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Allele Frequency Distribution of CYP2C19*3 and CYP1A1 Allelic Variants Associated with Clopidogrel Resistance in Cardiac Patients of Pakistan

Kashif-ur-Rehman[1,2], Ahmad Bakhsh[1], Muhammad Akram Tariq[3], Muhammad Khalil Ahmad Khan[4] and Sumaira Mehboob[1]*

[1]*School of Biochemistry, Minhaj University Lahore*
[2]*Parasitology and Molecular Biology Lab, Department of Zoology, University of the Punjab, Lahore*
[3]*Post Graduate College Town Ship, Lahore, Pakistan*
[4]*Department of Zoology, University of Okara, Okara, Pakistan*

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ABSTRACT

Drug resistance is a phenomenon that has received serious attention nowadays in medical practice. Such is the case with clopidogrel treatment response, which is variable inter-individually and inter-ethnically due to genetic polymorphisms in Cytochrome P450 (*CYP*) gene. Clopidogrel is an anti-platelet agent administered to cardiac patients in the form of a prodrug, which is further metabolized into an active form by the CYP enzymes. There are many allelic variants of the *CYP* gene which are involved in clopidogrel resistance but CYP2C19*3 has been proven one of the most significant loss-of-function alleles and the role of CYP1A1 has also been determined in reduced response to clopidogrel. We selected 100 cardiac patients with PCI or ACS who were on clopidogrel treatment and analyzed them for CYP2C19*3 and CYP1A1 allelic variants using gel-based screening of allele-specific amplified products. Sanger sequencing was utilized for the validation of our gel-based genotypes. The observed allelic frequency distribution of CYP2C19*3 variants were 35% heterozygous for A/G variants, 12% homozygous for A variant, and 53% homozygous for G wild type. While in the case of CYP1A1, the allelic frequency distribution of its variants were 5% heterozygous for A/C/G variants, 9% heterozygous for A/G variants, 7% heterozygous for A/C variants, and 79% homozygous for A/A wild type. The overall frequency of CYP1A1 G variants was 14% and C variants were 12%. Our results suggested that there are significant inter-ethnic variations in the allelic frequencies of CYP2C19*3 and CYP1A1 which may be responsible for variable clopidogrel treatment response in Pakistani cardiac patients.

INTRODUCTION

The phenomenon of drug resistance has become a burning issue in the recent era and in other words it is known as low responsiveness or 'non-responsiveness' as the patients show partial or no response to the medical treatment (Nishiyama *et al*., 2022). Globally, the cardiovascular diseases are ranked as the number one in causing death as compared to any other disease. The estimated deaths due to cardiovascular diseases during the year 2008 were 17.3 million which is about 30% of all the deaths worldwide. The countries having middle and low income status are being equally affected which accounts for about 80% of the deaths due to cardiovascular diseases (Mendis *et al*., 2015). From these 17.3 million deaths, about 7.3% deaths occurred due to coronary artery disease and 6.2 million deaths occurred due to stroke (Mendis *et al*., 2011). Now it is worth mentioning that the life saving drug clopidogrel is used to address the cardiovascular complications being an anti-platelet agent in patients who have undergone stents (PCI) and also in patients with acute coronary syndrome (ACS). But, the treatment response to this medicine is variable in different ethnic groups. So, the clopidogrel is recommended in combination with aspirin (Matsushita *et al*., 2022). The combination therapy of clopidogrel with aspirin provides additive advantages to the cardiac patients in significantly reducing the atherothrombotic events which either drug alone cannot provide (Yancy *et al*., 2016). The genetic

* Corresponding author: sumeirfan.sbs@gmail.com, drsumaira.biochem@mul.edu.pk
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variations in cytochrome P450 (*CYP*) gene are mainly responsible for the low response and non-responsiveness of the drug clopidogrel in cardiac patients belonging to different populations (Bergmeijer *et al*., 2018). Like most of the medicines, the clopidogrel is also being converted into its active metabolite i.e. 2-oxo-clopidogrel by the action of CYP isoenzymes in the liver. This gold standard anti-platelet agent blocks P2Y12 receptors on the platelet surface selectively and irreversibly and as a result ADP-induced platelet activation and aggregation is inhibited (Ding *et al*., 2003). The *ex-vivo* platelet aggregation tests have shown that patients having one or two loss of function alleles of CYP2C19 have lower plasma concentration of active metabolite of clopidogrel as compared with patients having no CYP2C19 allelic variant. This variability in inter-individual treatment response is due to the allelic variants of *CYP* (Sibbing *et al*., 2009). It has been elucidated that the hepatic metabolism of clopidogrel is being affected by the phenomenon of genetic polymorphism in CYP2C19 and therefore patients having at least one loss-of-function allele of CYP2C19 either *2 or *3 have low levels of active metabolite of clopidogrel and as a result inhibition of platelet activation is being reduced (Ferreiro and Angiolillo, 2009). Furthermore, it has been observed that there is strong association between the clopidogrel resistance and variable inter-ethnic allelic frequency of loss-of-function allele CYP2C19*3 (Chan, 2012). The significance of screening the loss of function allelic variants of CYP2C19*3 and their association with clopidogrel resistance has been discussed in detail in the review (Hasanzad *et al*., 2022). Such mutations in this variant allele are responsible for the inability of cytochrome P450 enzyme to convert clopidogrel into its active metabolite which result in the increased death risk, heart attack or stroke among patients who have undergone PCI (Abid *et al*., 2013). CYP1A1 is an allele of cytochrome p450 which is involved in the drug metabolism and phase I of xenobiotics. The fluoro-quinolones and macrolides inhibit this enzyme and aromatic hydrocarbons have ability to induce it. The other name of CYP1A1 is aryl hydrocarbon hydroxylase (AHH). It is involved in the metabolic activation of aromatic hydrocarbons to carcinogens (Khan *et al*., 2017). However, an *in vivo* experiment performed in gene-deficient mice has found that CYP1A1 is involved in hydroxylation of benzo (a) pyrene compound which can have protective effect on the DNA, rather than contributing to potentially carcinogenic DNA modifications. This effect is likely due to the fact that CYP1A1 is highly active in the intestinal mucosa, and thus infiltration of ingested benzo (a) pyrene carcinogen into the blood circulation is inhibited (Arlt *et al*., 2012). The gene expression of the CYP1A1 is being regulated by the hetero-dimeric transcription factor, aryl-hydrocarbon nuclear translocator and aryl-hydrocarbon receptor which is a ligand activated transcription factor along with CYP1A2/1B1 genes (Klomp *et al*., 2020). There are many polymorphisms in CYP1A1 which have been identified up till now and some of these polymorphisms result in highly inducible AHH activity of this enzyme. These polymorphisms have mutation 1 which involves substitution (T→C) at nucleotide position 3801 in the 3'-non-coding region, mutation 2 involves substitution (A→G) at position 2455 of nucleotide which leads to an amino acid change from isoleucine to valine at codon 462, mutation 3 involves (T→C) substitution at nucleotide position 3205 of 3'-non-coding region and mutation 4 involves (C→A) substitution at nucleotide position 2453 which leads to change of an amino acid from threonine to asparagine at 461codon (Petersen *et al*., 1991).

In this study, we aimed to determine the allele frequency distribution of allelic variants of CYP2C19*3 and CYP1A1 associated with clopidogrel resistance in Pakistani cardiac patients. We analyzed 100 cardiac patients with PCI or ACS who was on clopidogrel therapy by using Allele Specific Extension based PCR technique. We observed significant inter-ethnic variations in the allelic frequencies of CYP2C19*3 and CYP1A1 in our cardiac patients. Furthermore, we utilized Sanger sequencing to generate chromatograms for allelic variant of CYP2C19*3 and CYP1A1 for validation of our gel based results. The significant inter-ethnic variations in the allelic frequencies of both markers (CYP2C19*3 and CYP1A1) suggested their involvement in variable clopidogrel treatment response in these cardiac patients.

MATERIALS AND METHODS

Blood samples of 100 cardiac patients on clopidogrel therapy were collected in k3 EDTA vials from Punjab Institute of Cardiology Lahore and Mayo Hospital Lahore. These samples were processed for molecular analysis.

*PCR amplification of CYP2C19*3 and CYP1A1*

The DNA was extracted using genomic DNA Extraction Kit (Invitrogen, USA). The quality of DNA was determined on agarose gel electrophoresis. The DNA was quantified using spectrophotometer.

The allele specific and amplification primers for allelic variants of CYP2C19*3 and CYP1A1 were designed by using Primer 3 (http://frodo.wi.mit.edu) and sequences of designed primers are shown in Table I. Genomic DNA flanking the SNP was amplified with allele specific primers. Two different pairs of primers were used for SNP amplification of CYP2C19*3, one with

Table I. Allele specific primer sequences for CYP2C19*2, CYP2C19*3 and CYP1A1 Alleles and their Annealing Temperatures (Tm).

	Sequences of primers	Annealing temperature (Tm) ºC
CYP2C19*3 rs4986893		
A Forward	5′- AGG ATT GTA AGC ACC CCC TGA -3′	61.3
G Forward	5′- AGG ATT GTA AGC ACC CCC TGG -3′	63.3
Universal Reverse	5′-GGC TGT CTA GGC AAG ACT GTA G-3′	64.0
CYP1A1 rs1048943		
A Forward	5′-GAA GTG TAT CGG TGA GAC CA-3′	56.68
C Forward	5′-GAA GTG TAT CGG TGA GAC CC-3′	56.68
G Forward	5′-GAA GTG TAT CGG TGA GAC CG-3′	56.68
T Forward	5′-GAA GTG TAT CGG TGA GAC CT-3′	56.68
Universal Reverse	5′-CAG ACC AGG TAG ACA GAG TC-3′	

wild type allele specific primer and the other with mutant allele specific primer; the reverse primer is non-allele specific and identical in both wild and mutant genotypes. While four different pairs of primers were used for SNP amplification in CYP1A1, one wild type allele specific primer and other three mutant allele specific primers. The strategy for designing of allele-specific primers for both markers was followed by Hirotsu *et al*. (2010) and is depicted for marker CYP2C19*3 (rs4244285) in Figure 1 as an example.

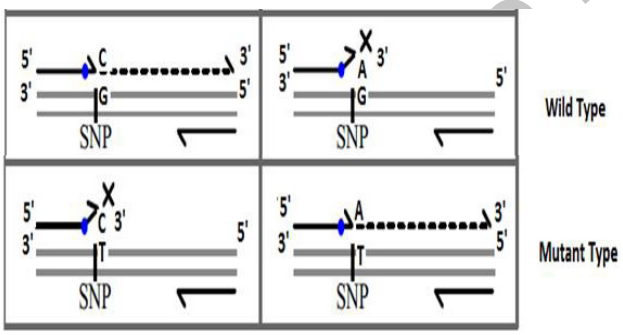

Fig. 1. Amplification strategy for allele specific extension of CYP2C19*3 (rs4244285) as an example.

PCR was performed in a 20µl reaction volume containing genomic DNA 10 ng, oligonucleotide primers 0.4ρM each, 1X PCR Buffer, dNTPs 200 µM, $MgCl_2$ 2mM and Taq polymerase 2U. The following PCR cycling conditions were carried out: 5 min at 95°C for 1 cycle, 32 cycles at 95°C for 30 seconds, with varied annealing temperatures (as given in Table I) for 30 seconds and 72°C for 30 seconds, followed by 1 cycle at 72°C for 5 min.

Amplified SNPs products were electrophoresed on 2% agarose gel stained with ethidium bromide (EtBr) and visualized on UV trans-illuminator. The cytochrome P4502C19 variant CYP2C19*3 and cytochrome P450 (CYP 1A1) variants were genotyped on gel based genotyping method mentioned above.

In order to validate the gel based method of SNP identification, we performed Sanger sequencing of purified PCR product of selected samples to confirm different allelic variants of both markers (CYP2C19*3 and CYP1A1). Sequencing of the purified products of heterozygous genotypes using reverse primers was done with BigDye Sequencing Kit according to the manufacturer's instruction (Applied Biosystems). The amplification consisted of a pre-denaturation at 96˚C for 1min, followed by 35 cycles of denaturing at 96˚C for 15s, annealing at 55˚C for 15s, and extension at 72˚C for 1 min, and a final extension at 72˚C for 5 min. Sequencing products were resuspended in 10 µl of formamide and denatured at 95°C for 5 min. The sequencing was performed on an ABI PRISM 3100Automated sequencer (Applied Biosystems). The sequencing results were assembled using ABI PRISM sequencing analysis software version 3.7 (Applied Biosystems) and analyzed with Chromas software (http://www.technelysium.com.au/chromas.html). The chromatograms of different SNP variants are given below.

RESULTS

We have screened out Pakistani cardiac patients (n=100) with acute coronary syndrome (ACS) and percutaneous coronary intervention (PCI) for alleles of CYP gene involved in reduced response to clopidogrel i.e. CYP2C19*3 and CYP1A1. The average age of patients without cardiac event outcome was 58.4 years while in patients with cardiac event outcome; the average age was 62.5 years. The cardiac event outcome includes

the occurrence of any one or more of the pathological conditions in a patient like myocardial infarction, heart failure or stroke. Out of these 100 cardiac patients, 69 were male and 34 were female. The inclusion criteria were the use of anti-platelet drug i.e. clopidogrel for more than one month, patients with or without cardiac outcome event, ACS or PCI, Hypertension, Diabetes mellitus and hypercholesterolemia. These were the baseline characteristics of the studied patients with their probability (p) values as given in Table II.

Table II. Baseline characteristics of the studied patients.

Demographic and clinical characteristics	Patients without cardiac outcome event	Patient with cardiac out-come event	p value
Male	66	03	0.04*
Female	30	01	0.03*
Average age (Yrs.)	58.4	62.5	0.9 #
ACS	79	05	0.06#
PCI	12	04	0.3 #
Hypertension	85	02	0.02*
DM	32	03	0.09#
Hypercholesterolemia	68	04	0.06#

Cardiac outcome event: It results in the form of myocardial infarction (MI), Heart Failure or stroke; *ACS, acute coronary syndrome; ** PCI, percutaneous coronary interventions; DM, diabetes mellitus; ***p-value, Probability value < 0.05 is statistically significant; *, Statistically Significant value; #, Statistically Non-Significant value.

We implemented the allele specific PCR extension strategy to detect allelic variants using agarose gel electrophoresis. The allele frequency distribution of each allelic variant was calculated and compared with the data available for different ethnic groups. Furthermore, the amplified product of each allelic variant was sequenced using Sanger sequencing method for selected samples to validate the gel based screening. The results of sequencing chromatograms showed 100% concordance of genotypes with gel electrophoresis method providing the evidence for specific amplification of each variant with its allele specific primer.

*CYP2C19*3 allele*

We have screened out Pakistani cardiac patients enrolled for clopidogrel therapy with loss of function allele of *CYP* gene i.e. CYP2C19*3 and analyzed them for allelic variant by using allele specific PCR extension method and visualized the amplified product using agarose gel electrophoresis. The electrophoresed PCR product of 150 base pairs (bp) for CYP2C19*3 allelic variants is shown in Figure 2. The amplified PCR products for CYP2C19*3 allelic variants were sequenced through Sanger sequencing method. The sequencing chromatograms for CYP2C19*3G wild type and A mutant type are shown in Figures 3 and 4, respectively. The results of sequencing chromatograms showed 100% concordance with gel electrophoresis method in the selected samples. The allele frequency distribution of CYP2C19*3 variant and wild type was calculated in Pakistani cardiac patients i.e. 35% were heterozygous for G/A variants, 12% were homozygous for A variant and 53% were homozygous for G wild type. The results of observed allelic frequency of CYP2C19*3 variants are given in Table III.

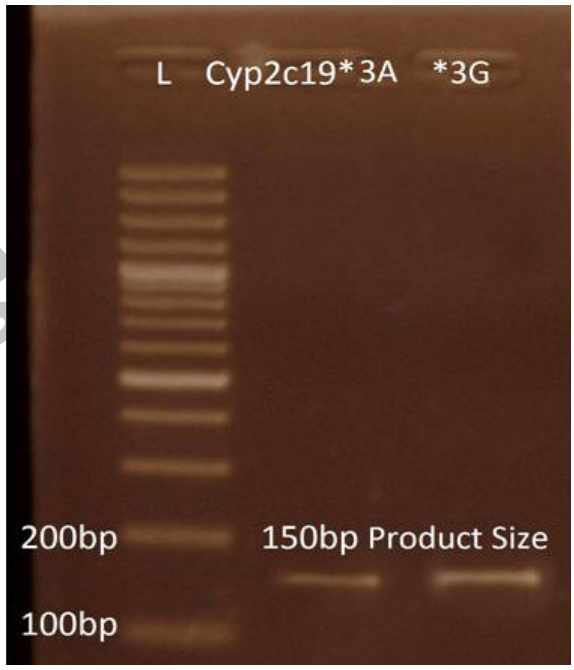

Fig. 2. Gel image showing amplified products for allelic variant A and G wild type of CYP2C19*3.

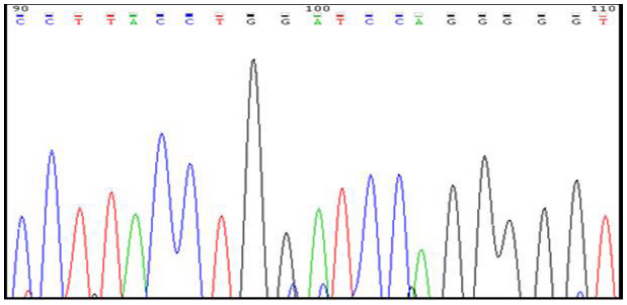

Fig. 3. Sanger sequence chromatogram for wild type CYP2C19*3G showing complementary SNP C at position 102 (reverse strand sequenced).

Table III. The comparison of allele frequency distribution of CYP2C19*3 variants associated with clopidogrel resistance among different populations.

SNP ID	Allelic variants of CYP2C19*3	Frequency distribution (%) in the		
		Studied population	Koreans population (Lee *et al*., 2009)	Caucasians population (Xie *et al*.,2001)
rs4986893	CYP2C19*3 A/A Variant	12	0.8	01
	CYP2C19*3 A/G variant	35	17.6	---
	CYP2C19*3 G/G wild type	53	81.6	99

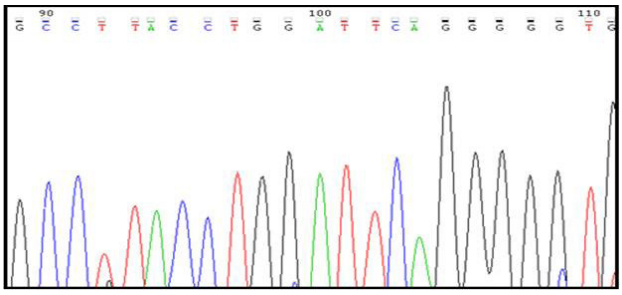

Fig. 4. Sanger sequence chromatogram for allelic variant CYP2C19*3A showing mutated SNP T at position 102 (reverse strand sequenced).

Fig. 5. Gel image showing amplified products for wild type A and allelic variants C, G of CYP1A1.

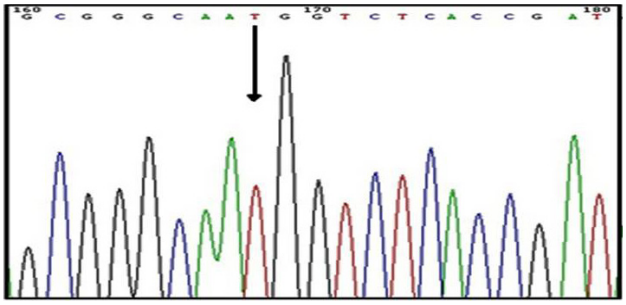

Fig. 6. Sanger sequence chromatogram for wild type A of CYP1A1 showing complementary SNP T at position 168 shown by arrow (Reverse strand sequenced).

CYP1A1 allele

One hundred selected cardiac patients enrolled in this study were analyzed for CYP1A1 allelic variants by using allele specific PCR extension method and visualized the amplified product using agarose gel electrophoresis. The electrophoresed PCR product of 218 base pairs (bp) for CYP1A1 allelic variants is shown in Figure 5. The amplified PCR products for CYP1A1 A wild type and C and G variant types were sequenced by Sanger sequencing method and their sequencing chromatograms are shown in Figures 6, 7 and 8, respectively. The results of sequencing chromatograms showed 100% concordance with gel electrophoresis method in the selected samples. The allele frequency distribution of CYP1A1 allelic variants and wild type was calculated in the selected samples i.e. 5% were heterozygous for A/C/G tri-allelic SNP variants, 9% were heterozygous for A/G bi-allelic variants, 7% were heterozygous for A/C bi-allelic variants and 79% were homozygous for A wild type. The overall frequency of CYP1A1 G variants was 14% and C variants were 12%. However, T SNP variant for CYP1A1 was not identified in any of the analyzed sample. The results of observed allelic frequency of different CYP1A1 variants are given in Table IV. For the first time, we have found C variant of CYP1A1 allele in Pakistani population which according to our knowledge has not been reported before in any other population.

Table IV. The comparison of allele frequency distribution of CYP1A1 variants associated with clopidogrel resistance among different populations.

SNP ID	Allelic variants of CYP1A1	Frequency distribution (%) in the	
		Studied population	Korean population (Lee *et al*., 2009)
rs1048943	A/A wild type	79	55
	A/G variant	09	40
	A/C variant	07	---
	A/C/G variant	05	---
	G/G variant	---	05

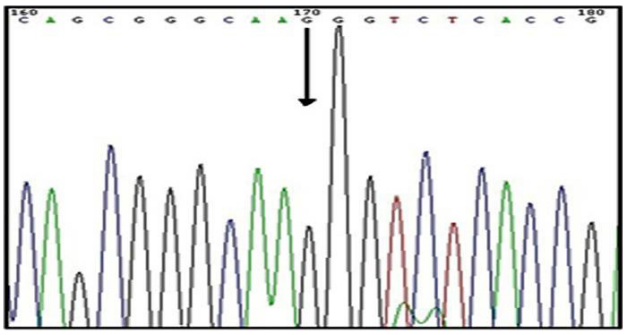

Fig. 7. Sanger sequence chromatogram for mutant type C of CYP1A1 showing complementary SNP G at position 170 shown by arrow (Reverse strand sequenced).

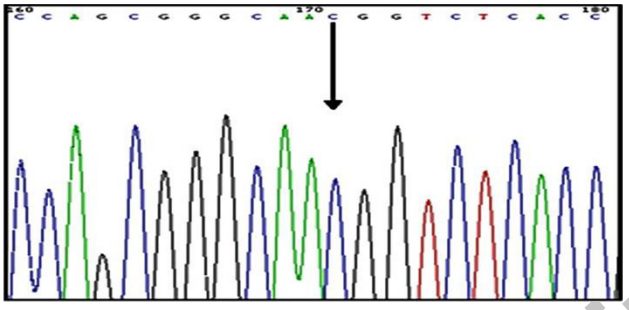

Fig. 8. Sanger sequence chromatogram for allelic variant CYP1A1 G showing mutated SNP C at position 171 shown by arrow (Reverse strand sequenced).

DISCUSSION

Clopidogrel is an anti-platelet agent administered to cardiac patients in the form of a pro-drug, which is further metabolized into active form by the CYP enzymes. The treatment response to clopidogrel is variable inter-individually and inter-ethnically due to genetic polymorphisms in cytochrome P40 (CYP) gene. We have screened out Pakistani cardiac patients (n=100) for alleles of *CYP* gene involved in reduced response to clopidogrel i.e. CYP2C19*3 and CYP1A1. We implemented the allele specific PCR extension strategy to detect allelic variants using agarose gel electrophoresis. The results of allelic variants were in concordance with Sanger sequencing chromatograms. CYP2C19*3 (CYP2C19m2) allele is characterized with a point mutation from G to A at 636 position of exon 4 and this transversion results in the production of stop codon prematurely (Demorais et al., 1994). It has been observed that CYP2C19*3A is involved in clopidogrel resistance in many ethnic groups (Akkaif *et al*., 2021; Harmsze *et al*., 2010; Valeria *et al*., 2021). The Koreans have allelic frequency 17.6% for heterozygous G/A variant, 0.52% for homozygous A variant and 81.7% for G wild type (Akkaif *et al*., 2021). While caucasians have allele frequency 1% for A variant and 99% for G wild type (Ramamoorthy *et al*., 2022). Compared to our results i.e. 35% were heterozygous for G/A variants, 12% were homozygous for A variant and 53% were homozygous for G wild. Our study is further providing the evidence for inter-ethnic differences in allele frequency distribution of CYP2C19*3 variants. Such inter-ethnic differences in the allelic frequencies of CYP2C19*3 variants are responsible for differential dose response to clopidogrel therapy in cardiac patients (Akkaif *et al*., 2021). According to Lee *et al*. (2009) and Harmsze *et al*. (2010) CYP2C19*3 polymorphism has been proven the most significant indicator for clopidogrel resistance as they have found that the carriers of CYP2C19*3A allelic variant had higher proportion of clopidogrel resistance. Furthermore, they have performed multiple logistic regression analysis for the data which demonstrated that CYP2C19*3A allelic variant is an independent predictor for clopidogrel resistance (Harmsze *et al*., 2010). The role of this mutant allele is more significant in the *CYP* activity compared to other studied alleles in Korean patients with coronary artery disease. That's why, CYP2C19*3 allele may have significant role in the metabolism of clopidogrel in Asian populations. Hulot *et al*. (2006) have reported that there is deficiency of CYP2C19*3A allele in young healthy white subjects, although the sample size was too small to represent the Caucasian population (Hulot *et al*., 2006). Xie *et al*. (2001) have presented in a study that the frequency of allelic variant CYP2C19*3A in Caucasians is 1% (Xie *et al*., 2001).

Our results suggest for the first time that allelic variant of CYP2C19*3 may be a significant risk factor for clopidogrel resistance and also it may have significant importance in the metabolism of clopidogrel in Pakistani cardiac patients. Further investigations are recommended to elucidate the functional influence of CYP2C19*3 allelic variant on the response to clopidogrel loading dose. The comparison of allele frequency distribution of CYP2C19*3 variants of Pakistani population with different other populations is given in Table III.

The CYP1A1 allele is mainly known due to its role in carcinogenesis. Although the studies regarding its role in drug metabolism, especially in clopidogrel metabolism are scarce as just one study is available in literature by Lee *et al*. (2009). The allelic variants of CYP1A1 involves the transition from A to G at position 4,889 in exon 7 which results change in amino acid from an isoleucine to valine (Ile-Val) at codon 462 (Ezzeldin *et al*., 2017) have demonstrated that CYP1A activity plays a key role in the metabolism of clopidogrel. Lee *et al*. (2009) have shown the comparison of percent platelet aggregation inhibition

according to each genotype including CYP1A1. The data of dominant models for CYP1A1 and CYP2C19*2 polymorphism have indicated a significant difference in percent platelet aggregation inhibition. This is the only study which demonstrates that allelic variants of CYP1A1 have some role in the reduced clopidogrel response. According to this study, the allele frequency distribution of CYP1A1 allelic variants in Korean population was 40% heterozygous for A/G variant, 5% homozygous for G/G variant and 55% homozygous for A/A wild type (Lee *et al*., 2009). In comparison to this, the allele frequency distribution of CYP1A1 variants in our population was 5% heterozygous for A/C/G tri-allelic SNP variants which has been reported for the first time according to our information, 9% heterozygous for A/G bi-allelic variants, 7% heterozygous for A/C bi-allelic variants and 79% homozygous for A wild type. The overall frequency of CYP1A1 G variants was 14% and C variants were 12%. The T SNP variant for CYP1A1 was not identified in any of the selected samples We have identified tri-allelic SNPs for the first time according to our information in CYP1A1 allele in Pakistani cardiac patients i.e. CYP1A1 A/C/G SNPs (5%). There was no evidence of tri-allelic SNP variants of CYP1A1 in different populations including Asian populations on clopidogrel therapy in the literature. Although bi-allelic SNP variants of CYP1A1 allele have been identified, some of these SNPs have potential to increase AHH enzymatic activity (Wongpratate *et al*., 2020). However, we observed significant frequency i.e. 12% of new allelic variant C at the same transition position from A to C as for A to G in heterozygous forms, in Pakistani cardiac patients as confirmed by Sanger sequencing method. However, the role of this new allelic variant still needs to be determined in clopidogrel responsiveness. Three different mutational mechanisms reported in literatures that are responsible to generate such an excess of tri-allelic sites are firstly hyper mutable regions in DNA, secondly simultaneous generation of two of the alleles at a tri-allelic site within a single individual and thirdly, subsequent mutations induced by a single SNP by the process of base mismatching in hetero-duplex DNA during recombination. For example, some sites may be hyper-mutable, and if the mutation rate of at least two pathways (e.g. C/T and C/A) is elevated at such sites, then there will be an excess of tri-allelic sites. The mutation rate of a site is known to depend upon the adjacent nucleotides, the best known example being the CpG dinucleotide at which the frequency of both transition and transversion mutations is elevated (Hodgkinson and Eyre, 2010). However, other adjacent nucleotides also influence the mutation rate (Tenaillon and Matic, 2020). Our results suggested that there are significant inter-ethnic variations in the allelic frequencies of CYP1A1 variants, which may be one of the factors responsible for variable clopidogrel response in cardiac patients. However, a large scale study with both CYP1A1 genotyping and platelet function assay is required to investigate its role in reduced clopidogrel response in Pakistani cardiac patients. The comparison of allele frequency distribution of CYP1A1 variants of Pakistani population with Koreans is given in Table IV.

Despite providing the informative genotyping data for CYP2C19*3 and CYP1A1 allelic variants in our population, there were several limitations to the present study. Only patients admitted to the hospitals were enrolled in the study. Therefore, some selection bias was likely. Small sample size of patients is insufficient to provide significant allele frequency distribution of these variants. A large scale study is required to strengthen the present issue regarding allele frequency distribution of these variants which contribute as a risk factor for stent thrombosis. Although it has been proven in many studies that CYP2C19*3 allele is an independent predictor of clopidogrel resistance (Hwang and Green, 2004; Meman and Seemab, 2014). However, we did not assess platelet aggregation assay in our population. Therefore, we suggest that there is a need to investigate the role of newly reported allelic variant CYP1A1C in inhibition of the anti-platelet response to clopidogrel.

CONCLUSION

In conclusion, we have screened out allelic variants of CYP2C19*3 and CYP1A1for the first time according to our information in Pakistani cardiac patients with ACS and PCI. We have observed significant inter-ethnic variability in the allelic frequencies of CYP2C19*3 and CYP1A1 providing the evidence that genetic polymorphism of CYP2C19*3 and CYP1A1 could be important in clopidogrel treatment response in Pakistani cardiac patients. Hence, the genetic screening of allele variants of CYP2C19*3 and CYP1A1 are emphasized as a suitable tool for selecting rationale anti-platelet drug to treat cardiac patients which will be a step towards personalized medicine. However, further studies are required to investigate other likely factors involved in clopidogrel resistance and there is also need of a larger study to better assess the role of genotyping in the evaluation of the phenomenon of clopidogrel resistance.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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