Analysis of the CYP3A4*1B gene variant in an Iranian Population: Pilot base study

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ABSTRACT. Various studies revealed that, CYP3A4*1B, as a variant allele of CYP3A4 gene, has variations in populations with different ethnic background. In this pilot research work, we studied 183 healthy non consanguinity men of an Iranian origin for this genetic variation. We applied RFLP-PCR technique for this research study. In contrast with other researches, in Asian country (such as China and Japan with 0% allele frequency) our study showed that frequency of this allele in Iranian men among an Asian continent is 3%. This finding is an important attempt in pharmacogenetics research endeavor.

1. INTRODUCTION

CYP450 gene super family enzyme products have major role in phase I of drug metabolism [1]. Among them, CYP3A4 is highly regarded and is considered, major CYP3A4 functional isoforms are located in human liver and intestine [2]. Moreover, CYP3A4 enzyme product is responsible for over 50% of all clinically drug products [3]. These drugs are included steroids immunosuppressive agent [4]. Also, CYP3A4 is responsible for the conversion of testosterone to less biologically active forms of this hormone, such as 2β, 6β, and 15β- hydroxy testosterone [5]. Studies have been shown variations of DNA sequences (specially polymorphisms) related to this enzyme, could be affected expression of gene [6],[7] and play a critical role in drug metabolism pathway of individual [8]. Cyp3A4 is located on 7q21. This gene has 27,205 bp in 13 exon and 12 intron [9]. In one study, Rebbeck et al. (1998) have detected, a genetic variant in promoter of this gene, as well that is known as nifedipine response element or CYP3A4*1B, with A/G transition in -285 (upstream of transcription site) is associated with higher clinical stage and Gleason score of pathology slides in men with prostate cancer, likely because of increased testosterone bioavailability [10]. The major role of this variant allele about in vivo has not been understood yet, but is shown, CYP3A4*1B is associated with 2-fold activity of enzyme [11]. This genetic variant is important in pharmacogenetics. Because, CYP3A4*1B has protective role for treatment-related leukemia, where as CYP3A*1A (as a wild type) has been associated with increased metabolism of epipodophyllotoxin as a chemotherapy agent [12], but pharmacokinetic studies have not confirm these [13].

Different studies show allele frequency variation of CYP3A4*1B in population with different racial background. These studies show the frequency of variant allele is higher in black strain (about 60%) than Caucasians (about 4%) and Japanese and Chinese men (0%) [14],[15]. Based on these finding in one review article allele frequency of CYP3A4*1B has been reported 0% in Asian population (China, Japan) so far. Since study of this allele in an Iranian population had not been done till now, we are present and determined allele frequency in our population.
2. MATERIALS AND METHODS

We studied 183 healthy non-consanguinity men. The mean age of the participant individual in this study was 68.31 (range of 50-85) peripheral blood samples were in EDTA K3 tube. Genomic DNA was extracted from peripheral blood with Diatom kit and salting out method. Forward primer: 5'-GGAATGAGGACAGCCATAGAGACAAGGG-3' and reverse Primer: 5'-CCTTTTCAGCTCTGTGTTGCTTTTGCTG-3' (described with Selma A. Cavalli, 2001) [16] were determined and aligned with sequence of gene in Ncbi.

PCR (with total volume 25 µlit) was carried out with components including: 15.75µlit distilled water, 1.5mM of 10X PCR buffer (KBC, Iran) with content 160mM (NH4)2SO4, 670 mM Tris HCL PH 8.8, 0.1% tween, 1.5mmol Mgcl2 (Bioscience, England), 200µmol of dNTPs (Bioscience, England), 8pmol of each primer (Bioneer, South Korea), 2.5IU of Taq polymerase enzyme (KBC, Iran) and 200ngr of genomic DNA. PCR condition was an initial denaturation step for 5min at 95ºC, 30 cycles at 95ºC for 40s as denaturation step, 52.4ºC for 40s as annealing step, and 72ºC for 40s as replication step, followed by a final replication step at 72ºC for 5min. Then, 0.3µgr of PCR products were digested with 1.25IU MboII restriction enzyme (described with Cavalli )[16], (Fermentas, Canada) and 10X reaction buffer (Fermentas, Canada) in 10µlit total volume for 16 hours at 37 ºC. 8µlit of digested products were loaded on to 3% agarose gel. Representative examples for PCR-RFLP of CYP3A4 genotypes are shown in Fig. 1.

We found 9 samples with a 210bp band -belonging to variant allele-. Pay attention to agarose gel electrophoresis restrictions related to separation of 175bp and 169bp fragments, digested samples with variant allele -which were known with 210bp band- were studied by nondenaturing 10% PAGE1 (table 1). Results of PAGE showed 1 sample has been homozygote for variant allele (GG) and other 8 samples with 210bp band has been heterozygote (AG). Results of PAGE are illustrated in Fig. 2.

Statistical Analysis

The results of the investigation were statistically analyzed, by applying chi square, where the statistical differences yielding p<0.05 were considered significant. Data analysis was performed by SPSS (version 11.5, Inc. USA) software.
3. RESULTS AND DISCUSSION

We found in 10% PAGE, one sample with homozygote genotype for variant allele and other 8 samples with heterozygote genotype. Results of statistical analysis are shown in Table 2.
CYP3A4 isoenzymes are responsible for the metabolism of > 50% of drugs such as steroid components, antidepressant, antibiotics, ... [4]. Pharmacogenetic studies show that genetic variations in this gene and other genes of CYP450 superfamily (special polymorphism) can affect metabolism process of drugs[1]. Peer review studies about CYP3A4*1B have shown different frequency of variant allele in population with different ethnic background [17],[18],[19],[15],[14],[20],[15']. We showed 3% allele frequency for variant allele in studied population as one of local country in Asian population. Furthermore to CYP3A4 enzyme product in drug metabolism, sequence variations in this gene might affect response to drug, efficacy and toxicity, therefore this genetic variant is important results for pharmacogenetic studies and further study of other polymorphism of this gene is suggested. Result of this study 1 non-denaturing polyacrylamide gel electrophoresis and other studies together in this area may present the information about pharmacogenetic significance and healthcare medicine in Iran.

Table 1. Components for 10% PAGE which is used for heterozygocity or homozygocity determination of samples with 210 bp bands.

| 10% PAGE       | Acry_bis aeryl 29:1(30% aq) | 10 mlit |
|----------------|-----------------------------|---------|
| TBE            | 4 mlit                      |
| DDW            | up to 45 mlit               |
| APS (30%)      | 120 µl                      |
| TEMED          | 40 µl                       |

Table 2. Genotype and allele frequency of CYP3A4*1B

| Genotype | N  | Genotype frequency | Allele frequency | X² |
|----------|----|--------------------|------------------|----|
| AA       | 174| 0.951              | A                |    |
| AG       | 8  | 0.044              | 0.97 0.03        | 5.77|
| GG       | 1  | 0.005              |                  |    |

Table 2. Show 0.03 (3%) allele frequency of variant allele and 0.97 (97%) of wild type allele in studied population. This population was studied about Hardy-Weinberg equilibrium. Genotype frequencies in this population were in line with Hardy-Weinberg equilibrium.
4. REFERENCES

[1] Eichelbaum, M., M. Ingelman-Sundberg, and W.E. Evans, Pharmacogenomics and individualized drug therapy. Annu. Rev. Med., 57: p. 119-137 (2006).
[2] Hu, Y.F., et al., CYP3A5*3 and CYP3A4*18 single nucleotide polymorphisms in a Chinese population. Clinica chimica acta., 353(1): p. 187-192(2005).
[3] Lamba, J.K., et al., Genetic contribution to variable human CYP3A-mediated metabolism. Advanced drug delivery reviews., 54(10): p. 1271-1294(2002).
[4] Shih, P.S. and J.D. Huang, Pharmacokinetics of midazolam and 1-hydroxymidazolam in Chinese with different CYP3A5 genotypes. Drug metabolism and disposition., 30(12): p. 1491-1496(2002).
[5] Keshava, C., E.C. McCanlies, and A. Weston, CYP3A4 polymorphisms—potential risk factors for breast and prostate cancer: a HuGE review. American journal of epidemiology, 160(9): p. 825-841(2004).
[6] Bork, R., et al., Characterization of mRNA species related to human liver cytochrome P-450 nifedipine oxidase and the regulation of catalytic activity. Journal of Biological Chemistry, 264(2): p. 910-919(1989).
[7] Beaune, P.H., et al., Isolation and sequence determination of a cDNA clone related to human cytochrome P-450 nifedipine oxidase. Proceedings of the National Academy of Sciences, 83(21):p. 8064(1989).
[8] Linder, M.W., R.A. Prough, and R. Valdes Jr, Pharmacogenetics: a laboratory tool for optimizing therapeutic efficiency. Clinical Chemistry., 43(2): p. 254-266(1997).
[9] Hashimoto, H., et al., Gene structure of CYP3A4, an adult-specific form of cytochrome P450 in human livers, and its transcriptional control. European Journal of Biochemistry, 218(2): p. 585-595(1993).
[10] Rebbeck, T.R., et al., Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. Journal of the National Cancer Institute, 90(16): p. 1225-1229(1998).
[11] Amirrmani, B., et al., Increased transcriptional activity of the CYP3A4*1B promoter variant. Environmental and molecular mutagenesis, 42(4): p. 299-305(2003).
[12] Felix, C.A., et al., Association of CYP3A4 genotype with treatment-related leukemia. Proceedings of the National Academy of Sciences, 95(22): p. 13176(1998).
[13] von Moltke, L.L., et al., Unusually low clearance of two CYP3A substrates, alprazolam and trazodone, in a volunteer subject with wild-type CYP3A4 promoter region. The Journal of Clinical Pharmacology, 40(2): p. 200-204(2000).
[14] Sata, F., et al., CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. Clinical Pharmacology & Therapeutics, 67(1): p. 48-56(2000).
[15] Ball, S.E., et al., Population distribution and effects on drug metabolism of a genetic variant in the 5 promoter region of CYP3A4. Clinical Pharmacology & Therapeutics, 66(3): p. 288-294 (1999).
[16] Cavalli, S.A., M.H. Hirata, and R.D.C. Hirata, Detection of MboII polymorphism at the 5 promoter region of CYP3A4. Clinical chemistry, 47(2): p. 348-351(2001).
[17] Arvanitidis, K., et al., Genetic polymorphisms of drug-metabolizing enzymes CYP2D6, CYP2C9, CYP2C19 and CYP3A5 in the Greek population. Fundamental & clinical pharmacology, 21(4): p.419-426 (2007).
[18] Miao, J., et al., Association of genotypes of the CYP3A cluster with midazolam disposition in vivo. The pharmacogenomics journal, 9(5): p. 319-326(2009).
[19] Oliveira, E., et al., Pharmacogenetically relevant polymorphisms in Portugal. Pharmacogenomics, 8(7): p. 703-712(2007).
[20] Wolbold, R., et al., Sex is a major determinant of CYP3A4 expression in human liver. Hepatology, 38(4): p. 978-988(2003).