İstanbul Ecz. Fak. Derg. / J. Fac. Pharm. Istanbul
46(1) 2016 pp.15-22

The evaluation of *CYP3A4* and *CYP3A5* genetic profiles in Turkish population

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Abstract: Adverse drug reactions are one of the major causes of death, amounting to the fifth leading cause in the United States, encompassing 100.000 deaths annually. Genetic polymorphism brings about significant inter-individual and inter-ethnic variability in the metabolism of numerous therapeutic agents, which results in differences in the clinical response of therapeutic agents and their adverse effects. Therefore, the crucial factor is major variability in the capacity of the metabolism and detoxification of drugs and other xenobiotics. Unites State Food and Drug Administration (FDA) has highlighted the potential of pharmacogenomic testing to create personalized drugs, and the agency aims to encourage both the public and private sector to develop pharmacogenetic products. Both of cytochrome P450 (CYP) 3A4 and 3A5, which are the most abundant and most important drug-metabolizing enzymes in humans, are responsible for the metabolism of more than 60% of therapeutic drugs. In present study, the genotype profiles of *CYP3A4*1B* and *CYP3A5*3,* very common and functional single-nucleotide polymorphisms (SNPs), were evaluated in Turkish healthy volunteers. The genotype distributions did not significantly deviate from the Hardy-Weinberg equilibrium analysis. The recessive allele frequencies of *CYP3A4*1B* and *CYP3A5*3* were 1% and 4% in the healthy group, respectively. According to the obtained results, it may be suggested that the carriers of *CYP3A5*3* variant allele should be taken higher doses for the drugs metabolizing this enzyme in Turkish population, while the carriers of *CYP3A4*1B* variant allele which do not generally have a risk should be taken normal doses.

Key words: Genetic polymorphism, Turkish population, *CYP3A4*, *CYP3A5*, Cytochrome *P450*

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Introduction

Pharmacogenetics is a vast field covering drug discovery research, the genetic basis of pharmacokinetics and dynamics, genetic testing and clinical management in diseases. Pharmacogenetic approach usually focuses on variations of drug transporters, drug targets, drug metabolizing enzymes and other biomarker genes (Ingelman-Sundberg et al., 2007; Spear et al., 2001). It is well known that inheritable changes in DNA sequence leading to two or more alleles of a certain gene within a population are called genetic polymorphism, which contributes to inter-individual and inter-ethnics variations in the metabolism of various drugs and other xenobiotics (Ginsberg et al., 2009). CYP enzymes, an essential source of variability in drug-response, play role not only phase I-dependent metabolism of xenobiotics but also metabolism of endogenous compounds such as steroids, vitamins and fatty acids (Daly et al., 1993; Danielson 2002). As it is well known, gene deletions, gene duplications, inversions, insertion and deleterious mutations are the parts of CYP enzyme polymorphisms, but SNPs are more common than those and important for inter-individual variations (Ingelman-Sundberg et al., 2007, Wright, 2005). Absence of enzyme, enzyme variants with high or low activity, altered substrate specificity, and decreased or increased enzyme expression can be resulted from those SNPs (Rodriguez-Antona and Ingelman-Sundberg, 2006).

Individuals in populations are classified into four phenotypes; as poor or slow metabolizers (*PMs*, having defective or deleted gene to lack functional enzyme activity), intermediate metabolizers (*IMs*, commonly having one functional and one defective allele leading to decrease enzyme activity), extensive or rapid metabolizers (*EMs*, having two functional genes) and ultra rapid metabolizers (*UMs*, having more than two active genes). It is considered as the important point for the inter-individual differences in drug response (Johansson and Ingelman-Sundberg, 2011; Scordo et al., 2004).

Because the determination of genotype and allele frequencies may provide a helpful support in the optimization of pharmacological therapies, we aimed to evaluate the genotype profile of *CYP3A4* and *CYP3A5* in Turkish population.

Material and Method

DNA was isolated from venous blood samples of unrelated 160 Turkish healthy volunteers (88 females and 72 males, aged 20-65 years) by High Pure PCR Template Preparation Kit (Roche, Germany). All participants provided informed consent and studies were approved by the ethics committee of Istanbul University (2014/1546). *CYP3A4*1B* (rs2740574, -392A>G) and *CYP3A5*3* (rs776746, 6986A>G) were genotyped on Roche LightCycler 480 RT-PCR platform with using LightCycler FastStart DNA Master HybProbe and appropriate Roche LightSNIP assay probes (Roche, Germany).

The Hardy-Weinberg equilibrium analysis was performed to compare the observed and expected genotype frequencies of subjects by using the chi-square (χ^2) test. Differences in the allele and genotype frequencies of *CYP3A4*1B* and *CYP3A5*3* genetic variants between Turkish and other ethnic populations were assessed by χ^2 test. A p value below 0.05 was considered statistically significant throughout the population comparisons. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (Version 17, Chicago, USA).

Result and Discussion

The human *CYP3A* gene subfamily consists of four known members; *CYP3A4, CYP3A5, CYP3A7* and *CYP3A43*, which are located on chromosome 7q22 (Sosa-Macías and Llerena, 2013). Both of CYP3A4 and CYP3A5, which are most abundant and important drug-metabolizing enzymes amongst those in humans, are responsible for the metabolism of more than 60% of therapeutic drugs (Alessandrini et al., 2013). It is considered that the substrates of CYP3A4 and CYP3A5 are almost similar. These consist of not only antidepressants, immunosuppressants, calcium channel blockers, cancer chemotherapeutics, antihistamines, sedatives but also several endogenous steroids, such as testosterone, progesterone, cortisol, bile acids (Liu et al., 2007; Zhou et al., 2009). Although more than 35 *CYP3A4* variants and sub-variants (*1A through *26) have been identified to date, the most common and studied variant is *CYP3A4*1B* which enhanced expression due to reduced binding of a receptor that may affect the transcriptional rate. There are approximately thirty *CYP3A5*

variants/sub-variants and the most investigated among them is *CYP3A5*3* leading to increase enzyme activity (http://www.cypalleles.ki.se; Amirimani et al., 2003; Kudzi et al., 2010).

In the present study, the frequency distributions of *CYP3A4*1B* A/A and A/G genotypes were 97.30% (144) and 2.70%, respectively (Table). The percentage of the genotype frequencies of *CYP3A5**3 A/G and G/G were 8.87% (14) and 91.03 % (142), respectively. However, *CYP3A4*1B* G/G and *CYP3A5**3 A/A were not observed in the studied population. The dominant allele frequencies for *CYP3A5*3* are 91-95, 73, 71, 70 and 27% in Caucasian, Chinese, Japanese, Korean, and African-American population, respectively (Van Schaik et al., 2002). The frequencies for *CYP3A4*1B* of Caucasian, African, European and Japanese were 3, 50, 3 and %0, respectively (International HapMap Consortium 2002-2010, http://hapmap.ncbi.nlm.nih.gov). According to our results, Turkish population are similar Caucasians with 0.96 frequencies of *CYP3A5*3* G allele and with 0.01 frequencies of *CYP3A4*1B* G allele.

Table. Genotype frequencies of the gene variants in the present study

SNPs	Genotype	Genotype frequency	Allele frequency
CYP3A4*1B (rs2740574)	A/A	144 (97.30)	A: 0.99
	A/G	4 (2.70)	
	G/G	0 (0)	G: 0.01
CYP3A5*3 (rs776746)	A/A	0 (0)	A: 0.04
	A/G	14 (8.97)	
	G/G	142 (91.03)	G: 0.96

There are many pharmacogenetics studies about the effects of CYP3A4 and CYP3A5 enzyme polymorphisms on drug-response. It is known that cyclosporine and tacrolimus, which are calcineurin inhibitors, have narrow therapeutic index and are most widely used in immunosuppressive agent for prevention of rejection following renal transplantation (Singh et al., 2009). A significant correlation was found between *CYP3A5*3* expressers (A/G, G/G) and dose adjustment for cyclosporine and tacrolimus in renal transplant patients in North India. While the expressers were needed

higher dose requirement at 1 month (7.43±1.58 vs. 7.13 ± 1.56 mg/kg/day, p=0.131) and 3 months (4.46 ± 1.26 vs. 4.19 ± 1.22 mg/kg/day, p=0.003), the dose-adjusted C_2 (2-h post oral dose) levels were lower for them at 1 month, not 3 months (0.22 ± 0.05 vs. 0.24 ± 0.06 μg/mL per mg/kg/day, p=0.058). On the contrary, there wasn't any correlation between *CYP3A4*1B* on cyclosporine/tacrolimus pharmacokinetics (Singh et al., 2009). Dai et al. (2004) suggested cyclosporine intrinsic clearance is approximately 2.3-fold higher for CYP3A4 than for CYP3A5. It was suggested that *CYP3A4*1B* allele carriers need to higher dose of tacrolimus contrast to *CYP3A4*1B* homozygotes wild type whereas *CYP3A5*3* homozygote mutant type patients in Netherlands (Hesselink et al., 2003). Another study about tacrolimus pharmacogenetics shows that AUC (the area under the curve) and C_{max} (maximum concentration) for the *CYP3A5*3* homozygous wild type (6.9%) or heterozygous variant type (48.3%) was much lower than *CYP3A5*3* homozygous variant type (44.8%) in a Korean population (Choi et al., 2007). It was also noted that the requirement of tacrolimus dose to maintain the target $dnAUC_{0-12}$ (dose-normalised area under the curve) was 2-fold higher in the individuals having a *CYP3A5*3* homozygous variant allele contrary to the individuals having wild type allele (Op den Buijsch et al., 2007).

Some studies indicated there wasn't any significant correlation between midazolam pharmacokinetics and *CYP3A* polymorphism (Hohmann et al., 2014; Miao et al., 2009; Stockis et al., 2015). Brown et al. (2012) suggested that *CYP3A5*3* allele was correlated with decreased AUC for neviparine, antiviral agent. They found that *CYP3A5*3* allele decreased $AUC_{0-12\ h}$ by 31% in Malawian populations. In a study investigated the correlation with SNPs and child patients with neuroblastoma, it was found that the risk of mortality in the individuals having *CYP3A5*3* homozygous variant genotype was 4-fold more than homozygous wild type or heterozygous variant individuals while the individuals having *CYP3A4*1B* homozygous and heterozygous variant genotype had a 52% lower risk of mortality than the others (Darwish et al., 2015).

According to the result of the study about association with *CYP3A5* polymorphism and clopidogrel resistance patients with coronary artery disease, the carriers of homozygous genotype of *CYP3A5*3* had 2.78 fold risk of developing clopidogrel resistance contrary to non-carriers of

the variant allele. Also, the individuals having *CYP3A5*3* heterozygous variant genotype had 2.45 fold risk of platelet hypo-responsiveness to clopidogrel (Priyadharsini et al., 2014). It was investigated whether there was any association between combined hormone replacement therapy including estrogen and progestin in postmenopausal breast cancer risk and *CYP3A4*1B* genetic polymorphism. It was observed an increased risk of estrogen receptor-negative tumors in women having *CYP3A4*1B* alleles in the therapy (Rebbeck et al., 2007).

In this study, the genotype profile of Turkish population about *CYP3A4*1B* and *CYP3A5*3* were investigated. The polymorphisms are considered as target for many therapeutic agents among various populations into their effects on enzymatic activity. Our findings indicated that Turkish population are similar Caucasians with 0.96 and 0.01 allele frequencies of *CYP3A5*3* G and *CYP3A4*1B* G, respectively. The characterization of polymorphisms in the enzymes may provide advantage for dosing adjustment of several drugs, so that occurrence of adverse effects and even death may be prevented or reduced. It may be suggested that the carriers of *CYP3A5*3* variant allele, not *CYP3A4*1B*, should be importantly taken in higher doses of the drugs metabolizing this enzyme in Turkish population.

Acknowledgement

This work was supported by the Research Fund of Istanbul University (Project No: 49558).

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