General background text Pharmacogenetics –

Thiopurine-S-methyltransferase (TPMT)

Last updated: 15 augustus 2023

Definitions in pharmacogenetics

The **genotype** is the hereditary information about a specific characteristic of an individual. This information is located in the genes, in the DNA that consists of nucleotides. The piece of the DNA that carries information for one specific hereditary characteristic is called a **gene.** The DNA is divided into chromosomes, which usually occur in pairs. This means that an individual has two copies (two **alleles**) of most genes. Each allele is located on one of the chromosomes of a chromosome pair.

The **phenotype** indicates what the final manifestation (phenotypic state) of a certain genotype is. This can involve the functionality of a protein (for example the enzyme or the receptor), but also the physical manifestation of a disease. The phenotype is a result of the genotype that a person possesses, the degree of expression of the gene in question and the combination with environmental factors such as co-medication, diet and disease conditions.

Variations can exist in a population within the DNA that encodes a protein. Variations can result in alleles that encode for proteins with no or reduced activity. The simplest form of variations are *"single-nucleotide polymorphisms" (SNPs)*, in which a certain part of a gene differs by only one nucleotide. If a gene variation occurs in at least 1% of the population, then this is referred to as a genetic **polymorphism. Wild-type** is the name given to the most common active allele. There can be a number of different polymorphisms for a certain allele.

The **promoter** is a section that precedes the gene, which is primarily responsible for regulating the activity (expression) of the gene.

Thiopurine metabolism

Thiopurines (azathioprine, mercaptopurine and thioguanine) are inactive pro-drugs, which are converted to the active metabolites in the body: thioguanine nucleotides.
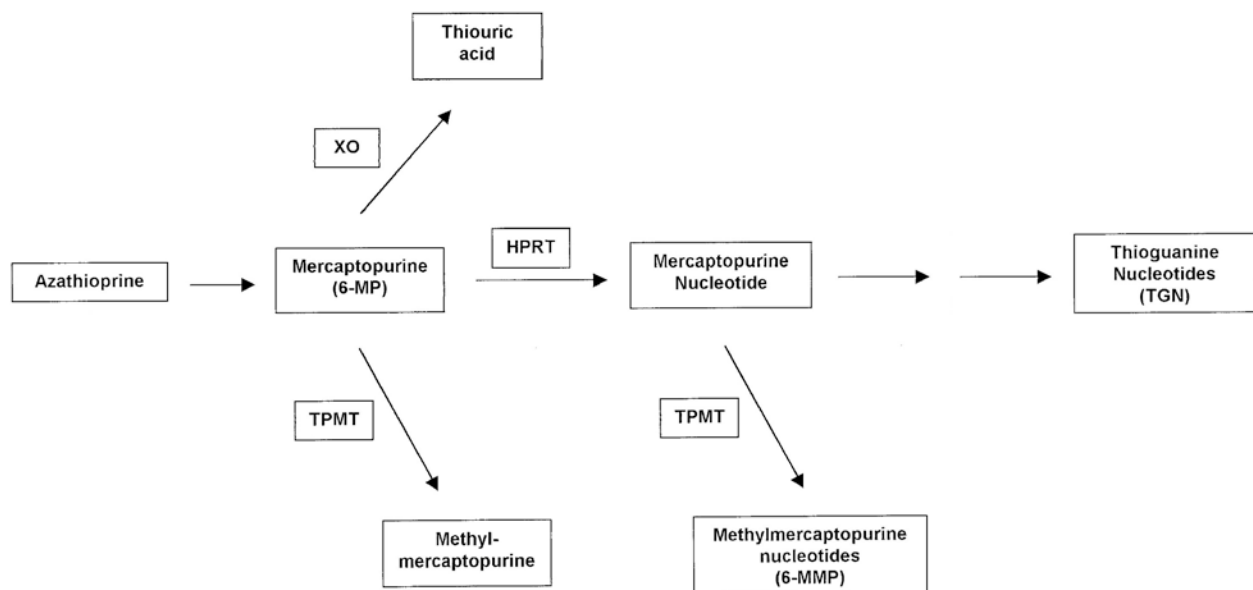

Figure 1. Schematic simplified overview of azathioprine and mercaptopurine metabolism.
Mercaptopurine nucleotides are 6-thio-inosine ribonucleotides.
(From: Corominas et al., 2000)

The active metabolites of azathioprine and mercaptopurine are formed in multiple steps. Azathioprine is non-enzymatically converted in the body to mercaptopurine and nitromethyl imidazole. Mercaptopurine is then converted to thioguanine nucleotides in three steps. The first of these steps is catalysed by the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT).

Two catabolic routes reduce mercaptopurine bioavailability for thioguanine nucleotide formation. In addition to this, mercaptopurine is oxidised to the inactive 6-thiouric acid by the enzyme xanthine oxidase (XO), which occurs primarily in the liver and intestines. Thiopurine methyltransferase (TPMT) catalyses S-methylation of both mercaptopurine and the metabolites formed by HPRT (mercaptopurine nucleotides or 6-thio-inosine ribonucleotides). The methylated 6-thio-inosine ribonucleotides contribute to the anti-proliferative properties of the thiopurines, probably through inhibition of *de novo* purine synthesis. High concentrations of methylated 6-thio-inosine nucleotides are also associated with a higher risk of hepatotoxicity.
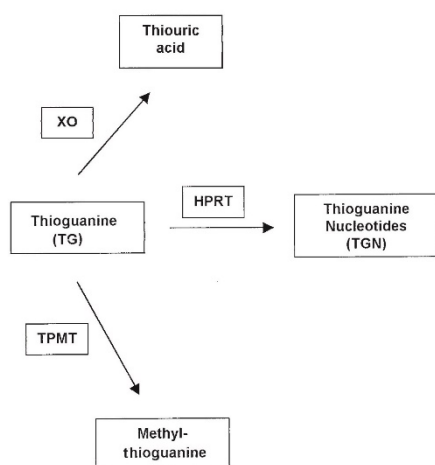

Figure 2. Schematic simplified overview of thioguanine metabolism.
(From: Corominas et al., 2000)

Thioguanine is converted to thioguanine nucleotides in one step by the enzyme HPRT. Catabolic routes play a lesser role in thioguanine metabolism than in mercaptopurine metabolism. The enzyme guanase converts thioguanine to the inactive metabolite 6-thioxanthine by deamination; 6-thioxanthine is subsequently converted to 6-thiouric acid by xanthine oxidase. S-methylation of thioguanine takes place by TPMT.

Absorption of all three thiopurines is incomplete and variable.

Altered metabolic capacity and clinical consequences
Variations in the gene that encodes the TPMT enzyme can result in reduced or absent enzyme activity.
The population can be divided into three phenotypes, based on the metabolic capacity of TPMT that is present:
- Poor metaboliser (PM), severely reduced or absent metabolic capacity (2 alleles with absent functionality);
- Intermediate metaboliser (IM), reduced metabolic capacity (1 allele with normal activity and 1 allele with absent functionality);
- Normal metaboliser (NM), "normal" metabolic capacity (2 alleles with normal activity);
There is also great variation in metabolic capacity within the IM and NM groups.
Tables 1 and 2 demonstrate to which of the three phenotypes a certain genotype leads.

The PM phenotype particularly leads to a strong increase in cellular concentration of the active metabolites (thioguanine nucleotides), which almost always leads to serious side effects such as bone marrow depression at normal thiopurine doses. In these cases, it may be necessary to adjust the standard dose or to choose an alternative. The need for changing the standard dose is less clear for IM.

As the genotype only determines part of the metabolic capacity, the guidelines for dose adjustment based on the genotype are no more than a tool that can be used to achieve the desired plasma concentration. Therapeutic drug monitoring (TDM) can be useful to help optimise the doses of substances that have pharmacogenetic guidelines. TDM is possible for thiopurines (analysis of concentrations of thioguanine nucleotides and methylated 6-thio-inosine nucleotides in RBCs), but is only used in the event of unexpected serious side effects. The concentrations found depend on the method. Although there is a relationship between

metabolite concentrations and therapeutic effect and side effects, there is a large grey area on either side of the therapeutic range. Various studies have found contradictory results.

A few studies report reduced therapeutic effects in the subgroup of patients with the NM genotype who have the highest metabolic activity. As the cause of the variation in metabolic capacity within the NM group is not known, genotyping cannot distinguish between NM patients with normal and very high metabolic capacity. Studies that differentiated using TPMT activity use variable limits between normal and very high metabolic activity. This makes it currently impossible to define an ultrarapid metaboliser phenotype (UM) for TPMT.

Genotyping
The process of genotyping is used to determine the genotype. It indicates which alleles of the gene for TPMT are present in the tested individual. Each allele has a name that consists of a star (*) and a number, an example of a possible TPMT genotype is TPMT*1/*3A.

Many variations exist for TPMT, more than 20 different allele variations have been identified/described in the literature. A number of these variations, including their functionality, are listed in Table 1. Genotyping usually screens for only the most common variant alleles. This may result in less common variants being missed and incorrectly being designated the wild-type. (see also the document "Uncertainties in genotyping outcomes" on www.knmp.nl).
The TPMT alleles *2, *3A, *3B and *3C together make up about 95% of the variant TPMT alleles. All other alleles are very uncommon.
Allele *3A has 2 SNPs, the SNP in allele *3B and the SNP in allele *3C. This makes it impossible to distinguish between the *1/*3A (2 variations in an allele) and *3B/*3C (2 variations over 2 alleles) using standard PCR techniques. Distinguishing is possible using special genotyping techniques or by phenotyping. However, the *3B/*3C phenotype is so rare in White populations that researchers generally assume the *1/*3A genotype when both variations are detected by PCR.

The promoter of the TPMT gene may contain a variable number of repeating sequences. This sequence is called variable number tandem repeat or VNTR. TPMT activity has been found to be influenced by the total number of VNTRs in both TPMT alleles. A the extent to which this variation in VNTRs contributes to the variation in TPMT activity in people with the same genotype is currently not known; table 1 does not distinguish between alleles with different numbers of VNTRs.

Table 1. TPMT alleles and metabolic capacity

Metabolic capacity	Allele number
Increased functionality	-
Fully functional (wild-type)	*1 *1S *1A
Reduced functionality	-
fully dysfunctional (null alleles) #	*2 *3A *3B *3C *3D *4 to *18

The TPMT alleles shown are almost fully dysfunctional. There is some metabolic activity, but this is generally below the detection limit of phenotyping methods.
The cause of the strongly reduced metabolic capacity for the TPMT alleles for which this has been determined, was found to be a very strong increase of the enzyme proteolysis causing a very strong decrease in the enzyme level.
Alleles *3D and *4 to *18 are very uncommon. They make up approximately 5% of the variant alleles.
The median TPMT activity and the median tolerated dose were significantly higher for 8 heterozygotes for *8 than for heterozygotes of *2, *3A or *3C, but not significantly lower than for *1/*1. The median TMPT activity was numerically higher for 2 heterozygotes for *12 than for *1/*1. This suggests that these alleles are not null alleles after all.

Phenotyping

The process of phenotyping is used to determine the phenotype, which means: measuring or estimating the activity of the TPMT enzyme. This involves measuring TPMT activity in red blood cells, which correlates well with TPMT activity in the thiopurine target organ (white blood cells).

Individual enzyme activity established in phenotyping depends on both genetic and environmental factors. There is significant variation in TPMT activity between individuals with the same genotype. TPMT activity may also be induced by thiopurines and other medicines. Phenotyping therefore supplies more information as compared with genotyping, although there is generally good correlation between genotyping and phenotyping of TPMT. The limits between the different TPMT phenotypes depend on the method used for phenotyping (see table 3 for the two most common methods used). As thiopurine can induce TPMT, the limits also depend on thiopurine usage.

A disadvantage of phenotyping versus genotyping is that recent blood transfusions can affect the result.

Table 2. Relationship between TPMT phenotype and genotype

Phenotype	Genotype	Example of genotype
NM	Fully functional/fully functional	*1/*1
IM	Fully functional/null allele	*1/*2, *1/*3A
PM	Null allele/null allele	*2/*3A, *3A/*3A

Table 3. Phenotyping of TPMT

Substrate used for phenotyping	Thiopurine usage at the time of phenotyping	Enzyme activity per phenotype[#]		
		NM	IM	PM
mercaptopurine[1]	no	≥ 10 U	≥ 5 U and < 10 U	< 5 U
mercaptopurine[2]	yes	≥ 13.7 U	≥ 5 U and < 13.7 U	< 5 U
thioguanine[3]	no	≥ 24 U	4-23 U	≤ 3 U

[#] 1 U is defined as the formation of 1 nmol methylmercaptopurine/mL packed red blood cells or 1 nmol methylthioguanine/g haemoglobin per hour.
[1] Weinshilboum et al., 1980.
[2] Lowry et al., 2001.
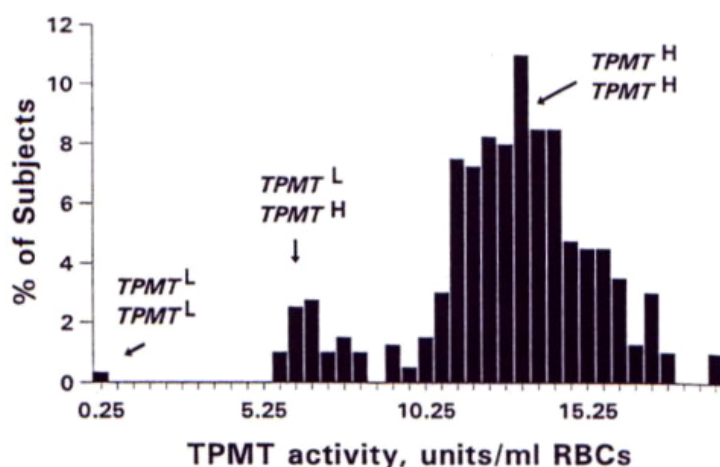[3] Schaeffeler et al., 2003.

Figure 3. Trimodal distribution of TPMT activity within the (White) population.
(Source: Weinshilboum et al., 1980)

Ethnic variation in prevalence of phenotypes and allele-and genvariant frequencies

The frequency of occurrence of the various TPMT alleles and the various phenotypes varies significantly between ethnic groups.

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Variant alleles are less common in the Asian population (2-5%) than in the European White and African population (7-14%). The Asian population therefore has a lower frequency of IM and PM phenotypes.

In the European White and South American populations, *3A is the most common variant allele. In Asian and African populations, *3C is the most common variant allele and *3A is very uncommon.

Allele frequencies and phenotypes per population are given in table 4 and genvariant frequencies found in Table 5.

Table 4. Ethnic variation in prevalence of phenotypes and allele frequency

Population	Region	Prevalence of predicted phenotype (%) #			Allele frequency (%)			
		PM	IM	NM	*2	*3A	*3B	*3C
White		0.1-0.5	7-13	86-93	0.2-0.5	3.2-5.7	0-0.2	0.2-0.8
	the Netherlands@	0.26	10	90	0.4	3.5	0.4	0.8
Mediteranian		0.14	7	93	0.4	2.5	0.4	0.5
Asian	South-West Asia	0.003-0.12	1-7	93-99	0-0.2	0.5-1	0	0-2.3
	East Asia	0.02-0.05	3-4	95-97	0	0	0	1.5-2.3
African		0.3-0.6	10-14	85-89	0	0-0.2	0	5.4-7.6
African-American		0.13	7	93	0.4	0.8	0	2.4
South-American		0.03-0.7	4-16	84-96	0.3-2.2	1.5-3.6	0-0.2	0-2.5

[#] The prevalence of the predicted phenotypes is calculated based on the allele frequencies.

[@] The allele frequencies given for the Netherlands are the weighted averages of three studies.

Note: According to the guideline of the Association for Molecular Pathology, at least the alleles *2, *3A, **3B, and *3C should be determined when genotyping TPMT.**

Table 5. Ethnic variation in gene variant frequency

Population	Area or subgroup	Genvariant frequency (%)		
		238G>C (*2)	460G>A (*3A en *3B)	719A>G (*3A en *3C)
White	Europa without Finland	0.2	3.8	4.3
	Finland	0.008	2.7	3.0
Asian	East Asia	0	0.02	1.4
	South Asia	0	0.7	1.8
African/Afro-American		0.04	0.7	5.5
Latin American/American, mixed ethnicity		0.4	4.2	4.7
Ashkenazi Jewish		0.2	1.3	1.3

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