

Genetic Polymorphism of Cytochrome p450 (2C9) Enzyme in Iranian Baluch Ethnic Group

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Abstract: The aim of the present study is to assess and compare the frequencies of the cytochrome P450 CYP2C9 variations in the Baluch ethnic group (n=110) with other ethnic groups. The allele frequencies of CYP2C9*1, CYP2C9*2 and CYP2C9*3 were 80.90%, 11.82% and 7.27%, respectively. 70.90%, 11.82%, 8.18%, 4.55%, 2.73% and 1.82% of subjects were with CYP2C9*1/*1, CYP2C9*1/*2, CYP2C9*1/*3, CYP2C9*2/*2, CYP2C9*2/*3 and CYP2C9*3/*3 genotypes, respectively. Different mutants may effect on prediction of drug dose requirements in different ethnic groups. Thus, CYP2C9 variants to be determined for findings high risk groups use optimal dosage of drugs metabolized by this polymorphic enzyme.

Keywords: Baluch ethnic group, CYP2C9 genetic polymorphism, polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP).

1. INTRODUCTION

The cytochrome P-450 (CYP) is responsible for the metabolism of many drugs, such as warfarin, phenytoin, losartan, irbesartan, tolbutamide, glipizide, torsemide, and different nonsteroidal anti-inflammatory drugs [1]. Various single nucleotide genetic polymorphisms for CYP2C9 have been recognized [2, 3]. There are 3 alleles, (CYP2C9*1 (wild-type allele), *2 and *3) in most different ethnic groups. People with the *2 and *3 alleles show decreased enzymatic activity, and these subjects have been indicated as “poor metabolizers” [4, 5] that they may tolerate drug toxicity. Therefore, it is necessary to determine CYP2C9 genotype in different ethnic groups to prevent drug toxicity. Study on the genotype distribution among Caucasian populations varies for the *1/*1, *1/*2 *1/*3*, 2/*2, *2/*3 and*3/*3 genotypes [2]. Caucasians populations have shown more allele frequencies of CYP2C9*2 and CYP2C9*3 when compared to African-American and Asian populations while Chinese and Japanese have not indicated the CYP2C9*2 allele [6]. There has not been any investigation on the genotype of CYP2C9 allelic variants in Baluch ethnic group in Iran. Different studies have shown that there are variation in the distribution of polymorphic alleles of CYP2C9 between Iranian ethnic groups in North and South of Iran. These studies have indicated that the frequency of CYP2C9*3 among southern Iranians (9.8%) and Caucasians (9.7%) were similar. The frequency of CYP2C9*3 among Africans (1%), Japanese (2.3%), and

northern Iranians (0%) were lower than southern Iranians [7]. Study of Azarpira *et al.* also showed that the frequency of CYP2C9*2 (25.3%) was higher than Caucasian (10%–13%), African (2%), and Asian (0%) populations [7]. The aim of the study was to assess and compare the frequencies of the cytochrome P450 CYP2C9 alleles and genotypes in the Baluch ethnic group with the frequencies in other different ethnic groups.

2. MATERIALS AND METHODS

The present study contained 110 unrelated healthy Baluch people (who speak Baluch as a native language and population inbreeding people) referred to Health Center in Chabahar, in Sistan and Baluchestan province (located in South East of Iran). The age ranges of Baluch ethnic group was 14-53 years old. The mean ages of subjects were 30/76 ±11/63 years old. A five milliliters venous blood was collected into EDTA tubes. DNA extraction from peripheral white blood cells was carried out by the method of salting out [8]. Sterilized distilled water was used to dissolve DNA extract and samples were stored in -20 °C until analyzed by polymerase chain reaction (PCR). Genotyping of CYP2C9 alleles was done by Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) technique [9]. The PCR was performed in a 25 microliter reaction mixture containing PCR buffer (2.5 µl (10 mM Tris-HCl, pH 9), 2 µl (40 Mm) MgCl₂ (Fermentas), 50 mM KCl (Fermentas), 12.5 mM deoxyribonucleotide triphosphate (dNTP) mix, 0.2 µl (5 U/µl) Taq polymerase (Fermentas), 1 µl (0.4 µM) of each primer (Bioneer), 2 µl (100 ng/µl) DNA (Genomic) and sterile distilled water). Genetix CG palm-thermocycler (India) was used to perform PCR. Restriction enzymes (Fermentas) were utilized to digest PCR products

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(10 μ l). Restriction enzymes, Ava II and Kpn I were used for CYP2C9*2 and CYP2C9*3 at 37°C for 16 hrs for complete digestion. Amplification of primers was done by De Morais *et al.* method [10]. Electrophoresis of the DNA fragments (Apelex, France) was performed on a 3% agarose gel and Ethidium bromide was utilized to stain the gel. Detected Bands were photographed using a Polaroid Gel Camera with black and white film (not shown). For detection of the CYP2C9*2 mutation, sense primer (5'-CACTGGCTGAAAGAGCTAACAGAG-3') and antisense primer (5'-GTGATATGGAGTAGGGTCACCCAC-3') were used. The CYP2C9*3 mutation detection was done using sense primer 5'-TGCACGAGGTCCAGAGGTAC-3' and antisense primer 5'-ACAACTTACCTTGGGAATGAGA-3'. The conditions of PCR amplification were as follows: For CYP2C9*2: Initial denaturation, Number of cycle(s), Denaturation, annealing, Extension and final extension step were 95°C, 10 min.; 40; 95°C, 5 sec.; 65 °C, 30 sec.; 72°C, 30 sec. and 72°C, 5 min. and for CYP2C9*3: 94°C, 5 min.; 30; 94 °C, 45 sec.; 63 °C, 45 sec.; 72 °C, 30 sec. and 72°C, 5 min., respectively. 95% confidence intervals (95% CI) were performed to determine the frequency of the variant alleles of each gene. Fisher exact test was used to evaluate variations in allele and genotype frequencies between Baluch ethnic groups and different other population.

3. RESULTS

The distribution of genotype and allelic frequencies of CYP2C9 among Baluch ethnic group are shown in Table 1. The allele frequency of CYP2C9*1 (Wild type), CYP2C9*2 and CYP2C9*3 were 80.90% (95% CI: 73.55-88.24), 11.82% (95% CI: 5.78-17.82) and 7.27% (95% CI: 2.44-12.16), respectively (Table 1). The frequencies of CYP2C9 genotypes in Baluch ethnic group were found to be 70.90% of subjects with CYP2C9*1/*1 genotype (95% CI: 62.41-79.38). 11.82%, 8.18%, 4.55%, 2.73 and 1.82 subjects with CYP2C9*1/*2 (95% CI: 5.78-17.84), CYP2C9*1/*3(95% CI: 3.06-13.30), CYP2C9*2/*2(95% CI: 0.65-8.43), CYP2C9*2/*3 (95% CI: 0-5.77) and CYP2C9*3/*3(95% CI: 0-4.30) genotypes, respectively (Table 1). Tables 2 and 3 show the distribution of CYP2C9 genotype and allele frequency in Baluch ethnic group and in different populations.

4. DISCUSSION

Variations in CYP expression among different ethnic groups make it important in drug response, activity and detoxification. It is been shown that there are three major variants of the CYP2C9 gene in different ethnic groups. It is reported that there are three major variants of the CYP2C9 gene in Caucasian populations [11]. CYP2C9*2 and CYP2C9*3 allelic variants show almost 10–40% and 5–15% of the activity of CYP2C9*1 for various drug, respectively [12-15]. The allelic frequency of CYP2C9*2 found in the present study was 11.82% (almost similar to the Iranian Fars) [16] which was higher than Japanese [24], African [25], Pakistanis [26], Tamilians [27], Keralans [28], Chinese [29] and Korean [30] populations (Table 2). Our results show that the CYP2C9*2 variant was more frequent than above mentioned populations. In our study group, the allelic frequency of CYP2C9*3 was 7.27%. The frequency of CYP2C9*3 was almost similar to the frequencies found in Italians [17], Greeks [18], Swedish [20], Pakistanis [26] and Keralans [28] (Table 2). Genetic differences in the CYP2C9 make it possible that some drugs such as warfarin metabolize differently. Single-nucleotide polymorphisms in CYP2C9 influence metabolism of this drug. Clinicians can appraise optimal warfarin dose by genotyping subjects for single-nucleotide polymorphisms. Subjects who have the CYP2C9*2 and/or CYP2C9*3 variants metabolize warfarin more in a different manner than subjects without these variants. It has been shown that CYP2C9 genotype estimates warfarin optimal dosage in subjects on warfarin treatment [30]. Distribution of CYP2C9 genotypes in Baluch and different ethnic groups has been shown in Table 3. Our genotype results have shown that frequency of *1/*2 and *1/*3 was 11.82% and 8.18% which was higher [24-30] and lower [16-21, 23 and 26-28] than other populations, respectively. The prevalence of CYP2C9*2/*2 (except Iranian Turkmen [16]), *2/*3 and *3/*3 genotypes in Iranian Baluch ethnic group were higher when compared with different other ethnic groups [16-30]. Many studies have indicated that *2/*2 [24-29, 6], *2/*3 [16, 22-29 and 6] and *3/*3 [16, 18, 21, 22, 25, 27 and 29, 6] genotype frequency was not detectable in some populations. CYP2C9*3/*3 genotype is contributed with some important clinical variations in the pharmacokinetics of CYP2C9 substrates.

Table 1. Genotype and Allelic frequencies of CYP2C9 in the Baluch ethnic group.

CYP2C9 genotypes	Number (%)	95% CI	CYP2C9 alleles	Number (%)	95% CI
*1/*1	78(70.90)	62.41-79.38	*1	89(80.90)	73.55-88.24
*1/*2	13(11.82)	5.78-17.84	*2	13(11.82)	5.78-17.82
*1/*3	9(8.18)	3.06-13.30	*3	8(7.27)	2.44-12.16
*2/*2	5(4.55)	0.65-8.43			
*2/*3	3(2.73)	0-5.77			
*3/*3	2(1.82)	0-4.30			
Total	110 (100)				

*Means separation of effective alleles.

Table 2. Comparison of allele frequencies of CYP2C9 in Baluch ethnic group with different populations.

Population study	Sample size (n)	Allele frequency of *1(%)	Allele frequency of *2(%)	Allele frequency of *3(%)	References
Iranian Baluch	110	80.90	11.82	7.27	Present study
Iranian Fars	140	83	11(NS)	6(NS)	16
Iranian Turkmen	140	88	8(NS)	4(NS)	16
Italian	360	77.7	12.5(NS)	9.7(NS)	17
Greek	283	79	12.8(NS)	8.1(NS)	18
Russians	290	82.7	10.5(NS)	6.7 (NS)	19
Swedish	430	81.9	10.6(NS)	7.4(NS)	20
Slovenia	129	81.7	12(NS)	6.2(NS)	21
UK	561	84.1	10.6(NS)	5.2 (NS)	22
Egyptians	247	81.7	11.8(NS)	6.2(NS)	23
Japan	828	97.6	0(0.001)	2.3(0.004)	24
African	47	9	2(0.004)	1 (<0.0001)	25
Pakistan	120	91.6	0.8(0.001)	7.5(NS)	26
Tamilians	135	90.7	2.6(0.0045)	6.7(NS)	27
Kerala	120	90	2.0(0.0011)	8.0 (NS)	28
Chinese	115	98.3	0(0.001)	1.7(<0.0001)	29
Korean	574	98.9	0 (0.001)	1.1(<0.0001)	30

Differences in the allele frequencies were determined by Fisher exact test. NS: No significant differences.

P-value versus Baluch ethnic group.

*Means separation of effective alleles.

Table 3. Comparison of CYP2C9 genotype frequency in Baluch ethnic group with different populations.

Population study	Sample size (n)	Genotype frequency of *1/*1(%)	Genotype frequency of *1/*2(%)	Genotype frequency of *1/*3(%)	Genotype frequency of *2/*2(%)	Genotype frequency of *2/*3(%)	Genotype frequency of *3/*3(%)	References
Iranian Baluch	110	70.90	11.82	8.18	4.55	2.73	1.82	Present study
Iranian Fars	140	70(NS)	14.55(NS)	10.91(NS)	2.73(0.011)	1.82(NS)	0(0.001)	16
Iranian Turkmen	140	37.9(<0.0001)	42.1(<0.0001)	9.3(NS)	9.3(<0.0001)	0	1.4(NS)	16
Italian	360	62(NS)	17.2(NS)	14.5(0.014)	2.7(0.011)	2.2(NS)	1.3(NS)	17
Greek	283	62(NS)	20(NS)	13.5(0.014)	1.5(0.001)	2.8(NS)	0(0.001)	18
Russians	290	68(NS)	18.2(NS)	10.3(NS)	0.6(<0.0001)	1.2(0.011)	0.3(0.0085)	19
Swedish	430	66.7(NS)	18.6(NS)	11.6(NS)	0.4(<0.0001)	1.6(0.011)	0.6(0.0095)	20
Slovenia	129	86.6(<0.0016)	19.3(NS)	10.8(NS)	1.5(0.001)	1.5(0.011)	0(0.001)	21
UK	561	69.9(NS)	19(NS)	0.06(<0.0001)	0.003(<0.0001)	0(<0.0001)	0(0.001)	22
Egyptians	247	66.3(NS)	19(NS)	12(NS)	2.4(0.011)	0(<0.0001)	0.4(0.0088)	23
Japan	828	95(<0.0001)	0(<0.0001)	4(<0.001)	0(<0.0001)	0(<0.0001)	1(NS)	24
African	47	93.6(<0.0001)	4.2(0.014)	2.1(<0.001)	0(<0.0001)	0(<0.0001)	0(0.001)	25

(Table 3) contd....

Population study	Sample size (n)	Genotype frequency of *1/*1(%)	Genotype frequency of *1/*2(%)	Genotype frequency of *1/*3(%)	Genotype frequency of *2/*2(%)	Genotype frequency of *2/*3(%)	Genotype frequency of *3/*3(%)	References
Pakistan	120	85.8(<0.0011)	0(<0.0001)	11.7(NS)	0.8(<0.0001)	0(<0.0001)	1.7(NS)	26
Tamilians	135	82.3(NS)	4.4(0.017)	12.7(NS)	0(<0.0001)	0.7(<0.0001)	0(0.001)	27
Kerala	120	81.0(NS)	4.0(0.011)	14.0(0.014)	0(<0.0001)	0(<0.0001)	1.0(NS)	28
Chinese	115	97.0(<0.0001)	0(<0.0001)	3.0(<0.001)	0(<0.0001)	0(<0.0001)	0(0.001)	29
Korean	574	97.7(<0.0001)	0(<0.0001)	2.3(<0.001)	0(<0.0001)	0(<0.0001)	0(0.001)	30

Differences in the genotype frequencies were determined by Fisher exact test. NS: No significant differences. P-value versus Baluch ethnic group.

*Means separation of effective alleles.

CONCLUSION

In conclusion, our results show that there are ethnic variations in the *CYP2C9* allele and genotype frequencies. Genetic differences in the *CYP2C9* were seen in Iranian Baluch. The presence of different mutant genotypes and alleles may effect on prediction of drug dose requirements in different ethnic groups. Thus, it requires that *CYP2C9* variants determination in different populations may help high risk groups to use optimal dosage of drugs metabolized by this polymorphic enzyme.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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