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Clopidogrel Pharmacogenetics in Iranian Patients Undergoing Percutaneous Coronary Intervention

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Abstract

Clopidogrel is used in patients with coronary syndromes and at risk of thrombotic events or receiving percutaneous coronary intervention (PCI) for reducing heart attack and stroke. Here we present genotype and phenotype study of Iranian patients undergoing PCI treated with clopidogrel during a 6-month period of follow-up; common variants of *CYP2C19, CYP3A5, CYP3A4*, and *ABCB1* genes were determined as well as the patients' cardiovascular outcomes to find out the effect of these variants individually and in combination. 388 individuals receiving PCI were enrolled in this study. Different pretreatment doses of clopidogrel were prescribed under the interventional cardiologists' guidance. The patients were followed for a duration of 1 month, and 6 months. Six SNPs were selected for genotyping including *CYP2C19**2 (c.681G > A), *CYP2C19*3* (c.636G > A), *CYP2C19**17 allele (c.−806C > T), *ABCB1* (c.3435C > T), *CYP3A5* (c.6986A > G), and *CYP3A4* (c.1026 + 12G > A). The mean loading dose was 600 mg/day in 267 (68.8%) individuals, 300 mg/day in 121 (31.2%). 8 patients had cardiovascular events such as thrombosis, unstable angina, and non-STEMI. The studied alleles and genotypes were in Hardy–Weinberg equilibrium. None of the SNPs individually were significantly associated with outcome events. Our results indicate that combinations of different alleles of genes are involved in pharmacokinetic variability and joint factors are important; this means that genotyping and analysis of an individual variant may not be as straightforward in risk assessment and pharmacogenetics. This highlights the importance of personalized medicine in risk assessment and treatment.

Keywords Genetic variants · Polymorphism · Clopidogrel · Cardiovascular event

Introduction

Genetic variability of metabolizing enzymes promotes our understanding of pharmacogenetics and personalized medicine [1]. Genetic analysis promises to determine the susceptible and resistance variants, translating the genetic into clinical practice. The genes and genetic variability of platelet aggregation pathway has been well recognized including cytochrome 450 enzymes *CYP2C19, CYP3A5, CYP3A4* (active metabolizer), *ABCB1* (absorption regulation) [2], and *P2RY12* (receptor on platelet) [3]. Metabolism of drugs for example clopidogrel occurs in liver in two oxidative steps using different enzymes; for this pro-drug, first step would produce 2-oxo-clopidogrel, which is then mainly converted to active metabolites by *CYP3A4* enzyme [4, 5]. *CYP2C19* appears in phases 1 and 2 [6]. 85% of drug metabolites are converted to inactive form while the remaining having thiol group binds to $P2RY_{12}$ (*ITGA2* gene) receptor and blocks ADP binding and its activation. Platelet aggregation is, therefore, inhibited, and then fibrinogen receptor (glycoprotein gp-IIb/IIIa receptor encoded by *ITGB3*) is blocked [7]. *CYP3A4* and *CYP3A5* are hepatic cytochrome P450 3A which generates an active metabolite inhibiting platelet activation. Also, *ABCB1* is the protein used for transportation of drugs into intestine.

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Clopidogrel is prescribed in patients with coronary syndromes and at risk of thrombotic events or receiving percutaneous coronary intervention (PCI) for reducing heart attack and stroke [8]. The pharmacodynamic response to clopidogrel is variable, depending on being responders, poor responders, and resistance to the drug. Although, clopidogrel is effective in most patients, allelic variations influence the drug response in some individuals [9]. Non-response ranges from 4 to 30% [10] due to drug interaction, under dosing, genetic, and non-genetic factors [11, 12].

Here, we present genotype and phenotype study of Iranian patients undergoing PCI treated with clopidogrel during a 6-month period of follow-up; common variants of *CYP2C19, CYP3A5, CYP3A4*, and *ABCB1* genes were determined as well as the patients' cardiovascular outcomes to find out the effect of these variants by themselves and in combination.

Materials and Methods

Study Population

The study was performed on 416 individuals receiving PCI at Rajaie Cardiovascular Research center, as a tertiary referral center. Written consent was signed by each patient. This study was approved by ethics committee of Shahid Rajaie Hospital, Iran University of Medical Sciences. We gathered the data on outcome of patients with acute coronary syndrome (ACS) for 1- and 6-month periods. Demographic information was analyzed. The following information was documented for each patient: family history of ACS, hypertension, dyslipidemia, smoking status, positive family history of coronary artery disease, history of renal failure, diabetes, coronary artery bypass graft (CABG), MI, and presentation of unstable angina, non-ST-elevation myocardial infarction (non-STEMI), and ST-elevation myocardial infarction (STEMI).

Stenting Procedure

For the patients, different pretreatment doses of clopidogrel were prescribed according to guideline [8]; the patients received a loading dose of 75–300 mg of clopidogrel at least 24 h before PCI or loading dose of 600 mg of clopidogrel at least 6 h before PCI. On the day of PCI loading dose was 300 and 600 mg (according to guideline); 150 mg/day for 7 days followed by 75 mg after discharge. All patients were on aspirin (81–325 mg daily). Statin therapy was used for 368 patients. Patients also received heparin. Angioplasty and stent placement were performed by the experienced operators using femoral or radial approaches. All the orders were performed under the interventional cardiologists' guidance.

Follow-up

The patients followed for duration of 1 month, and 6 months. If coronary event such as PCI, CABG, MI or stroke, stent thrombosis, and death occurred they were considered as the endpoints. All patients had checkups with a specialist in cardiology.

Gene and SNP-Selection

In our study, we considered the genes in the metabolizing pathway of drug used in thromboembolic events. The selection criteria for the SNPs were the frequency in Caucasians and functional consequences of the SNPs based on literature in cardiac patients. One of the active metabolizer of clopidogrel pro-drug is cytochrome 450 especially, *CYP2C19**1. *CYP2C19**2 (p.Pro227Pro) was selected because of its high frequency i.e., it is the common variant with loss of function effect producing an aberrant splice site leading to a truncated protein [13]; *CYP2C19**3 (p.Trp212Ter) was also selected as it was recognized in Asians [14]. *CYP2C19**17 was another variant which has been reported to be associated with increased transcription function i.e., it acts as a gain of function variant [9].

*CYP3A4**1G (c.1026 + 12G > A) variant and *CYP3A5**3, which are loss of function alleles [5, 15], were also selected. *CYP3A4*1G* has been described to associate with reduced platelet receptor activation in patient during their clopidogrel treatment and no association for ADP-mediated aggregation after PCI [12] but there was association with CYP3A5. *ABCB1* T allele (c.3435C > T; p.Ile1145Ile) was also selected to genotype in our patients; it is associated with decrease metabolizer activation [16]. It affects the production of *p*-glycoprotein, the structure, folding, and function of protein [17]. Annotation of the SNPs was based on nomenclature of the Human Genome Variation Society.

Genotyping

Blood was collected in EDTA collecting tube after PCI and DNA were extracted following standard salting out protocols. Six SNPs were selected namely, *CYP2C19*1–CYP2C19**2 (c.681G > A; rs4244285), *CYP2C19*3* (c.636G > A; rs4986893), *CYP2C19**17 allele (c.-806C > T; rs12248560), *ABCB1* (c.3435C > T; rs1045642), *CYP3A5* (c.6986A > G; rs776746) and *CYP3A4* (c.1026 + 12G > A; rs2242480). The six selected SNPs were genotyped for 388 individuals undergoing PCI with drug eluting stent using RFLP-PCR, and tetra ARMS-PCR (primers and enzyme are provided on request).

Statistical Analysis

All statistical tests were analyzed by SPSS version 24 and Excel. All SNPs were tested for deviation from Hardy–Weinberg equilibrium with the use of Chi-Square test. The frequency of all SNPs was evaluated in the studied population. The frequencies of SNPs of genes were also compared using Chi-Square in PCI patients and the patients with cardiovascular events.

Results

A total of 416 patients were enrolled whom 388 were followed and genotyped. No one had event within 1 month after intervention. The clinical demography and history of 388 cases were available (Table 1). They all received the clopidogrel. Treatment was based on clopidogrel in combination with aspirin. The mean loading dose was 600 mg/day in 267 (68.8%) individuals, 300 mg/day in 121 (31.2%). The mean maintenance dose a day after discharge was 75 mg/day.

Genotyping

The distribution of *CYP2C19*1, CYP2C19**2 (c.681G > A; rs4244285), *CYP2C19*3* (c.636G > A; rs4986893), *CYP2C19**17 allele (c.−806C > T; rs12248560), *ABCB1* (c.3435C > T; rs1045642), *CYP3A5* (c.6986A > G; rs776746 defined as *CYP3A5*3*), and *CYP3A4* (c.1026 + 12G > A; rs2242480 defined as *CYP3A4G or CYP3A4*1G*) were in Hardy–Weinberg equilibrium. The frequencies are shown in individuals with and without cardiovascular event (Table 2).

Frequency of the Alleles

*CYP2C19*2* allele frequency is ~ 15% in Caucasians and Africans, and 29–35% in Asians; CYP2C19*3 frequency is typically < 1% and CYP2C19*17 is around 3–21% [9]. Though in our studied population the frequency of *CYP2C19*2* is 16.5%. Also, the allele frequency of *CYP2C19*17* was 21.78%.

Allele frequency of C allele of *ABCB1* at position 3435 is 59.3% and T 40.7%. The allele frequency of G for *CYP3A4*1* is 83.6 and 16.4% for A allele. The frequency

Table 1 Demographic data of patients

Characters	No. (%)	Characters	No. (%)
Male	274 (70.6)	Cardiac history and/or presentation	
Age	60.5 (± 10.40)	Myocardial infarction	72 (18.8)
Weight (kg)	78.06 (± 12.35)	PCI	82 (21.7)
Height (cm)	167.47 (± 9.04)	Unstable angina	181 (46.6)
Body mass index	27.81 (± 3.67)	Non-STEMI	57 (14.7)
Present comorbidity		STEMI	10 (2.6)
Diabetes	150 (38.6)	CAD	112 (28.9)
Dyslipidemia	144 (37.1)	CABG	48 (12.7)
Hypertension	178 (45.9)	Angioplasty	
History of opium	14 (3.6)	LAD	133 (25.7)
History of CKD	12 (3.1)	LAD/LCX	18 (4.4)
C/S	71 (19.1) (10 past)	LAD/RCA	47 (6.2)
Family history	85 (21.9)	LCX	58 (9.5)
Ejection fraction	45.89 (± 9.19)	RCA	79 (15.2)
Angiography no. vessel disease		LAD/RCA/LCX	5 (0.8)
Single	149 (38.4)	PDA	2 (0.5)
2VD	120 (30.9)	RCA/LCX	14 (2.6)
3VD	119 (30.7)	Left main disease	4
Medication		LM/LAD	2
Aspirin	388 (100)	LM/LAD/LCX	3
Statin	377 (97.2)	LM/LAD/RCA	1
CCB	50 (12.9)	30 days mortality	0
Amlodipine	16 (4.1)		
PPI	378 (97.4)		

PPI proton pump inhibitors, *CCB* calcium channel blockers, *C/S* cigarette smokers

Table 2 Genotypes of patients undergoing PCI

Gene	Genotype	Individuals $N=388$	%	p value	Individuals with cardiovascular outcome (nine patients)	%	p value *
CYP2C19	*1/*1	151	38.9		5	55.6	
	*1/*2	73	18.8		1	11.1	
	*1/*3	1	0.3		0		
	*1/*17	102	26.3		2	22.2	
	*2/*2	10	2.6		1	11.1	
	*2/*3	0	0		0		
	*2/*17	35	9.0		0		
	*17/*17	16	4.1		0		
	*3/*17	0	0	4.31787E−61	0		0.4974
ABCB1	C/C	151	39		1	11.1	
	C/T	158	40.8		7	77.8	
	T/T	79	20.4	3.7742E−07	1	11.1	0.4701
CYP3A5 (362 patients available)	G/G	320	81.2		8	88.9	
	G/A	50	13.3		1	11.1	
	A/A	4	1.1		0		0.2408
CYP3A4	G/G	269	69.3		8	88.9	
	G/A	111	28.6		1	11.1	
	A/A	8	2.1	8.95105E−59	0		0.6140

*p value in PCI patients compared to individuals with cardiovascular event

of G for *CYP3A5*3* is 92.2 and 7.7% for A. The allele frequency of *CYP2C19*3* was less than 0.1%.

Genotype and Clinical Outcomes

Total of eight patients had cardiovascular events such as thrombosis, unstable angina, non-STEMI, and PCI. None of the selected SNPs were associated with death in our study. Although after 6 months follow-up we investigated a patient with stent thrombosis; non-STEMI was observed in 5 patients. Unstable angina was also seen in three patients.

We observed that *CYP2C19*2* variant presented in one patient with non-STEMI and one patient with cardiovascular events. To note, 4 out of 13 patients (30.8%) with *CYP2C19*2* variant previously had PCI referred for stent restenosis. Basically, *CYP2C19*3* was not observed in our population except one who was originally from Afghanistan. For example, individual (case 4) with 1* and 17* also had high risk of cardiovascular events which did not follow the guideline. Also, stent thrombosis was seen in one patient (case 1) with the strongest association to *ABCB1* variant, *CYP3A4*1G and *CYP3A5*3* but not to *CYP2C9* (Table 3).

*CYP2C19*2/*2* variant as poor metabolizer (PM), C/T in *ABCB1* gene, *CYP3A5*3, and *CYP3A4*1G showed association for unstable angina in patient 6 (Table 3). Case 8, presented unstable angina with *CYP2C19*1* as extensive metabolizer (EM) despite case 6 having *CYP2C19*2/*2*, but had *ABCB1* C/T, *CYP3A5*3*, and *CYP3A4*1G* variants (Table 3). Another patient (case 7) as EM had TT variant in *ABCB1, CYP3A5*3*, and *CYP3A4*1G* variants presented unstable angina (Table 3). As it is shown other genes may be involved in presentation of cardiovascular events; although other risk factors and non-genetic factors may be involved. That is, 25% of them were cigarette smokers, 50% had DM, and 50% DLP.

Other patients had undergone PCI as seen carried EM, and UM, though other genes were candidates of carrying loss of function alleles (Table 3). Among those who did not have outcome event 13 patients had history of PCI and have undergone PCI due to restenosis (Table 4).

None of the SNPs were significantly associated with outcome events. However, the associated SNPs appeared in the patients with clinical outcomes. As shown in the study, the presence of risk alleles in the patients with clinical outcome was higher which associated with the significance and risk events.

Discussion

The study aimed to determine the occurrence of six SNPs in *CYP2C19, CYP3A4*, and *CYP3A5*, and *ABCB1* genes affecting clopidogrel metabolism which plausibly could be associated with stent thrombosis or restenosis or cardiovascular events among patients undergoing PCI during a 6-month period of follow-up in Iranian population. This is

Table 3 Patients with cardiovascular event after 6 months follow-up:

	Sex	*CYP2C9* genotype	Clinical significant[a]	*ABCB1* genotype	*CYP3A4* genotype	*CY3PA5* genotype	Risk factors	Family history	Previous history of CVD	Angioplasty	Angiography	Follow-up
1	m	*1/*1	EM	C/T	G/G	G/G	C/S		Non-STEMI	LAD	2VD	In-stent thrombosis, Non-STEMI
2	m	*1/*1	EM	C/C	G/G	G/G	DM		Non-STEMI	LAD, RCA	3VD	Non-STEMI, patent LAD RCA stent, PCI on LCX, OM
3	m	*2/*1	IM	C/T	G/A	G/A			Non-STEMI CABG	Ramus diagonal	3VD	Non-STEMI, patent stent
4	f	*1/*17	UM	C/T	G/G	G/G	DM, Htn, DLP		UA, PCI, CABG	RCA	3VD	Non-STEMI-PCI on RCA
5	m	*1/*1	EM	C/T	G/G	G/G	DM, DLP	+	CAD	LCX	3VD	Non-STEMI,PCI on LAD & RCA, patent privilege stent
6	m	*2/*2	PM	C/T	G/G	G/G				LAD	3VD + LM	Significant stent resteno-sis, UA, PCI on SVG on OM, PCI on LAD
7	m	*1/*1	EM	T/T	G/G	G/G	DM, DLP		Non-STEMI	LAD, RCA	2VD	Patent previous stent and moderate LCX, UA
8	m	*1/*1	EM	C/T	G/G	G/G	DLP, C/S		UA, MI	LAD	SVD	UA, mfu (viability RCA and LAD)

EM normal, *PM* poor metabolizer, *UM* ultra metabolizer, *IM* intermediate metabolizer, *DM* diabetes mellitus, *Htn* hypertension, *DLP* dyslipidemia, *PCI* percutaneous coronary intervention, *UA* unstable angina, *CAD* coronary artery disease, *FH* family history, *CABG* coronary artery bypass graft, *m* male, *f* female

[a]Clinical significant is based on CYP2C19 gene metabolizing effect

Table 4 Patient referred due to restenosis of previous PCI

	Sex	*CYP2C19* genotype	Clinical significant[a]	*ABCB1* genotype	*CYP3A4* genotype	*CYP3A5* genotype	Risk factor	Family history	Cardiovascular history	Angioplasty	Angiography	In-stent restenosis	6 months follow-up
1	m	*1/*1	EM	C/C	G/A	G/G	DM		PCI, CAD	LCX	2VD	Mild	No
2	f	*1/*1	EM	C/T	G/A	G/G	DM, Htn, DLP	FH+	PCI, CAD	PDA	2VD	Significant	No
3	f	*1/*2	IM	T/T	G/G	G/G	DLP, Htn		PCI (LAD, in-stent restenosis Ramus), UA	LAD	3VD	Significant	No
4	m	*1/*2	IM	C/T	G/A	G/G		FH+	CABG (LIMA), PCI on SVG on PDA, non-STEMI	SVG on OM	3VD	Significant	No
5	m	*1/*1	EM	C/C	G/G	G/G	C/S	FH+	CAD, PCI, CABG	LAD	3VD	Mild	No
6	m	*17/*1	UM	T/T	G/A	G/G	Htn	FH+	CAD, PCI (RCA LAD)	RCA	3VD	Moderate	No
7	m	*1/*2	IM	T/T	G/A	G/A	C/S	FH+	PCI, MI, UA	RCA	3VD, LCX, D1 D2 patent	Significant (LAD)	No
8	m	NA	NA	NA	NA	NA	C/S	FH+	PCI (LAD, RCA), MI, UA	LAD	2VD	Moderate	No
9	m	*17/*17	UM	C/C	G/A	G/G	C/S		UA, PCI	RCA	SVD	Significant	No
10	m	*17/*1	UM	C/C	G/A	G/G	DM, Htn, DLP		UA, PCI(LAD SVG in-stent)	LAD	SVD	Significant	No
11	m	*1/*2	IM	C/C	G/G	G/G	Htn, DLP		PCI, non-STEMI	LAD	SVD	Significant	No
12	f	*1/*1	EM	C/C	G/G	G/G	DM, Htn, DLP		UA, PCI	LAD	SVD	Moderate	No
13	f	*1/*17	UM	C/T	G/G	G/G	Htn, DLP	FH+	CAD, PCI	LCX	SVD	Moderate	No

EM normal, *PM* poor metabolizer, *UM* ultra metabolizer, *IM* intermediate metabolizer, *DM* diabetes mellitus, *Htn* hypertension, *DLP* dyslipidemia, *PCI* precutanus coronary intervention, *UA* unstable angina, *CAD* coronary artery disease, *FH* family history, *CABG* coronary artery bypass graft

[a]Clinical significant is based on CYP2C19 gene metabolizing effect

the first large study analyzing association of six variants in PCI patients in the population.

As previously shown, the association of variant candidates was determined with clopidogrel absorption (*ABCB1* gene) and metabolism (*CYP2C19, CYP3A4* and *CYP3A5* gene) with increased risk of cardiovascular events [16, 18–20]. However, clopidogrel responsiveness is based on expression of active enzymes and their genetic variability. Of course, beside the genetic background, environmental variability may influence the expression of the enzymes.

*CYP2C19*2* (c.681G > A; rs4244285), T/T and C/T *ABCB1* variants, *CYP3A4*1G* and *CYP3A5*3* were chosen to find the association between these and the cardiovascular events. To explain more about the results and the association of variants we presented case by case. Briefly, the evaluation of the genotype of loss of function *CYP2C19* allele in patients with outcome event did not significantly follow the clinical significant in guidelines. However, the patients had other risk alleles in *ABCB1* gene, *CYP3A4*, and *CYP3A5* gene (Table 3).

ABCB1 gene is a transporter *P*-glycoprotein modulating the metabolism of drugs. The C3435T (rs1045642) variant would hinder the absorption of drugs [20]. Although C3435T is a silent variant it may alter the splicing activity and expression and function of glycoprotein [21], it is reported to affect mRNA stability [22]. C/T *ABCB1* genotype was observed in six patients with cardiovascular event, four of which showed non-STEMI. In addition one patient was homozygous (TT) at this position.

Allele G of IVS10 + 12G > A (*CYP3A4*1G*, rs2242480) decreases transcriptional activity compared to A allele. The *CYP3A4 GG* non-response genotype was observed in 88.9% of patients; seven patients were homozygous for *CYP3A4 GG* and one patient with *CYP3A4 GA* genotype showed cardiovascular events. In all these patients the *CYP3A5* was also a non-expresser.

Despite the use of clopidogrel in patients undergoing PCI, stent thrombosis and clinical cardiovascular outcomes are seen in patients with *CYP2C19* extreme/normal, and ultra metabolizers which may be due to other risk factors and other genetic variants' effects. The *CYP2C19*1* patients had at least one loss of function allele in *CYP3A or ABCB1* gene. The patient with ultra metabolizing (*CYP2C19*17*) had *CYP3A* and *ABCB1* risk alleles. We should keep in mind that there might be other genetic variants which were not evaluated in this study may affect the drug metabolism. For instance, *P2Y12* gene variants were not presented in this study. 7 patients with event carried *ABCB1* T allele, 100% carried *CYP3A5*3* and *CYP3A4 G* variant as poor metabolizer and non-expressers.

However, since *CYP3A, ABCB1* polymorphisms occurred within PCI patients during our follow-up period, a larger patient population, or longer follow-up duration needs to be established to determine the impact of these polymorphisms. Also, residual platelet aggregation was not determined in our study to find the correlation of the polymorphisms with phenotypic effect. In addition, determination of clopidogrel and its metabolite concentration in plasma (as a factor of dose) of patients could be correlated with genetic variability and blood clotting [23–25]. Further studies are required as an obvious next step for personalized therapy.

Pharmacogenetics

The cytochromes have the role for metabolism of drugs in the liver. The proportion of non-responders to clopidogrel may be due to many reasons. Factors such as poor compliance and polymorphisms in different genes may be involved. In our study, with 94.1% compliance, different variants were investigated to find the association of the genotypes to the clopidogrel effectiveness.

CYP2C19 also is the enzyme for proton pump inhibitors (PPIs) metabolism and clearance and inactivation of many drugs. The genotype of *CYP2C19* affects inhibitory effect of PPIs [26–28]. As presented out of 378 individuals consuming PPI, 71 had genotype *1/*2, one case *1/*3, 8 individuals *2/*2, 35 cases had *2/*17 which are poor metabolizer of PPI (30.4%). *CYP2C19*17* allele causes drug failure of treatment such as clopidogrel in patients using PPIs as well as having risk of bleeding [29, 30]. 7 patients out of 30 (23.3%) who underwent PPI consumption carried *CYP2C19*17* (*p* value 0.3681) with one patient showing cardiovascular event during the follow-up period which may have conflicting drug treatment based on previous investigations. 4 patients carried *CYP2C19*17* with restenosis referred for PCI. Genotyping would help patients' management for other drug effects e.g., bleeding during longer follow-up time.

CYP3A4 and *CYP3A5* are responsible for the metabolism of clopidogrel into active form [5]. *CYP3A4* is dominant but it is easily inhibited and the *CYP3A5* compensates its function. Usually, both *CYP3A4* and *CYP3A5* contribute to total *CYP3A* activity, if *CYP3A4* works properly, there is no difference in *CYP3A5* expresser genotypes [*CYP3A5 GA* (**3/*1*) *or CYP3A5 AA* (**1/*1*)] or non-expresser genotypes [*CYP3A5 GG* (**3/*3*)]. In the presence of multiple inhibitors, *CYP3A4* is easily inhibited and *CYP3A5* becomes contributor. Therefore, if *CYP3A5* level is decreased (loss of function allele G), the system would have problem for drug inhibitory effects [31, 32]. Therefore individuals with *CYP3A5*3* which has decreased activity [15] would have a problem in drug metabolism.

Calcium channel blockers (CCBs) decrease the antiplatelet effect of clopidogrel [33] but some state that this may not be true [34, 35]. CCBs are inhibitors of *CYP3A4*; in our study two patients out of 50 patients using CCB had

non-STEMI and one with staged PCI. The variants for both these patients were *CYP3A5*3* (p value 0.738) and *CYP3A4*1* bringing decreased enzyme function.

Drug–drug interaction and inactivation of metabolic enzymes may cause unsatisfactory results for clinicians. Therefore, use of safe drug combination is of importance [36]. Studies showed that amlodipine also decreases response to clopidogrel in PCI patients [37] i.e., our patients who consumed amlodipine should be followed for cardiovascular events. 46 (92%) patients had functionally decreased expression variants *CYP3A4* out of which 15 patients also have used amlodipine. In addition, 45 patients (p value 0.965) had decreased allele variant for *CYP3A5* and 13 used amlodipine.

Other Factors and Testing Strategies

The risk of non-genetic factors was evaluated in the eight patients with cardiovascular event. 25% of them were cigarette smokers, 50% had DM, and 50% DLP.

Our results indicate that genotyping of different genes involved in pharmacokinetics variability are important and it may not be as straightforward in risk assessment and pharmacogenetics. This highlights the important of personalized medicine in risk assessment and treatment [1]. Platelet aggregation or platelet reactivity may help but should not be the clue for responsiveness, because they may vary. For clinical management of patients, genetic analysis and platelet aggregation would be beneficial depending on the situation. Therefore, close follow-up is helpful for the patients. Geisler et al., reports a PREDICT score which is used to evaluate the risks [11], it includes the genetic and non-genetic factors. In our study the genotypes and non-genetic factors were evaluated and for the next studies we have to assay the RPA to define an algorithm.

On 2010 FDA released a warning on pharmacogenetic testing of clopidogrel but it elucidated the reaction of clinicians that there is no meta-analysis of this statement. However, *CYP2C19* accounts for 12–20% of response variability [38] but studies showed that clopidogrel response is highly heritable (70%) [19] and other genes including *ABCB1* gene are also accounted for this heritability [16]. Chan and colleagues also mentioned the role of biomarker analysis in clopidogrel therapy [39]. Our interactome analysis using STRING (Fig. 1) showed that at least eleven proteins interact with each other to metabolize clopidogrel; so multi-interaction of genes which could influence the drug interaction should not be ignored.

PM patients should be aware of using clopidogrel; hence, genetic testing should be performed for *CYP2C19* gene to manage the patients with different antiplatelet drugs or different dosing, though other high risk polymorphisms should be investigated to inform clinicians about the risk of having cardiovascular events.

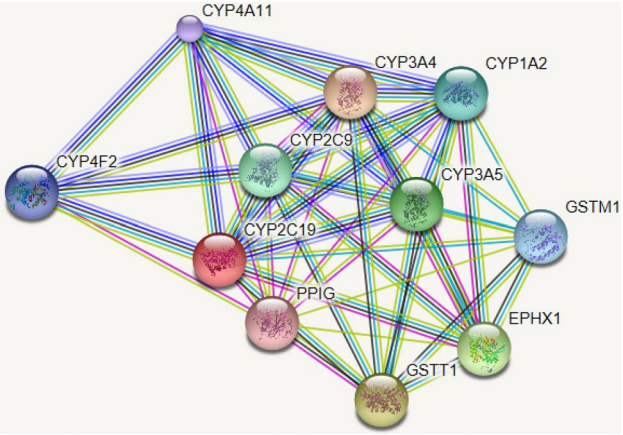

Fig. 1 Protein–protein interaction of clopidogrel metabolizing enzymes. *CYP3A4* cytochrome P450, family 3, subfamily A, polypeptide 4; *GSTT1* glutathione *S*-transferase theta 1; *EPHX1* epoxide hydrolase 1, microsomal (xenobiotic); *GSTM1* glutathione *S*-transferase mu 1; *PPIG* peptidylprolyl isomerase G (cyclophilin G)

Prasugrel and ticagrelor are alternative drugs with more effectiveness than clopidogrel but with higher costs and risk of bleeding [30, 40]. If pharmacogenetics is used to identify the genetic variability of the patients, to some extent the appropriate therapy is planned for the patients and large number of the patients are not put through the risk events [18].

Although, this may be hard to establish and provide such genotype–phenotype correlation for the benefit of patients but is could be established for some SNPs with proved events to reduce the risk occurrence. Personalized treatment should be considered as different strategies and stent profile is used for each person; however pharmacogenetics usage in clinic is laborious and costly but could be enrolled to overcome the consequences.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest to declare in relation to this manuscript.

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