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Original Article

Cytochrome P4502C19*3 allelic variant frequency in Iranian healthy Azeri Turkish population

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Abstract

Introduction: Cytochrome P4502C19 (CYP2C19) is well-known to be one of the determinants responsible for the pronounced interethnic and individual differences in response and character of clinically important drugs. CYP2C19*3 arises from a G to A transition at position 636 in exon 4 of CYP2C19. These individuals be situated poor metabolizers (PMs) of a wide range of medications including omeprazole (OMP). In this study, we determined genotypes of CYP2C19*3 in Iranian Azeri Turkish population to compare allele frequencies with previous findings in other ethnic groups.

Methods: CYP2C19*3 allelic variant was determined in 200 unrelated healthy Iranian volunteers by polymerase chain reaction-restriction fragment length polymorphism assays (PCR-RFLP).

Results: The frequencies of CYP2C19*3 in healthy volunteers reported in this study (P = 0.569, χ^2 = 2.35). This is not higher than that would be predicted from the genotypic status of these cases in CYP2C19*3 allelic variants. Our results revealed that 95.46% had wild type allele, they did not carry any of the tested mutations and 9 (4.54%) had mutant alleles.

Conclusion: Our data recommend that genotyping for CYP2C19*3 is interest in using pharmacokinetics to individualize medicine, but results of this study demonstrated that CYP2C19*3 genetic polymorphism is not important determinant of the efficacy of PM of drugs, such as OMP, which may be metabolized by this enzyme.

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Introduction

Cytochrome P450 (CYP) enzymes in humans are the main cause of differences in the metabolic activities of therapeutic drugs. Eradication of Helicobacter pylori (H. pylori) infection is now performed for the treatment of upper gastrointestinal disorders such as peptic ulcer diseases.[1-3] The first-line course of therapy for H. pylori eradication includes proton pump inhibitor (PPI), 1 or 2 antibacterial agents such as amoxicillin (AMX), clarithromycin (CAM), and metronidazole. However, the failure rate of triple anti-pylori therapies has increased up to 30%.[4,5]

CYP2C19 is an isoenzyme that has been shown to metabolize various pharmacologically important therapeutic agents including barbiturates, diazepam, omeprazole (OMP), proguanil, and propranolol.[1-4] The recognized factors include antibiotic resistance, poor compliance, high gastric acidity, high bacterial load, and CYP2C19 genotype status and bacterial susceptibility to CAM were

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each significantly related to eradication rates of H. pylori by the triple therapy with a PPI, CAM, and AMX,[6] Hence, the triple regimen would be abandoned while the CAM-resistance rate in the region is more than 15-20%, because several studies published recently have confirmed that the intention to treatment eradication rate is falling short of 80%.[6-8]

In humans, the CYP2C gene subfamily is a cluster of four genes on chromosome 10q24, arranged in the consecutive order CYP2C8, CYP2C9, CYP2C19, and CYP2C18.[9,10] All members of this subfamily are genetically polymorphic. Clinically, CYP2C19 is the most important among the CYP2C gene subfamily. The predominant genetic polymorphisms in CYP2C19 are 2 variant alleles, CYP2C19*2 and CYP2C19*3 (c.G636A; rs4986893), which result in impaired metabolism of CYP2C19 substrates.[8-10] The substitution of G681A in exon 5 of the CYP2C19*2 variant allele creates an aberrant splice site while the substitution of G636A in exon 4 of the CYP2C19*3 allele results in the emergence of a premature stop codon.[9,10]

Poor metabolism of drugs is associated with genetic variants of the CYP2C19 enzyme (CYP2C19*2 and CYP2C19*3).[9] There is a gene dose-dependent decrease in drug metabolism, and individuals who are homozygous wild type, heterozygous or homozygous variant for these null alleles are termed extensive, intermediate or poor metabolizers (PMs). Even though an indigenous difference in its distribution of the CYP2C19 genotype has been defined, it is not well-known whether there is an ethnic heterogeneity of the structure and expression of the CYP2C19 enzyme in the Iranian Azeri population.

In this study, we determined genotypes of CYP2C19*3 in Iranian Azeri Turkish population to compare allele frequencies with previous findings in other ethnic groups.

Methods

Venous blood samples and DNA purification

The study population consisted of 200 healthy adult volunteers had no drug administration (aged 27-54 years), all unrelated Iranian Azeri from East Azerbaijan of Iran. Informed consent was obtained from all volunteers. Blood samples were collected from volunteers and placed in tubes that contained an ethylenediamine tetraacetic acid anticoagulant. Then, genomic DNA was extracted from peripheral blood cells by the "salting out" technique. DNA concentrations were determined by an ultraviolet spectrophotometer at 260 nm.[11]

*Polymerase chain reaction (PCR) amplification of CYP2C19*3 region*

Genomic DNA was amplified in 2× PCR Master Mix (Ampliqon, Denmark) containing nuclease-free water in a total volume of 20 µl, with PCR primers at a concentration of 0.2 µM. The nucleotide sequence of PCR primers is listed in table 1.

Amplification of this region was performed with a Multigene OPTImax Labnet PCR system (Labnet, USA) using an initial denaturation step of 95 °C for 2 minutes; 32 cycles of 94 °C for 30 seconds, 59 °C for 30 seconds, and 72 °C for 50 seconds. The amplified PCR products were analyzed on a 3% agarose gel with a 50 bp ladder (Fermentase, Carlsbad, CA) as a molecular weight marker.

Restriction enzyme digestion of PCR product

The restriction fragment length polymorphism (RFLP) PCR analysis of these variant alleles is widely used and well validated. To detect the CYP2C19*3 defect, 10 µl PCR product was digested with 0.5 U BamHI (Fermentase, Carlsbad, CA) in a complemented reaction buffer in a total volume of 20 µl at 37 °C for 18 hours.

Table 1. Sequences of primers used in polymerase chain reactions (PCRs)

Allele	Primers sequence	Orientation
CYP2C19*3	5'- TATTATTATCTGTTAACTAA-3'	F
	5'- ACTTCAGGGCTTGGTCAATA -3'	R

CYP2C19: Cytochrome P4502C19

Digested product was analyzed on a 12% polyacrylamide gel. The wild type appears as two bands of digestion products (137 and 96 bp for CYP2C19*3) On the other hand, the homozygous mutated type appears as a single band of undigested product (233 bp for CYP2C19*3). If all products (undigested and digested) appeared on the gel, the subject was a heterozygote.

Results

Data were compiled according to the genotype and allele frequencies estimated from the observed numbers of each specific allele. The frequency of each allele in our subjects is given together with the 95% confidence interval (CI). Differences in allele frequencies were measured using the χ^2 test and Fisher's exact test. A $P < 0.050$ was considered to be statistically significant throughout the population comparisons.

The frequencies of CYP2C19*3 in healthy volunteers reported in this study ($P = 0.569$, $\chi^2 = 2.35$). This is not higher than that would be predicted from the genotypic status of these cases in CYP2C19*3 allelic variants. Our results revealed that 191 of 200 (95.46%) had wild type allele (G), they did not carry any of the tested mutations and 9 (4.54%) had mutant alleles (A). There were no significant differences with regard to sex, age, smoking status in the group. Demographic data are presented in table 2.

Discussion

CYP2C19 is a xenobiotic metabolizing enzyme that metabolizes foreign compounds such as clinically used drugs and other environmental chemicals. Allelic variants CYP2C19*2/*3 are the most important detrimental alleles of this isoenzymes.[12-16] This study contributes significantly toward a better understanding of the prevalence of CYP2C19*3 in the Iranian Azeri Turks population and consequently of the pharmacological management of a large number of patients with such ethnic background. By providing new data on the pattern of CYP2C19 polymorphism and the relationship between phenotype and genotype in the ethnic population, this study contributes significantly toward a better understanding of the prevalence of CYP2C19 in the Iranian Azeri population.

The RFLP-PCR analysis of CYP2C19 variant alleles is a widely used and confirmed method. We have adapted this assay for determination of the CYP2C19 genotype from archival serum using published methods for DNA extraction from serum.

Most of the CYP2C19 polymorphism studies in Oriental populations were performed in East Asian Populations. The frequency of PMs of CYP2C19 varies between 18 and 23% in Asians, 2-5% in Caucasians and 4% in a Shona population of Zimbabwe.[12] We did not find CYP2C19 *3/*3 (AA) genotype, as they are rare in several ethnic groups (about 3% of Caucasians, 4-7% of Afro-Americans),[14,17] but they are more prevalent in Korean (12-16%)[15] Japanese (18-23%).[13] In previous measured, two mutations, CYP2C19*2 and CYP2C19*3 have been shown to account for > 99% of Oriental but only 88% of 37 Caucasian PM alleles, which suggests that other defective alleles contribute to the PM phenotype in Caucasians.[16,17]

Table 2. Demographic characteristics of the subjects

Characteristics	Characteristics of samples		
Age (mean) (year)	44		
Men (percentage of patients)	53		
Women (percentage of patients)	47		
Smoking status (%)	49		
Omeprazole usage history (%)	84.2		
Family history of duodenal ulcer (%)	14.8		
H. pylori serologic test (%)	Positive for IgM	Positive for IgG	Negative
	11.3	76.5	12.3

H. pylori: Helicobacter pylori, IgM: Immunoglobulin M; IgG: Immunoglobulin G

CYP2C19*2 accounts for 75% of CYP2C19 defective alleles in Orientals, and 93% in Caucasians,[13,14] although, there are other reports about the frequency of this mutation in Caucasian populations.[15,17] The well-characterized allele (CYP2C19*3) discovered in Japanese PMs,[13,15] accounts for approximately 25% of all inactive forms in Orientals, being by converse extremely rare in non-Oriental populations.[16] The results of this study may be helpful for the determinant of the efficacy of PM of drugs, such as OMP, which may be metabolized by this enzyme.

Conclusion

Our data recommend that genotyping for CYP2C19*3 is interest in using pharmacokinetics to 'individualize medicine, but results of this study demonstrated that CYP2C19*3 genetic polymorphism is not important determinant of the efficacy of PM of drugs, such as OMP, which may be metabolized by this enzyme.

Conflict of Interests

Authors have no conflict of interest.

Acknowledgments

All of the authors made equal and significant contributions to acquisition of data, analysis and interpretation of data, writing the manuscript and final decision to submit for publication. "The author(s) declare(s) that there is no conflict of interest regarding the publication of this manuscript."

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