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Brief communication on allele frequency of CYP3A4*22 in Indian population

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***Abstract*---**Cytochrome p450 3A4 polymorphism at CYP3A4*22 has become clinically significant due to its role in metabolizing various drugs. Therefore, in this study we established the frequency of CYP3A4*22 variant in Indian population. The allele frequency distribution was determined in 350 healthy subjects of Indian origin by Real time polymerase chain reaction method. We found that 97% of subjects has AA variant and 3% subjects has AG variant of CYP3A4*22. We did not find any homozygous minor allele AA variant. However, studies conducted in other population also did not show any homologous minor allele with exception to Belgium. The p-value of Hardy Weinberg equilibrium is observed as 0.9, which was calculated using the chi-square model. This study shows significant difference in the distribution of allelic variants of CYP3A4*22 among various ethnicities. Therefore, the study results may provide an insight in genotype distribution of the CYP3A4*22 in Indian population for further studies.

***Keywords*---**CYP3A4*22, allele distribution, Cyp allele

1. Cytochrome p450 3A4, commonly termed as CYP3A4, metabolizes around 30-50% of drugs belonging to various therapeutic classes[1]. It occupies about 20% of CYP enzymes in the liver and plays a significant role in the oral first pass effect[2]. A study by Rodríguez-Antona C et al shows that there is a 10-100 fold variation in the expression of this enzyme in liver samples among different individuals[3,4]. However, genetic contributors, which are usually suspected of this high variability of expression, remain uncertain and need to be elucidated.

Meta-analysis study by Guttman Y et al. identified 856 mutations in CYP3A4 and showed that one third of these mutations are capable of modifying the protein structure[5]. However, one of the important revelations of CYP3A4 variants by Wang D et al. showed that intronic polymorphism at rs35599367 (CYP3A4*22) reduces the length of functional mRNA during splicing[6]. This causes a reduction in the CYP3A4 enzyme activity, which was confirmed in other studies conducted in-vivo with midazolam and erythromycin[7,8]. The genetic polymorphism of CYP3A4*22 also found to increase the plasma concentrations of simvastatin in a clinical trial conducted in 555 whites and 275 African-Americans[9]. Although, the frequency distribution of this enzyme is studied in Europeans, ad mixed Americans and other Asians[10,11], the data on the allelic frequency of this gene is not available in the Indian population. Therefore, for the first time, the study was carried out to determine the distribution of CYP3A4*22 allele in the Indian population.

350 healthy subjects were volunteered in this study. The subjects of Indian origin and unrelated to each other are only included in the study. The subjects with chronic diseases like hypertension, diabetes mellitus, liver disorders, etc., were excluded. The study was conducted after obtaining the ethical approvals from the ICMR-National Institute of Nutrition and Osmania general hospital. Written informed consent was obtained from the subjects after explaining the purpose of the study. The subject details were taken in a pretested questionnaire. Subject details include demographic details, medical history, personal habits like smoking, drinking, etc. The fasting blood sample was collected following safety precautions. The biochemical parameters, which include liver function tests, renal function tests, and lipid levels, were analyzed in serum using Cobas c311 system. DNA isolation from whole blood was done by using a DNA purification kit. The allelic discrimination of CYP3A4*22 single nucleotide polymorphism was done using the TaqMan probes, which are predesigned and obtained from Applied Biosystems by the real-time polymerase chain reaction method.

Data compilation and analysis was done by SPSS Version 19.0. The categorical variables were assessed using the chi-square test, and the t-test was used to analyze the biological parameters. The difference in variables is considered significant, provided the p-value is >0.05. Among 350 subjects, only 11 were heterogenic, and 339 subjects had wild-type homozygous genotypes. However, we did not find any sample with a homozygous minor allele genotype. (Table 1) The minor allelic frequency was estimated to be 0.02% in the samples studied. The p-value of Hardy Weinberg equilibrium is observed as 0.7, which was calculated using the chi-square model.

We noted that there is a significant change in the distribution of allelic variants among different ethnicities. The minor allele frequency in Indian population is significantly lower as compared to American and Caucasian population[7]. The allelic distribution is also varied among the Asian countries. Studies conducted by Okubo M et al.[10] in 53 Japanese subjects and Shi et al.[11] in 216 Chinese subjects did not find minor allele in their subjects, whereas, 11 subjects are found to be heterozygous in Indian population. However, the distribution of minor allele is still low in Asian countries as compared to Americans and Caucasians and homozygous minor allele was found only in Belgian population[8].

Although the significant role of change of CYP3A4*22 in altering the enzyme activity was established in many studies, the minor allele frequency is low to make an impact in Indian population. However, it may impair the metabolism in individuals carrying the homologous minor allele. The significant difference in the allele frequency of CYP3A4*22 in various ethnic groups may help in understanding the difference in drug metabolism in various ethnic groups. Furthermore, this study may give a future insight for the evaluation of the genetic association studies.

Table 1: Frequency of CYP3A4*22 gene allele variants

SNP Type	Genotype	N (%)	MAF	P_{HWE}
CYP3A4 (rs35599367)	AA	339(97%)	0.02	0.9
	AG	11(3%)		
	GG	-		

MAF- minor allele frequency
PHWE- P value of hardy weinberg equilibrium

Table 2: Ethnic differences in CYP3A4*22 gene allele frequencies

Ethnicity	N	Genotypes			MAF
		AA	AG	GG	
Indians (current study)	350	339	11	-	0.02
Americans	555	513	42	-	0.08
African-americans	275	267	8	-	0.02
Caucasian	41	36	5	-	0.08
Japanese	53	53	-	-	0.00
Chinese	216	216	-	-	0.00
Belgians	185	173	11	1	0.04

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3. References

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