

Clinical Pharmacogenomics of Thiopurine S-methyltransferase

Shufeng Zhou*

Department of Pharmacy, Faculty of Science, National University of Singapore, Singapore

Abstract: Thiopurine methyltransferase (TPMT) catalyzes the S-methylation of thiopurine drugs such as 6-mercaptopurine (6-MP), thioguanine and azathioprine (AZA). These drugs are used to treat conditions such as acute lymphoblastic leukemia, inflammatory bowel disease, rheumatoid arthritis, and organ transplant rejection. This review highlights the polymorphisms of *TPMT* gene and their clinical impact on the use of thiopurine drugs. To date, there are 18 known mutational *TPMT* alleles. The three main *TPMT* alleles, namely *TPMT* *2, *3A and *3C, account for 80 – 95% of the intermediate and low enzyme activity. The *TPMT* gene exhibits significant genetic polymorphisms among all ethnic groups studied. Patients who inherited very low levels of TPMT activity are at greatly increased risk for thiopurine-induced toxicity such as myelosuppression, when treated with standard doses of these drugs, while subjects with very high activity may be undertreated. Moreover, clinical drug interactions may occur due to TPMT induction or inhibition. Identification of the *TPMT* mutant alleles allows physicians to tailor the dosage of the thiopurine drugs to the genotype of the patient or to use alternatives, improving therapeutic outcome.

Keywords: Thiopurine methyltransferase, Thiopurine, Single nucleotide polymorphism (SNP), Genetic polymorphism, toxicity.

1. INTRODUCTION

Current concepts in drug therapy often attempt drug treatment of large patient populations as groups, irrespective of the potential for individual, genetically based differences in drug response [1]. It is well recognized that most medications exhibit wide interpatient variability in their efficacy and toxicity. Clinical pharmacogenomics is the study of how genetic basis affects variability in drug response [2-4]. The traditional pharmacogenetic approach relies on studying sequence variations in candidate genes that probably affect drug response. On the other hand, the advent of highly efficient and specific genomic technologies enables the search for relevant genes and their variants in the genome, and these new technologies have essentially spawned a new discipline, termed pharmacogenomics. Pharmacogenomics emphasizes the identification of the network of genes that govern drug response in individual patients using genome-wide approaches [2]. Moreover, pharmacogenomics analysis can identify disease susceptibility genes representing potential new drug targets. Numerous genes, in particular those encoding drug metabolizing enzymes, drug transporters and drug targets, have been identified to play a role in drug response and toxicity [2]. All of this will lead to novel approaches in drug discovery, individualized dosing of medications, and new insights into disease susceptibility and prevention [5].

The discovery that levels of thiopurine S-methyltransferase (TPMT, EC2.1.1.67) activity in human tissues are predominantly controlled by a common genetic polymorphism represents one of the best examples of the potential importance of clinical pharmacogenomics for rational pharmacotherapy. TPMT is a cytosolic enzyme that S-methylates thiopurine drugs such as azathioprine (AZA), 6-mercaptopurine (6-MP)

and thioguanine (TG) (Fig. 1), forming inactive methylated metabolites [6, 7]. AZA is converted mainly in the liver into

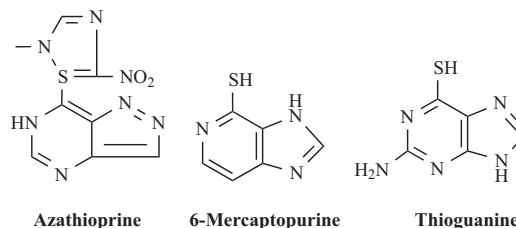


Fig. (1). Chemical structures of thiopurine drugs.

6-MP, possibly as a result of a glutathione-S-transferase catalyzed reaction [8]. Further conversion of 6-MP occurs in the liver and the gut. Thiopurines as prodrugs require multi step metabolic activation, which is initiated by hypoxanthine guanine phosphoribosyl transferase (HGPRT), resulting in cytotoxic thioguanine nucleotides (TGNs) [9], which subsequently incorporate into DNA and RNA (Fig. 2) [10, 11]. TGNs act as metabolic analogs and are responsible for both the immunosuppressive activity and the toxicity of AZA. On the other hand, S-methylation by TPMT or oxidation by xanthine oxidase inactivates thiopurines, generating 6-methylmercaptopurine and thiouric acid, respectively. In addition, 6-thioinosine 5' monophosphate (TIMP) is also S-methylated by TPMT to form methyl-TIMP, which can inhibit the *de novo* purine synthesis, and thus represents an alternative mechanism for cytotoxicity [11].

Thiopurine drugs have been widely used for the treatment of leukemia, autoimmune diseases and organ transplants. For example, oral 6-MP is routinely used in maintenance treatment of acute lymphoblastic leukemia in children, which contributes to the high cure rates achieved [12-14]. Thioguanine is indicated in the management of acute myeloid leukemia and childhood acute lymphoblastic leukemia. AZA is widely used for the treatment of

*Address correspondence to this author at the Department of Pharmacy, Faculty of Science, National University of Singapore, Science Drive 4, Singapore 117543, Singapore; Tel: 0065 6874 2931; Fax: 0065 6779 1554; E-mail: phazsf@nus.edu.sg

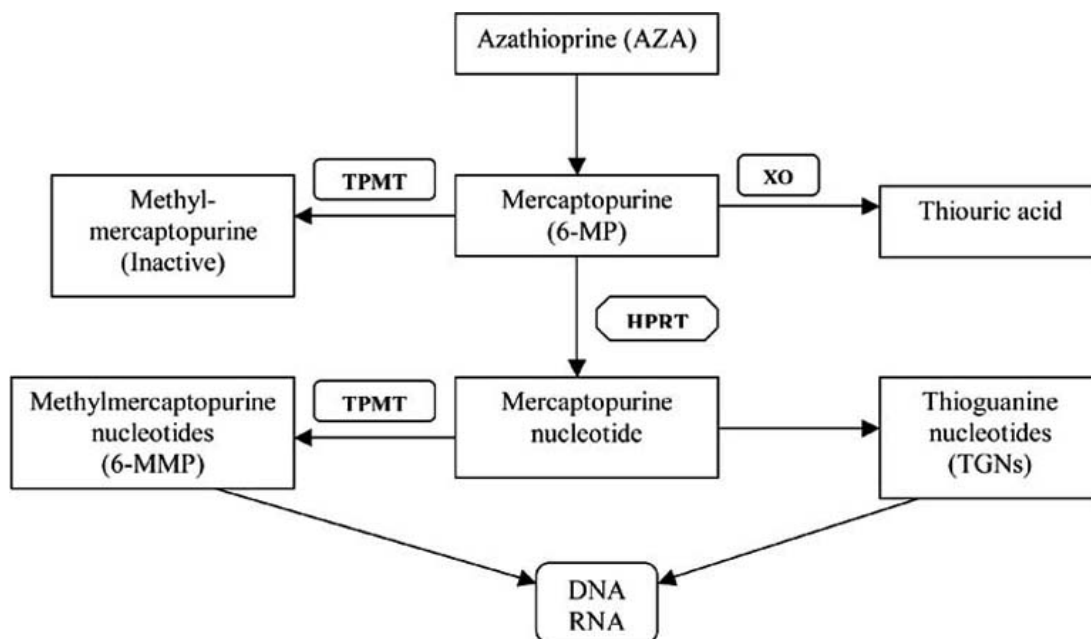


Fig. (2). Metabolism of thiopurine drugs. Azathioprine (AZA) is converted into 6-mercaptopurine (6-MP) which is subsequently metabolized by 3 pathways: the first *via* TPMT to form methyl-mercaptopurine, which is inactive; the second path is *via* oxidation to thiouric acid by xanthine oxidase (XO); and the third, catabolization to 6-thioguanine nucleotides (TGNs), which are cytotoxic when incorporated into the DNA/RNA, by hypoxanthine phosphoribosyl transferase (HPRT). TPMT is also involved in the conversion of mercaptopurine nucleotide to form 6-methylmercaptopurine nucleotides (6-MMP), which cause the inhibition of *de novo* purine synthesis leading towards cytotoxicity.

inflammatory bowel disease (IBD), autoimmune hepatitis, systemic lupus erythematosus (SLE), rheumatoid arthritis, dermatological conditions and organ transplantation [15, 16]. This review highlights the polymorphisms of TPMT and their impact on the clinical use of thiopurine drugs.

2. POLYMORPHISM OF TPMT GENE

2.1. Polymorphism of TPMT Activity

TPMT is a cytoplasmic transmethylase found in mammalian and avian species. Using S-adenosyl-L-methionine as a methyl donor, this enzyme catalyzes methylation of sulphur atoms in aromatic and heterocyclic compounds. No endogenous substrate is known for this enzyme and its principal biological role remains obscure. TPMT is present in most tissues such as heart, blood cells, placenta, pancreas and intestine. TPMT has a molecular mass of 28 kDa, and contains 246 amino acid residues and is not metal-dependent. The conventional method for monitoring TPMT activity in patients is based on a biochemical assay that measures the methylation of 6-mercaptopurine with [¹⁴C-methyl]-S-adenosylmethionine as the methyl donor. With this assay, erythrocytes are typically used as surrogate cells for drug metabolizing tissues, and a strong correlation between TPMT activity in erythrocytes and other tissues has been confirmed.

There is a large interindividual variability in the activity of TPMT. Caucasians show a trimodal distribution, with 89-94% possessing high enzyme activity, 6-11% intermediate activity due to heterozygosity at the TPMT locus and 0.33% low activity [17, 18]. However, a unimodal distribution of TPMT activity in the mainland Chinese was found [19-21].

Surprisingly, this is somewhat different from what has been reported in a population sample of Chinese adults from Singapore, which had a bimodal distribution in TPMT activity with a clear antimode [22]. The reason for such a difference in the mode of distribution is unknown, but may be related to the differences in the study populations used. It has been shown that differences in assay conditions can cause significant differences in TPMT activity measured in different laboratories [23].

Nongenetic factors could also affect TPMT activity. Patients that have received a recent red blood cell transfusion could get misleading results when TPMT activity is assessed from their erythrocytes. Thiopurine drugs have also been shown to induce TPMT activity, and patients on thiopurine drugs might have increased enzyme activity. The TPMT activity is induced by AZA treatment during the first month after transplantation [24]. On the other hand, some drugs (e.g., sulfasalazine and olsalazine) are potent inhibitors of TPMT, resulting in potential drug interactions (see Section 3.5).

There appear to be some gender differences in TPMT activity. In a Korean study, the TPMT activity was shown to be higher than the TPMT activity in females [25]. In another study of the Bulgarian population, a significant gender-related difference in TPMT activity ($P = 0.02$) was observed with 6.2% higher values in men than in women [26]. However, other studies indicate that there may be no significant gender difference in TPMT activity [27]. In a study which showed that the Saami population had a 29% higher TPMT activity than the Caucasian population,

Table 1. TPMT Mutant Alleles

TPMT Allele	Gene Mutations Involved	Amino Acid Substitutions	Ref.
*1	Wild-type		
*2	G238C	Ala80→Pro	[30]
*3A	G460A A719G	Ala154→Thr Tyr240→Cys	[31]
*3B	G460A	Ala154→Thr	[32]
*3C	A719G	Tyr240→Cys	[32]
*3D	G460A A719G G292T	Ala154→Thr Tyr240→Cys Glu98Stop	[35]
*4	G→A transition at the intron 9-exon 10 junction		[35]
*5	T146C	Leu49→Ser	[35]
*6	A539T transversion in exon 8	Tyr180→Phe	[35]
*7	T681G transversion in exon 10	His227→Glu	[43]
*8	G644A	Arg215→His	[44]
*10	G430C	Gly144→Arg	[41]
*11	G395A	C132→Y	[45]
*12	C374T	Ser125→Leu	[41]
*13	A83T	Glu28→Val	[41]
*14	A→G transition in the start codon (exon 3)	Met→Val	[40]
*15	G→A transition in the acceptor splice site in intron 7/exon 8 (IVS7 -1G→A)		[40]
*16	G488A	Arg163→His	[42]
*19	A365C	Lys122→Thr	[42]

differences in chronic diseases, drug consumption, age, or gender could not explain the interethnic difference in red blood cell TPMT activity [28]. Age does not seem to have any correlation with the level of TPMT activity [26].

2.2. Polymorphism of TPMT Gene

TPMT is encoded by a 34 kb gene consisting of 10 exons and 9 introns with a cDNA of ~3000 bp and an open reading frame of 735 bp that encodes a 245-amino acid peptide with a molecular mass of ~35 kDa [29]. TPMT gene is localized to chromosome 6p22.3. TPMT activity is inherited as an autosomal codominant trait with genetic polymorphism in all large populations studied to date [6, 7]. From molecular genetic and familial studies, more was learnt about the hereditary nature of TPMT deficiency in humans. Patients with intermediate activity are heterozygous at the TPMT gene locus and the TPMT deficient subjects are homozygous for low activity alleles. Altered TPMT activity predominantly results from single nucleotide polymorphisms. The wild-type

allele is designated as TPMT*1 and to date, at least 18 variant alleles of TPMT gene have been reported.

The common mutant alleles include TPMT*2 [30], TPMT*3A [31], TPMT*3B and TPMT*3C [32]. These four mutant alleles are detected in over 80-95% of Caucasians with low or intermediate TPMT activity [33]. The mutant allele TPMT*2 is defined by a single nucleotide transversion (G238C) in the open reading frame, leading to an amino acid substitution at codon 80 (Ala→Pro), resulting in a more than 100-fold reduction in the TPMT activity relative to wild type cDNA, despite a comparable level of mRNA [34]. The second and more prevalent mutant allele, TPMT*3A, contains two nucleotide transition mutations (G460A and A719G) in the open reading frame, leading to amino acid substitutions at codon 154 (Ala→Thr) and codon 240 (Tyr→Cys) [31]. When COS-1 cells expressed heterologously, TPMT*3A had > 200-fold lower TPMT activity and immuno-detectable protein compared to wild type cDNA. There was an enhanced rate of proteolysis of mutant TPMT proteins

Table 2. Frequencies of *TPMT* Variant Alleles in Different Ethnic Groups

Ethnicity	No. of Alleles	Frequency (%)			Ref.
		*3A	*3C	*6	
Chinese	400	0	3.0	0	[68]
Indian	400	0	2.3	0.3	[68]
Malay	400	0.5	0.8	0	[68]
Japanese	384	0	0.8	0	[85]
Chinese	384	0	2.3	0	[67]
Egyptian	400	0	0.3	1.3	[86]
Blacks					
African-American ^a	496	0.8	2.4	0	[38]
Ghanaian	434	0	7.6	0	[87]
Kenyan	202	0	5.4	0	[66]
Caucasians					
Caucasian-American ^a	564	3.2	0.2	0	[33, 38]
British	398	4.5	0.3	0	[67]
French	382	5.7	0.8	0	[6]
Italian	412	3.9	1.0	0	[88]
Norwegian	132	3.4	0.3	0	[89]
Saami-Norwegian	388	0	3.3	0	[89]

^a Calculated.

encoded by *TPMT**2 and *TPMT**3A alleles, with degradation half lives of 15 min for both mutant proteins compared with 18 hr for the wild type protein [34].

A number of rare mutant *TPMT* alleles (*TPMT**3D, *4, *5, *6, *7, *8, *10, *11, *12, *13, *14, *15, *16, and *19) have been identified [35-42]. *TPMT**4 has a G→A transition at the intron 9–exon 10 junction, which disrupts the final nucleotide of the intron at the 3' acceptor splice site sequence [35]. *TPMT**5 was identified as a T146C transition in a heterozygous individual and has intermediate *TPMT* activity [35]. This mutation results in a Leu→Ser amino acid substitution at codon 49. *TPMT**6 results in intermediate activity [35]. This A539T transversion in exon 8 results in a Tyr→Phe substitution at codon 180. *TPMT**7 results in intermediate activity [43]. This allele contains a T681G transversion in exon 10, which results in a His→Glu amino acid substitution at codon 227. *TPMT**8 contains a single nucleotide transition (G644A), leading to an amino acid change at codon 215 (Arg→His) [44]. This also resulted in intermediate enzyme activity. A few new missense mutations, *TPMT**10 (G430C, codon 144 Gly→Arg), *TPMT**12 (C374T, codon 125 Ser→Leu) and *TPMT**13 (A83T, codon 28 Glu→Val), were identified in the 10 exons of the *TPMT* gene when DNA samples from four leucopenic patients were screened by PCR analysis [41]. *TPMT**11 is a missense

mutation (G395A) in exon 6, resulting in an amino acid exchange C132Y with reduced enzyme activity [45]. *TPMT**14 and *TPMT**15 were recently reported [40]. *TPMT**14 contained an A→G transition in the start codon (exon 3, Met→Val), whereas *TPMT**15 had a G→A transition in the acceptor splice site in intron 7/exon 8 (IVS7 -1G→A) [40]. Both *TPMT**14 and *TPMT**15 resulted in the loss of enzyme activity. Recently, two novel missense mutations, *TPMT**16 (G488A, Arg163→His) and *TPMT**19 (A365C, codon 122 Lys→Thr) were identified in a Caucasian and a Moroccan patient, respectively [42]. The Lys122→Thr exchange did not significantly affect the intrinsic clearance value (V_{max}/K_m) of the variant enzyme, whereas the Arg163→His substitution significantly decreased the intrinsic clearance value by 3-fold. The frequencies and contributions of these alleles to reduced *TPMT* activity in different ethnic groups have not been defined yet.

Polymorphisms in the 5'-flanking promoter region of *TPMT* gene have also been identified due to a variable number of tandem repeats (VNTR) with three kinds of motifs (A, B, and C) differing by the length of the unit core and nucleotide sequence [7, 46-52]. Each repeat consists of 17 or 18 bp unit and contains a potential binding site for the transcription factor Sp1. The polymorphic tandem repeat within the 5'-flanking promoter region of the *TPMT* gene

Table 3. Drugs that can Interact with Thiopurine Drugs

Drugs	Usage	Mechanism of Interaction	Ref.
Olsalazine	Crohn's disease, ulcerative colitis	Inhibition of TMPT	[82]
Sulfasalazine	Rheumatic disease	Inhibition of TMPT	[81]
Aspirin	Analgesic, anti-platelet agent	Inhibition of TMPT	[80]
Disulfiram	Alcoholism	Inhibition of TMPT	[84]
Diuretics (furosemide, bendroflumethiazide, & trichlormethiazide)	Diuretics	Inhibition of TMPT	[83]

appears to participate in the regulation of level of erythrocyte TPMT activity [46]. Allele VNTR*6 was found to be consistently associated with decreased levels of TPMT activity in humans [52]. However, those effects are probably small in a quantitative sense [37]. A few recent studies demonstrated that the variable number tandem repeats (VNTR*3 to VNTR*9) had no significant impact on enzyme activity in British Asians and Caucasians [48, 49].

It has been shown that there might be a negative correlation between the variable number of tandem repeats within the 5'-flanking region of the *TPMT* gene and the level of TPMT activity. TPMT VNTR length varied from three to nine repeats (*V3 to *V9), but the most commonly occurring were *V4 and *V5. The lowest levels of TMPT activity were found with genotypes that included an allele with more than 5 repeat elements. The *V4/*V5 were associated with higher activity levels than *V4/*V4 and *V5/*V5 [46]. In another study, the *V6 was found to be consistently associated with decreased levels of TPMT activity [52]. However, the effect of VNTR length may not be as drastic as TPMT deficiency caused by genetic mutations. The mechanistic effect of VNTR on TPMT activity remains to be elucidated.

2.3. Studies of Genotype-Phenotype Relationship

Both TPMT activity measurement and genotyping methods can be used to diagnose TPMT deficiency [53]. A simple activity assay by HPLC or radiochemical methods would allow the identification of "rapid" or "slow" metabolizers. Proper dose adjustment is needed for "rapid" metabolizers and they should be treated with an alternative therapeutic agent if drug resistance is highly possible [54, 55], whereas dose reduction is certainly necessary for avoiding toxicity in "slow" or deficient metabolizers who are intolerant to thiopurine therapy. However, the standard activity assay is associated with a number of significant limitations. For example, this method cannot be conducted on patients who have received a blood transfusion, because the donor erythrocytes may affect the result [56]. On the other hand, genotyping methods can reliably detect the major and rare mutant allele at human *TPMT* locus, in particular when genetic polymorphism is highly likely to provide an explanation for TPMT deficiency in individuals [53]. To date, it has become possible to detect TPMT inactivating mutations with more than 95% concordance between genotype and phenotype [56].

About 1/300 of the population who inherit complete TPMT deficiency as an autosomal recessive trait, if treated with standard doses of thiopurines, will accumulate excessive TGNs in hematopoietic tissues, leading to severe hematological toxicity that can be fatal. However, TPMT-deficient patients can be successfully treated with a 10- to 15-fold lower dosage of these medications. The molecular basis for altered TPMT activity has been defined, with rapid and inexpensive assays available for the three signature mutations which account for the majority of mutant alleles. TPMT genotype correlates well with *in vivo* enzyme activity within erythrocytes and leukemic blast cells and is clearly associated with the risk of toxicity.

TPMT polymorphisms have been associated with the therapeutic efficacy and toxicity of thiopurine drugs. The impact of 6-mercaptopurine dose intensity is also being clarified as an important determining factor of event-free survival in childhood leukemia. Acute lymphoblastic leukemia patients with at least one mutant *TPMT* allele tend to have an improved response to mercaptopurine therapy and better chances of being cured, compared with patients who have two wild-type *TPMT* alleles [57, 58]. Patients with inherited low levels of TPMT activity are at greatly increased risk for thiopurine-induced toxicity such as myelosuppression, when treated with standard doses of these drugs, and require doses to be reduced to as little as a tenth of the normal dose in order to tolerate therapy [57, 59, 60]. The mutation, however, might be a double-edged sword, as a reduced TPMT expression will increase the risk of developing a thiopurine-related second tumor, including brain tumors and acute myelogenous leukemia.

In this regard, a number of studies have indicated that there is a significant negative correlation between the intracellular concentration of TGNs and TPMT activity in erythrocytes [57, 61], and that the TGN concentrations are associated with the efficacy and toxicity of thiopurines in various diseases [58, 62-65].

2.4. Interethnic Comparison of *TPMT* Polymorphism

The pattern and frequency of mutant TPMT alleles is different among various ethnic populations [23]. The most prevalent TPMT mutant allele in the Caucasian and Latin-American population is *TPMT**3A [33, 66], while *TPMT**3C is predominant in Chinese, Egyptians and African-Americans [38, 67]. For the Caucasian, Black and Latin

American populations, trimodal or bimodal distributions have been largely observed. For the East Asian and Israel populations, studies showed that a unimodal distribution was observed.

The genetic basis and molecular mechanisms for inherited differences in TPMT activity have recently been elucidated in healthy Chinese newborns ($n = 200$) and compared with other ethnic groups including Malays ($n = 200$) and Indians ($n = 200$) in Singapore [68]. In the cord blood study, the *TPMT*3C* variant was detected in all three ethnic groups; Chinese, Malays, and Indians had allele frequencies of 3%, 2.3%, and 0.8%, respectively. The *TPMT*3A* variant was found only among the Indians at a low allele frequency of 0.5%. The *TPMT*6* variant was found in one Malay sample. Among 100 children with acute lymphoblastic leukemia, two whites and one Chinese were heterozygous for the *TPMT*3A* variant and showed intermediate sensitivity to 6-mercaptopurine during maintenance therapy [68]. Three Chinese patients and one Malay patient were heterozygous for the *TPMT*3C* variant. Mercaptopurine sensitivity could be validated in only one out of four *TPMT*3C* heterozygous patients. The overall allele frequency of the TPMT variants in this multiracial population was 2.5%. The *TPMT*3C* was the most common variant allele, whereas the *TPMT*3A* and *TPMT*6* were rare in these Asian populations.

3. CLINICAL SIGNIFICANCE OF TPMT POLYMORPHISM

3.1. Cancer Therapy

Oral 6-MP is used as maintenance therapy for childhood acute lymphoblastic leukemia. Chemotherapy in childhood acute lymphocytic leukemia often involves administering 6-MP dose that is close to the maximum tolerable dose. High TGN levels in the erythrocytes correspond to the levels of leucopenia achieved and hence a good therapeutic response, whereas low TGN levels correspond to the risk of relapse. Hence it is important to identify whether the patient is an intermediate or deficient metabolizer and correspondingly reduce the doses to avoid hematopoietic toxicity.

It is found that only higher dose intensity of 6-MP for acute lymphocytic leukemia is a significant predictor of event-free survivor. Lower TPMT activity is associated with better outcome. Increasing the dose intensity in children with homozygous *TPMT* wild-type alleles would increase the event-free survivor rate amongst these patients, but the dose increase should not be too great that it should cause neutropenia, which is the worst clinical outcome [58].

Generally, TPMT-deficient patients or low methylators (homozygous mutant or compound heterozygote) can be treated with 6–10% of the standard dose (i.e. 10- to 15-fold decrease of standard dose), while patients with heterozygous phenotypes / genotypes can be treated with 65% of the standard dose (i.e. 2-fold decrease of standard dose). If patients are clearly tolerating these doses without toxicity, it is desirable to carefully increase the dose to avoid sub-therapeutic drug exposure. Nevertheless, prospective validation for each disease indication is required before this

approach can be recommended for broad application to the thiopurine therapy.

Influence of *TPMT* genotype is most dramatic for homozygous mutant patients but is also of clinical relevance for heterozygotes. Besides acute toxicity, TPMT activity and subsequent TGN level is also an important determinant of delayed toxicity, e.g. event-free survival after extensive 6-MP therapy for childhood acute lymphocytic leukemia. Patients with increased TPMT activity (low TGN concentrations in erythrocytes) are more prone to relapse after standard MP therapy. Care should also be taken as higher intensity of pulse chemotherapy during the consolidation phase may affect bone marrow reserve and unmask the influence of a heterozygous *TPMT* genotype on 6-MP myelosuppression. TPMT genotype may also affect the risk of developing secondary malignancies, e.g. TPMT-deficient or heterozygous patients treated with 6-MP while receiving cranial irradiation for brain leukemia are at a higher risk for developing secondary brain tumors.

3.2. Inflammatory Bowel Disease (IBD) and Crohn's Disease

In the treatment of corticosteroid-dependent or -resistant IBD, AZA and 6-MP are used widely for induction and maintenance of remission. In a prospective study of 92 pediatric patients with IBD, higher TGN concentration due to a *TPMT* mutation was associated with better therapeutic response regardless of other potential influencing factors [69, 70]. Although leucopenia was associated with higher TGN levels, it was observed in only 1/13 of responders [69]. Hence, individuals with lower TPMT activity may benefit from better therapeutic efficacy than individuals with high TPMT activity [71].

Heterozygous TPMT genotypes did not predict adverse reactions of AZA, but were significantly associated with a subgroup of IBD patients experiencing nausea and vomiting [72]. Adverse drug reactions to AZA occur in 15% to 28% of patients and the majority is not explained by TPMT deficiency. However, a recent pharmacogenetic study indicated that the adverse reaction to AZA in patients may be associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase) in 62 patients with IBD [72]. The ITPA 94C→A allele resulted in low of deficient ITPase and was significantly associated with adverse AZA reactions such as flu-like symptoms, rash and pancreatitis. The reason for such association is not clear. It is known that ITPase deficiency results in the benign accumulation of the inosine nucleotide ITP. 6-MP, the metabolite of AZA, is activated through a 6-thio-IMP intermediate and potentially toxic 6-thio-ITP is likely to accumulate in ITPase deficient patients and thus cause toxicity.

A clinical study in patients with Crohn's disease found that only 27% had mutant TPMT alleles associated with enzyme deficiency [73]. However, the delay between administration of AZA and occurrence of myelosuppression was less than 1.5 months in the 4 patients with 2 mutant *TPMT* alleles, and ranged from 1 to 18 months in patients with 1 mutant allele and from 0.5 to 87 months (median

13.7) in patients with normal genotype [73]. Myelosuppression is more often caused by other factors such as drug combination and concomitant viral infection.

Alternative immunosuppressive drugs, particularly 6-TG, should be considered for AZA-intolerant patients. 6-TG has a less genetically controlled metabolism and skips genetically determined metabolic steps. Theoretically, 6-TG might therefore have a more predictable profile than AZA and 6-MP [74]. However, the use of 6-TG has been associated with an increased risk of nodular regenerative hyperplasia of the liver and veno-occlusive disease. Further study is warranted before 6-TG can be considered as a treatment option for inflammatory bowel disease.

3.3. Rheumatoid Arthritis and Systemic Lupus Erythematosus (SLE)

In a clinical study, six patients (9%) with AZA treatment experienced adverse reactions to the drug [75]. Of these 6 patients with adverse side effects, three were heterozygous for the *TPMT*3A* allele and developed severe nausea and vomiting. Patients with wild type TPMT received AZA therapy for a median of 39 weeks compared with a median of 2 weeks in patients with mutant alleles [75]. These results indicate that TPMT activity and genotypes should be monitored, so that adequate doses would be given to patients that have the normal *TPMT* alleles and the dose be reduced or discontinued for those with deficient *TPMT* alleles so as to avoid adverse reactions. In a group of Japanese patients with rheumatic disease, all three patients with *TPMT*3C* discontinued AZA therapy due to severe leucopenia, but only four wild type patients (12%) experienced leucopenia [76].

Mutant TPMT alleles were identified in seven patients out of 120 (5.8%) unselected patients with treated with AZA for SLE [77]. Severe marrow toxicity occurred in the single homozygote identified. AZA was generally well tolerated, but 11 drug-associated neutropenias were detected. One of the 11 patients with *TPMT*3A* developed severe myelosuppression, while three patients with heterozygous mutations tolerated AZA therapy [77]. It appears that TPMT genotype does not explain the observed myelosuppression in SLE patients treated with AZA.

AZA is used to treat autoimmune rheumatic disorders such as rheumatoid arthritis and SLE. Patients under such drug regimens showed varied responses; some underwent clinical remission due to unsatisfactory response while others suffered from severe toxicity. However, AZA is seldom used to treat rheumatoid arthritis nowadays, mainly due to lack of response to the drug probably due to low dose regimens for safety reasons and the development of severe adverse drug effects such as severe nausea and vomiting. TPMT genotyping could allow higher doses of AZA to be used to treat patients homozygous for the wild type allele.

3.4. Organ Transplantation

If treated with AZA, TPMT polymorphism may affect rejection of transplanted organ. High TPMT activity is associated with an increased risk of rejection [65]. This is also observed in adult kidney transplant recipients [78].

Higher TPMT activity may accelerate the catabolism of AZA, resulting in less TGNs.

However, when TPMT is induced following long-term AZA therapy, less acute rejection episodes were observed in patients with renal allografts, compared to the patients whereby TPMT activity was not induced [79]. Among TPMT inducitors, an acute rejection episode was observed in 34% of the patients versus 69% among non-TPMT inducitors ($P = 0.002$). TPMT activity induction was observed in 57% of renal transplant recipients who received AZA. These results indicate that TPMT induction by AZA and/or its metabolite 6-MP is associated with better graft outcome. The appropriate conversion from AZA, which is a pro-drug, into 6-MP could explain both better graft outcome and TPMT induction.

3.5. Drug Interactions

TPMT activity can potentially influence a number of drugs that could be co-administered with thiopurine drugs (Table 3) [80]. For example, aspirin within therapeutic doses can lead to the inhibition of TPMT. Also, sulfasalazine and its metabolite 5-aminosalicylic acid inhibited TPMT [81]. Sulphasalazine as well as 3-, 4- and 5-aminosalicylic acid inhibited recombinant human TPMT, with IC_{50} values of 78, 99, 2600 and 1240 μ M, respectively. Olsalazine and olsalazine-O-sulfate are potent noncompetitive inhibitors of recombinant human TPMT, and there was a case report where a patient with refractory Crohn's disease had two separate episodes of bone marrow suppression while receiving 6-MP and olsalazine [82]. The diuretics furosemide, bendroflumethiazide and trichlormethiazide had inhibitory effect on TPMT, with IC_{50} values of 170, 360 and 1000 μ M, respectively [83]. TPMT S-methylates the diethyldithiocarbamate metabolite involved in disulfiram activation and could affect disulfiram treatment of alcoholics [84]. TPMT activity can potentially affect the levels of drugs in drug interactions. It remains to be investigated if genetic differences lead to a different susceptibility to these drug interactions.

4. Conclusions and Future Perspectives

Pharmacogenetics studies the great heterogeneity in the way individuals respond to medication, in terms of efficacy and also adverse reactions. Although polymorphism is known to be the underlying reason and influence for pharmacodynamics and pharmacokinetics of a variety of drugs, it is only in recent years that new genetic technology has enabled us to draw correlations between genetics and drug response. The usefulness of prospective determination of functional TPMT status to prevent 6-MP and AZA toxicity in patients with childhood leukemia, rheumatic diseases, IBD and in transplant recipients is becoming increasingly recognized. With future quantification of the predictive power of TPMT genotype for preventing toxicity and determination of specific dosage recommendations for thiopurine drugs in the various disease areas and with further defining of data on TPMT genotype being associated with secondary malignancies, these molecular diagnostics will become more widely used. The end result will be the optimal selection of medications and their dosages based on the individual and not treatment based on the average patient.

The frequency and pattern of mutant TPMT alleles are different among various ethnic populations. More extensive research is to be conducted amongst the different ethnic populations as different dosage range is required for the various ethnic groups. In particular, Asians appear to have low levels of TPMT variants and thus dosage adjustment is needed in patients who have inherited with these mutations. The clinical relevance of the variation found in TPMT genotype on the therapeutic efficacy of thiopurine drugs now needs to be evaluated in different ethnic groups to facilitate the use of molecular based assays to guide therapeutics. Hence, the widespread use of thiopurines as antileukemic agents and immunosuppressive therapy, and the potential for fatal toxicities in TPMT-deficient patients who do not have a substantial reduction in their dosage of these medications, underscore the importance of fully elucidating the molecular mechanisms of this genetic polymorphism in drug metabolism.

The number of clinically important applications of TPMT molecular genetics is constantly increasing, from the initial application of TPMT polymorphism screening in ALL patients to prevent toxicity, to current interest emerging in TPMT phenotyping/genotyping of transplant patients, patients with IBD, Crohn's disease, SLE, or other autoimmune diseases. Of note, there is no known phenotype for TPMT deficient patients unless they are treated with thiopurine medications, and we have no clue about what biological function this enzyme might have.

Clinical studies involving thiopurine drugs showed that an average of 78% of the adverse side effects seen in patients heterozygous for the TPMT gene were not due to TPMT polymorphisms. Thus, caution must be employed towards dose increases in patients who are homozygous for the wild type alleles, as there could be toxicity due to accumulation of toxic by-products of the S-methylation of the thiopurine drugs. Importantly, polymorphisms in several other enzymes involved in the disposition of thiopurines may also contribute to the toxicity and altered clinical responses. It is now well documented that drug responses are the result of polygenic interactions instead of monogenic. Considering TPMT pharmacogenetics alone may not be sufficient to explain the differences in drug responses in patients, polygenic interactions in the thiopurine metabolic pathway may be addressed in the future studies.

Despite significant advances in the research of genetic polymorphism of TPMT, many issues important to the biological functions and the therapeutic implications of this enzyme remain to be addressed. For example, there are still some unexplained variations in response to thiopurine drugs. Hence, clinicians still have to monitor the patient carefully for signs of adverse drug reactions. There may be additional molecular genetic mechanisms that participate in regulating levels of TPMT activity that has yet to be discovered and the question of additional 5'-flanking region variants beyond the polymorphic VNTR that presently known remains unanswered. Finally, the issue of the mechanism responsible for the induction of TPMT activity during long-term therapy of patients remains to be addressed. Therefore, our current level of understanding of TPMT molecular biology is far

from complete. The translation of pharmacogenetic information into useful, cost-effective and practical clinical reality holds huge potential for our future. The acceleration of the discovery process as well as such a translation would likely be the primary challenge for us in the near future.

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ABBREVIATIONS

AZA	=	Azathioprine
HGPRT	=	Hypoxanthine guanine phosphoribosyl transferase
HPRT	=	Hypoxanthine phosphoribosyl transferase
IBD	=	Inflammatory bowel disease
ITPase	=	Inosine triphosphate pyrophosphatase
6-MP	=	6-mercaptopurine
SLE	=	Systemic lupus erythematosus
TG	=	Thioguanine
TIMP	=	6-thioinosine 5' monophosphate
TPMT	=	Thiopurine S-methyltransferase
VNTR	=	Variable number of tandem repeat

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