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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, Mohannah, Parsi and Sindhi. This diversity demands to study the distribution of CYP2D6 polymorphisms in individual groups since this have not

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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, Brahui, 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'TCCCAGCTGGAA TCCGGTGTCG3') and 2-New (5'GGAGCTCGCCCTG CAGAGACTCCT3') primer pairs, followed by second round of PCR using CYP 7 (5'CGAAAGGGGCGTCC3') and B-Mut (5'TCTCCCACCCCCAA3') 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), Brahui (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 Kashmiri, 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 Sstatistical 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, Brahui, 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.

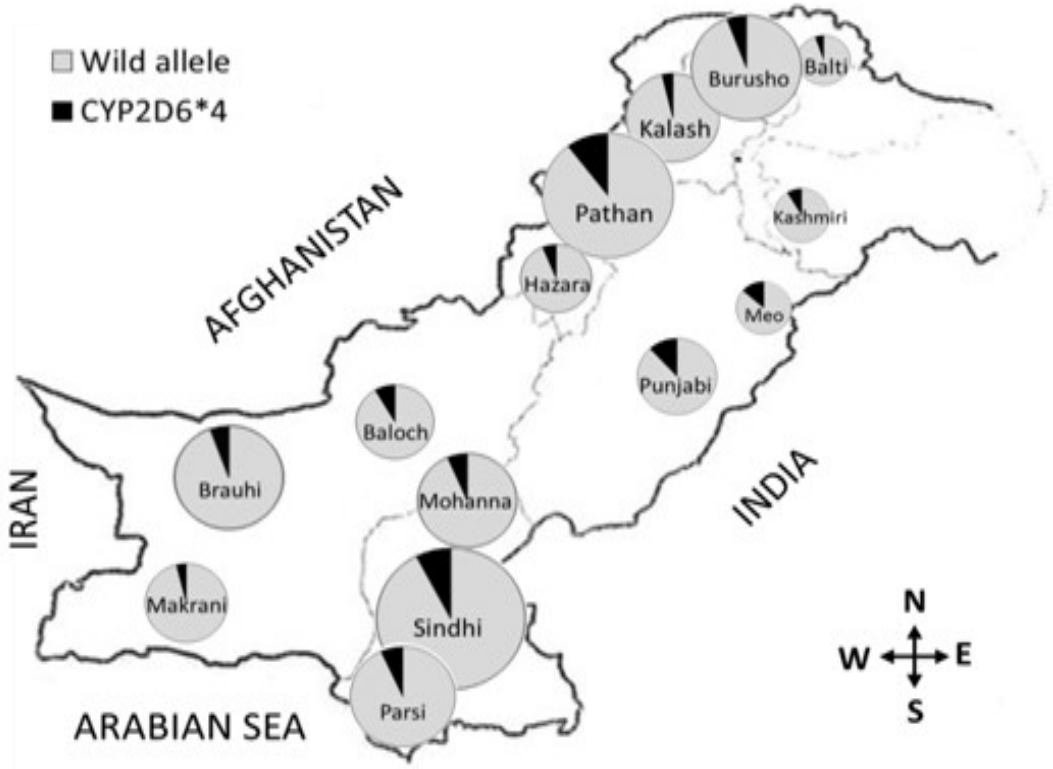

Fig. 1: Location of different ethnic groups of Pakistan. The radius of circles represents the percentage of total alleles included in study population whereas the pie area shows *4 allelic frequency.

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

No	Population	Total alleles	Allele *4(%)	Genotype frequencies			HW equi
				wild/wild	wild/*4	*4/*4	P value
1	Balti	38	5.26	17 (89.47%)	2 (10.53%)	0 (0%)	0.8
2	Burusho	172	5.81	76 (88.37%)	10 (11.63%)	0 (0%)	0.56
3	Hazara	80	6.25	35 (87.50%)	5 (12.50%)	0 (0%)	0.67
4	Kalash	134	3.73	62 (92.54%)	5 (7.46%)	0 (0%)	0.75
5	Kashmiri	46	8.70	20(86.96%)	2 (8.70%)	1 (4.35)	0.03
6	Meo	44	13.64	16 (72.73%)	6 (27.27%)	0 (0%)	0.45
7	Pathan	240	10.42	96 (80.00%)	23(19.17%)	1 (0.83%)	0.76
8	Punjabi	92	11.96	36 (78.26%)	9 (19.57%)	1 (2.17%)	0.63
9	Balochi	94	8.51	40 (85.11%)	6(12.77%)	1 (2.13%)	0.21
10	Brauhi	178	5.62	80 (89.89%)	8 (8.99%)	1 (1.12%)	0.15
11	Makrani	102	3.92	47 (92.16%)	4 (7.84%)	0 (0%)	0.77
12	Mohanna	138	6.52	63 (91.30%)	3 (4.35%)	3 (4.35%)	0
13	Parsi	160	6.88	70 (87.50%)	9 (11.25%)	1 (1.25%)	0.22
14	Sindhi	312	7.69	139 (89.10%)	10 (6.41%)	7 (4.49%)	0
15	Syed	72	6.94	31 (86.11%)	5 (13.89%)	0 (0%)	0.6
16	Somali	50	4.00	23 (8.00%)	2(8.00%)	0 (0%)	0.835
	Total	1952	7.22	851 (87.17%)	109 (1.64%)	16 (1.64%)	

*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.

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REFERENCES

Bradford LD (2002). CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics*., **3**(2): 229-243.

Cai WM, Chen B and Zhang WX (2007). Frequency of CYP2D6*10 and *14 alleles and their influence on the metabolic activity of CYP2D6 in a healthy Chinese population. *Clin. Pharmacol. Ther*., **81**(1): 95-8.

De Leon J, Dinsmore L and Wedlund P (2003). Adverse drug reactions to oxycodone and hydrocodone in CYP2D6 ultrarapid metabolizers. *J. Clin. Psychopharmacol*., **23**(4): 420-421.

Fuselli S, Dupanloup I, Frigato E, Cruciani F, Scozzari R, Moral P, Sistonen J, Sajantila A and Barbujani G (2004). Molecular diversity at the CYP2D6 locus in the Mediterranean region. *Eur. J. Hum. Genet*., **12**(11): 916-924.

Gaedigk A, Gotschall RR, Forbes NS, Simon SD, Kearns GL and Leeder JS (1999). Optimization of cytochrome P4502D6 (CYP2D6) phenotype assignment using a genotyping algorithm based on allele frequency data. *Pharmacogenetics*., **9**(6): 669-82.

Gaedigk A, Bradford LD, Marcucci KA and Leeder JS (2002). Unique CYP2D6 activity distribution and genotype-phenotype discordance in black Americans. *Clin. Pharmacol. Ther*., **72**(1): 76-89.

He X, He Na, Ren L, Ouyang Y, Zhang N, Ma Y, Yuan D, Kang L and Jin T (2016). Genetic polymorphisms analysis of CYP2D6 in the Uygur population. *BMC Genom*., **17**: 409.

Hersberger M, Marti-Jaun J, Rentsch K and Hanseler E (2000). Rapid detection of the CYP2D6*3, CYP2D6*4 and CYP2D6*6 alleles by tetra-primer PCR and of the CYP2D6*5 allele by multiplex long PCR. *Clin. Chem*., **46**(8): 1072-7.

Ingelman-Sundberg M (2002). Polymorphism of cytochrome P450 and xenobiotic toxicity. *Toxicology*., **181-182**: 447-452.

Kirchheiner J, Brosen K, Dahl ML, Gram LF, Kasper S, Roots I, Sjoqvist F, Spina E and Brockmoller J (2001). CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: A first step towards subpopulation-specific dosages. *Acta. Psychiatr. Scand*., **104**(3): 173-192.

Kouhi H, Hamzeiy H, Barar J, Asadi M and Omidi Y (2009). Frequency of five important CYP2D6 alleles within an Iranian population (Eastern Azerbaijan). *Genet. Test. Mol. Biomarkers*., **13**(5): 665-670.

Marez D, Legrand M, Sabbagh N, Lo Guidice JM, Spire C, Lafitte JJ, Meyer UA and Broly F (1997). Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: Characterization of 48 mutations and 53 alleles, their frequencies and evolution. *Pharmacogenetics*., **7**(3): 193-202.

McLellan RA, Oscarson M, Seidegard J, Evans DA and Ingelman-Sundberg M (1997). Frequent occurrence of CYP2D6 gene duplication in Saudi Arabians. *Pharmacogenetics*., **7**(3): 187-91.

Mendoza R, Wan YJ, Poland RE, Smith M, Zheng Y, Berman N and Lin KM (2001). CYP2D6 polymorphism in a Mexican American population. *Clin. Pharmacol. Ther*., **70**(6): 552-60.

Mizutani T (2003). PM frequencies of major CYPs in Asians and caucasians. *Drug. Metab. Rev*., **35**: 99-106.

Naveen AT, Adithan C, Soya SS, Nathalie G and Krishnamoorthy R (2006). CYP2D6 genetic polymorphism in South Indian populations. *Biol. Pharm. Bull*., **29**(8): 1655-1658.

Rau T, Wohlleben G, Wuttke H, Thuerauf N, Lunkenheimer J, Lanczik M and Eschenhagen T (2004). CYP2D6 genotype: Impact on adverse effects and nonresponse during treatment with antidepressants - A pilot study. *Clin. Pharmacol. Ther*., **75**(5): 386-393.

Sachse C, Brockmoller J, Bauer S and Roots I (1997). Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am. J. Hum. Genet*., **60**(2): 284-295.

Sambrook J, Fritsch EF and Maniatis T (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor laboratory press. New York, pp.931-957.

Sistonen J, Sajantila A, Lao O, Corander J, Barbujani G, Fuselli S (2007). CYP2D6 worldwide genetic variation shows high frequency of altered activity variants and no continental structure. *Pharmacogenet Genomics*., **17**(2): 93-101.

Tharanga TDP, Jinadasa CMV, Risama MF, Galappatthy P, Jayakody RL, Dissanayake VHW (2013). Genetic variants in the cytochrome P450 2D6 gene in the Sri Lankan population. *Indian J. Hum. Genet*., **19**(4): 392-396.

Wijnen PAHM, Op den Buijsch RA, Drent M, Kuijpers PM, Neef C, Bast A, Bekers O and Koek GH (2007). Review article: The prevalence and clinical relevance of cytochrome P450 polymorphisms. *Aliment. Pharmacol. Ther*., **26**(2): 211-219.

Wilkinson GR (2005). Drug metabolism and variability among patients in drug response. *N. Engl. J. Med*., **352**(21): 2211-2221.