

## Thio-Purine Methyl Transferase Gene Single Nucleotide Polymorphisms in Inflammatory Bowel Disease

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The thiopurine drugs azathioprine (AZA), 6-mercaptopurine (6-MP) and thioguanine (TGN) are widely used in medicine, including inflammatory bowel disease (IBD).<sup>1</sup> These agents undergo S-methylation catalyzed by thiopurine methyltransferase (TPMT) to methylmercaptopurine or oxidation to thiouric acid via xanthine oxidase (XO).<sup>2</sup> Genetic studies over the last two decades have established that a polymorphism at the TPMT gene locus plays a significant role in the occurrence of life-threatening myelosuppression, a serious dose-related toxicity of thiopurine drugs.<sup>3-4</sup> In addition to toxicity, clinical implications of the TPMT polymorphism may include variations in therapeutic efficacy and drug interactions.<sup>5</sup>

The hereditary nature of the TPMT deficiency in humans was initially identified in a study of TPMT activity in red blood cells (RBC). This and subsequent studies determined the distribution of TPMT activity in RBC to be trimodal; 90% of persons have high activity, 10% have intermediate activity and 0.3% have low or no detectable enzyme activity [Figure 1].<sup>6</sup>

Individual differences in TGN accumulation after drug therapy have been shown to be associated with bone marrow toxicity. The cellular accumulation of TGN nucleotides is inversely proportional to TPMT activity, since high TPMT activity results in more drugs to the methyl-

ation pathway, leaving less for activation to cytotoxic TGNs. Conversely, TPMT-deficient patients accumulate very high TGN concentrations in tissues, including RBC.<sup>1</sup> Subsequently the use of standard doses of thiopurine drugs in patients with complete TPMT deficiency could be hazardous or even fatal due to bone marrow toxicity.<sup>7</sup>

Altered TPMT activity predominantly results from single nucleotide polymorphisms (SNPs). To date, eight TPMT alleles have been identified, including three alleles (TPMT\*2, TPMT\*3A and TPMT\*3C) which account for 80-95% of intermediate or low enzyme activity cases in Caucasians. The mutant allele TPMT\*2 is defined by a single nucleotide transversion (G238C) in the open reading frame (ORF), leading to an amino acid substitution at codon 80 (Ala80Pro). The second and more prevalent mutant allele, TPMT\*3A, contains two nucleotide transition mutations (G460 and A719G) in the ORF, leading to amino acid substitutions at codon 154 (Ala154Thr) and codon 240 (Tyr240Cys) [Figure 2].<sup>2</sup> It should be noted that the frequency of pattern of mutant TPMT alleles is different among various ethnic populations.<sup>6</sup>

**Key words:** TPMT, azathioprine, polymorphism, inflammatory bowel disease, ulcerative colitis, Crohn's disease.

### Abbreviations

**AZA**=Azathioprine

**6-MP**=6-Mercaptopurine

**CD**=Crohn's Disease

**IBD**=Inflammatory Bowel Disease

**ORF**=Open Reading Frame

**RBC**=Red Blood Cells

**TPMT**=Thio-Purine Methyl Transferase

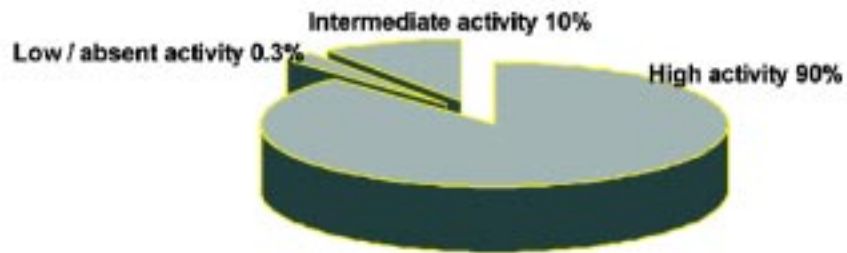
**SNP(s)**=Single Nucleotide Polymorphism(s)

**UC**=Ulcerative Colitis

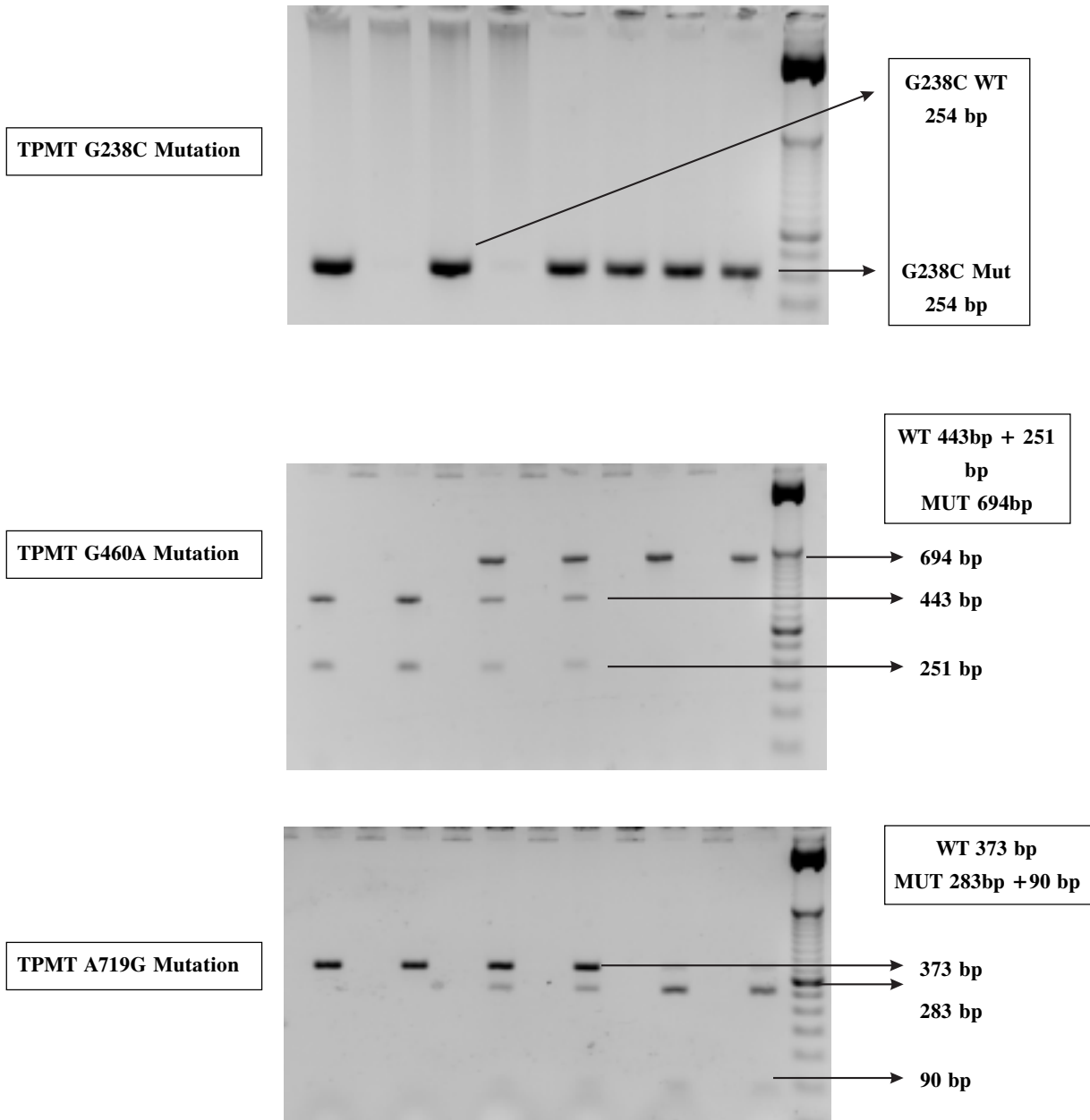
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**Fig. 1.** TPMT activity in red blood cells.



**Fig. 2.** Thio-Purine Methyl Transferase (TPMT) gene single nucleotide polymorphisms G238C, G460A and A719G. Agarose gel electrophoresis of PCR product. (WT= Wild type? Mut=Mutated type, bp=base pairs)

In addition to a high degree of variability in TPMT activity within both the homozygous wild type and heterozygous groups, some individuals with a heterozygous genotype exhibit high activity whereas some homozygous wild type subjects exhibit an intermediate phenotype; attention has to be paid also to transfused individuals.<sup>8</sup> Such discrepancies are due to the fact that the SNPs discussed so far are not the only factors regulating catalytic activity. Population genetic studies have shown that the genotype which regulates TPMT activity accounted for only approximately two-thirds of the total variance in the level of RBC enzyme activity. Other factors such as promoter polymorphisms, drug interactions and environmental factors could play an important role in the final TPMT activity phenotype.<sup>9</sup>

However, TPMT polymorphism and activity is at present one of the best models for the translation of genomic information to guide IBD patient therapeutics and seems an effective method for tailored thiopurine drug therapy in IBD patients.

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