

CYP2C19 Genetic Polymorphism in the East of Iran: It's Association with the Severity and Pattern of Coronary Artery Disease

Mahdi Zahedi^{1*}, Mahmoud Mohammadzadeh Shabestari², Hossein Ayatollahi³, Arash Gholoobi²

¹ Interventional Cardiologist, Ischemic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran

² Interventional Cardiologist, Atherosclerosis Prevention Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

³ Hematopathologist, Department of Pathology, Mashhad University of Medical Sciences, Mashhad, Iran

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 22 Feb 2019
Revised: 06 Mar 2019
Accepted: 09 Mar 2019

Keywords:
Clopidogrel
Coronary Artery Disease
CYP2C19 Polymorphism

ABSTRACT

Introduction: Since there has been a dearth of research on the assessment of CYP2C19 polymorphism in the east of Iran (Khorasan provinces), this study aimed to detect, CYP2C19*2 and CYP2C19*3 allele frequencies among patients with coronary artery disease. The participants were selected among those referring to Emam Reza Hospital, Mashhad, Iran. Furthermore, the current research was motivated to elucidate the association of CYP2C19 polymorphism with the severity and pattern of coronary artery disease.

Material and Methods: This study was conducted on 84 patients who were subjected selective coronary angiography. The participants of the present study were from Khorasan, Iran. The Genotyping of extracted crude DNA for CYP2C19*2 (rs4244285) and CYP2C19*3 (rs4986893) alleles was performed through PCR-RFLP method.

Results: The obtained results of the current study revealed three different allelic band patterns. Out of the 84 individuals, 71 were homozygous for the wild type allele in both exon 5 and exon 4 (wt/wt; 84.5%), 15 were homozygous for the CYP2C19*2 polymorphism (*2/*2; 14.3%), and 1 subject was homozygous for the CYP2C19*3 (*3/*3; 1.2%). No subjects were heterozygous for the CYP2C19*2 (wt/*2; 0.0%) or CYP2C19*3 (wt/*3; 0.0%) or heterozygous for the CYP2C19*2 and the CYP2C19*3 mutations (*2/*3; 0.0%).

Conclusion: The findings of the current study confirmed the existence of CYP2C19 polymorphism among people of Khorasan.

► Please cite this paper as:

Zahedi M, Mohammadzadeh Shabestari M, Ayatollahi H, Gholoobi A. CYP2C19 Genetic Polymorphism in the East of Iran: Its Association with the Severity and Pattern of Coronary Artery Disease. J Cardiothorac Med. 2019; 7(1): 417-421.

Introduction

The P2Y₁₂ receptor inhibition by thienopyridines is one of the most important antiplatelet strategies to prevent stent thrombosis in patients undergoing percutaneous coronary intervention (PCI) (1).

The R130964 is an active metabolite of

clopidogrel that is produced by a 2-step process in the liver. It involves several CYP450 isoenzymes (2). CYP2C19 isoenzyme is required for half of the first step formation (3, 4).

The decreased rates of clopidogrel activation are associated with CYP2C19 polymorphisms (5,

*Corresponding author: Mahdi Zahedi, Ischemic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran. Tel and Fax: +98 5138022711; Email: dr_zahedi@hotmail.com

© 2019 mums.ac.ir All rights reserved.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

6). On the other hand, the increased rates of thrombosis and decreased antiplatelet response to clopidogrel are related to polymorphisms in the CYP2C19*2 and CYP2C19*3 alleles (7).

There are ethnic differences in the prevalence of these loss-of-function alleles among Caucasians, African Americans, Asians, and Latinos; however, these groups have different enzyme expression (7).

Iran is a country with a large number of ethnic groups, such as Khorasani, Fars population, Azari, Gilaki and Mazandarani, Kurd, Arab, Lor, Balouch, Turkmen.

Regarding the dearth of investigations on the assessment of CYP2C19 polymorphism in the east of Iran (Khorasani population), this study aimed to detect CYP2C19*2 and CYP2C19*3 allele frequencies in patients with coronary artery disease referring to Emam Reza Hospital, Mashhad, Iran (referral cardiology center of east of Iran), Mashhad, Iran. Furthermore, this study moved further to elucidate the association of CYP2C19 polymorphism with the severity and pattern of coronary artery disease.

Materials and Methods

A total number of 84 patients from Khorasan participated in this prospective study. The sample size was determined based on the sample size of previous studies. The participants were selected from those subjected to selective coronary angiography.

About 5 ml of blood was taken from each subject and DNA was extracted from leucocytes. The genotyping of the extracted crude DNA for CYP2C19*2 (rs4244285) and CYP2C19*3 (rs4986893) alleles was performed by the PCR-RFLP method as described previously (8).

The PCR amplification was performed using the forward and reverse primers, including 5'-AATTACAACCAGAGCTTGGC-3' and 5'-TATCAC TTTCCATAAAAAGCAAG-3'. The primers used for the analysis of CYP2C19*3 mutant allele were 5'-TATTATTATCTGTAACTAATATGA-3' and 5'-ACTTCAGGGCTTGGTCAATA-3'.

After selective coronary angiography, the syntax score for each patient was calculated retrospectively by scoring all coronary lesions with diameter stenosis 50% and vessel diameter of 1.5 mm, using the SYNTAX (SX) score algorithm (9, 10).

The CYP2C19 polymorphism was also assessed for six patients with drug-eluting stent thrombosis (five patients with acute and one patient with sub acute stent thrombosis).

The study protocol was approved by the ethics committee of the university and written informed consent was obtained from the volunteers.

Statistical analysis

All statistical analyses were performed using the Statistical Package of Social Science (SPSS) software (version 24, IBM Corporation) and the means of variables were calculated in the current study. Data were presented as mean±SD for quantitative variables and percentages for qualitative variables (frequency).

Allele and genotype frequencies were calculated and compared between groups.

The Chi-square test, Fisher's exact, and t-tests were performed to assess the relationship among genotype, SX score, and pattern of coronary artery disease. P-values less than 0.05 was considered statistically significant.

Results

A total of 84 patients (41 males and 43 females) with the age range of 35-83 years were included in this study. The mean age of the investigated participants was 59.87±11.45. All patients were subjected to selective coronary angiography. The genotyping of CYP2C19*2 and CYP2C19*3 were performed for all the patients. Furthermore, the SX score was calculated for all study population.

CYP2C19 Genotyping

The findings revealed three different allelic band patterns. Out of the 84 individuals investigated in this study, 71 were homozygous for the wild type allele in both exon 5 and exon 4 (wt/wt; 84.5%), 15 were homozygous for the CYP2C19*2 polymorphism (*2/*2; 14.3%), and 1 subject was homozygous for the CYP2C19*3 (*3/*3; 1.2%).

No subject was heterozygous for the CYP2C19*2 (wt/*2; 0.0%) or CYP2C19*3 (wt/*3; 0.0%) or heterozygous for the CYP2C19*2 and the CYP2C19*3 mutations (*2/*3; 0.0%).

The allele frequencies of the CYP2C19*2 and CYP2C19*3 mutations in the investigated subjects were 14.3% and 1.2%, respectively (Table 1).

There was no significant difference between patients with regard to age (60.1±11.6, 58.0±10.6, and 68 for wt/wt, *2/*2, and *3/*3 genotype, respectively). Moreover, as can be seen in Table 2, the subjects were homogenous in

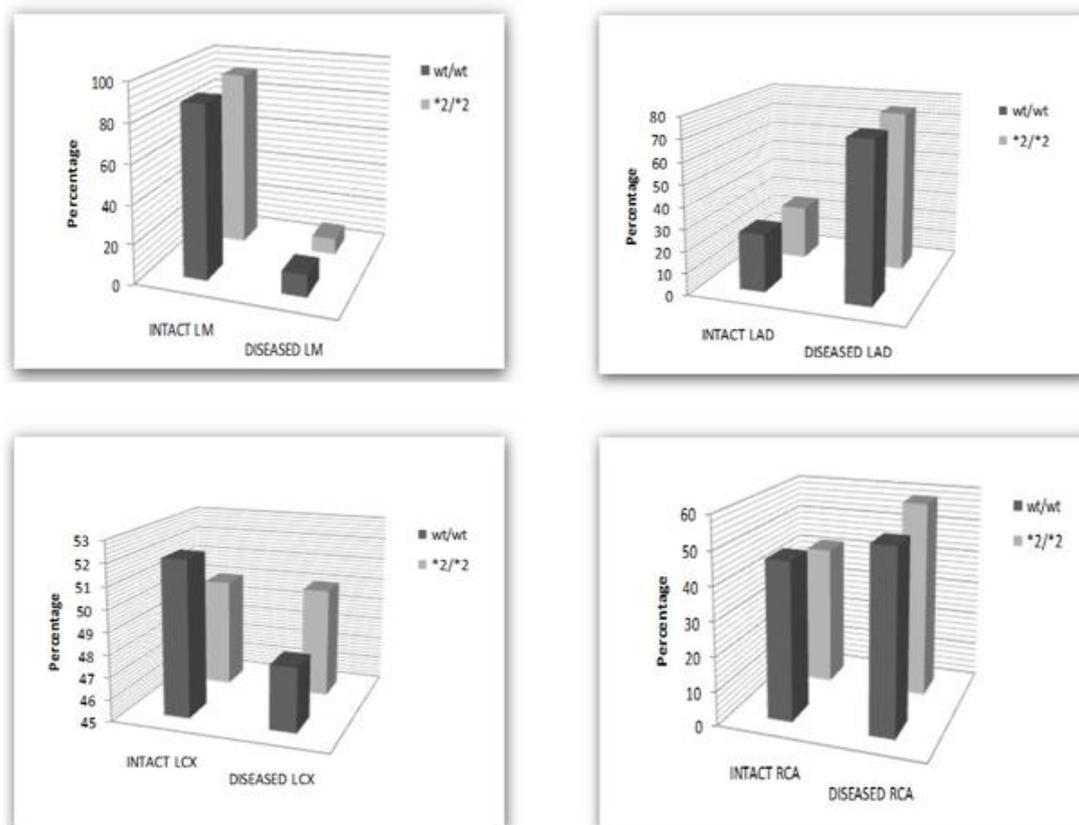
Table 1. Genotype and allele frequencies of CYP2C19 (n=84)

Genotype	n (%)
*1/*1 (wild type)	71 (84.5)
*1/*2	0 (0.0)
*1/*3	0 (0.0)
*2/*2	12 (14.3)
*2/*3	0 (0.0)
*3/*3	1 (1.2)
*1 allele	142 (84.5)
*2 allele	24 (14.3)
*3 allele	2 (1.2)

Table 2. Demographic data according to Genotype

Parameter	Wild Type (wt/wt) (n=71)	Cyp2c19*2 Homozygote (*2/*2) (n=12)	P Value
Age (years) ^a	60.1±11.6	58.0±10.6	0.56
Male	33(46.5%)	7(58.3%)	0.44
Female	38(53.5%)	5(41.7%)	

^a Depicted as mean ± SE.

**Figure 1.** Association of CYP2C19 polymorphism and pattern of coronary artery disease**Table 3.** SYNTAX score according to genotype

Parameter	Wild Type (wt/wt) (n=71)	Cyp2ca9*2 Homozygote (*2/*2) (n=12)	P Value
SX score	12.6±1.4	12.2±3.7	0.95

Depicted as mean ± SE. SX., SYNTAX

terms of sex (33 male, 38 female; 7 male, 5 female; and 1 male, 0 female for wt/wt, *2/*2, and *3/*3 genotype, respectively).

Selective coronary angiography

All 84 patients were subjected to selective coronary angiography. Figure 1 indicates the involvement of left main (LM), left circumflex (LCX), left anterior descending (LAD), and right coronary artery (RCA), in patients with wt/wt and *2/*2 genotypes.

All three major epicardial coronary arteries were involved in the patient with *3/*3 genotype.

There was no significant correlation between the pattern of coronary artery involvement and CYP2C19 polymorphism (P=0.9).

SYNTAX score

As can be seen in Table 3, the mean SX score in patients with CYP2C19 *1/*1 (wt/wt), *2/*2 and *3/*3 genotype were 12.6±1.4, 12.9±3.7, and 35 respectively (P =0.9).

CYP2C19 Genotyping in patients with stent thrombosis

The CYP2C19 genotyping was observed in five

Table 4. Genotype of patients with stent thrombosis

	Thrombosis	Genotype
Case 1	Subacute	wt/wt
Case 2	Acute	*2/*2
Case 3	Acute	wt/wt
Case 4	Acute	wt/wt
Case 5	Acute	wt/wt
Case 6	Acute	wt/wt

patients with acute and one patient with subacute drug-eluting stent thrombosis. The patient with subacute stent thrombosis had *1/*1(wt/wt) genotype. Among patients with acute stent thrombosis, one subject had the *2/*2 genotype and four subjects had *1/*1 (wt/wt) genotype (Table 4).

Discussion

The interethnic differences in the frequency of defective CYP2C19 alleles or diminished CYP2C19 catalytic activity is an important research subject. The reason is that interethnic differences may result in various CYP expression and marked variability in drug response, drug activity, or detoxification. Therefore, it is essential to understand the genetic factors that influence CYP levels and activities.

Furthermore, CYP2C9 is the most abundant isoform, which represents approximately 18% of total hepatic CYPs. The CYP2C19 indicates about 3% of the total hepatic CYPs. Several reports of CYP2C genetic polymorphism demonstrate its potential clinical role in the determination of both inter-individual and inter-ethnic differences in drug efficacy (11-15).

In this study, the distribution of CYP2C19 common variants in the Iranian (Khorasani) population was determined and the obtained results of these data with those from other populations were compared.

CYP2C19 genotyping in Khorasanian population

In our study three different allelic band patterns were observed. The allele frequencies of the CYP2C19*2 and CYP2C19*3 mutations in our subjects were 14.3% and 1.2%, respectively.

In a study conducted by Zand et al., the frequency of CYP2C19*1, CYP2C19*2, and CYP2C19*3 were respectively 86.4%, 13.7%, and 0% among the Persian population. There was no statistically significant difference between Khorasani population and Persian population in the study by Zand et al. regarding the frequency of these alleles (16).

Table 5 depicts comparison of the frequency of CYP2C19*2 and *3 alleles in other Asian populations versus Khorasanian population.

CYP2C19 genotype regarding coronary artery disease

All three major epicardial coronary arteries were involved in the patient with *3/*3 genotype. There was no statistically significant difference between patients with CYP2C19*2/*2 and those with wt/wt genotypes regarding the involvement of epicardial coronary arteries.

It seems that CYP2C19 genotyping does not affect the pattern and severity of epicardial coronary artery involvement.

CYP2C19 Genotyping in patients with stent thrombosis

Regarding the rarity of stent thrombosis among the investigated patients, CYP2C19 genotyping was assessed (only in five patients with acute and one patient with subacute drug-eluting stent thrombosis). The patient with subacute stent thrombosis had *1/*1 genotype. Among patients with acute stent thrombosis, one subject had the *2/*2 genotype and four subjects had *1/*1 genotype.

Table 5. Frequency of CYP2C19*2 and *3 alleles in other Asian populations versus Khorasanian population

Population	Sample size	Cyp2c19*2	Significance	Cyp2c19*3	Significance
		Allele frequency (%)		Allele frequency (%)	
Iranian- Khorasanian	84	14.3		1.2	
Iranian- (Fars province)	404	13.7	NS	0	NS
Jordan	78	16	NS	0	NS
Saudi Arabia	97	15	NS	0	NS
Phillipines	52	39	S	8	S
India	121	29	S	0	NS
Japan	217	27.4	S	10.8	S
Korea	103	20.9	S	11.7	S
Australian	227	35.5	S	14.3	S

S: Significant, NS: Not significant

Conclusion

The obtained results of the current study confirmed the existence of CYP2C19 polymorphism among the selected patients from Khorasan. However, further studies are required to assess the clinical significance of the

polymorphism for treatment outcome and optimal dosage of drugs metabolized by this polymorphic enzyme. It is of potential clinical importance to be able to identify Khorasani individuals who have altered pharmacokinetics

for CYP2C19 substrates. As a result, an appropriate dosage strategy for these drugs (e.g., clopidogrel) can be adopted, and adverse drug reactions (e.g., stent thrombosis) can be avoided.

Acknowledgments

Non

Conflict of Interest

The authors declare that they have no conflicts of interests.

References

1. Wang H, Qi J, Li Y, Tang Y, Li C, Li J, et al. Pharmacodynamics and pharmacokinetics of ticagrelor vs. clopidogrel in patients with acute coronary syndromes and chronic kidney disease. *Br J Clin Pharmacol*. 2018; 84:88-96.
2. Holmes DR Jr, Dehmer GJ, Kaul S, Leifer D, O'Gara PT, Stein CM. ACCF/AHA clopidogrel clinical alert: approaches to the FDA "boxed warning". a report of the American College of Cardiology Foundation Task Force on clinical expert consensus documents and the American heart association endorsed by the society for cardiovascular angiography and interventions and the society of thoracic surgeons. *J Am Coll Cardiol*. 2010; 56:321-41.
3. 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.
4. Luo HR, Poland RE, Lin KM, Wan YJ. Genetic polymorphism of cytochrome P4502C19 in Mexican Americans: a cross-ethnic comparative study. *Clin Pharmacol Ther*. 2006; 80:33-40.
5. Man M, Farmen M, Dumauval C, Teng CH, Moser B, Irie S, et al. Genetic variation in metabolizing enzyme and transporter genes: comprehensive assessment in 3 major East Asian subpopulations with comparison to Caucasians and Africans. *J Clin Pharmacol*. 2010; 50:929-40.
6. Mirabbasi SA, Khalighi K, Wu Y, Walker S, Khalighi B, Fan W, et al. CYP2C19 genetic variation and individualized clopidogrel prescription in a cardiology clinic. *J Community Hosp Intern Med Perspect*. 2017; 7:151-6.
7. Anichavezhi D, Chakradhara Rao US, Shewade DG, Krishnamoorthy R, Adithan C. Distribution of CYP2C19*17 allele and genotypes in an Indian population. *J Clin Pharm Ther*. 2012; 37:313-8.
8. Serruys PW, Morice MC, Kappetein AP, Colombo A, Holmes DR, Mack MJ, et al. Percutaneous coronary intervention versus coronary-artery bypass grafting for severe coronary artery disease. *N Engl J Med*. 2009; 360:961-72.
9. 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.
10. Brown SA, Preira N. Pharmacogenomic impact of CYP2C19 variation on clopidogrel therapy in precision cardiovascular medicine. *J Pers Med*. 2018; 8:E8.
11. Novkovic M, Matic D, Kusic-Tisma J, Antonijevic N, Radojkovic D, Rakicevic L. Analysis of the CYP2C19 genotype associated with bleeding in Serbian STEMI patients who have undergone primary PCI and treatment with clopidogrel. *Eur J Clin Pharmacol*. 2018; 74:443-51.
12. Ge H, Lv X, Ren H, Jin H, Jiang Y, He H, et al. Influence of CYP2C19 genetic polymorphisms on clinical outcomes of intracranial aneurysms treated with stent-assisted coiling. *J Neurointerv Surg*. 2017; 9:958-62.
13. Zand N, Tajik N, Hoormand M, Moghaddam AS, Milanian I. Allele frequency of CYP2C19 gene polymorphisms in a healthy Iranian population. *Iran J Pharmacol Ther*. 2005; 4:124-8.