

Association between *NUDT15* polymorphisms and thiopurine-
induced leukopenia in pediatric acute lymphoblastic leukemia
patients: a systematized review

by

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Abstract

Background: Thiopurines are commonly used to treat acute lymphoblastic leukemia (ALL). A recent genome-wide association study shows the variants of *NUDT15*, an enzyme involved in thiopurine metabolism, are associated with thiopurine-induced leukopenia. This study aims to organize the literature on the relationship between the occurrence of leukopenia and the *NUDT15* gene polymorphism in pediatric ALL patients and to examine the differences in associations due to ethnicity.

Methods: A literature search was performed for *NUDT15* as a keyword using PubMed. Eligible studies included the following inclusion criteria: 1) participants are pediatric ALL patients, 2) ethnic group of the participants is clear, 3) analyzing the association between *NUDT15* gene polymorphism and leukopenia. Minor allele frequency (MAF) of rs116855232 was calculated based on each result of the study.

Results: A total of 16 studies were included. All of them were reported from Asian countries, except Ethiopia and Sweden. All studies in Asian countries showed a strong positive association between *NUDT15* variants and thiopurine-induced leukopenia, while a Swedish report indicated no relationship between *NUDT15* variants and leukopenia. MAF of rs116855232 in Asia is between 0.050 and 0.168, while MAF in Non-Asian countries is relatively low, and there is no allele in studies in Ethiopia. The difference in the association between Asians and non-Asians seems to be from the difference in MAF.

Conclusion: The present study summarized the association between thiopurine-induced leukemia and *NUDT15* gene polymorphisms in pediatric ALL patients. It is hoped that the

distribution of *NUDT15* polymorphisms will be clarified and that treatment guidelines will be established for each ethnicity.

Keywords: ALL, NUDT15, thiopurine-induced leukopenia

List of abbreviations

6-MP	6-mercaptopurine
IBD	inflammatory bowel diseases
ALL	acute lymphoblastic leukaemia
TPMT	Thiopurine S-methyltransferase
GWAS	genome-wide association study
NUDT15	nudix hydrolase 15
Rs	reference number
MAF	minor allele frequency
OR	odds ratio
CI	confidential interval
HR	hazard ratio

1. INTRODUCTION

1.1. Background Information

Thiopurines, such as azathioprine and 6-mercaptopurine (6-MP), are commonly used in the treatment of various autoimmune disorders, inflammatory bowel diseases (IBD) and acute lymphoblastic leukaemia (ALL) ⁽¹⁾. In the treatment of childhood ALL, 6-MP is often used in the maintenance phase and is a major component of the therapeutic approach ⁽²⁾. However, there are known adverse effects of thiopurines typically including leukopenia, thrombocytopenia, hair loss, and hepatotoxicity.

It has long been known that the polymorphisms of thiopurine S-methyltransferase (TPMT), a gene involved in thiopurine metabolism, are associated with the risk of developing thiopurine-induced leukopenia. However, TPMT variants are more common in Caucasian and African American populations than in Asian populations⁽³⁾. The frequency of myelosuppression did not differ by ethnicity, suggesting another genetic predisposition for the development of thiopurine-induced leukopenia in Asians.

Recent genome-wide association studies (GWAS)⁽⁴⁾ have indicated that genetic polymorphisms in *nudix hydrolase 15* (*NUDT15*) are also associated with the risk of developing thiopurine-induced leukopenia. *NUDT15* is also an enzyme involved in 6-MP metabolism, and its genetic polymorphisms, including the most common variant, rs116855232 (*NUDT15**3), are known to reduce the enzyme activity. *NUDT15* variants are more common in Asians than in Caucasians or African Americans and were expected to be a major genetic cause of the development of thiopurine-induced leukopenia in Asians.

Several studies have investigated the relationship between *NUDT15* variants and thiopurine-induced leukopenia in pediatric ALL patients, mainly in Asia, since the seminal Yang et al. study reporting the primary discovery GWAS⁽⁴⁾. In the past few years, there have

also been reports on non-Asian patients, and there is interest in whether the frequency and effect of *NUDT15* variants varies by ethnicity, as in the case of TPMT.

1.2. Objectives

In this systematized review, we aimed to search the literature for the relationship between the occurrence of leukopenia and the *NUDT15* gene polymorphism in pediatric ALL patients treated with 6-MP and to determine whether there are differences in the frequency of leukopenia and the *NUDT15* gene polymorphism between different ethnic groups. This work was intended to serve as the beginning to more extensive systematic activities in the future which may include a broader range of outcome definitions spanned across different disease populations.

2. METHODS

2.1. Search strategy and study selection

We conducted literature search for *NUDT15* as a keyword using PubMed. We limited the search term to be able to maximally identify all relevant studies. There was no restriction placed by publication date. Studies were included if the following inclusion criteria were met: 1) participants were pediatric ALL patients (18 years old and under), 2) ethnic group of the participants was clear, 3) analysis of the association between *NUDT15* gene polymorphism and leukopenia induced azathioprine treatment, 4) literature language is restricted to English. We excluded studies that fall into the following criteria: 1) review studies, meta-analysis, commentaries, editorials, opinion, letters and case reports, 2) duplicated studies, 3) not human study, 4) no full text articles, 5) no abstract. This study has not been registered into PROSPERO.

2.2. Data extraction

From eligible studies, we collected general information on study characteristics and association result. General information were as follows: first author's name, year of publication, ethnicity, sample size, study design, age, sex, genetic polymorphisms, treatment drug, genotyping methods, and definition of leukopenia. Minor allele frequency (MAF) of rs116855232 was calculated based on each result of the study. The calculation formula was as follows:

$$\text{MAF} = \frac{\text{frequency of heterozygotes} + \text{frequency of homozygotes} \times 2}{(\text{Total sample numbers} \times 2)}$$

3. RESULTS

3.1. Study selection

In all, 311 articles were retrieved from the literature search up to January 7, 2024. In the

screening phase, 207 articles were excluded, and 104 studies were selected for assessing eligibility (**Figure 1**). We further removed 88 articles which included 51 reviews, 32 case reports, 8 comments, 6 editorials, 4 letters after the retrieving process. Finally, 15 studies were included in this systematized review.

3.2. Study characteristics and results

Table 1 shows the characteristics of the 15 studies included. The articles were published from 2015 to 2023. All of them were reports from Asian countries (five from China, four from Thailand, one from Japan, two from Vietnam and one from Korea), except Ali et al.⁽⁵⁾ from Ethiopia and Wahlund et al.⁽⁶⁾ from the Nordic countries. Most studies adopted $WBC < 2.0 \times 10^9/L$ or $ANC < 1000 \text{ cells/mm}^3$ as a leukopenia criteria. MAF of rs116855232 in Asians was reported as being between 0.050 and 0.168, while MAF in non-Asian countries was relatively low and there was no allele in studies in Ethiopia. All the studies from Asian countries showed a positive association between *NUDT15* variants and thiopurine-induced leukopenia, while those from non-Asian countries did not (**Table 2**).

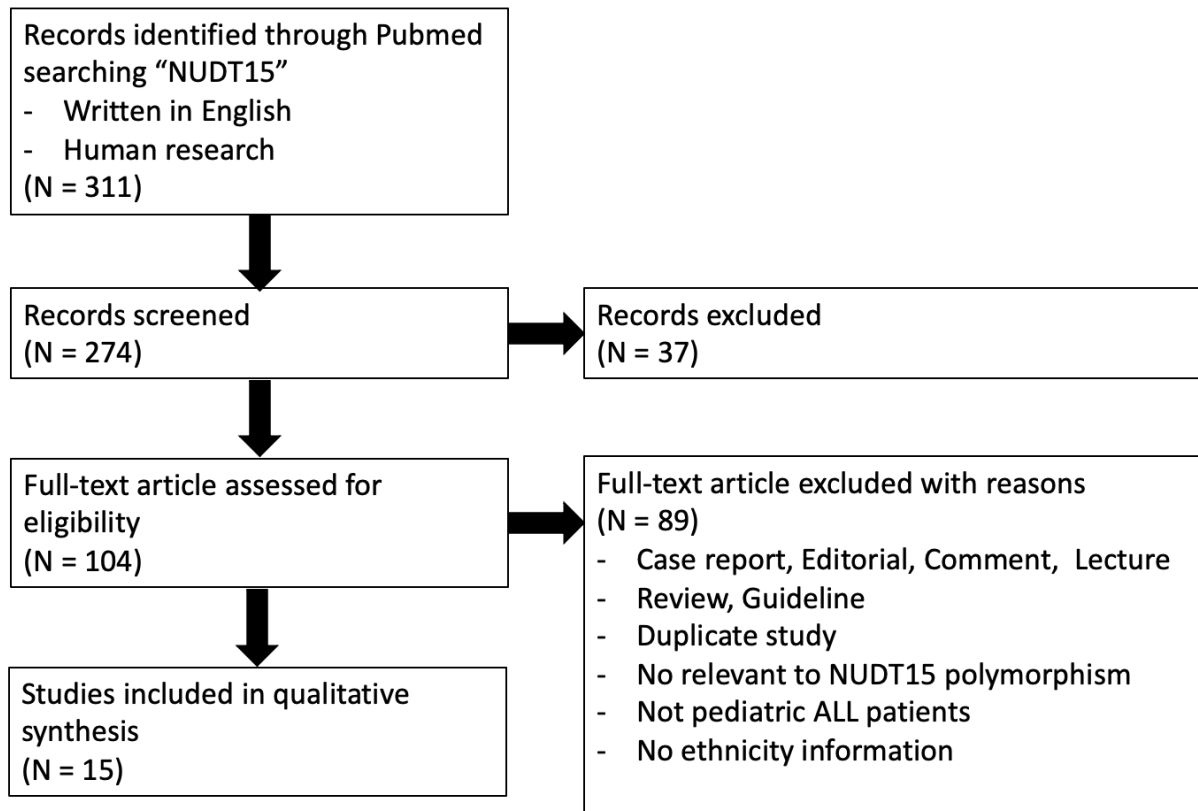


Figure 1: Study selection process

Table 1: Study Characteristics of studies included in this systematized review

Sources	Ethnicity	Sample size (male/female)	SNP	Leukopenia criteria	MAF
Tanaka et al. (2018) ⁽⁷⁾	Japanese	95 (47/48)	<i>NUDT15*3</i> <i>NUDT15*5</i>	WBC < 2.0 × 10 ⁹ /L or ANC < 1000 cells/mm ³	0.168
Zhou et al. (2018) ⁽⁸⁾	Chinese	105 (66/39)	<i>NUDT15*3</i>	WBC < 2.0 × 10 ⁹ /L	0.157
Kim et al. (2018) ⁽⁹⁾	Korean	185 (110/75)	<i>NUDT15*3</i>	ANC < 500 cells/mm ³	0.122
Buaboonnam et al. (2019) ⁽¹⁰⁾	Thai	102 (58/44)	<i>NUDT15*3</i>	ANC < 500 cells/mm ³	0.127
Puangpetch et al. (2020) ⁽¹¹⁾	Thai	100 (54/46)	<i>NUDT15*3</i>	ANC < 500 cells/mm ³	0.050
Wahlund et al. (2020) ⁽⁶⁾	Swedish	102 (51/51)	<i>NUDT15*3</i>	ANC < 500 cells/mm ³	0.020
Cao et al. (2020) ⁽¹²⁾	Chinese	173 (88/85)	<i>NUDT15*3</i>	NA	0.104
Zhou et al. (2020) ⁽¹³⁾	Chinese	60 (26/34)	<i>NUDT15*3</i>	WBC < 2.0 × 10 ⁹ /L	NA
Boonyawat et al. (2021) ⁽¹⁴⁾	Thai	99 (55/44)	<i>NUDT15*3</i>	ANC < 1500 cells/mm ³	0.085
Mao et al. (2021) ⁽¹⁵⁾	Chinese	149 (85/64)	<i>NUDT15*3</i>	WBC < 2.0 × 10 ⁹ /L	0.141

Nghia et al. (2022) ⁽¹⁶⁾	Vietnamese	70 (38/32)	<i>NUDT15*2</i> <i>NUDT15*3</i> <i>NUDT15*6</i>	WBC < 2.0 × 10 ⁹ /L	0.100
Khaeso et al. (2022) ⁽¹⁷⁾	Thai	169 (90/79)	<i>NUDT15*3</i>	ANC < 500 cells/mm ³	0.077
Fan et al. (2022) ⁽¹⁸⁾	Chinese	145 (81/64)	<i>NUDT15*3</i>	WBC < 2.0 × 10 ⁹ /L	0.079
Ali et al. (2023) ⁽⁵⁾	Ethiopian	142 (92/50)	<i>NUDT15*3</i>	WBC < 1.0 × 10 ⁹ /L or ANC < 500 cells/mm ³	0.000
Nguyen et al. (2023) ⁽¹⁹⁾	Vietnamese	102 (64/38)	<i>NUDT15*2</i> <i>NUDT15*3</i> <i>NUDT15*5</i> <i>NUDT15*6</i>	NA	0.170

SNP, single nucleotide polymorphism; MAF, minor allele frequency of rs116855232 (*NUDT15*3*); WBC, white blood cell; ANC, absolute neutrophil count; NA, not available.

Table 2: Major results reported by each study

Sources	Ethnicity	Major results
Tanaka et al. (2018) ⁽⁷⁾	Japanese	Significant association between leukopenia and rs116855232 (HR 2.79 [1.80 - 4.31], p = 4.41×10 ⁻⁶) with Cox regression analysis
Zhou et al. (2018) ⁽⁸⁾	Chinese	Significant association between leukopenia and rs116855232 (OR 3.617 [1.377 - 9.051], p = 0.009) with logistic regression analysis

Kim et al. (2018) ⁽⁹⁾	Korean	Significant association between leukopenia and TT genotypes of rs116855232 (HR 11.42 [3.35 - 38.97], $p < 0.001$) with Cox regression analysis. Not significant association between leukopenia and CT genotypes of rs116855232 (HR 1.25 [0.78 - 2.01], $p = 0.358$) with Cox regression analysis.
Buaboonnam et al. (2019) ⁽¹⁰⁾	Thai	Significant association between leukopenia within the first three months and rs116855232 (OR 12 [3.781 – 38.085], $p < 0.001$) with Fisher's exact test
Puangpetch et al. (2020) ⁽¹¹⁾	Thai	Significant association between leukopenia during the first 60 days and rs116855232 (OR 17.862 [14.198 - 75.992], $p = 9.5 \times 10^{-5}$) with logistic regression analysis
Wahlund et al. (2020) ⁽⁶⁾	Swedish	Not significant association between leukopenia and rs116855232 (HR 0.98 [0.69 - 1.39], $p = 0.90$) with a proportional hazard model
Cao et al. (2020) ⁽¹²⁾	Chinese	Significant association between leukopenia and rs116855232 ($p = 6.37 \times 10^{-11}$) with logistic regression analysis
Zhou et al. (2020) ⁽¹³⁾	Chinese	Significant association between leukopenia and rs116855232 (OR 6.44 [1.02 – 40.66], $p = 0.048$) with logistic regression analysis
Boonyawat et al. (2021) ⁽¹⁴⁾	Thai	The number of ANC counts were significantly decreased among patients with rs116855232 ($p < 0.001$)
Mao et al. (2021) ⁽¹⁵⁾	Chinese	Significant association between leukopenia and rs116855232 (OR 2.743 [1.305 – 5.768], $p = 0.008$) with logistic regression analysis
Nghia et al. (2022) ⁽¹⁶⁾	Vietnamese	The number of ANC during the maintenance therapy was significantly different among patients with normal, intermediate, low function of NUDT15 ($p = 0.0001$) with Analysis of Variance
Khaeso et al. (2022) ⁽¹⁷⁾	Thai	Significant association between leukopenia and rs116855232 (OR 10.35 [1.79 – 59.85], $p < 0.01$) with logistic regression analysis
Fan et al. (2022) ⁽¹⁸⁾	Chinese	The number of leukopenia episode was significantly different among patients with normal, intermediate, low function of NUDT15 ($p = 0.012$) using Kruskal-Wallis test and posthoc

Dunn's multiple test

Ali et al. (2023) ⁽⁵⁾	Ethiopian	No variant alleles were identified for rs116855232 of NUDT15.
Nguyen et al. (2023) ⁽¹⁹⁾	Vietnamese	Leukopenia during the first 60 days was significantly associated patients with intermediate function of NUDT15 (OR 7.4 [1.2 – 20], p = 0.0001) with Fisher's exact test

HR, Hazard ratio; OR, Odds ratio; ANC, absolute neutrophil count; NA, not available.

4. DISCUSSION

Since Yang et al.⁽⁴⁾ reported the association between *NUDT15* variants and thiopurine-induced leukopenia in pediatric ALL patients, there have been multiple reports which showed the relationship, especially from Asian countries. The studies retrieved in this study were also mostly from Asian countries, with seven reports from East Asia and six from South-East Asia. Although the studies from Asian countries differed in the definition of leukopenia and other detailed outcome settings, they all reported a strong positive association between *NUDT15* variants and thiopurine-induced leukopenia, and none reported a negative association or no association. On the other hand, a report from Sweden⁽⁶⁾ with 102 ALL patients reported an HR [95% CI] adjusted for age at diagnosis of 0.77 [0.50 - 1.18] for leukopenia and *NUDT15* variants, which indicated no significant relationship between *NUDT15* variants and leukopenia.

In addition, a report from Ethiopia⁽⁵⁾ of 142 pediatric ALL patients showed no association between rs116855232, as the most common variant of *NUDT15* variants, and leukopenia. These may all be the result of low MAF, and the contribution of *NUDT15* variants to thiopurine-induced leukopenia is likely to be low in regions with low MAF. However, Wahlund et al. ⁽⁶⁾ also examined the relationship between *NUDT15* variants and thiopurine dose and noted that patients with *NUDT15* variants had significantly lower doses of thiopurine ($p = 0.0097$). Based on this fact, they argue that *NUDT15* variants may be involved in unexplained leukopenia during treatment, even in Europeans with low MAF, and that testing for the *NUDT15* gene polymorphism may be recommended.

MAF of rs116855232 is similar to previous large-scale genetic polymorphism study⁽²⁰⁾ with results of 0.105 in East Asians, 0.001 in African American, and 0.023 in Finnish (Nordic). Within East Asia, alleles were found at a relatively high frequency in Japan, China, and Korea (from 0.122 to 0.168). In contrast, the frequency tended to be slightly lower in Southeast Asian

countries such as Thailand and Vietnam, at 0.050 to 0.127. Although it is known that *NUDT15* mutations are more prevalent amongst Asians and Latin Americans compared to European and African Americans, there have been no studies examining intra-Asian differences in MAF in detail and few reports examining the relationship between mutations and leukopenia in non-Asian regions. In recent years, reports have emerged from other Asian countries such as India⁽²¹⁾ and Lebanon⁽²²⁾, Latin America such as Colombia⁽²³⁾ and Brazil⁽²⁴⁾, and Africa such as Zimbabwe⁽²⁵⁾ and Ethiopia⁽⁵⁾. It is hoped that further clarification of regional differences in *NUDT15* genetic polymorphisms and the frequency of azathioprine-induced leukopenia will enable the provision of personalized medicine tailored to the genetic characteristics of different ethnic groups.

A limitation of this study is that a quantitative assessment could not be carried out due to the varying conditions of individual studies, such as the definition of leukemia and treatment protocols. In addition, because leukemia was used as the primary outcome, we could not evaluate studies that used outcomes such as thiopurine dosage when the dosage of thiopurine was changed according to neutrophil counts during treatment.

4.1. Conclusions

The present study qualitatively summarized the reported association between thiopurine-induced leukemia and *NUDT15* gene polymorphisms in pediatric ALL patients. The association between *NUDT15* variants and leukemia has been reported mainly from Asian countries, but in recent years, there has been an increase in studies from non-Asian countries. It is hoped that the distribution of *NUDT15* polymorphisms will be clarified, and that treatment guidelines will be established for each ethnicity.

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