Pharmacogenetics of Thiopurine S-Methyltransferase and Thiopurine Therapy

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Abstract: Most medications exhibit wide interpatient variability in their efficacy and toxicity. For many medications, these interindividual differences result in part from polymorphisms in genes encoding drug-metabolizing enzymes, drug transporters, and/or drug targets (eg, receptors, enzymes). Pharmacogenomics is a burgeoning field aimed at elucidating the genetic basis of differences in drug efficacy and toxicity, using genome-wide approaches to identify the network of genes that govern an individual's response to drug therapy. For some genetic polymorphisms, such as thiopurine S-methyltransferase (TPMT), monogenic traits have a marked effect on the pharmacokinetics of medications, such that individuals who inherit an enzyme deficiency must be treated with markedly different doses of the affected medications (eg, 5–10% of the standard thiopurine dose). This review uses the TPMT polymorphism and thiopurine therapy (eg, azathioprine, mercaptopurine) to illustrate the potential of pharmacogenomics to elucidate genetic determinants of drug response, and optimize the selection of drug therapy for individual patients.

Key Words: pharmacogenomics, thiopurine S-methyltransferase, thiopurine

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It is clear that patients respond differently when treated with the same medication, and such differences are often considerably greater across a population of patients than within the same person (or between monozygotic twins).[1] This indicates that a component of interindividual differences in drug response is inherited; indeed, genetics can account for 20–95% of variability in drug disposition and effects.[2] There are now numerous examples where interindividual differences can be attributed, at least in part, to polymorphisms in genes encoding drug-metabolizing enzymes, drug transporters, and/or drug targets.[3–5] It is also clear that many nongenetic factors influence the effects of medications, including age, organ function, concomitant therapy, drug interactions, and the nature and severity of disease. However, inherited determinants of drug response remain stable for an individual's lifetime, and the effects can be profound.

Clinical observations of inherited differences in drug effects were first documented in the 1950s, giving rise to the field of "pharmacogenetics," which has now been rediscovered by a broader spectrum of academia and industry, giving birth to "pharmacogenomics." Pharmacogenomics aims to elucidate the genetic basis for interindividual differences in drug response, using genome-wide approaches to identify genetic polymorphisms that govern an individual's response to specific medications.

The initial reports from the human genome project described over 1.4 million single nucleotide polymorphisms (SNPs) in the human genome,[6] with over 60,000 of these in the coding region of human genes, and the number of SNPs continues to grow as more humans are studied. Some of these SNPs have already been associated with significant changes in the metabolism or effects of medications and are beginning to make their way into clinical medicine as molecular diagnostics.[3–5] Because most drug effects are determined by the interplay of several gene products that determine the pharmacokinetics and pharmacodynamics of medications, pharmacogenomics is increasingly focused on polygenic determinants of drug effects.[5] The human genes involved in many pharmacogenetic traits have now been identified, their molecular mechanisms defined, and their clinical importance more clearly elucidated.[3–5] This review uses the genetic polymorphism of thiopurine S-methyltransferase (TPMT) and its influences on thiopurine therapy to illustrate the potential of pharmacogenetics to provide a powerful set of molecular diagnostics to individualize and optimize drug therapy.

GENETIC POLYMORPHISMS OF DRUG METABOLISM: THE TPMT EXAMPLE

There are more than 30 families of drug-metabolizing enzymes in humans,[3] and essentially all have genetic variants,

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many of which translate into functional changes in the proteins encoded. One of the most developed examples in pharmacogenetics is the genetic polymorphism of thiopurine methyltransferase (TPMT). TPMT catalyzes the S-methylation (inactivation) of azathioprine, mercaptopurine, and thioguanine,[7–9] medications that are widely used to treat leukemia, inflammatory bowel diseases, severe rheumatic diseases, and for immunosuppression following solid organ transplantation. TPMT represents the predominant inactivation pathway of thiopurines in hematopoietic cells; thus, patients who inherit TPMT deficiency accumulate excessive concentrations of the active thioguanine nucleotides in blood cells when treated with conventional doses of these medications.

TPMT activity exhibits genetic polymorphism; approximately 90% of individuals inherit high activity, 10% have intermediate activity because of heterozygosity, and 0.3% have low or no detectable enzyme activity because they inherit 2 nonfunctional TPMT alleles (Fig. 1).[10] Numerous studies have shown that TPMT-deficient patients are at high risk for severe, and sometimes fatal, hematologic toxicity,[11–15] whereas patients who are TPMT heterozygotes have an intermediate risk of hematologic toxicity.[16,17] Of note, hepatotoxicity has not been linked to TPMT activity,[15] probably because there are alternative inactivation pathways in hepatic tissue (eg, xanthine oxidase). As depicted in Figure 1, patients who inherit 2 nonfunctional variant TPMT alleles should be treated with 5–10% of the standard dose of thiopurines, whereas heterozygous patients can usually start on full doses but have a significantly higher probability of requiring a dose reduction to avoid toxicity (compared with TPMT homozygous wild-type patients). TPMT deficiency has also been associated with a higher risk of irradiation-induced brain tumors in patients treated concomitantly with thiopurines and CNS irradiation.[18] The molecular basis for polymorphic TPMT activity has now been defined (see below), and TPMT genotyping is now available as a CLIA-certified molecular diagnostic from reference laboratories (eg, *http://www.prometheuslabs.com*).

TPMT activity is inversely related to the accumulation of TGNs in hematopoietic cells and to thiopurine hematopoietic toxicity.[19] Mercaptopurine and thioguanine, their nucleosides, and the principal nucleotide metabolites (ie, thioinosine monophosphate and thioguanosine monophosphate) are all substrates for human TPMT.[20] The product of TPMT-catalyzed methylation of thioinosine monophosphate, methylthioinosine monophosphate, is an inhibitor of de novo purine biosynthesis; therefore, methylation of mercaptopurine nucleotide metabolites may contribute to its pharmacologic effects.[21,22]

Genetic Polymorphism and Variant Alleles of TPMT

The initial finding of inherited differences in TPMT activity in humans[7] has now been defined at the molecular level

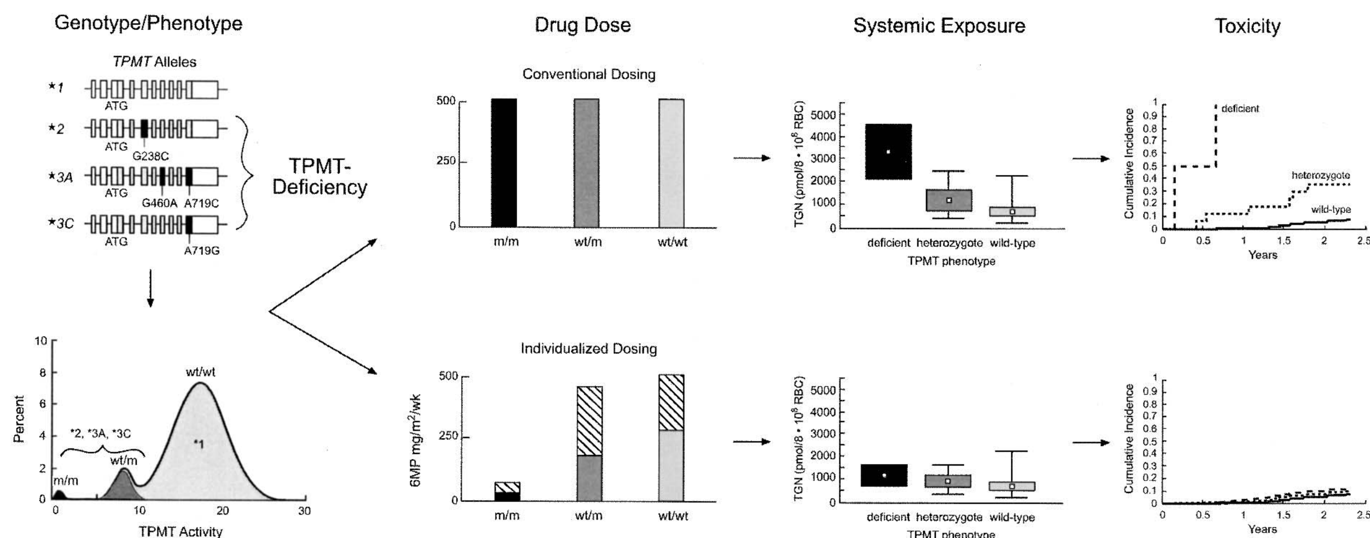

FIGURE 1. Genetic polymorphism of thiopurine methyltransferase and its role in determining response to thiopurine medications (azathioprine, mercaptopurine, thioguanine). The left panels depict the predominant TPMT mutant alleles causing autosomal codominant inheritance of TPMT activity in humans. As depicted in the subsequent top 3 panels, when uniform (conventional) dosages of thiopurine medications are administered to all patients, TPMT-deficient patients accumulate markedly higher (10-fold) cellular concentrations of the active thioguanine nucleotides (TGN), and heterozygous patients accumulated about 2-fold higher TGN concentrations, translating into a significantly higher frequency of hematopoietic toxicity (far right panels). As depicted in the bottom 3 panels, when genotype-specific dosing of thiopurines is administered, comparable cellular TGN concentrations are achieved, and all 3 TPMT phenotypes can be treated without acute toxicity. Reproduced with permission from Evans, *Pharmacogenetics* 2002;12:421–423.

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by the identification of inactivating mutations in the human TPMT gene.[10,23–30] The first identified variant allele, *TPMT*2,* contains a 238G→C transversion leading to the substitution of a rigid proline for a more flexible alanine residue (Ala80Pro). As a result, the tertiary structure of the TPMT protein is changed, leading to protein instability and decreased catalytic activity.[23]

The second variant allele isolated, *TPMT*3A,* contains 2 transition polymorphisms,[24] one in exon 7(460G→A) and the other in exon 10 (719A→G), each of which causes an amino acid change, whereas *TPMT*3C* contains only the single transition in exon 10 (719A→G).[27] Consequences of these amino acid substitutions in proteins encoded by the *2, *3A, *3B, and *3C alleles have been extensively characterized in both in vitro and in vivo experiments, revealing enhanced proteolysis of the variant proteins.[31,32] *TPMT*3A* is the most prevalent mutant allele in whites,[10,26,33] whereas *TPMT*3C* is the predominant TPMT mutant allele in Asian,[34] African,[33] and African-American[29] populations.

Currently, approximately 11 variant alleles have been associated with low TPMT enzymatic activity in humans. Similar to variant alleles of other polymorphic enzymes, these alleles contain SNPs leading to amino acid substitutions (*TPMT*2, *3A, *3B, *3C, *3D, *5, *6, *7, *8*), formation of a premature stop codon (*TPMT*3D*), or destruction of a splice site (*TPMT*4*). Furthermore, based on studies in numerous world populations to date,[9] *TPMT*3C*, **3A,* and **2* are the predominant variant alleles, accounting for over 95% of inherited TPMT deficiency, with the other variant alleles having been observed in very few individuals (in some cases, in only a single person, suggesting that the variant nucleotide may be a mutation and not a true polymorphism).

TPMT Assay and Clinical Genotyping Methods

Erythrocytes are typically used as surrogate cells for measuring TPMT activity in humans, and a strong correlation has been established between TPMT activity in erythrocytes and other tissues, including leukemia cells.[35–38] It should be noted that in patients who have received an RBC transfusion within 30–60 days, the TPMT activity in erythrocytes can be spuriously altered if a deficient or heterozygous patient has been transfused with blood from a homozygous wild-type person. This is illustrated by one of the TPMT-deficient patients treated St. Jude Children's Research Hospital (genotype TPMT*3A/*3A), who had a TPMT activity of 9.8 U/mL pRBC 12 days after receiving 2 units of packed erythrocytes, compared with undetectable activity 4 months after the heterologous erythrocyte transfusion. Because it is not uncommon for newly diagnosed leukemia patients or organ transplant recipients to receive allogeneic erythrocyte transfusions, molecular genetic methods have been developed that are not affected by donor erythrocytes, providing a more robust method to diagnose patients with TPMT-deficiency or heterozygosity.

Several methods have been developed for determining TPMT genotype, involving amplification of genomic DNA by PCR (or RT-PCR of mRNA) around the functionally important SNPs. Detection of individual SNPs is then achieved by a variety of methods, including RFLP, allele-specific amplification, direct sequencing, SSCP, or DHPLC analysis.[10,26,28,39–41] DNA-based genotyping offers a clinically important strategy to prospectively diagnose TPMT deficiency and minimize the risk of potentially life-threatening hematopoietic toxicity in patients treated with these medications.[10,17,42,43] Genotyping for the 3 most common TPMT SNPs (238G→C, 460A→G, 719G→A) provides a molecular diagnostic with >95% concordance between TPMT genotype and phenotype. This level of concordance has now been achieved in different populations at several institutions,[10,44,45] demonstrating the feasibility of genotyping for this genetic polymorphism, although it must be recognized that other rare SNPs exist and additional mechanisms of variable TPMT activity remain to be elucidated.[45–47]

Molecular Haplotyping of TPMT Alleles and Population Studies

Detection of individual SNPs within the TPMT coding region is a well-established and robust procedure, but certain combinations of alleles present a source of potential ambiguity with conventional genotyping methods. As noted above, the most common nonfunctional variant allele of TPMT in whites is *TPMT*3A*, with the heterozygous genotype **1/*3A* found in about 10% of whites. Individuals with this genotype have 1 active *TPMT* allele, and their risk of thiopurine hematopoietic toxicity is greater than that of patients who have a homozygous wild-type TPMT genotype (~35% versus ~7%), but not as great a risk as those who have 2 nonfunctional TPMT alleles (~100%).

Alternatively, individuals with the **3B/*3C* genotype, who are compound homozygotes, are TPMT-deficient and thus are at a very high risk of thiopurine toxicity. Although the **3B* allele is very rare (probably <1% of variant alleles), it has been reported by several investigators; thus, the **3B/*3C* genotype cannot be completely ruled out when individuals are heterozygous for both the 460G→A and 719A→G SNPs. Using the conventional PCR genotyping analysis, it is not possible to distinguish between the **1/*3A* and **3B/*3C* genotypes, posing a potential diagnostic problem.[48]

To resolve the ambiguity of conventional genotyping methods, we developed a molecular haplotyping method using genomic DNA, which permits determination of SNPs located kilobases apart.[49] This method uses long-range PCR to amplify regions of genomic DNA that include distantly spaced SNPs of interest at the opposite ends of the generated fragments, then intramolecular ligation (circularization) brings these SNPs into close physical proximity, followed by the use of RFLP, allele-specific amplification (ASA), or allele-

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specific oligonucleotide ligation (ASO) analysis to determine whether the SNPs reside on the same or different alleles (Fig. 2). Using this new haplotype-specific method of detecting TPMT SNPs, it is possible to discriminate the *TPMT*1/*3A* genotype from the **3B/*3C* genotype using genomic DNA, without cloning, physical separation of chromosomes or sophisticated instrumentation. However, many investigators simply assume that patients who are heterozygous at both the 460 and 719 nucleotide positions have the SNPs on the same allele and are thus a **1/*3A* genotype because the *3B allele is so rare (probability that this would be a **3B/*3C* genotype is estimated to be only 1 in 120,000).

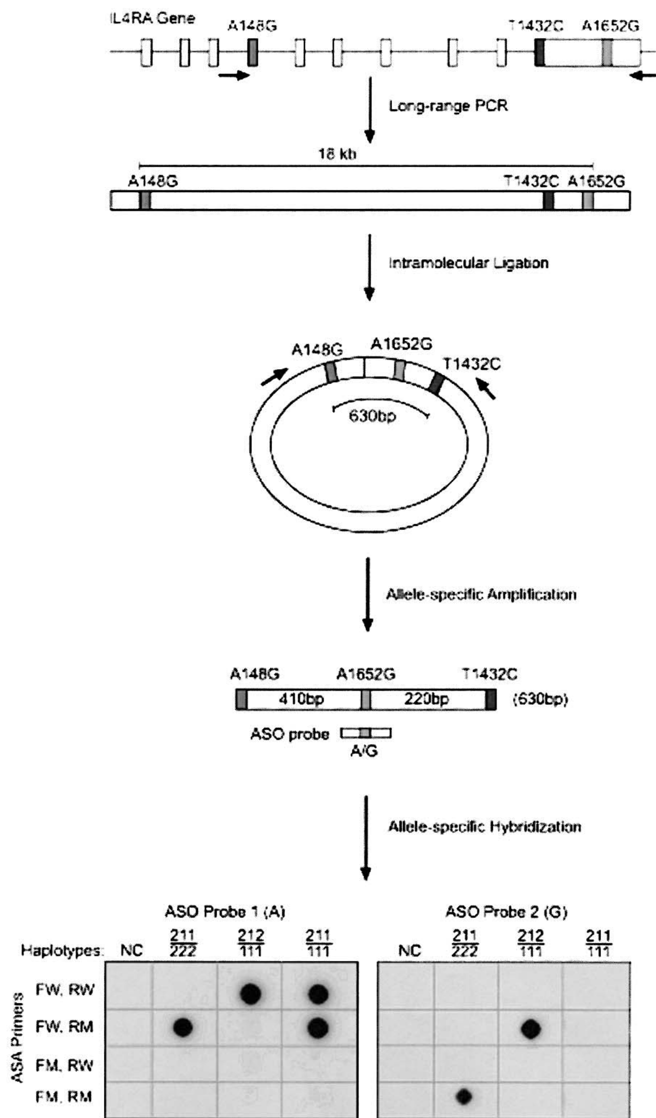

FIGURE 2. Strategy to haplotype 3 single-nucleotide polymorphisms (SNPs) in a 13-kb region at the human *TPMT* gene locus. Results of allele-specific amplification (ASA)/RFLP analyses for all of the possible haplotype combinations are shown at the bottom. Allele-specific primers discriminate the 460G→A (4W, 4M) and 238G→C (2W, 2M) SNPs. The presence of a mutant 719G in the polymerase chain reaction product introduces an *AccI* restriction site. Undigested products (850 bp) and digested products (600 bp and 250 bp) indicate the presence or absence of the 719 SNP. ASA primer combinations are 4W, 2W in lane 1; 4W, 2M in lane 2; 4M, 2W in lane 3; and 4M, 2M in lane 4, as we have described elsewhere.[49] Boxes denote exons, with color boxes denoting exons containing SNPs. Reproduced with permission from McDonald et al, *Pharmacogenetics* 2002;12:93–99.

CONCLUSIONS

The TPMT genetic polymorphism serves as a good example of the potential importance of pharmacogenetics because it has been well characterized at the molecular, biochemical, and clinical levels.[8] Its importance is readily appreciated because the effect of this genetic polymorphism is very highly penetrant when TPMT-deficient patients are treated with standard doses of one of the affected thiopurine medications (ie, essentially 100% of TPMT-deficient leukemia patients develop hematopoietic toxicity). Interestingly, the natural substrate for TPMT is not known, although the absence of a detectable phenotype (unless patients are treated with thiopurines) suggests that TPMT is not involved in an essential pathway for endogenous substrates. This does not preclude TPMT from playing an important role in the metabolism of additional environmental substrates beyond thiopurine medications, but few such environmental substrates have been discovered to date. However, it is clear that the genetic polymorphism of TPMT plays a critical role in the metabolism of widely prescribed thiopurine medications, and this is now well recognized as the mechanism for excessive toxicity of these medications in patients who inherit a deficiency in this metabolic pathway. Knowledge of the genetic basis for this inherited pharmacogenetic trait has allowed the development of new molecular diagnostics for identifying patients who need alternative doses of these medications and who are likely to benefit from careful therapeutic monitoring to guide treatment. The availability of CLIA-certified TPMT genotyping from clinical reference laboratories (eg, Prometheus Labs, San Diego, CA) has made routine clinical use of TPMT genotyping a feasible approach for prospectively optimizing thiopurine therapy. Furthermore, cost–benefit analyses have established that this approach is a cost-effective strategy to optimize treatment with thiopurine medications.[50]

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