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 sulphydryl compounds, which include anticancer thiopurine groups such as mercaptopurine, thioguanine, and azathioprine.² Three of these medications are currently used to treat many diseases, such as cancers, autoimmune diseases and inflammatory diseases.

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
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. The TPMT*3B contains single nucleotide transition G>A at position 460 that lead to the amino acid substitution at codon 154 Ala>Thr. The TPMT*3C contains A>G transversion at position 719 that leads to the amino acid substitution at codon 240 Tyr>Cys.

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.

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 Lüksante 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>
<thead>
<tr>
<th>Table I. Demographic Features of the Patients.</th>
<th>Range-Mean</th>
<th>Age±SD</th>
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<tbody>
<tr>
<td>Age, years</td>
<td>1-15</td>
<td>7.8±3.76</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Type of disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALL</td>
<td>151</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>BAL</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; BAL: Biphenotype acute leukemia
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 χ² 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

### Discussion

TPMT catalyzes the methylation of thiopurine drugs such as azathioprine and 6-mercaptopurine. TPMT enzyme activity is influenced by polymorphisms in the TPMT gene. Thus, heterozygous alleles have intermediate TPMT activity, homozygous alleles have low TPMT activity and both of them have greater risk of developing thiopurine-induced
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. Sayitoglu et al. 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 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. 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. 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. 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. 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. 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. (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.

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

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


