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Original

Frequency of thiopurine S-methyltransferase gene variations in Turkish children with acute leukemia

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In this study we aim to determine the genotype distribution and allele frequencies of common TPMT (*2, *3A, *3B and *3C) polymorphisms in Turkish children with acute leukemia. The study population consisted of 169 patients aged between 1 and 15 years who were admitted to Losante Pediatric Hematology and Children's Hospital with the diagnosis of acute leukemia. Genotyping of TPMT polymorphisms was screened with real-time PCR using fluorescence melting curve detection analysis. We found that the frequencies of four allelic variants of TPMT are *2 (238 G>C) (0,0%), *3A (460G>A and 719A>G) (1.7%), *3B (460G>A) (1,7%) and *3C (719A>G) (2.4%). Frequency of TPMT alleles increases the efficacy of leukemia treatment. Thus, TPMT genotyping can be useful for optimizing 6-MP therapy.

Key words: TPMT, leukemia, childhood, polymorphism.

Drug metabolizing enzymes play a role in the neutralizing of xenobiotics and biotransformation of drugs.[1] Polymorphisms in the drug-metabolizing enzyme coding genes change the activity of these enzymes for their substrates. Thiopurine S-methyltransferase (*TPMT*) is a cytosolic methylating enzyme that preferentially catalyzes the S-methylation (inactivation) of aromatic and heterocyclic sulfhydryl compounds, which include anticancer thiopurine groups such as mercaptopurine, thioguanine, and azathioprine.[2,3] These medications are currently used to treat many diseases, such as cancers, autoimmune diseases and inflammatory diseases.[4,5]

6-Mercaptopurine (6-MP) and 6-thioguanine (6-TG) have been important components of curative therapy for childhood acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML), respectively.[6,7] 6-MP is a prodrug which is activated by intracellular multistep pathway to cytotoxic thioguanine nucleotides (TGNs) that are incorporated into DNA repair pathway and trigger cell death.[8]

TPMT displays genetic polymorphisms in different ethnic groups, including Caucasians, Africans, African-Americans, and Asians. Additionally *TPMT* has been associated with high levels of 6-MP metabolite levels and toxicity.[9] The *TPMT* is localized on chromosome 6p22.3 and consists of 10 exons.[10] To date more than 20 SNPs for *TPMT* have been reported.[11-13] Four alleles (*TPMT*2, *3A, *3B, and *3C*) describe for ~95% of inherited TPMT deficiency and have been biochemically characterized.[14-16] *TPMT *3A* allele results in an about 400- fold decrease; *TPMT*3B* allele results in a fourfold decrease; TPMT*2 allele results 100-fold decrease; *TPMT*3C* allele results in a 1.4-fold in protein levels.[17,18] The wild-type allele, *TPMT*1*, encodes the fully active enzyme.[19] The frequencies of *TPMT*2* (238G>C), *TPMT*3A* (719A>G-460G>A) *TPMT *3B* (460G>A) and *TPMT *3C* (719A>G) alleles have been reported in different populations as shown in Table I. The molecular defect in *TPMT*2* contains G>C transversion at position 238 that leads to an amino acid substitution at codon 80 Ala>Pro.[20] The *TPMT*3A* contains

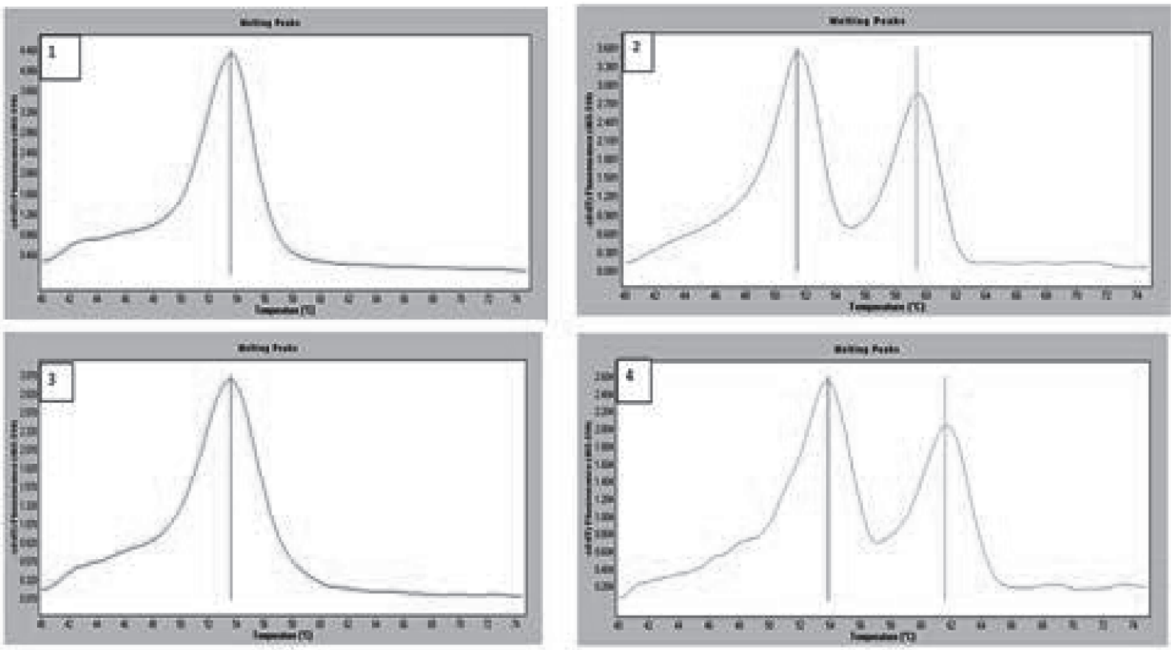

Fig. 1. Melting Curve analysis with the Light Cycler 480 II. These dual peaks show heterozygous samples. (1) TPMT *3C wildtype, (2) TPMT *3C heterozygote (rs1142345), (3) TPMT *3B wildtype, (4) TPMT *3B heterozygote (rs1800460).

two nucleotide transitions G>A and A>G at positions 460 and 719 that lead to the amino acid substitution at codon 154 Ala>Thr and at codon 240 Tyr>Cys.[21] The *TPMT*3B* contains single nucleotide transition G>A at position 460 that lead to the amino acid substitution at codon 154 Ala>Thr.[22] The *TPMT*3C* contains A>G transversion at position 719 that leads to the amino acid substitution at codon 240 Tyr>Cys.[23]

These alleles are accounted for more than 80% of *TPMT* in Caucasians [24]. The frequencies of four variant *TPMT* alleles (*TPMT *2, TPMT *3B, TPMT *3C, TPMT *3A*) accounting for more than 80% of all low activity cases were determined in children with ALL in Turkish population.[25]

The aim of this study is to determine the genotype distribution and allele frequencies of common *TPMT*2,*3A, 3B,* and *3C* polymorphisms by melting curve detection analysis with Light Cycler 480 II System in Turkish children with acute leukemia.

Material and Methods

Patients and sample collections

Study population consisted of 169 patients (99 males and 70 females) aged between 1 and 15 years who were admitted to Lösante Pediatric Hematology and Children's Hospital and diagnosed with childhood acute leukemia. Patient characteristics were shown in Table I.

An informed written consent was obtained from all the patients' parents. The study was carried out in accordance with the code of Ethics of

Table I. Demographic Features of the Patients.

	Range-Mean	Age±SD
Age, years	1-15	7.8±3.76
Gender		
Male	97	
Female	72	
Type of disease		
ALL	151	
AML	14	
BAL	4	

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; BAL: Biphenotype acute leukemia

Table II. Genotype Distributions and Frequencies of *TPMT* (1,2,3A,3B,3C)* Gene Polymorphisms in Turkish Children with Acute Leukemia.

TPMT Allele	SNP Position	Ref SNP number	Amino acid change	Genotype distributions (%) n:169	Allele frequencies (%) n:169
TPMT *1	Wild Type			165 (97.6)	1
TPMT*2	238G>C	rs1800462	Ala80Pro	0.0	0.0
TPMT*3A	460G>A- 719A>G	rs1800460- rs1142345	Ala154Thr- Tyr240Cys	3 (1.7)	G/0.99-A/0.01 A/0.98-G/0.02
TPMT*3B	460G>A	rs1800460	Ala154Thr	3 (1.7)	G/0.99-A/0.01
TPMT*3C	719A>G	rs1142345	Tyr240Cys	4 (2.4)	A/0.98-G/0.02

SNP: single nucleotide polymorphism

the World Medical Association (Declaration of Helsinki) for experiments involving humans. The Ankara University, School of Medicine Ethics Committee approved the study protocol (Project No.14-646-14/2014) and informed consent was provided by the patients' parents. Blood samples were collected with EDTA-containing tubes and DNA was extracted from peripheral blood and bone marrow leukocytes with MagNA Pure automatic DNA isolation instrument (Roche Diagnostics, Manheim, Germany).

TPMT genotyping

TPMT polymorphisms analyses were performed by real-time PCR (RT-PCR). Genotyping of *TPMT* mutations were screened with real time PCR using fluorescence melting curve detection analysis by means of the Light Cycler 480 II System (Roche Diagnostics, Manheim, Germany). For G238C (*2), primers are used which flank the region around the potential mutation place and produce an amplicon of 197 bp. For G460A (*3A, *3B) and A719G (*3A, *3C), two primer pairs are used generating amplicons of 159 bp and respectively 177 bp. The melting-point curve analysis allows a clear identification of wildtype, heterozygous or homozygous genotypes.

Statistical analysis

The chi-square test was used to compare categorical variables. P value of <0.05 was considered statistically significant. Allelic frequencies were calculated by gene-counting method and the genotype distribution with Hardy- Weinberg expectations were determined by χ^2 and Fisher's exact tests.

Results

In this study, we performed Real Time PCR method to detect three types of polymorphisms of the human *TPMT*. Three *TPMT* polymorphisms; G238C substitution located in exon 5 region, G460A substitution located in exon 7 region, and A719G substitution located in exon 10 region. *TPMT* genotypes of the most prevalent mutant allele's (*TPMT *2, *3A, *3B and *3C*) variant genotypes were determined in 169 Turkish children with leukemia who were diagnosed with ALL and AML.

As presented in Table II, three (1.7%) patients carried G460A polymorphism in heterozygote state, thus named as carriers of *TPMT*3B*. Three (1.7%) patients carried both G460A and A719G polymorphisms and were named *TPMT*3A*. Four (2.4%) patients carried polymorphism A719G indicating to have heterozygote state *TPMT*3C*. *TPMT*2* polymorphism was not detected in 169 patients. The patients that carried none of these variants were named as *TPMT*1* and one hundred sixty-five samples (165 of 169 subjects) carried the *TPMT*

The genotype frequencies of *TPMT* polymorphisms in healthy individuals from different countries were given in Table III.

Discussion

TPMT catalyzes the methylation of thiopurine drugs such as azathioprine and 6-mercaptopurine.[26] TPMT enzyme activity is influenced by polymorphisms in the *TPMT* gene.[27-29] Thus, heterozygous alleles have intermediate TPMT activity, homozygous alleles have low TPMT activity and both of them have greater risk of developing thiopurine-induced

Table III. The Genotype Distribution (%) of *TPMT* Polymorphism in Healthy Individuals From Different Country.

Study Group(n)	Population	Genotype distribution (%)			References
		TPMT*2	TPMT*3A	TPMT*3C	
382	France	0.7	3.0	0.4	[9]
296	Turkey	2.0	1.0	1.4	[30]
398	England	0.5	4.5	0.3	[37]
564	America	0.2	3.2	0.2	[38]
2428	Germany	0.2	4.4	0.4	[39]
206	Italy	0.4	3.9	0.9	[40]
384	Japan	0.0	0.0	1.6	[41]
254	Iran	3.93	0.87	1.57	[42]
400	Chinese	0	0	3	[43]
654	Kazakhstan	0.0	0.3	0.9	[43]

myelosuppression. Therefore, it is indispensable to have knowledge about *TPMT* SNP frequencies in a population to evaluate the safety and efficacy of ALL treatment. Accordingly, this study was undertaken to determine the prevalence of the *TPMT* mutations in Turkish children with acute leukemia. We found that genotype distribution of the most relevant *TPMT* polymorphisms in Turkish children with acute leukemia were 2.4%.

Differences in *TPMT* polymorphisms change among ethnic groups, ranging from 2% to 14% prevalence. *TPMT*2* and **3* alleles are the most common mutant alleles in Caucasians [30]. Sayitoglu et al.[31] detected a prevalence of the *TPMT*2, TPMT*3B* and **3C* in Turkish healthy individuals, 2%, 0%, and 1.4%, respectively. However, we did not detect *TPMT*2* alleles in Turkish children with leukemia. When their data in Turkish healthy individuals compared to ours in present study, 3B and 3C genotype prevalence were found higher in our Turkish children with leukemia. Tumer et al's.[25] data on the frequency of *TPMT* variants in the Turkish population on 106 Turkish children with ALL revealed the frequency of the *TPMT*2, TPMT*3B* and *3C** in their study group, %0, 0.9%, and 0.9%, respectively. Their genotype frequencies were found lower than our results.

A study by Hongeng et al.[32] analyzed *TPMT* genotype status of 75 Thai children with ALL. They detected only *3C allele (11%). They reported that there was no difference in the genetic variation of *TPMT* between Asian and North American Caucasian populations. Lennard et al.[33] who studied 1320 children with ALL in England reported that *TPMT *3B* allele was not detected, *3A and *3C allele frequencies were 4.5% 0.7% respectively. Ayesh et al.[29] studied 56 children with ALL in Gaza Strip and *TPMT*2, TPMT* 3B* and *TPMT*3C* were not found. *3A allele frequency in their study group was found 0.89%. Zgheib et al.[34] who analyzed 127 children with ALL in Lebanon have reported that none of the patients were homozygous alleles while three patients were heterozygous *TPMT** 3A (2.4%) alleles. Farfan et al.[35] who studied 103 Chilean children with leukemia have reported that the total frequency of *TPMT* alleles was 8%. *TPMT*2, TPMT*3A* and *TPMT*3B* alleles were found in 0%, 7%, and 1%, respectively. In the study performed by Chrzanowska et al.[36] (included 98 children with ALL) they have shown a frequency of *TPMT*1* 94.4 %, *TPMT*3A* 5.1 % and *TPMT*2* 0.5 %.

Genotype and phenotype determination studies are needed to identify the predictive power of *TPMT* genotyping. These results can help to coordinate pretreatment strategies in patients with leukemia requiring thiopurine medication in their standard therapy.

We hope that our results show the presence of genetic causes of TPMT hypoactivity in Turkish population and help to provide genetic strategies to analyze these patients prior to anticancer therapy.

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