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Frequency of Two Genetic Polymorphisms of CYP1A2 Gene in Iranian Population

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Authors' contributions

This work was carried out in collaboration between both authors. Author MSS performed the experiments, statistical analysis and wrote the first draft of the manuscript. Author AR managed the analyses of the study and revised the final manuscript. Both Authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: The present study was conducted to explore the allelic and genotypic frequencies of *CYP1A2* gene polymorphisms, *CYP1A2*1C* (−3860 G>A) and *CYP1A2*1F* (−163 C>A), among 200 healthy Iranian volunteers.
Study Design: Completely randomized design.
Place and Duration of Study: Place – Biotechnology Department, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran. Duration – June, 2011 to May, 2013.
Methodology: DNA was extracted from the blood samples by salting-out method. Primers and probes were designed by the Primer Express® Software v3.0.1. Allelic discrimination of the *CYP1A2* alleles was performed by Real-Time PCR, and the data were analyzed by the SPSS 16.00 software.
Results: The frequency of each polymorphism in Iranian population was found to be 0.05 and 0.3 for *CYP1A2**1C and *CYP1A2*1F*, respectively. Our data did not show any difference between the Iranian, Turkish, and Egyptian populations in *CYP1A2*1C* polymorphism; also, *CYP1A2*1F* polymorphism analysis did not show any differences between Iranian, Turkish, Egyptian, Chinese, Serbian, and Japanese populations. Allele frequency in our population was in equilibrium according to Hardy–Weinberg principle (*P*=0.05).
Conclusion: This is the first study analyzing the *CYP1A2* clinically important allelic

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variants in Iranian population. Our results indicate that functional significance of these alleles should be evaluated in various populations. It is necessary to determine the relationship between these variants and *CYP1A2*-related drug metabolism in associated groups.

Keywords: SNP; CYP1A2; real time PCR; pharmacogenomics; Iran.

1. INTRODUCTION

The cytochromes P450 (CYP450) are a large family of cysteinato-heme enzymes that catalyze the oxidation of organic molecules and are present in all forms of life in plants, bacteria, and mammals [1,2]. The *CYP1A2* (CYP450, family 1, subfamily A, polypeptide 2) plays an important role in the endogenous and exogenous molecules oxidative transformation [3-4]. *CYP1A2* bioactivates a number of procarcinogens including polycyclic aromatic hydrocarbons (e.g., benzo[a]pyrene), heterocyclic aromatic amines/amides (e.g., 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine), mycotoxins (e.g., aflatoxin B(1) and some natural compounds such as aristolochic acids [5]. This enzyme also metabolizes some endogenous compounds including retinols, melatonin, steroids, uroporphyrinogen and arachidonic acids.

Like other CYPs, *CYP1A2* can be induced and inhibited by a number of compounds. Several therapeutic drugs can inhibit *CYP1A2*, including antofloxacin, carbamazepine, dihydralazine, furafylline, isoniazid, rofecoxib, clorgyline, thiabendazole, and zileuton. The enzyme reversible and irreversible inhibition by some drugs may explain some clinical drug–drug interactions [4-5]. *CYP1A2* can be subjected to reversible and/or irreversible inhibition or induction by several drugs, natural substances and other compounds [4]. Smoking is the most important inducer of *CYP1A2.* Rifampin and barbiturates can increase the activity of the enzyme. Co-administration of the drugs can increase or decrease the rate of metabolism by altering the metabolic pathways of CYPs [3,6]. Induction of *CYP1A2* activity depends on several factors such as smoking, diet, drugs, presence of chronic hepatitis and exposure to chemicals such as polybrominated biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [3,7]. Thus, for efficient drug development, it is essential to identify if the new drug is a substrate, inducer, or inhibitor [4,8-9].

Genetic polymorphisms in *CYP1A2* may cause variable susceptibility to carcinogenesis [10]. Understanding *CYP1A2* inhibition or induction is important for drug discovery and interindividual variations to drug response, which are major challenges in clinical setting [11]. Common variant alleles of *CYP1A2* gene may have variable distribution among different ethnic populations [4,12-14]. Phenotypes of metabolizers in populations can be classified to poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM), and ultra-rapid metabolizers (UM).

CYP1A subfamily consists of *CYP1A1* and *CYP1A2*. The *CYP1A* gene cluster has been mapped on to chromosome 15q24.1, with close link between *CYP1A1* and 1A2 sharing a common 5′-flanking region [15]. The human *CYP1A2* gene encodes a 515-residue protein with a molecular mass of 58,294 Da. It comprises seven exons and six introns.

Until now, 39 variants of the *CYP1A2* gene have been reported (www.cypalleles.ki.se/cyp1a2.htm). The *CYP1A2*1C* (−3860 G>A) in the 5′-flanking region

and the *CYP1A2*1F* (-163 C>A) in the first intron are associated with decreased and increased inducibility of *CYP1A2* gene, respectively [6]. Various techniques for SNP genotyping have been developed [16-17]. The choice of the method is dependent on the number of SNPs and the number of samples to be genotyped. For large-scale projects with high SNPs and small population, the Affymetrix SNP GeneChip and Illumina GoldenGate BeadChip assays are the ideal techniques. For projects involving a small number of SNPs and a large population, the TaqMan® assay is the method of choice, because it is high throughput and highly accurate, time-saving, and cost-effective [18]. There are several studies on the frequency of *CYP1A2* variants in different ethnic populations [19]. This is the first report on common *CYP1A2* allelic variants in the Iranian population. This study aimed to identify the clinically important *CYP1A2* (*1C and *1F) allelic variants in 200 unrelated healthy Iranian volunteers by TaqMan® genotyping approach.

2. MATERIAL AND METHODS

2.1 Subjects

In this study, peripheral blood samples were collected from June 2010 to May 2011, from 200 unrelated healthy Iranian volunteers belonging to different ethnic backgrounds such as Persians, Kurds, Gilakis, Turks, Tats, and Lurs; the participants had no history of diseases. Out of 200 participants, 108 were women and 92 were men (54% females and 46% males) aged between 19–57 years, with mean±SD=28.72. Our participants included 44 Persian, 21 Gilak, 20 Taat, 40 Turk, 40 Kurd, and 35 Lur. Signed informed consent was obtained from each participant, and the study was conducted after being approved by the Human Ethics Committee from the Zanjan University of Medical Sciences (ZUMS).

2.2 DNA Extraction

Genomic DNA was extracted from 5 ml of peripheral blood samples by the modified salting-out method [20]. Blood cell lyses buffer containing SDS (Sodium Dodecyl Sulfate), proteinase K and sodium chloride (NaCl) was used for DNA extraction. DNA samples were concentrated by Ethanol (70%), recovered by centrifugation and re-suspended in TE buffer (Tris-HCl EDTA pH=8.8). The DNA Concentration and purity was determined spectrophotometrically (Eppendorf, Germany) by reading absorbance at 260 and 280 (A_{260} and A_{280}). DNA samples were stored at 4ºC.

2.3 Genotyping of the CYP1A2 Variant Alleles

Real-time polymerase chain reactions were performed to detect the *CYP1A2*1C* and *CYP1A2*1F* allelic variants. TaqMan® conventional probes and primers were designed by Primer Express® Software v3.0.1 (Applied Biosystems, USA), and purchased from Bioneer Company (South Korea). TaqMan® probes labeled with a fluorophore and a quencher were used in the real-time PCR assays to detect the SNP target genes. The fluorophore, FAM (6-carboxyfluorescein) was used as the probe at 5′ end for the wild type probes and HEX (hexachloro-6-carboxyfluorescein) for the variant. The fluorophore TAMRA (tetramethylrhodamine) was used as the quencher at 3′ end for both wild type and variant probes. Allelic discrimination was performed using TaqMan® SNP Genotyping Assay on the Rotor-Gene 6000 (Corbett, Australia).

Real-time PCR was performed in a 20-µL reaction volume containing 10-µL qPCR probe Master Mix (Jena Bioscience, Germany), 200 nM of each probe (wild type and variant allele), 10 pM of each specific forward and reverse primers and 50 ng of extracted DNA. These concentrations were applied for two allelic variants. The following amplification condition was used: First, initial heat activation and denaturation at 95ºC for 10 min, followed by 45 cycles at 95ºC for 10 s and 60ºC for 1 min. For allelic variant genotyping quality control, 5% of the sample was genotyped in duplicate and confirmed by sequencing (Bioneer, South Korea). The nucleotide sequence of the PCR primers and probes used in this study are shown in Tables 1 and 2.

2.4 Statistical Analysis

Differences in allele frequency between different populations were determined using chi square test. Deviations from Hardy–Weinberg Equilibrium were also assessed using a chi square test. All analyses were performed using the SPSS 16.0 for windows.

Table 1. The nucleotide sequence of all PCR primers and probes for CYP1A2*1C detection

Oligo name	Sequence (5'-3')	T_m	%GC	Amplicon Size (bp)
Forward Primer	TGGTGCGATCTTGGCTCAC	59.4	58	
Reverse Primer	ATGGTGCACACCTGTAATTCCAG	59.8	48	
Probe (Wild-type allele)	FAM-CGCCTCTCgGATTCAAGCAATTGT-TAMRA	66.2	50	97
Probe (Variant allele)	HEX-CCTCCGCCTCTCaGATTCAAGCAA-TAMRA	66.5	54	

SNP sites in the probe sequences indicated by lower case letters

Table 2. The nucleotide sequence of all PCR primers and probes for CYP1A2*1F detection

Oligo name	Sequence (5'-3')	Tm	%GC	Amplicon Size (bp)
Forward Primer	TGGATACCAGAAAGACTAAGCTCCAT	69.6	42	
Reverse Primer	CTCAGATTCTGTGATGCTCAAAGG	59	46	
Probe (Wild-type allele)	FAM-ACCATGCGTCCTG**t**GCCCAC-TAMRA	65.1	65	81
Probe (Variant allele)	HEX-ATGCGTCCTGgGCCCACAG-TAMRA	65.2	68	

SNP sites in the probe sequences indicated by lower case letters

3. RESULTS AND DISCUSSION

The frequencies of *CYP1A2*1C* (−3860 G>A) and *CYP1A2*1F* (−163 C>A) alleles in Iranian populations were found to be 0.05 and 0.3, respectively. Genotype frequencies of *CYP1A2*1C* allele for normal homozygous wild type (G/G), heterozygous (G/A), and homozygous variant (A/A) were 90%, 10%, and 0%, respectively. Genotype frequencies of *CYP1A2*1F* allele for homozygous wild type (A/A), heterozygous (C/A), and homozygous variant (C/C) were 50%, 40%, and 10%, respectively. *CYP1A2*1F* was more frequent

among Iranian population compared with *CYP1A2*1C*. No significant difference was noticed between the male and female subjects (P=0.05). Frequency of genotypes for *CYP21A2* variants was consistent with Hardy Weinberg equilibrium (P=0.05) among studied population (P=0.05). Comparisons of the allelic frequencies of *CYP1A2*1C* and *CYP1A2*1F* between Iranian population and other populations are shown in Tables 3 and 4.

Table 3. Allele frequencies of *CYP1A2*1C* variant in Iranian populations and other populations

Population	Number	1C		*P* Value	References
		G	A		
Iran	200	0.95	0.05		(Present study)
Turkey	110	0.96	0.04	0.2809	[19]
Egypt	212	0.93	0.07	0.1464	[21]
China	422	0.79	0.21	0.0001*	[22]
Japan	159	0.79	0.21	0.0001*	[23]
China	139	0.75	0.25	0.0001*	[19]
Serbia	126	0.996	0.004	0.0001*	[24]

**Statistically significant difference. The P value show the statistically differences in allele frequency between present study and other populations*

Table 4. Allele frequencies of CYP1A2*1F variant in Iranian population and other populations

Population	Number	*1F		*P* Value	References
		C	A		
Iran	200	0.3	0.7		(Present Study)
Turkey	110	0.27	0.73	0.5423	[19]
Egypt	212	0.32	0.68	0.5533	[21]
China	422	0.64	0.36	0.0334*	[22]
Japan	159	0.39	0.61	0.3453	[23]
China	139	0.33	0.67	0.3745	[19]
Serbia	126	0.389	0.611	0.3355	[24]
Korea	250	0.68	0.32	0.0001*	[25]
Ovambos	177	0.54	0.46	0.0001*	[25]
Mongol	153	0.79	0.21	0.0003*	[25]
Caucasian	495	0.682	0.318	0.0001*	[26]
British	114	0.667	0.333	0.0001*	[27]
Ethiopia	173	0.504	0.496	0.0001*	[28]
Japan	250	0.372	0.628	0.4576	[29]

**Statistically significant difference. The P value show the statistically differences in allele frequency between present study and other populations.*

Inter-individual variation in *CYP1A2* level in different populations may be explained by variety of factors, including genetic differences and environmental factors. It has been demonstrated that some *CYP1A2* polymorphisms are associated with individual differences in *CYP1A2* enzyme activity. Thus, it may influence individual's susceptibility to the risk of

cancer and therapeutic efficacy of the related drug. *CYP1A2* polymorphisms have ethnic variability in many populations. Therefore, the detection of these variants is essential to identify the relationship between the polymorphisms and therapeutic efficacy of related drugs [30-31]. In the current study, we indentified the two clinically important polymorphisms in *CYP1A2* by TaqMan® genotyping assay. The frequency of *CYP1A2*1C* was relatively similar in Iranian and Turkish population, whereas, it was lower compared with Egyptian population. Overall, no significant difference was observed between Iranian, Turkish, and Egyptian populations Table 3. Although the frequency of *CYP1A2*1F* was significantly lower in Iranian population than that in Chinese, Korean, Caucasians, and British Caucasian populations, it was almost similar to Turkish, Egyptian, Chinese, Serbian, and Japanese. *CYP1A2*1F* was more frequent among Iranian population compared with *CYP1A2*1C*. This finding may be of clinical importance, as previous study showed that smokers homozygous for the C-allele had, on average, 40% lower *CYP1A2* activity in comparison with those with the A/A genotype. This functional relationship of the C→A polymorphism and *CYP1A2* activity could not be found in nonsmokers [32]. Such a genetic variation may have a major influence in altering the response and/or toxicity of a large number of drugs metabolized by CYP1A2, although this needs further investigation.

4. CONCLUSION

This is the first study analyzing the CYP1A2 clinically important allelic variants in Iranian population. Our results indicate that functional significance of these alleles should be evaluated in various populations. It is necessary to determine the relationship between these variants and CYP1A2-related drug metabolism in associated groups. Further studies are needed to detect the functional roles of these polymorphisms to identify associated with the clinical challenges.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have no conflicts of interest to disclose.

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