Drug metabolizing enzymes participate in the neutralization 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 bioavailability 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 azathioprine (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 CYP3A5 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.

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

<table>
<thead>
<tr>
<th></th>
<th>Chinese&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Japanese&lt;sup&gt;a&lt;/sup&gt;</th>
<th>British&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Caucasian&lt;sup&gt;a&lt;/sup&gt;</th>
<th>African-American&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Turkish&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 366)</td>
<td>(n = 366)</td>
<td>(n = 200)</td>
<td>(n = 366)</td>
<td>(n = 366)</td>
<td>(n = 372)</td>
</tr>
<tr>
<td></td>
<td>27.0%</td>
<td>29.0%</td>
<td>6.5%</td>
<td>5.0%</td>
<td>73.0%</td>
<td>7.5%</td>
</tr>
</tbody>
</table>

<sup>a</sup>Hustert et al., <sup>b</sup>King et al., <sup>c</sup>present study

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

<table>
<thead>
<tr>
<th></th>
<th>American-African&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Caucasian&lt;sup&gt;b&lt;/sup&gt;</th>
<th>British&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Finnish&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Chinese&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Turkish&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 80</td>
<td>n = 106</td>
<td>n = 200</td>
<td>n = 118</td>
<td>n = 108</td>
<td>n = 186</td>
</tr>
<tr>
<td></td>
<td>35.0%</td>
<td>6.5%</td>
<td>6.5%</td>
<td>4.2%</td>
<td>0.0%</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

<sup>a</sup>Kuehl et al., 2001, <sup>b</sup>Lamba et al., 2002, <sup>c</sup>King et al., 2003, <sup>d</sup>Sata et al., 1999, <sup>e</sup>present study

### 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
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).
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.

### Acknowledgement

The Research Fund of the Istanbul University, Project Numbers, supports this work: 1768/21122001 and 01544/16012001. Müge Aydin Sayitoğlu was supported in part by grants from the American Lebanese Syrian Associated Charities (ALSAC) through the International Outreach Program and the Pharmaceutical Sciences Department of St. Jude Children’s Research Hospital, Memphis, TN, USA. The authors would also like to thank Dr. Mary Relling for kindly providing detailed protocols for genotyping TPMT mutant alleles.

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

<table>
<thead>
<tr>
<th>Allele</th>
<th>SNP position</th>
<th>Aminoacid substitution</th>
<th>Frequency (%) (n=296)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT*1</td>
<td>wild type</td>
<td></td>
<td>94.6</td>
</tr>
<tr>
<td>TPMT*2</td>
<td>238G&gt;C</td>
<td>Ala80Pro</td>
<td>2.0</td>
</tr>
<tr>
<td>TPMT*3A</td>
<td>460G&gt;A and 719A&gt;G</td>
<td>Ala154Thr and Tyr240Cys</td>
<td>1.0</td>
</tr>
<tr>
<td>TPMT*3B</td>
<td>460G&gt;A</td>
<td>Ala154Thr</td>
<td>0.0</td>
</tr>
<tr>
<td>TPMT*3C</td>
<td>719A&gt;G</td>
<td>Tyr240Cys</td>
<td>1.4</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th></th>
<th>CYP3A4(n=296)</th>
<th>CYP3A5(n=186)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4*1</td>
<td>98.6%</td>
<td>92.5%</td>
</tr>
<tr>
<td>CYP3A4*1B(-288A&gt;G)</td>
<td>1.4%</td>
<td>7.5%</td>
</tr>
</tbody>
</table>

*n, observed number of alleles
References


