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Very important pharmacogene summary: thiopurine S-methyltransferase

Liewei Wang^a, Linda Pelleymounter^a, Richard Weinshilboum^a, Julie A. Johnson^a, Joan M. Hebert^b, Russ B. Altman^{b,c}, and Teri E. Klein^b

^aMayo Clinic, Rochester, Minnesota, Stanford University, Stanford, California, USA

^bDepartment of Genetics, Stanford University, Stanford, California, USA

^cDepartment of Bioengineering, Stanford University, Stanford, California, USA

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Thiopurine S-methyltransferase (TPMT, S-adenosyl-L-methionine : thiopurine S-methyltransferase; EC 2.1.1.67) catalyzes the S-methylation of thiopurine drugs such as 6-mercaptopurine (6-MP) and azathioprine as well as other aromatic and heterocyclic sulfhydryl compounds [1,2].

Weinshilboum and Sladek [3] reported trimodality for level of red cell *TPMT* among 298 randomly selected Caucasians: 88.6% had high enzyme activity, 11.1% had intermediate activity, and 0.3% had undetectable activity. This distribution conformed to Hardy–Weinberg expectations for a pair of autosomal codominant alleles for low and high activity, *TPMT*-L and *TPMT*-H, with frequencies of 0.059 and 0.941, respectively. Segregation analysis was consistent with this hypothesis. This genetic polymorphism has been shown to be an important factor in individual variations in response to thiopurine drug therapy. 6-MP is inactivated, in part, by S-methylation, catalyzed by *TPMT*. An alternative ‘metabolic activation’ process leads to the formation of cytotoxic 6-thioguanine nucleotides (6-TGN). In addition, 6-MP is metabolized to methyl-thioinosine monophosphate, which inhibits *de novo* purine synthesis, adding another mechanism of cytotoxicity [4,5].

Lennard *et al.* [6,7] showed that, in the children with acute lymphoblastic leukemia who were treated with 6-MP, red cell 6-TGN correlated inversely with RBC (red blood cell) *TPMT* activity (i.e. the lower the level of S-methylation, the more drug would be available for metabolism to form the cytotoxic 6-TGNs). It was also shown that individuals with very low RBC *TPMT* (i.e. those homozygous for that trait) were at greatly increased risk for life-threatening myelosuppression when they were treated with ‘standard’ doses of thiopurine drugs [8,9]. Conversely, patients with acute lymphoblastic leukemia who had 6-TGN concentrations below the group mean had higher *TPMT* activities and a higher subsequent relapse rate. Individuals heterozygous for functional variants tolerate 6-MP intermediate

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Correspondence to Teri E. Klein, PhD, Department of Genetics, Stanford University Medical Center, 300 Pasteur Drive, L301, Stanford, CA 94305-5120, USA Tel: +1 650 725 0659; fax: +1 650 725 3863; feedback@pharmgkb.org.

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between homozygous deficient and homozygous wild-type patients [10]. RBC *TPMT* levels have been found to correlate with levels in other tissues such as liver, kidney, and lymphocytes [11,12]. Therefore, the *TPMT* genetic polymorphism is a significant factor responsible for serious adverse drug reactions (myelosuppression) in patients treated with thiopurines and may also contribute to individual variation in therapeutic efficacy [13]. *TPMT* has become one of a small number of examples in pharmacogenomics to be 'translated' into routine clinical care.

The human *TPMT* gene is 34 kb in length, consists of 10 exons, maps to chromosome 6p22.3 [14] and has a pseudo-gene located on chromosome 18 [15]. Twenty-eight variant alleles have been identified [16], most of which have been associated with decreased activity *in vitro* [17]. Most of these involve nonsynonymous single-nucleotide polymorphisms (SNPs) [17,18]. Among those, *TPMT**2, *3A, *3B, and *3C have been intensively studied both with regard to their clinical implications and/or molecular mechanisms. Szumlanski *et al.* [14] and Tai *et al.* [19] described *TPMT**3A (Online Mendelian Inheritance in Man 187680.0002, <http://www.ncbi.nlm.nih.gov/omim/>), the most common variant allele associated with low *TPMT* activity in Caucasians (frequency approximately 5%). *TPMT**3A contains two nonsynonymous cSNPs, one in exon 7 and another in exon 10 that result in Ala154Thr and Tyr240Cys alterations in encoded amino acids. *TPMT**3B occurs rarely and contains only the exon 7 SNP while *TPMT**3C contains only the exon 10 SNP and is the most common variant allele in East Asian, African-American and some African populations (frequency approximately 2%) [20–23]. More extensive population testing shows that *8 also occurs at a frequency of approximately 2% in some African populations [24].

*TPMT**2, the first variant allele described, results in an Ala80Pro amino acid substitution (*2; Online Mendelian Inheritance in Man 187680.0001) [25]. This allele is much less common than either *TPMT**3A or *3C. It was also shown that expression of *TPMT**2 and *3A were comparable in wild-type and mutant cDNAs, but that wild-type had an approximately 100-fold higher enzymatic activity than mutant *TPMT* [26]. Gene expression did not correlate with protein activity in *TPMT**2 and *3 [25,26]. Tai *et al.* [26] showed that enhanced degradation of *TPMT* allozymes encoded by the *TPMT**2 and *3A alleles is the mechanism for decreased levels of *TPMT* protein and catalytic activity inherited as a result of these alleles.

Subsequent studies performed by Wang *et al.* [27,28] demonstrated that the rapid degradation of *TPMT**3A involves molecular chaperones such as the heat shock proteins hsp70 and hsp90 and that *TPMT**3A can also form intracellular aggregates; both processes contribute to the low levels of protein and activity observed in the tissues of individuals with this allele.

This series of observations suggested that a dynamic balance might exist among *TPMT**3A protein folding, protein degradation, and protein aggregation and raised the question of the identity of the proteins involved in these cellular processes. In an effort to answer that question, a *Saccharomyces cerevisiae* yeast gene-deletion library was used to identify genes required for the degradation/aggregation of *TPMT**3A (Li *et al.* [29]). Twenty-four genes that fell into several functionally related categories were identified. The classes of genes involved included those affecting ubiquitin-dependent protein degradation (E2 ubiquitin-conjugating enzymes, E3 ubiquitin ligases, and proteasome subunits), vesicle trafficking, and vacuolar (lysosomal) degradation. The presence of genes involved in vesicular transport and vacuolar degradation suggested a possible role for autophagy in *TPMT**3A degradation. *UBE2G2*, the human homologue of the E2 ubiquitin-conjugating enzyme identified by the yeast genetic screen, was shown to be involved in the degradation of *TPMT**3A in

mammalian cells. Further, expression of *TPMT**3A induced autophagy and small interfering RNA-mediated knockdown of the expression of *ATG7*, an autophagy-related gene, enhanced *TPMT**3A aggregation in mammalian cells, indicating that autophagy is also involved in *TPMT**3A degradation.

*TPMT**4 and *15 involve alterations in canonical mRNA splice site sequences [30,31], resulting in alternative *TPMT* mRNA splicing and decreased enzyme expression.

Recently, trinucleotide repeat variants in the *TPMT* promoter region have been described which may explain the 1–2% of Caucasians who show ultrametabolizer phenotype [32]. In addition, a promoter region variable number tandem repeat which may have functional significance has been investigated [33–37].

Important variants:

For detailed mapping information, see
<http://www.pharmgkb.org/search/annotatedGene/tpmt/variant.jsp>

TPMT: *2 (Ala80Pro; rs1800462)

TPMT: *3B (Ala154Thr; rs1800460)

TPMT: *3C (Tyr240Cys; rs1142345)

TPMT: *4 (rs1800584).

For other variants of known or suspected functional importance, see Table 1.

***TPMT*: *2 (Ala80Pro; rs1800462)**

TPMT cDNA was cloned from a *TPMT*-deficient patient who had developed severe hematopoietic toxicity during mercaptopurine therapy. Sequencing of the mutant *TPMT* cDNA revealed a single point mutation (G238 → C, where 238 refers to the coding sequence), leading to an amino acid substitution at codon 80 (Ala80 → Pro). When assessed in a yeast heterologous expression system, this mutation led to a 100-fold reduction in *TPMT* catalytic activity relative to the wild-type cDNA, despite a comparable level of mRNA expression. *2 is a relatively rare allele. It is degraded rapidly when transiently expressed in both yeast and mammalian cells. This allele results in low *TPMT* protein and catalytic activity in blood and cell culture. Therefore, if patients who carry this allele are treated with standard dose of thiopurine drugs, they might have increased 6-TGN levels and develop drug-related toxicity such as myelosuppression.

***TPMT*: *3B (Ala154Thr; rs1800460)**

The *TPMT**3B allele is rare and has only the codon 154 SNP. It is usually in tight linkage disequilibrium with the *3C SNP, resulting in the common allele, *3A. When expressed in the yeast and mammalian cells such as COS-1, *3B is degraded rapidly, resulting in significantly decreased levels of enzyme activity and protein [14]. It is also associated with thiopurine drug-related toxicity [14].

***TPMT*: *3C (Tyr240Cys; rs1142345)**

This SNP results in the *TPMT**3C allele. *3C is the most common allele in African-American and East Asian populations. It is associated with decreased enzyme activity and protein quantity as a result of accelerated degradation of *3C in mammalian cells. This allele is associated with decreased levels of protein and enzyme activity in blood cells, but less than is the case with *TPMT**3A and *TPMT**3B [18].

***TPMT*: *4 (rs1800584)**

The *TPMT**4 allele includes a G to A transition at the final splice acceptor nucleotide in *TPMT* intron 9 [31]. This mutation disrupts the intron 9–exon 10 acceptor splice site and results in two abnormal transcripts, one that results from the activation of a cryptic splice site in intron 9, leading to the inclusion of 330 nucleotides of intron sequence, and another that uses a novel splice acceptor site located one nucleotide 3′ downstream from the original splice junction. The latter situation results in a single nucleotide deletion and a frameshift in the portion of the mRNA encoded by exon 10 [31]. Presence of *TPMT**4 in an extended kindred uniformly resulted in very low *TPMT* activity in individuals carrying this allele [31].

Important haplotype

***TPMT*: *3A**

This haplotype contains two nonsynonymous SNPs, *3B and *3C, and is the most common *TPMT* variation occurring in the Caucasian population [14]. The haplo-type results in significant decreases in *TPMT* enzymatic activity resulting in toxicity when thiopurine therapy is administered [19]. Supplemental digital content for the *TPMT* gene (PA356) and VIP is available at <https://www.pharmgkb.org/search/annotatedGene/tpmt/>

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Other alleles of known or suspected functional importance

Allele	Common name(s)	Variant information
<i>TPMT*3D</i>	292G >T +460G> A + 719A >G (Glu98Stop + Ala154Thr +Tyr240Cys)	Identified in a clinical sample showing intermediate <i>TPMT</i> activity [38].
<i>TPMT*5</i>	146T >C (Leu49Ser)	Present in a patient (*1/*5) showing intermediate <i>TPMT</i> activity [38]. When *5 was expressed in COS-1 cells, enzyme activity level was almost undetectable [18].
<i>TPMT*6</i>	539A > T (Tyr180Phe)	Present in a Korean individual showing low RBC <i>TPMT</i> activity [38]. When *6 was expressed in COS-1 cells, enzyme activity level was about a third of wild-type [18].
<i>TPMT*7</i>	681T >G (His227Gln)	First detected in a European individual (*1/*7) who was an intermediate methylator [39]. Present in a European patient (*1/*7) treated with azathiopurine who developed severe leukopenia. In a recombinant yeast expression system, intrinsic clearance rate for the *7 allele was about 10-fold lower than that for *1 [40]. In another report, enzyme activity when expressed in COS-1 cells was essentially at wild-type level [18].
<i>TPMT*8</i>	644G>A (Arg215His)	*8 found in one African–American heterozygote (*1/*8) who showed intermediate <i>TPMT</i> activity [20].
<i>TPMT*9</i>	356A >C (Lys119Thr) c. 356A >C (p.Lys119Thr)	*1/*9 German–Caucasian individual had intermediate <i>TPMT</i> activity [41]. Noted in a Caucasian with intermediate <i>TPMT</i> activity [16]. However, when *9 was expressed <i>in vitro</i> , the catalytic activity of the variant protein was not significantly affected [16,18], and *9 was found in one individual defined as a normal methylator [41]
<i>TPMT*10</i>	430G>C (Gly144Arg)	Present in a patient (*1/*10) treated with azathiopurine who developed severe leukopenia. In a recombinant yeast expression system, intrinsic clearance rate for the *10 allele was about 3.5-fold lower than that for *1 [40]. In another report, expression in COS-1 cells resulted in enzyme activity at about 70% that of wild-type [18].
<i>TPMT*11</i>	395G >A (Cys132Tyr)	Identified in a patient of Spanish origin (*3A/*11) who showed very low RBC <i>TPMT</i> activity [42]. Expression of the *11 allele in COS-1 cells resulted in low enzyme activity [18].
<i>TPMT*12</i>	374C >T (Ser125Leu)	Present in an azathiopurine-treated patient (*1/*12) who developed severe leukopenia. In a recombinant yeast expression system, intrinsic clearance rate for the *12 allele was about 3.5-fold lower than that for *1 [40]. In another report, expression in COS-1 cells resulted in enzyme activity of about 40% of wild-type [18].
<i>TPMT*13</i>	83A > T (Glu28Val)	Identified in an azathiopurine-treated patient (*1/*13) who developed severe leukopenia. <i>In vitro</i> , enzyme activity for the *13 allele was about 60% that of *1 [18,40].
<i>TPMT*14</i>	1A >G (Met1Val)	Identified in a Northern European patient who demonstrated low RBC <i>TPMT</i> activity [30].
<i>TPMT*15</i>	intron VII/exon VIII (IVS7-1G >A)(splice site)	Identified in a Northern European patient who showed low RBC <i>TPMT</i> activity [30].
<i>TPMT*16</i>	488G>A (Arg163His)	<i>In vitro</i> , decreased the intrinsic clearance value with respect to 6-TG methylation three-fold. A Moroccan patient (*1/*16) was an intermediate methylator [43]. *1/*16 German–Caucasian heterozygote had intermediate <i>TPMT</i> activity [41].
<i>TPMT*17</i>	124C >G (Gln42Glu)	*1/*17 German–Caucasian subject had intermediate <i>TPMT</i> activity [41].
<i>TPMT*18</i>	211G >A (Gly71Arg)	*1/*18 German–Caucasian individual had intermediate <i>TPMT</i> activity [41].
<i>TPMT*20</i> (vs.1-[44]) (this is termed *24 in [17])	106G> A (Gly36Ser)	Identified in a Japanese individual (<i>TPMT</i> status not indicated) [44]. When expressed in COS-7 cells, the intrinsic clearance of 6-TG S-methylation was less than 10% of wild-type <i>TPMT</i> [17].
<i>TPMT*20</i> (vs.2-[16917910])	712A >G (Lys238Glu) (c. 712A >G)	Identified in a Caucasian individual with intermediate RBC <i>TPMT</i> activity [45].
<i>TPMT*21</i>	205C >G (Leu69Val) c.205C >G (p.Leu69Val)	Noted in a Caucasian with intermediate <i>TPMT</i> activity [45]. When *21 was expressed in a recombinant yeast expression system, the resulting

Allele	Common name(s)	Variant information
		enzyme had significantly reduced intrinsic clearance when compared to wildtype protein [16].
<i>TPMT*22</i>	488G>C (Arg163Pro) (c.488G>C)	Identified in a Caucasian individual with intermediate RBC TPMT activity [45].
<i>TPMT*23</i>	500C>G (Ala167Gly)	Identified in a Caucasian individual with very low RBC TPMT activity [46].
<i>TPMT*24</i> [16] also see *20 information	537G>T (Gln179His) c.537G>T (p.Gln179His)	Identified in a Caucasian individual having intermediate methylator phenotype. However, when *24 was expressed in a recombinant yeast expression system, the catalytic activity of the variant protein was not significantly affected [16].
<i>TPMT*25</i>	634T>C (Cys212Arg) c.634T>C (p.Cys212Arg)	Identified in two Caucasian individuals showing intermediate thiopurine drug methylation activity. In a recombinant yeast expression system, the recombinant enzyme had significantly decreased intrinsic clearance rate when compared to wild-type protein [16].

RBC, red blood cell; TG, thioguanine; TPMT, thiopurine S-methyltransferase.