Polymorphism of the thiopurine S-methyltransferase gene in African-Americans

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The molecular basis for the genetic polymorphism of thiopurine S-methyltransferase (TPMT) has been established for Caucasians, but it remains to be elucidated in African populations. In the current study, we determined TPMT genotypes in a population of 248 African-Americans and compared it with allele frequencies in 282 Caucasian Americans. TPMT genotype was determined in all individuals with TPMT activity indicative of a heterozygous genotype (≤10.1 U/ml pRBC, n = 23 African-Americans, n = 21 Caucasians) and a control group with TPMT activity indicative of a homozygous wild-type genotype (>10.2 U/ml pRBC, n = 23 African-Americans, n = 21 Caucasians). No mutant alleles were found in the high activity control groups. The overall mutant allele frequencies were similar in African-Americans and Caucasians (4.6 and 3.7% of alleles, respectively). However, while TPMT*3C was the most prevalent mutant allele in African-Americans (52.2% of mutant alleles), it represented only 4.8% of mutant alleles in Caucasians (P < 0.001). In contrast, TPMT*3A and TPMT*2 were less common in African-Americans (17.4 and 8.7% of mutant alleles), whereas TPMT*3A was the most prevalent mutant allele in Caucasians (85.7% of mutant alleles). A novel allele (TPMT*8), containing a single nucleotide transition (G644A), leading to an amino acid change at codon 215 (Arg→His), was found in one African-American with intermediate activity. These data indicate that the same TPMT mutant alleles are found in American black and white populations, but that the predominant mutant alleles differ in these two ethnic groups.

INTRODUCTION

Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyzes the S-methylation of aromatic and heterocyclic sulphydryl compounds, including 6-mercaptopurine, 6-thioguanine and azathioprine (1). Thiopurines are largely used as antineoplastic agents and as immunosuppressants for the treatment of inflammatory bowel diseases, rheumatoid arthritis and other autoimmune diseases. The parent thiopurine drugs are metabolized to nucleotide intermediates by hypoxanthine-guanine phosphoribosyltransferase (HPRT) and then further metabolized to the principal active metabolites, thioguanine nucleotides (TGN) (2,3). Alternatively, the thiopurine bases (parent drugs) and most of their nucleotide metabolites can be methylated by TPMT (4), yielding inactive S-methylated metabolites, thereby decreasing the amount of drug available for activation to TGN. In this regard, studies have shown a significant negative correlation between the intracellular concentration of TGN and the activity of TPMT in erythrocytes (5,6). Because TGN concentrations have been associated with the efficacy and toxicity of thiopurines in various diseases (7–12), inter-patient differences in TPMT activity can have a significant impact on the effects of these medications.

TPMT activity is inherited as an autosomal codominant trait, exhibiting genetic polymorphism in all large populations studied to date (13,14). Genetic polymorphism in the TPMT gene is such that ~90% of Caucasians have high TPMT activity, ~10% have intermediate activity and 1 in 300 individuals has low activity (13). Polymorphism in TPMT activity has also been established in African-Americans (14,15) and Chinese (16). The range of TPMT activity varies among ethnic groups; the highest median activity was reported in a small Saami population (17) and Caucasians exhibited higher TPMT activity than African-Americans [median activity 16.8 versus 14.4 U/ml packed red blood cells (pRBC), P < 0.001] (14).

The genetic basis and molecular mechanisms for inherited differences in TPMT activity have recently been elucidated in Caucasians (18–22), but have not been established in other ethnic groups. To date, a total of nine non-functional mutant alleles have been reported (18–22) TPMT*2–*7, with TPMT*3A, TPMT*2 and TPMT*3C being the most prevalent mutant alleles (23). These variant alleles result from point mutations in the TPMT open reading frame or at intron/exon splice sites. These TPMT mutant alleles have been documented only in Caucasians (23) or individuals of unknown ethnic origin (20), except TPMT*6.
Figure 1. Allelic variants at the human TPMT locus. Boxes depict exons in the human TPMT gene. White boxes are untranslated exonic regions and black boxes represent exons in the ORF. Grey boxes represent exons that contain mutations that result in changes to amino acids.

which was found in a single Korean individual (20). TPMT*3A is the most prevalent mutant allele in Caucasians, with TPMT*3C and TPMT*2 being more rare (23). Genotyping for these mutant alleles yielded ∼95% concordance between genotype and phenotype in a Caucasian population (23). The current study was undertaken to elucidate the genetic basis for the TPMT polymorphism in African-Americans and determine whether there are differences in this gene polymorphism in black and white populations.

RESULTS

For the African-Americans, TPMT phenotypes were determined in 248 unrelated individuals [196 healthy adult blood donors and 52 children with acute lymphoblastic leukemia (ALL)]. In these 248 individuals, 23 subjects (9.3%, 95% CI 5.7–12.9%) had intermediate TPMT activity (5–10.1 U/ml pRBC), and no TPMT homozygous deficient individual was found. TPMT genotypes were determined for all 23 individuals with intermediate TPMT activity (i.e. presumed heterozygotes, <10.2 U/ml pRBC), using PCR methods to detect the predominant mutant alleles (TPMT*2, TPMT*3A, TPMT*3B and TPMT*3C). These allelic variants at the human TPMT locus are shown in Figure 1. Twelve black subjects were found to have the TPMT*3C allele, four TPMT*3A and two TPMT*2. Mutations at nucleotides 238, 460 and 719 were not detected in the remaining five black individuals with intermediate activity. These mutations were not found in any of the 23 control individuals with high TPMT activity (i.e. presumed homozygous wild-type, >10.1 U/ml pRBC).

To identify other potential mutations of the TPMT gene, direct sequencing of PCR-amplified exons in the TPMT coding region was performed in the two black individuals with the lowest TPMT activity but without mutations at nucleotide 238, 460 or 719. One individual with an activity of 6.8 U/ml pRBC had no detectable mutation throughout the entire open reading frame (ORF) and intron/exon splice sites. The other individual, with an activity of 5.8 U/ml pRBC, had a novel mutation in exon 10 of one TPMT allele. A G→A transition was found at nucleotide 644, changing codon 215 from arginine to histidine. Existence of this new allele (TPMT*8) was confirmed by TaqI restriction analysis and by cloning and sequencing of the amplified exon 10 after nested PCR of genomic DNA. With the wild-type TPMT sequence, a TaqI restriction site spans nucleotide 644. Presence of the G644A mutation disrupts this restriction site, permitting its detection in genomic DNA by PCR–RFLP analysis (Fig. 2). Using this method to detect G644A, the individual carrying this new mutation was confirmed to be heterozygous at this locus, but none of the other subjects with intermediate TPMT activity had the G644A mutation.

Table 1 summarizes the different TPMT mutant alleles found in African-Americans, and compares these data with those for Caucasians. Results of TPMT genotyping in Caucasians are from 21 individuals with intermediate TPMT activity identified from 282 unrelated white subjects (7.4%, 95% CI 4.7–11.2%), as previously described (23). TPMT*3C was the most prevalent mutant allele found in African-Americans (12 of 23 subjects,
Figure 2. An ethidium bromide stained gel that depicts DNA fragments of amplified exon 10 after digestion by TaqI restriction enzyme. Wild-type DNA yields fragments of 132 and 161 bp and mutant DNA yields an uncleaved fragment of 293 bp. b.p., base pairs; wt, wild-type; mut, mutant.

52.2%), whereas it was found significantly less frequently in Caucasians (one of 21 subjects, 4.8%, \( P < 0.001 \)). In contrast, \( TPMT^*3A \) occurred less often in African-Americans (four of 23 subjects, 17.4%), but it was the most prevalent mutant allele in Caucasians (18 of 21 subjects, 85.7%, \( P < 0.001 \)). \( TPMT^*2 \) was rare in both populations, with a frequency of 8.7% in blacks and 4.8% in whites (not significant). \( TPMT^*8 \) was found in one black subject out of 23 individuals (4.3%) with intermediate TPMT activity. TPMT activity was similar in Caucasian and African-American heterozygotes who inherited either the \( TPMT^*3A \) or \( TPMT^*3C \) allele. The median TPMT activity for the 12 African-American \( TPMT^*3C \) heterozygotes was 7.8 U/ml pRBC, comparable with TPMT activity in the one Caucasian \( TPMT^*3C \) heterozygote (7.0 U/ml pRBC). Likewise, the median TPMT activity for 18 Caucasian \( TPMT^*3A \) heterozygotes was 8.8 U/ml pRBC, a value similar to the median TPMT activity in four African-American \( TPMT^*3A \) heterozygotes (8.0 U/ml pRBC). As a negative control, 23 African-Americans with high TPMT activity (median 17.6, range 12.1–24.3 U/ml pRBC) and 21 high activity Caucasians (median 16.5, range 11.3–25.4 U/ml pRBC) were genotyped by the same methods and no mutant alleles were detected.

Table 2 summarizes the estimated frequencies of different \( TPMT \) alleles for African-Americans and Caucasians. The wild-type allele, \( TPMT^*1 \), was the predominant \( TPMT \) allele in both populations, with mutant alleles comprising 4.6% and 3.7% of all \( TPMT \) alleles in black and white populations, respectively. Among all mutant alleles, there was a significant difference in frequency between African-Americans and Caucasians for the \( TPMT^*3A \) (0.8 versus 3.2%, \( P = 0.007 \)) and \( TPMT^*3C \) (2.4 versus 0.2%, \( P < 0.001 \)) alleles.

Table 1. \( TPMT \) allelic variants in African-Americans and Caucasians with heterozygous phenotypes

<table>
<thead>
<tr>
<th>Allele</th>
<th>African-Americans (n = 23)</th>
<th>Caucasians (n = 21)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n )</td>
<td>Mutant alleles (%) [95% CI]</td>
<td>( n )</td>
</tr>
<tr>
<td>( TPMT^*2 )</td>
<td>2</td>
<td>8.7 [0–20.2]</td>
<td>1</td>
</tr>
<tr>
<td>( TPMT^*3A )</td>
<td>4</td>
<td>17.4 [1.9–32.9]</td>
<td>18</td>
</tr>
<tr>
<td>( TPMT^*3C )</td>
<td>12</td>
<td>52.2 [31.8–72.6]</td>
<td>1</td>
</tr>
<tr>
<td>( TPMT^*8 )</td>
<td>1</td>
<td>4.3 [0–12.7]</td>
<td></td>
</tr>
<tr>
<td>( TPMT^*X^b )</td>
<td>4</td>
<td>17.4 [1.9–32.9]</td>
<td>1</td>
</tr>
</tbody>
</table>

\( ^a \)Caucasian results from our laboratory, as previously reported (23).

\( ^b \)Represents as yet undefined \( TPMT \) mutant alleles.

95% CI, 95% confidence interval for each estimate; NS, not significant.

Table 2. Estimated \( TPMT \) allelic frequencies for the African-American and Caucasian populations

<table>
<thead>
<tr>
<th>Allele</th>
<th>Estimated allelic frequency in population (%) [95% CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>African-American</td>
<td>Caucasian</td>
</tr>
<tr>
<td>( TPMT^*2 )</td>
<td>0.4 [0–1.0]</td>
<td>0.17 [0–0.5]</td>
</tr>
<tr>
<td>( TPMT^*3A )</td>
<td>0.8 [0–1.6]</td>
<td>3.20 [1.7–4.6]</td>
</tr>
<tr>
<td>( TPMT^*3C )</td>
<td>2.4 [1.1–3.8]</td>
<td>0.17 [0–0.5]</td>
</tr>
<tr>
<td>( TPMT^*8 )</td>
<td>0.2 [0–0.6]</td>
<td></td>
</tr>
<tr>
<td>( TPMT^*X^b )</td>
<td>0.8 [0–1.6]</td>
<td>0.17 [0–0.5]</td>
</tr>
<tr>
<td>Total mutant</td>
<td>4.6</td>
<td>3.7</td>
</tr>
</tbody>
</table>

\( ^a \)Allele frequencies in the total population were calculated based on a 9.3 and 7.4% prevalence of heterozygotes in the African-American and Caucasian populations, respectively. Thus, the percentage of each allele in the heterozygotes was multiplied by 0.0465 (i.e. 9.3% \( \times 2 \) alleles/person) for African-Americans and by 0.037 (i.e. 7.4 \( \times 2 \)) for Caucasians. 95% CI, 95% confidence interval for each estimate; NS, not significant.
DISCUSSION

The genetic polymorphism of TPMT activity can have a profound effect on the risk of thiouprine-induced hematopoietic toxicity (1), which can be fatal if TPMT-deficient patients are treated with conventional dosages of these medications (12). Although TPMT activity is known to exhibit genetic polymorphism in most populations studied to date, the genetic basis for this inherited trait has been elucidated only in Caucasians, in whom the TPMT*2 and TPMT*3 alleles account for the great majority of non-functional mutant alleles (23), and in Chinese (24), in whom TPMT*3C was the only mutant allele detected. Other rare mutations have recently been identified (20), but these alleles were identified in individuals of unknown ethnic origin (TPMT*5), a single family of ‘Northern European ancestry’ (TPMT*4) (21) or a single Korean individual (TPMT*6). While a polymorphism of TPMT activity has been established in American blacks (14), the current study is the first to elucidate the genetic basis for this inherited trait in African-Americans.

The current study has documented that the overall frequency of mutant TPMT alleles is similar in American white and black populations and that the same mutations are found in these two ethnic groups. However, there are significant differences in specific mutant allele frequencies in these two populations, with the TPMT*3C allele being the most prevalent mutant TPMT allele in African-Americans, whereas the TPMT*3A allele is the most prevalent mutant allele in the American white population. In the current study, TPMT*3C accounted for 52% of the mutant alleles in African-Americans, but only 5% of mutant alleles in American whites. In contrast, TPMT*3A accounted for >80% of mutant alleles in American whites, but only 17% of mutant alleles in African-Americans. These findings indicate that the same genotyping methods can be utilized to identify the majority of TPMT heterozygotes and homoyzgous deficient individuals in both black and white populations in the USA, but that different frequencies can be anticipated for the known mutant alleles.

From an anthropological point of view, it appears likely that the A719G transition was the first TPMT mutation to arise in humans, as this was the only TPMT mutation found in a Chinese population (24) and in a Ghanaian population (25) and this mutation is also found in Caucasians, either alone in the TPMT*3C allele or in combination with the G460A mutation in the TPMT*3A allele (19). Thus, the ancestral TPMT*3C allele likely evolved to contain a second mutation (G460A) after the divergence of African and non-African populations, giving rise to the TPMT*3A allele which predominates in US (23) and European Caucasians (24). Interestingly, it was recognized three decades ago that Caucasian genes are being introduced into the African-American genome (26) and this integration of genomes may be the source for the small number of TPMT*3A alleles found in African-Americans. Such genome integration has been postulated to explain the nature of the CYP2D6 genetic polymorphism in African-Americans; the relative absence of CYP2D6 mutations in African populations and a 25% admixture of Caucasian-derived genes into the African-American genome was postulated to explain the low frequency of CYP2D6 mutant alleles in the American black population (27). In this regard, if TPMT*3A represents 3.2% of Caucasian TPMT alleles, as was the case in our population (i.e. 85.7% of mutant alleles), and there has been 25% introduction of Caucasian-derived alleles into the African-American genome, then the expected frequency of TPMT*3A alleles would be 0.8%, a number identical to the 0.8% frequency in our African-American population.

The current study also revealed a novel TPMT mutation in an African-American individual with a heterozygous phenotype (TPMT*8), in whom one TPMT allele contained a single nucleotide transition (G644A) leading to an amino acid change at codon 215 (Arg→His). This allele was established by direct sequencing of PCR-amplified TPMT exons and intron/exon boundaries from genomic DNA and by PCR–RFLP analysis of genomic DNA. Using the PCR–RFLP method, this mutation was not found in any other African-American individual and the mechanism by which this mutation is associated with low TPMT activity remains to be elucidated. It is also noteworthy that four African-Americans (17%) with a putative heterozygous phenotype (6.8, 8.4, 9.3 and 9.8 U/ml pRBC) did not have the TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C or TPMT*8 alleles. It is possible that some of these four individuals are indeed homozygous wild-type at the TPMT locus, but have TPMT activities slightly below the cut-off point (10.1 U/ml pRBC) for discriminating heterozygotes and homozygous wild-type phenotypes. A putative cut-off point of 8.2 U/ml pRBC was identified by maximum likelihood estimation of TPMT activity distribution in an African-American population (14). However, it should be noted that seven of 10 African-Americans with TPMT activity between 8.2 and 10.1 U/ml pRBC in the current study had one TPMT mutant allele, while no mutations were found in those with TPMT activity >10.1 U/ml pRBC, indicating that 10.1 U/ml pRBC was an appropriate cut-off point for both populations and that genotype displayed greater utility than maximum likelihood for establishing such cut-off points. In the individual with the lowest TPMT activity (6.8 U/ml pRBC), direct sequencing of all TPMT coding exons and intron/exon boundaries failed to reveal any mutations, raising the possibility of mutations in the TPMT promoter region as an alternative explanation. The recent isolation and characterization of the TPMT gene promotor (28,29), should facilitate future studies to investigate this as a site for mutations associated with low TPMT activity.

Previous studies have established enhanced proteolysis of proteins encoded by the mutant TPMT*2 and TPMT*3 alleles (30). Because the present study has established that the same mutant alleles are largely responsible for the genetic polymorphism of TPMT in both African-Americans and Caucasians, the mechanism for loss of TPMT activity is principally the same in these two populations, even though the mutant allele frequencies differ. The absence of immunodetectable TPMT protein in those inheriting these mutant alleles (30,31) means that TPMT-deficient individuals will be unable to metabolize the as yet undiscovered endogenous and/or environmental substrates for TPMT, as well as thiopurine medication.

MATERIALS AND METHODS

Selection of samples for TPMT genotyping

Blood samples were obtained from Caucasians and African-Americans and DNA was isolated from all subjects with TPMT activity indicative of a heterozygous genotype (i.e. 5–10.1 U/ml pRBC) or homozygous deficiency (i.e. <5 U/ml pRBC), plus a comparable number of unrelated individuals with high TPMT activity (i.e. >10.2 U/ml pRBC) as homozygous wild-type controls. Samples from Caucasians and African-Americans were...
obtained from healthy volunteers and children with ALL who were treated at or referred to St Jude Children’s Research Hospital. The studies were approved by the institutional review board for clinical trials at St Jude Children’s Research Hospital and informed consent was obtained from the patients or their guardians. Erythrocytes and leukocytes were isolated from peripheral blood for determination of TPMT activity and genotype analysis. TPMT activity in erythrocytes was determined by the modified radiochemical assay (32), as previously described (14). TPMT phenotypes were classified as high, intermediate or low activity according to the criteria of Weinshilboum and Sladek, in which the cut-off values for activity were based on the frequency distribution of a randomly selected Caucasian population (13), and subsequently confirmed in our laboratory (14). The same value was used to identify putative heterozygotes (<10.2 U/ml RBC) in both populations, because the estimated cut-off points did not differ significantly in our previous population study (14), because this was established as a reliable cut-off point in our previous study of TPMT genotype in Caucasians (23) and to facilitate comparison with other studies of TPMT polymorphism. Homozygous wild-type genotype was expected for individuals with high TPMT activity (>10.2 U/ml pRBC), thus our studies focused on individuals with a presumed heterozygous TPMT genotype, based on intermediate TPMT activity (i.e. 5–10.1 U/ml pRBC). The lowest measured TPMT activity was used to assign phenotype for each subject. Individuals were classified as Caucasians or African-Americans based on their self-declaration of race and its agreement with skin color, as previously described (27). Genomic DNA was isolated by phenol/chloroform extraction using the method of Chomczynski and Sacchi (33) and PCR-based techniques (described below) were performed to detect TPMT gene mutations in these individuals, as previously described (23). Genotype was determined in an equal number of African-Americans or Caucasians with high TPMT activity (>10.2 U/ml pRBC), as wild-type controls for TPMT genotype.

Determination of TPMT genotype in different ethnic groups by PCR-based techniques

PCR-based methods were used to detect the three principal TPMT mutations (Fig. 1), as previously described in detail (23). In brief, allele-specific PCR amplification was used to detect the G238C transition in exon 5, while PCR amplification and restriction enzyme digestion were used to detect the G460A and A719G mutations. Individuals with heterozygous phenotypes in whom both G460A and A719G mutations were detected in combination with a wild-type nucleotide in the other allele were presumed to have the TPMT*3A allele and a wild-type allele, although compound heterozygotes TPMT*3B/TPMT*3C would produce the same genotyping results. However, we have yet to find any individual with the TPMT*3B allele.

Detection of other potential TPMT gene polymorphisms

For selected individuals with an intermediate TPMT activity but no known mutations, direct sequencing was performed to examine the sequence of the entire TPMT ORF. Exons 3–10 (ORF) were amplified with intron-based primers using nested PCR, as previously described (28). One hundred nanograms of genomic DNA was used for the first round amplification. The product obtained was diluted 1:100 with water and second round amplification was performed with another set of primers encoding a shorter inner fragment. The final product was desalted by ultrafiltration through a Centricon 30 filter (Amicon, Beverly, MA) and then purified on 5% polyacrylamide gels. The corresponding fragment was excised from the gel under UV visualization and eluted with a GE 200 Sixpax gel eluter (Hoefer Scientific Instruments, San Francisco, CA). The eluted fragment was further concentrated and desalted twice with Amicon 10 (Amicon). Sequence was then determined by the fluorescence-tagged dye terminators sequencing technique (Applied Biosystem, Foster City, CA).

Detection of the G644A mutation in an African-American

To confirm a G644A mutation that was found in one African-American by direct sequencing of PCR-amplified TPMT exons, exon 10 was PCR amplified and cloned into a PCR2.1 vector (Invitrogen, Carlsbad, CA). Plasmids were purified with the Qiagen plasmid kit (Qiagen, Santa Clarita, CA) and positive clones with the insert were sequenced by the dye terminators sequencing technique. A PCR–RFLP method was also developed to detect the G644A mutation in genomic DNA. Exon 10 was amplified using the same primers and conditions described for the A719G mutation (23). The PCR product was desalted by filtration with a Centricon 30 filter, treated with 1 U heparinase at room temperature for 2 h, digested with TaqI restriction enzyme for 1 h at 65°C and analyzed by gel electrophoresis. Because the G644A mutation eliminates a TaqI restriction site, wild-type DNA yielded fragments of 132 and 161 bp and the mutant DNA yielded an uncut fragment of 293 bp (Fig. 2).

Data analysis

The University of Wisconsin Genetics Computer Group software package was used to analyze sequence information (CGC, Madison, WI). The differences in the frequency of mutant alleles between African-Americans and Caucasians were analyzed by the \( \chi^2 \) or Fisher’s exact test. All reported \( P \)-values were two tailed.

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