Genotype and allele frequency of CYP2C19*17 in a healthy Iranian population

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Abstract
Background: Cytochrome P450 2C19 (CYP2C19) is important in metabolism of a wide range of drugs. CYP2C19*17 is a novel variant allele which increases gene transcription and therefore results in ultra-rapid metabolizer phenotype (URM). Distribution of this variant allele has not been well studied worldwide. The aim of present study was to investigate allele and genotype frequencies of CYP2C19*17 in a healthy Iranian population and compare them with other ethnic groups.

Methods: One hundred eighty healthy unrelated Iranian volunteer took part in this study and were genotyped for CYP2C19 *2, *3, *17 (-3402) by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and CYP2C19*17 (-806) by a nested-PCR assays. The distribution of CYP2C19*17 polymorphism in Iranian population was then compared with other ethnic groups.

Results: The CYP2C19*17 allele frequency was 21.6% in Iranian population. Among studied subjects 5.5% were homozygous for CYP2C19*17 and phenotyped as ultra-rapid metabolizers; 28.8% were genotyped as CYP2C19*1*17 (extensive metabolizers) and 3.3% as CYP2C19*2*17 (intermediate metabolizers).

Conclusion: The CYP2C19*17 genetic distribution in Iranian population is similar to Middle East or European countries. The high frequency of CYP2C19*17 in Iranian population highlights the importance of this new variant allele in metabolism of CYP2C19 substrates. Thus, future association studies are required to reveal clinical consequence of this genetic polymorphism in carrier individuals.

Keywords: CYP2C19, Genotyping, Iranian, Polymorphism, Polymerase chain reaction.


Introduction
The cytochrome P450 2C19 (CYP2C19) is important in metabolism of a wide range of drugs including proton pump inhibitors (omeprazole), tricyclic antidepressants (amitriptyline), selective serotonin reuptake inhibitors (citalopram), anticonvulsant S-mephenytoin and anti-platelet drugs (clopidogrel) (1-3). Genetic polymorphism of CYP2C19 was identified by its considerable effect in metabolism of S-mephenytoin, resulting in poor metabolizer (PM) and extensive metabolizer (EM) (4). The EM phenotype is attributed to wide type allele (CYP2C19*1). The PM is assigned to CYP2C19*2 which is defined as a mutation in exon 5 (CYP2C19, 681G>A) and causes a splice

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site and CYP2C19*3 (CYP2C19, 636G>A) which creates a stop codon in exon 4 (5). In 2006 a novel variant allele, CYP2C19*17 was reported by Sim et al. (6) which is specified by a double mutation in promoter region (CYP2C19 -806C>T and -3402C>T) (2), these mutations lead to increased gene transcription and therefore ultra-rapid metabolizer phenotype (URM) (7-9).

Few studies have reported allelic frequency of CYP2C19*17 and its effect on clinical response to CYP2C19 substrates (8,10-13). CYP2C19*17*17 genotype has been found to increase clinical response to clopidogrel treatment by better anti-platelet activity and therefore was associated with higher risk of bleeding (14). In treatment of peptic ulcer, URM phenotype, caused the therapeutic failure by increasing proton pump inhibitor metabolism (15) and PM phenotype led to greater acid suppression (16,17).

Several studies show inter-ethnic differences in distribution of this variant allele (1,6,17). The allele frequency of CYP2C19*17 was estimated 18% in Swedish, 4% in Chinese (6) and 1.7% in Japanese people (1). To our best knowledge, no study has been conducted to evaluate distribution of this new variant allele in Iranian population. Thus, the present study aims to investigate allele and genotype frequencies of CYP2C19*17 variant in the Iranian population and compare these frequencies with other ethnic groups. These findings would help us to estimate importance of this new polymorphism in variable response to CYP2C19 substrates between Iranian individuals.

**Methods**

**Subjects and DNA extraction**

One hundred and eighty unrelated healthy Iranian volunteers (60 women and 120 men) with the mean age of 36 years (ranging between 20 and 55) and average body weight of 63 kg (ranging between 45 and 89) took part in this study. A randomize prospective cross sectional design was used to recruit participants. All subjects were students or stuffs of faculty of pharmacy from four different cities in Iran. They signed written informed consent before attending this project. The study protocol was confirmed by ethics committee of Tehran University of Medical Sciences.

Five ml venous blood sample was taken from each subject and transferred into tubes containing 10 µl of 10% ethylene diamine tetra-acetic acid (EDTA). Genomic DNA was obtained from peripheral blood leucocytes by salting out method (using supersaturated 6M NaCl solution) (18). The extracted DNA was dissolved in sterile distilled water and stored at 4°C until the day of analysis.

**Genotyping of CYP2C19*2 (681G>A), CYP2C19*3 (636G>A) and CYP2C19*17 (-3402C>T)**

The genotyping of CYP2C19*2 (681G>A), CYP2C19*3 (636G>A), and CYP2C19*17 (-3402C>T) polymorphisms was performed by modified PCR-RFLP assays as originally described by De Morais et al. (19) and Sim et al. (6). The PCR reaction mixture (25 µl) was consisted of 250 ng template DNA, 1× PCR buffer, 2 mM MgCl2, 0.2-0.8 µM of each specific forward and reverse primers, 0.2 mM dNTPs, 1 Unit of Taq DNA polymerase (CinnaGen, Iran) and sterile distilled water. The PCR reaction was carried out in an Eppendorf PCR system gradient master cycler (Hamburg, Germany). Thermal profile for DNA amplification was as follows: the initial denaturation at 94°C for 2 min; then 35 cycles of denaturation at 94°C for 45 seconds, annealing at 53°C for 40 seconds, and extension at 72°C for 30 seconds; final extension at 72°C for 5 min eventually applied to cycling. All three PCR reactions were amplified by similar thermal profile. The PCR product of each reaction was digested by specific restriction endonuclease (New England Biolabb, UK). The details of primers sequence and restriction enzymes are summarized in Table 1. SmaI digested the 169 bp fragment to 120 and 49 bp fragments in wild type (wt) carriers of...
**CYP2C19**. BamHI yielded 233 and 96 bp fragments in wild type carriers of **CYP2C19**. MnlI resulted in 280 and 224 bp fragments in wt subjects for **CYP2C19**. While the homozygous carriers of **CYP2C19**2, *3 and *17 were resistant to digestion by related enzymes and resulted in single band in the 2.5% agarose gel stained with ethidium bromide (Table 1).

**Genotyping of CYP2C19**(-806C>T)

The **CYP2C19**(-806C>T) mutation was analyzed by semi-nested PCR approach as described by Sim et al. with a few modifications (6). The first PCR reaction mixture and thermal protocol (PCR I) was identical to what described earlier for **CYP2C19**2, *3 and *17(-3402); R1 and F1 primers (Table 1) yielded a 470 bp product. 0.5 µl of PCR I product was then included in second allele-specific PCR reactions (PCR II). Primer F2 (wt) or primer F3 (mt) were used with primer R1 to differentiate between -806C (**CYP2C19**I) and -806T (**CYP2C19**I7) alleles, respectively. The reaction mixture (15 µl) of PCR II (one reaction for each allele) was similar to that of first reaction, except for using 2.5 mM MgCl2. The second reaction (PCR II) was performed with initial denaturation at 94°C for 1 min, and then 15 cycles at 94°C for 15 seconds, annealing at 53°C for 20 seconds and extension at 72°C for 30 seconds. The 200 bp PCR products (wt or mt) were separated by 2.5% agarose gel.

**Statistical analysis**

The allele frequency differences between populations were estimated using Chi-square test and two tailed Fisher’s exact test. The 95% confidence intervals (CIs) were calculated using Confidence Interval Analysis software. The relation of sex and genotype was analyzed by two tailed Fisher’s exact test. The observed and expected frequencies were calculated using Hardy-Weinberg equation. The two tailed Fisher’s exact test was used to assess genotype frequencies deviation in the studied population from Hardy-Weinberg equilibrium. In all statistical analysis p<0.05 was considered as significant difference.

**Results**

The **CYP2C19**I7 allele was identified in 78 out of 180 volunteer with the frequency of 21.6% (95% CI: 17.5 – 26.3). 10 subjects were homozygous for **CYP2C19**I7 (5.5%, 95% CI: 2.7-10 %), 52 subjects (28.8%, 95% CI: 22.4-36.1 %) were heterozygous for **CYP2C19**I7 and 75 subjects (41.7%, 95% CI: 34.4-49.2%) were found to be homozygous for **CYP2C19**I. The genotype and allele frequencies of CYP2C19 are reported in Table 2. The dis-

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### Table 1. Primer sequences, PCR products, restriction endonucleases, and digested fragments

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>PCR product (bp)</th>
<th>Restriction endonuclease, T (°C)</th>
<th>Restriction Pattern (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19*2</td>
<td>5’-AATTACAAACCAGCTTGCC-3’ (F) 5’-TATCACTTTCCATAAAAAAGCAAG-3’ (R)</td>
<td>169</td>
<td>Smal, 25</td>
<td>Wt:120, 49</td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>5’-TATTATTATCTGTTAACTAATATGA-3’ (F) 5’-ACTTCAAGGGCCATTGCAATA-3’ (R)</td>
<td>329</td>
<td>BamHI, 37</td>
<td>Wt:233, 96</td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td>5’-ATATAAGATGACCTTGAATCCTGG-3’ (F) 5’-CTTCTGAAGTGTCTGACAT-3’ (R)</td>
<td>500</td>
<td>MnlI, 37</td>
<td>Wt:280, 224</td>
</tr>
<tr>
<td>(-3402C&gt;T)</td>
<td>5’-GCCCTTAGACCAATTTCTC-3’ (F1) 5’-ATTTAACCCCCTAAAAAAACCG-3’ (R1)</td>
<td>470</td>
<td>–</td>
<td>Het:500,280,224</td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td>5’-TCTGTTCACAAACG-3’ (F2 wt)</td>
<td>200</td>
<td>–</td>
<td>Mt: 500</td>
</tr>
<tr>
<td>(-806C&gt;T)II</td>
<td>5’-TCTGTTCCAAAGT-3’ (F2 mt)</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

F (Forward primer), R (Reverse primer), bp (Base pair), Wt (wild type), Mt (mutant), Het (heterozygous)

CYP2C19I7(-806C>T) genotyping was performed by semi-nested PCR technique. R1 + F1 primers were used for first PCR (I) amplification (470 bp). In second PCR (II), R1 + F2 primers were used for amplification of -806C (wide type) allele and R1 + F3 primers were used for amplification of -806T (mutant) allele (200 bp).
CYP2C19*17 polymorphism in Iranian population

Table 2. CYP2C19 genotype and allele frequencies and expected phenotype in 180 healthy Iranian individuals

<table>
<thead>
<tr>
<th>CYP2C19 Genotype</th>
<th>Number of subjects</th>
<th>Frequency (%)</th>
<th>95% CI</th>
<th>Predicted Phenotype</th>
<th>Expected frequency by Hardy-Weinberg low* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men (120)</td>
<td>Women (60)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>17</em>17</td>
<td>5</td>
<td>5</td>
<td>5.5</td>
<td>2.7-10.0</td>
<td>URM</td>
</tr>
<tr>
<td><em>1</em>17</td>
<td>37</td>
<td>15</td>
<td>28.8</td>
<td>22.4-36.1</td>
<td>EM</td>
</tr>
<tr>
<td><em>1</em>1</td>
<td>53</td>
<td>22</td>
<td>41.7</td>
<td>34.4-49.2</td>
<td>EM</td>
</tr>
<tr>
<td><em>1</em>2</td>
<td>20</td>
<td>13</td>
<td>18.3</td>
<td>13.0-24.7</td>
<td>IM</td>
</tr>
<tr>
<td><em>2</em>17</td>
<td>4</td>
<td>2</td>
<td>3.3</td>
<td>1.23-7.1</td>
<td>IM</td>
</tr>
<tr>
<td><em>2</em>2</td>
<td>2</td>
<td>2</td>
<td>2.2</td>
<td>0.6-5.5</td>
<td>PM</td>
</tr>
<tr>
<td>Alleles</td>
<td>No. of alleles</td>
<td>Frequency (%)</td>
<td>95% Confidence Interval</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19*17</td>
<td>78</td>
<td>21.6</td>
<td>17.5-26.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19*1</td>
<td>235</td>
<td>65.3</td>
<td>60.1-70.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19*2</td>
<td>47</td>
<td>13.1</td>
<td>9.7-16.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2C19*3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: UM, Ultra-rapid metabolizer; Het-EM, Heterozygous extensive metabolizer, EM, Extensive metabolizer; IM, Intermediate metabolizer; PM, Poor metabolizer; CI: Confidence Interval

*Observed and expected frequencies were not statistically different (p>0.05).

Distribution of CYP2C19*17 and CYP2C19*2 polymorphisms were not statistically different in the studied men and women (p>0.05). The expected frequencies of CYP2C19 genotypes in Iranian population in this study, had no significant deviation from the Hardy-Weinberg equilibrium (p>0.05). Complete linkage disequilibrium was observed in -806 C>T with that of -3402C>T and this observation was in accordance with previously published studies (1,6,13,20). Both -806 C>T and -3402C>T mutations were not observed in subjects homozygous for CYP2C19*2 and accordingly CYP2C19*2 allele was not detected in subjects homozygous for CYP2C19*17.

CYP2C19*2 allele was present in 43 individuals (with the frequency of 13.1%; 95% CI: 9.7-16.9 %). Four subjects were homozygous for CYP2C19*2 (2.2%, 95% CI: 0.6-5.5%) and 39 subjects were heterozygous for CYP2C19*2 (21.6%, 95% CI: 15.8-28.4%). Six out of these 39 subjects had both CYP2C19*17 and CYP2C19*2 alleles (3.3%, 95% CI: 1.2-7.1%) and were genotyped as CYP2C19*2*17, and the rest of 33 subjects were combination of CYP2C19*1 and CYP2C19*2 alleles (18.3%; 95% CI: 13.0-24.7%) and were genotyped as CYP2C19*1*2. CYP2C19*3 was not identified in our study population.

Based on the CYP2C19 genotypes, subjects were divided into 4 previously defined phenotypes (2); the summary of predicted phenotypes is shown in Table 2. Homozygous carriers of CYP2C19*17 allele were categorized as ultra-rapid metabolizers (URM). Subjects with CYP2C19*1*17 and CYP2C19*1*17 genotypes were designated as extensive metabolizer (EM). Individuals with CYP2C19*2*17 and CYP2C19*1*2 genotypes were classified as intermediate metabolizers (IM) and homozygous carrier of CYP2C19*2 were stratified as poor metabolizers (PM).

The allele and genotype frequencies of CYP2C19 in this study were also compared with previously published reports. Data are summarized in Table 3 and Table 4. According to the data in the Table 3, the allele frequency of CYP2C19*17 in this study was not statistically different from European and Middle East countries. For example, the frequency of CYP2C19*17 allele was 20% in Swedish, 20.1 % in Danish, 22% in Norwegian, 19.6% in Greek, 24.4% in Turkish and 25.7% in Saudi Arabian people (6,9,21,23) while in the East and South Asia like Korea, Thailand and Japan, there was an statistically significant difference in allele frequency of CYP2C19*17 with European and Middle East countries (p< 0.05) (1,24,25).

Discussion

CYP2C19 is an important factor in the pharmacokinetic of CYP2C19 substrates like clopidogrel, imipramine, escitalopram and proton pump inhibitors (PPIs) (12,13,15,20, 28). CYP2C19*17 variant
that was identified by Sim et al. and reported to be associated with higher metabolic activity of CYP2C19. This variant allele has a different genetic distribution in various ethnic groups (6). Several studies have shown the effect of CYP2C19*17 polymorphism on treatment of peptic ulcer disease (10,15,20,30). The data in the different studies indicate that CYP2C19*17*17 is associated with 2.1 fold lower omeprazole plasma concentration and therefore the efficacy of PPIs like omeprazole is decreased in CYP2C19*17*17 individuals (10,15,20). Musumba et al. investigated the effect of CYP2C19*17 allele on occurrence of peptic ulcer disease (PUD) and showed increased prevalence of PUD in patient with CYP2C19*17*17 genotype in comparison to CYP2C19*1*1 genotype (30). CYP2C19*17 allele also leads to lower risk of breast cancer by increasing estrogen metabolism (31).

The distribution of CYP2C19*17 polymorphism has not been well estimated worldwide. The main goal of present study was to investigate allele and genotype frequencies of CYP2C19*17 in Iranian population. In this study the allele frequency of CYP2C19*17, *2 and *1 were 21.6%, 13.1% and 65.3% respectively which is in agreement with previous study in our lab for CYP2C19*2 and *1 (32). In our previ-
ous study subjects that were genotyped as CYP2C19*1*17 and CYP2C19*17*17 in this study were identified as CYP2C19*1*17 and CYP2C19*17*17 (28.8% and 5.5%) is deduced from CYP2C19*1*17 genotype frequency in previous report (75%), the similar frequency for CYP2C19*1*1 (40.55%) is attained. Accordingly, people with CYP2C19*2*17 in this study were defined as CYP2C19*1*2 in previous report, so, if we recalculate the CYP2C19*1*2 genotype frequency by deduction of the CYP2C19*2*17 frequency in this study (3.3%) form CYP2C19*1*2 genotype frequency in the previous study (22%), the frequency of 18.7% for CYP2C19*1*2 is obtained which is similar to the result of this study (18.3%).

Based on the results of this study, there is not a significant difference in distribution of CYP2C19*17 variant allele in Iranian population compared with European and other Middle East countries. In contrast, east and south Asian people have significantly lower frequency of this polymorphism than Iranians.

CYP2C19*2 allele reduces enzyme activity and CYP2C19*17 allele increases enzyme activity (6). In this study, subjects with CYP2C19*2*17 were categorized as IM. Few studies have reported effect of CYP2C19*2*17 on metabolic capacity of this enzyme (2,13,21,26). In the study by Gurbel et al. (2) the CYP2C19 activity in stented patients treated with clopidogrel was similar in both CYP2C19*2*17 and CYP2C19*1*2 genotypes. Therefore, it was concluded that in patients carrying both *2 and *17 alleles the reduction of enzyme activity by *2 allele is more predominant than induction of enzyme activity by *17 allele, consequently, in this study CYP2C19*2*17 genotype was classified as IM.

Finally, although genotype-based phenotype prediction is not the best and complete way for estimation of protein activity because other factors like sex, age and associated disease, combination therapy and environmental factors may influence this relationship, the high frequency of CYP2C19*17 in Iranian population makes this new variant allele suitable candidate for association studies and help us to reveal clinical consequences of this genetic polymorphism in carrier individuals.

Conclusion
The allele frequency of CYP2C19*17 in Iranian population is 21.6% and is similar to Middle East or European countries. The high frequency of CYP2C19*17 allele in Iranian population highlights the importance of this new variant allele in metabolism of CYP2C19 substrates. Future association studies are required to reveal clinical consequence of this genetic polymorphism in carrier individuals.

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References


CYP2C19*17 polymorphism in Iranian population