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Detection of CYP2C18 m1, and m2 Alleles within an Iranian Population (Mazandaran) Using Denaturing High-Performance Liquid Chromatography (DHPLC)

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Abstract

Background: Genetic polymorphisms of cytochrome p450 in humans are the main cause of differences in the drug metabolism. The allele and genotype frequencies of CYP2C19 and CYP2C9 have been studied in some Iranian populations. The aim of present study was to examine the frequencies of CYP2C18 m1, and m2, alleles in the Mazandarani ethnic group among Iranian Population.

Materials and Methods: In this study, genomic DNA was extracted from leucocytes of one hundred unrelated healthy volunteers. The prevalence of the common variants CYP2C18 m1 and m2 alleles were studied by using high fidelity polymerase chain reaction (HF-PCR) - DHPLC methods.

Results: The frequency of CYP2C18 m1 and m2 alleles were 0.0% and 1.5%, respectively. CYP2C18 genotypes wt/wt, wt/m1, wt/m2, m1/m1, m1/m2, and m2/m2 frequencies were 98.5 %, 0.0 %, 1.5%, 0.0%, 0.0%, and 0.0%, respectively.

Conclusion: The result of the current study shows that impaired CYP2C18 activity in 1.5% of our sample population may decrease extra hepatic metabolism of some important drugs such as Phenytoin. It may also affect transdemal delivery of drug substrate for this isoenzyme.

Keywords: CYP2C18; Genotype; Alleles; Mazandaran; Iranian

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Introduction

The most of Cytochromes P450 (CYPs) enzymes exhibit variability in their expression level and activity, and genetic polymorphisms of these enzymes in humans are the main cause of differences in the metabolism of drugs and could influence drug plasma levels and drug response (1, 2). In human, about 20% of clinically used drugs are metabolized by the CYP2C subfamily (3). CYP 2C8, 2C9, and 2C19 are the isozymes of the 2C subfamily which are expressed at the highest level in the human liver (4-6). However, CYP 2C18, appears to be expressed at a very low level in the human liver (7, 8) and this enzyme seems to be a major P450 2C in the skin and in the lung as judged from its mRNA levels (9, 10).

Two Polymorphisms of CYP2C18 have been described previously (11, 12). Komai *et al* identified a single nucleotide change (T to A) at position 204 of exon 2 (CYP2C18m1) which creates a stop codon that yields a protein lacking the heme-binding site (12). Tsuneoka *et al* discovered a single nucleotide change (T to C) at position A478T of the (5'-UTR) 5'-flanking region of CYP2C18 or CYP2C18m2 (11).
For CYP2C subfamily, the allele and genotype frequencies of CYP2C19 and CYP2C9 have been studied in some Iranian population (13-16). In the present study, we examined the frequencies of CYP2C18 m1, and m2, alleles and CYP2C18 genotypes in the Mazandarani ethnic group among

Iranian Population.

Materials and Methods

Subjects

One hundred unrelated healthy volunteers of Mazandarani origin residing in Mazandaran, a northern province of Iran in the south of Caspian Sea, were enrolled in the study. All subjects were included in the study after giving written informed consent.

Genomic DNA extraction and PCR amplification

A 10 milliliters venous blood was obtained from each subject and transferred into sterile plastic tubes containing Na-EDTA anticoagulant and stored at -25 °C until processed. Lymphocytic genomic DNA was extracted by High Pure Nucleic Acid Extraction Kit (Roche Applied Sciences, Germany) according to the manufacturer's instructions. The genomic DNA concentrations were measured by NanoDrop (Biowave, UK) and the DNA samples were stored at −20 °C.

Sequences and primers

Cyp2C18 m1 anomaly yielded a truncated 67-amino-acid CYP2C18 lacking a haem-binding region. This SNP was at position 204 of exon 2 (T>A); changing at position 68 from Tyr to a TAA stop codon (Tyr 68X). The sequence was determined as rs41291550 accession number through NCBI databank. Cyp2C18 m2 was a single nucleotide change (T to C) at position - 478 of the 5'-flanking region (5'-UTR) and was available through the accession number rs12570771 at NCBI.

For designing specific primers (Table 1), GeneRunner software was used and then the primers were checked and finalized via a primer- BLAST program at NCBI.

Table 1. Primers for Cyp2C18 m1 and Cyp2C18 m2 alleles.

Allele name	Forward primer	Reverse primer	PCR band (bp)	Accession No.
Cyp2C18 m1	(9197)5'AATATCAGTCTGAATCA CGGACA3' (9219)	(9354)5'CAATCAGGGCCTCCTTC ACT3'(9373)	177	NG_008373.1 RefSeqGene on chromosome 10
Cyp2C18 m2	(4959)5'ACACAGATTGCCCTCAA AGTC3'(4679)	(5103)5'TAGTCTGAAGCAGAGCC CAG3'(5084)	465	NG_008373.1 RefSeqGene on Chromosome 10

Polymerase chain reaction and Gel electrophoresis

The total volume of the PCR was 25 µl containing 1 µl of each primer (0.1-1 µM), 1 µl DNA template (50–100 ng), 12.5 µl PCR master mix (2X, Thermo Scientific), up to 25 µl dH2O. The PCR reactions were carried out by personal thermocycler (Bio-Rad, USA) according to the Thermo product information.

Six microliters of the PCR products was visualized on 2% agarose gel containing 1X SyberGold stain and the concentration of DNA samples was measured by BioWave Spectrophotometer equipped with Helma Tray Cell cuvette (UK).

Denaturing High-Performance Liquid Chromatography (DHPLC) analysis

DHPLC was performed on the WAVE fragment analysis system (Transgenomic Inc., USA) to detect the m1 and m2 variants in the Cyp2C18 gene. In order to generate the hetero- and homoduplexes, an equal amount of the PCR products from patients and the wild type were mixed. Heteroduplex formation step was performed using Mastercycler gradient thermal cycler (Eppendrof, Germany) under the following program: heating at 95 °C for 5 min, 95 ° C for 22 sec and re-annealing by decreasing 1 °C every 22 s until the temperature reached 25 °C. Eight to ten microliters of cooled PCR products was automatically injected by DHPLC instrument into the DNA Sep column and the eluted DNA fragments were detected with UV absorption at wavelength 260 nm. The optimal melting temperature for each PCR product was determined by the WAVE utility software (17-19) .

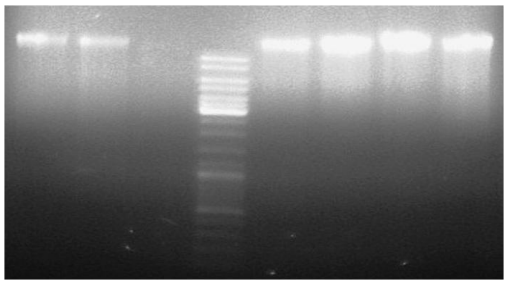

Figure 1. High molecular weight DNAs extracted from blood patients (From left side lanes 1,2,5-8); lane 3, empty; lane 4, 100-10000 bp DNA ladder (Thermo, USA). 2% agarose gel contained 1X SyberGold stain at 0.5X TBE.

The optimal column temperatures for resolution of PCR products were 57 °C for Cyp2C18 m1 and 60 °C for Cyp2C18 m2, respectively. The gradient of DHPLC buffer B was 44.1–54.5% for Cyp2C18 m1 and 50.0–62.7% for Cyp2C18 m2, respectively. We

used the Low Range Mutation Standard (Transgenomic Inc., USA) to confirm the appropriate conditions of the instrument, buffer A (consists of 0.1 mM TEAA in H2O, pH=7), and buffer B (consists of 0.1 mM TEAA in dH2O, 25% acetonitrile). A wild DNA control for each variant was also used to confirm the positive and negative results.

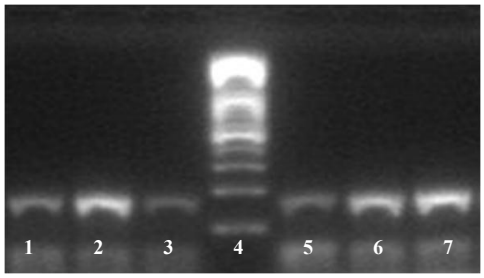

Figure 2. The 177 bp PCR products of Cyp2C18 m2 gene (From left side, lanes 1-3 and 4-6); lane 4, 100-10000 bp DNA ladder (Thermo, USA). 2% agarose gel contained 1X SyberGold stain at 0.5X TBE.

Ethics Statement

The protocol of this study was approved by Ethic Committee of Mazandaran University of Medical Sciences. Every subject signed an informed written consent form.

Results

The quality of isolated DNAs from blood of healthy volunteers for PCR amplification of Cyp2C18 m1 and m2 variants are shown in Figure 1.

The results of PCR amplicons for the CYP2C18m1 and m2 alleles in representative subjects are shown in Figures 2 and 3, respectively.

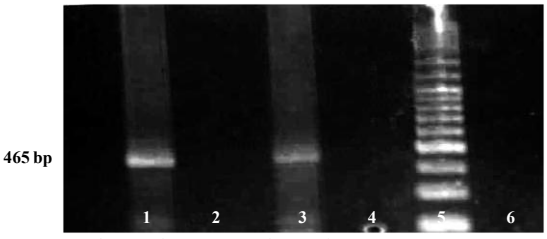

Figure 3. The 465 bp PCR products of Cyp2C18 m2 gene (From left side, lanes 1, 3). lane 5, 100-10000 bp DNA ladder (Thermo, USA). 2% agarose gel contained 1X SyberGold stain at 0.5X TBE.

DHPLC chromatograms

The results of DHPLC analysis of one hundred samples from m1 and m2 alleles of CYP2C18 gene are shown in Figures 4 and 5, respectively.

As shown in Figure 4, the chromatograms of CYP2C18m1 amplicons were not shown different elution times. However, 3 different chromatograms were shown in samples checked according to CYP2C18m2 variant (Figure 5).

The frequency of CYP2C18 m1 and m2 alleles were 0.0% and 1.5%, respectively. CYP2C18 genotypes wt/wt, wt/m1, wt/m2, m1/m1, m1/m2, and m2/m2 frequencies were 98.5 %, 0.0 %, 1.5%, 0.0 %, 0.0%, and 0.0%, respectively (Table 2).

Table 2. Allele and genotype frequencies of CYP2C19 in Mazandarani ethnic group among Iranian Population.

Variant allele	**Allele frequency (%)**	
CYP2C18 m1	0	
CYP2C18m2	3	
Genotype	**Frequency (%)**	**Anticipated phenotype (based on genotype)**
CYP2C18 wt/CYP2C18 wt	98.5	Homozygous-EM)
CYP2C18 wt/CYP2C18 m1	0	(Heterozygous-IM)
CYP2C18 wt/CYP2C18 m2	1.5	(Heterozygous-IM)
CYP2C18 m1/CYP2C18m1	0	(Homozygous-PM)
CYP2C18 m1/CYP2C18m2	0	(Heterozygous-PM)
CYP2C18 m2/CYP2C18m2	0	(Homozygous-PM)

PM, poor metabolizer; EM,extensive metabolizer; IM, intermediate metabolizer

Discussion

There are at least eight ethnic groups living in various parts of Iran (16). To our knowledge, this is the first study that we analyzed the allelic variation of CYP2C18 gene in the Mazandarani ethnic group among Iranian population.

The human CYP2Cs are important subfamily of P450 enzymes that metabolize approximately 20% of clinically used drugs (20). Almost, all members of this subfamily which are expressed at the highest level in human liver (4-6) are known to be highly polymorphic and leading to changes in enzyme activity (20).

From isozymes of the 2C subfamily, only CYP 2C18 is expressed poorly in the liver and seems to be major enzyme involve in extra-hepatic (skin and lung) metabolism of drugs such as phenytoin (21) as judged from its mRNA levels (9, 10). Polymorphisms of CYP2C18 have also been described previously (11, 12). Komai *et al* (12) identified a single nucleotide change(T to A) at position 204 of exon2 (CYP2C18m1)which creates a stop codon and yields a protein lacking the heme-binding site.

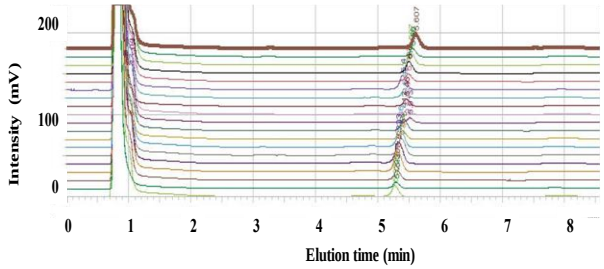

Figure 4. DHPLC chromatograms of Cyp2C18 m1 177 bp amplicons. The chromatograms show no heteroduplexes formations.

Tsuneoka *et al* (11) discovered a single nucleotide change (T to C) at position 478 of the 5'-flanking region (CYP2C18m2).

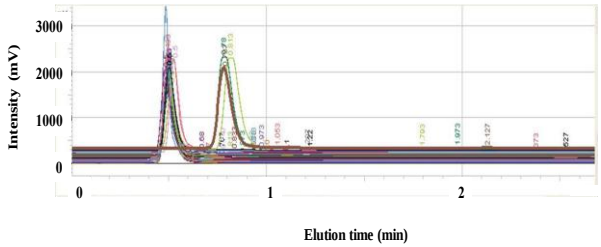

Figure 5. DHPLC chromatograms of Cyp2C18 m2 456 bp amplicons. The chromatograms show 3 heteroduplexes formations.

The frequency of CYP2C18m1 allele found in our subjects was (0%) similar to those showed in Xhosa (n=109), Yoruba (n=60) and was comparable to Cape Mixed Ancestry (n=97; 0.09%), Luhya (n=89: 0.01%), Maasai (n=143: 0.06%), Caucasian (n=60: 0.35%), Chinese (n=45: 0.23%), Hispanic (n=23: 0.28%), and African American (n=24: 0.09%). By contrast, the CYP2C18m2 alleles occur at a relatively higher frequencies in the East Asian countries (11).
The results of present study will aid in understanding the ethnic diversity of the Iranian population and offer a preliminary basis for more rational use of drugs that are substrates for CYP2C18 in this population.

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Authors' contributions
Adele Rafati and Pooria Gill: laboratory analysis and preparation of lab report and contribution to first draft of paper and methodology design. Mehdi shabani and Maryam peyrovei: sampling, preparation of first draft of their thesis and paper Seyed Mohammad Bagher Hashemi-Soteh and Mohammad Reza Shiran: study design, data analysis, writing and revising the paper and Correspondence

Conflict of interest
No conflict of interest

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