Brief Report

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LYMPHOID NEOPLASIA

Novel variants in *NUDT15* and thiopurine intolerance in children with acute lymphoblastic leukemia from diverse ancestry

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Key Points

- We identified 3 novel loss-of-function variants in *NUDT15* linked to thiopurine intolerance.
- Our findings extended the importance of *NUDT15* variation in thiopurine pharmacogenetics in diverse populations.

Prolonged exposure to thiopurines (eg, mercaptopurine [MP]) is essential for curative therapy in acute lymphoblastic leukemia (ALL), but is also associated with frequent dose-limiting hematopoietic toxicities, which is partly explained by inherited genetic polymorphisms in drug metabolizing enzymes (eg, *TPMT*). Recently, our group and others identified germ line genetic variants in *NUDT15* as another major cause of thiopurine-related myelosuppression, particularly in Asian and Hispanic people. In this article, we describe 3 novel *NUDT15* coding variants (p.R34T, p.K35E, and p.G17_V18del) in 5 children with ALL enrolled in frontline protocols in Singapore, Taiwan, and at St. Jude Children's Research Hospital. Patients carrying these variants experienced significant toxicity and reduced tolerance to MP across treatment protocols. Functionally, all 3 variants led to partial to complete loss of NUDT15 nucleotide diphosphatase activity and negatively influenced protein stability. In particular, the p.G17_V18del variant protein showed extremely low thermostability and was completely void of catalytic activity, thus likely to confer a high risk of thiopurine intolerance. This in-frame deletion was only seen in African and European patients, and is the first *NUDT15* risk variant identified in non-Asian, non-Hispanic populations. In conclusion, we discovered 3 novel loss-of-function variants in *NUDT15* associated with MP toxicity, enabling more comprehensive pharmacogenetics-based thiopurine dose adjustments across diverse populations. (*Blood*. 2017;130(10):1209-1212)

Introduction

Thiopurines (eg, mercaptopurine [MP]) are critical components of treatment regimens of acute lymphoblastic leukemia (ALL) in children and adults.[1-4] Although efficacious as antileukemic agents, thiopurines also have narrow therapeutic indices with common dose-limiting hematopoietic toxicity. In addition to polymorphisms in the *TPMT* gene,[5,6] we recently identified NUDT15 as a novel thiopurine metabolizing enzyme, genetic variation of which strongly influences MP tolerance in children with ALL[7,8] and patients with inflammatory bowel diseases.[9] As a nucleotide diphosphatase, NUDT15 negatively affects MP cytotoxicity by converting the thioguanine triphosphate (TGTP) to thioguanine monophosphate (TGMP), reducing DNA incorporation of thioguanine, and thus attenuating DNA damage–induced apoptosis.[8,10] Thus far, 4 *NUDT15* variants have been associated with loss of diphosphatase activity, excessive MP activation, and myelosuppression in patients.[8] Interestingly, these hypomorphic variants are largely restricted to Asian people or Asian-related ancestral groups (Native Americans),[7,8] suggesting their potential origin in Asia. *NUDT15*-guided thiopurines dosing was considered of limited relevance in European or African patients for which *NUDT15* risk variants had hitherto been rarely reported. However, a substantial proportion of patients do not have the currently known risk variants in *TPMT* or *NUDT15*, yet experience severe MP toxicity,[7-9] pointing to novel variants in these or other genes that are still to be discovered. In this article, we report 3 novel *NUDT15* loss-of-function variants that were associated with MP intolerance in children with ALL from diverse ancestry.

Methods

Study subjects were children treated on frontline ALL clinical trials at National University Singapore, Singapore,[11] National Taiwan University Hospital,

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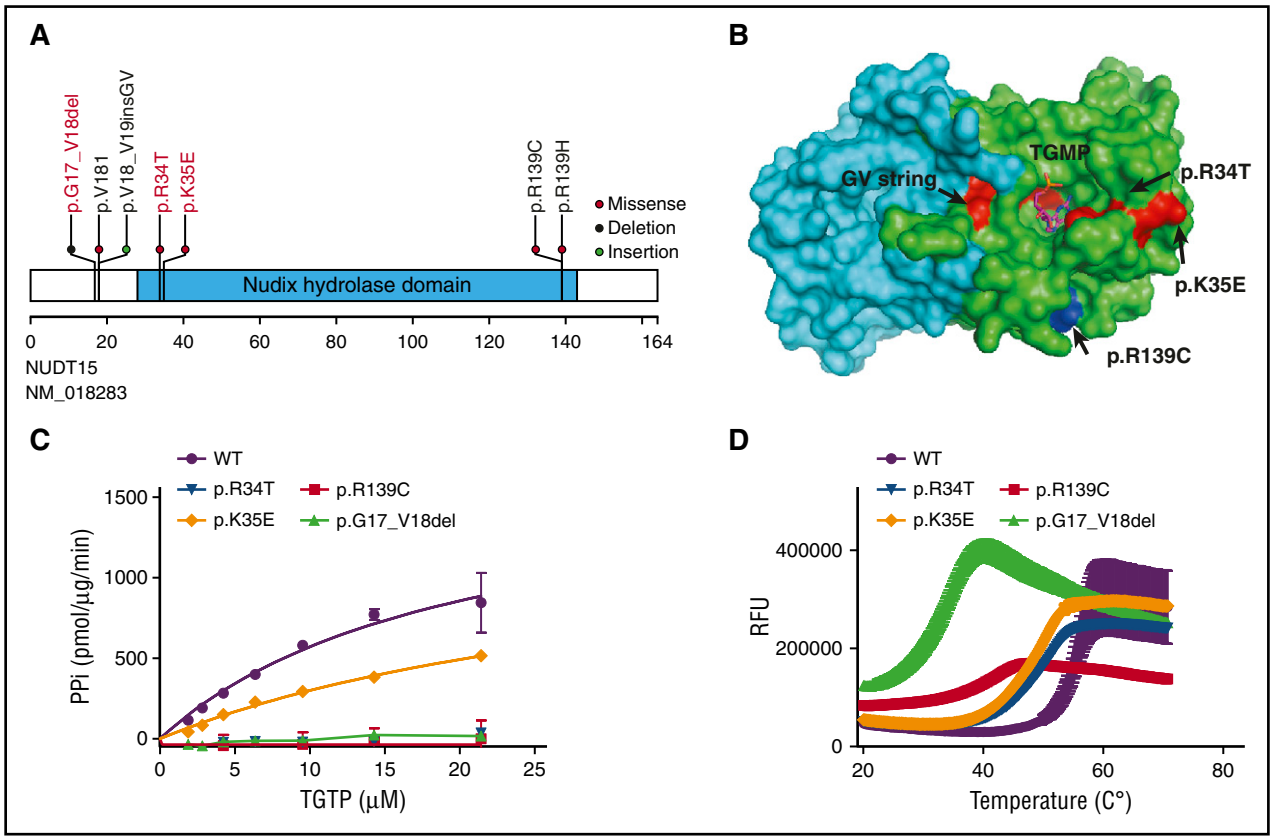

Figure 1. Functional characterization of 3 novel *NUDT15* risk variants. (A) Shown are the positions of novel (red text) and known risk variants (black text) in the coding region of the *NUDT15* gene. (B) Shown is the spatial distribution of amino acid residues affected by risk variants in the human NUDT15 protein. Presented as a homodimer, chains A and B are discriminated by color (green and cyan, respectively). Each risk variant is identified by letters as well as colors, and TGMP is shown in stick-ball presentation. The 3-dimensional structure was drawn by using PyMOL software (accession code 5LPG of the Protein Data Bank, http://www.rcsb.org/pdb). (C) NUDT15 nucleotide diphosphatase activity was determined by using the PiPer Pyrophosphate Assay (Life Technologies) for each of the 3 novel variants, with wild-type (WT) and p.R139C proteins included as controls. (D) NUDT15 protein thermostability was measured by using SYPRO Orange (Molecular Probes) for each of the 3 variants, with wild-type and the p.R139C variant included as controls. The inflection point of each curve indicates the temperature for protein unfolding and is thus a measurement of stability. RFU, relative fluorescence unit.

Taiwan,[12] or St. Jude Children's Research Hospital, Memphis, TN[13,14]. This study was approved by the Institutional Review Board at St. Jude Children's Research Hospital and participating institutions of respective frontline ALL protocols.

MP tolerance was defined as the average stable MP dosage (milligram per square meter per day) over >4 weeks during maintenance therapy of the Malaysia-Singapore (MaSpore) 2003 and St. Jude Total Therapy Study (TOT) XVI low-risk protocols.[11,14] TOT XIIIB high-risk maintenance therapy consisted of weekly rotation of drug pairs during which MP was given 1 week for every 4 weeks at 75 mg/m^2 per day.[13] The patient in Taiwan did not complete maintenance therapy because of relapse, and his MP tolerance was defined on the basis of other therapy phases of continuous MP treatment. The MP dose was clinically titrated to a target white blood cell count in each clinical protocol.

Details of *NUDT15* sequencing, variant functional characterization, and thiopurine metabolite assay are provided in supplemental Methods, available on the *Blood* Web site.

Results and discussion

In total, we identified 3 novel coding variants in the *NUDT15* gene: p.R34T, p.K35E, and p.G17_V18del, in 5 children with ALL of Asian, African, or European descent (Figure 1A-B; Table 1). The p.R34T missense variant (c.101G>C, resulting in an arginine-to-threonine conversion at the amino acid residue 34) was recurrent in 2 patients of East Asian ancestry enrolled in the MaSpore 2003 ALL protocol in Singapore and the TPOG-2002 infantile ALL protocol in Taiwan, respectively. Both patients tolerated very low doses of MP (17.9 and 16.4 mg/m^2 per day, respectively) relative to that observed previously in Asian children who were heterozygous at the common risk variant p.R139C (33.1 ± 11.5 mg/m^2 per day).[8] The p.K35E variant substitutes the lysine residue 35 with glutamic acid and was observed in a single East Asian patient in Singapore who also had the known p.V18_V19insGV and p.R139C variants. Subsequent haplotyping confirmed that he was compound heterozygous for *NUDT15* risk alleles (ie, 1 allele with the p.V18_V19insGV and p.R139C variants and the other allele with the p.K35E variant). He was exquisitely sensitive to MP with a tolerated dosage of 8.5 mg/m^2 per day, similar to those homozygous at the p.R139C variant (4.3 ± 2.4 mg/m^2 per day).[7,8] Finally, the in-frame deletion variant, p.G17_V18del, was observed in 2 patients at St. Jude, both of whom were non-Asian (ie, of European or African descent, respectively). The patient on the TOT XVI protocol tolerated 43.5 mg/m^2 per day of MP during the maintenance phase, which was comparable to patients with the heterozygous p.R139C variant, as we reported previously (47.3 ± 19.1 mg/m^2 per day).[7,8] The second patient with this deletion variant was treated on the TOT XIIIB protocol, for which maintenance therapy consisted of drug pairs administered in weekly rotation. Thus, for this patient, MP was given for only 1 week of every 4-week period.[13] Likely because of the short duration of her MP exposure, this patient did not experience significant

Table 1. Patient characteristics and MP tolerance

	NUDT15 novel variant				
	c.101G>C p.R34T		c.103A>G p.K35E	c.37_42delGGAGTC p.G17_V18del	
Position at chr13	48037847		48037849	48037783-48037788	
rsID	rs766023281		NA	rs746071566	
	Subject 1	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**
Sex	Male	Male	Male	Female	Male
Age, y	13.8	0.3	4.4	13.7	6.3
Race	East Asian	East Asian	East Asian	African	European
Diagnosis	B-ALL	B-ALL	B-ALL	T-ALL	B-ALL
Protocol	MaSpore 2003 SR	TPOG-2002-infantile ALL	MaSpore 2003 IR	TOT XIIIB HR	TOT XVI LR
NUDT15 diplotype*	*1/p.R34T	*1/p.R34T	*2/p.K35E	*1/p.G17_V18del	*1/p.G17_V18del
TPMT genotype	WT	WT	WT	WT	WT
4-wk tolerated MP dosage, mg/m^2 per day	17.9	16.4†	8.5	82.5 for a 1-wk period‡	43.5
Protocol MP dosage, mg/m^2 per day	50	25†	50	75 for a 1-wk period‡	75

B-ALL, B-cell acute lymphoblastic leukemia; HR, high risk; IR, intermediate risk; LR, low risk; NA, not applicable; rsID, reference SNP ID; SR, standard risk; T-ALL, T-cell acute lymphoblastic leukemia; TPOG, Taiwan Pediatric Oncology Group; WT, wild-type.

*The *1 represents the *NUDT15* wild-type haplotype, and *2 represents the haplotype with both p.V18_ V19insGV and p.R139C variants.

†Patient received MP at 17.9 and 15.2 mg/m^2 per day for 17 and 22 days, respectively, during remission, but did not complete the entire treatment regimen due to relapse.

‡Maintenance therapy (120 weeks) for TOT XIIIB HR consisted of drug pairs administered in weekly rotation. Therefore, the standard MP exposure was 75 mg/m^2 per day for only 1 week, followed by other drug pairs for the subsequent 3 weeks. With MP dosed for this short duration, the patient did not experience toxicity. It is likely her actual long-term MP tolerance would be low if MP were given in a continuous fashion similar to other ALL treatment protocols.

toxicity, and her tolerated dosage was 82.5 mg/m^2 per day for that week. This is consistent with our experience with *TPMT*-deficient patients who also tolerated full MP dosages for short durations.[15] The actual MP tolerance for this patient could have been different if she had been challenged with sustained MP dosing.

Next, we characterized the enzymatic activity of the 3 variant NUDT15 proteins (Figure 1C). Compared with wild-type NUDT15 that efficiently converts TGTP to TGMP (V_{max}/K_m [catalytic efficiency, pmol/μg enzyme/min/μM TGTP] of 85.6 ± 37.2), all 3 novel variant proteins showed loss of nucleotide diphophatase activity: the p.K35E variant had a V_{max}/K_m of 38.2 ± 12.0, whereas activity could not be detected for the p.R34T and p.G17_V18del variants. A thermal stability assay showed reduced (the temperature midpoint for the protein unfolding transition) values for all 3 variant NUDT15 proteins (48.7 ± 0.04, 47.7 ± 0.03, 31.3 ± 0.2, and 38.4 ± 0.1°C for p.R34T, p.K35E, p.G17_V18del, and p.R139C, respectively) compared with wild-type NUDT15 (54.8 ± 0.1°C; Figure 1D). In fact, the p.G17_V18del variant was even more unstable than the p.R139C variant, which had the most severe loss of function in our previous report,[8] raising the possibility that p.G17_V18del is a very high-risk variant for profound toxicity in patients. These results were in line with the reduced MP tolerance observed in patients (Table 1), providing functional evidence for genotype-guided thiopurine dose reductions.

Although there is a growing appreciation of *NUDT15* variants as the primary genetic cause for thiopurine toxicity in Asian patients,[7-9,16-22] the importance of this gene in thiopurine pharmacogenetics was less understood in European or African patients, probably due to the rarity of the main p.R139C risk variants in these populations. As the first *NUDT15* risk variant identified in non-Asian and non-Hispanic patients with ALL, characterization of the p.G17_V18del variant extends the importance of *NUDT15* polymorphism to additional populations. Although still relatively rare (0.26% and 0.05% in the Exome Aggregation Consortium database of 36 677 European and 5203 Africans, respectively; supplemental Figure 1; supplemental Table 1), this variant resulted in highly damaging effects on NUDT15 activity and therefore predisposition to severe toxicity in patients. Interestingly, red blood cell thioguanine nucleotides in the patient with the p.G17_V18del variant (subject 5 in Table 1) were comparable to those from ALL patients with different genotypes at the p.R139C variant after normalization to MP dosage[7,23,24] (supplemental Figure 2), which is consistent with our recent report that total thioguanine nucleotides are not influenced by *NUDT15* genotype and thus may not be an informative pharmacological marker for toxicity in *NUDT15*-deficient patients.[25] Our findings indicate that *NUDT15*-related toxicity can occur in all major race/ethnic groups, and thus *NUDT15*-guided thiopurine dosing has clinical implication across diverse populations. However, it should be noted that the 3 novel variants reported in this article were rare, and our sample size was limited for definitive analyses of their exact effects on MP intolerance, even though our functional characterization provides strong evidence corroborating their association with MP toxicity. Surveying the entire Exome Aggregation Consortium database of whole-exome sequences of 60 706 individuals, there are 85 coding nonsynonymous *NUDT15* variants, the vast majority of which are rare. Systematic functional assays of these variants are warranted in the future to understand and anticipate their potential effects on MP toxicity. Because our current study focused only on *NUDT15* and *TPMT*, it is also possible that novel risk variants in other genes might have been missed. In fact, a significant proportion of interpatient variability in thiopurine intolerance remains unexplained by *NUDT15* or *TPMT*,[8] suggesting that genome-wide sequencing studies are needed in the future to discover additional genetic risk factors.

The NUDT15 protein has 3 glycine-valine (G-V) repeats at amino acid positions from 13 to 18, and this region of the gene appears to be particularly vulnerable to genetic polymorphism (Figure 1B). Either amino acid insertion or deletion at this position resulted in significant perturbation of NUDT15 activity. In fact, this G-V string is located in the NUDIX fold, a key catalytic motif for the diphosphatase activity,[10,26,27] with these G-V repeats directly facing the substrate binding pocket (Figure 1B),[10,27] suggesting that the amino acid substitutions at this position might negatively affect catalytic activity by altering the conformation of the substrate binding pocket.

In conclusion, our findings provide further rationale for preemptive *NUDT15* genotyping to enable individualized thiopurine dose reduction and safer use of this important antileukemic agent.

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Authorship

Contribution: J.J.Y. conceived the research project, collected data, analyzed data, interpreted data, wrote the manuscript, and gave final approval; T.M. performed experiments, collected data, analyzed data, interpreted data, and wrote the manuscript; M.V.R., W.E.E., R.N., T.-N.L., C.L., W.Y., D.-T.L., C.-H.Y., and S.K. collected data, analyzed data, interpreted data, and critically reviewed the manuscript; and Y.-L.Y., H.A., S.J., C.-H.P., and A.E.-J.Y. evaluated patients, collected data, analyzed data, and critically reviewed the manuscript.

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