vitro data provide conflicting evidence for the contributions of CYP2C19 and PON1. Ther Adv Drug Saf 2011; 2: 253-261.
47) Yi X, Wang Y, Lin J, Cheng W, Zhou Q, Wang C. Interaction of CYP2C19, P2Y12, and GPIIIa variants associates with efficacy of clopidogrel and adverse events on patients with ischemic stroke. Clin Appl Thromb Hemost 2017; 23: 761-768.
48) Lev EI, Patel RT, Guthikonda S, Lopez D, Bray PF, Kleiman NS. Genetic polymorphisms of the platelet receptors P2Y12, P2Y1 and GP IIIa and response to aspirin and clopidogrel. Thromb Res 2007; 119: 355-360.
49) Floyd CN, Ferro A. The PlA1/A 2 polymorphism of glycoprotein IIIa in relation to efficacy of antiplatelet drugs: a systematic review and meta-analysis. Br J Clin Pharmacol 2014; 77: 446-457.
50) Saydam F, DeĐirmenci İ, Birdane A, Özdemir M, Ulus T, Özbayer C, Çolak E, Ata N, GüneĐ HV. The CYP2C19* 2 and CYP2C19* 17 polymorphisms play a vital role in clopidogrel responsiveness after percutaneous coronary intervention: a pharmacogenomics study. Basic Clin Pharmacol Toxicol 2017; 121: 29-36.
51) Fontana P, Dupont A, Gandrille S, Bachelot-Loza C, Reny JL, Aiach M, Gaussem P. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. Circulation 2003; 108: 989-995.