

**STUDY THE FREQUENCY OF CYP2D6\*4 NULL ALLELE IN IRANIAN POPULATION**

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**Summary**

Many of the clinically important drugs are CYP2D6 substrates. Polymorphisms of variants of CYP2D6 affect enzyme function causing different drug responses. This mutated allele shows a very high degree of inter-individual variability. The frequency of CYP2D6\*4 defects is less than 1% in Asian population and up to 10% of Caucasians. The CYP2D6\*4/CYP2D6\*4 genotype has a nonfunctional P450 protein product without enzyme activity and is concerned as “poor metabolizer” (PM) phenotype which is failure to use CYP2D6-dependent metabolic pathways for 30% of drugs. The aim of this study was to estimate the frequency of CYP2D6\*4 defects in the Iranian population. The wild-type allele of CYP2D6 and the mutated allele CYP2D6\*4 for three hundred ninety-one unrelated healthy volunteers were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The frequency of CYP2D6\*4 allele, characterized by loss of *BstNI* site, was observed in 9% of volunteers. The CYP2D6\*4/CYP2D6\*4 genotype was observed in only 2.3% of volunteers. According to this study, the prevalence of allelic variants in the Iranian population is higher than other populations in Asia.

**Keywords:** CYP2D6\*4 polymorphism, Iranian population, PCR-RFLP

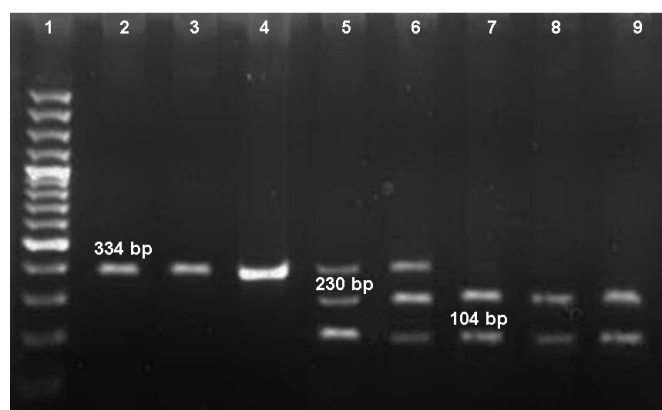
### Introduction

The CYP2D6 enzyme responsible for the oxidative metabolism of environmental chemicals and 20-25% of many clinically important drugs which are substrates for it, including beta-adrenoceptor blockers, antidepressants, neuroleptics, antiarrhythmics, antipsychotics, selective serotonin reuptake inhibitors and many of common anti-cancer (1-4). CYP2D6 gene is located on chromosome 22q13.1. Until now more than 100 variant alleles have been detected. Variation in CYP2D6 alleles affects enzyme's function associated with different patient's responses to drug. Function and expression of CYP2D6 enzyme are influenced by genetic polymorphism which varies widely in inter-individual and interethnic population (1, 2). Mutation in alleles leads to three clinically distinct phenotypes; poor metabolizer (PM) phenotype is caused by defect alleles, rapid metabolizers are result of duplicated or multiduplicated, and normal metabolizers (2, 3). The most common null alleles which include 70 to 90 % of all PMs is Mutant CYP2D6\*4 with transition G1934A that cause premature stop codon with the result of defect enzyme and produce nonfunctional CYP2D6 protein (nucleotide 3465, Genbank accession No. M33388). In this phenotype, therapeutic failure has been observed (2, 6). Allele frequencies of CYP2D6\*4 defective is 12–21 % in Caucasians, 1% in Asians, 7% black Africans, 1–4% Ethiopians and Saudi Arabians (2, 7-10). Since this polymorphism indicates efficacy in the clinical of drug therapy, we studied the frequency of CYP2D6\*4 allele in Iranian population.

### Methods

**Studied Population:** Three hundred ninety-one donor healthy volunteers (120 males (39%) mean age=35.81 years SD=±9.7 and 191 females (61%) mean age = 38.15 years SD=±12.13) participated for genotype study. They were referred from blood transfusion center (Mashhad, Iran). This research project was approved by research ethics board of Mashhad University of Medical Sciences.

**Molecular analysis:** Genomic DNA was extracted by commercially DNA extraction kit (Biogene, Mashhad, Iran) using salting-out method from 10 ml of whole blood. Genotyping of CYP2D6\*4 was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), applied primer sets were as follows; sense primer 5'-GCTTCGCCAACCCTCCG-3'; and antisense 5'-AAATCCTGCTCTCCGAGGC- 3' (11). PCR was performed in a T3 Thermocycler (Biometra, Germany). Each 20µl of PCR mixture contained 100ng of genomic DNA, 0.5 unit of Taq DNA polymerase, 1x PCR reaction buffer (10mM/L Tris-HCl, 50 mM/L KCl, 1.5 mM/L MgCl<sub>2</sub>), 0.2 mM each dNTP, 0.5µM each primer and 1.1 mM MgCl<sub>2</sub>. The reaction mixture was initially denatured at 95°C for 3 min, followed by 35 cycles of 95°C for 1 min, 59°C for 1 min, 72°C for 2 min and a final extension at 72°C for 7 minutes. 334 bp amplified were analyzed on 1.5% agar's gel stained with ethidium bromide before digestion. One unit of *Bst*NI restriction enzyme was added to each PCR product (5µl) and incubated at 37°C for 16 hours. Digested products were analyzed on 2.5% agar's gel. G to A transition at position 1934 (G1934 →A) abolishes the restriction site and a fragment of 334 bp is observed. Heterozygous individuals (IM) show one normal allele (230, 104 bp) and one mutated allele of 334 bp and homozygous individuals (PM) show 334 bp band while normal individuals show only 230 and 104 bp fragments. Heterozygous individuals (IM) show one normal allele (230, 104 bp) and one mutated allele of 334 bp and homozygous individuals (PM) show 334 bp band while normal individuals show only 230 and 104 bp fragments (Figure 1).



**Fig. 1.** Analysis of the CYP2D6\*4(G1934A) polymorphism. Lane 1: DNA size marker (100bp); Lane2-4: \*4/\*4 genotype (Poor metabolizer -334 base pairs); Lane 5and 6: wt/\*4 genotype (Intermediate metabolizer -334, 230 and 104 base pairs); Lane 7-9: wt/wt genotype (Extensive metabolizer -230 and 104 base pairs)

**Statistical Analysis:** The allele for wild type genotype is referred as “WT” and for mutated genotype as “\*4” . The frequency of WT allele was calculated by introducing the total amount of the EM genotypes (Extensive Metabolizer) and half of the IM genotypes (Intermediate Metabolizer), which was divided using total number of individuals, \*4 allele frequencies were calculated as WT allele frequency. SPSS software was used. For calculating chi-sq Hardy-Weinberg equilibrium test, OEGE (2006) was used (12).

### Results

Chi-sq Hardy-Weinberg equilibrium test was calculated for CYP2D6\*4 ( $\chi^2=8.3$ , d.f=1, P value <0.005). Heterozygous individuals express one normal allele (230, 104 bp) and one mutated allele of 334 bp and homozygous individuals show 334 bp band while normal individuals show only 230 and 104 bp fragments . 83.9% of volunteers (n=293) had wild-type “WT” allele. 2.3% of cases (n=7) were carriers of two \*4 (mutated) alleles, being homozygous for CYP2D6. 14.19% of subjects (n= 44) were carriers of one \*4 allele, being heterozygous for CYP2D6\*4. The frequency of the CYP2D6\*4 allele was 9% in group (Table1).

**Table 1.** genotype and allele CYP2D6\*4 frequency in the Iranian population, N ; total number ; WT ,wild type allele ; MUT, mutant allele ; PM, refer to homozygous mutant status; EM, refer to homozygous normal status; IM, heterozygote.

Genotype Frequency (n=311) N%			Allele Frequency (n=622)	
PM	EM	IM	WT	MUT
7(2.3)	260(83.9)	44(14.19)	0.91	0.09

### **Discussion**

CYP2D6 are responsible for the biotransformation of 25-30% important drugs as beta-blockers, antiarrhythmics, opioids, antidepressant, antipsychotic and anticancer agents (1-4). Drug response and effective treatment are influenced by CYP2D6 polymorphisms (4). Genetic variations at CYP2D6 are exhibited interethnic and inter-individual differences. More than 90 known allelic variants and subvariants reported (2, 5 and 13). As a result, three clinical metabolic phenotypes have been known in individuals: poor metabolizers (PM) bear two nonfunctional CYP2D6 alleles and has nonfunctional CYP2D6 enzyme, extensive metabolizer (EM) phenotype, intermediate metabolizers (IM) that carry two and one of the functional CYP2D6 alleles have normal enzyme activity and ultrarapid metabolizers (UM) that carry multiple copies of functional alleles. These phenotypes happen with varying frequency in different populations (3, 4). Many variant alleles include CYP2D6\*3, \*4, and \*5 produce nonfunctional enzyme and these variants most probably concerned as the PM phenotype (2, 9). PM phenotype has been estimated 7 to 10% in European Caucasians and 1% of Chinese, Japanese, and Koreans populations (14-17). The most common defective variant allele is CYP2D6\*4 in Caucasians (allele frequency ~21%). But in Chinese, Japanese, Korean, and Filipino have reported its absence or incidence about 1% (2, 7, 9, 14, and 17). The outcome of \*4 mutation is losing of enzyme function and therapeutic failure, for example in treatment with Tamoxifen (TAM). Studies have reported beneficial function of TAM in Breast cancer patients who heterozygous (wt/\*4) or homozygous (\*4/\*4) is less than other patients having wild-type (wt/wt) genotype. Also the severe and mild toxicities were observed among patients carrying mutated allele (18, 19). The genotype of CYP2D6 \*4 has not been studied in Persian people. In this study we only determined CYP2D6\*4 gene of 311 unrelated subjects in the Iranian population inhabited in the North-East of Iran using PCR-RFLP, as this polymorphism produces a non functional enzyme that effects on drug therapy. Our results showed a frequency of 9% CYP2D6\*4 allele in the volunteers (Table 1). That is near to frequency\*4 allele (12.5%) in Eastern Azerbaijan of Iran (21). The frequencies of the prevalent CYP2D6\*4 allele was lower than other Caucasian populations (12–21 %) and higher than Orientals (1%) and African (7%). This finding was close to the ones found in previous studies in central/South Asia (8.1%) and higher than other populations in which were the East Asia (2.7%) and Middle East (6.8%) (9, 14-17). The major racial groups in Iran are Persians (51%), Azeris (24%), Gilaki and Mazandarani (8%), Kurds (7%), Arabs (3%), Baluchi (2%), Lurs (2%), Turkmens (2%), and others (1%) (20). Most of the people in the North-East of Iran are Persian; who participated in this study (20). This study presents the results of CYP2D6\*4 mutant allele distributions in Iran and provides a therapeutic approaches.

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