



The impact of genetic polymorphisms of *P2Y12*, *CYP3A5* and *CYP2C19* on clopidogrel response variability in Iranian patients

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ABSTRACT

Clopidogrel is an inhibitor of platelet ADP *P2Y12* receptors and currently used for prevention of stent thrombosis. Despite certain clinical benefit using this drug in patients undergoing percutaneous coronary intervention (PCI), some patients do not attain adequate antiplatelet effects. In this study, we investigated the role of three genetic factors (*P2Y12*, *CYP3A5*, *CYP2C19*), demographic characteristics, and pathologic condition on clopidogrel response variability in Iranian patients after PCI.

Patients who were candidate for elective PCI were enrolled in this study. All patients had received aspirin 80–325 mg daily for ≥ 1 week before PCI. Blood samples were taken from patients at baseline, 2 h after taking a 600-mg loading dose of clopidogrel, 24 h and 30 days after PCI. Platelet aggregation was measured by turbidimetric aggregation assay with two different concentrations of ADP (5 and 20 μ M). *CYP2C19**2(rs4244285), *CYP2C19**3(rs4986893), *CYP3A5* (A6986G), and *P2Y12* (T744C) genotypings were performed by PCR-RFLP.

One hundred and twelve patients were included in this study. Maximum clopidogrel non-responsiveness (25.90%) occurred at 2 h after taking 600 mg of the loading dose of clopidogrel. Although there were no significant associations between clopidogrel responsiveness and polymorphisms of *CYP2C19*, *CYP3A5*, and *P2Y12* ($P > 0.05$), subjects who were *CYP3A5* genotype expressor had a greater inhibition of platelet aggregation. No significant associations were observed between environmental factors and clopidogrel responsiveness ($P > 0.05$).

Our results showed that *P2Y12*, *CYP3A5*, and *CYP2C19* polymorphisms along with non-genetic factors were not responsible for the interindividual variability in response to clopidogrel in Iranian population.

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1. Introduction

Percutaneous coronary intervention (PCI) has improved acute coronary syndrome (ACS) outcomes, but thrombosis after stent placement is a major complication that can affect the outcome and survival of ACS patients. Although prolonged dual antiplatelet therapy with aspirin and clopidogrel in such patients has been associated with better long-term clinical outcomes [1,2], adequate antiplatelet effects of clopidogrel are not achieved in 4–30% of patients [3,4]. Individual differences in response to clopidogrel therapy have been established in many studies. Several drug (pharmacokinetic and pharmacodynamic) and patient (e.g. diabetes

mellitus, smoking, obesity, and genetics) related factors have been investigated that can be responsible for this inappropriate response to clopidogrel [5,6]. However the true mechanism of inter-individual variability of clopidogrel responsiveness has remained unknown.

Clopidogrel is a prodrug which is rapidly absorbed following oral administration. Two sequential oxidative steps by hepatic cytochrome P450 (CYP) are required to generate a metabolite that is an active inhibitor of platelet ADP *P2Y12* receptors [7]. *CYP1A2*, *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP3A4/5*, and glycoprotein paraoxinase-1 [8] are enzymes regulating clopidogrel transformation into the active metabolite. However, only a small proportion of the administered clopidogrel is metabolized by *CYP450*. The majority of the drug is hydrolyzed by esterases to an inactive carboxylic acid derivative that accounts for 85% of the clopidogrel-related compounds circulating in plasma [9,10]. *CYP3A5* is expressed polymorphically and may account for up to 50% of the total hepatic *CYP3A* content in one-third of the white and in half-of the black subjects [11,12]. Several variant alleles have been delineated for the *CYP3A5* gene. Only *CYP3A5**3 is at linkage disequilibrium. This

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allele shows inter racial group differences and has functional relevance. Other variant alleles are either very rare or irrelevant for enzyme activity (*2, *4, *5 *6, *7, *8 and *9) [13,14]. CYP3A5*3/*3 a non-expressor genotype is strongly correlated with a decreased CYP3A5 activity, whereas the wild-type CYP3A5*1 allele (an expressor), present in either homozygous or heterozygous form, is correlated with a high CYP3A5 activity. Thus, CYP3A5 expression may be the most important factor determining the total CYP3A content of the human liver [15,16].

CYP2C19 isoenzyme is involved in both sequential metabolic steps and is also the most important indicator of the pharmacokinetic and pharmacodynamic responses to clopidogrel [17]. At least 25 single nucleotide polymorphisms (SNPs) in the gene coding for CYP2C19 have been reported, and the “loss-of-function” allele, CYP2C19*2 in exon 5, is the most common and the most investigated polymorphism. Carriers of at least one loss-of-function allele show a reduced platelet aggregation response to clopidogrel as compared with non-carriers [18–21], whereas the presence of CYP2C19*17 is associated with a raised risk of the adverse effects of clopidogrel such as bleeding [22].

P2Y12 receptor is a purinergic receptor. It has a pivotal role in platelet aggregation. Activation of the P2Y12 receptor leads to the activation of the glycoprotein (GP) IIb/IIIa receptor, which results in the enhancement of platelet aggregation, and the stabilization of the platelet aggregate [23]. P2Y12 gene is expressed polymorphically. T744C polymorphism is in total linkage disequilibrium with the other 3 polymorphisms of the P2Y12 receptor gene [24]. Two functional haplotypes (H1 and H2) were determined by Fontana et al. The more rare variant is named H2 haplotype, whereas the wild type is denoted as H1 haplotype [25]. According to Fontana et al., the H2 haplotype (a C in position 744) is associated with a higher maximal aggregation in response to ADP [25].

Using data from previous studies showed that polymorphisms of both of the hepatic CYP450 system (CYP3A5, CYP3A4, CYP2C19) or within the platelet P2Y12 receptor were more investigated among genes and may contribute to the variability in antiplatelet activity of clopidogrel [20,25–30]. These pharmacogenetic studies were mainly investigated in Caucasians and to a lesser extent in Asians [30]. We could not find any published research on this subject in Iran. Iran is a Middle Eastern country with many emigrant ethnic groups [31]. Multivariate gene polymorphisms and different phenotypes in enzymatic/receptor activities could be observed in the Iranian population. Therefore, we conducted this survey to determine the role of the three mentioned important genetic factors (CYP3A5, CYP2C19, and P2Y12) in antiplatelet response variability of clopidogrel in Iranian population undergoing elective PCI.

2. Methods

2.1. Patients

The patients admitted to Kowsar Hospital in Shiraz and scheduled for elective PCI between September 2007 and October 2008 were enrolled in this study. PCI was performed for all patients with drug-eluting stents. All patients gave written informed consent. This cross-sectional study was approved by the Ethics Committee of Shiraz University of Medical Sciences. All patients had received aspirin 80–325 mg daily for ≥ 1 week before PCI and had not received thienopyridine derivatives in the week prior to enrollment. Patients were over 18 years old. Exclusion criteria were acute myocardial infarction (AMI) within one week, any contraindications to aspirin or clopidogrel, thrombocytopenia (platelet $< 100 \times 10^3$ cells/mm³), anemia (hemoglobin < 10 g/dL), or renal failure (serum creatinine > 2.5 mg/dL). Demographic characteristics, lab data, clinical information, diameter and length

of stent, and drug history of the patients were recorded from their files or face-to-face interview. These factors included: age, sex, smoking, diabetes mellitus, hypertension (BP $\geq 140/90$ mmHg), hyperlipidemia (LDL-C ≥ 100 mg/dL), body mass index (BMI), white blood cell (WBC) count, platelet (PLT) count, multi vessel disease (involving 2 or more coronary arteries), multi vessel intervention (Intervention involving 2 or more coronary arteries), and LVEF $< 45\%$.

2.2. Medications

All patients received a loading dose (LD) 600 mg clopidogrel (Plavix[®], Sanofi-Aventis) at least 24 h before PCI, followed by 150 mg/day of clopidogrel for two weeks and 75 mg/day thereafter for 12 months after PCI. Aspirin was prescribed as 325 mg/day for one week then 80 mg/day for an indefinite period of time after PCI. Unfractionated heparin (50–70 IU/kg) was administered as a bolus to all patients in the catheterization laboratory immediately before stenting. Subjects receiving other drugs affecting platelet activity or had a major effect on CYP3A5 and CYP2C19 metabolism (i.e. abciximab, dipyridamole, warfarin, phenytoin, omeprazole, and phenobarbital) were excluded [32].

2.3. Blood sampling

Blood samples were collected in tubes containing 3.8% Na-Citrate. Samples were obtained before coronary intervention from patients on aspirin alone (baseline sample), 2 h after taking LD 600 mg clopidogrel, 24 h and later 30 days after stenting.

Laboratory procedures (platelet aggregation and hematology assays) were done within 2–3 h after sampling to minimize environmental effects.

2.4. Platelet aggregation

Platelet aggregation was evaluated by light transmittance aggregometry (LTA). The blood-citrate mixture was centrifuged at 800 rpm for 8 min to recover platelet-rich plasma (PRP) and further subjected to centrifugation at 4000 rpm for 20 min to recover the platelet-poor plasma (PPP). The PRP and PPP were stored at room temperature and used in 2 h. Platelets in PRP were stimulated with a final concentration of either 5 or 20 μ M ADP (Helena BioSciences Europe, Sunderland) using a Helena laboratories PACKS-4. Platelet aggregation was expressed as the maximal percent change in light transmittance from baseline, using PPP as a reference.

2.5. Genotyping

Genomic DNA was extracted from buffy coat by DNA extraction kit according to the manufacture's instruction (Cinagen, Tehran, Iran).

2.5.1. P2Y12

Genotyping of the T744C polymorphism in intron 1 of the P2Y12 receptor gene (rs2046934) was performed according to Angiolillo et al. [24]. Briefly, polymerase chain reaction (PCR) included a hot start at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 51 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min, using the sense primer 5'-TCTGGTGAATAAAAAGATTACGTA-3' and the anti-sense primer 5'-GTCAGAAATGGCCTGTGTATATATG-3'. After PCR and restriction digestion with Rsa1 (Fermentase, Lithuania), two fragments of 196 and 24 bp for the C allele, and a single fragment of 220 bp for the T allele were obtained.

The C allele of the T744C polymorphism is a constituent of the minor genotype of the P2Y12 previously described by Fontana et al. [25].

2.5.2. CYP3A5

Genotyping of the A6986G polymorphism in intron 3 of the CYP3A5 gene (A → CYP3A5*1 and G → CYP3A5*3) (rs776746) was performed according to Thervet et al. [33]. Amplification involved 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 70 °C for 1 min followed by a final extension at 72 °C for 7 min using the sense primer 5'-CCTGCCTTCAATTTTTCCT-3' and the anti-sense primer 5'-GGTCCAAACAGGGAAGAGGT-3'. A product of 196 bp was obtained. After PCR and restriction digestion with Rsa1 (Fermentase, Lithuania), three fragments of 103, 73 and 20 bp for the CYP3A5*1/*1 allele, and two fragments of 103, 93 for the CYP3A5*3/*3 allele and four bands of 103, 93, 73, and 20 bp for CYP3A5*1/*3 were obtained.

2.5.3. CYP2C19

For the CYP2C19*2(rs4244285), and CYP2C19*3(rs4986893) alleles, the protocol described by Lamba et al. [34] was used. The forward primer of 5'-AATTACAACCAGAGCTTGGC-3' and the reverse primer of 5'-TATCACTTTCCATAAAAGCAAG-3' were used for the amplification of the CYP2C19*2 allele. The PCR was performed with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 30 s, elongation at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The amplified products (169 bp) were digested with MspI (Fermentase, Lithuania), restriction enzyme.

For the CYP2C19*3 variant, the forward primer was 5'-AAATGTTTCCAACATTTAGCT-3' and the reverse primer was 5'-ACTTCAGGGCTTGGTCAATA-3'. The reaction conditions were the same as those for the other alleles, except that annealing was performed at 55 °C and the 271 bp fragment was digested with BamHI (Fermentase, Lithuania).

Samples from each genotype (CYP3A5, CYP2C19, and P2Y12) were selected randomly and submitted for direct DNA sequencing which confirmed our results.

2.6. Statistical analysis

Continuous variables are presented as mean ± SD. Categorical variables are reported as counts (percentage). The patients were divided into non-responders, semi-responders and responders on the basis of the relative platelet inhibition (RI). The Kolmogorov–Smirnov test was used to assess conformity with a normal distribution.

Comparisons were made between continuous variables and groups by one way ANOVA. Pearson Chi-Square and Fisher's exact tests were utilized for comparisons between categorical variables and the groups. The t-test was used to compare the degree of platelet inhibition between the expressor and the non-expressor genotype groups. The association between length and diameter of stent and clopidogrel responsiveness was found by Mann–Whitney *U* test. Since the patients might have received more than one stent, length and diameter of stent were indicated as average of maximal stent length (or diameter), average of minimal stent length (or diameter) and also average of total stent length (or diameter).

$P < 0.05$ was considered significant. Analyses were performed using SPSS version 13 statistical software.

2.7. Definition of clopidogrel responsiveness

Responsiveness was defined as the relative platelet inhibition (RI) induced by the addition of clopidogrel. $RI = [(pre-treatment\ aggregation - posttreatment\ aggregation) / (pre-treatment\ aggregation)] \times 100$.

For both 5 and 20 μM ADP-induced aggregation, non-responders were defined as those patients with $RI < 10\%$, semi-responders as those with $RI = 10-30\%$, and responders as those with $RI > 30\%$ [35,36].

3. Results

3.1. Patient and procedural characteristics

One-hundred and twelve patients were enrolled in this study. Demographic characteristics, clinical information, lab data, and procedural information of the patients are shown in Table 1.

According to our results, 25.90% and 28.60% of our patients obtained the maximum rate of clopidogrel nonresponsiveness at 2 h after taking 600 mg of clopidogrel measured by 5 and 20 μM ADP, respectively. A strong correlation between the two concentrations of ADP agonist ($P < 0.001$, $r = 0.88$) was observed.

There were no significant differences in patient characteristics between non-responders, semi responders and responders ($P > 0.05$). Ninety-three of our patients had received at least one drug that could affect clopidogrel metabolism. But the results of LTA showed that these drugs had no significant effects ($P > 0.05$) on clopidogrel responsiveness.

3.2. P2Y12 genetic polymorphism

In the whole population of 112 patients, the genotype distribution of the T744C polymorphism of the P2Y12 gene was as follows: 104 patients (92.9%) were CC homozygotes, 8 (7.1%) were CT heterozygotes and was TT homozygotes. Since, no patient was a wild type and the number of heterozygote patients was very few, statistical analysis on this population was impossible. Therefore, the relation between genetic polymorphism and clopidogrel responsiveness was not investigated. The allele frequencies were in Hardy–Weinberg equilibrium.

3.3. CYP3A5 genetic polymorphism

The frequencies of CYP3A5 *1/*1, CYP3A5 *1/*3, and CYP3A5 *3/*3 genotypes were 8.00% (9/112), 37.50% (42/112), and 54.50% (61/112), respectively. There was no significant difference in CYP3A5 gene polymorphism among clopidogrel non-responders, semi-

Table 1

Demographic, clinical, laboratory and procedural information of the patients ($N = 112$).

Age (years), mean ± SD	58 ± 11
Sex	
Male, n (%)	79 (70)
Female, n (%)	33 (29)
Smoking, n (%)	47 (42)
Diabetes mellitus, n (%)	21 (19)
HTN ^a (BP ≥ 140/90 mmHg), n (%)	57 (51)
Hyper lipidemia (LDL-C ≥ 100 mg/dL), n (%)	76 (68)
BMI ^b (kg/m ²), mean ± SD	26.3 ± 5.0
Multi vessel disease ^c , n (%)	34 (30)
LVEF ^e < 45%, n (%)	10 (9)
WBC ^f (×1000/μL), mean ± SD	7.2 ± 1.9
PLT ^g (×1000/μL), mean ± SD	230 ± 64
Length of drug-eluting stent (mm), mean ± SD	
Average of the lengths	21.6 ± 7.2
Average of the smallest lengths	19.2 ± 8.2
Average of the largest lengths	24.0 ± 8.1
Diameter of drug-eluting stent (mm), mean ± SD	
Average of the diameters	3.1 ± 1.3
Average of the smallest diameters	2.9 ± 0.7
Average of the largest diameters	3.3 ± 2.7

^a Hypertension.

^b Body mass index.

^c Involving 2 or more coronary arteries.

^d Intervention involving 2 or more coronary arteries.

^e Left ventricular ejection fraction.

^f White blood cells.

^g Platelets.

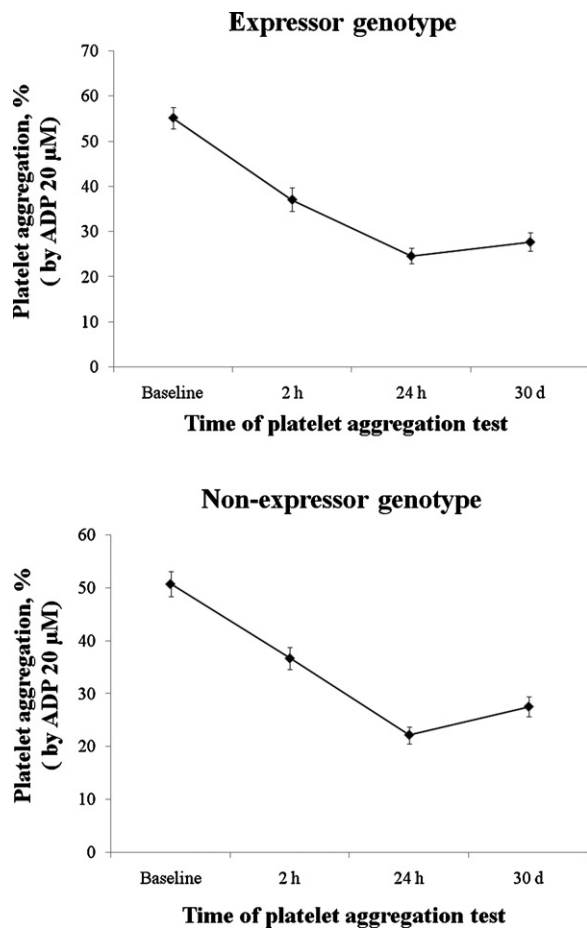


Fig. 1. Plot of % platelet aggregation at 20 μ M ADP vs. time for the expressor and the non-expressor groups. There was significant differences at all points compared with the baseline in both groups ($P < 0.001$).

responders, and responders ($P > 0.05$). The allele frequencies were in Hardy–Weinberg equilibrium.

Since, the *CYP3A5* $*1/*1$ genotype was observed in 9 patients, the subjects were divided into two genotype groups; those with expressor alleles ($*1/*1$ and $*1/*3$ alleles) and those with non-expressor alleles ($*3/*3$ alleles). The two groups did not differ significantly in terms of age, BMI and sex ($P > 0.05$). There were also no significant differences between these groups and clopidogrel

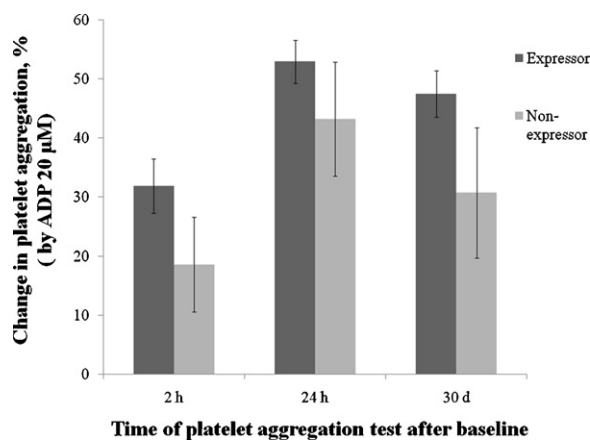


Fig. 2. The percentage of platelet aggregation inhibition vs. time after taking 600 mg clopidogrel (ADP concentration = 20 μ M). There was no significant difference between the *CYP3A5* expressor and the non-expressor groups ($P > 0.05$).

non-responsiveness ($P > 0.05$). The inhibition of platelet aggregation after clopidogrel treatment was evaluated in both the expressor and the non-expressor groups and was found to be significant at all points compared with the baseline in the both groups ($P < 0.05$) (Fig. 1). Thereafter, the efficacy of clopidogrel in terms of the change in aggregation from baseline (RI) was compared between these two groups. Although, there were no significant differences between them ($P > 0.05$), *CYP3A5* expressor group showed a higher inhibition of platelet aggregation (Fig. 2).

3.4. *CYP2C19* genetic polymorphism

The allele frequencies of *CYP2C19* $*1$, *CYP2C19* $*2$, and *CYP2C19* $*3$ were 88.99%, 10.09%, and 0.91%, respectively. Due to the low frequency of *CYP2C19* $*3$, the statistical analysis was based on the comparison between the patients with the wild type $*1/*1$ and the patients with ≥ 1 variant allele (*CYP2C19X/Y*). The significant difference between the mentioned groups and the clopidogrel responsiveness was not observed ($P > 0.05$). The allele frequencies were in Hardy–Weinberg equilibrium.

4. Discussion

Individual variability in the rate of platelet reactivity obviously influences normal hemostasis and the pathological outcome of thrombosis. Such individual variability is largely determined by environmental and genetic factors [5,6]. A significant association between environmental factors and anti platelet effect of clopidogrel was not observed in our patients. This finding was in line with other studies performed in various populations [36–38].

Besides environmental parameters, genetic factors also contribute to the variability in response to clopidogrel [25–30,39]. The effects of genetics can be defined by either pharmacokinetic or pharmacodynamic parameters. In our survey the polymorphism of *P2Y12* receptor gene in the pharmacodynamic site was examined. Since no patient was a wild type and more than 92% of our patients had CC genotype for *P2Y12* receptor gene, statistical analysis was impossible. In the case of a larger sample size, we could probably find more gene variations and could probably find different statistical results. A study by Angiolillo et al. showed that there were no significant differences between TT genotype and C allele carriers (CT and CC) in clopidogrel response in a Spanish population. In their survey, the genotype distribution was 22/36 (61.1%) TT genotype, and 14/36 (38.9%) C allele carriers [24]. Another study by Alessi et al. in a French population also did not show any influence of the *T744C* polymorphism of the *P2Y12* receptor gene in response to clopidogrel, whereas in the whole population of 597 patients, 13 patients (2%) were CC, 125 (21%) were CT and 459 (77%) were TT [40]. The *P2Y12* genotype distribution in a study by Lee et al. in a Korean population was 258/385 (67%) TT genotype and 127/385 (33%) C allele carriers and they found no significant association between *P2Y12* receptor gene and clopidogrel responsiveness [30]. In a different study in a French population, Fontana et al. indicated that carriers of the minor haplotype (H2 haplotype) had increased ADP-induced platelet aggregation [41]. However, previous studies on the *T744C* polymorphism of the *P2Y12* receptor gene, did not report any relationship between the non-carriers and the carriers of C allele [24,30,40]. As indicated above, TT genotype of *P2Y12* had the highest frequency among other populations, while this genotype was not found in our patients. Interestingly, clopidogrel non-responsiveness was in the range (4–30%) reported by other studies [3,4], though all our patients carried C allele.

Another genetic factor evaluated in this study was *CYP3A5* gene. In our study, most of the patients had *CYP* $*3/*3$ genotype. Due to

the effect of this genotype in metabolizing clopidogrel to its active metabolite, it could lead to a poor clopidogrel effect.

There are several studies investigated the influence of *CYP3A5* polymorphisms on antiplatelet effect of clopidogrel. Suh et al., investigating a Korean population, indicated that atherothrombotic events occurred more frequently among the patients with the non-expressor genotype. In their study, *CYP3A5* expressor group showed more inhibition of platelet aggregation than *CYP3A5* non-expressors after clopidogrel treatment, though it was not significant [27]. Kim et al., in a study in South Korea, showed that the presence of *CYP3A5**3 allele was not a factor causing inter-individual variability in the clopidogrel pharmacokinetics and its antiplatelet effect [28]. Moreover, the results of Frere et al.'s study in a French population showed that *CYP3A5**3 polymorphism did not influence the post-treatment platelet reactivity and the clopidogrel response [42]. In our study, the *CYP3A5* expressor genotype showed a higher inhibition of platelet aggregation, though it was not significant. Therefore, the lack of association of *CYP3A5* polymorphism in response to clopidogrel in our patients was consistent with other studies performed in various populations [27,28,42], whereas *CYP3A5* allele frequencies in Iranian population were different compared to Asians and Caucasians [43].

Pervious studies conducted in different populations including Japanese [44,45], Chinese [18,46], Korean [47,48], Italian [49], Germany [50], and French [51], reported that among the healthy cases and patients treated with clopidogrel, carriers with at least one variant of *CYP2C19* alleles (2*, 3*) had a reduced clopidogrel response due to decreased formation of the active metabolite. Therefore, this is translated into the higher relative risk of adverse cardiovascular events by a factor of 1.53–3.69 among the carriers of the loss-of function alleles as compared with the non-carriers [19,52–54]. Moreover, these fundamental findings have been validated by a recent meta analysis of data from nine pharmacogenetic investigations of clopidogrel involving 9685 cases who had an ACS or underwent PCI [55]. On the contrary to these mentioned studies, our study and other limited surveys demonstrated that *CYP2C19* loss-of function alleles did not have a significant effect on clopidogrel response. Wallentin et al. in a sub-study of the PLATO Trial (PLATElet inhibition and patient Outcomes) demonstrated that the relationship between *CYP2C19**2 polymorphism and clopidogrel effect is time dependent and the *CYP2C19**2 variant had no significant effect on clopidogrel response in a long-term therapy [56]. In a randomized, double blind, placebo-controlled trial performed in patients of European and Latin American ancestry, Pare G et al. reported that *CYP2C19* genotype did not have a significant influence on clopidogrel effect and consequently did not show adverse cardiovascular events [57]. In another study carried out in 79 patients with CAD, *CYP2C19**2 loss-of function had no significant impact on clopidogrel anti-platelet effect [58].

Numerous factors can explain the discrepancies of the results, including the study design, the participation of ACS patients undergoing or not undergoing PCI, the method of platelet aggregation assay, the duration of follow-up, the variation of clopidogrel maintenance or loading doses, the efficacy outcome, and finally the population or the race studied. Different distributions of *CYP2C19**2 allele frequency have been observed in different racial groups. The allelic frequency of *CYP2C19**2 is significantly higher in the Asian (~30%) than in Caucasian (~13%) and the Africo-American (~18%) populations. The *CYP2C19**3 variant is also presented with more frequency in the Asians (~10%) compared with other racial groups (~<1%) [44–48,59–61]. In our study, the frequency of *CYP2C19**2 allele (10.09%) in patients was lower than other populations. Therefore, considering that *CYP2C19* polymorphisms to be only responsible for approximately 12% of the variability in clopidogrel antiplatelet effect [53] and the low

frequency of the *CYP2C19**2 variant in our population, we believed that this genetic polymorphism does not have a significant impact on clopidogrel responsiveness in the Iranian population. Moreover, the results of other studies conducted in Iran, indicated that the allele frequencies of *CYP2C19* were more similar to Caucasians in spite of Iran location in Asia [62,63].

Our study had some limitations. Firstly, the sample size was relatively limited. In a larger population, the results would have been more reliable. Secondly, there was limitation in access to flow cytometer; and finally, we did not determine the active metabolite of clopidogrel in patient's plasma directly, although we used platelet aggregation test with ADP as an indirect marker of the active metabolite.

In conclusion, the goal of our study was to find out the effective and consequently the predictive parameters acting on clopidogrel responsiveness and thus post stent thrombosis in an Iranian population. We studied non-genetic factors, which showed no significant association. We also studied three genetic factors which were more investigated in other populations and were found to have no significant association with clopidogrel non-responsiveness. Due to the importance of post stent thrombosis, the effect of other factors such as glycoprotein paraoxinase-1, *GP1Ib/IIIa*, and also gene modulating clopidogrel absorption (*ABCB1*) should be investigated.

Conflict of interest

All authors had not any conflict of interest.

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References

- [1] Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, et al. Clopidogrel in Unstable angina to prevent Recurrent Events trial (CURE) Investigators. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. *Lancet* 2001;358:527–33.
- [2] Steinhubl SR, Berger PB, Mann JT, Fry ETA, DeLago A, Wilmer C, et al. CREDO Investigators. Clopidogrel for the Reduction of Events during Observation. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial (CREDO). *J Am Med Assoc* 2002;288:2411–20.
- [3] Nguyen TA, Diodati JG, Pharand C. Resistance to clopidogrel: a review of the evidence. *J Am Coll Cardiol* 2005;45:1157–64.
- [4] Barsky AA, Arora RR. Clopidogrel resistance: myth or reality. *J Cardiovasc Pharmacol Ther* 2006;11:47–53.
- [5] Camilleri E, Jacquin L, Paganelli F, Bonello L. Personalized antiplatelet therapy: review of the latest clinical evidence. *Curr Cardiol Rep* 2011;13:296–302.
- [6] Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Alfonso F, Macaya C, Bass TA, et al. Variability in individual responsiveness to clopidogrel: clinical implications, management, and future perspectives. *J Am Coll Cardiol* 2007;49:1505–16.
- [7] Sibbing D, Kastrati A. Risk of combining PPIs with thienopyridines: fact or fiction. *Lancet* 2009;374:952–4.
- [8] Campo G, Miccoli M, Tebaldi M, Marchesini J, Fileti L, Monti M, et al. Genetic determinants of on-clopidogrel high platelet reactivity. *Platelets* 2011;22:399–407.
- [9] Clarke TA, Waskell LA. The metabolism of clopidogrel is catalyzed by human cytochrome P450 3A and is inhibited by atorvastatin. *Drug Metab Dispos* 2003;31:53–9.
- [10] Antiplatelet Drug Clopidogrel Pathway (PK). <http://www.pharmgkb.org/search/pathway/platelet/platelet-clopidogrel-pk.jsp> [accessed 20.11.09].

- [11] Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* 2001;27:383–91.
- [12] Evans WE, McLeod HL. Pharmacogenomics—drug disposition, drug targets, and side effects. *N Engl J Med* 2003;348:538–49.
- [13] Xie HG, Wood AJ, Kim RB, Stein CM, Wilkinson GR. Genetic variability in CYP3A5 and its possible consequences. *Pharmacogenomics* 2004;5:243–72.
- [14] Balram C, Zhou Q, Cheung YB, Lee EJ. CYP3A5*3 and *6 single nucleotide polymorphisms in three distinct Asian populations. *Eur J Clin Pharmacol* 2003;59:123–6.
- [15] Jounaïdi Y, Hyraïlles V, Gervot L, Maurel P. Detection of CYP3A5 allelic variant: a candidate for the polymorphic expression of the protein. *Biochem Biophys Res Commun* 1996;221:466–70.
- [16] van Schaik RH, van der Heiden IP, van den Anker JN, Lindemans J. CYP3A5 variant allele frequencies in Dutch Caucasians. *Clin Chem* 2002;48:1668–71.
- [17] Steinhubl SR. Genotyping, clopidogrel metabolism, and the search for the therapeutic window of thienopyridines. *Circulation* 2010;121:481–3.
- [18] Chen H, Yan W, Wu XY. Relationships of blood stasis syndrome, CYP2C19 gene polymorphism with clopidogrel resistance and post-PCI prognosis. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 2010;30:1245–9.
- [19] Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, et al. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009;360:354–62.
- [20] Pettersen AA, Arnesen H, Opstad TB, Seljeflot I. The influence of CYP 2C19*2 polymorphism on platelet function testing during single antiplatelet treatment with clopidogrel. *Thromb J* 2011;9:4.
- [21] Jeong YH, Kim IS, Park Y, Kang MK, Koh JS, Hwang SJ, et al. Carriage of cytochrome 2C19 polymorphism is associated with risk of high post-treatment platelet reactivity on high maintenance-dose clopidogrel of 150 mg/day: results of the ACCEL-DOUBLE (Accelerated Platelet Inhibition by a Double Dose of Clopidogrel According to Gene Polymorphism) study. *JACC Cardiovasc Interv* 2010;3:731–41.
- [22] Sibbing D, Koch W, Gebhard D, Schuster T, Braun S, Stegherr J, et al. Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation* 2010;121:512–8.
- [23] Cattaneo M. Platelet P2 receptors: old and new targets for antithrombotic drugs. *Expert Rev Cardiovasc Ther* 2007;5:45–55.
- [24] Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, Ramirez C, Cavallari U, Trabetti E, et al. Lack of association between the P2Y12 receptor gene polymorphism and platelet response to clopidogrel in patients with coronary artery disease. *Thromb Res* 2005;116:491–7.
- [25] Fontana P, Dupont A, Gandrille S, Bachelot-Loza C, Reny JL, Aiach M, et al. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. *Circulation* 2003;108:989–95.
- [26] 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;119:355–60.
- [27] Suh JW, Koo BK, Zhang SY, Park KW, Cho JY, Jang JJ, et al. Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. *CMAJ* 2006;174:1715–22.
- [28] Kim KA, Park PW, Park JY. Effect of CYP3A5*3 genotype on the pharmacokinetics and antiplatelet effect of clopidogrel in healthy subjects. *Eur J Clin Pharmacol* 2008;64:589–97.
- [29] Trenk D, Hochholzer W, Fromm MF, Chialda LE, Pahl A, Valina CM, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol* 2008;51:1925–34.
- [30] Lee JM, Park S, Shin DJ, Choi D, Shim CY, Ko YG, et al. Relation of genetic polymorphisms in the cytochrome P450 gene with clopidogrel resistance after drug-eluting stent implantation in Koreans. *Am J Cardiol* 2009;104:46–51.
- [31] Farjadian S, Ota M, Inoko H, Ghaderi A. The genetic relationship among Iranian ethnic groups: an anthropological view based on HLA class II gene polymorphism. *Mole Biol Rep* 2009;36:1943–50.
- [32] Lacy CF, Armstrong LL, Goldman MP, Lance LA. Drug information handbook, 19th ed., Hudson, Ohio: Lexi-comp, Inc; 2010–2011.
- [33] Therivet E, Anglicheau D, King B, Schlageter MH, Cassinat B, Beaune P, et al. Impact of cytochrome P450 3A5 genetic polymorphism on tacrolimus doses and concentration-to-dose ratio in renal transplant recipients. *Transplantation* 2003;76:1233–5.
- [34] Lamba JK, Dhiman RK, Kohli KK. CYP2C19 genetic mutations in North Indians. *Clin Pharmacol Ther* 2000;68:328–35.
- [35] Müller I, Besta F, Schulz C, Massberg S, Schönig A, Gawaz M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost* 2003;89:783–7.
- [36] Samara WM, Bliden KP, Tantry US, Gurbel PA. The difference between clopidogrel responsiveness and posttreatment platelet reactivity. *Thromb Res* 2005;115:89–94.
- [37] Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003;107:2908–13.
- [38] Lev EI, Patel RT, Maresh KJ, Guthikonda S, Granada J, DeLao T, et al. Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention: the role of dual drug resistance. *J Am Coll Cardiol* 2006;47:27–33.
- [39] Rozalski M, Boncler M, Luzak B, Watala C. Genetic factors underlying differential blood platelet sensitivity to inhibitors. *Pharmacol Rep* 2005;57:1–13.
- [40] Cuisset T, Frere C, Quilici J, Morange PE, Saut N, Lambert M, et al. Role of the T744C polymorphism of the P2Y₁₂ gene on platelet response to a 600-mg loading dose of clopidogrel in 597 patients with non-ST-segment elevation acute coronary syndrome. *Thromb Res* 2007;120:893–9.
- [41] Fontana P, Gaussem P, Aiach M, Fiessinger JN, Emmerich J, Reny JL. P2Y₁₂ H2 Haplotype is associated with peripheral arterial disease: a case–control study. *Circulation* 2003;108:2971–3.
- [42] Frere C, Cuisset T, Morange PE, Quilici J, Camoin-Jau L, Saut N, et al. Effect of cytochrome P450 polymorphisms on platelet reactivity after treatment with clopidogrel in acute coronary syndrome. *Am J Cardiol* 2008;101:1088–93.
- [43] Azarpira N, Namazi S, Khalili A, Tabesh M. The investigation of allele and genotype frequencies of CYP3A5 (1*/3*) and P2Y12 (T744C) in Iran. *Mol Biol Rep* 2010.
- [44] Yamamoto K, Hokimoto S, Chitose T, Morita K, Ono T, Kaikita K, et al. Impact of CYP2C19 polymorphism on residual platelet reactivity in patients with coronary heart disease during antiplatelet therapy. *J Cardiol* 2011;57:194–201.
- [45] Sawada T, Shinke T, Shite J, Honjo T, Haraguchi Y, Nishio R, et al. Impact of cytochrome P450 2C19*2 polymorphism on intra-stent thrombus after drug-eluting stent implantation in Japanese patients receiving clopidogrel. *Circ J* 2010;75:99–105.
- [46] Liu XL, Wang ZJ, Yang Q, Ge HL, Gao F, Liu YY, et al. Impact of CYP2C19 polymorphism and smoking on response to clopidogrel in patients with stable coronary artery disease. *Chin Med J* 2010;123:3178–83.
- [47] Oh IH, Park KW, Kang SH, Park JJ, Na SH, Kang HJ, et al. Association of cytochrome P450 2C19*2 polymorphism with clopidogrel response variability and cardiovascular events in Koreans treated with drug-eluting stents. *Heart* 2011.
- [48] Hwang SJ, Jeong YH, Kim IS, Koh JS, Kang MK, Park Y, et al. The cytochrome 2C19*2 and *3 alleles attenuate response to clopidogrel similarly in East Asian patients undergoing elective percutaneous coronary intervention. *Thromb Res* 2011;127:23–8.
- [49] Sofi F, Giusti B, Marcucci R, Gori AM, Abbate R, Gensini GF. Cytochrome P450 2C19*2 polymorphism and cardiovascular recurrences in patients taking clopidogrel: a meta-analysis. *Pharmacogenomics J* 2011;11:199–206.
- [50] Sibbing D, Stegherr J, Latz W, Koch W, Mehilli J, Dorrlor K, et al. Cytochrome P450 2C19 loss-of-function polymorphism and stent thrombosis following percutaneous coronary intervention. *Eur Heart J* 2009;30:916–22.
- [51] Bonello L, Armero S, Ait Mokhtar O, Mancini J, Aldebert P, Saut N, et al. Clopidogrel loading dose adjustment according to platelet reactivity monitoring in patients carrying the 2C19*2 loss of function polymorphism. *J Am Coll Cardiol* 2010;56:1630–6.
- [52] Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet* 2009;373:309–17.
- [53] Shuldiner AR, O'Connell JR, Bliden KP, Gandhi A, Ryan K, Horenstein RB, et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *J Am Med Assoc* 2009;302:849–57.
- [54] Simon T, Verstruyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, et al. French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009;360:363–75.
- [55] Mega JL, Simon T, Collet JP, Anderson JL, Antman EM, Bliden K, et al. Reduced-function CYP2C19 genotype and risk of adverse clinical outcomes among patients treated with clopidogrel predominantly for PCI: a meta-analysis. *J Am Med Assoc* 2010;304:1821–30.
- [56] Wallentin L, James S, Storey RF, Armstrong M, Barratt BJ, Horrow J, et al. PLATO investigators. Effect of CYP2C19 and ABCB1 single nucleotide polymorphisms on outcomes of treatment with ticagrelor versus clopidogrel for acute coronary syndromes: a genetic substudy of the PLATO trial. *Lancet* 2010;376:1320–8.
- [57] Pare G, Mehta SR, Yusuf S, Anand SS, Connolly SJ, Hirsh J, et al. Effects of CYP2C19 genotype on outcomes of clopidogrel treatment. *N Engl J Med* 2010;363:1704–14.
- [58] Smith SMG, Buckland R, Daly ME, Storey RF. Inhibition of platelet aggregation by clopidogrel is unaffected by the CYP2C19 681G>A polymorphism in patients with coronary artery disease (abstr). *J Am Coll Cardiol* 2007;49:375A.
- [59] Desta Z, Zhao X, Shin JG, Flockhart DA. Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin Pharmacokinet* 2002;41:913–58.
- [60] Xie HG, Kim RB, Wood AJ, Stein CM. Molecular basis of ethnic differences in drug disposition and response. *Annu Rev Pharmacol Toxicol* 2001;41:815–50.
- [61] Wedlund PJ. The CYP2C19 enzyme polymorphism. *Pharmacology* 2000;61:174–83.
- [62] Azarpira N, Namazi S, Hendijani F, Banan M, Darai M. Investigation of allele and genotype frequencies of CYP2C9, CYP2C19 and VKORC1 in Iran. *Pharmacol Rep* 2010;62:740–6.
- [63] Zand N, Tajik N, Moghaddam AS, Milanian I. Genetic polymorphisms of cytochrome P450 enzymes 2C9 and 2C19 in a healthy Iranian population. *Clin Exp Pharmacol Physiol* 2007;34:102–5.