

INTERVENTION OF PHARMACOGENETIC PROFILING IN THERAPEUTICS OF KASHMIRI POPULATION, A LESSON LEARNED FROM CYP3A5*3 VARIANT

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ABSTRACT

Human Cytochrome P₄₅₀ 3A, a variant of Cytochrome P-450-dependent monooxygenases is a major drug metabolizing enzyme. CYP3A has evolved to catalyze the biotransformation of its substrate. A great deal of inter-individual variation in expression levels exists for CYP3A5 gene. The common polymorphism in CYP3A5 that induces a cryptic splice site has been shown to cause a significant reduction in its expression. Variation in CYP3A enzyme activity contributes greatly to variation in systemic clearance of CYP3A substrates and its oral bioavailability. The present study was designed to study the CYP3A5*3 polymorphism in the Kashmiri population. CYP3A5*3 genotypic status of 400 unrelated individuals of the Kashmiri population was evaluated by PCR-RFLP method. The frequency of CYP3A5 variant in Kashmiri population was found similar to Caucasian but higher than that in Chinese and Japanese population. Our findings suggest that approximately 89.5% of study population carry CYP3A5*3 genotype and thus might be at an increased risk. So, detection of CYP3A5 variant alleles and knowledge about their allelic frequency in ethnic Kashmiris might play a key player in improved therapeutic intervention in Kashmiri population.

Keywords: Cytochrome P₄₅₀, CYP3A5*3, Cyclosporina, PCR-RFLP

1. INTRODUCTION

Carcinogenesis is a multi factorial disease with genetic aberrations being at the heart of this dreadful disease. Chemical carcinogenesis has been earmarked as one of the main contributors to cause genetic instability. Most of the toxic substances are essentially procarcinogens and therefore require metabolic conversion to form highly reactive carcinogens. In humans, xenobiotic metabolizing enzymes which comprises of phase I and II enzymes, play a central

role in the detoxification and eventually in systemic elimination of various toxic substances from the body. Human CYP3A super family of the phase I enzymes is chiefly responsible for the elimination of various endogenous as well as exogenous compounds (Eichelbaum *et al.*, 2006). CYP3A subfamily of CytochromeP₄₅₀ consists of CYP3A4, CYP3A5, CYP3A7 and CYP3A43 are chiefly expressed in human liver and intestines. Among these, CYP3A4 and CYP3A5 are active forms of CYP3A in human liver and intestine (Gonzalez *et al.*, 1988; Aoyama *et al.*,

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1989; Komori *et al.*, 1989; Domanski *et al.*, 2001). Several studies have intimated that 30-85% of the inter-individual difference have in constancy in CYP3A prevalence due to genetic factors, such as Single Nucleotide Polymorphisms (SNPs) (Thummel and Wilkinson, 1998; Evans and Relling, 1999; Ozdemir *et al.*, 2000). CYP regulated drug metabolism is prone to genetic in constancy that can ensue in enzyme's normal, low or no activity. The result of differences in CYP3A activity may be a clinical significance with respect to either efficacy of drug or biotransformation of drug-drug interactions as well as efficacious toxic substrates.

CYP3A5*3 (6986A → G) mutant allele is the prime defective allele among known alleles so far investigated in the 3rd intone of CYP3A5 (Kuehl *et al.*, 2001). Results have also revealed that the mutant allele lead to production of variant mRNA by alternative splicing which translates in the synthesis of truncated protein, resulting in the absence of CYP3A5 from some individuals. CYP3A5 may appear to contribute up to 50% of total hepatic CYP3Aprotein in individual carrying at least one allele of CYP3A5*1.

Thus, CYP3A5*3 mutant allele may play a prime role in inter-individual and inter-ethnic differences in the metabolic profile of many drugs used in therapeutics (Shin and Huang, 2002; King *et al.*, 2003). Because evaluating the frequency of CYP3A5*3 allele in a large scale Kashmiri population is likely to be instrumental in pharmacogeneticsresearch. Therefore, the present study was designed to evaluate the allelic frequency of CYP3A5*3 among a representative 400 Kashmiri individuals.

2. MATERIALS AND METHODS

2.1. Subject Recruitment

This hospital based cross sectional study was conducted over a period of fourteen months starting from May, 2012 to September, 2013, following approval by the ethical committee of Government Medical College (GMC), Srinagar, India. Informed consent was obtained from all the subjects.

Briefly, the patients recruited in the study were those who didn't have any previous cancer history and visited the hospital for some minor disease like hernia surgery, intestinal infection, skin burn, gastritis. The volunteer group consisting of 400 individuals (207 male and 193 female), aged between 35-60 years were

recruited from the Department of Medicine, GMC Srinagar.

2.2. Genomic DNA Isolation

Five ml of venous blood was collected from each subject in a sterile EDTA coated vials and were subsequently stored at -80°C for future use. Genomic DNA was isolated from the blood samples following Phenol-Chloroform method (Blin and Stafford, 1976). Integrity of the isolated genomic DNA was checked by running 5 µL of the sample on 1% agarose gel.

2.3. Genotyping of CYP 3A5 *3

CYP3A5*3 polymorphism in intron 3rd was analyzed through PCR-RFLP (restriction fragment length polymorphism) method.

PCR reactions contained 100 ng of DNA, a 0.25 mM dNTPs (Sigma-Aldrich), 1× PCR buffer, 2.5 units of Taq polymerase (Ferment's) and 15 pmol of forward 5/-CATGACTTAGTAGACAGATGAC-3/and reverse 5/-GGTCCAAACAGGGAAGAAATA-3/Primers in a total volume of 25 µL. PCR cycling conditions included a 5-min initial denaturation step at 95°C followed by 35 cycles of the following: 50 s at 95°C, 50 s at an appropriate annealing temperature, 50 s at 72°C and a final extension step at 72°C for 5 min. Quality of amp icons was assessed by running it in 2% agarose gel.

The PCR products so obtained were subjected to restriction digestion using SspI enzyme (Ferment as). The digested products of each subject were genotyped by running on 3% agarose gel (**Fig. 1**). CYP3A5*3 wild type allele possess the restriction site and thus produces three fragments of size 148, 125 and 20 bp whereas mutant allele lacks restriction site and on digestion produces two fragments of size 168 and 125 bp.

2.4. Statistical Analysis

All the statistical analyses were performed with Statistical Package for the Social Science (SPSS version 15.0.). Chi square test was put in use for comparison of the allele and genotypic frequency variations between different populations. The divergences from the Hardy-Weinberg equilibrium for allele and genotype frequencies for the various SNPs were assessed by Fisher's exact test. The 95% confidence intervals were enumerated for all observed allele frequencies. A $p < 0.05$ was considered statistically significant.

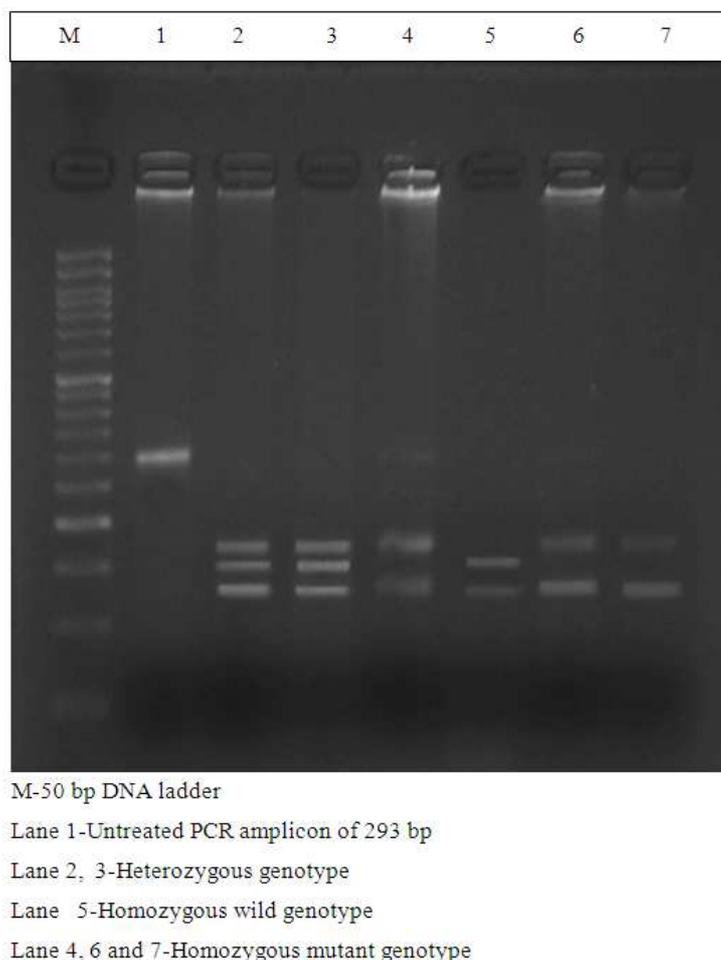


Fig. 1. PCR-Restriction Fragment Length Polymorphism analysis (PCR-RFLP) of CYP3A5*3 polymorphism

3. RESULTS

The distribution of genotypic frequencies of CYP3A5*3 of the present cohort of four hundred volunteer patients is summarized in **Table 1**. The genotypic frequency observed for homozygous wild type (1*/1*) was 3% (12/400). The genotypic frequencies analyzed for heterozygous genotype (1*/3*) and homozygous mutant type (3*/3*) were 7.5% (30/400) and 89.5% (358/400) respectively (**Table 1**). The genotypic distribution of CYP3A5*3 SNP at 3rd intone position is in Hardy-Weinberg equilibrium in the present study.

The frequency of wild allele in the patients under study was 0.067(54/800). The mutant allele frequency was 0.93(746/800). Therefore, the percentage calculated

for wild and mutant allele of CYP3A5*3 in the present patients was 6.75 and 93.25 respectively.

Comparison of the allelic and genotypic frequency of the present cohort of the Kashmiri population with other ethnic populations was done. We observed that the allelic frequency of CYP3A5*3 in the Kashmiri population was found to be 93%, which is similar to Dutch Caucasian (91.7%) $p = 0.80$, $\chi^2 = 0.06$, but significantly higher than in Chinese (77.8 %) $p = 0.02$, $\chi^2 = 5.14$, Japanese population (76.8%) $p = 0.03$ $\chi^2 = 4.55$ and African Americans (47.5%) $p = 0.01$ $\chi^2 = 5.87$. Also, CYP3A5*3 heterozygous and homozygous mutant genotype was found to be similar in Dutch Caucasian, but was higher as compared to the Chinese, Japanese and African American (**Table 2**).

Table 1. Allelic and genotypic Frequency of CYP3A5 variant alleles in the Kashmiri population (n = 400)

| Polymorphism | Effect | Allele | Frequency | Genotypic | Frequency |
|-----------------|--------|--------|-----------|-----------|-----------|
| CYP3A5*3 | 1* | 3* | 1*/1* | 1*/3* | 3*/3* |
| Splicing defect | 0.067 | 0.933 | 3 | 7.5 | 89.5 |

Table 2. Allelic and genotype frequency of the CYP3A5 in Kashmiri population and other ethnic population

| Population | Na | Allelic frequency | | Genotype frequency (%) | | References |
|---------------------|-------|-------------------|-------|------------------------|-------|---------------------------------------|
| | ----- | ----- | ----- | ----- | ----- | |
| | 1* | 3* | 1*/1* | 1*/3* | 3*/3* | |
| Kashmiri population | 800 | 0.067 | 0.900 | 37.5 | 89.5 | in this study |
| Chinese | 604 | 0.222 | 0.778 | 7.3 | 29.8 | 62.9 (Yong-Fang <i>et al.</i> , 2005) |
| Japanese | 400 | 0.233 | 0.768 | 7.0 | 32.5 | 60.5 (Kuehl <i>et al.</i> , 2001) |
| Dutch Caucasian | 1000 | 0.083 | 0.917 | 0.2 | 16.7 | 83.1 (Komori <i>et al.</i> , 1989) |
| African American | 40 | 0.450 | 0.475 | 25.0 | 25.0 | 35.0 (First <i>et al.</i> , 1996) |

4. DISCUSSION

Despite the marked advances in pharmacokinetics and drug therapy, fractions of patients don't respond favorably or experiences severe adverse drug side effects. Pharmacogenetic studies have revealed the essence of polymorphism in drug metabolizing enzymes, transporters and receptors which contribute to variable drug response as per the individual's genetic makeup (Eichelbaum *et al.*, 2006). CYP3A5 is forms are responsible for metabolizing about 50% of all clinically approved drugs (Arvanitidas *et al.*, 2007). Thus polymorphism of CYP family may have most impact on the fate of these and other therapeutic drugs whose metabolism they regulate. Therefore, the present study was designed to evaluate the genetic status of the CYP3A5 gene in the large ethnic Kashmiri population.

Our results showing high prevalence (89.5%) of the defective CYP3A5*3 allele in Kashmiri population is in agreement with the other studies conducted in India and elsewhere (Bajpai *et al.*, 2010; Fukuen *et al.*, 2002). The present study reveals a marked interethnic variation in CYP3A5 allelic and genotypic frequencies between Kashmiri population and other ethnic populations like African Americans, Dutch Caucasians and Chinese. The CYP3A5*3 variant allele and homozygote are more frequent in Kashmiri population and Dutch Caucasians than in Chinese and is much less common in African Americans (Ozdemir *et al.*, 2000; Van Schaik *et al.*, 2002). Interethnic variations in polymorphism in drug metabolizing enzymes like CYP3A5 may contribute to drug efficiency and its toxicity. For instance, Cyclosporine A (CsA), an immunosuppressive agent widely administrated in the patients who receive organ transplants which inhibits

the activation of naïve T cells is metabolized by CYP3A4 and CYP3A5. It has been reported that the bioavailability of CsA was found significantly lower in African-American patients with renal transplantation than those in Caucasian-American patients, in contrast to dosing requirement of CsA, African-American patients had significantly higher dosing requirement compared with Caucasian and American patients, when the same therapeutic regimen was maintained (First *et al.*, 1996). This may be due to the frequency of the CYP3A5*3 mutant allele is higher in Caucasian-American than African-American. So in the wake of above evidences, our study may represent the basis for the intervention of pharmacogenetic profiling in therapeutic options in Kashmiri population.

5. CONCLUSION

The present study shows that genotypic screening for CYP3A5*3 alleles in the ethnic Kashmiri population is highly relevant. Thus population based screening for CYP3A5*3 mutant allele will help understand the disease risk as well as pharmacokinetics of many drugs.

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