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CYP2C19 Genetic Polymorphism of Cytochrome p450 Enzyme in Iranian Fars Ethnic Group

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Authors' contributions

Author AM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RGT and OAA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: There are differences in the frequency of *CYP2C19* mutant alleles among different ethnic group. The aim of present study was to estimate the distribution of *CYP2C19* allele and genotypic variants in Iranian Fars ethnic group and compare it with other populations.
Study Design: Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) technique was used to determine Genotyping of *CYP2C19* alleles.
Place and Duration of Study: Biochemistry and Biophysics, Metabolic Disorders Research Center, Gorgan Faculty of Medicine, Golestan University of Medical Sciences, between September 2012 and August 2013.
Methodology: To determine the genotype and allelic frequency of *CYP2C19*, 140 unrelated healthy Fars origin people who were referred to Health Center, were included this study.
Results: The allele frequency of *CYP2C19*1*, *CYP2C19*2* and *CYP2C19*3* were 77.8%, 19.2% and 2.8%, respectively. 75% of subjects were with *CYP2C19*1/*1* genotype. 22.1%, 1.4%, 1.4%, 0% and 0% subjects were with *CYP2C19*1/*2*, *CYP2C19*1/*3*,

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*CYP2C19**2/*2, *CYP2C19**2/*3 and *CYP2C19**3/*3 genotypes, respectively. Poor metabolizer, intermediate metabolizer and extensive metabolizers genotype frequencies were seen in 75%, 23.5% and 1.4% of subjects, respectively.

Conclusion: Ethnic differences in *CYP2C19* genetic polymorphism of cytochrome *p450* enzyme may cause variation in drug response, activity or detoxification. This study suggests the further study of this polymorphic enzyme in Fars population to determine the clinical significance and optimal dosage of some drugs in this ethnic group.

Keywords: *CYP2C19* polymorphism; fars; polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP).

1. INTRODUCTION

Cytochrome *P450 2C19* (*CYP2C19*) forms an isoform of the *CYPs*. It shows a correlation with the metabolism of some significant drugs, such as omeprazole, lansoprazole, and pantoprazole that have been shown to exhibit a greater cure rate for gastric ulcers with *Helicobacter pylori* infections in poor metabolizers (PMs) than in extensive metabolizers (EMs) [1]. It has been shown that there are three allelic variants of *CYP2C19* (*CYP2C19**1, *CYP2C19**2, *CYP2C19**3) with different enzymatic activities [2].

The most common polymorphisms of *CYP2C19* gene are *CYP2C19**2 and *CYP2C19**3 alleles [3]. These polymorphisms are considered the PMs and EMs phenotypes for malfunction of *CYP2C19* alleles. The frequency of the PM phenotype is higher in Asian populations (18 – 23%) than in Caucasians (2 – 5%) [4]. It has been shown that the *CYP2C19**2 allele is the most common variant among the normal Iranian population. *CYP2C19**3 is absent in the normal Iranian population [5].

Some studies showed that wider frequency of poor metabolizer phenotypes in oriental subjects is presented. Some studies indicated that 18-23%, 15-17%, 12-16% and 4-7% of Japanese [6-7], Chinese [8-9], Koreans [10-11] and Black African [12] shown to have poor metabolizer phenotype, respectively. *CYP2C19**2 is the major alleles among the Oriental. These defective alleles for the Oriental and the Caucasians account 75% and 93% of populations, respectively [4,13]. *CYP2C19**3 phenotype includes about 25% of defective gene among the Orientals. It has been initially shown in Japanese poor metabolizer population [14], but this phenotype was significantly rare among non-Oriental subpopulation [15]. Some studies have shown that the prevalence of poor metabolizer phenotype was 3-5% and 70% in European white [16] and the inhabitants of Vanuatu Island in Melanesia populations [17], respectively. The prevalence of *CYP2C19**2 and *CYP2C19**3 alleles were 29.7% and 0.00% among north Indian populations, respectively [18].

Some other study has shown that the frequency of *CYP2C19**2 and *CYP2C19**3 alleles among South Indian of Tamil, Telgu, Kannada and Malayalam were 35% and 1%, respectively [19]. The main aim of present study was to estimate the distribution of *CYP2C19* variants in Iranian Fars ethnic group.

2. MATERIALS AND METHODS

To determine the allelic frequency of *CYP2C19*, 140 unrelated healthy Fars origin people (people who speak Persian as a native language) who were referred to Health Center (They

were referred to Health Center before they perform a wedding) in Gonbadkavoos (located in North East of Iran, South East of Caspian Sea), 2012-2013. Fars people who had no history of sickness were included. The exclusion criterion was the coexistence of any other serious illness.

DNA was extracted from peripheral white blood cells using salting out method [20]. Samples were stored in -20°C until analysis was done. Detection of the *CYP2C9*2* and *CYP2C9*3* variant alleles were done by Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) technique [4]. 25 microliter used PCR mixture include PCR buffer (10 mM Tris-HCl, pH 9, 1.5 mM MgCl_2 , 50 mM KCl, 10 mM deoxyribonucleotide triphosphate mix, 5 U/ μl Taq polymerase, 5 pM of each primer, 500 ng DNA and sterile distilled water. The PCR was carried out in this mixture. Genetix CG palm-thermocycler was used to carry out PCR. Restriction enzymes (SmaI for *CYP2C19*2* and BamHI for *CYP2C19*3*) were used to digest PCR products at 30°C and 37°C for 16 hrs for complete digestion, respectively. The method of De Morais et al. [4] was used for primers amplification. Electrophoresis of the DNA fragments were done on a 2% (for *CYP2C19*3*) and 3% (for *CYP2C19*2*) agarose gel. The gels were stained with Ethidium bromide. A short wavelength UV transluminator was used to detect bands. Polaroid Gel Camera was used to photograph bands. Sense primer 5'-AATTACAACCAGAGCTTGGC-3' and antisense primer 5'-TATCACTTTCCATAAAAGCAAG-3' were used to detect the *CYP2C19*2* mutation. Sense primer 5'-AAATTGTTTCCAATCATTAGCT-3' and antisense primer 5'-ACTTCAGGGCTTGGTCAATA-3' were also used to detect the *CYP2C19*3* mutation. The PCR amplification conditions for findings out of *CYP2C19*2* and *CYP2C19*3* were as follow: Initial denaturation: 94°C , 300 sec., Number of cycle(s): 37, Denaturation: 94°C , 60 sec., Extension: 72°C , 30 sec., Final extension step: 72°C , 300 sec. Annealing conditions for findings out of *CYP2C19*2* and *CYP2C19*3* were 55°C , 30 sec. and 52°C , 45 sec., respectively. This study was approved by the Ethical Committee of Golestan University of Medical Sciences (No: 127692050610) and all patients signed a fully informed written consent before including to the study. 95% confidence intervals (95% CI) were determined for the frequency of the variant alleles of each gene. The observed *CYP2C19* genotype frequencies were determined and compared with expected frequencies (by HWE equilibrium). Allele and PM genotype frequencies differences between Fars ethnic group and different populations were determined by Fisher exact test. SPSS-16 version software was used to analysis the statistical data. Statistical significance was considered at $P < 0.05$.

3. RESULTS

The mean age of subjects (47.1% males and 52.9% females) was 28.03 ± 8.21 years. Table 1 shows the distributions of the *CYP2C19* alleles and genotype frequencies. The allele frequency of *CYP2C19*1*, *CYP2C19*2* and *CYP2C19*3* were 77.8% (95% CI: 70.29-83.94), 19.2% (95% CI: 13.61-26.61) and 2.8% (95% CI: 1.45-7.56), respectively. The frequency of *CYP2C19*2* (19.2%) was higher than Caucasian populations (10–16%) and in northern Iranians (14%), but it was lower than Egyptian (37.5%) and Indians (31%). 75% of subjects were with *CYP2C19*1/*1* genotype (95% CI: 67.22-81.44). 22.1%, 1.4%, 1.4%, 0% and 0% subjects were with *CYP2C19*1/*2* (95% CI: 16.60-29.71), *CYP2C19*1/*3* (95% CI: 0.3-5), *CYP2C19*2/*2* (95% CI: 0.3-5), *CYP2C19*2/*3* (95% CI: 0) and *CYP2C19*3/*3* (95% CI: 0) genotypes, respectively. There were no significant differences between frequencies of different Observed number of genotypes when compared with Expected number which is determined by Hardy-Weinberg law. PM, IM and EM genotype frequencies were seen in 1.4%, 23.5% and 75% of subjects, respectively.

Table 1. Genotype and allelic frequency of *Cyp2c19* among fars ethnic group

Genotype	Observed number and frequency (%)	95% CI	Expected number and frequency (%) by Hardy-Weinberg law	P-value	Allele	Number (%)	95% CI
*1/*1	105(75)	67.2-81.4	105.44(75.3)	0.361	CYP2C19*1	109(77.8)	70.3-83.9
*1/*2	31(22.1)	16.6-29.7	30.38(21.7)	0.564	CYP2C19*2	27(19.2)	13.6-26.6
*1/*3	2(1.4)	0.3-5	1.74(1.2)	0.483	CYP2C19*3	4(2.8)	1.45-7.56
*2/*2	2(1.4)	0.3-5	2.19(1.5)	0.247			
*2/*3	0(0)	0	0.25(0.1)	0.866			
*3/*3	0(0)	0	0.01(0.01)	-			
Total	140 (99.9)		140.1			140 (99.8)	

4. DISCUSSION

The results of this study have shown that there are differences in *CYP2C19* genotype in Fars ethnic group when compared with other populations. Several studies have shown that *CYP2C19*2* and *CYP2C19*3* alleles reveal significant differences in the distribution of allelic variants among different ethnic groups.

The presence of the *CYP2C19*2* allele in our study group was comparable to the results of other populations. The *CYP2C19*2* polymorphism has been shown in different populations. Its prevalence was 13% in Southern Iranian [21], 9.4% in Italian [22], 13% in Greek [23], 15.9% in Slovenia [24], 37.5% in Egyptian [25] ($P < 0.05$), 31% in Indian [18] ($P < 0.05$), 8.8% in Colombian [26], 14% in Iranian [5] and 11.3% in Russian [27]. The prevalence of *CYP2C19*2* in Egyptian and Indian were high when compared with our findings ($P < 0.05$). The prevalence of *CYP2C19*3* in Slovenia [24] (0.3%), Egyptian [25] (0.2%), Russian [27] (0.3%), Southern Iranian [21] (1%) and Italian [22] (0.8%) was lower than our study (2.8%), ($P > 0.05$). *CYP2C19*3* was absent in the Greek [23] (0%), Indian [18] (0%), Colombian [26] (0%) and Iranian [5] (0%) populations as it has been shown in Table 2.

A study has shown that *CYP2C19*1* is the most common allele while *CYP2C19*2* may be considered as the most common mutation for this gene. The *CYP2C19*3* allele is very rare or totally absent in the Caucasian population, respectively. *CYP2C19*3* allele is common in Asian population [15]. Many studies have shown that *CYP2C19*2, *3* polymorphisms may affect metabolism of some drugs [28]. Distribution of *CYP2C19* genotypes has been shown in Fars and different ethnic groups in Table 3. The prevalence of *CYP2C19*1/*1* in this study was higher [21,24-27] and lower [22-23,18,29] when compared with different other ethnic groups.

Table 2. Distribution of Cyp2c19 allele in fars and different ethnic groups

Genotype	This study (%)	Southern part of Iran (%)	Italian (%)	Greek (%)	Slovenia (%)	Egyptian (%)	Indian (%)	Colombian (%)	Iranian (%)	Russian (%)
*1	77.8	86	89.7	86.9	83.7	87.8	62.5	91.2	86	88.2
2	19.2	13	9.4	13	15.9	37.5 (P<0.05)	37.5* (P<0.05)	8.8	14	11.3
*3	2.8	1	0.8	0	0.3	0.2	0	0	0	0.3
References	-	21	22	23	24	25	18	26	5	27

Table 3. Distribution of Cyp2c19 genotype in fars and different ethnic groups

Genotype	This Study (%)	Southern part of Iran (%)	Italian (%)	Greek (%)	Slovenia (%)	Egyptian (%)	Indian (%)	Colombian (%)	Iranian (%)	Russian (%)	Chinese (%)
*1/*1	75	74	79.4* (P=0.002)	76	68.2	78.5* (P=0.001)	35	83.5	75	76.6	36.7* (P<0.001)
*1/*2	22.1	25	18.8	22* (P<0.001)	30	20	55* (P=0.005)	15.3	22	19	38.2* (P=0.02)
*1/*3	1.4	0.6	1.6	0	0.7	0.4	0	0	0	0.3	5.8
*2/*2	1.4	0.6	0	2	0.7	0.8	10	1	3	1.7	5.8
*2/*3	0	0	0	0	0	0	0	0	0	0.3	11
*3/*3	0	0	0	0	0	0	0	0	0	0	1.4
Total	140	147	360	283	129	247	20	189	200	290	68
References	-	21	22	23	24	25	18	26	5	27	28

Comparison of *CYP2C19**1/*2 genotype with other populations has been also shown in Table 3. Prevalence of *CYP2C19**1/*2 genotype was lower [21,24,18,29] and higher [22-23,25-26,5,27] in comparison with other ethnic groups. The results of this study show that *CYP2C19**1/*3 genotype in this study was lower [22,29] and higher [5,21,18,23-27] when compared with other populations. The prevalence of *CYP2C19**2/*2 genotype in Fars (1.4%) ethnic group was lower and higher than different other populations, respectively [5, 18,23,28,21-22,24-26]. The prevalence of *CYP2C19**2/*3 genotype (0%) was lower [27-29] and the same [5,18,21-26] in comparison with other populations Table 3. The prevalence of *CYP2C19**3/*3 genotype (0%) was lower than Chinese [28]. Many studies have indicated that *CYP2C19**3/*3 genotype frequency was very low and/or not detectable. Its prevalence is the same as when compared with other populations [5,18,21-27]. *CYP2C19**3/*3 genotype is collaborated with clinical alterations in the pharmacokinetics of *CYP2C19* substrates. These allelic differences may influence the enzyme activity and required drug amount by different ethnic groups. The frequency of defective *CYP2C19* alleles and decreased enzyme activity stay to be significant study subject in different ethnic groups [30-31].

The frequency of poor metabolizers changes significantly among different ethnic groups. The frequency of 1.4% PM of *CYP2C19* was found in the present study. Many studies have shown a higher prevalence of PM than our study such as in Orientals, up to 18–23% in Japanese, 15–17% in Chinese, 12–16% in Koreans [6], 2 to 7% in Caucasians and 60% in the Vanuatu [32-33]. They are uncommon in the Turkish (1%), German (4.3%) [34] and black Tanzanians (1.5%) ethnic groups [35]. People who are PMs probably develop side-effects to the drugs when they use the normal doses. The PMs are more likely to develop side-effects to drugs when they use the normal doses. People with PMs may benefit less therapeutic effect of some drugs.

5. CONCLUSION

Our findings in the present study have been indicated that ethnicities in different area show unique allelic frequency of *CYP2C19*. Our study approves ethnic differences in the frequencies of *CYP2C19* allele and genotype. Ethnic differences in *CYP2C19* genetic polymorphism of cytochrome p450 enzyme may cause variation in drug response, activity or detoxification. This study suggests the further study of this polymorphic enzyme in Fars population to determine the clinical significance and optimal dosage of some drugs in this ethnic group.

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COMPETING INTERESTS

No conflict of interest.

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