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ARTICLE

The influence of *NUDT15* variants on 6-mercaptopurine-induced neutropenia in Vietnamese pediatric acute lymphoblastic leukemia

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Summary

6-Mercaptopurine (6-MP) serves as the backbone of maintenance therapy in acute lymphoblastic leukemia. The nucleoside diphosphate-linked moiety X-type motif 15 genes (*NUDT15*) affects the metabolism of 6-MP and thiopurine-related neutropenia in the Asian population. This study reports the influence of these variants on 6MP-induced neutropenia in children with acute lymphoblastic leukemia (ALL). A total of 102 children were enrolled in this retrospective cohort study. *NUDT15* variants on exon 1 and exon 3 were identified by Sanger sequencing. We divided the intermediate metabolizer group and the normal metabolizer group base on *NUDT15* diplotypes. During the first 3 months of maintenance treatment, medical reports measured treatment-related toxicity (neutropenia) and 6-MP dose decreases. *NUDT15* genotyping showed two categories of mutations: wild type (75.5%) and heterozygous variant (24.5%). Neutropenia during the early phase of maintenance therapy in the intermediate metabolizer group (68%) was significantly higher than the normal metabolizer group (18.2%) with 10-fold greater odds. Especially, the c.415C>T heterozygous variant was extremely associated with neutropenia compared with the C>C genotype (odds ratio [OR]: 12; 95% confidence interval [CI]: 3.5–41.7). The tolerated doses of 6-MP after the first 3 months of maintenance therapy related to the intermediate metabolizer group and the normal metabolizer group were 48.7 and 64.3 mg/m^2/day, respectively ($p < 0.001$). One-fourth of individuals had NUDT15 variations. All NUDT15 heterozygous mutations cause neutropenia and need 6-MP dose optimization. Given the frequency of NUDT15 mutations in Vietnamese children and their connection with early neutropenia, testing is indicated.

Introduction

Acute lymphoblastic leukemia (ALL) is the major type of leukemia in children, making up approximately 25% of newly diagnosed cancers in patients under 15 years old.[1] Pediatric ALL treatment is divided into three main distinct phases: induction, consolidation, and maintenance. The maintenance phase is the longest, lasting for approximately 2–3 years in pediatric patients. Daily 6-mercaptopurine (6-MP) serves as the backbone of maintenance therapy. Ideally, chemotherapeutic drugs administered for such an extended length of time would have minimal or mild adverse effects.[2]

Thiopurines, such as 6-MP, are prodrugs that require enzymatic conversion into thioguanine nucleotides (TGNs), which in turn are incorporated into DNA (forming DNA-TG) to induce cell apoptosis. Previous studies have reported that inherited genetic polymorphisms of thiopurine methyltransferase (*TPMT*) and nucleoside diphosphate-linked moiety X-type motif 15 (*NUDT15*) genes could influence -thiopurine toxicity as these genes encode vital enzymes involved in thiopurine metabolism, particularly the formation of TGN and DNA-TG, respectively.[3] The toxicity of 6-MP has been well documented, with neutropenia being one of the most significant adverse effects. This risk is amplified in patients with *TPMT* and *NUDT15* allelic mutations, leading to increased infection and mortality risk.[4] To minimize this risk, pre-emptive testing for *TMPT* and *NUDT15* mutations and subsequent genotype-guided dose adjustment is the standard of care in high-resource settings.[5] According to the Clinical Pharmacogenetics Implementation Consortium (CPIC) 2018, *NUDT15* phenotypes were assigned to normal metabolizer (*1/*1), intermediate metabolizer (*1/*2, *1/*3), possible intermediate metabolizer (*2/*5, *2/*6), and poor metabolizer (*2/*2, *2/*3, *3/*3). The initial doses of thioguanine should be adjusted based on the *NUDT15* phenotypes.[6]

Recently, germline genetic variants in *NUDT15* have been identified as major causes of thiopurine-related myelosuppression, particularly in Asian and Hispanic populations.[7] Previous studies have reported the proportion of *NUDT15* variants in a variety of populations, including those in Japanese (20%), Korean (26.4%),[8] Guatemalan (12.6%), Singaporean (24%),[3] and Thai (15%) genetic ancestries.[9] A meta-analysis review of 26 studies showed the prevalence of *NUDT15* variants in South Asia was 16.5%.[10] For example, one in every four people carries at least one allele of *NUDT15* variants in the Japanese population. Moreover, one in every 50 people is homozygous or compound heterozygous for these defective variants.[11] In Vietnam, a previous study showed 32.9% *NUDT15*

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variants and 2.8% *TPMT* variants in patients with ALL.[12] There are limited trials on *NUDT15* variants and their impact on toxicity in pediatric patients with ALL in Vietnam. Pre-therapy genotyping of *NUDT15* variants was not used to guide the initial dose reduction of the 6-MP in our hospital. Therefore, this study aims to determine the percentage of *NUDT15* variants and their effects on 6-MP neutropenia toxicity for pediatric ALL in Vietnam.

Material and methods

Study participants and ALL treatment protocol

A total of 220 pediatric patients with ALL were treated from June 2020 to May 2021 at Children's Hospital 2. In this retrospective cohort study, 102 patients with ALL who had completed at least the first 3 months of maintenance treatment were recruited. This study was approved by the institutional ethics committees of Children's Hospital 2, Ho Chi Minh City, Vietnam (number: 743/ND2-CDT). Written informed consent was obtained from the parents or guardians of the patients.

We used Children's Oncology Group (COG) Protocol AALL0434, AALL0932, and AALL1131 to guide the treatment of these patients, each of whom was under 16 years old.[13,14] Patients with ALL were stratified to the standard-risk group if the patient was between 1 and 10 years of age, had an initial white blood cell (WBC) count of less than 50,000/μL, and had an absence of central nervous system and testicular disease status. We did not use molecular classification due to limited financial resources. The other patients were considered high risk and were treated with more intensive chemotherapy than the standard-risk group in the induction phase. The standard-risk group was reevaluated for minimum residual disease in peripheral blood and bone marrow on days 8 and 29 of induction therapy, respectively. In 2–3 years of the maintenance phase, both the standard- and high-risk groups received oral 6-MP at 75 mg/m^2/day, oral methotrexate (MTX) at 20 mg/m^2/week, and intravenous vincristine at 1.5 mg/m^2/month. The standard-risk group also received oral dexamethasone at 6 mg/m^2/day, while the high-risk group also received prednisolone at 40 mg/m^2/day for the first 5 days of the month.

The initial daily 6-MP dose on maintenance therapy was 75 mg/m^2/day in this study. The 6-MP dosage was adjusted to maintain an absolute neutrophil count (ANC) of 0.5–1.5 10^9/L. The 6-MP therapy was discontinued if the patients had severe neutropenia (ANC $< 0.5\ 10^9$/L) for at least 1 week. Then, the patients were followed up with to check their ANC every week until recovery. The 6-MP dosage modifications for myelotoxicity are listed in the supplemental information. The early neutropenia was identified by obtaining WBC count every 4 weeks, or when patients had a fever, during the first 3 months of maintenance therapy. To evaluate the relationship between *NUDT15* variants and 6-MP dosage, the 6-MP doses required during the early maintenance treatment were obtained from past medical records. Therefore, the adjustment of the 6-MP dose could be an indicator of 6-MP tolerance. The tolerated dose of 6-MP was calculated as the mean of 14 daily doses over at least 9 weeks of a stable dose of 6-MP during maintenance therapy.[3]

Genotyping

The *NUDT15* variants were identified using the Sanger sequencing technique. We collected 0.2 mL peripheral blood with EDTA anticoagulant, then genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Waltham, MA, USA), according to the manufacturer's instructions. Exons 1 and 3 of the *NUDT15* gene were first amplified by polymerase chain reaction (PCR) using two primer pairs: NUD-1F (5′-AGTGAGCGCGTCACTTCCTTG-3′) and NUD-1R (5′- AGATGACCTCCAGGGAGTTG-3′) for exon 1 and NUD-3F (5′-GGTTGGGAGTGGGTTCCTTG-3′) and NUD-3R (5′- CAAATCTTCTCGGCCACCTA-3′) for exon 3. PCR was done at an annealing temperature of 60°C in separate 25 μL reactions consisting of 1× PCR Buffer, 200 μM each dNTP, 1.5 mM $MgCl_2$, 0.5U Taq Hot Start Polymerase (Takara Bio, Shiga, Japan), 0.1 μM each forward and reverse primer, and 100 ng genomic DNA. After being analyzed on agarose gel electrophoresis, the PCR products were purified with the ExoSAP-IT reagent (Thermo Scientific) and directly sequenced with BigDye Terminator v.3.1 Cycle Sequencing Kit on an ABI 3500 Genetic Analyzer (Applied Biosystems, Waltham, MA, USA). *NUDT15* variants were identified using the CLC Main Workbench, based on referenced genomic and coding sequences of *NUDT15* (NG_047021.1 and NM_018283.4).[3]

The variants were identified at *NUDT15* locus: *1 (wild type), *2 (p.Val18_Val19insGlyVal and p.Arg139Cys), *3 (p.Arg139Cys only), *4 (p.Arg139His), *5 (p.Val18Ile), and *6 (p.Val18_Val19insGlyVal only). The other alleles were unidentified in our study (example: *7, *8, *9 …). This study could not detect *NUDT15* alleles on separate chromosomes or the same chromosome. Therefore, we temporary assigned the patients with p.Val18_Val19insGlyVal and p.Arg139Cys to the *1/*2 diplotype due to the *3/*6 diplotype being very rare. We classified them into three groups based on their *NUDT15* diplotype: normal metabolizer group (wild type), intermediate metabolizer group (heterozygous variant), and poor metabolizer group (homozygous or compound heterozygous variant *2/*3).[3]

Statistical analysis

Data regarding the demography, diagnosis, risk stratification, and treatment phase of all patients with ALL were collected. In addition, we studied *NUDT15* variants presented in the participants to determine the percentage of *NUDT15* gene mutations in patients with ALL. Data were entered and analyzed using SPSS v.26 (IBM, Armonk, NY, USA). Correlations between genetic variations and severe neutropenia, the mean dose of 6-MP in the first 3 months of maintenance therapy, and the tolerated dose were analyzed using Pearson chi-squared test or Fisher's exact test (categorical) or the Mann-Whitney U-test (continuous variables). A statistically significant p value was less than 0.05.

Results

Frequency of NUDT15 variants in children with ALL

The study population was shown in Figure 1. We identified a total of 102 patients with ALL who had a median age of 6 years. There was a slight male predominance. Patients with pre-B cell ALL (B-ALL) were predominant compared with T cell ALL (T-ALL; 93.2% vs. 6.8%). Patients with pre-B-ALL were stratified into standard- and high-risk groups based on age, presenting WBC, testicular, and/or CNS involvement, and end induction measurable residual disease (MRD). The patient's features are listed in Table 1. Sanger sequencing analysis detected 3 types of *NUDT15*

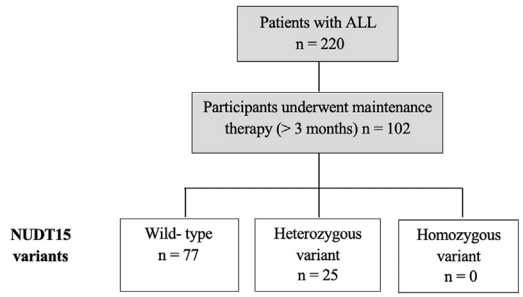

Figure 1. Flow chart of population
The patients were classified into wild-type, heterozygous, and homozygous variant groups.

variants in our patients, namely p.Val18_Val19insGlyVal and p.Val18Ile in exon 1 and p.Arg139Cys in exon 3. Accordingly, five types of alleles were identified at the *NUDT15* locus: *1, *2, *3, *5, and *6. However, we did not identify *4 in this study. There were 25 patients (24.5%) with *NUDT15* heterozygous variants including *1/*2, *1/*3, *1/*5, and *1/*6. The homozygous variant was not found in the participants (Table 1).

The relationship between NUDT15 variants and early severe neutropenia, median dose, and tolerated dose of 6-MP

The participants were divided into two groups based on *NUDT15* variants: the normal metabolizer group (wild type) and the intermediate metabolizer group (heterozygous). This study did not have a poor metabolizer patient. There were 31 patients (30.4%) who developed early neutropenia after the first 3 months of maintenance treatment. The intermediate metabolizer group was at an increased odds of neutropenia compared with the wild-type group (odds ratio [OR]: 10; 95% confidence interval [CI]: 3.4–26; $p < 0.001$). Notably, the c.415C>T (p.Arg139Cys) was the most common variant at 16.7%. It was extremely associated with neutropenia (OR: 12; 95% CI: 3.5–41.7; $p < 0.001$). The intermediate metabolizer group had 5.7-fold higher odds of febrile neutropenia than the normal metabolizer group (OR: 5.7; 95% CI: 1.9–17; $p < 0.001$). The association of *NUDT15* variants with neutropenia during the first 3 months of maintenance therapy is shown in Table 2.

The median starting dose of 6-MP was comparable between groups during the maintenance phase ($p = 0.23$). Regarding the median doses of 6-MP after the first month and the second month of maintenance therapy, the intermediate metabolizer group was administered a lower dose than the normal metabolizer group (both $p < 0.001$). The median 6-MP doses after 3 months of maintenance therapy for the *NUDT15* heterozygous group and the normal metabolizer group were 47.6 and 65.9 mg/m^2/day, respectively. The tolerated doses after 3 months of maintenance therapy of the intermediate metabolizer group and the normal metabolizer group were 48.7 and 64.7 mg/m^2/day, respectively ($p < 0.001$) (Figure 2).

Table 1. Clinical characteristics and frequency of *NUDT15* variants in the patients with ALL

Characteristics	Subjects (n = 102)	Proportion (%)
Age (years)	6 (2–14)[a]	-
Sex		
Female	38	37.3
Male	64	62.7
Leukemia type, risk group		
B-ALL standard risk	54	53
B-ALL high risk	41	40.2
T-ALL	7	6.8
Therapy		
Initial 6-MP dose, mg/m^2/day	67.7 (44–80)[a]	-
Early neutropenia	31	30.4
NUDT15 genotyping		
Wild type	77	75.5
Heterozygous variant	25	24.5
*1/*2	5	4.9
*1/*3	12	11.8
*1/*5	5	4.9
*1/*6	3	2.9
Homozygous variant	0	0

[a]Median (interquartile range).

Discussion

This study showed that approximately one-fourth of Vietnamese patients had *NUDT15* heterozygous variants. Its prevalence is lower than the 32.9% found in a recent study of 70 Vietnamese patients with ALL. They may vary due to variations in the enrolled population.[12] *NUDT15* variation prevalence is one of the highest in the Asian population. The proportion of *NUDT15* variants was high in Asia, including those of Japanese (20%), Korean (26.4%),[8] Guatemalan (12.6%), Singaporean (24%),[3] and Thai (15%) genetic ancestries.[9] A meta-analysis review of 26 studies showed that the prevalence of *NUDT15* variants in South Asia was 16.5%.[10] This result was extremely high compared with Europe. The most common variant was c.415C>T at 16.7%, like the other reports in Asia. This variant played an important role in thiopurine-induced toxicity.[14] We identified five variants of *NUDT15*, including *1, *2, *3, *5, and *6. A similar percentage was found in the study of Vietnamese, Korean, and Thai children with ALL.[8,9,12] However, we did not identify homozygous variants or compound heterozygous for two *NUDT15* variant alleles in the study.

The maintenance therapy analysis was based on two groups: 25 participants in the intermediate metabolizer group and 77 participants in the normal metabolizer

Table 2. Association between *NUDT15* variants and the early neutropenia, the median 6-MP dose of patients with ALL on maintenance therapy

Characteristics	The intermediate metabolizer group, n = 25	The normal metabolizer group, n = 77	OR (95% CI)	p value
Age (year)	6 (2–11)[a]	6 (2–14)[a]	-	0.8
Sex (%)			0.7 (0.3–1.9)	0.53
Female	36.7	38.9	-	-
Male	63.3	61.1	-	-
Leukemia type, risk group (%)				0.85
B-ALL standard risk	50	48.3	-	-
B-ALL high risk	38.9	41.7	-	-
T-ALL	11.1	10	-	-
Severe neutropenia, n (%)				
1 month	9 (36)	8 (10.4)	4.9 (1.6–14)	0.003
2 months	15 (60)	13 (16.9)	7.4 (1.2–20)	0.0001
3 months	17 (68)	14 (18.2)	10 (3.4–26)	0.0001
Febrile neutropenia, n (%)	10 (40)	8 (10.4)	5.7 (1.9–17)	0.003
Median 6-MP dose (mg/m^2/day)[a]				
First month	67	68.5	–	0.23
Second month	55	66.7	–	0.0001
Third month	47.6	65.9	–	0.0001
Median 6-MP adjusted dose (mg/m^2/day) [+]	48.7	64.3	–	0.0001

OR, odds ratio; 95% CI, 95% confidence interval. p values were calculated using the Mann-Whitney U-test. The statistical significance of $p < 0.05$.
[a]Median (interquartile range).

group. Thirty-one patients developed neutropenia in the early phase of maintenance, which accounted for 30.4%. Within the intermediate metabolizer group, 17 out of 25 patients developed neutropenia during this period, which accounted for 68%, or an increased odd of 10-fold, compared with the normal metabolizer group. In other words, the intermediate metabolizer group was significantly associated with an increased risk of 6-MP-related neutropenia. Our finding was comparable to that of Thai population research, which had shown that *NUDT15* variants were associated with a 17.8-fold increased risk of developing neutropenia compared with wild type.[9] Among *NUDT15* variants, the c.415C>T variant was the major predictor for thiopurine-related neutropenia.[12] In our study, 76.5% of patients with the c.415C>T variant had neutropenia. This variant significantly increased the odds of neutropenia compared with the others (OR: 12; 95% CI: 3.5–41.7; $p < 0.001$). These results are consistent with a systematic review and meta-analysis in Asia that provided evidence that *NUDT15* c.415C>T was significantly correlated with high incidences of thiopurine-induced leukopenia.[14] Patients with severe neutropenia had a higher risk of severe infectious disease. In this study, febrile neutropenia related to the intermediate metabolizer group was 5.7-fold higher than the normal metabolizer group. This was an important complication that affected the result of treatment.

Several previous reports have demonstrated thiopurine intolerance in patients with *NUDT15* variants in ALL. The Thai population study observed that 80% of patients carrying *NUDT15* variants required 6-MP dose reduction or discontinuation due to neutropenia within the first 8 weeks of maintenance therapy.[9] According to the CPIC, the initial doses of thioguanine should be adjusted based on *NUDT15* genotypes.[6] In our experiences, pre-therapy genotyping of *TPMT* or *NUDT15* variants were not used to guide the initial dose reduction of the 6-MP. We regulated the 6-MP doses according to the patient's neutrophil count and hepatotoxicity status. In clinical practice, the median 6-MP doses after 2 months in the intermediate metabolizer group and the normal metabolizer group were 55 and 66.7 mg/m^2/day, respectively ($p < 0.001$). The median 6-MP doses after 3 months in patients with the intermediate metabolizer group and the normal metabolizer group were 47.6 and 65.9 mg/m^2/day, respectively ($p < 0.001$). This finding differed from a Japanese study, which had shown that the average 6-MP doses during maintenance therapy were 40.0 and 29.3 mg/m^2/day for the wild-type group and the heterozygous group, respectively.[15] One of the causes might be the initial dose of 6-MP at 50 mg/m^2/day based on Japan's protocol. In our study, we determined that the tolerated dose of 6-MP after 3 months in the intermediate metabolizer group was significantly lower than the normal metabolizer group (48.7 vs. 64.3 mg/m^2/day; $p < 0.001$).

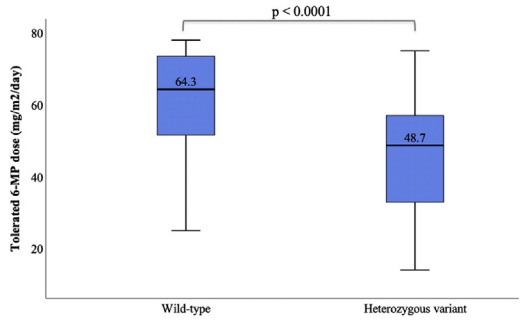

Figure 2. Tolerated dose of 6-MP after 3 months of maintenance phase in patients with *NUDT15* variant
At the early stage of maintenance phase, the tolerated dose of 6-MP for the intermediate metabolizer group was significantly higher than the normal metabolizer group (48.7 vs. 64.3 mg/m^2/day; $p < 0.0001$).

This finding was that the tolerated dose of 6-MP in the intermediate metabolizer group was 75% of the wild-type group. Our results were similar to a previous study in Vietnam that showed that the tolerated dose of 6-MP in the intermediate metabolizer group was significantly lower than the normal metabolizer group (55.2 vs. 69.5 mg/m^2/day; $p < 0.001$),[12] It was comparable to the aforementioned Thai study, which demonstrated that the tolerated dose of 6-MP in patients with NUDT15 heterozygous variations was lower than in those without the variant (36.6 vs. 50 mg/m^2/day).[9] Our results matched the CPIC's recommendations.[6]

This study had some limitations regarding assessing the association between *NUDT15* variants and 6-MP treatment. First, this was a single-institution study. Findings might be different for children in other regions of Vietnam. Second, this study could not detect NUDT15 alleles on separate chromosomes or the same chromosome. Therefore, the *1/*2 diplotype could not be distinguished from the *3/*6 diplotype. Third, we did not check the *TPMT* mutations due to limited financial resources and because the proportion of *TPMT* mutations has been reported to be insignificant in patients of Asian ancestry.

Conclusions

This study found a significant incidence of *NUDT15* variations in children with ALL from Vietnam. *NUDT15* variants were associated with neutropenia in the early phase of maintenance therapy. We suggest *NUDT15* variants testing for all children with ALL before treatment with 6-MP to optimize the effectiveness of the treatment and subsequently reduce recurrent admission due to febrile neutropenia. We also highly recommend untested Vietnamese patients exhibiting toxicities related to thiopurine exposure be considered for *NUDT15* variants testing and dose adjustments.

Data and code availability

The datasets supporting the current study have not been deposited in a public repository because of privacy and ethical concerns but are available from the corresponding author upon request.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.xhgg.2023.100183.

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Declaration of interests

The authors declare no competing interests.

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References

1. Chiang, M.Y., Radojcic, V., and Maillard, I. (2016). Oncogenic Notch signaling in T-cell and B-cell lymphoproliferative disorders. Curr. Opin. Hematol. *23*, 362–370.
2. Rudin, S., Marable, M., and Huang, R.S. (2017). The promise of pharmacogenomics in reducing toxicity during acute lymphoblastic leukemia maintenance treatment. Dev. Reprod. Biol. *15*, 82–93.
3. Moriyama, T., Nishii, R., Perez-Andreu, V., Yang, W., Klussmann, F.A., Zhao, X., Lin, T.N., Hoshitsuki, K., Nersting, J., Kihira, K., et al. (2016). NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat. Genet. *48*, 367–373.
4. Wang, R., Liu, B., Li, J., Xu, J., Wang, X., Zhao, Z., and Zhao, L. (2019). Association between the c.415C > T, c.52G > A, and 36_37insGGAGTC polymorphisms of NUDT 15 and thiopurine-induced leukopenia, thiopurine intolerance, and severe hair loss: an updated meta-analysis. Drug Des. Dev. Ther. *13*, 2729–2744.
5. Schaeffeler, E., Jaeger, S.U., Klumpp, V., Yang, J.J., Igel, S., Hinze, L., Stanulla, M., and Schwab, M. (2019). Impact of NUDT15 genetics on severe thiopurine-related hematotoxicity in patients with European ancestry. Genet. Med. *21*, 2145–2150.
6. Relling, M.V., Schwab, M., Whirl-Carrillo, M., Suarez-Kurtz, G., Pui, C.H., Stein, C.M., Moyer, A.M., Evans, W.E., Klein, T.E., Antillon-Klussmann, F.G., et al. (2019). Clinical Pharmacogenetics implementation Consortium guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update. Clin. Pharmacol. Ther. *105*, 1095–1105.
7. Cargnin, S., Genazzani, A.A., Canonico, P.L., and Terrazzino, S. (2018). Diagnostic accuracy of NUDT15 gene variants for thiopurine-induced leukopenia: a systematic review and meta-analysis. Pharmacol. Res. *135*, 102–111.
8. Yi, E.S., Choi, Y.B., Choi, R., Lee, N.H., Lee, J.W., Yoo, K.H., Sung, K.W., Lee, S.Y., and Koo, H.H. (2018). NUDT15 variants cause hematopoietic toxicity with low 6-TGN levels in children with acute lymphoblastic leukemia. Cancer Res. Treat. *50*, 872–882.
9. Puangpetch, A., Tiyasirichokchai, R., Pakakasama, S., Wiwattanakul, S., Anurathapan, U., Hongeng, S., and Sukasem, C.

(2020). NUDT15 genetic variants are related to thiopurine-induced neutropenia in Thai children with acute lymphoblastic leukemia. Pharmacogenomics *21*, 403–410.

10. Jena, A., Jha, D.K., Kumar-M, P., Kasudhan, K.S., Kumar, A., Sarwal, D., Mishra, S., Singh, A.K., Bhatia, P., Patil, A., and Sharma, V. (2021). Prevalence of polymorphisms in thiopurine metabolism and association with adverse outcomes: a South Asian region-specific systematic review and meta-analysis. Expet Rev. Clin. Pharmacol. *14*, 491–501.
11. Yang, J.J., Landier, W., Yang, W., Liu, C., Hageman, L., Cheng, C., Pei, D., Chen, Y., Crews, K.R., Kornegay, N., et al. (2015). Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. J. Clin. Oncol. *33*, 1235–1242.
12. Nghia, H., Than, H.H., Dong, C.V., Oanh, T.T.K., Truc, V.T.T., Ngan, C.T.T., Ngon, H.T., Binh, N.T., Dung, P.C., Anh Vu, H., and Xinh, P.T. (2022). The effects of NUDT15 and TPMT variants on mercaptopurine treatment in Vietnamese pediatric acute lymphoblastic leukemia patients. Pediatr. Hematol. Oncol. *39*, 561–570.
13. Angiolillo, A.L. (2010). AALL0932-Treatment of patients with newly diagnosed standard risk B-lymphoblastic leukemia (B-ALL) or localized B-lineage lymphoblastic lymphoma (B-LLy). In Children's Oncology Group (CureSearch).
14. Salzer, W. (2012). AALL1131-A phase III randomized trial for newly diagnosed high risk B-precursor acute lymphoblastic leukemia (ALL) testing clofarabine in the very high risk stratum. In Children's Oncology (CureSearch).
15. Tanaka, Y., Nakadate, H., Kondoh, K., Nakamura, K., Koh, K., and Manabe, A. (2018). Interaction between NUDT15 and ABCC4 variants enhances intolerability of 6-mercaptopurine in Japanese patients with childhood acute lymphoblastic leukemia. Pharmacogenomics J *18*, 275–280.