ORIGINAL ARTICLE

Frequency Evaluation of the CYP3A4*4 Polymorphism in Iranian Healthy Volunteers

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ABSTRACT

Purpose: CYP3A4 (cytochrome P450, family 3, subfamily A, polypeptide 4) is an important enzyme in the body. The purpose of this enzyme is to oxidize small foreign organic molecules such as drugs or toxins. The different genetic variants CYP3A4 present in individuals such as CYP3A4*4. The cytochrome P450 3A subfamily have an important role in the catabolic reactions like many of peroxidative, oxadative, and reductive biotransformation reactions of common drugs such as Carbamazepine, Hydroxylations and Nevirapine.
Methods: In this study, the prevalence of CYP3A4*4 in healthy subject of Iran were analyzed. Three hundred healthy unrelated subjects were chosen. After DNA extraction, genotypes were analyzed by PCR-RFL Pand PCR-sequencing.
Results: No mutation was detected for CYP3A4*4 (Ile118Val) in these individuals. This study can be a background for future studies specially pharmacogenomic investigations and association studies. In addition, this data could be help clinicians optimize therapy or recognition persons who have risk of adverse drug reactions.
Conclusions: Our results show that the frequencies of the CYP3A4*4 polymorphism in Iranian population were almost similar to the other populations such as Malaysian, Indian, Taiwanese, Tepehuan Amerindians and Mestizo Mexicans. CYP3A4*4 mutation causes decrease enzyme activity in vivo because the Ile118Val mutation may affect the substrate binding and cause decrease in CYP3A4 activity. Therefore, lack of the CYP3A4*4 mutation among Iranian population renders the consumption of drugs whose metabolismis done by CYP3A4, harmless.

INTRODUCTION

Pharmacogenetics is the science that explains how different genetic polymorphisms in drug metabolic pathways which cause different responses to drugs in individuals.[1] These genetic polymorphisms can lead to varied individual responses to drugs, toxins and environmental factors.[2,3] Cytochrome P450 (CYP) enzymes are the most important enzymes in mammals and primarily responsible for the metabolism (degradation and elimination) of drugs.[4]

CYP is an important enzyme in the body and finds in the liver, intestine, gut, colon, prostate, breast and brain.[5-8] The enzyme activity is to oxidize small foreign organic molecules (xenobiotics) such as toxins or drugs, in order that they can be removed from the body.[9]

CYP3A subfamily is a member of CYP family of oxidizing enzymes that catalyzes many reactions such as synthesis of steroids and other lipids components.[10,11] In human, the CYP3A subfamily is consisted of CYP3A4, CYP3A5, CYP3A7; CYP3A43.[12] In addition, several members of this family involved in drug metabolism that CYP3A4 is the most common one.[5] CYP3A4 was originally named nifedipineoxidase for its ability to metabolize the antianginal drug nifedipine.[13] Until now more than 30 genetic variations were identified that most of them do not have an influence on level of expression and activation of this enzyme but several of them cause decrease of these.[14] Different variation in CYP3A4 have a major role in modulation of sex hormone metabolite levels therefore it can play role in breast and prostate carcinogenesis.[7] In addition, CYP3A4 presents

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in mammary epithelial cells and involves in activation of many environmental carcinogens.[1]

Gender dependent expression of CYP3A4 has studied in several researches.[15-17] These evidences show an increased drug clearance by CYP3A4 in females.[16] Many endogenous steroids can inhibit drugs metabolism whose they are catalyzed by CYP3A4, but some of them can active it.[18] Therefore, difference of endogenous steroid level between females and males can cause of increase drug clearance by CYP3A4 in females.[18]

Genetic variability in these enzymes may influence a patient response to commonly prescribed drug classes, including beta blockers and antidepressants.[19] So far, several polymorphic CYP3A isoforms have been reported from different ethnicities, particularly the CYP3A4,CYP3A5, CYP3A7 and CYP3A43 isoforms.[14,20] CYP3A4*4 allele of CYP3A4 was identified by polymerase chain reaction and restriction enzyme.[11] The CYP3A4*4 allele has an Ile118Val change in exon 5.[11] For the first time, this allele has been reported in 2.9% of the Chinese population.[11] CYP3A4 gene was cloned and determined that it contains 13 exons.[11] This gene is located on chromosome 7q21.3 and also it has 27592 base pairs.[7]

MATERIALS AND METHODS

In this study, 300 blood samples of unrelated and healthy donors from Iranian population (132 men and 168 women with range age 16-58) from the Special Medical Centre, Tehran, Iran were obtained. Blood samples (2 ml) were taken and collected into tubes with ethylenediaminetetraacetic acid (EDTA). DNA was extracted from whole blood using genomic extracting DNA kit (Diatom DNA extraction kit, Gene Fanavaran, Iran). CYP3A4*4 were analyzed by PCR-RFLP and PCR-sequencing. Primer sequences CYP3A4*4 were ordered according to ones described by Kun- Pin Hsieh include:

IN5(F): CAGCTGAGGATGAAGAATGGAAGAGAT

IN5(R): CCCGCCTCAGATTTCTCACCAAC.[11]

Restriction Fragment Length Polymorphism (RFLP)

Genotyping of the CYP3A4*4 alleles was performed by polymerase chain reaction and they were analyzed with methods of sequencing and RFLP. For each PCR reaction, a 25 μL volume of solution was prepared in the PCR tube. The solution for the PCR reaction included 1 μL of each primer, 2.5 μL buffers 10X, 0.8 μL mgcl2, 0.5 μLdNTP, 1 μL of DNA, 0.3 μLTaq and 17.9 μL of ultra-pure water. The conditions for amplifications were as follows: an initial denaturing step of 94°C for 4 min, followed by 32 cycles of 94°C for 50 s, 61.3 for 50 s, 72°C for 50 s, and a final elongation step of 72°C for 10 min. In the next stage, the PCR product was sequenced and compared with the Gen Bank database (no. AF209389). Finally, the products were resolved in 12.5% gel to ensure amplification of the specific products. The resulting 249-base pair product was assessed by PCR and RFL Panalysis using BsmA I that its cleavage site sequence is

5′... G T C T C $(N)_1\downarrow$... 3′

3′... C A G A G $(N)_5\uparrow$... 5′

Next, these productions run on a12.5% polyacylamid gel. The fragments produced for each of the two CYP3A4 alleles are as follows:

The wild allele (A_{13989}): 118Ile = 141 bp + 94 bp + 14 bp

The mutant allele (G_{13989}): 118Val = 94 bp+ 47 bp + 14 bp

RESULT

Allele and genotype frequency distribution of the Ile118Val (A>G) variant was analyzed in blood samples of 300 unrelated healthy volunteers from Iranian population. Only the wild type homozygous was observed in all the subjects (Figure 1). This result is not a surprising observation because this mutation is rare.

DISCUSSION

The CYP3A4 has important role in many drugs metabolism like domperidone, antidepressantsand antipsychotic.[21-23] Therefore, the information about genotypes variation of CYP3A4 and their protein function is very essential in pharmacology. Until recently, many studies have done about distribution and pharmacology of CYP3A4 variations. In addition, CYP3A4*4 is one of variants CYP3A4 that this variant causes a decrease in the level of enzyme activity.[24,25] For example, CYP3A4*4 carriers have shown a higher decline of the cholesterol and triglycerids levels with

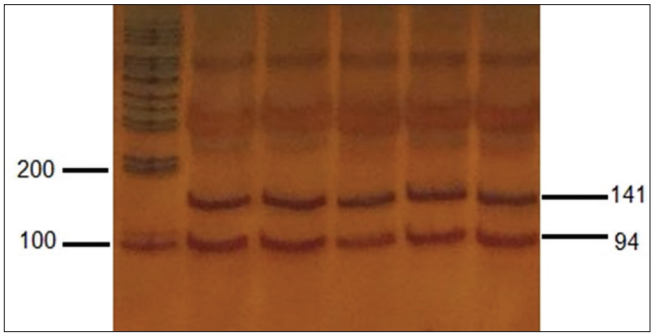

Figure 1: RFLP results for wild-typeCYP3A4 after digestion with BsmA I

Table 1: Frequency of CYP3A4*4 in Iranian and several other populations

Population	n	Frequency	References
Iranian	300	0.00	This study
Malaysian	121	0.00	3
Taiwanese	102	0.015	11
Chinese	211	0.0233	24
Indian	200	0.00	26
Mestizo Mexicans	100	0.00	27
Tepehuan Amerindians	100	0.00	27
Han Chinese	451	2.4	30

consuming simvastatin than non-carrier.[24] The frequency of CYP3A4*4 was low in the majority of populations that elevated its frequency (Table 1).[3,11,24,26,27] Also, it has been studied to have association with some diseases that according to these, no information are about the association of CYP3A4*4 with these diseases.[24,28,29]

In this study, we determined the frequencies of CYP3A4*4 (Ile118Val) in the individuals and we did not found this allele among them. Therefore, our result shows similar significance between our population and the frequencies of other populations like Malaysian, Indian, Taiwanese, Tepehuan Amerindians and Mestizo Mexicans (Table 1).[3,24,26,27] Also, these results indicate which the allelic frequency of CYP3A4*4 is rare in these populations. Although, CYP3A4*4 in the most of studies is rare, this variation has higher frequency in Han Chinese.[30]

CONCLUSION

In conclusion, we did not detect CYP3A4*4 in these subjects of Iranian. Following this result, consumption of drugs whose metabolism is done by CYP3A4, for Iranian is not harmful. Studies of frequency of CYP3A4 polymorphisms in healthy individuals from each ethnicity could help to have a valuable guideline for consumption of these drugs.

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