Thiopurine-S-Methyltransferase Gene Polymorphism and Drug-related Toxicity in Children Treated for Acute Leukemia and Non-Hodgkin’s Lymphoma

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ABSTRACT

Aim: Thiopurine S-methyltransferase (TPMT) is an essential enzyme in the metabolism of thiopurine drugs, and its activity may change due to different polymorphisms in the TPMT gene. The TPMT gene has different genetic polymorphisms in different ethnic groups. This study aimed to determine the frequency of TPMT polymorphisms in children with acute leukemia/non-Hodgkin lymphoma (AL/NHL) and healthy children and to evaluate their association with severe toxicities in the study population.

Materials and Methods: Sixty-seven pediatric AL/NHL patients and 84 healthy children were evaluated. Genotyping for the TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C, TPMT*4, TPMT*5, TPMT*6, and TPMT*7 alleles were performed by the real-time polymerase chain reaction technique. The number of grade 3 or higher hematologic and hepatic toxicities were recorded from the patient charts.

Results: In the AL/NHL patients, we found that the patients had generally wild-type TPMT*1 allele in 80.6%, whereas TPMT*2 (238G>C) was seen in 1.5%, TPMT*3A (c.460G>A and c.719A>G) in 0%, and TPMT*3B polymorphisms (460G>A) in 17.9%. We found wild-type TPMT*1 allele in 98.8% and TPMT*3B polymorphisms (460G>A) in 1.2% of the healthy volunteers. Grade ≥3 myelosuppression developed in 22/54 patients with the wild type allele, and it developed in 5/12 patients with TPMT*3B allele. Six (8.9%) patients had grade ≥3 aspartate aminotransferase elevations, 17 (25%) patients had grade ≥3 alanine transaminase elevations (1-5 times), and 42 patients had (62.6%) grade ≥3 total bilirubin elevations.

Conclusion: TPMT*3B polymorphism was the most common allele detected in our study group. This allele frequency is very high in comparison to other studies from our country and it was over-represented in comparison to the healthy volunteers. We did not find any relationship between severe hematologic/hepatic toxicities and TPMT gene polymorphisms.

Keywords: Thiopurine S-methyltransferase, leukemia, lymphoma, polymorphism, toxicity, childhood
Introduction

Thiopurine drugs, such as 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG), are cytotoxic agents used to treat acute leukemia and non-Hodgkin’s lymphoma (NHL). Oral 6-MP is a mainstay of the maintenance therapy of acute lymphoblastic leukemia (ALL) and lymphoblastic lymphomas (LLs). Oral 6-TG is another thiopurine drug used in the maintenance treatment of acute myeloid leukemia (AML). One of the most frequent adverse effects of thiopurine drugs is myelosuppression, which is usually reversed by decreasing the dosage of the drug.

Thiopurine S-Methyltransferase (TPMT) is a cytosolic enzyme that converts thiopurine drugs into inactive metabolites via methylation reactions and it provides protection against 6-MP/6-TG drug toxicity (1). Usually, 90% of patients have normal TPMT activity. However, in 10% of patients, TPMT enzyme activity may be different due to different \( \text{TPMT} \) gene polymorphisms. Patients who carry homozygous \( \text{TPMT} \) gene polymorphisms with two non-functional \( \text{TPMT} \) genes (0.3%) have no TPMT enzyme activity and may experience life-threatening myelosuppression when taking thiopurine drugs (1,2). Patients with heterozygous mutations with one non-functional allele have intermediate enzyme activity and have a higher risk for toxicity than normal patients, but 40-70% can tolerate these drugs. Due to these life-threatening toxicities, the Clinical Pharmacogenetics Implementation Consortium (2013) and the US Food and Drug Administration (2015) recommended testing for \( \text{TPMT} \) gene polymorphism before starting mercaptopurine treatment and adjusting dosages according to gene status (2).

The \( \text{TPMT} \) gene is located on chromosome 6p22.3, and more than 23 single nucleotide polymorphisms have been reported (3). TPMT 1 (wild-type) allele codes the normal active enzyme. The most common alleles responsible for deficiency are TPMT 2 (c.238G>C), TPMT 3A (c.460G>A and c.719A>G), and TPMT 3C (c.719G>A). These account for 80-95% of deficient cases. TPMT 3A and 3B polymorphisms may result in an apparent lack of TPMT enzyme activity and may experience life-threatening myelosuppression when taking thiopurine drugs (1,2). Patients with heterozygous mutations with one non-functional allele have intermediate enzyme activity and have a higher risk for toxicity than normal patients, but 40-70% can tolerate these drugs. Due to these life-threatening toxicities, the Clinical Pharmacogenetics Implementation Consortium (2013) and the US Food and Drug Administration (2015) recommended testing for \( \text{TPMT} \) gene polymorphism before starting mercaptopurine treatment and adjusting dosages according to gene status (2).

In the maintenance protocols, patients were given 100% of the estimated drug dosage if white blood cell (WBC) counts were between 2,000-3,000/mm\(^3\) and 50% of the dosage if WBC counts were between 1,000-2,000/mm\(^3\). The drug is withdrawn in cases where WBC is less than 1,000/mm\(^3\). Similarly, the drug is withdrawn in cases where hepatotoxicity is ≥3 grade. The number of grade ≥3 hematologic (Hemoglobin, WBC, platelet counts) and hepatic toxicities [serum alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin levels] were obtained from the patient charts by the investigators.

We used the Common Toxicity Criteria version 5 recommended by the National Cancer Institute for hematological and hepatic toxicity. The grades explain the severity of toxicity; Grade 1: Mild, Grade 2: Moderate, Grade 3: Severe, Grade 4: Life-threatening, Grade 5: Death (6).

The patients were followed up at the outpatient service bi-weekly. The highest value measured in each month was considered to be the toxicity value.

DNA Isolation and TPMT Genotyping

Genomic DNA was extracted from all subjects’ peripheral blood leukocytes using the high pure polymerase chain reaction (PCR) template preparation kit (Roche Applied Science, Mannheim, Germany) and stored at -20 °C until use. Genotyping for the TPMT*2, TPMT*3A,
TPMT*3B, TPMT*3C, TPMT*4, TPMT*5, TPMT*6 and TPMT*7 alleles was performed according to the modified protocol of Tai et al. (4) by means of real-time PCR using the Light Cycler® v.2.0 instrument (Roche Applied Science, Mannheim, Germany). For this purpose, specific primers and hybridization probes (TIB MOLBIOL, Berlin, Germany) for the analyzed alleles were used combined with the Light Cycler DNA Master Hybridization Probes Kit (Roche Applied Science, Mannheim, Germany). Polymorphic alleles were identified by the specific melting temperature (Tm) of the resulting amplicons.

**Statistical Analysis**

Data were analyzed using SPSS software (version 21.0). The means of the groups were analyzed using non-parametric tests. Correlation of the TPMT polymorphisms with different parameters was performed using the chi-square or Fisher’s exact tests, and a p-value of <0.05 was considered statistically significant.

**Results**

The study group consisted of 67 patients (43 male, 24 female), and the mean age was 8.1 years (1-18 years). The diagnosis was ALL in 42, AML in 5, and LL in 20 patients. The control group consisted of 84 healthy children (34 male and 50 female) with a mean age of 9.5 years (1-18 years). Patient characteristics are given in Table I.

In the genotyping, we found that the patients in the study group had generally wild-type TPMT (*1) allele at a prevalence of 80.6%, TPMT*2 (G238C) at a prevalence of 1.5% and TPMT*3B polymorphisms (G460A) at a prevalence of 17.9% (Table II). The polymorphisms detected were heterozygous mutations, and no homozygous mutations were detected. Other polymorphisms including TPMT*3A, TPMT*3C (A719G), TPMT*3D, TPMT*4 (G-A), TPMT*5 (T146C), TPMT*6 (A539T) and TPMT*7 (T681G) were not detected.

In the control group, wild-type TPMT (*1) was 98.8% and TPMT*3B was 1.2%. Other polymorphisms were not detected. The leukemia-lymphoma patients were found to have less wild-type TPMT but more TPMT*3B polymorphism (p=0.0001 and 0.0001) (Table II).

As for hematologic toxicity, only one patient (1.5%) developed grade ≥3 anemia (2 times) during maintenance treatment, and this patient had a wild-type TPMT allele. However, 28 patients (41.8%) developed grade ≥3 leukopenia. Of these patients, 22 of them had wild-type TPMT allele, 5 of them had TPMT*3B polymorphism, and one of them had TPMT*2 polymorphism. None of the patients developed grade ≥3 thrombocytopenia (Table III).

As for severe hepatic toxicity, six (8.9%) patients had grade ≥3 AST elevations, while 17 (25%) patients had grade ≥3 ALT elevations (1-5 times), and 42 patients (62.6%) had grade ≥3 total bilirubin elevations (1-23 times) (Table III).

We did not find any relationship between the numbers of severe (grade ≥3) hematologic/hepatic toxicities and TPMT gene polymorphisms in the statistical analysis.

**Discussion**

Thiopurine drugs, such as 6-MP and 6-TG are a mainstay of the maintenance therapy of acute leukemia and LLs. TPMT enzyme catalyzes the methylation and inactivation of thiopurine drugs. The enzyme activity is influenced by polymorphisms in the TPMT gene and TPMT has different genetic polymorphisms in different ethnic groups. It is important to know TPMT polymorphisms in the population.

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<thead>
<tr>
<th>Table I. Characteristic features of the patients</th>
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<tbody>
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<td>Age, years</td>
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<td>8.1 (1-18)</td>
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<table>
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<th>Patients (n)</th>
<th>Control group (n)</th>
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<tbody>
<tr>
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<td>43</td>
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<tr>
<td>Female</td>
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<th>Disease</th>
<th>Patients (n)</th>
<th>Control group (n)</th>
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<tbody>
<tr>
<td>ALL</td>
<td>42</td>
<td>-</td>
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<tr>
<td>AML</td>
<td>5</td>
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<td>NHL</td>
<td>20</td>
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<th>Table II. TPMT polymorphism distribution of the patients</th>
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<td>TPMT allele</td>
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<td>TPMT 1</td>
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<tr>
<td>TPMT 2</td>
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<tr>
<td>TPMT 3B</td>
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TPMT: Thiopurine S-methyltransferase, SNP: Single-nucleotide polymorphism
to evaluate the safety of these drugs. Therefore, we aimed to determine the frequency and type of TPMT polymorphisms in Turkish children. The most common polymorphism was TPMT*3B allele in healthy Turkish children (1.2%). Other polymorphisms (TPMT*2, TPMT*3B, TPMT*3A, TPMT*3C, TPMT*3D, TPMT*4, TPMT*5, TPMT*6, TPMT*7) were not determined. There is a limited data about the TPMT polymorphisms in healthy Turkish children. Sayitoğlu et al. (7) analyzed TPMT genotypes in healthy Turkish individuals and detected the prevalence of the TPMT*2 to be 2%, TPMT*3B to be 0%, and TPMT*3C to be 1.4%. However, this is different from our results. We did not detect TPMT*2 and TPMT*3C alleles.

In the leukemia-lymphoma group, we found that leukemic children had wild type TPMT allele (TPMT*1) in 80.6%, TPMT*3B in 17.9%, and TPMT*2 in 1.5%. Unlike other studies, TPMT*3B allele frequency was very high in our patients. TPMT*3B is a rare allele that is usually absent in many populations. Tumer et al. (8) studied 106 Turkish children with ALL and detected TPMT*2 in 0%, TPMT*3B in 0.9%, and TPMT*3C in 0.9%. Similarly, Akın et al. (9) studied 169 children with leukemia and detected TPMT*2 in 0%, TPMT*3A in 1.7%, TPMT*3B in 1.7%, and TPMT*3C in 2.4%. We did not detect TPMT*3C and TPMT*3A in our population. This difference may be related to our study population. Our population was recruited from Western Anatolia. However, other studies were recruited from the Marmara region of Central Anatolia. Only a few studies have reported TPMT*3B polymorphism as high in the literature. Moreno-Guerrero et al. (10) studied Mexican children with different cancer types and reported TPMT*3B polymorphism at a rate of 7.5%. However, another study from Mexico reported TPMT*3B to be 0.1% in leukemia patients (11). According to these results, we thought there might be regional differences within the same country. In Europe, TPMT-3B was only reported in the Spanish population to be 1.5%, and other studies from our country at a prevalence of 0.9-1.7% (7-9,12). Among Asian countries, an Iranian study reported TPMT*3B allele to be 1.6% in the healthy population (13).

The increased TPMT*3B allele frequency in the patient group compared to healthy children is striking in our study group. This result raises the question of whether these polymorphisms might affect leukemia susceptibility. We know that folate metabolism plays an important role in DNA synthesis and methylation. Deviations in the folate metabolism resulting from polymorphisms in genes encoding folate-dependent enzymes may affect susceptibility to leukemia. One of the most extensive series from Mexico studied 849 patients (428 ALL, 421 non-ALL), and they found TPMT polymorphism frequency to be higher in ALL patients but did not find it statistically significant (11). Previous studies from the Mexican population also reported TPMT polymorphisms are not a risk factor for ALL (14,15).

In our study, TPMT*3C allele was not detected. However, TPMT*3C is the most frequent allele in Asian and African populations (16). Leukemic children from Singapore were reported to have TPMT*3C allele at a rate of 3% in the Chinese population, 2.3% in Malaysians, whereas children from Thailand have TPMT*3C at a rate of 11% (17,18).

TPMT*3A is the most common allele in the Caucasian and American populations but was not detected in our study. Lennard et al. (19) evaluated 1,320 children with ALL in England and detected TPMT*3A at a rate of 4.5%, and TPMT*3B at a rate of 0%. For the Serbian population, TPMT*3A allele frequency was reported as 3.2% while TPMT*2 was 0.2%, and TPMT*3B was 0.5% (20).

TPMT*2 is another less common allele. This allele has been detected 0-0.1% in Asian populations and 0.1-0.85% in European populations (11,13). On the other hand, two larger sample-sized studies in Iran reported this allele at a rate of 2.16-3.93% (13,21). In our study, TPMT*2 frequency was 1.5%.

As for drug toxicity, theoretically, it can be assumed that children with heterozygous or homozygous polymorphisms might experience more hematologic or hepatic toxicities of 6-MP and 6-TG as a result of decreased enzyme activity, decreased thiopurine clearance from plasma, and accumulation of the drug in the body. There are many studies regarding thiopurine dosage and its relevance with TPMT alleles, but this research is very limited in the pediatric age group.

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<th>Table III. Grade≥3 hematologic/hepatic toxicities in patients according to TPMT polymorphism (n=67)</th>
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<tr>
<td>TPMT 1</td>
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<td>TPMT 2</td>
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<td>TPMT 3B</td>
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TPMT: Thiopurine S-methyltransferase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase
The authors declared that this study received no financial support.

Ethics

Ethics Committee Approval: The Local Ethics Committee of Ege University Faculty of Medicine approved this study (approval number: 05-4/5, date: 28.04.2021).

Informed Consent: Informed consent was obtained from the parents.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Conflict of Interest: The authors declare that they have no conflict of interest.

Financial Disclosure: The authors declared that this study received no financial support.

References


