Pharmacogene Variation Consortium Gene Introduction: *NUDT15*

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The Pharmacogene Variation (PharmVar) Consortium is the successor of the Human Cytochrome P450 (*CYP*) Allele Nomenclature website that served the pharmacogenetics community by designating *CYP* star (*) alleles. The aim of PharmVar is to continue the mission of serving as an official allele designation authority for the global pharmacogenetics community.[1] Herein, we describe the introduction of the first non-*CYP* gene to PharmVar. Pharmacogenetic variation of *NUDT15* plays a significant role in thiopurine response variability and toxicity.

THE *NUDT15* GENE

NUDT15 is a member of a large phosphatase protein family that shares a common NUDIX catalytic domain and metabolizes a wide range of nucleotide substrates (**Table 1**). Originally characterized as a pyrophosphatase, NUDT15 converts oxidized GTP to its monophosphate form, preventing the integration of the damaged purine nucleotides into DNA and subsequent mismatch repair. However, 8-oxo-GTP is a weak substrate for NUDT15 compared with its main metabolizer NUDT1; thus, the physiological functions of NUDT15 remain unclear. NUDT15 has been linked to xenobiotic drug metabolism in genome-wide association studies of drug toxicity, and subsequent mechanistic studies have demonstrated that it plays a key role in the conversion of the active thiopurine metabolite thioguanosine triphosphate to thioguanosine monophosphate.[2] *NUDT15* variants encoding no or severely decreased function predispose patients to excessive thiopurine activation and hematopoietic toxicities when receiving this class of drugs for either benign (e.g., inflammatory bowel diseases) or malignant (e.g., acute lymphoblastic leukemia) conditions. Given the compelling underlying biology and clinical relevance of this pharmacogenetic association, there is a growing interest in preemptive *NUDT15*-guided thiopurine dosing to avoid severe adverse events. The importance of including *NUDT15* genetic information in dosing recommendations for preventing thiopurine toxicity is further evidenced by the updated Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline on *TPMT*-guided thiopurine dosing recommendation that includes *NUDT15* in addition to *TPMT*.[3]

The initial genome-wide studies associated only a single nonsynonymous single-nucleotide polymorphism in exon 3 of *NUDT15* with thiopurine toxicity.[4] There is, however, growing evidence that additional functional variants exist. A total of 16 coding region variants have been reported to date (**Figure 1**), most of which affect function and/or thermal stability of the NUDT15 protein. On the basis of NUDT15 pyrophosphatase activity with thioguanosine triphosphate as the substrate, haplotypes containing p.R139C (c.414C>T, *rs116855232*) or the p.G17_V18del (c.55_56insGAGTCG, *rs869320766*) variant showed a severe decrease in activity[2] and were thus classified as "no function" alleles by CPIC.[3]

There are substantial racial and ethnic differences in the population frequencies of *NUDT15* variants. For example, alleles containing p.R139C (*NUDT15*2* and *NUDT153*) are predominantly found among Asians, but have also been detected in US Hispanics, especially those with high Native American ancestry. In contrast, the allele containing a five-nucleotide deletion in exon 1 (*NUDT15*9*) is almost exclusively found in patients with European ancestry.[4]

WHY NOMENCLATURE IS NEEDED

Clinical pharmacogenetic testing can be performed by numerous platforms and approaches, including targeted genotyping, sequencing, and array-based technologies. Molecular genetic testing results typically use the Human Genome Variation Society (HGVS) nomenclature system for reporting sequence variants and the International System for Human Cytogenetic Nomenclature system for reporting copy number variants. Although haplotype nomenclature is available within the HGVS framework, results of pharmacogenetic testing for genes involved in drug metabolism, such as *CYPs*, are most commonly reported using the star allele (*) nomenclature system. In addition to the identified variants and/or haplotypes, the interpretation of these results includes variant

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allele function and inferred phenotype, which can vary between testing laboratories. As such, it is imperative to establish clearly defined haplotype nomenclature as new pharmacogenes are discovered and subsequently offered by clinical testing laboratories and included in dosing guidelines.

Because of its pivotal role in thiopurine metabolism, clinical laboratories will likely incorporate preemptive *NUDT15* genotyping in concert with *TPMT* to avoid potentially life-threatening toxicity in patients carrying *TPMT* and/or *NUDT15* risk alleles. Although nomenclature databases focusing on haplotype definitions exist for *TPMT*, *UGTs*, and *NATs*, there are no centralized resources for other important pharmacogenes, including *NUDT15*, *DPYD*, or drug transporters. Standardized allele nomenclature will allow all professionals "to speak the same language" and facilitate the communication and dissemination of allelic variation. As discussed by Kalman *et al.*,[5] there is a need to increase transparency and standardization of clinical pharmacogenetic result reporting as well as the dissemination of research findings. To that end, PharmVar is now developing nomenclature for important non-*CYP* pharmacogenes, such as *NUDT15*, with an initial focus on genes with available CPIC guidelines.

Some of the *NUDT15* allelic variants were assigned star allele numbers when published (*NUDT15*1-NUDT15*6*, *NUDT15*10*, and *NUDT15*11*), whereas others were reported without a star allele designation or designations were assigned *post hoc* (*NUDT15*7- NUDT15*9*) (**Figure 1**). The absence of a centralized and widely accepted naming system not only makes it difficult to facilitate standardized test reporting, but also to interpret test results and compare research findings. Furthermore, reports from different clinical laboratories may not be consistent and may lead to confusion regarding dosing recommendations. Consequently, standardized nomenclature, ideally established when allelic variation is first described, and subsequent consistent use of agreed-on terminology are pivotal for clinical reporting of test results to reduce potential errors in drug selection and dosing.

PHARMVAR NOMENCLATURE FOR *NUDT15*

Experts representing global research, clinical testing, and implementation interests were recruited from PharmVar members to serve on the *NUDT15* expert panel and tasked to establish a formal nomenclature, according to PharmVar allele designation principles. The panel also included a Pharmacogenomics Knowledge Base/CPIC representative to ensure that the nomenclature is consistent with CPIC guidelines and to facilitate dissemination to a greater audience through the PharmGKB knowledgebase as well as other databases, such as ClinGen. The panel met via teleconferences and communicated by email. A literature search was performed on the PubMed database (beginning of PubMed to July 13, 2018, keyword "NUDT15") to compile records for published allelic variants. Most of the 38 articles only tested for p.R139C; there were only three articles describing allelic variants (**Figure S1**).

Because of the relatively few known allelic variants and the fact that most haplotypes are characterized by a single defining nucleotide variant, the review of haplotypes with star allele names was straightforward. Discussions centered mostly on the alleles initially published as *NUDT15*2* and *NUDT15*3*, carrying either p.R139C alone or p.R139C and p.G17-V18ins in *cis*. *NUDT15*2* and *NUDT15*3* share c.415C>T (p.R139C), which causes a severe decrease in *in vitro* function assays and predisposes carriers to a high risk of developing toxicity when exposed to thiopurines. Hence, the question was raised whether c.415C>T dictates the function of both alleles and, therefore, the allele published as *NUDT15*2* should be designated as a suballele of *NUDT15*3* to be consistent with PharmVar criteria for allele designation. According to those criteria, all haplotypes/alleles that carry a sequence variation causing no function are listed under the same star number (see ALLELE DESIGNATION CRITERIA under the SUBMISSIONS menu tab). The panel also discussed concerns regarding a potential merge of the two alleles into a single star allele and suballele designation because this may cause confusion with existing literature and among the clinical laboratories that already offer *NUDT15* testing, an argument that ultimately drove the decision to keep these two designations separate. More important, this issue of *NUDT15* star allele revision underscores the need to develop systematic haplotype nomenclature when variants are initially reported.

Figure 1 provides an overview of all variants published or submitted to PharmVar at the time this report was prepared. Allele frequencies can be accessed on the PharmGKB website at http://www.pharmgkb.org/page/nudt15RefMaterials. Function is assigned on the basis of *in vitro* activity data and/or thermal stability (details are available through the *NUDT15* READ ME document on the PharmVar *NUDT15* gene page and **Figure S1**). Allelic variants containing a frameshift or premature stop codon are predicted to be nonfunctional. The panel's recommendation was reviewed and approved by the PharmVar Steering Committee.

PharmVar maps all coordinates of *NUDT15* allelic variation to the most recent genomic and transcript reference sequences, GRCh37 and GRCh38 (**Table 1**). The *NUDT15* gene page which was released in September 2018 includes READ ME and CHANGE LOG documents that provide important information,

Table 1 Gene summary

Gene data	Description
Alias	NUDT15D (nudix nucleoside diphosphate linked moiety X–type motif 15), MTH2, MutT homolog 2
Gene IDs	HGNC: 23063 Entrez gene: 55270 Ensembl: ENSG00000136159 OMIM: 615792 UniProtKB: Q9NV35 PharmGKB accession ID: PA134963132
Genomic RefSeq	NG_047021.1
Transcript RefSeq	NM_018283.3
Protein RefSeq	NP_060753.1
LRG	NA

NA, not applicable; LRG, Locus Reference Genomic record

rs ID		rs181638201	rs368390627	rs769369441	rs941255227	rs61746486	rs746071566	rs186364861	rs869320766	rs777311140	rs1202487323	rs766023281	n/a	rs138959770	n/a	rs149436418	rs1457579126	rs761191455	rs1368252918	rs116855232	rs147390019	rs139551410	rs61973267	
NM_018283.3 (cDNA)		−79	−20	2	3	36	37	52	55	79	88	101	123	103	139	156	217	342	352	415	416	467	*7	
Sequence variation		G>A	G>A	T>C	G>C	A>C	delGGAGTC	G>A	insGAGTCG	insGCGG	C>T	G>C	A>G	C>A	G>A	C>G	delA	insG	G>T	C>T	G>A	T>A	G>A	
Amino acid change or impact on expression		unknown	unknown	M1T	M1I	Pro12=	G17_V18del	V18I	V18_V19insGV	C28G fs	L30V	R34T	K35E	G41=	G47R	F52L	N74M fs	E115G fs	E118X	R139C	R139H	L156Q	unknown	
		5'UTR		exon 1													exon 2			exon 3				
Assigned allele name	**as**																							**Impact**
NUDT15*1.01	*1																							none
NUDT15*1.02	_					■																		Unknown
NUDT15*1.03	_	■																						Unknown
NUDT15*1.04	_		■																					Unknown
NUDT15*1.05	_																						■	Unknown
NUDT15*1.06	_													■										Unknown
NUDT15*2.01	*2								■											■				V18_V19insGV, R139C
NUDT15*3.01	*3																			■				R139C
NUDT15*4.01	*4																				■			R139H
NUDT15*5.01	*5							■																V18I
NUDT15*6.01	*6								■															V18_V19insGV
NUDT15*7.01	_											■												R34T
NUDT15*8.01	_												■											K35E
NUDT15*9.01	_						■																	G17_V18del
NUDT15*10.01	*10	▨	▨	■																				M1T
NUDT15*11.01	*11	▨	▨												■									G47R
NUDT15*12.01	_															■								F52L
NUDT15*13.01	_																	■						E115G fs
NUDT15*14.01	_									■														C28G fs
NUDT15*15.01	_																					■		L156Q
NUDT15*16.01	_										■													L30V
NUDT15*17.01	_																		■					E118X
NUDT15*18.01	_																■							N74M fs
NUDT15*19.01	_				■																			M1I

Figure 1 Allele nomenclature summary. The graph contains a graphical summary of all *NUDT*15 allelic variants described to date. Of the published variants now designated *NUDT*1* through *NUDT*19*, eight have been published using star nomenclature (**1-*6*, **10*, and **11*); those designated **7* to **9* were named as such *post hoc*. Six novel haplotypes were designated **14* to **19*. Red boxes indicate SNVs that confer an amino acid change; black boxes indicate SNVs in noncoding regions of unknown functional consequence or synonymous SNPs in coding regions. Gray shaded boxes indicate the gene regions not covered by sequencing. SNV *rs* IDs, their positions on the cDNA reference sequence, nucleotide changes, and impact (amino acid change, frameshift (fs), or stop codon (X)) within a haplotype are shown in the top panels and the right-hand column. An extended figure with additional information, including references, and PharmVar IDs is provided as **Supplemental Material**.

including details regarding allele functionality; these documents will be updated as new information becomes available. All updates will also be coordinated with PharmGKB to provide unified information to the pharmacogenomics community. Furthermore, PharmVar will be announcing the incorporation of standardized *NUDT15* nomenclature to stakeholders via blogs, social media, and targeted announcements/email communications to promote the use of standardized nomenclature because testing is being offered by an increasing number of laboratories.

NOVEL *NUDT15* ALLELIC VARIANTS

Since the inception of the *NUDT15* expert panel in April 2018, six novel previously unpublished allelic variants containing nonsynonymous sequence variations were submitted, reviewed, and

designated *NUDT15*14* through *NUDT15*19* (**Figure 1**). An additional five haplotypes were designated as *NUDT15*1* suballeles because of the presence of single-nucleotide variants in noncoding regions or harboring synonymous single-nucleotide variants. It remains unknown whether these variants have any functional consequences.

PharmVar strongly encourages submissions by investigators before publication (submission requirements and details of how to submit are provided at http://www.PharmVar.org). All information is regarded as confidential and will not be shared outside of the expert panel and Steering Committee. Designated haplotypes can be held for up to 6 months to allow timing of release on PharmVar and through article publication. PharmVar also welcomes direct submissions to make information available to the public independent of a publication.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Figure S1. NUDT15 nomenclature.

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CONFLICT OF INTEREST

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1. Gaedigk, A. *et al.* The Pharmacogene Variation (PharmVar) Consortium: incorporation of the human cytochrome P450 (CYP) allele nomenclature database. *Clin. Pharmacol. Ther.* **103**, 399–401 (2018).
2. Moriyama, T. *et al.* NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. *Nat. Genet.* **48**, 367–373 (2016).
3. Relling, M.V. *et al.* Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update. *Clin. Pharmacol. Ther.* **105**, 1095–1105.
4. Yang, J.J. *et al.* Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. *J. Clin. Oncol.* **33**, 1235–1242 (2015).
5. Kalman, L.V. *et al.* Pharmacogenetic allele nomenclature: international workgroup recommendations for test tesult teporting. *Clin. Pharmacol. Ther.* **99**, 172–185 (2016).