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Allele Frequency of CYP2C19 Gene Polymorphisms in a Healthy Iranian Population

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ABSTRACT

Cytochrome P450 2C19 (CYP2C19) plays an important role in the metabolism and elimination of a wide range of medications. The polymorphisms of this enzyme give rise to substantial inter-individual and inter-ethnic variability in drug excretion rates and final serum concentrations. For this reason, therapeutic responses and adverse drug reactions may vary from one person to another. In this study we determined genotypes of CYP2C19 in Iranian population to compare allele frequencies with previous findings in other ethnic groups. CYP2C19 (*1/*2/*3) allelic variants were determined in 200 unrelated healthy Iranian volunteers by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays. Six subjects (3%) were homozygous for CYP2C19*2, while 44 individuals (22%) were heterozygous. In the remainder (75%) no CYP2C19*2 was found. In addition, no CYP2C19*3 was detected in the population studied. Based on our data, there was no difference between frequency of CYP2C19 allelic variants in our study and other evaluated Caucasians ($p > 0.05$).

Keywords: *CYP2C19, Iranian population, PCR-RFLP, Pharmacogenetics, Polymorphism*

Serious attention to inter-ethnic or inter-racial differences in drug response, started with the rise of pharmacogenetics. Therefore, genotype analysis before drug therapy seems to be a promising approach to reduce adverse effects and to enhance efficacy of drugs.

The cytochrome P450 (CYP) enzymes play an important role in the metabolism and elimination of a wide range of medications as well as other xenobiotics. CYP mediates biotransformation of lipophilic compounds to polar metabolites, which can be eliminated through urine or bile. The human hepatic CYP system consists of over 30 related isoenzymes, with different, sometimes overlapping substrate specificities [1]. Among these, CYP2C19 exhibits genetic polymorphism [2] and can cause variability in drug response. Genetic variation in a gene coding for a drug-metabolizing enzyme can cause enzyme variants with high, low or no activity. Thereby, population can be divided into phenotypes of poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and finally, ultra-rapid metabolizers (UM).

The human CYP2C subfamily consists of at least four isoforms, 2C8, 2C9, 2C18 and 2C19, whose genes are located together on chromosome 10. CYP2C19 metabolizes some drugs such as S-mephenytoin, diazepam, omeprazole, proguanil, citalopram, R-warfarin and many antidepressants [2]. The genetic defect first reported, m_1 (CYP2C19*2), is a single base pair mutation in exon 5 which creates an aberrant splice site. The next deficient allele m_2 (CYP2C19*3), is also a single base variation in exon 4 creating a premature stop codon [3]. The wild-type allele is referred to as *1. Allelic variants CYP2C19*2/*3 are the most important detrimental alleles of this isoenzyme.The frequency of poor metabolizers of CYP2C19 varies between 18-23% in Asians, 2-5% in Caucasians and 4% in a Shona population of Zimbabwe [4].

CYP2C19*2 accounts for 75 % of CYP2C19 defective alleles in Orientals [5], and 93 % in Caucasians [6], although, there are other reports about the frequency of this mutation in Caucasian populations [7].

The other well characterized detrimental allele (CYP2C19*3) discovered in Japanese PMs [7], accounts for approximately 25% of all inactive forms in Orientals, being by converse extremely rare in non-Oriental populations [8]. In Caucasians, additional deficient CYP2C19 alleles have been subsequently found [9].

Table 1. Techniques for genetic analysis of the human CYP2C19 gene.

Allele	Primer set	Size (b.p)	Detection	Refs.
2C19*2	(1) 5'-AATTACAACCAGAGCTTGGC-3' (F) (2) 5'-TATCACTTTCCATAAAAGCAAG-3'(R)	169	SmaI, 25ºC Wt: 120, 49; v: uncut	(5;7)
2C19*3	(1)5'-TATTATTATCTGTTAACTAATATGA-3'(F) (2) 5′ -ACTTCAGGGCTTGGTCAATA-3' (R)	329	BamHI, 37ºC Wt: 233, 96; v: uncut	(5;7)

F: Forward primer; R: reverse primer; wt: wild-type allele; v: variant allele

MATERIALS AND METHODS

Study Population

Iran (Persia) is a Middle Eastern country located in southwest Asia. The name Iran is a cognate of the Aryan meaning "Land of the Aryans". There are a number of ethnic groups living in various parts of Iran. These ethnic groups include Persian (Parsi), Azari, Gilaki & Mazandarani, Kurd, Arab, Lor, Balouch, Turkmen and others. In Present study, we estimated the distribution of CYP2C19 common variants in a sample of Iranian population and compared these data with those from other populations.

Two hundred unrelated healthy Iranian volunteers, (85 males and 115 females, aged between 17-48 years, mean±SD = 26.16±6.98) from different ethnic groups were involved in this study. Our participants were referred to the Medical Laboratory Sciences Research Center, Tehran/Iran, for routine marriage lab tests. The study protocol was approved by the ethics committee of the university and written informed consent to participate in the study was obtained from the volunteers.

DNA Extraction and Genotyping

Ten mL venous blood was obtained from each subject. DNA was manually extracted from peripheral blood leukocytes by a simple salting-out method [10]. The extracted DNA was dissolved in sterile distilled water and stored at -20ºC until PCR analysis. PCR was performed in a 50 µL reaction mixture containing QIAGEN PCR buffer and 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2-0.8 µM of each specific forward and reverse primers, 1.25 unit of Taq DNA polymerase (QIAGEN GmbH, Germany) and template DNA (500 ng). These concentrations were applied for two allelic variants. The amplification condition was as follows: First, initial denaturation at 94ºC for 5 min, second, 35 cycles of denaturation at 94ºC for 45 seconds, annealing at 53ºC for 40 seconds, extension at 72ºC for 30 seconds. Final extension at 72ºC for 5 min was also performed. The amplification procedure was done in an Eppendorf PCR system (Mastercycler Gradient).

PCR products of each allelic variant were digested with a specific restriction endonuclease. PCR products of CYP2C19*2 and CYP2C19*3 were digested with SmaI and BamHI respectively, as previously described by De Morais [7].

Digested PCR products were analyzed by 3% agarose gels stained with ethidium bromide and then documented with a video system. The nucleotide sequence of all PCR primers and restriction patterns are listed in Table 1.

RESULTS

The CYP2C19 alleles as well as the genotype frequencies in the Iranian population are summarized in Table 2.

6 individuals (3%) carried two mutated alleles being homozygous for CYP2C19*2, thus could be classified as poor metabolizers, while 44 subjects (22%) carried one mutated allele (CYP2C19*1/*2). The remainder had wild type allele (75%). No CYP2C19*3 was found in the evaluated population. Based on our genotype frequencies, allele frequencies will be 86% wild-type, (CYP2C19*1) and 14% CYP2C19*2. Allele frequency of CYP2C19*3 was zero.

DISCUSSION

The human CYP2Cs are an important subfamily of Cytochrome P450 enzymes which metabolize approximately 20% of clinically used drugs [11]. Among these, the CYP2C9 is the most abundant isoform which represents approximately 18% of total hepatic CYPs. CYP2C19 represents about 3% of total hepatic CYPs [12]. Several reports of CYP2C genetic polymorphism demonstrate its potential clinical role in determining both inter-individual and inter-ethnic differences in drug efficacy. We estimated the distribution of CYP2C19

Table 2. Allele (A) and genotype (B) frequencies of CYP2C19 among Iranian volunteers (n=200).

Gene	Variant allele	Frequency (%)	95%confidence interval
A			
CYP2C19	CYP2C19*1	86	(81.19-90.8) %
	CYP2C19*2	14	(9.19-18.8) %
	CYP2C19*3	0	_
	Genotype	**n**	**Frequency (%)**
B			
CYP2C19	CYP2C19*1/*1	150	75
	CYP2C19*1/*2	44	22
	CYP2C19*1/*3	0	_
	CYP2C19*2/*2	6	3
	CYP2C19*2/*3	0	_
	CYP2C19*3/*3	0	_

Table 3. Distribution of CYP2C19 variant alleles among different ethnic groups.

Ethnicity	Allele frequency (%)			Refs.
	WT (*1)	m1 (*2)	m2 (*3)	
Iranian	86	14	0	Present study
Japanese	67	23	10	[3, 11]
Filipinos	54	39	7	[3, 11]
Chinese-Taiwanese	63	32	5	[3, 11]
Sum for Asians	62	32	6	
		$\chi2$=80.5 p = 0.0001		
Saudi Arabian	85	15	0	[3, 11]
		$\chi2$=0.90 p > 0.05		
African American	75	25	0	[3, 11]
		$\chi2$=11.89 p = 0.005		
European American	87	13	0	[3, 11]
		$\chi2$=0.15 p > 0.05		

common variants in the Iranian population and compared these data with those from other populations.

For CYP2C19 allelic variants, the CYP2C19*2 is the most common variant among Caucasians [13].The frequency of 14% found in our study (Table 3), was consistent with findings in other Caucasians [5]. It is interesting to note that this defect is distributed in different ethnic groups at a similar and relatively high frequency, implying that this detrimental mutation is relatively old and occurred before the Black, Oriental and Caucasian racial groups split [13]. By contrast, the absence of CYP2C19*3 in our study further illustrates the ethnical difference between Caucasian and Oriental populations, by confirming the Asian specificity of this allelic variant, whose frequency is very low, or totally absent, in different Caucasian populations [14]. These findings clearly suggest that the CYP2C19*3 mutation occurred quite recently, after the differentiation of the Caucasian and Oriental racial groups [13]. The frequency of 3% PMs of CYP2C19 (CYP2C19*2/*2), found in our study, was similar to other Caucasians [15]. By contrast, several independent studies have shown a much higher incidence of poor metabolisers in Orientals, up to18-23% in Japanese, 15-17% in Chinese and 12-16% in Koreans [4]. No CYP2C19*3 was detected in our study. This allele is extremely rare in non-Oriental populations.

In brief, the frequency of CYP2C19 allelic variants in Iranians was similar to other Caucasian populations. Thus, future studies on genetic profile of this subfamily and other CYPs, further illustrates distribution of different allelic variants in Iranian population.

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