Azathioprine Therapy and *TPMT* Genotype

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Azathioprine is an immunosuppressive antimetabolite that belongs to the drug class thiopurines. Azathioprine is used to prevent kidney transplant rejections and to treat rheumatoid arthritis when other treatments have not been effective ([1]). Off-label uses include the treatment of inflammatory bowel disease and myasthenia gravis ([2]).

Azathioprine is a prodrug for mercaptopurine. Thiopurine S-methyltransferase (TPMT) is involved in the metabolism of all thiopurines and is one of the main enzymes that inactivates mercaptopurine. In all patients receiving azathioprine, there is a risk of bone marrow suppression. This adverse effect is dose-dependent and may be reversed by reducing the dose of azathioprine. However, individuals who inherit two nonfunctional *TPMT* alleles universally experience life-threatening myelosuppression ([3, 4]).

The FDA recommends *TPMT* genotyping or phenotyping before starting treatment with azathioprine. This allows patients who are at increased risk for toxicity to be identified, and for the starting dose of azathioprine to be reduced, or for an alternative therapy to be used ([1]). The Clinical Pharmacokinetics Implementation Consortium (CPIC) has made recommendations on the dosing of azathioprine based on an individual's *TMPT* phenotype (see Table 1) ([4]).

Table 1.

TPMT phenotypes and the therapeutic recommendations for azathioprine therapy

Phenotype	Phenotype details	Genotype	Examples of diplotypes	Therapeutic recommendations for azathioprine
Homozygous wild-type ("normal")	High enzyme activity. Found in ~86–97% of patients.	Two or more functional alleles	**1/*1*	Start with normal starting dose. Adjust doses of azathioprine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment.
Heterozygous	Intermediate enzyme activity. Found in ~3–14% of patients.	One functional allele plus one nonfunctional allele	**1/*2* **1/*3A* **1/*3B* **1/*3C* **1/*4*	Consider starting at 30–70% of the full dose, if treatment of the disease normally starts with a full dose. Titrate dose based on tolerance. Allow 2–4 weeks to reach steady state after each dose adjustment.

Phenotype	Phenotype details	Genotype	Examples of diplotypes	Therapeutic recommendations for azathioprine
Homozygous variant	Low or deficient enzyme activity. Found in ~1 in 178 to 1~3736 patients.	Two nonfunctional alleles	*3A/*3A *2/*3A *3C/*3A *3C/*4 *3C/*2 *3A/*4	Consider alternative agents. If using azathioprine, start with drastically reduced doses (reduce daily dose by 10-fold and dose thrice weekly instead of daily) Adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment.

The strength of therapeutic recommendations is “strong” for all phenotypes.

Table is adapted from Relling M.V. et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. Clinical pharmacology and therapeutics. 2011;89([3]):387–91 ([4]).

Drug: Azathioprine

Thiopurines are antimetabolites—as purine analogues, they exert their immunosuppressive effects partly by blocking purine synthesis. Three thiopurines are used clinically: azathioprine, mercaptopurine, and thioguanine. All are prodrugs that give rise to the major active metabolites, thioguanine nucleotides (TGNs).

Azathioprine is first metabolized to mercaptopurine. Activation of mercaptopurine occurs via the enzyme HPRT1 (hypoxanthine phosphoribosyltransferase) followed by a series of reactions to form TGNs. The cytotoxicity of azathioprine is due, in part, to the incorporation of TGNs into DNA.

Mercaptopurine is inactivated by two different pathways, one being catalyzed by TPMT. Individuals who inherit two nonfunctional *TPMT* alleles (~1 in 178 to 1 in 3,736) experience life-threatening myelosuppression, due to high levels of TGNs, if they receive conventional doses of azathioprine ([4]).

Individuals who are heterozygous for nonfunctional *TPMT* alleles (~3–14%) are at a significantly higher risk for toxicity than individuals with two functioning alleles. However, some of these individuals (~40–70%) can still tolerate the full dose of azathioprine. This may be because heterozygous-deficient individuals have lower concentrations of less active metabolites, such as MeMPN (methylmercaptopurine nucleotides), than homozygous-deficient individuals ([4]).

Another mercaptopurine inactivation pathway is oxidation, which is catalyzed by xanthine dehydrogenase, XDH (also known as xanthine oxidase). If this pathway is inhibited, catabolism of mercaptopurine is reduced leading to mercaptopurine toxicity. Therefore, a lower dose of azathioprine is required in patients taking the XDH inhibitor, allopurinol ([1]).

Gene: *TPMT*

The *TPMT* gene encodes one of the main enzymes involved in the metabolism of thiopurines, such as azathioprine. TPMT activity is inherited as a monogenic, codominant trait.

TPMT is highly polymorphic—more than 25 variants are known ([5, 6]). The wild-type allele *TPMT*1* is associated with normal enzyme activity. Individuals who are homozygous for

*TPMT*1* have a phenotypically normal response to azathioprine and a lower risk of myelosuppression. This accounts for the majority of patients (~86–97%) ([4]).

The following nonfunctioning alleles are associated with reduced levels of TPMT activity ([7]):

- *TPMT*2* (238G>C)
- *TPMT*3A* (contains two SNPs, **3B* and **3C*)
- *TPMT*3B* (460G>A)
- *TPMT*3C* (719A>G)

The frequency of *TPMT* alleles varies among different populations. In the United States, the most common low-activity variant in the Caucasian population is *TPMT*3A* (~5%). This allele is also found in individuals who originate from India and Pakistan, but less frequently ([5, 7, 8]).

In East Asian, African-American, and some African populations, the most common variant is *TPMT*3C* (~2%), although *TPMT*8* may be more common in African populations than previously thought (~2%). In general, *TPMT*2* occurs much less commonly, and *TPMT*3B* occurs rarely ([3, 5]).

Genetic Testing

Genetic testing is available for several *TPMT* variant alleles. Most commonly *TPMT*2*, **3A*, and **3C* are tested for, which account for >90% of inactivating alleles. Rare or previously undiscovered variants will not be detected by variant-specific genotyping methods ([4]).

Phenotype testing is also available. For example, the TPMT activity in red blood cells can be measured directly. However, the results will not be accurate in patients who have received recent blood transfusions ([1]). Measures of azathioprine metabolites (TGN and MeMPN) are also available.

Therapeutic Recommendations based on Genotype

This section contains excerpted[1] information on gene-based dosing recommendations. Neither this section nor other parts of this review contain the complete recommendations from the sources.

Statement from the US Food and Drug Administration (FDA): TPMT testing cannot substitute for complete blood count (CBC) monitoring in patients receiving azathioprine. TPMT genotyping or phenotyping can be used to identify patients with absent or reduced TPMT activity. Patients with low or absent TPMT activity are at an increased risk of developing severe, life-threatening myelotoxicity from azathioprine if conventional doses are given. Physicians may consider alternative therapies for patients who have low or absent TPMT activity (homozygous for non-functional alleles). Azathioprine should be administered with caution to patients having one non-functional allele (heterozygous) who are at risk for reduced TPMT activity that may lead to toxicity if conventional doses are given. Dosage reduction is recommended in patients with reduced TPMT activity. Early drug discontinuation may be considered in patients with abnormal CBC results that do not respond to dose reduction.

Please review the complete therapeutic recommendations that are located here: ([1]).

Statement from the Clinical Pharmacogenetics Implementation Consortium (CPIC): Testing for *TPMT* status is recommended prior to starting azathioprine therapy so that the starting dosages can be adjusted accordingly—see Table 1 for dosing recommendations. In homozygous variant individuals, either an alternative agent should be used, or the doses of azathioprine should be drastically reduced. In heterozygous individuals, depending on the disease being treated, starting doses should be reduced. In both patient groups, a longer period of time should be left after each dose adjustment to allow for a steady state to be reached.

Please review the complete therapeutic recommendations that are located here: ([4]).

The FDA labels specific drug formulations. We have substituted the generic names for any drug labels in this excerpt. The FDA may not have labelled all formulations containing the generic drug.

Nomenclature

Common allele name	Alternative names	HGVS reference sequence		dbSNP reference identifier for allele location
		Coding	Protein	
*TPMT*2*	238G>C Ala80Pro	NM_000367.2:c.238G>C	NP_000358.1:p.Ala80Pro	rs1800462
*TPMT*3A*	This allele contains two SNPs, *TPMT*3B* and *TPMT*3C*			
*TPMT*3B*	460G>A Ala154Thr	NM_000367.2:c.460G>A	NP_000358.1:p.Ala154Thr	rs1800460
*TPMT*3C*	719A>G Tyr240Cys	NM_000367.2:c.719A>G	NP_000358.1:p.Tyr240Cys	rs1142345

Guidelines for the description and nomenclature of gene variations are available from the Human Genome Variation Society (HGVS): http://www.hgvs.org/content/guidelines

Acknowledgments

The Pharmacogenomics Knowledgebase: http://www.pharmgkb.org

The Clinical Pharmacogenetics Implementation Consortium: http://www.pharmgkb.org/page/cpic

References

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Tests in GTR by Condition

Azathioprine response

Tests in GTR by Gene

TPMT gene