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EXPERIMENTAL / LABORATORY STUDIES

Common Cytochrome p4503A (CYP3A4 and CYP3A5) and Thiopurine S-Methyl Transferase (TPMT) Polymorphisms In Turkish Population

Müge AYDIN SAYİTOĞLU[1], İnci YILDIZ[2], Özden HATIRNAZ[1], Uğur ÖZBEK[1]

[1]Department of Genetics, Institute for Experimental Medical Research (DETAE), İstanbul University, İstanbul - Turkey

[2]Division of Pediatric Hematology/Oncology, Cerrahpasa Faculty of Medicine, İstanbul University, İstanbul - Turkey

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Abstract: Polymorphisms in the genes encoding cytochrome p450 (CYP) and thiopurine S-methyl transferase (TPMT) enzymes catalyze the metabolic reactions of several drugs. These polymorphisms might be responsible for adverse drug reactions. Turkish population data for these genes still needs to be elucidated. We aimed to detect the allele frequencies of thiopurine S-methyl transferase *(TPMT)*, cytochrome *p4503A4*1B (CYP3A4*1B)* and cytochrome *p4503A4*3 (CYP3A5*3)* gene variants in the Turkish population. We examined the *TPMT (*1, *2, *3A, *3C), CYP3A4*1B* and *CYP3A5*3* variant allele frequencies in a group of healthy Turkish Caucasian blood donors by using PCR-RFLP, allele-specific PCR and direct sequencing techniques. The frequencies of four allelelic variants of *TPMT* gene, are **2 (238G>C)(2.0%), *3A (460G>A* and *719A>G)(1.0%), *3B (460G>A)(0.0%)* and **3C (719A>G) (1.4%)*. We observed *CYP3A4*1B* allele frequency in 1.4% and *CYP3A5*3* allele frequency in 7.5% of our population. This study provides the first analysis of *TPMT, CYP3A4*1B* and *CYP3A5*3* mutant allele frequencies in the Turkish population.

Key Words: pharmacogenetics, TPMT, CYP3A4, CYP3A5

Introduction

Drug metabolizing enzymes participate in the neutralizing of xenobiotics and biotransformation of drugs. Polymorphisms in the drug-metabolizing enzyme coding genes alter the activity of these enzymes for some substrates (1). Cytochrome P4503A (CYP3A) mediated metabolism is associated with drug-drug interactions and chemically induced carcinogenesis in several cancers (2, 3). CYP3A is the most highly expressed subfamily in the liver and small intestine tissues. The isoforms of CYP3A, which include the CYP3A4, CYP3A5, CYP3A7 and CYP3A43, comprise the largest portion of the liver (30% of all CYP's). Steroids, antidepressants, benzodiazepines, immunosuppressive agents, macrolide antibiotics and toxins are the most common substrates of CYP3A enzymes (4). Moreover, dexamethasone, rifampicin and clotrimazole increase the expression of *CYP3A* genes (5). CYP proteins are encoded by distinct genes (6). CYP3A4 and CYP3A5 are the predominant hepatic p450 forms in adults and their expression varies among individuals. They metabolize approximately 45-60% of drugs currently in use and their catalytic activity is important in bioavailibility and drug-drug interactions (3, 7).

Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyzes the S-methylation of aromatic and heterocyclic sulfhydryl compounds like 6-mercaptopurine (6MP), which is used to treat patients with acute lymphoblastic leukemia (ALL), thioguanine a widely used drug in acute myeloblastic leukemia, and azathiopurine (AZA), which is used in rheumatic diseases effects but also myelosuppression (8). TPMT activity is related to the outcome and/or toxicity of therapy. Patients with inherited very low levels of TPMT activity are at increased risk for thiopurine-induced toxicity, when treated with standard doses of these drugs (9).

More than 30 single nucleotide polymorphisms have been identified in the *CYP3A4* gene. *CYP3A4*1B* is an A to G transition in the 5' flanking region of the gene, and it has been speculated that this variant has a reduced activity (10). Significant ethnic differences occur in the allele frequencies of *CYP3A* variants (Tables 1 and 2). To date, nine mutant *TPMT* alleles have been reported (11). The wild type allele is *TPMT*1*, which shows high TPMT activity, has a frequency of 90% in Caucasians, while 10% of the individuals show intermediate activity (12). The frequencies of *TPMT *2 (238G>C), TPMT*3A (719A>G-460G>A)* and *TPMT *3C (719A>G)* alleles have been demonstrated in different populations in Table 3. These alleles account more than 80% of *TPMT* gene in Caucasians.

Testing for the most common *TPMT* and *CYP3A* genotypes are used to modify doses of thiopurines such as 6-MP and AZA that are used to treat ALL and steroids, antidepressants, benzodiazepines, immunosuppressive agents and macrolide antibiotics respectively. There have been no previously published studies on *TPMT, CYP3A4* and *CYPA5* genes in the Turkish Population. In the current study, we investigated allele frequency of *TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C, CYP3A4*1B* and *CYP3A5*3* in the Turkish population.

Material and Methods

Blood samples were collected from members of the faculty and hospital staff and students (n = 148; 83 females and 65 males; age range of 16 years and 59 years (mean age, 28.7 ± 8.3)). The Institutional Review Board approved the research, and informed consents were obtained from all participants.

Genomic DNA was extracted from peripheral blood by using proteinase K/salting out method. Primer design and restriction enzyme analysis were performed according to previous studies (13, 14). The samples without any TPMT*2, *3A, *3B and *3C mutations were genotyped as TPMT wild type allele (TPMT*1), the samples with one deficient allele (TPMT*1/*2, *1/*3C, *1/*3B, *1/*3A) were genotyped as heterozygous and the samples with two deficient alleles (TPMT*2/*3C, *2/*3B, *3C/*3B, *2/*3A etc.) were genotyped as homozygous. The samples that carried both the G460A and A719G mutations were named TPMT *3A.

The analysis of A→G transversion at the 5'untranslated region of *CYP3A4*1B* gene was performed by a two-step PCR-based restriction fragment polymorphism assay as described by Wandel *et al.* (10). *CYP3A5*3* gene was analyzed by direct sequencing as described by Kuehl *et al.* (15). We performed the

Table 1. CYP3A5*3 variant allele frequencies in different populations.

Chinese[a] (n = 366)	Japanese[a] (n = 366)	British[b] (n = 200)	Caucasian[a] (n = 366)	African-American[a] (n = 366)	Turkish[c] (n = 372)
27.0%	29.0%	6.5%	5.0%	73.0%	7.5%

[a]Hustert et al., [b]King et al., [c]present study

Table 2. CYP3A4*1B variant allele frequencies in different populations.

American-African[a] n = 80	Caucasian[b] n = 106	British[c] n = 200	Finnish[d] n = 118	Chinese[d] n = 108	Turkish[e] n = 186
35.0%	6.5%	6.5%	4.2%	0.0%	1.4%

[a] Kuehl et.al., 2001, [b] Lamba et.al., 2002, [c] King et.al., 2003,
[d] Sata et al., 1999, [e] present study.

Table 3. Frequencies of TPMT variants in different populations.

Population	Allele frequencies (%)			Reference
	TPMT*2	TPMT*3A	TPMT*3C	
British Caucasian(n=398)	0.5	4.5	0.3	Ameyaw et al., 1999
American Caucasian(n=564)	0.2	3.2	0.2	Hon et.al., 1999
French Caucasian(n=608)	0.7	3.0	0.4	Ganiere-Monteil et al., 2004
German Caucasian(n=2428)	0.2	4.4	0.4	Elke et al., 2004
African-American(n=496)	0.4	0.8	2.4	Hon et.al.,1999
Italian(n=206)	0.4	3.9	0.9	Rossi et.al, 2001
Japanese(n=302)	0.0	0.0	1.6	Kumagai et.al., 2001
South-east Asian(n=698)	0.0	0.0	1.0	Chang et al., 2002
Brazil(n=408)	2.2	1.5	1.0	Boson et.al., 2003
Turkish(n=296)	2.0	1.0	1.4	present study

n, observed number of alleles

sequencing in 93 individuals (46 females and 47 males) of the population. The primers are designed according to previously published sequences (15). Amplified fragments were purified with PCR purification columns (Qiagen) and sequenced on PE ABI 310 capillary sequencer, using the Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer Kit).

Results

*TPMT *2, *3A, *3B* and **3C* variant genotypes were determined in 148 unrelated individuals for the Turkish population. The individuals that carried none of these variants were named as *TPMT*1* and one hundred thirty five samples (135 of 148 subjects) carried the *TPMT* *1/*1 genotype. Six *TPMT *2* heterozygotes and four *TPMT*3C* heterozygotes were found in the 148 Turkish subjects. Three samples were observed as carriers (3 of 148 subjects) of both G460A and A719G mutations and they were named *3A. *TPMT*3B* variant was not detected in Turkish subjects. The allele frequencies of *TPMT*2* (2.0%), *TPMT*3A* (1.0%), *TPMT*3B* (0.0%) and *TPMT*3C* (1.4%) were given in Table 4.

We did not observe any homozygous variant for *CYP3A4*1B* and *CYP3A5*3* genes in the studied population (Table 5). Four *CYP3A4*1B* heterozygotes in 148 subjects and fourteen *CYP3A5*3* heterozygotes in 93 subjects were determined in our subjects. Allele frequencies of *CYP3A4*1B* and *CYP3A5*3* were 1.4% and 7.5% respectively, in the Turkish Population.

Discussion

It has been demonstrated that the presence of the mutant alleles is predictive of the enzyme activity, so that heterozygous individuals have intermediate activity and homozygous individuals have low activity, although a variability can seen between these groups (16-18). *TPMT*2* was the most common mutation (45.4%) among the examined variants and the other mutations were *TPMT*3C* (31.8%), *TPMT*3A* (22.7%) and *TPMT*3B* (0.0%). These alleles have been identified as responsible for enzyme deficiency and account for more than 80% of the *TPMT* gene in Caucasians (11).

The current study has documented that the overall frequency of *TPMT* alleles is 4.4% in Turkish Population. *TPMT*2* and **3* alleles are the most common mutant alleles in Caucasians (11). *TPMT*2* was found the most prevalent mutation among Turks as well as the Brazilian population (19), whereas TPMT*3A seems the most common variant in American and European Caucasians (11, 20). TPMT*3C allele is the only mutation found in Japanese (21) and the south-east Asians (22).

Table 4. Allele frequencies of common TPMT variants in a group of 148 Turkish subjects.

Allele	SNP position	Aminoacid substitution	Frequency (%) (n=296)
TPMT*1	wild type		94.6
TPMT*2	238G>C	Ala80Pro	2.0
TPMT*3A	460G>A and 719A>G	Ala154Thr and Tyr240Cys	1.0
TPMT*3B	460G>A	Ala154Thr	0.0
TPMT*3C	719A>G	Tyr240Cys	1.4

None of analysed mutations was detected.
*n, observed number of alleles

Table 5. Allelic frequencies of CYP3A4*1B and CYP3A5*3 variants in the Turkish population.

	CYP3A4(n=296)		CYP3A5(n=186)	
	CYP3A4*1	CYP3A4*1B(-288A>G)	CYP3A5*1	CYP3A5*3(6986A>G)
Allele frequency	98.6%	1.4%	92.5%	7.5%

*n, observed number of alleles

The CYP3A subfamily plays a particularly important role, between 45% and 60% of all currently used drugs are substrates for CYP3A enzymes (6). Our findings showed that in the Turkish population, CYP3A5*3 allele frequency is similar to the previously reported data, whereas CYP3A4*1B allele frequency seems to be lower than other studies that were reported before. For comparison, the CYP3A5 allele was detected in 29% Japanese, 6.5% British, 73% African-American and 7.5% Turkish (23, 24) and the CYP3A4 allele was in 0.0% Chinese, 6.5% British, 65% African-American and 1.4% Turkish (15, 24-26).

Given that all major TPMT and CYP3A mutant alleles are present in the Turkish population, it would be good clinical practice for the rational use of individual drug therapy. This current study is the first to elucidate the genetic basis for TPMT, CYP3A4 and CYP3A5 enzyme deficiencies in Turkish Population.

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Corresponding author:
Uğur ÖZBEK
Institute for Experimental Medicine (DETAE),
Department of Genetics İstanbul University,
Vakıf Gureba Cad. 34093 Fatih, İstanbul - Turkey
E-mail: uozbek@istanbul.edu.tr

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