Purpose

This document provides educational content and reference information about TPMT and NUDT15 genetic testing for thiopurine precision therapeutics.

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I. Introduction

The genes *TPMT* and *NUDT15* are involved in the metabolism of thiopurines (azathioprine, mercaptopurine, and thioguanine), which are used in the treatment of immunologic disorders, lymphoid malignancies, and myeloid leukemias. Germline variants in these genes thus affect drug exposure, reducing the metabolism of these drugs into inactive metabolites. Consequences of conventional doses given to individuals carrying loss-of-function variants could result in life-threatening toxicities such as myelosuppression; underdosing may also lead to subtherapeutic outcomes.

II. Reported Alleles

Variant nomenclature is based on the following transcripts:

Gene	Genomic Coordinates (GRCh38)	Coding	Protein Consequence
TPMT	NC_000006.12	NM_000367.5	NP_000358.1
NUDT15	NC_000013.11	NM_018283.4	NP_060753.1

The DRAGEN™ v4.0 Star Allele Caller (Illumina, San Diego, CA, USA) is a bioinformatics tool used to make automatic allele calls from sequencing results. The genomic positions of variants

describing insertions, duplications, and deletions may vary slightly from what is listed for the tool depending on the direction of the sequencing alignment (3' or 5'). These are annotated in the footnotes below for reference.

No Function Alleles

Gene	Allele	Genomic Coordinate(s)	Coding (c.)	Protein consequence (p.)
TPMT	**2*	chr6:18143724C>G	238G>C	Ala80Pro
TPMT	**3A*	chr6:18138997C>T chr6:18130687T>C	460G>A 719A>G	Ala154Thr Tyr240Cys
TPMT	**3B*	chr6:18138997C>T	460G>A	Ala154Thr
TPMT	**3C*	chr6:18130687T>C	719A>G	Tyr240Cys
TPMT	**4*	chr6:18130781C>T	626-1G>A	Splice Acceptor Variant
TPMT	**11*	chr6:18139689C>T	395G>A	Cys132Tyr
TPMT	**14*	chr6:18149127T>C	1A>G	Met1Val
TPMT	**15*	chr6:18133890C>T	495-1G>A	Splice Acceptor Variant
TPMT	**23*	chr6:18133884G>C	500C>G	Ala167Gly
TPMT	**29*	chr6:18149126A>G	2T>C	Met1Thr
TPMT	**41*	chr6:18130687T>G	719A>C	Tyr240Ser
TPMT	**42*[1]	chr6:18149034dup[2]	95dup	Trp33Valfs*26
NUDT15	**2*	chr13:48037796_48037801dup[3] chr13:48045719C>T	50_55dup 415C>T	Gly17_Val18dup Arg139Cys
NUDT15	**3*	chr13:48045719C>T	415C>T	Arg139Cys
NUDT15	**9*	chr13:48033796_48037801del[4]	50_55del	Gly17_Val18del

(1) *TPMT*42* is characterized by an insertion of a single nucleotide in exon 2, causing a frameshift in the open reading frame. There is no CPIC-assigned clinical function for this allele; however, it is likely a no-function allele due to the frameshift resulting in a premature stop codon predicted to result in a nonfunctional truncated TPMT protein or loss of protein expression via mRNA nonsense mediated decay. This allele is observed at a frequency of 0.11% in the Finnish European population (https:// gnomad.broadinstitute.org/variant/6-18149032-C-CT, last accessed November 21, 2023).
(2) chr6:18149032C>CT
(3) chr13:48037782A>AGGAGTC
(4) chr13:48037782AGGAGTC>A

Unknown or Uncertain Function Alleles-*TPMT*

Allele	Genomic Coordinate(s)	Coding (c.)	Protein consequence (p.)
**5*	chr6:18147910A>G	146T>C	Leu49Ser
**6*	chr6:18133845T>A	539A>T	Tyr180Phe
**7*	chr6:18130725A>C	681T>G	His227Gln
**8*	chr6:18130762C>T	644G>A	Arg215His
**9*	chr6:18143606T>G	356A>C	Lys119Thr
**10*	chr6:18139027C>G	430G>C	Gly144Arg
**12*	chr6:18139710G>A	374C>T	Ser125Leu
**13*	chr6:18149045T>A	83A>T	Glu28Val
**16*	chr6:18138969C>T	488G>A	Arg163His
**17*	chr6:18149004G>C	124C>G	Gln42Glu
**18*	chr6:18147845C>T	211G>A	Gly71Arg
**19*	chr6:18143597T>G	365A>C	Lys122Thr
**20*	chr6:18130694T>C	712A>G	Lys238Glu
**21*	chr6:18147851G>C	205C>G	Leu69Val
**22*	chr6:18138969C>G	488G>C	Arg163Pro
**24*	chr6:18133847C>A	537G>T	Gln179His
**25*	chr6:18130772A>G	634T>C	Cys212Arg
**26*	chr6:18132136A>G	622T>C	Phe208Leu
**27*	chr6:18143643A>C	319T>G	Tyr107Asp
**28*	chr6:18143613C>G	349G>C	Gly117Arg
**30*	chr6:18149022C>T	106G>A	Gly36Ser
**31*	chr6:18132147A>G	611T>C	Ile204Thr
**32*	chr6:18143622C>T	340G>A	Glu114Lys
**33*	chr6:18138970G>A	487C>T	Arg163Cys
**34*	chr6:18143718G>A	244C>T	Arg82Trp
**35*	chr6:18147856A>G	200T>C	Phe67Ser
**36*	chr6:18132163C>T	595G>A	Val199Ile
**37*	chr6:18130758A>T	648T>A	Cys216*
**38*	chr6:18133870A>G	514T>C	Ser172Pro
**39*	chr6:18147838G>A	218C>T	Ala73Val
**40*	chr6:18130729C>T	677G>A	Arg226Gln
**43*	chr6:18143728C>A	234G>T	Trp78Cys
**43*	chr6:18143700C>T	262G>A	Gly88Ser
**44*	chr6:18133887T>C	497A>G	Tyr166Cys

Unknown or Uncertain Function Alleles-*NUDT15*

Allele	Genomic Coordinate(s)	Coding (c.)	Protein consequence (p.)
**4*	chr13:48045720G>A	416G>A	Arg139His
**5*	chr13:48037798G>A	52G>A	Val18Ile
**6*	chr13:48037796_48037801dup[1]	50_55dup	Gly17_Val18dup
**7*	chr13:48037847G>C	101G>C	Arg34Thr
**8*	chr13:48037849A>G	103A>G	Lys35Glu
**10*	chr13:48037748T>C	2T>C	Met1Thr
**11*	chr13:48037885G>A	139G>A	Gly47Arg
**12*	chr13:48037902C>G	156C>G	Phe52Leu
**13*	chr13:48041104dup[2]	343dup	Glu115Glyfs*4
**14*	chr13:48037826_48037827insCGGG[3]	80_81insCGGG	Cys28Glyfs*28
**15*	chr13:48045771T>A	467T>A	Leu156Gln
**16*	chr13:48037834C>T	88C>T	Leu30Phe
**17*	chr13:48041113G>T	352G>T	Glu118*
**18*	chr13:48040982del[4]	221del	Asn74Metfs*8
**19*	chr13:48037749G>C	3G>C	Met1Ile
**20*	chr13:48045690C>G	386C>G	Pro129Arg

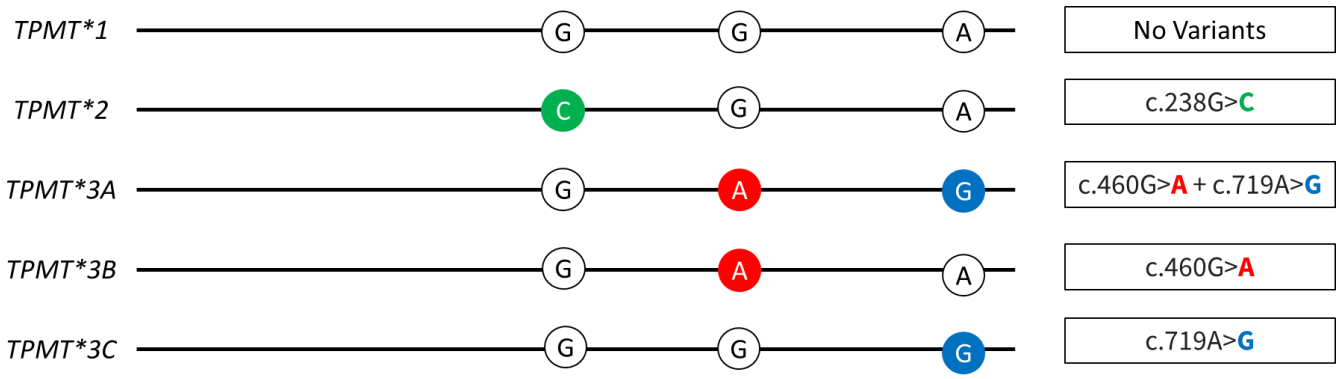
(1) chr13:48037782A>AGGAGTC
(2) chr13:48041103T>TG
(3) chr13:48037825C>CGCGG
(4) chr13:48040977GA>G

III. Complex Allele Determination

TPMT

Three single nucleotides variants: c.238G>C p.(Ala80Pro), c.460G>A p.(Ala154Thr), and c.719A>G p.(Tyr240Ser), account for over 90% of low activity phenotypes.[1] In the figure, these variants are displayed in their respective haplotype arrangements. Variants are described using the coding reference sequence NM_000367.5, and protein reference sequence, NP_000358.1.

- *TPMT*1* represents the "reference" allele. No variants detected.
- *TPMT*2* contains only c.238G>C
- *TPMT*3A* contains both c.460G>A and c.719A>G
- *TPMT*3B* contains only c.460G>A
- *TPMT*3C* contains only c.719A>G



*TPMT*3A* Allele Interpretation

The phenotype for a *TPMT*1/*3A* is Intermediate Metabolizer and for *TPMT*3B/*3C* is Poor Metabolizer.

Compound heterozygous individuals for c.460G>A and c.719A>G *without* confirmation of phasing is reported as "*TPMT*1/*3A* OR *TPMT*3B/*3C*". "*TPMT*1/*3A*" indicates c.460G>A and c.719A>G are on the same chromosome (*cis*). "*TPMT*3B/*3C*" indicates the two variants on separate chromosomes (*trans*). While the two variants are more likely to be in *cis*, they can also occur in *trans*, depending on the population.[2] See the table below for selected *TPMT* allele frequencies by ancestry.

Genotyping the patient's biological parents could aid in assessing the patient's metabolizer status. Prior to initiating thiopurines, consider evaluating TPMT enzyme activity to measure the ability of the patient's erythrocytes to generate thiopurine metabolites. However, erythrocyte TPMT activity test results can be influenced by disease and are unreliable in the case of a recent red blood cell transfusion. Thiopurine metabolite testing is also available after thiopurines are

initiated. This test will provide the concentration of the metabolites in the red blood cells after treatment with thiopurines.

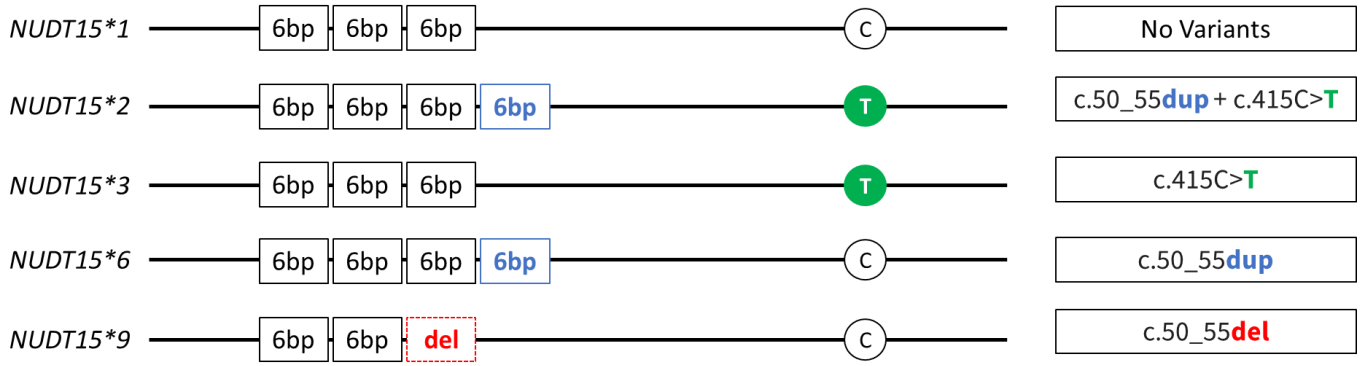
TPMT Allele	African American / Afro-Caribbean	Central/ South Asian	East Asian	European	Latino	Near Eastern	Sub-Saharan African
*1	92.341%	98.137%	97.963%	95.343%	94.346%	96.561%	92.149%
*2	0.531%	0.023%	0.008%	0.206%	0.346%	0.719%	0.000%
*3A	0.800%	0.422%	0.031%	3.384%	4.173%	1.307%	0.162%
*3B	0.000%	0.173%	0.000%	0.283%	0.238%	0.472%	0.000%
*3C	2.400%	1.122%	1.637%	0.492%	0.583%	0.940%	5.288%

This table is adapted from the supplemental "TPMT frequency table" document from the CPIC Guideline for Thiopurines and TPMT and NUDT15.

NUDT15

The complex haplotype definitions involving the variants: c.415C>T p.(Arg139Cys), c.50_55dup (p.Gly17_Val18dup), and c.50_55del p.(Gly17_Val18del), are displayed in the figure below. Of note, there are two variants described at position c.50_55. While the reference sequence contains three repeats of a 6-base pair (bp) motif [GAGTCG], the "c.50_55dup" variant is an insertion of an additional 'GAGTCG' (four repeats total). Variant "c.50_55del" is a deletion of one of the repeats (two repeats total). Variants are described using the coding reference sequence, NM_018283.4, and protein reference sequence, NP_060753.1

- *NUDT15*1* represents the "reference" allele. No variants detected. The 6bp [GAGTCG] sequence motif is repeated three times.
- *NUDT15*2* contains both c.50_55dup and c.415C>T. The 6bp [GAGTCG] sequence motif is repeated four times.
- *NUDT15*3* contains only c.415C>T. The 6bp [GAGTCG] sequence motif is repeated three times.
- *NUDT15*6* contains only c.50_55dup. The 6bp [GAGTCG] sequence motif is repeated four times.
- *NUDT15*9* contains only c.50_55del. The 6bp [GAGTCG] sequence motif is repeated two times.



*NUDT15*2* Allele Interpretation

The phenotype for a *NUDT15*1/*2* is Intermediate Metabolizer and for *NUDT15*3/*6* is Intermediate or Poor Metabolizer.

Compound heterozygous individuals for c.50_55dup and c.415C>T *without* confirmation of phasing are reported as "*NUDT15*1/*2* OR *NUDT15*3/*6*". "*NUDT15*1/*2*" indicates c.50_55dup and c.415C>T are on the same chromosome (*cis*). "*NUDT15*3/*6*" indicates the two variants on separate chromosomes (*trans*). *NUDT15*6* is currently assigned "uncertain function" by CPIC. The two variants are more likely to be in *trans*, however they can also occur in *cis*, depending on the population. See the table below for selected *NUDT15* allele frequencies by ancestry.

Genotyping the patient's biological parents could aid in assessing the patient's metabolizer status.

NUDT15 allele	Central/ South Asian	East Asian	European	Latino
*1	93.002%	87.874%	99.313%	93.638%
*2	0.000%	3.500%	0.000%	3.650%
*3	6.700%	6.050%	0.200%	0.750%
*6	0.200%	1.300%	0.300%	0.150%
*9	0.049%	0.000%	0.183%	0.026%

This table is adapted from the supplemental "NUDT15 frequency table" document from the CPIC Guideline for Thiopurines and TPMT and NUDT15.

IV. Helpful Resources

- PharmGKB (Pharmacogenomics Knowledge Base): https://www.pharmgkb.org/
    - TPMT https://www.pharmgkb.org/gene/PA356
    - NUDT15 https://www.pharmgkb.org/gene/PA134963132
- CPIC (Clinical Pharmacogenetics Implementation Consortium): https://cpicpgx.org/
    - https://cpicpgx.org/guidelines/guideline-for-thiopurines-and-tpmt/
- NIH-GTR (National Institutes of Health – Genetic Testing Registry): https://www.ncbi.nlm.nih.gov/gtr/x
- TPMT Nomenclature Site (Linköping University, Sweden) https://liu.se/en/research/tpmt-nomenclature-committee
- NUDT15 Nomenclature Site (Pharmacogene Variation Consortium "PharmVar") https://www.pharmvar.org/gene/NUDT15

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