REPORT

CYP2D6*4 null allele frequency in sixteen Pakistani ethnic groups

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Abstract: CYP2D6 belongs to a family of Cytochrome P450 and is involved in metabolism of a number of commonly prescribed drugs. This study was designed to identify *4 allelic frequency of CYP2D6 in Pakistani population. The ethno-geographic variations in the CYP2D6 alleles are responsible for varied expression of this enzyme and thus influence the metabolic rate and efficacy of prescribed drugs. In total, 976 volunteers belonging to 16 different ethnic groups of Pakistan were screened for CYP2D6*4 polymorphism. The *4 allele was detected in all the ethnic groups with varied frequency ranging from 3.73%-13.64% and an overall average of 7.22% in different ethnic groups of the population. Maximum frequency was detected in northern Pakistani population including Meo (13.64%), Punjabi (11.96%) and Pathan (10.42%). Low frequency (<4%) of*4 polymorphism was observed in Kalash and Makrani groups, whereas an intermediate frequency (5-9%) was observed in all the other ethnic groups. The data indicates that despite ethnic diversity poor metabolizers in Pakistani population are expected to carry CYP2D6*4 allele at a relatively higher frequency than most other Asian populations. (Word count = 186)

Keywords: Cytochrome P450, metabolism, polymorphism, genotyping, ethnicity.

INTRODUCTION

Cytochrome P450 system predominantly found in liver is responsible for metabolism of several endogenous and exogenous compounds. Cytochrome 2D6 (CYP2D6) is one of the well-studied member of this family involved in metabolism of more than 25% of the marketed drugs including β-blockers, antidepressants, chemotherapeutics, neuroleptics and opioids (Kirchheiner et al., 2001; Wilkinson, 2005). Metabolic activity of this enzyme greatly varies in different individuals and are classified accordingly as ultra-rapid metabolizer (UM), extensive metabolizer (EM), intermediate metabolizer (IM) and poor metabolizer (PM) (Sachse et al., 1997). This variability of metabolic rate may be due to allelic variations in the CYP2D6 gene. So far, more than 70 alleles of the CYP2D6 gene have been reported, indicating that this gene is highly polymorphic (Marez et al., 1997). These polymorphisms affect CYP2D6 gene expression and thus pharmacokinetics, metabolism, safety and efficacy of clinically important drugs (Ingelman-Sundberg, 2002). Allele *1 codes for extensive metabolizing enzyme; alleles *10, *17, *36 and *41 results in partial loss of enzymatic activity; alleles *3, *4, *5, *6, *7, *8, *11, *12, *13 and *14 have complete loss of enzymatic activity and therefore are called as null alleles (Marez et al., 1997).

A number of studies have shown ethnic and racial differences in drug metabolism because the frequencies of alleles vary from population to population. High frequency of CYP2D6*4 (complete loss of enzymatic activity/poor metabolizer allele) was observed in Asians, Black Africans and African American and are more frequent in Whites of European origin. Whereas, CYP2D6*10 allele (partial loss of enzymatic activity) was highly prevalent in Asians and CYP2D6*17 (partial loss of enzymatic activity) was more in African population (Bradford, 2002). Caucasians and Asians represents (5-10 %; 1 %) of CYP2D6*4 allele (He et al., 2016). Extremes in CYP2D6 gene expression are associated with important therapeutic consequences including predisposing patients to severe adverse drug effects, drug toxicity, diminished therapeutic response and even death (de Leon et al., 2003; Rau et al., 2004). For these reasons there is significant interest in genetic variations that affect CYP2D6 gene expression and function, racial differences in alleles and methods to identify alleles both for commercial and clinical genetic testing (Gaedigk et al., 1999; Gaedigk et al., 2002; Fuselli et al., 2004).

Pakistan is rich with diverse ethnicities as people from Middle, Mediterranean and Central Asia have settled after invasion during various eras. The country consists of more than 18 ethnic groups confined to different areas of the country. The populations residing in the northern region of the country include Balti, Burusho, Hazara, Kalash, Kashmiri, Meo, Pathan and Punjabi, whereas ethnic groups residing in the south includes Balochi, Brahui, Makrani, Mohanah, Parsi and Sindhi. This diversity demands to study the distribution of CYP2D6 polymorphisms in individual groups since this have not
been studied yet. Therefore, present study was designed to determine the frequency of CYP2D6*4 null allele in Pakistani ethnic groups that can be used as a predictor of poor drug metabolism in carriers.

MATERIALS AND METHODS

Study population
Nine hundred and seventy-six unrelated healthy individuals from 16 different ethnic groups of Pakistan were enrolled in the study. The ethnic groups included were Balti, Burusho, Hazara, Kalash, Kashmiri, Meo, Pathan and Punjabi from the northern part of the country and Balochi, Brahu, Makrani, Mohanna, Parsi and Sindhi from southern region of the country. In addition to these, Syed were also included in the study as a separate group, they claim Arab ancestry and some of the Somali samples were also included as they reside in the northern parts of the country as an outgroup. Written informed consent was obtained from all the participants. The study was conducted following ethical guidelines of the Helsinki-II Declaration, and formal approvals from the Ethical Review Committee of the Institute of Biomedical and Genetic Engineering, Islamabad was obtained.

DNA extraction and target gene amplification
Genomic DNA was isolated from 5ml of venous blood samples using standard phenol/chloroform method (Sambrook et al., 1989). Primers and cyclic conditions used for the amplification of CYP2D6*4 genotyping was performed according to Hersberge et al., (2000) with slight modifications (Hersberger et al., 2000). PCR consisted of two rounds of amplifications: first round amplification with 1-New (5’TCCAGCTGGGAA TCCGTTGCTC3’) and 2-New (5’GGAGCTCGCCTG CAGAGACTCCT3’) primer pairs, followed by second round of PCR using CYP 7 (5’CGAAAGGGGCGTCC3’) and B-Mut (5’TCTCACCACCCCAA3’) primer pairs. Alleles were assigned on the basis of presence or absence of allele specific band of 560 bp (wild type) and 217 bp CYP2D6*4 (mutant type) resolved on 2% (w/v) agarose gels. Individual samples with only 560 bp fragments were considered homozygous, with both 560 and 217 fragments were considered heterozygous whereas, the ones with only 217 bp fragments were considered homozygous for the defective null allele.

RESULTS

Non-functional *4 allele of CYP2D6 was genotyped in the Pakistani population of different ethnic background. Predominant localization of these ethnic groups is shown in the map (fig. 1).

Frequency (%) of *4 allele in Pakistani population
Among ethnic groups, highest frequency of *4 allele was found in Meo population (13.64%), followed by Punjabi (11.96), Pathan (10.42%), Kashmiri (8.7%), Balochi (8.51%), Sindhi (7.69%), Syed (6.94%), Parsi (6.88%), Mohanna (6.52%) and Hazara (6.25%). On the contrary, lowest allele frequency of *4 was present in Kalash (3.73%) followed by Makrani (3.92%), Somali (4.0%), Balti (5.26), Brahu (5.62%) and Burusho (5.81%) respectively (table 1). Regarding PM genotype (*4/*4), prevalence is maximum in case of Sindhi (4.49%) however, Pathan shows minimum frequency (0.83%). Chi-square distribution showed that all the ethnic groups studied were in Hardy-Weinberg equilibrium for the genotype distribution except Kashmir, Mohanna and Sindhi. These three populations showed higher frequency of the null allele in homozygous state.

STATISTICAL ANALYSIS

The % age frequencies were calculated using MS Excel software and Vassar Statistical Program was used to calculate P values. Hardy-Weinberg equilibrium was calculated to check the genotype distribution.

DISCUSSION

It is well known that most drugs currently used in clinical practice are effective in only 25% to 60% of patients (Wijnen et al., 2007). Genetic and environmental factors explain only a part of the inter-individual variability whereas other factors that contribute to this variability are largely unknown. Previous studies have shown that there is a marked ethno-geographic variance among populations in cytochrome P450 drug metabolizing enzyme system throughout the world. Genetic basis for this variation is not well known but there are known differences in allelic representation for various P450 genes among different world populations. It is also clear from a number of studies that there is marked diversity in the distribution of CYP2D6 of this system where the allele frequencies and representation of alleles is known to have multi-continent segregation (Cai et al., 2007). Null alleles *3, *4, *5 and *6 play a major role in all populations studied to date. For example, about 8-15 % whites of European descent are PM with low CYP2D6 frequency whereas these four alleles account for 93-97% of the PM phenotypes. The allele frequency of CYP2D6*4 in whites is up to 23.0%. Asians represents wider range of allelic frequency distribution from 4-24% (Mizutani, 2003) than the European descent or the Africans about 1% (Bradford, 2002). Results of present study shows that frequency of *4 varies considerably among all the ethnic groups studied starting from lowest percentage in Kalash (3.74%) to the highest in Meo (13.64%). We can further classify ethnic groups into three categories with low, intermediate and high frequencies. Kalash and Makrani showing low frequencies (<4%); Balti, Burusho, Hazara, Kashmiri, Baluchi, Brahu, Mohanna, Parsi, Sindhi and Syed with
intermediate (5-9%) whereas, Meo, Pathan and Punjabi showing higher frequencies (>10%). Analyzing by the Northern and Southern population the high frequency populations belong to the Northern region but the low and intermediate frequency population exhibit a mixed picture of ethnic group distribution. Lower and higher frequencies do not show a Northern or Southern divide.

Fig. 1: Location of different ethnic groups of Pakistan. The radius of circles represents the percentage of total alleles included in study population whereas the pie area shows *4 allelic frequency.

Table 1: CYP2D6*4 allele and genotype frequencies in different ethnic groups of Pakistani population.

<table>
<thead>
<tr>
<th>No</th>
<th>Population</th>
<th>Total alleles</th>
<th>Allele *4(%)</th>
<th>Genotype frequencies</th>
<th>HW equi</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>wild/wild</td>
<td>wild/*4</td>
<td>*4/*4</td>
</tr>
<tr>
<td>1</td>
<td>Balti</td>
<td>38</td>
<td>5.26</td>
<td>17 (89.47%)</td>
<td>2 (10.53%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>2</td>
<td>Burusho</td>
<td>172</td>
<td>5.81</td>
<td>76 (88.37%)</td>
<td>10 (11.63%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>3</td>
<td>Hazara</td>
<td>80</td>
<td>6.25</td>
<td>35 (87.50%)</td>
<td>5 (12.50%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>4</td>
<td>Kalash</td>
<td>134</td>
<td>3.73</td>
<td>62 (92.54%)</td>
<td>5 (7.46%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>5</td>
<td>Kashmiri</td>
<td>46</td>
<td>8.70</td>
<td>20 (86.96%)</td>
<td>2 (8.70%)</td>
<td>1 (4.35)</td>
</tr>
<tr>
<td>6</td>
<td>Meo</td>
<td>44</td>
<td>13.64</td>
<td>16 (72.73%)</td>
<td>6 (27.27%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>7</td>
<td>Pathan</td>
<td>240</td>
<td>10.42</td>
<td>96 (80.00%)</td>
<td>23 (19.17%)</td>
<td>1 (0.83%)</td>
</tr>
<tr>
<td>8</td>
<td>Punjabi</td>
<td>92</td>
<td>11.96</td>
<td>36 (78.26%)</td>
<td>9 (19.57%)</td>
<td>1 (2.17%)</td>
</tr>
<tr>
<td>9</td>
<td>Balochi</td>
<td>94</td>
<td>8.51</td>
<td>40 (85.11%)</td>
<td>6 (12.77%)</td>
<td>1 (2.13%)</td>
</tr>
<tr>
<td>10</td>
<td>Brauhi</td>
<td>178</td>
<td>5.62</td>
<td>80 (89.89%)</td>
<td>8 (8.99%)</td>
<td>1 (1.12%)</td>
</tr>
<tr>
<td>11</td>
<td>Makrani</td>
<td>102</td>
<td>3.92</td>
<td>47 (92.16%)</td>
<td>4 (7.84%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>12</td>
<td>Mohanna</td>
<td>138</td>
<td>6.52</td>
<td>63 (91.30%)</td>
<td>3 (4.35%)</td>
<td>3 (4.35%)</td>
</tr>
<tr>
<td>13</td>
<td>Parsi</td>
<td>160</td>
<td>6.88</td>
<td>70 (87.50%)</td>
<td>9 (11.25%)</td>
<td>1 (1.25%)</td>
</tr>
<tr>
<td>14</td>
<td>Sindhi</td>
<td>312</td>
<td>7.69</td>
<td>139 (89.10%)</td>
<td>10 (6.41%)</td>
<td>7 (4.49%)</td>
</tr>
<tr>
<td>15</td>
<td>Syed</td>
<td>72</td>
<td>6.94</td>
<td>31 (86.11%)</td>
<td>5 (13.89%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>16</td>
<td>Somali</td>
<td>50</td>
<td>4.00</td>
<td>23 (80.00%)</td>
<td>2 (8.00%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1952</td>
<td>7.22</td>
<td>851 (87.17%)</td>
<td>109 (1.64%)</td>
<td>16 (1.64%)</td>
</tr>
</tbody>
</table>

*No. 1 - 8 Northern population; No. 9 - 14 Southern population
Based on various types of molecular marker analysis, Pakistani ethnic groups show close evolutionary association with the populations of European origin but there is a wide difference in *4 allele frequency between European and any of the Pakistani ethnic group studied. This shows that allele frequency for this locus is not evolutionarily related and may have some other local selection pressures but it does reflect marked inter-ethnic variation for the allelic distribution among various groups studied. The allele frequencies for the higher frequency group i.e., Punjabi, Meo and Pathan is similar to Iranian (12.5%), North Indian (11.5%) and Mexican (10%) populations (Mendoza et al., 2001; Kouhi et al., 2009). The intermediate group is similar to Central/South Indian, Srilankan Tamils and Moor populations (Naveen et al., 2006; Tharanga et al., 2013). Low frequency group is similar to Saudi, African and some Asian populations including sub-Saharan Africans and Hans (McLellan et al., 1997; Sistonen et al., 2007). Genotype distributions for all the ethnic groups were in Hardy-Weinberg equilibrium except three populations (Kashmiri, Mohanna and Sindhi) probably due to some selection pressure for this allele.

CONCLUSION

Our study elucidates that there is a significant difference in the prevalence of CYP2D6*4 alleles in different Pakistani ethnic groups. To sum up, CYP2D6*4 allele frequency in Pakistan is higher compared to African and some other Asian populations but lower than the European population. Population frequency shown in this study will be helpful to recommend CYP2D6*4 typing as a pre-treatment test to monitor drug dosage (personalized medicine) to avoid adverse drug reactions as a personalized medicine approach. The study results might allow us in future to detect genetic variations in drug-metabolizing enzymes which are useful for clinicians to suggest right dosage and efficacy of drugs metabolized by this polymorphic enzyme to avoid adverse drugs reactions. However for some ethnic groups the sample number is small as in the case of Balti’s, Meo’s and Kashmiri’s. For these ethnic groups further studies would be needed before coming to some conclusion.

ACKNOWLEDGEMENT

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REFERENCES


