General background text Pharmacogenetics – Nudixhydrolase 15 (NUDT15)

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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 variant 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.

Thiopurine metabolism
Thiopurines (azathioprine, 6-mercaptopurine and thioguanine) are inactive pro-drugs, which are converted to the active metabolites in the body: thioguanine nucleotides (see figure 1). The triphosphate forms of these thioguanine nucleotides are the fully activated metabolites. 6-Thiodeoxyguanosine triphosphate (6-thio-dGTP) is incorporated in DNA, resulting in cytotoxicity. 6-Thioguanosine triphosphate (6-thio-GTP) contributes to cytotoxicity and immunosuppression because it is incorporated in RNA and inhibits the enzyme Rac1 in T-cells. The Rac1 inhibition results in a reduced response of these immune cells.
NUDT15 reverses the complete activation of the thiopurines by converting 6-thio(deoxy)guanosine triphosphate to 6-thio(deoxy)guanosine monophosphate (6-thio-dGMP and 6-thio-GMP) (refer to the right side of figure 1). It thereby reduces the effect and toxicity of the thiopurines.
NUDT15 ("nucleoside diphosphate-linked moiety X-type motif 15" or nudixhydroxylase 15) is a member of a family of enzymes that hydrolyse chemical bonds, with the general structure of a nucleoside diphosphate bound to another unit X (a thio group in the case of NUDT15). The enzyme is also called MTH2 (mutatorT homolog2). This was based on a previous name, assigned to the group of enzymes because they were identified as proteins encoded by genes, of which mutant versions in bacteria increased the mutagenic effect and toxicity of cytotoxic purines and pyrimidines.

The absorption of all three thiopurines is incomplete and variable.

Altered metabolic capacity and clinical consequences
Variations in the gene that encodes the NUDT15 enzyme can result in reduced or absent enzyme activity. There are indications that this reduced enzyme activity is caused by a combination of a lower enzyme activity and a lower stability of the resulting enzyme variants.
The population can be divided into three phenotypes, based on the metabolic capacity of the encoded NUDT15 enzyme that is present:
1. poor metaboliser (PM), severely reduced or absent metabolic capacity (2 alleles with reduced or absent activity)
2. intermediate metaboliser (IM), reduced metabolic capacity (1 allele with normal activity and 1 allele with reduced or absent activity)
3. normal metaboliser (NM), "normal" metabolic capacity (2 alleles with normal activity)

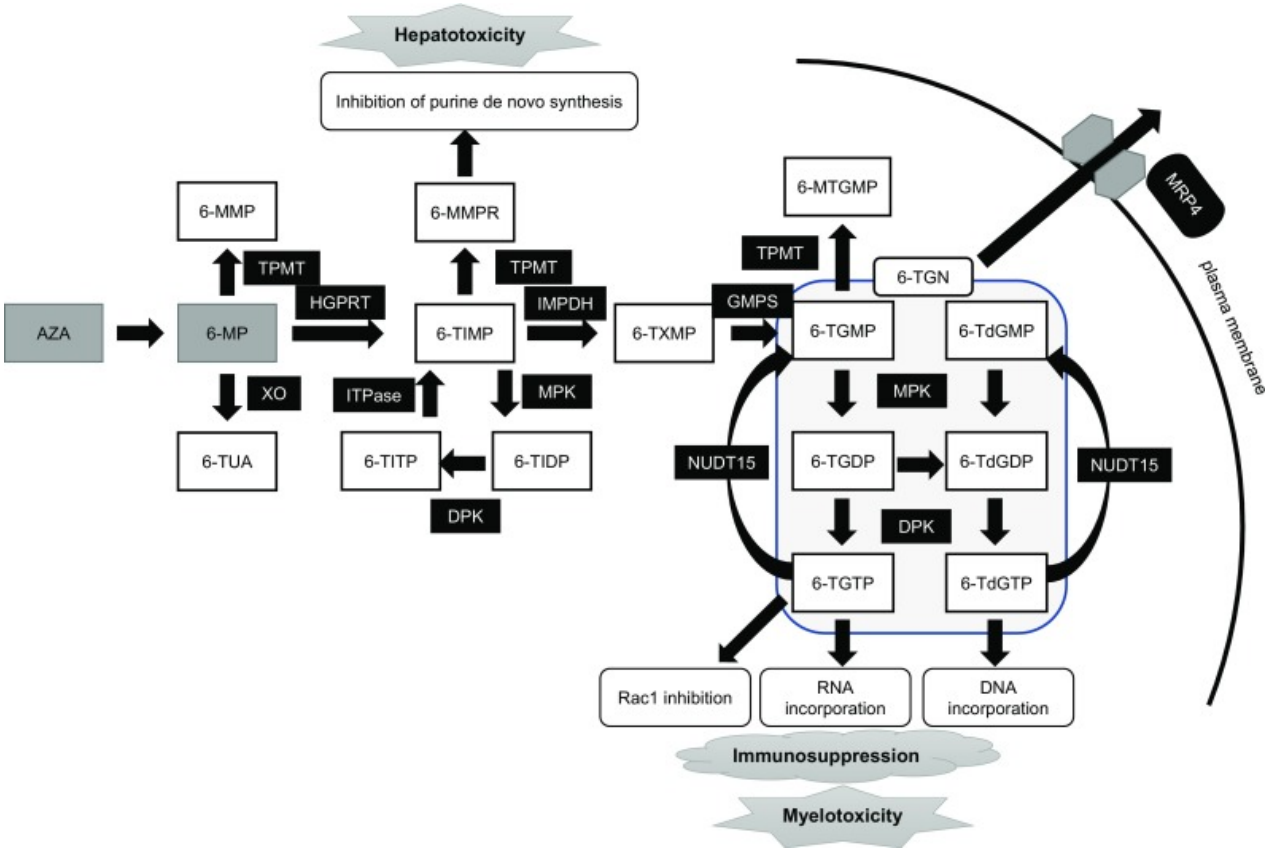

Figure 1. Schematic overview of azathioprine and 6-mercaptopurine metabolism. Thioguanine is directly converted by HGPRT to 6-thioGMP (6-TGMP, top left in the large, light-grey square).
The grey rectangles represent medicines: AZA = azathioprine, 6-MP = mercaptopurine.
The white rectangles represent metabolites: 6-MMP = 6-methylmercaptopurine, 8-OHMP = 8-hydroxy-6-mercaptopurine, 6-TUA = 6-thiouric acid, 6-MMPR = 6-methylmercaptopurine ribonucleotide, 6-TIMP = 6-thioinosinemono-phosphate, 6-TIDP = 6-thioinosine diphosphate, 6-TITP = 6-thioinosine triphosphate, 6-TXMP = 6-thioxanthosine monophosphate, 6-TGMP = 6-thioguanosine monophosphate, 6-TGDP = 6-thioguanosine diphosphate, 6-TGTP = 6-thioguanosine triphosphate, 6-TdGMP = 6-thiodeoxyguanosine monophosphate, 6-TdGDP = 6-thiodeoxyguanosine diphosphate, 6-TdGTP = 6-thiodeoxyguanosine triphosphate, 6-MTGMP = 6-methylthioguanosine monophosphate, 6-TGN = 6-thioguanine nucleotides.
The black rectangles represent enzymes or transporters: XO = xanthineoxidase, TPMT = thiopurine-S-methyltransferase, HGPRT = hypoxanthine-guanine phosphoribosyltransferase, IMPDH = inosine-monophosphate dehydrogenase, GMPS = guanosine monophosphate synthetase, MPK = monophosphate kinase, DPK = diphosphate kinase, ITPase = inosine triphosphate pyrophosphatase, MRP4 = multi-drug resistance-associated protein 4.
(Derived from: Kakuta et al., 2018)

There is also great variation in metabolic capacity within the IM and NM groups.
Table 1 provides an overview of the various alleles and the resulting enzyme activity.

The PM phenotype particularly leads to a strong increase in cellular concentration of the active metabolites (6-thio-dGTP and 6-thio-GTP), which almost always leads to severe side effects such as leukopaenia at normal thiopurine doses. In these cases, it is necessary to adjust the standard dose or to choose an alternative. The risk of leukopaenia is also increased for IM, but more than half of the patients who start with a standard dose do not develop leukopaenia.

As the genotype only determines a part of the metabolic capacity, the recommendations 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. In the case of thiopurines, TDM consists of determining the concentrations of thioguanine nucleotides in erythrocytes. However, determination of the concentrations of the total thioguanine nucleotides does not provide any useful information for patients with a NUDT15 variation. The NUDT15 variations alter the ratio of thioguanine nucleotide triphosphates and thioguanine nucleotide monophosphates, without changing the total quantity of thioguanine nucleotides. Therefore, patients with a NUDT15 variation develop toxicity within the therapeutic range defined for patients without NUDT15 variations. The therapeutic range has not been determined for patients with one or more NUDT15 variations. The currently available TDM is not useful

for patients with a NUDT15 variation. The dose can only be adjusted based on toxicity (monitoring of blood counts) and efficacy.

Genotyping

The process of genotyping is used to determine the genotype. It indicates which alleles of the gene for NUDT15 are present in the tested individual. Many alleles have a name that consists of a star (*) and a number. An example of a possible NUDT15 genotype is NUDT15 *1/*2. Standard nomenclature is not available for all discovered alleles. This is why some alleles are still referred to by nucleotide numbers followed by nucleotide changes (or by amino acid number preceded by symbol of the amino acid in the wild-type protein and followed by the symbol of the amino acid in the variant).

More than 10 different allele variations have been identified/described in the literature for NUDT15 [1-3]. These variations, including their functionality, are listed in Table 1. The variant 415C>T (alleles *2 and *3) has the most evidence to suggest that this results in a fully dysfunctional enzyme. An *in vitro* decrease in functionality by 75-100% has been observed for 416G>A (*4), 52G>A (*5) and 36_37insGGAGTC (*2 and *6), with indications that the reduction is strongest for 36_37insGGAGTC (*2 and *6) [3]. No significant difference was found *in vitro* between heterozygotes from *2 through to *6 [3]. *In vivo*, *1/*5 also increased the risk of thiopurine-induced leukopaenia, whilst a numerically slightly increased risk was found for *1/*6 compared to *1/*5 (significance not tested per genotype) [5].

Genotyping usually screens for only the most common allele variants. This may result in less common variants being missed and incorrectly being designated the wild-type allele. (see also the document “Uncertainties in genotyping outcomes” on www.knmp.nl).

In most population groups, *2 and/or *3 are the most common alleles. As the genotype *3/*6 occurs very rarely compared to *1/*2 (0-3.4% of the frequency of *1/*2), a patient with both the polymorphism 415C>T and the polymorphism 36_37insGGAGTC generally has the genotype *1/*2. NUDT15 gene variants occur very rarely in Europeans and Africans and only *3, *6 and *9 are found [1,6].

Note: In population groups in which *2 is found (East Asian, American and Latin American), a patient with both the polymorphism 415C>T and the polymorphism 36_37insGGAGTC will have the genotype *1/*2 in 96.7-100% of cases and only 0-3.3% of the cases have genotype *3/*6. As the tolerated dose is much lower and the risk of thiopurine-induced leukopaenia is much higher in the case of *3/*6 than in the case of *1/*2, the laboratory performing the tests should initially report the determined genotype to the healthcare provider as “probably *1/*2, possibly *3/*6. The healthcare provider can take this into consideration, for example by performing blood counts at an earlier stage if the patient is receiving a dose for IM or by initially giving the patient the dose for PM and then increasing the dose based on the results of the blood counts. The genotype/phenotype involved can only be determined with certainty if the dose tolerated by the patient is known (*3/*6 and PM if the dose that is tolerated most closely matches 10% of the dose tolerated by patients without a gene variant and *1/*2 and IM if the dose that is tolerated most closely matches 50% of the dose tolerated by patients without a gene variant).

Table 1. Overview of the notations used and metabolic activity for wild-type and variant NUDT15 alleles [1,2,3,4,7]

Notations for the variant and the accompanying polymorphism				**Functionality**
star notation	**amino acid change**	**nucleotide change**	**rs number**	
*1	-	-	-	normal
*2	Arg139Cys en Gly17_Val18dup	415C>T en 50_55dupGAGTCG	116855232 and 746071566	absent
*3	Arg139Cys	415C>T	116855232	absent
*4	Arg139His	416G>A	147390019	strongly decreased
*5	Val18Ile	52G>A	186364861	strongly decreased
*6	Gly17_Val18dup	50_55dupGAGTCG	869320766	strongly decreased
*7	Arg34Thr	101G>C	766023281	strongly decreased
*8	Lys35Glu	103A>G		decreased
*9	Gly17_Val18del	50_55delGAGTCG	746071566	strongly decreased
-	Met1Thr			unknown
-	Arg10Trp			unknown
-	Gly47Arg			unknown

Note: Pharmvar currently indicates that the functionality of *4-*8 is uncertain following CPIC, despite the fact that *4-*7 reduced nucleotide diphosphatase activity by 74-100% in vitro and thiopurine toxicity has been observed in vivo with *4-*8 [3]. Another study found no significant difference in tolerated dose between 29 PM with only *2 and/or *3 and 8 PM with at least 1 allele other than *2 and *3 (*5, *6 or *7) [8].

As no clear difference was found in the extent to which different variant alleles increase the risk of thiopurine-induced leukopaenia, the classification of the genotypes based on the predicted phenotypes does not distinguish between the different variant alleles (see Table 2).

Table 2. Relationship between NUDT15 genotype and the predicted NUDT15 phenotype

genotype	**phenotype**
fully functional allele / fully functional allele	normal metaboliser (NM)
fully functional allele / allele with reduced function or non-functional allele	intermediate metaboliser (IM)
allele with reduced function or non-functional allele / allele with reduced function or non-functional allele	poor metaboliser (PM)

Phenotyping

Phenotyping is the process of determining the phenotype, which involves: measuring or estimating the activity of the NUDT15 enzyme. However, for NUDT15, there is no reliable phenotyping method other than determining the maximum thiopurine dose tolerated by the patient.

Therapeutic drug monitoring for thiopurines normally consists of determining the concentrations of thioguanine nucleotides in erythrocytes. However, determination of the concentrations of the total thioguanine nucleotides does not provide any useful information for patients with a NUDT15 variation. The NUDT15 variants change the ratio of thioguanine nucleotide triphosphates and thioguanine nucleotide monophosphates without changing the total amount of thioguanine nucleotides. Therefore, patients with a NUDT15 variation develop toxicity within the therapeutic range defined for patients without NUDT15 variations. The therapeutic range has not been determined for patients with one or more NUDT15 variants. The currently available TDM is not useful for patients with a NUDT15 variation.

One phenotyping method that can be used for NUDT15 is to measure the amount of thioguanine incorporated in DNA (DNA-TG) [9]. However, this phenotyping method still requires prospective validation. It is currently not a reliable phenotyping method therefore. Another option would be a phenotype ring assay that distinguishes between the mono-, di- and triphosphate guanosine nucleotides [10]. However, this option is also currently insufficiently developed and validated. Direct measurement of the enzyme activity of NUDT15 measures the amount of pyrophosphate released [3]. As pyrophosphate can be derived from many sources *in vivo*, this measurement can only be performed *in vitro* using purified enzyme. Use in (cells of) patients is not possible. Phenotyping of NUDT15 in patients is therefore currently only possible by means of determining the maximum tolerated thiopurine dose.

Ethnic variation in prevalence phenotypes and allele and gene variant frequencies.

The frequency of occurrence of the different NUDT15 alleles and the various phenotypes differs strongly between population groups.

NUDT15 variants are common in patients of East Asian, South-East Asian or (Latin) American ethnicity. The most common alleles in these population groups are *2 and/or *3.

NUDT15 variants occur rarely in patients of European or African ethnicity. Only the alleles *3, *6 and *9 are found in these population groups. Prevalence data have not been determined for the Dutch population.

Allele frequencies and phenotypes per population are given in Table 3 and found genvariant frequencies in Table 4.

Table 3. Ethnic variation in prevalence of genotypes and allele frequency [3,6]

	prevalence of genotype (%)											allele frequency (%)					
	PM				IM						NM						
population group	*2/*3	*2/*5	*3/*3	*3/*6	*1/*2	*1/*3	*1/*4	*1/*5	*1/*6	*1/*9	*1/*1	*2	*3	*4	*5	*6	*9
European	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.6	0.5	98.5	0.0	0.2	0.0	0.0	0.3	0.3
East Asian	0.4	0.1	0.4	0.2	6.1	10.7	0.2	2.5	2.3	0.0	76.7	3.5	6.1	0.1	1.4	1.3	0.0
South-East Asian	0.0	0.0	0.4	0.0	0.0	12.4	0.0	0.2	0.4	0.1	86.4	0.0	6.7	0.0	0.1	0.2	0.1
African	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.4	0.1	99.3	0.0	0.1	0.0	0.0	0.2	0.1

American	0.1	0.0	0.0	0.0	7.0	1.5	1.5	0.0	0.4		89.3	3.7	0.8	0.8	0.0	0.2	
Latin American	0.1	0.0	0.0	0.0	5.4	3.5	3.5	0.0	0.0	0.0	87.0	2.9	1.9	1.9	0.0	0.0	0.0

The prevalences of the genotypes were calculated from the allele frequencies found.
Note: According to the Association for Molecular Pathology guideline, genotyping of NUDT15 requires at least allele *3 to be determined [11].

Table 4. Ethnic variation in genvariant frequency [12]

population group	Area or subgroup	genvariantfrequentie (%)				
		415C>T (*2 en *3)	50_55dup GAGTCG (*2 en *6)	416G>A (*4)	52G>A (*5)	50_55del GAGTCG (*9)
White	Europe without Finland	0.35	0.26	0.002	0.002	0.21
	Finland	2.3	1.3	0	0	0.05
Asian	East Asian	10	6.1	0.11	1.1	0
	South Asian	6.7	0.15	0.003	0.05	0.05
African/African-American		0.10	0.22	0.02	0	0.02
Latin-American/ American,mixed ethnicity		6.0	4.9	1.8	0	0.03
Ashkenazi Jewish		0.38	0.03	0	0.01	0

Literature

1. Kakuta Y et al. Pharmacogenetics of thiopurines for inflammatory bowel disease in East Asia: prospects for clinical application of NUDT15 genotyping. J Gastroenterol 2018;53:172-80. PubMed PMID: 29192347.
2. https://www.pharmgkb.org/, geraadpleegd op 12 juni 2018.
3. Moriyama T et al. NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet 2016;48:367-73. PubMed PMID: 26878724.
4. Zhu Y et al. Combination of common and novel rare NUDT15 variants improves predictive sensitivity of thiopurine-induced leukopenia in children with acute lymphoblastic leukemia. Haematologica 2018;103:e293-e295. PubMed PMID: 29519865.
5. Chao K et al. Combined detection of NUDT15 variants could highly predict thiopurine-induced leukopenia in Chinese patients with inflammatory bowel disease: a multicenter analysis. Inflamm Bowel Dis 2017;23:1592-9. PubMed PMID: 28570428.
6. Moriyama T et al. Novel variants in NUDT15 and thiopurine intolerance in children with acute lymphoblastic leukemia from diverse ancestry. Blood 2017;130:1209-1212. PubMed PMID: 28659275.
7. https://www.pharmvar.org/gene/NUDT15, geraadpleegd op 31 juli 2023.
8. Tanaka Y et al. An international retrospective study for tolerability of 6-mercaptopurine on NUDT15 bi-allelic variants in children with acute lymphoblastic leukemia. Haematologica 2021;106:2026-9. PMID: 33504140.
9. Nielsen SN et al. Measures of 6-mercaptopurine and methotrexate maintenance therapy intensity in childhood acute lymphoblastic leukemia. Cancer Chemother Pharmacol 2016;78:983-994. PubMed PMID: 27600880.
10. Vikingsson S et al. Novel assay to improve therapeutic drug monitoring of thiopurines in inflammatory bowel disease. J Crohns Colitis 2014;8:1702-9. PubMed PMID: 25239576.
11. Pratt VM et al. TPMT and NUDT15 genotyping recommendations: a joint consensus recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, and Pharmacogenomics Knowledgebase. J Mol Diagn 2022; 24:1051-63. PMID: 35931343.
12. genome aggregation database (gnomAD) v2.1.1, https://gnomad.broadinstitute.org.