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Genetic Polymorphism of Cytochrome P450 2C9 (CYP2C9) in Two Ethnic Groups in Iran

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Abstract

Cytochrome P450 2C9 is considered as a vital enzyme for drugs and toxic detoxification in human body. The present study was carried out to assess the distribution of *CYP2C9* allele and genotypic variants in two ethnic groups (Fars and Turkman) in the North East of Iran. The present study contained 110 unrelated healthy Turkman origin and 110 unrelated healthy Fars origin people who were referred to the Health Center. CYP2C9 genotyping were done by Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) technique. The allele frequency of CYP2C9*1, CYP2C9*2 and CYP2C9*3 in Turkman ethnic group were 88%, 8% and 4%, respectively. The frequency of above alleles in Fars ethnic group was 83%, 11% and 6%, respectively. 15.45%, 7.27%, 0%, 0.9 and 0% Turkman ethnic group belong to CYP2C9*1/*2, CYP2C9*1/*3, CYP2C9*2/*2, CYP2C9*2/*3 and CYP2C9*3/*3 genotypes, respectively. 76.36% and 70% of Turkman and Fars ehtnic groups were with CYP2C9*1/*1 genotype, respectively. 14.55%, 10.91%, 2.73%, 1.83% and 0% of Fars ethnic group was with CYP2C9*1/*2, CYP2C9*1/*3, CYP2C9*2/*2, CYP2C9*2/*3 and CYP2C9*3/*3 genotypes, respectively. There were no significant differences in allele and genotype frequencies between two (Turkman and Fars) ethnic groups (P>0.05).It is demonstrated that there is a significant *CYP2C9* alleles and genotypes difference among the worldwide populations based on the collective data from published work and our results. These differences might cause variations in drug dose necessities. These findings may help clinicians to make a choice and suitable plan for the optimal dosage of some drugs and reduce drugs side effects and intoxification.

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Keywords: CYP2C9 polymorphism, Iranian Turkman and Fars ethnic groups, polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP).

1. Introduction

Cytochrome P450 2C9 is considered as a vital enzyme for the drugs and toxic detoxification in human body. Many studies indicated that the drug metabolism in the liver is genetic and ethnic dependent [1-3]. CYP2C9 is believed to be one of the important enzymes which are involved in the metabolism of 10–20% of clinically important drugs [3-10]. Enzyme which is responsible for drug metabolism is sub-grouped according to the genetic bases, and they are divided into high, low and non-active enzymes [11].

According to definition of pharmacology, the polymorphism of cytochrome P450 are divided into poor (or slow) metabolizers (PM), extensive (or rapid) metabolizers (EM), and ultra-rapid metabolizers (UM) [12]. Those with PM definition require drug therapeutic regiment which has a longer duration or they are adversely affected by the routine dose of prescribed drugs, due to the drug susceptibility. These subjects are prone to drugs negative side effects. The PM individuals require much longer time for the drug activation with eventual delay in the drug response. The UMs are those patients, which the normal prescribed drugs are not adequate for the drug expected metabolic response, and therefore they require an extra dosage for the normal defined response to the specific drug. In addition to, in cases where the enzyme activates the drug, elevated plasma levels of the active metabolite can be expected in UMs. The safe drug prescription particularly for those with narrow therapeutic index can be obtained by the assessment of patient metabolic capacity through the determination of genetic polymorphism pattern.

Various studies indicated that it seems three allelic variants of CYP2C9*1, CYP2C9*2, CYP2C9*3, which codes for the 3 isoenzymes (with different catalytic activities), responsible for the drug metabolism [10]. Some studies have shown that the Caucasians seems to have *CYP2C9*2* and *CYP2C9*3* frequency at about 8-12% and 3-8%, respectively, but other studies indicate lower frequency among Orientals and Black Africans population [12-13]. Although the CYP2C9*3 allele established among Japanese and Taiwanese populations, to be 2.1% and 1.7%, respectively, but it seems the CYP2C9*2 allele has not been detected among the Han Chinese, Japanese and Taiwanese populations [14].

Clinical studies indicated that those population carrying CYP2C9*1/*2 and *1/*3 genotypes should have been given 10–20% and 20–50% lower average maintenance doses of some drug for example warfarin, respectively, when they are compared to wild type individuals [15]. Aithal et al also reported that those individual with CYP2C9 heterozygous with either CYP2C9*2 or CYP2C9*3 alleles recommended to be prescribed lower therapeutic doses of warfarin than wild type homozygous patients [16].

These findings indicate that CYP2C9 phenotype is genetically expressed and this is the gene mutation which dictates which CYP2C9 type is available in one individual. Therefore, the recommended phenotype and genotype determination should be established for an individual. Prior to some given therapeutic drug regiment, particularly for those narrow therapeutic index drugs to pave the way for a safer and careful drug administration for those patients who are prone to some adverse effect of drugs due to their susceptibility. This present study was carried out to assess the distribution of CYP2C9 allele and genotypic variants in two ethnic groups (Turkman and Fars) in the South East of Caspian Sea, North East of Iran.

2. Materials and Methods

2.1 Demographic Information

The present study contained 110 unrelated healthy Turkman origin and 110 unrelated healthy Fars origin people (people who speak Turkman and Farsi as a native language and population inbreeding people, respectively) who

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were referred to Health Center in Gonbadkavoos (located in South East of Caspian Sea, North East of Iran). The frequency of CYP2C9 variants in Turkman and Fras ethnic groups were determined and compared with each other and different populations. The age ranges of Turkman and Fras ethnic groups were 16-56 years old.

2.2 PCR- RFLP analysis

A 5 ml venous blood was collected from each subject into EDTA tubes. DNA extraction was carried out by salting out method from peripheral white blood cells [17]. Extracted DNA was dissolved in sterilized distilled water and samples were stored at -20 ^{o}C. CYP2C9 alleles genotyping (CYP2C9*1, CYP2C9*2, and CYP2C9*3 alleles) were done by Polymerase Chain Reaction (PCR)-Restriction Fragment Length Polymorphism (RFLP) technique [18]. The PCR was done in a 25 microliter reaction mixture containing PCR buffer (10 mM Tris–HCl, pH 9, 2 mM $MgCl_2$ (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 sterile distillated water)**.** Genetix CG palm-thermocycler (India) was used to carry out PCR. Restriction enzymes (Fermentas) were used to digest PCR products (10µl). Restriction enzymes, Ava II and Kpn I were utilized for CYP2C9*2 and CYP2C9*3 at 37^{o}C for 16 hrs for complete digestion. Amplification of primers was described before by De Morais et al. [16].

The PCR amplification conditions for CYP2C9*2 and CYP2C9*3 were as follow: For CYP2C9*2: Initial denaturation, Number of cycle(s), Denaturation, annealing, Extention and final extention step were 95^{o}C, 10 min.; 45; 95^{o}C, 5 sec.; 67^{o}C, 10 sec.; 72^{o}C, 15 sec. and 72^{o}C, 5 min. and for CYP2C9*3: 94^{o}C, 5 min.; 33; 94 ^{o}C, 45 sec.; 66 ^{o}C, 45 sec.; 72 ^{o}C, 60 sec. and 72^{o}C, 5 min., respectively.The Products of PCR before and after restriction enzyme digestion for CYP2C9*2 and CYP2C9*3 genotypes are summarized in Figure 1.

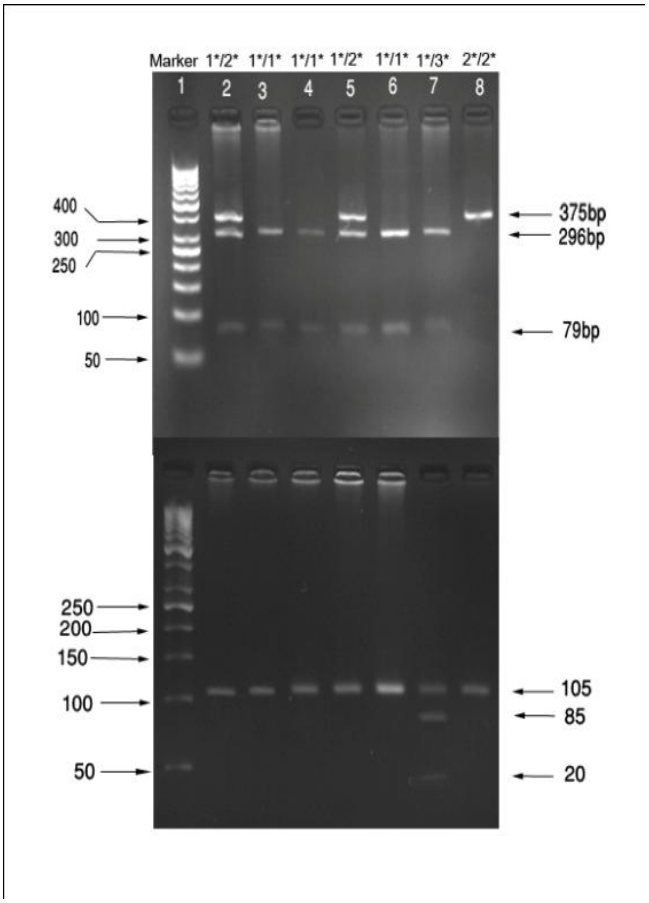

Figure 1 PCR-RFLP analysis of CYP2C9*2(A) and CYP2C9*3 (B) genotypes. Ethidium bromide was used for the agarose gel staining of fragmented patterns of PCR-restriction enzyme for CYP2C9*2 and CYP2C9*3 samples (Ava II and Kpn I, respectively) corresponding for *1/*2, *1/*1, *1/*1, *1/*2, *1/*1, *1/*3 and *2/*2 genotypes (From 2 to 8 wells), Lane 1 is DNA marker.

2.3 Electrophorese of DNA

Electrophorese of the DNA fragments (Apelex, France) was done on a 3% (for CYP2C9*2 and CYP2C9*3) agarose gel and Ethidium bromide was used to stain the gel. Detected Bands were photographed using a Polaroid Gel Camera with black and white film. Sense primer (5'-AATTACAACCAGAGCTTGGC-3') and antisense primer (5'-TATCACTTTCCATAAAAGCAAG-3') were

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used to detect the CYP2C9*2 mutation. The detection of CYP2C9*3 mutation was done using sense primer 5'-AAATTGTTTCCAATCATTTAGCT-3' and antisense primer 5'-ACTTCAGGGCTTGGTCAATA-3'.

2.4 Statistical analysis

The Chi-squared test was used to compare the allele and genotype frequencies of CYP2C9 in Turkman and Fars ethnic groups. 95% confidence intervals (95% CI) were also used to determine the frequency of the variant alleles of each gene. Hardy–Weinberg law was used to compare the observed genotype frequencies of CYP2C9 with expected frequencies. Variations in allele and genotype frequencies between Turkman and Fars ethnic groups and different other population mentioned in tables 3 and 4 were evaluated by Fisher exact test. SPSS version 16.0 was used to do the analysis of the statistical data. $P < 0.05$ was considered statistically significant.

3. Results

The distributions of the *CYP2C9* alleles and genotype frequencies in Turkman and Fars ethnic groups are shown in Tables 1 and 2. The allele frequency of CYP2C9*1, CYP2C9*2 and CYP2C9*3 in Turkman ethnic group were 88% (95% CI: 80.19-93), 8% (95% CI: 4-15) and 4% (95% CI: 1.57-9.84), respectively. The frequency of these alleles in Fars ethnic group were 83% (95% CI: 74.45-89.11), 11% (95% CI: 6.25-18.63) and 6% (95% CI: 2.78-12.48), respectively.

Differences in allele and genotype frequencies between two (Turkman and Fars) ethnic groups and other ethnic populations were determined by Fisher exact test (Table 3 and 4). Table 3 is shown that there was no significant differences in CYP2C9*2 allele of these ethnic groups. The prevalence of CYP2C9*2 was 8% in Turkman, 11% in Fars, 25.3% in Southern Iran, 12.5% in Italian, 12.8% in Greek, 10.5% in Russian, 10.6% in Sweden, 12% in Slovenia, 10.6% in UK, 11.8% Egyptian, 0% in Japanese, 25% in African and 12.7% in Iranian.

CYP2C9*1 was the most frequently determined allele in Turkman (88%) and Fars (83%) ethnic groups. 76.36% of Turkman ethnic group was with CYP2C9*1/*1 genotype (95% CI: 67.62-83.33). 15.45%, 7.27%, 0%, 0.9 and 0% Turkman ethnic group was with CYP2C9*1/*2 (95% CI: 9.88-23.36), CYP2C9*1/*3(95% CI: 3.73-13.7), CYP2C9*2/*2(95% CI: 0-3.37), CYP2C9*2/*3 (95% CI: 0.16-4.97) and CYP2C9*3/*3(95% CI: 0-3.37) genotypes, respectively.

Table 3 is also shown that the prevalence of CYP2C9*1/*1 among Turkman (76.36%) and Fars (70%) ethnic groups was higher when compared with Southern Iran (41.2%), Italian (62%), Greek (62%), Russian (68%), Sweden (66.7%), UK (69.9%) and Egyptian (66.3%), but it was lower in comparison with Slovenia (86.6%), Japanese (95%), African (93.6%) and Iranian (82%).

Table 1 CYP2C19 allele frequencies of Turkman and Fars ethnic groups.

Variant allele	**Turkman (n=110)**		**Fars (n=110)**	
	Frequency n (%)	**95% CI**	**Frequency n (%)**	**95% CI**
CYP2C19*1	**97(88)**	**80.19-93**	**91(83)**	**74.45-89.11**
CYP2C19*2	**9(8)**	**4-15**	**12(11)**	**6.25-18.63**
CYP2C19*3	**4(4)**	**1.57-9.84**	**7(6)**	**2.78-12.48**

Differences in the allele frequencies between Turkman and Fars ethnic groups were determined by the Chi-squared test. P>0.05

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Table 2 CYP2C9 genotype frequencies of Turkman and Fars ethnic groups.

Genotype	Turkman (n=110)			Fars (n=110)		
	Observed frequency n (%)	95% CI	Expected frequency % by Hardy–Weinberg law	Observed frequency n (%)	95% CI	Expected frequency % by Hardy–Weinberg law
CYP2C19*1/*1	84(76.36)	67.62-83.33	73.18	77(70)	60.88-77.77	73.18
CYP2C19*1/*2	17(15.45)	9.88-23.36	15	16(14.55)	9.16-22.33	15
CYP2C19*1/*3	8(7.27)	3.73-13.7	9.1	12(10.91)	6.35-18.10	9.1
CYP2C19*2/*2	0(0)	0-3.37	1.36	3(2.73)	0.93-7.71	1.36
CYP2C19*2/*3	1(0.9)	0.16-4.97	1.36	2(1.82)	0.5-6.39	1.36
CYP2C19*3/*3	0(0)	0-3.37	0	0(0)	0-3.37	0

Differences in the genotype frequencies between Turkman and Fars ethnic groups were determined by the Chi-squared test. P>0.05

Table 3 Distribution of CYP2C9 alleles among Turkman, Fars and comparison of them with different other populations

CYP2C9 Genotypes	Southern Iran (%)	Italian (%)	Greek (%)	Russian (%)	Swedan (%)	Slovenia (%)	UK (%)	Egyptian (%)	Japan (%)	African (%)	Iran (%)	The present study (Turkman) (%)	The present study (Fars) (%)
*1	64.8[1,2]	77.7[1,2]	79[1,2]	82.7	81.9[1]	81.7	84.1	81.7[1]	97.6[1,2]	9[1,2]	87.3[1]	88	83
*2	25.3[1,2]	12.5	12.8	10.5	10.6	12	10.6	11.8	0[2]	2	12.7	8	11
*3	9.8	9.7	8.1	6.7	7.4	6.2	5.2	6.2	2.3[1]	1	0[1,2]	4	6
Total sample	147	360	283	290	430	129	561	247	828	47	200	110	110
Reference	25	26	27	28	29	30	31	32	33	34	35	-	-

1: Ccomparison of Fars ethnic group with other different ethnic groups ([1]P<0.05), 2: Ccomparison of Turkman ethnic group with other different ethnic groups ([2]P<0.05). Differences in the allele frequencies were determined by Fisher exact test.

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Table 4 Distribution of CYP2C9 genotypes among Turkman, Fars and comparison of them with different other populations

CYP2C9 Genotypes	Southern Iran (%)	Italian (%)	Greek (%)	Russian (%)	Sweden (%)	Slovenia (%)	UK (%)	Egyptian (%)	Japan (%)	óAfrican (%)	Iran (%)	The present study (Turkman) (%)	The present study (Fars) (%)
***1/*1**	**41.2[1,2]**	**62[2]**	**62[2]**	**68**	**66.7**	**86.6[1, 2]**	**69.9**	**66.3**	**95[1,2]**	**93.6[1,2]**	**82[1]**	**76.36**	**70**
***1/*2**	**37.8[1,2]**	**17.2[1]**	**20[1]**	**18.2[1]**	**18.6[1]**	**19.3[1]**	**19[1]**	**19[1]**	**0[1,2]**	**4.2**	**10.5[1]**	**15.45**	**14.55**
***1/*3**	**9.5**	**14.5**	**13.5**	**11.3**	**11.6**	**10.8**	**0.06[1,2]**	**12[1]**	**4[1]**	**2.1**	**0[1,2]**	**7.27**	**10.91**
***2/*2**	**10.1[1,2]**	**2.7**	**1.5**	**0.6**	**0.4**	**1.5**	**0.003[1]**	**2.4**	**0[1]**	**0**	**7.5[2]**	**0**	**2.73**
***2/*3**	**1.3**	**2.2**	**2.8**	**1.2**	**1.6**	**1.5**	**0.006**	**0**	**0**	**0**	**0**	**0.9**	**1.82**
***3/*3**	**0**	**1.3**	**0**	**0.3**	**0.6**	**0**	**0**	**0.4**	**1**	**0**	**0**	**0**	**0**
Total sample	**147**	**360**	**283**	**290**	**430**	**129**	**561**	**247**	**828**	**47**	**200**	**110**	**110**
Reference	25	26	27	28	29	30	31	32	33	34	35	-	-

1: Ccomparison of Fars ethnic group with other different ethnic groups ([1]P<0.05), 2: Ccomparison of Turkman ethnic group with other different ethnic groups ([2]P<0.05). Differences in the genoytpe frequencies were determined by Fisher exact test.

CYP2C9*1/*2 allele was the most frequently (15.45%) observed mutant allele. 70% of Fars ethnic group was with CYP2C9*1/*1 genotype (95% CI: 60.88-77.77). 14.55%, 10.91%, 2.73%, 1.83% and 0% Fars ethnic group was with CYP2C9*1/*2 (95% CI: 9.16-22.33), CYP2C9*1/*3(95% CI: 6.35-18.10), CYP2C9*2/*2(95% CI: 0.93-7.71), CYP2C9*2/*3 0.9%(95% CI: 0.5-6.39) and CYP2C9*3/*3(95% CI: 0-3.37) genotypes, respectively.

Table 4 is indicated that Prevalence of CYP2C9*1/*2 genotype was lower than Southern Iranian (37.8%), Italian (17.2%), Greek (20%), Russian (18.25%), Sweden (18.6%), Slovenia (19.3%), UK (19%) and Egyptian (19%), but it was higher than Japanese (0%), African (4.2%) and Iranian (10.5%).

CYP2C9*1/*2 allele was the most frequently (14.45%) observed mutant allele. There were no significant differences in allele and genotype frequencies between two (Turkman and Fars) ethnic groups (P>0.05).

The results of this study show that CYP2C9*1/*3 genotype in two ethnic groups was lower than Italian (14.5%), Greek (13.5%), Russian (11.3%), Sweden (11.6%) and Egyptian (12%). It was higher than UK (0.006%), Japanese (4%), African (2.1%) and Iranian (0%)(Table 4).

The prevalence of CYP2C9*2/*2 genotype in Turkman ethnic group (0%) was lower than Southern Iran (10.1%), Italian (2.7%), Greek (1.5%), Russian (0.6%), Sweden (0.4%), Slovenia (1.5%), UK (0.003%), Egyptian (2.4%) and Iranian (7.5%)(Table 4).

The prevalence of CYP2C9*2/*3 genotype in Turkman (0.9%) ethnic group was lower than Southern Iran (1.3%), Italian (2.2%), Greek (2.8%), Russian (1.2%), Sweden (1.6%) and Slovenia (1.5%) and higher than (UK (0.006%), Egyptian (0%), Japanese (0%), African (0%) and Iranian (0%)(Table 4).

4. Discussion

The human cytochrome P450 metabolizes many drugs [7]. The most abundant of the CYP2C enzymes is CYP2C9 [19]. The presence of the CYP2C9*2 and CYP2C9*3 alleles reduce the metabolism of some drugs [20–24]. The findings of this study and other studies done in different ethnicities in other countries, shows a

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unique allelic and genotypic frequency of CYP2C9 and alteration in CYP 2C9 genetic polymorphism can influence the drug response and activity. CYP2C9*1 (wild type allele) was the most prevalent allele with high frequencies in Turkman (88%) and Fars (83%) ethnic groups. CYP2C9*2 and CYP2C9*3 were detected in both ethnic groups but the frequency of these alleles were lower in Turkman (8% and 4%, respectively) when compared with Fars ethnic group (11% and 6%).

There are important differences in the frequencies of CYP2C9*2 and CYP2C9*3 allelic variants between different ethnic groups (Table 3). The findings of CYP2C9*2, CYP2C9*3 show significant differences in the frequency distribution of allelic variants among different ethnic groups. In the present study, CYP2C9*2 allele was detected in two ethnic groups.

The prevalence of CYP2C9*2 in Southern Iranian [25] was high when compared with Turkman and Fars ethnic groups. The prevalence of CYP2C9*2 in Fars ethnic group was almost the same as other ethnic groups [26-32, 35] (except Japanese and African [33-34]), but the prevalence of CYP2C9*2 in Turkman ethnic group was lower than other ethnic groups [25-32, 34-35](except Japanese [33]) as it has been shown in Table 3.

Table 3 has indicated that the prevalence of CYP2C9*3 polymorphism in Turkman (4%) and Fars (6%) ethnic groups was lower than Southern Iran (9.8%), Italian (9.7%), Greek (8.1%) and Sweden (7.4%) [25-27 and 29].The prevalence of CYP2C9*3 in two ethnic groups was higher than Japanese (2.3%), African (1%) and Iranian (0%) [33-35]. Prevalence of CYP2C9*3 is almost the same in Fars (6%) ethnic group when compared with Russian (6.7%), Slovenia (6.2%), UK (5.2%) and Egyptian (6.2%) [28, 30-32] (Table3). It has been shown that that the CYP2C9*3 and CYP2C9*2 alleles are responsible for the most and intermediate decrease in metabolic activity when compared with CYP2C9*1 (wild type), respectively [36].

Genetic polymorphisms of CYP2C9 affected pharmacokinetics of some drugs such as glibenclamide and glimepiride in healthy people [37-38]. Heterozygous subjects with the CYP2C9*3 allele, the pharmacokinetics of glibenclamide was changed to 280% [38], and in CYP2C9*3 homozygotes the pharmacokinetics was reduced by more than 50% when compared with the wild-type genotypes [36]. Subjects with the CYP2C9*3 allele, the pharmacokinetics of glimepiride was increased by 267% when compared with the wild-type (CYP2C9*1*1) [38].

These may show that the effects of these drugs may be different in subjects with the various genotypes when compared with the different other genotypes [37]. The effect of other drugs such as glipizide on healthy homozygous subject for CYP2C9*3 was shown to have a 5.5 times increase which have serious complications of hypoglycemia when compared with normal subjects [39].

Distribution of CYP2C9 genotypes has been shown in Turkman, Fars and different ethnic groups in Table 4. The prevalence of CYP2C9*1/*1 in two ethnic groups was higher [25-29, 31-32] and lower [30, 33-35] when compared with different other ethnic groups.

Comparison of CYP2C9*1/*2 genotype in two ethnic groups with other populations has been shown in Table 4. Prevalence of CYP2C9*1/*2 genotype was lower [25-32] and higher [33-35] in comparison with other ethnic groups.

The results of this study show that CYP2C9*1/*3 genotype in two ethnic groups was lower [26-29 and 32] and higher [32-35] when compared with other populations. The prevalence of higher CYP2C9*1/*3 genotype in Turkman (7.27%) and Fars (10.91%) ethnic groups was lower and when compared with Southern Iran (9.5%) [25], respectively. Its prevalence in Fars (10.91%) ethnic group is almost the same as Slovenia (10.8%) [30] (Table 4).

The prevalence of CYP2C9*2/*2 genotype in Turkman (0%) and Fars (2.73%) ethnic groups were lower than different other populations, respectively [25-32 and 35; 34-35]. Its prevalence was the same as Japanese (0%) and African (0%) [33-34]. Prevalence of CYP2C9*2/*2 genotype in Fars ethnic group (2.73%) was lower than Southern Iran (10.1%) and Iranian (7.5%) and higher than other ethnic groups (Table 4). Its

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prevalence in Fars (2.73%) ethnic group is almost the same as Slovenia (2.7%) [30].

The prevalence of CYP2C9*2/*3 genotype in Turkman (0.9%) ethnic group was lower [25-30] and higher [31-35] in comparison with other populations. The prevalence of CYP2C9*2/*3 genotype in Fars ethnic group (1.82%) was lower than Italian (2.2%) and Greek (2.8%) [26-27] (table 4).

The prevalence of CYP2C9*3/*3 genotype in both ethnic groups (0%) was lower than Italian (1.3%) [26], Russian (0.3%) [28], Sweden (0.6%) [29], Egyptian (0.4%) [32] and Japanese (1%) [33]. Its prevalence in both (0%) ethnic groups is the same as Southern Iran (0%) [25], Greek (0%) [27], Slovenia (0%) [30], UK (0%) [31], African (0%) [34] and Iranian (0%) [35]. Many studies have shown that CYP2C9*3/*3 genotype frequency was very low and/or not detectable [28-29, 32 and 25, 27, 30-31, 34-35]. CYP2C9*3/*3 genotype is collaborated with significant clinical changes in the pharmacokinetics of CYP2C9 substrates.

5. Conclusion

There are a different studies and different results about the Genetic polymorphism of CYP2C9 in different populations. Thus it is important to determine this polymorphism in different ethnic groups. The results of this study approved that there are significant differences in *CYP2C9* alleles and genotypes frequencies with some different other populations. These differences might cause differences in drug dose necessities. These findings may help clinicians to make a choice of suitable plan for optimal dosage of some drugs and decrease drugs side effects and intoxification.

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Guarantor: I am the guarantor as the head of the team and corresponding author

Contributorship:

Abdoljalal Marjani (was researched literature and conceived the study, wrote the first draft of the manuscript and involved in protocol development and data analysis),

Ogholdondy Agh Ataby (was involved in gaining ethical approval and protocol development),

Robabeh Ghiyas Tabari (was involved in gaining ethical approval),

Azad Reza Mansourian (was involved in protocol development and edited the manuscript) and

Nader Mansour Samai (was involved in protocol development)

All authors reviewed and edited the manuscript and approved the final version of the manuscript

References

1. Daly, A.K., Cholerton, S., Gregory, W., Idle, J.R. (1993) Metabolic polymorphisms. Pharmacol. Ther., 57, 129–160.
2. Bertilsson, L. (1995) Geographical/interracial differences in polymorphic drug oxidation. Current state of knowledge of cytochromes P450 (CYP) 2D6 and 2C19. Clin. Pharmacokinet., 29,192–209.
3. Rogers, J.F., Nafziger, A.N., Bertino, Jr J.S. (2002) Pharmacogenetics affects dosing, efficacy, and toxicity of cytochrome P450-metabolized drugs. Am. J. Med., 113,746–750. DOI: 10.1016/S0002-9343(02)01363-3.

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4. Daly, A.K., King, B.P. (2003) Pharmacogenetics of oral anticoagulants. Pharmacogenetics., 13, 247–252. DOI: 10.1097/01.fpc.0000054071.64000.bd.
5. Takahashi, H., Wilkinson, G.R., Nutescu, E.A., et al. (2006) Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra- and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African–Americans. Pharmacogenet. Genomics, 16, 101–110. DOI: 10.1097/01.fpc.0000184955.08453.a8
6. Goldstein, J.A., de Morais, S.M. (1994) Biochemistry and molecular biology of the human CYP2C subfamily. Pharmacogenetics., 4: 285–299.
7. Xie, H.G., Kim, R.B., Wood, A.J., Stein, C.M. (2001) Molecular basis of ethnic differences in drug disposition and response. Annu. Rev. Pharmacol. Toxicol., 41, 815–850. DOI: 10.1146/annurev.pharmtox.41.1.815
8. Kirchheiner, J., Brockmoller, J. (2005) Clinical consequences of cytochrome P450 2C9 polymorphisms. Clin. Pharmacol. Ther., 77, 1–16. DOI:10.1016/j.clpt.2004.08.009
9. Garcia-Martin, E., Martinez, C., Ladero, J.M., Agundez, J.A. (2006) Interethnic and intraethnic variability of CYP2C8 and CYP2C9 polymorphisms in healthy individuals. Mol. Diagn. Ther., 10, 29–40.
10. Miners, J.O., Birkett D.J. (1998) Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. Br. J. Clin. Pharmacol., 45, 525–538.DOI: 10.1046/j.13652125.1998.00721.x
11. Meyer, U.A. (1994) Pharmacogenetics. The slow, the rapid, and the ultrarapid. Proc Natl. Acad. Sci. USA, 91,1983–4.
12. Meyer, U.A. (2000) In: Carruthers GS, Hoffmann BB, Melmon KL, Nieremberg DW, editors. Drugs in special patient groups: clinical importance of genomics in drug effects. New York: McGraw Hill, pp. 1179–205.
13. Scordo, M.G., Aklillu, E., Yasar, U., Dahl, M.L., Spina, E., Ingelman-Sundberg, M. (2001) Genetic polymorphism of cytochrome P450 2C9 in a Caucasian and a Black African population. Br. J. Clin. Pharmacol., 52, 447–50. DOI: 10.1046/j.0306-5251.2001.01460.x
14. Obayashi, K., Nakamura, K., Kawana, J., Ogata, H., Hanada, K., Kurabayashi, M., et al. (2006) VKORC1 gene variations are the major contributors of variation in warfarin dose in Japanese patients. Clin Pharmacol Ther., 80, 169-178. DOI:10.1016/j.clpt.2006.04.010
15. Sanderson, S., Emery, J., Higgins, J. (2005) CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. Genet. Med., 7, 97–104. DOI:10.1097/01.GIM.0000153664.65759.CF
16. Aithal, G.P., Day, C.P., Kesteven, P.J., Daly, A.K. (1999) Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet., 353, 717–719. DOI: S0140673698044742
17. Chang, M., Dahl, M-L., Tybring, G., Gotharson, E., Bertilsson, L. (1995) Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: comparison with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype. Pharmacogenetics, 5, 358–63.
18. Brosen, K., de Morais, S.M.F., Meyer, U.A., Goldstein, J.A. (1995) A multifamily study on the relationship between CYP2C19 genotype and S-mephenytoin oxidation phenotype. Pharmacogenetics, 5, 312–7.
19. Lapple, F., On Richter, O., Fromm, M.F., et al. (2003) Differential expression and function of CYP2C isoforms in human intestine and liver. Pharmacogenetics,13, 565–575.DOI: 10.1097/01.fpc.0000054122.14659.1e
20. Scordo, M.G., Pengo, V., Spina, E., et al. (2002) Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. Clin. Pharmacol. Ther., 72, 702–710. DOI: 10.1111/j.1742-7843.2005.pto_194.x
21. Kamali, F., Khan, T,.I., King, B.P., et al. (2004) Contribution of age, body size, and CYP2C9 genotype to anticoagulant response

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to warfarin. Clin. Pharmacol. Ther., 75, 204–212. DOI: 10.1016/j.clpt.2003.10.001
22. Kirchheiner, J., Seeringer, A. (2007) Clinical implications of pharmacogenetics of cytochrome P450 drug metabolizing enzymes. Biochim. Biophys. Acta, 1770, 489–494. DOI.org/10.1016/j.bbagen.2006.09.019
23. Lindh, J.D., Holm, L., Andersson, M.L., Rane, A. (2008) Influence of CYP2C9 genotype on warfarin dose requirements—a systematic review and meta-analysis. Eur. J. Clin. Pharmacol., 65, 365–375. DOI: 10.1007/s00228-008-0584-5
24. Becquemont, L. (2008) Evidence for a pharmacogenetic adapted dose of oral anticoagulant in routine medical practice. Eur. J. Clin. Pharmacol., 64, 953–960. DOI: 10.1007/s00228-008-0542-2
25. Azarpira, N., Namazi, S., Hendijani, F., Banan, M., Masumeh Darai, M. (2010) Investigation of allele and genotype frequencies of *CYP2C9*, *CYP2C19* and *VKORC1* in Iran. Pharmacological Reports, 67, 740-746.
26. Scordo, M.G., Caputi, A.P., D'Arrigo, C., Fava, G., Spina, E.: (2004) Allele and genotype frequencies of *CYP2C9*, *CYP2C19* and *CYP2D6* in an Italian population. Pharmacol Res., 50, 195–200. DOI. org/10.1016/j.phrs.2004.01.004
27. Arvanitidis, K., Ragia, G., Iordanidou, M., Kyriaki, S., Xanthi, A., Tavridou, A., Manolopoulos, V.G.: (2007) Genetic polymorphisms of drug-metabolizing enzymes CYP2D6, CYP2C9, CYP2C19 and CYP3A5 in the Greek population. Fundam Clin Pharmacol., 21, 419–426. DOI: 10.1111/j.1472-8206.2007.00510.x
28. Gaikovitch, E.A., Cascorbi, I., Mrozikiewicz, P.M., Brockmöller, J., Frötschl, R., Köpke, K., Gerloff, T., et al. (2003) Polymorphisms of drug-metabolizing enzymes CYP2C9, CYP2C19, CYP2D6, CYP1A1, NAT2 and of P-glycoprotein in a Russian population. Eur. J. Clin. Pharmacol., 59, 303–312. DOI: 10.1007/s00228-003-0606-2
29. Yasar, U., Eliasson, E., Dahl, M.L., Johansson, I., Ingelman- Sundberg, M., Sjöqvist, F. (1999) Validation of methods for CYP2C9 genotyping: frequencies of mutant alleles in a Swedish population. Biochem. Biophys. Res. Commun, 254, 628–631. DOI.org/10.1006/bbrc.1998.9992
30. Herman, D., Dolzan, V., Breskvar, K. (2003) Genetic polymorphism of cytochromes P450 2C9 and 2C19 in Slovenian population. Zdrav. Vestn., 72, 347–351.
31. Sconce, E.A., Khan, T.I., Wynne, H.A., Avery, P., Monkhouse, L., King, B.P., Wood, P., et al.: (2005) The impact of *CYP2C9* and *VKORC1* genetic polymorphism and patient characteristics upon warfarin dose requirements: proposal for a new dosing regimen. Blood, 106, 2329–2333. DOI: 10.1182/blood-2005-03-1108
32. Hamdy, S.I., Hiratsuka, M., Narahara, K., El-Enany, M., Moursi, N., Ahmed, M.S., Mizugaki, M. (2002) Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. Br. J. Clin. Pharmacol., 53, 596–603. DOI: 10.1046/j.1365-2125.2002.01604.x
33. Mushiroda, T., Ohnishi, Y., Saito, S., Takahashi, A., Kikuchi, Y., Saito, S., Shimomura, H., et al. (2006) Association of *VKORC1* and *CYP2C9* polymorphisms with warfarin dose requirements in Japanese patients. J. Hum. Genet., 51, 249–253. DOI: 10.1007/s10038-005-0354-5
34. Isaza, C., Henao, J., Martínez, J.H., Arias, J.C., Beltrán, L. (2007) Phenotype-genotype analysis of *CYP2C19* in Colombian mestizo individuals. BMC. Clin. Pharmacol., 7, 6. doi:10.1186/1472-6904-7-6
35. Zand, N., Tajik, N., Moghaddam, A.S., Milanian, I. (2007) Genetic polymorphisms of cytochrome P450 enzymes 2C9 and 2C19 in a healthy Iranian population. Clin. Exp. Pharmacol. Physiol., 34, 102–105. DOI: 10.1111/j.1440-1681.2007.04538.
36. Lee, C.R., Goldstein, J.A., Pieper, J.A. (2002) Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. Pharmacogenetics, 12, 251–63.

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37. Kirchheiner, J., Brockmoller, J., Meineke, I., Bauer, S., Rohde, W., Meisel, C., et al. (2002) Impact of CYP2C9 amino acid polymorphisms on glyburide kinetics and on the insulin and glucose response in healthy volunteers. Clin Pharmacol Ther, 71, 286–96. DOI: 10.1067/mcp.2002.122476
38. Niemi, M., Cascorbi, I., Timm, R., Kroemer, H.K., Neuvonen, P.J., Kivisto, K.T. (2002) Glyburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. Clin Pharmacol Ther, 72, 326–32. DOI:10.1067/mcp.2002.127495
39. Kidd, R.S., Straughn, A.B., Meyer, M.C., Blaisdell, J., Goldstein, J.A., Dalton, J.T. (1999) Pharmacokinetics of chlorpheniramine, phenytoin, glipizide and nifedipine in an individual homozygous for the CYP2C9*3 allele. Pharmacogenetics, 9, 71–80.

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