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ORIGINAL ARTICLE

Genetic Polymorphism of CYP2C9 Among Sistani Ethnic Group in Gorgan

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Abstract Cytochrome P450 2C9 (CYP2C9) is involved in metabolism of many important drugs and its genotype variations is thought to affect drug efficacy and the treatment process. The aim of this study was to assess the distribution of CYP2C9 allele and genotypic variants in Sistani ethnic group, living in Gorgan, South East of Caspian Sea and North East of Iran. This study included 140 Sistani, referred to the health center of Gorgan. CYP2C9 genotyping was carried out by polymerase chain reaction–restriction fragment length polymorphism technique. The allele frequency of CYP2C9*1, CYP2C9*2 and CYP2C9*3 was 76.1, 16.1 and 7.8%, respectively. The frequency of CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*1/*3, CYP2C9*2/*2, CYP2C9*2/*3 and CYP2C9*3/*3 genotypes was 53.9, 22.1, 11.4, 2.9, 4.3% and nil, respectively. In this study the genotypic variations of the CYP2C9 allele among the Sistani ethnic group was investigated and great differences were observed in comparison to other populations. Our findings suggest that different genotypes of *CYP2C9* may influence the pharmacokinetics of some drugs. More studies on the pharmacokinetic effects of *CYP2C9* genotypes may help physicians choose optimal dosage of some drugs for treatment and prevention of their side effects. Since different ethnic groups from all over the world use medications, it suggests to investigate the pharmacokinetic effects of *CYP2C9* genotypes in different populations.

Keywords Polymorphism of CYP2C9 · Sistanee ethnic group · Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP)

Introduction

Cytochrome P450 2C (CYP 2C) subfamily of enzymes metabolizes approximately 20–30% of most pharmaceutical drugs and CYP2C9 is known as its main isoform [1, 2]. This enzyme is encoded by a gene located on chromosome 10q24 which shows genetic polymorphism [3]. Variations during genetic polymorphism of this enzyme may change catalytic activity of the enzyme in drug response [3, 4]. The CYP2C9 allele frequencies vary among different populations and many studies on CYP2C9 polymorphism in different populations have shown a significant difference in the frequency of alleles and genotypes [5]. This may be the cause of possible ethnic differences in drug response. Variations in genotype of enzyme have shown that the effect of some genotype is the most acute with S-warfarin metabolism when compared to the wild type genotype. Clinical studies have also indicated that subjects with the mutated genotypes need 10–20 and/or 20–50% lower doses of S-warfarin, when compared to wild type subjects [5]. CYP2C9 is involved in the metabolism of important drugs such as (S)-warfarin, losartan, rosuvastatine, tolbutamide, glipizide, glibenclamide, the diuretic torsemide, phenytoin, flurbiprofen, ibuprofen and diclofenac [6, 7]. The mutated alleles of CYP2C9 are CYP2C9*2 and CYP2C9*3, CYP2C9*6, CYP2C9*15, and CYP2C9*25, but CYP2C9*2 and CYP2C9*3 are the most prevalent variants

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[8, 9]. These alleles are also the most common variants in white populations (CYP2C9*2 = 0.08–0.14% and CYP2C9*3 = 0.04–0.16%) while, some studies indicated a significantly lower frequency of these alleles among Asian and African populations [10, 11]. Some studies have shown that the CYP2C9*2 allele is considered in European, Middle Eastern and Central and South Asian populations. This allele was absent or considered at very low frequencies in Africa, East Asia, Oceania and the Americas. The CYP2C9*3 allele is the highest allele frequencies in European and Central/South Asian populations [12]. Some other studies have revealed that the frequency of the CYP2C9*3 allele among Japanese and Taiwanese populations are 2.1 and 1.7%, respectively. Meanwhile, the CYP2C9*2 allele was not detected among the Han Chinese, Japanese and Taiwanese populations [13]. As mentioned earlier, these alleles may change the catalytic activity of CYP2C9 [6] and on the other hand, many studies have shown the variations among different ethnic groups. We have previously studied the genetic polymorphism of CYP2C9 gene among different ethnic groups in Golestan province [14, 15]. This study aimed to assess the distribution of CYP2C9 allele and its genotypic variants in Sistani ethnic group in the city of Gorgan, South East of Caspian Sea, and North East of Iran.

Table 1 CYP2C9 genotype and allele frequencies of Sistani ethnic group

Genotypes	Observed frequency (%)	95% CI
CYP2C9*1/*1	59.30	51–67.60
CYP2C9*1/*2	22.10	15.10–29.10
CYP2C9*1/*3	11.40	6–16.80
CYP2C9*2/*2	2.90	0.10–5.70
CYP2C9*2/*3	4.30	0.90–7.70
CYP2C9*3/*3	0	0
Total number	100	–
Alleles		
CYP2C9*1	76.10	71–81.20
CYP2C9*2	16.10	11.70–20.50
CYP2C9*3	7.80	4.60–11
Total number	100	

Results

The distributions of the *CYP2C9* alleles and genotype frequencies in Sistani ethnic groups are shown in Table 1. The allele frequency of CYP2C9*1, CYP2C9*2 and CYP2C9*3 were 76.10% (95% CI 71–81.20), 16.10% (95% CI 11.70–20.50) and 7.80% (95% CI 4.60–11), respectively (Table 1). Tables 2 and 3 demonstrate the differences in allele and genotype frequencies between the Sistani ethnic group and other populations using the Fisher exact test. Table 2 shows a significant difference in CYP2C9*1 allele of the Sistani ethnic group with Iranian Turkmen (88%) [18], Southern Iranians (64.88%) [19], Mongolian in China (97%) [21], Japenese (97.30%) [22], Russians (82.70%) [25], Macedonians (83.10 [27] %) and Mestizo in Mexicans (91%) [29] (Table 2). There were no significant differences in all allele's frequencies between the Sistani and Saudi Arabian, Greek and Italian [20, 23, 24]. The results also showed that The genotype frequency of CYP2C9*1/*1, CYP2C9*1/*2 CYP2C9*1/*3, CYP2C9*2/*2, CYP2C9*2/*3 and CYP2C9*3/*3 were 59.30% (95% CI 51–67.60), 22.10% (95% CI 115.10–29.10), 11.40% (95% CI 6.0–16.80), 2.90% (95%

Table 2 Distribution of CYP2C9 alleles among Sistani ethnic group and comparison of them with different other population

CYP2C9 alleles	CYP2C9*1	CYP2C9*2	CYP2C9*3	Ref.
Present study (%)	76.1	16.10	7.80	–
Turkmen ethnic (%)	88*	8*	4	[15]
Fars ethnic (%)	83	11	6	[15]
Southern Iran (%)	64.88*	25.34*	9.80	[19]
Saudi Arabia (%)	79.20	11.70	9.10	[20]
Mongolian in China (%)	97*	0*	3*	[21]
Japan (%)	97.30*	0*	2.70*	[22]
Greek (%)	78.97	12.90	8.13	[23]
Italian (%)	77.70	12.50	9.70	[24]
Russian (%)	82.70*	10.50*	6.70	[25]
Hungarian (%)	78.70	12.50	8.80	[26]
Macedonian (%)	83.10*	10.10*	6.80	[27]
Southeastern Europe (%)	79.40	11.30*	9.30	[28]
Mestizo in Mexico (%)	91*	5.10*	3.90*	[29]

* $p < 0.05$

Table 3 Distribution of CYP2C9 genotypes among Sistani ethnic group and comparison of them with different other population

CYP2C9 genotypes	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3	Total sample number	Ref.
Present study (%)	59.30	22.10	11.40	2.90	4.30	0	140	–
Turkmen ethnic (%)	76.36*	15.54	7.27	0	0.90	0	110	[15]
Fars ethnic (%)	70	14.55	10.91	2.73	1.82	0	110	[15]
Southern Iran (%)	41.21*	37.83*	9.46	1.35	10.13	0	148	[19]
Saudi Arabia (%)	64.10	17.20	13	2.10	2.10	1.60	192	[20]
Mongolian in China (%)	93*	0*	7	0*	0*	0	280	[21]
Japanese (%)	94.70*	0*	5.30*	0*	0*	0	1017	[22]
Greek (%)	62.19	20.14	13.43	1.41	2.83	0	283	[23]
Italian (%)	62	17.20	14.50	2.70	2.20	1.30	360	[24]
Russian (%)	68	18.20	11.30	0.60	1.20	0.30	290	[25]
Hungarian (%)	62	19.50	13.90	2.10	1.50*	1.10	535	[26]
Macedonian (%)	79.80*	18.60	0*	1.60	0*	0	124	[27]
Southeastern Europe (%)	63	18.70	14.10	0.60*	2.70	0.90	332	[28]
Mestizo in Mexico (%)	83*	8.70*	6.80*	0.40*	0.70*	0.20	947	[29]

* $p < 0.05$

CI 0.10–5.70), 4.30% (95% CI 0.9–7.70) and 0% (95% CI 0), respectively (Table 1).

Table 3 is shown that the prevalence of CYP2C9*1/*1 among Sistanee (59.30%) ethnic group was higher when compared with Southern Iran (41.21%) [19], but it lower in comparison with IranianTurkman (76.36%) [18], Mongolian in China (93%) [21], Japanese (94.70%) [22], Macedonians (79.80%) [27], and Mestizo Mexicicans (83%) [29]. The most frequently observed mutant allele was CYP2C9*1/*2 allele (22.10%). Our results showed that CYP2C9*1/*3 genotype (11.4%) was higher in the Sistani ethnic group compared to the Japanese (5.3%) [22], Macedonians (0%) [27] and Mestizo Mexicans (6.8%) [29] (Table 3).

The prevalence of CYP2C9*2/*2 genotype (2.90%) was higher than Mongolians in China (0%) [21], Japanese (0%) [22], Southern Europe (0.60%) and Mestizo Mexicans (0.40%) [29].The prevalence of CYP2C9*2/*3 genotype (4.30%) was higher than Mongolians in China (0%) [21], Japanese (0%) [22], Hungarians (1.50%) [26] and Mestizo Mexicans (0.70%) [29] (Table 3).

Methods

This study involved 140 healthy Sistani (people who speak Sistani as native language) who were referred to a health center in Gorgan (South East of Caspian Sea, North East of Iran). The sample size was determined under supervision of statistical expert. This study was approved by the Ethical Committee of Research Deputy of Golestan University of Medical Sciences. Written consent was obtained from all the study subjects. The distribution of CYP2C9 allele and its genotypic variants in Sistani ethnic group were determined and compared with other populations. First, 5 ml of blood was obtained from all the subjects into EDTA tubes. Peripheral white blood cells were used for DNA extraction by salting-out method [16].

Two times distillated water was used to dissolve the extracted DNA. Collected samples were stored at −20 °C. CYP2C9*1, CYP2C9*2, and CYP2C9*3 alleles genotyping were performed by Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) technique using Genetix CG palm-thermocycler (India) [17]. The PCR was done in a 25 µl reaction mixture including: PCR buffer (10 mM Tris–HCl, pH 9, 2 mM MgCl2 (Fermentas), 50 mM KCl (Fermentas), 0.2 mM deoxyribonucleotide triphosphate (dNTP) mix, 1 U/µl Taq polymerase (Fermentas), 0.4 µM of each primer (Bioneer), 100 ng DNA (Genomic) and two times distillated water. Digestion of PCR products (10 µl) was performed using restriction enzymes (Fermentas), Ava II for CYP2C9*2 and Kpn I for CYP2C9*3 at 37 °C for 16 h. Primer amplification was done using the method explained previously by Aithal et al. [18].

The PCR amplification conditions for CYP2C9*2 included: Initial denaturation (95 °C, 10 min), number of cycle (45 cycles at 95 °C for 5 s), denaturation (67 °C, 10 s), annealing (72 °C, 15 s), extension and final extension (72 °C, 5 min). The parameters for CYP2C9*3 were 94 °C, 5 min.; 33; 94 °C, 45 s.; 66 °C, 45 s.; 72 °C, 60 s and 72 °C, 5 min, respectively.

Figure 1a, b show the PCR products before and after digestion with the restriction enzyme for CYP2C9*2 and CYP2C9*3 genotypes. Agarose gel (3%) was used to perform electrophoresis of (Apelex, France) CYP2C9*2 and CYP2C9*3 DNA fragments. The gel was stained with

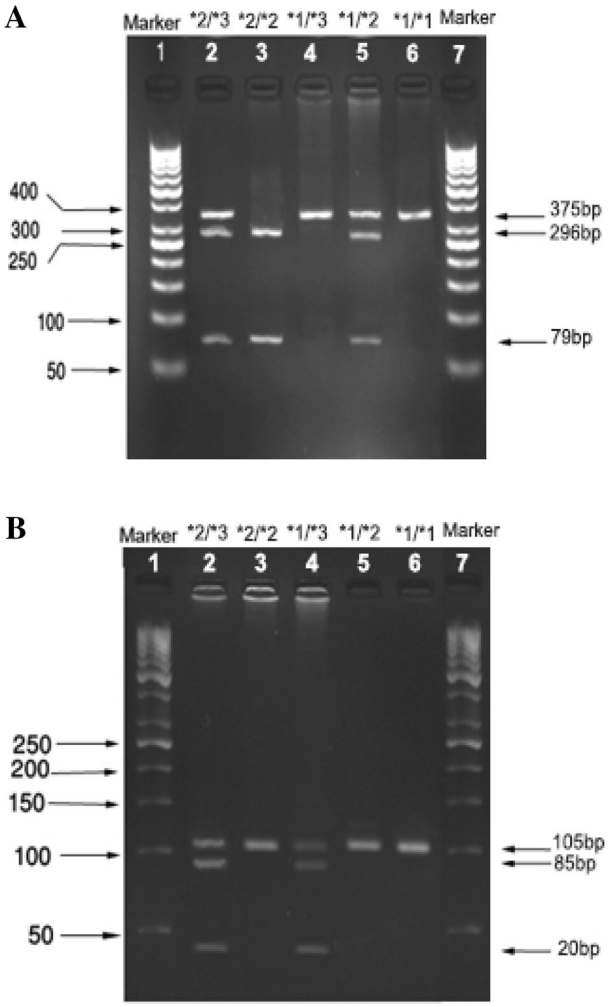

Fig. 1 **a** PCR–RFLP analysis of CYP2C9*2 genotypes. *2/*3, *2/*2, *1/*3, *1/*2 and *1/*1 genotypes were shown from 2 to 6 wells. Lanes *1* and *7* are DNA marker. **b** PCR–RFLP analysis of CYP2C9*3 genotypes. *2/*3, *2/*2, *1/*3, *1/*2 and *1/*1 genotypes were shown from 2 to 6 wells. Lanes *1* and *7* are DNA marker

ethidium bromide, and a Polaroid Gel Camera with black and white film was used to detect the bands. Detection of CYP2C9*2 and CYP2C9*3 mutations were done using sense and antisense primers as described below:

For CYP2C9*2 detection:

Sense primer: 3′-CAC TGG CTG AAA GAGS CTA ACA GAG-5′
Antisense primer: 3′-GTG ATA TGG AGT AGG GTC ACC CAC-5′

For CYP2C9*3 detection:

Sense primer: 5′-AATTACAACCAGAGCTTGGC-3′
Antisense primer: 5′-TATCACTTTCCATAAAAG-CAAG-3′

Chi squared test was carried out to determine and compare the allele and genotype frequencies of CYP2C9. The frequency of alleles variant was determined using 95% confidence intervals (95% CI). Fisher exact test was used to compare the variations of allele and genotype frequencies between Sistani ethnic group and other populations. The analysis of data was done using SPSS version 16.0 and p value of less than 0.05 was considered as statistically significant.

Discussion

Genotype identification is the most important challenges in the future. There are different ethnic groups in various areas of Iran. Many drugs are metabolized by the cytochrome P450 2C9 enzyme [7, 19]. Decreased metabolism of some drugs may be associated with the presence of CYP2C9*2 and CYP2C9*3 alleles [20–24]. Alteration in CYP 2C9 genetic polymorphism may affect drug response and enzyme activity. The results of this study showed CYP2C9*1 as the most prevalent allele in the Sistani ethnic group (76.1%).

Table 3 shows significant differences in the frequencies of CYP2C9*2 and CYP2C9*3 allelic variants between different populations. The prevalence of CYP2C9*2 in Southern Iranian [25] was higher compared with the Sistanis, but the prevalence of CYP2C9*2 in the Sistani ethnic group was higher than other ethnic groups [21, 22, 25, 27–29]. The prevalence of CYP2C9*3 polymorphism in Sistanis (7.8%) was higher than other populations [21, 22, 29]. Studies have shown that the CYP2C9*3 and CYP2C9*2 alleles are responsible for reduction in metabolic activity when compared with CYP2C9*1 [1]. Metabolism of some drugs such as glibenclamide and glimepiride may affect by different genetic polymorphisms of CYP2C9 [37]. Studies have also shown that metabolism of glibenclamide in heterozygous and homozygotes subjects with the CYP2C9*3 allele, was decreased 2.8 and 0.5 folding in comparison to the wild-type genotypes, respectively [1, 30]. Comparison of different genotypes in various populations may affect drug metabolism [31]. Some other studies on glipizide have revealed that homozygotes subjects with the CYP2C9*3 allele may be involved in complications of hypoglycemia [38]. The prevalence of CYP2C9*1/*1 in the Sistani ethnic group was higher than Southern Iranian (41.21%) [19] and lower than Iranian Turkmen (76.36%), Mongolian in China (93%), Japanese (94.70%), Macedonian (79.80%) and Mestizo Mexican (83%) [21, 22, 29].The prevalence of CYP2C9*1/*2 genotype was lower [19] and higher [21, 22, 29] in comparison with other ethnic groups. Our results show that CYP2C9*1/*3 genotype was higher [22, 27, 29] than other populations. The prevalence of CYP2C9*2/*2 [21, 22, 28, 29] and CYP2C9*2/*3

[21, 22, 26, 27, 29] genotypes were higher than different other populations.

In our study, the prevalence of CYP2C9*3/*3 genotype (0%) showed no significant differences with other populations. Numerous studies have demonstrated that frequency of CYP2C9*3/*3 genotype is either very low or undetectable [19, 23, 25, 32–37]. Variation of CYP2C9*3/*3 genotype may affect drug effectiveness in some ethnic group. Genetic polymorphisms, especially the homozygous with *CYP2C9*3* allele, can lead to a significant impairment in metabolic activity of CYP2C9 in different ethnic groups.

Conclusion

In this study the genotypic variations of the CYP2C9 allele among the Sistani ethnic group was investigated and great differences were observed in comparison to other populations. Our findings suggest that different genotypes of *CYP2C9* may influence the pharmacokinetics of some drugs. More studies on the pharmacokinetic effects of *CYP2C9* genotypes may help physicians choose optimal dosage of some drugs for treatment and prevention of their side effects. Since different ethnic groups from all over the world use medications, it suggests to investigate the pharmacokinetic effects of *CYP2C9* genotypes in different populations.

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Compliance with Ethical Standards

Conflict of interest The authors declared no conflicts of interest.

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