ORIGINAL ARTICLE

Pathway genes and metabolites in thiopurine therapy in Korean children with acute lymphoblastic leukaemia

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Aims: We aimed to investigate the impact of various genetic polymorphisms affecting thiopurine metabolism pathways and toxicity in paediatric acute lymphoblastic leukaemia patients for the first time in Korea.

Methods: From May 2006 to September 2016, 139 paediatric acute lymphoblastic leukaemia patients treated with combination chemotherapy including 6-mercaptopurine were included in the study. One hundred and twenty-three variants in 43 genes, including *TMPT* and *NUDT15*, were screened using targeted genotyping, such as a MassARRAY system, direct sequencing and polymerase chain reaction-restriction fragment length polymorphism methods. Among the polymorphisms screened, 103 polymorphisms of 43 genes were included for further analyses.

Results: The genetic polymorphisms in the *ABCC4*, *AHCY*, *ATIC*, *FAM8A6P*, *GART*, *GNG2*, *GSTA1*, *MTHFD1*, *MTHFR*, *NUDT15*, *PACSIN2*, *TYMS* and *XDH* genes, and an intronic polymorphism between *HIVEP2* and *AIG1*, and *TPMT* genotype were associated with thiopurine metabolism ($P < 0.05$). Genetic polymorphisms in the *ABCC4*, *ADK*, *ATIC*, *GART*, *GMPS*, *GSTP1*, *IMPDH1*, *ITPA*, *KCNMA1*, *MOCOS*, *MTRR*, *NUDT15*, *SLC19A1*, *SLC28A3*, *SLC29A1*, *SLCO1B1*, *TYMP* and *XDH* genes were associated with thiopurine-related toxicities; neutropenia, hepatotoxicity and treatment interruption ($P < 0.05$).

Conclusions: Findings of this study may provide basic knowledge for personalized medicine for thiopurine treatment in paediatric acute lymphoblastic leukaemia patients.

KEYWORDS

acute leukaemia, genome-wide association study, paediatric, polymorphisms, thiopurine

1 | INTRODUCTION

Mercaptopurine (6-MP), the thiopurine, is used in combination chemotherapy as a myelosuppressive maintenance therapy and is required for more than 2 years in paediatric patients with acute lymphoblastic leukaemia (ALL) to achieve a long-term cure.¹⁻³ However, adverse effects of 6-MP, including myelotoxicity which can lead to life-threatening infection, and hepatotoxicity often lead to decreased thiopurine dosage or treatment interruption.² The pharmacokinetic and pharmacogenetic characteristics of thiopurine drugs are complicated (Figure S1), with high intra- and inter-individual variability.^{4,5}

Thiopurine S-methyltransferase (TPMT) is one of the well-known key enzymes involved in the metabolism of thiopurine drugs and its activity is largely influenced by polymorphisms of the *TPMT* gene.⁶ 6-MP is converted into active or inactive metabolites, such as 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine (6-MeMPN) by TPMT intracellularly.⁷ Patients with inherited TPMT deficiency influenced by the polymorphism in *TPMT* gene present with higher 6-TGN level and have an increased risk of myelotoxicity and/or with higher 6-MeMPN level and have an increased risk of hepatotoxicity, respectively.⁸ Despite a lower frequency of *TPMT* variant alleles in Asians, the incidence of thiopurine-induced leukopenia is higher in Asians than in individuals of European descent.⁹ Studies have found an association between several genetic polymorphisms and toxicity in paediatric ALL patients, but the association varies among ethnic groups.^{5,10} For example, thiopurine-related toxicity is frequently observed in Asian populations, with importance of the Nudix hydro-lase 15 (*NUDT15*) genotype rather than the thiopurine methyltransferase (*TPMT*) genotype.¹¹

It has been suggested that pharmacogenomics should be incorporated as a dosage-calibrating tool in the treatment of paediatric ALL in order to predict and minimize the occurrence of serious toxicity in these patients.⁵ However, to the best of our knowledge, there is a lack of pharmacogenetic studies focused on the various genetic polymorphisms in association with thiopurine metabolism and toxicity in Korean paediatric ALL patients. Therefore, the aims of this study were to explore the genetic polymorphisms of genes involved in the thiopurine and folate metabolism pathways and their relationships with thiopurine metabolism and toxicity in Korean paediatric ALL patients. These findings may serve as a basis for thiopurine therapy in paediatric ALL patients.

2 | METHODS

2.1 | Study population and definition

This retrospective study included paediatric ALL patients who were treated with 6-MP at the Paediatric Department of Samsung Medical Center (Seoul, Korea) from May 2006 to September 2016. Clinical information including 6-MP dosage (mg/body surface area/day), duration of 6-MP treatment, and adverse effects related to treatment were

What is already known about this subject

- Thiopurine-related toxicity is frequently observed in Asian populations, with more importance of the *NUDT15* genotype rather than the *TPMT* genotype.
- There is a lack of study focused on the genetic polymorphisms and metabolites, which can provide information on toxicity in Korean patients.

What this study adds

- This is the first study focused on the various genetic polymorphisms in association with thiopurine metabolism in Korean paediatric leukaemia patients.
- Among the 103 SNPs analysed, 32 SNPs in 24 genes (including *NUDT15*) other than *TPMT* genotype were associated with either or both thiopurine metabolism and any adverse effects.

investigated. The treatment protocol has been described in detail previously.¹² Patients who were over 10 years of age or under 12 months or who had initial white blood cell (WBC) count of $50 \times 10^9 \text{ L}^{-1}$ or more were considered as high-risk ALL patients (the others were standard risk ALL patients) according to the risk stratification criteria of the National Cancer Institute.¹³ Laboratory data including 6-TGN and 6-MeMPN levels, WBC count, neutrophil count, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin were collected.

We defined the terms used to describe the adverse effects of thiopurine treatment as follows: leukopenia, WBC count $<1.5 \times 10^9 \text{ L}^{-1}$; neutropenia, absolute neutrophil count $<0.5 \times 10^9 \text{ L}^{-1}$; hepatotoxicity, ALT or AST enzyme activity >3 times the upper limit of the reference range and bilirubin >1.5 times the upper limit of the reference range after starting 6-MP and during a stable disease state; and treatment interruption, any period in which 6-MP therapy was discontinued due to an adverse drug reaction. Treatment was interrupted when patients showed significant hematopoietic toxicity (absolute neutrophil count below $0.5 \times 10^9 \text{ L}^{-1}$ or platelet count below $50 \times 10^9 \text{ L}^{-1}$) or serious infectious events.¹² This study was approved by the Institutional Review Board of Samsung Medical Center (IRB file No. SMC 2012-04-106-014).

2.2 | Measurement of thiopurine metabolites

Measurements of RBC levels of thiopurine metabolites (6-TGN and 6-MeMPN) were performed using a liquid chromatography–tandem mass spectrometry as described previously.^{4,14,15} The lower limit of quantification (LOQ) was $0.1 \mu\text{mol L}^{-1}$ for 6-TGN and $0.5 \mu\text{mol L}^{-1}$ for 6-MeMPN.^{4,15} The details of the measurement method have been described previously.¹⁵ For each individual patient, the ratio of 6-MeMPN to 6-TGN and the ratio of the 6-TGN to 6-MP dose was calculated to analyse the patient's pharmacological phenotype.¹⁶

2.3 | SNP selection and targeted genotyping

Genetic polymorphisms associated with thiopurine metabolism were reviewed through a literature review and public databases, such as the PubMed and PharmGKB databases (<http://www.pharmgkb.org/pathway>, Figure S1) using search keywords in various combinations of the following words: 'leukaemia/leukemia', 'thiopurine' and 'polymorphism'.¹⁷ A total of 123 genetic polymorphisms of 43 genes including the *TPMT* gene and the *NUDT15* gene were included for genetic analyses.

Single nucleotide polymorphisms (SNPs) were genotyped using the MassARRAY[®] system with SpectroTYPER[™] software (Sequenom, Inc., CA, USA).⁴ The *TPMT* genotype and *NUDT15* genotype of each patient were determined by direct sequencing analysis as described previously.^{4,18} The *TYMS* polymorphism (28-bp repeat) was analysed using polymerase chain reaction as described previously.^{4,19} Among 121 SNPs in 43 genes, after excluding 18 SNPs of 13 genes with a call rate < 90%, a minor allele frequency (MAF) < 0.01, or a Hardy–Weinberg equilibrium *P*-value < 0.001, 103 autosomal genetic polymorphisms in 43 genes and the *TPMT* genotype were included for statistical analysis (Table S1). The variant of c.416G>A in the *NUDT15* gene (rs147390019, also reported as the *4 allele¹¹) was excluded in the final analysis because of its low allele frequency in this study (<0.01).

2.4 | Statistical analysis

Quantitative non-normally distributed variables are expressed as medians and interquartile ranges (IQR). Categorical variables are presented as frequencies and percentages. Nonparametric methods were used for non-normally distributed data. The association between mean dose of 6-MP and metabolites (6-TGN and 6-MeMPN) was analysed using the generalized estimating equations. Linear regression analysis was performed under genotype, dominant and recessive genetic models in order to evaluate the association of thiopurine metabolites and ratios with each polymorphism with a frequency of more than 10% in this study population.²⁰ The *P*-value was corrected with the false-discovery rate (FDR) for candidate polymorphisms according to the linear step-up procedure.²¹ Fisher's exact test was performed under genotype, dominant and recessive genetic models in order to evaluate the association of adverse effects of thiopurine treatment.²⁰ The FDR correction was used to adjust the *P*-values for multiple comparisons. Statistical analyses were performed using R 3.1.0 (R Foundation for Statistical Computing, Vienna, Austria) and SAS version 9.3 (SAS Institute, Cary, NC). *P*-values less than 0.05 were considered to be significant.

2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.²²

3 | RESULTS

3.1 | Demographic characteristics

A total of 139 patients (64 boys and 75 girls) treated with 6-MP (median dose, 30.1 mg m⁻² per day) were included in this study. The median (range) duration of treatment with 6-MP to measure thiopurine metabolites used for analysis was 23.7 months (1.4–91.3 months). The follow-up period for these patients ranged from 1.5–10.7 years (median

TABLE 1 Patient characteristics (139 paediatric patients with acute lymphoblastic leukaemia)

Sex, n (%)	
Male	64 (46)
Female	75 (54)
Age, median (IQR), years	5.1 (3.7–10.6)
Follow-up period, median (IQR), years	5.9 (3.6–7.9)
Body weight, median (IQR), kg	19.4 (15.2–37.5)
Body surface area, median (IQR), m ²	0.79 (0.66–1.19)
Diagnosis	
B-lymphoblastic leukaemia, n (%)	131 (94)
T-lymphoblastic leukaemia, n (%)	8 (6)
Risk group	
Standard risk, n (%)	86 (62)
High risk, n (%)	53 (38)
Number of thiopurine metabolite measurements per patient, median (range)	7 (1–14)
6-MP daily dose, median (IQR), mg m ⁻² per day	30.1 (22.2–38.9)
6-TGN level, median (IQR), pmol per 8 × 10 ⁸ RBCs	388.5 (301.1–555.2)
6-MeMPN level, median (IQR), pmol per 8 × 10 ⁸ RBCs	5089.0 (2064.2–9363.7)
6-MeMPN/6-TGN ratio, median (IQR)	13.9 (5.2–23.5)
6-TGN/6-MP dose ratio, median (IQR)	12.8 (9.3–18.1)
<i>TPMT</i> genotype, n (%)	
*1/*1	133 (96)
*1/*3C	2 (1)
*1/*6	2 (1)
*1/*32	1 (1)
*1/? (c.532T>C) (novel allele)	1 (1)
Toxicity, n (%) ^a	
Leukopenia (WBC < 1.5 × 10 ⁹ L ⁻¹)	38 (27)
Neutropenia (ANC < 0.5 × 10 ⁹ L ⁻¹)	40 (29)
Hepatotoxicity	64 (46)
Treatment interruption, n (%)	114 (82)

Abbreviations: ANC, absolute neutrophil count; IQR, interquartile range; WBC, white blood cell.

^aAny toxicity experienced during the follow-up period.

5.9 years). The length of treatment duration ranged from 1.4 to 92.9 months (median 25.6 months). During the follow-up period, 1–14 times (median 7 times) of thiopurine metabolites measurement were performed for each individual patient. Among the 139 patients, six had a *TPMT* variant (two patients heterozygous for *3C, two heterozygous for *6, one heterozygous for the *32 allele, and one heterozygous for the variant of uncertain significance, c.532T>C²³). The length of treatment interruptions (median, IQR) in the first year of maintenance therapy was 20.0 (7.0–32.0) days. Patient characteristics are summarized in Table 1.

3.2 | Association between 6-MP dosage and thiopurine metabolites

The levels of thiopurine metabolites were significantly associated with 6-MP dosage: 6-TGN, $p = 0.2085$ (95% CI 0.1502–0.2667, $Z = 7.01$, $P < 0.0001$) and 6-MeMPN, $p = 0.7687$ (95% CI 0.6107–0.9268, $Z = 9.53$, $P < 0.0001$, Figure 1).

3.3 | Genetic polymorphisms associated with thiopurine metabolites and 6-TGN level/6-MP dose ratio

Table 2 shows the SNPs associated with thiopurine metabolites. Different genetic polymorphisms were associated with 6-TGN and 6-MeMPN levels. Among 139 patients, 36 (25.9%) patients had one or more variant alleles of *NUDT15*. The allele frequencies were 86.0% for *NUDT15**1, and 4.3, 7.6, 1.4 and 0.7% for *2, *3, *5 and *6, respectively. The *TPMT* variants had significantly lower 6-MeMPN levels and 6-MeMPN/6-TGN ratios. Figure 2A shows the 6-MeMPN/6-TGN ratios among patients with different *TPMT* and *NUDT15* genotypes. Patients who had *NUDT15* variants showed variable 6-MeMPN/6-TGN ratios.

Table 3 shows the genetic polymorphisms associated with the 6-TGN/6-MP dose ratio. Among the genetic polymorphisms, rs2274407 in the *ABCC4* gene, rs819146 in the *AHCY* gene,

rs2070388 in the *GART* gene, rs2413739 in the *PACSLN2* gene, rs34743033 in the *TYMS* gene, and rs116855232 in the *NUDT15* gene were significantly related to the 6-TGN/6-MP dose ratio ($P < 0.05$). Univariate analysis did not show statistical significance for genetic polymorphisms after FDR correction, except for *NUDT15* rs116855232. Figure 2B shows the 6-TGN/6-MP dose ratios among patients with different *TPMT* and *NUDT15* genotypes. The *TPMT* variants were associated with a higher 6-TGN/6-MP dose ratio while *NUDT15* variants were associated with a lower 6-TGN/6-MP dose ratio ($P < 0.05$).

3.4 | Genetic polymorphisms associated with toxicity

Table 4 shows the genetic polymorphisms associated with neutropenia, hepatotoxicity and treatment interruption. The results of this study are summarized in Table 5 and Table S2 with findings from previous studies performed in paediatric ALL patients or in patients with other diseases, in vitro studies or theoretical studies.

4 | DISCUSSION

Among the genetic polymorphisms analysed, 32 SNPs in 24 genes (including rs116855232 and rs186364861 of the *NUDT15* gene) other than *TPMT* genotype were identified as having an association with either or both the levels of thiopurine metabolites and any adverse effects. Among the 32 SNPs, results of 16 SNPs were comparable with previous studies, while the other 16 SNPs were needed to validate through further confirmatory clinical or biochemical studies (Table S2). Among these polymorphisms, four SNPs (rs2070388 in the *GART* gene, rs3744900 in the *MOCOS* gene, rs116855232 and rs186364861 in the *NUDT15* gene) and *TPMT* genotype were statistically significant in this study (Table 5).

In this study, patients with *TPMT* variant alleles had significantly higher 6-TGN/6-MP dose ratios (Figure 2) which confirmed previous findings on the importance of *TPMT* in patients who are treated with

FIGURE 1 Correlation between the 6-TGN measurements and 6-MP dose (A) and 6-MeMPN measurements and 6-MP dose (B)

6-MeMPN/6-TGN ratio																
ABCC4	rs2274407	C	A	0.98 ± 0.58	1.08 ± 0.49	-0.09 ± 0.00	0.0932	0.9573	1.01 ± 0.55	-0.09 ± 0.00	0.0481	0.9388	0.98 ± 0.58	1.05 ± 0.51	0.5103	0.9371
AT1C	rs3821353	T	G	0.88 ± 0.72	1.18 ± 0.43	0.92 ± 0.57	0.0329	0.9573	1.03 ± 0.61	0.92 ± 0.57	0.3294	0.9388	0.88 ± 0.72	1.06 ± 0.51	0.1006	0.9371
FAM8A6P	rs1040637	G	A	1.04 ± 0.55	0.74 ± 0.78	0.75 ± 0.77	0.1339	0.9573	1.01 ± 0.58	0.75 ± 0.77	0.4579	0.9550	1.04 ± 0.55	0.74 ± 0.75	0.0446	0.9371
GN2	rs12886319	T	C	1.14 ± 0.50	0.89 ± 0.65	1.10 ± 0.43	0.0507	0.9573	0.99 ± 0.61	1.10 ± 0.43	0.4289	0.9550	1.14 ± 0.50	0.93 ± 0.61	0.0481	0.9371
MTHFD1	rs2236225	G	A	0.91 ± 0.66	1.10 ± 0.41	1.41 ± 0.28	0.0905	0.9573	0.98 ± 0.58	1.41 ± 0.28	0.2080	0.9388	0.91 ± 0.66	1.12 ± 0.41	0.0457	0.9371
NUDT15	rs147390019	G	A	1.02 ± 0.56	0.37 ± 1.17		0.0269	0.9573								
NUDT15	rs186364861	G	A	1.00 ± 0.60	1.03 ± 0.39		0.0269	0.9864								
TPMT		*1	Other	1.03 ± 0.57	0.39 ± 0.50		0.0085	0.8871								

^aIntronic SNP between the *AI*G1 and *HIVE*P2 gene.

FIGURE 2 The 6-TGN/6-MP dose ratio among patients with *TPMT* genotypes and *NUDT15* variants: The 6-TGN/6-MP dose ratio (A) at all metabolite measurements and (B) at the metabolite measurement used to study the association with genetic polymorphisms

thiopurines.^{17,29} *TPMT* genotyping before administering thiopurines is recommended to determine the proper initial dosing and consideration of an alternate agent or extreme dose reduction of azathioprine for patients with low or deficient *TPMT* activity by clinical guidelines (start at 30–70% of target dose for patients with intermediate enzyme activity).^{8,29} In this study only 4.3% of patients (6/139) had *TPMT* variant alleles. This finding was comparable to previous studies performed in Asian populations (variant allele frequency 2.3–5.0%)⁶ which was lower than for individuals of European descent.^{8,29} Meanwhile, in this study, the frequency of *NUDT15* variant alleles was 14.0% which was comparable to a previous study in the Korean population.¹⁸ *NUDT15*, another important gene with a similar pathway as *TPMT*, was first identified as a candidate gene by a genome-wide association study (GWAS) of inflammatory bowel disease patients⁹ and in children with ALL.³⁰ *NUDT15* was associated with thiopurine-related hematopoietic toxicity and has been confirmed in several other studies to be associated with thiopurine tolerance and/or thiopurine-related toxicity in leukaemia patients.^{11,12,31–33} In this study, two SNPs of *NUDT15* (rs116855232 and rs186364861) were identified in association with thiopurine metabolites and therapeutic interruption which confirmed and reflected previous findings on its association with thiopurine-related hematopoietic toxicity (Tables 2–4). Because the frequency of the variant alleles range from 0.2–17.2% in different ethnic populations and the variant alleles are more common in East Asian populations, standard guidelines including thiopurine dosage adjustment on the basis of *NUDT15* genotyping is needed for safe thiopurine therapy.¹⁸ In this study, patients with *NUDT15* variants showed significantly lower 6-TGN/6-MP dose ratios (Figure 2). It has been suggested that *NUDT15* may prevent incorporation of 6-TGN into DNA and loss of function variants in the *NUDT15* gene cause thiopurine-induced cytotoxicity.¹¹ Another study identified that DNA-6-TGN accumulated more efficiently in vivo with an increasing number of variant alleles of *NUDT15* and suggested that DNA-6-TGN is a more relevant metabolite rather than 6-TGN in RBCs because 6-TGN levels were significantly lower in patients with

NUDT15 deficiency, likely because of toxicity-related 6-MP dose reduction.² In this study, clinical implementation of *NUDT15* genotype-guided 6-MP dose individualization was performed by measuring levels of RBC 6-TGN. Future studies are needed to clarify the relationship between *NUDT15* and metabolites including DNA-6-TGN and 6-TGN in RBCs.

Because the folate cycle is important in treatments using antimetabolite drugs including analogues of purines (thiopurines such as 6-MP, 6-thioguanine and azathioprine), pyrimidines (fluoropyrimidines such as 5-fluorouracil, tegafur and capecitabine), and antifolates (methotrexate, pemetrexed and raltitrexed) and co-medication is widely used in order to improve clinical outcomes in paediatric ALL patients, future studies assessing information about co-medication in association with various genetic polymorphisms are needed.¹⁷

Most polymorphisms identified in this study were not statistically significant after FDR correction; however, rs2070388 in the *GART* gene and rs3744900 in the *MOCOS* gene showed a possible trend toward an association (corrected $P = 0.07$) with treatment interruption (Tables 2–5 and S2). In previous research on possible *trans* expression associations with SNPs, rs200148 in the region between *HIVEP2* and *AIG1* showed the association with reduced *TPMT* activity and increased sensitivity to 6-TGN and 6-MP.³⁴ This SNP was associated with expression of the thiopurine pathway genes *ADA* (reduced expression) and *GART* (increased expression).^{25,34,35} The protein encoded by the *GART* gene (glycinamide ribonucleotide transformylase) is a trifunctional polypeptide (phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase and phosphoribosylaminoimidazole synthetase activity) required for de novo purine biosynthesis in association with folate metabolism and has long been considered a potential target for the development of anti-neoplastic therapeutics.²⁶ In the present study, rs200148 in the region between *HIVEP2* and *AIG1* was associated with decreased 6-TGN levels, in contrast to the findings of previous studies.^{25,34,35} The complex mechanisms of these SNPs and their clinical impacts should be elucidated

TABLE 3 Predictors of log-transformed 6-TGN/6-MP dose ratio (mean ± standard deviation)

Genes	rsID	Maj	Min	Total number of patients				Genotype			Dominant				Recessive				
				Maj hom	Het	Min hom	Maj hom	Het	Min hom	P	FDR P	Maj hom + het	Min hom	P	FDR P	Maj hom	Het + min hom	P	FDR P
ABCC4	rs2274407	C	A	89	42	1	1.14 ± 0.28	1.12 ± 0.21	1.79	0.0406	0.8253	1.13 ± 0.26	1.79	0.0124	0.5312	1.14 ± 0.28	1.14 ± 0.23	0.9277	0.9873
AHCY	rs819146	T	G	78	39	9	1.18 ± 0.26	1.06 ± 0.27	1.06 ± 0.23	0.0484	0.8253	1.14 ± 0.27	1.06 ± 0.23	0.3666	0.9941	1.18 ± 0.26	1.06 ± 0.26	0.0137	0.6891
GART	rs2070388	G	C	93	35	9	1.11 ± 0.23	1.24 ± 0.31	1.05 ± 0.32	0.0369	0.8253	1.15 ± 0.26	1.05 ± 0.32	0.2739	0.9941	1.11 ± 0.23	1.20 ± 0.32	0.0878	0.7726
NUDT15	rs116855232	C	T	109	27	3	1.15 ± 0.24	1.14 ± 0.30	0.55 ± 0.24	0.0003	0.0335	1.15 ± 0.25	0.55 ± 0.24	<0001	0.0052	1.15 ± 0.24	1.08 ± 0.34	0.1959	0.8077
PACSLN2	rs2413739	C	T	116	13		1.12 ± 0.26	1.28 ± 0.32		0.0386	0.8253								
TPMT		*1	Other	133	6		1.12 ± 0.25	1.47 ± 0.37		0.0016	0.0847								
TYMS	rs34743033	≥3 R	2 R	91	45	3	1.18 ± 0.28	1.07 ± 0.21	1.07 ± 0.09	0.0556	0.8253	1.14 ± 0.27	1.07 ± 0.09	0.6575	0.9941	1.18 ± 0.28	1.07 ± 0.20	0.0160	0.6891

Abbreviations: FDR, false-discovery rate; Het, heterozygote; Hom, homozygote; Maj, major allele; Min, minor allele; R, repeats.
P-values < 0.05 calculated by linear regression analysis are shown in bold.

TABLE 4 Influence of genetic polymorphisms on leukopenia, neutropenia, hepatotoxicity, and treatment interruption (number and %)

Category	Adverse effect	Gene	rsID	Maj	Min	Total number of patients				Genotype			P	FDR P
						Maj hom	Het	Min hom	Het	Maj hom	Het	Min hom		
Significant	Leukopenia	NUDT15	rs116855232	C	T	109	27	3	3 (11)	2 (2)	3 (11)	2 (67)	<0.0001	0.0013
Possible trend toward significance ^a	Treatment interruption	GART	rs2070388	G	C	93	35	9	20 (57)	83 (89)	20 (57)	9 (100)	0.0522	0.7622
		MOCOS	rs3744900	G	A	114	23	1	13 (57)	99 (87)	13 (57)	1 (100)	0.0027	0.2839
		NUDT15	rs116855232	C	T	109	27	3	27 (100)	84 (77)	27 (100)	3 (100)	0.0056	0.2931
Others	Leukopenia	ABCC4	rs2274405	C	T	42	65	29	1 (2)	2 (5)	1 (2)	4 (14)	0.1481	0.8886
		ABCC4	rs2274406	C	T	51	56	30		3 (6)		4 (13)	0.2858	0.8886
		SLC29A1	rs9394992	C	T	86	49	4		7 (8)			0.0419	0.8886
	Neutropenia	NUDT15	rs116855232	C	T	109	27	3	1 (4)	4 (4)	1 (4)	2 (67)	0.0063	0.6501
		ABCC4	rs4773866	C	T	124	13	2	1 (8)	5 (4)	1 (8)	1 (50)	0.0242	1
		SLC28A3	rs7867504	C	T	52	68	18	7 (10)		7 (10)		0.3167	1
	Hepatotoxicity	MOCOS	rs3744900	G	A	114	23	1	16 (70)	47 (41)	16 (70)	1 (100)	0.0067	0.3501
		MTRR	rs1801394	A	G	77	51	8	15 (29)	44 (57)	15 (29)	3 (38)	0.0067	0.3501
		SLCO1B1	rs11045879	T	C	47	62	25	28 (45)	16 (34)	28 (45)	16 (64)	0.0166	0.5754
		IMPDH1	rs2278294	A	G	36	74	28	32 (43)	22 (61)	32 (43)	10 (36)	0.0370	0.7396
		ADK	rs10824095	C	T	58	63	12	34 (54)	19 (33)	34 (54)	6 (50)	0.0427	0.7396
		XDH	rs494852	G	A	101	27	2	16 (59)	43 (43)	16 (59)	2 (100)	0.0375	0.7396
	Treatment interruption	TYMP	rs470119	C	T	82	48	8	21 (44)	36 (44)	21 (44)	7 (88)	0.1327	0.8625
		ATIC	rs3821353	T	G	45	46	40	37 (80)	34 (76)	37 (80)	38 (95)	0.0178	0.4351
		ATIC	rs4673993	T	C	83	44	8	39 (89)	63 (76)	39 (89)	8 (100)	0.0251	0.4351
		GMPS	rs61750368	T	C	102	31	5	24 (77)	87 (85)	24 (77)	2 (40)	0.0202	0.4351
		KCNMA1	rs12765834	C	T	118	20	0	20 (100)	93 (79)	20 (100)		0.0242	0.4351
		ABCC4	rs1678387	C	T	86	46	7	39 (85)	72 (84)	39 (85)	3 (43)	0.1141	0.8681
		SLC19A1	rs1051266	G	A	41	66	26	58 (88)	33 (81)	58 (88)	17 (65)	0.2264	0.8753

Abbreviations: FDR, false-discovery rate; Het, heterozygote; Hom, homozygote; Maj, major allele; Min, minor allele.

Polymorphisms were arranged in a sequence starting from the lowest *P*-value and were corrected using the false-discovery rate of genotype mode analysis for each adverse outcome.*P*-values < 0.05 are shown in bold.^aPossible trend toward significance with *P* < 0.10 corrected with a false-discovery rate.

TABLE 4 (Continued)

Category	Dominant			Recessive			FDR P
	Maj hom + het	Min hom	P	Maj hom	Het + min hom	P	
Significant	5 (4)	2 (67)	0.0064	2 (2)	5 (17)	0.0053	0.4565
Possible trend toward significance ^a	103 (81)	9 (100)	0.2112	83 (89)	29 (66)	0.0017	0.0717
	112 (82)	1 (100)	1	99 (87)	14 (58)	0.0025	0.0717
	111 (82)	3 (100)	1	84 (77)	30 (100)	0.0022	0.0717
Others	3 (3)	4 (14)	0.0373	2 (5)	5 (5)	1	1
	3 (3)	4 (13)	0.0410	3 (9)	4 (5)	0.7113	1
	7 (5)		1	7 (8)		0.0440	0.9770
	5 (4)	2 (67)	0.0064	4 (4)	3 (10)	0.1714	1
	6 (4)	1 (50)	0.0985	5 (4)	2 (13)	0.1662	1
	7 (6)		0.5942	1	7 (8)	0.0448	1
	63 (46)	1 (100)	0.4638	47 (41)	17 (71)	0.0123	0.5291
	59 (46)	3 (38)	0.7273	44 (57)	18 (31)	0.0030	0.2585
	44 (40)	16 (64)	0.0442	16 (34)	44 (51)	0.0720	0.8654
	54 (49)	10 (36)	0.2886	22 (61)	42 (41)	0.0518	0.8654
	53 (44)	6 (50)	0.7651	19 (33)	40 (53)	0.0223	0.6405
	59 (46)	2 (100)	0.2182	43 (43)	18 (62)	0.0906	0.8654
	57 (44)	7 (88)	0.0247	36 (44)	28 (50)	0.4927	1
	71 (78)	38 (95)	0.0207	34 (76)	75 (87)	0.1380	0.9974
	102 (80)	8 (100)	0.3507	63 (76)	47 (90)	0.0413	0.8875
	111 (84)	2 (40)	0.0414	87 (85)	26 (72)	0.1285	0.9974
	111 (84)	3 (43)	0.0200	72 (84)	42 (79)	0.5052	1
	91 (85)	17 (65)	0.0461	33 (81)	75 (82)	1	1

TABLE 5 Identified SNPs in this study in comparison with previous studies

Gene	rsID	Maj	Min	MAF in this study	Related drug pathway	Results of this study (identified associations)	Results of previous studies in paediatric ALL patients	Comments (studies in other diseases)	Comparable with previous study?
GART	rs2070388	G	C	0.193	Folate cycle	GG genotype: ↑treatment interruption? ^a	Overexpression of GART gene was reported in association with late relapse in children with ALL. ²⁴ Polymorphisms in the GART gene showed increased sensitivity to 6-TGN and 6-mercaptopurine but the significance disappeared after applying a Bonferroni correction. ²⁵	Glycinamide ribonucleotide transferase (GART) is an important trifunctional enzyme involved in purine synthesis. ²⁶ This gene is involved in the methotrexate pathway. ¹⁷ This variant has been studied in paediatric IBD patients and showed no significant association between this variant and thiopurine metabolite concentrations or any adverse outcomes including leukopenia, neutropenia, hepatotoxicity, gastrointestinal intolerance, alopecia, arthralgia, rash, and hair loss in patients treated with azathioprine. ⁴	Unknown
MOCOS	rs3744900	G	A	0.091	Thiopurine metabolism	GG genotype: ↑treatment interruption? ^a	– ^b	Molybdenum cofactor sulfurylase (MOCOS) is a protein-coding gene which sulfurates the molybdenum cofactor in XDH and AOX1, key enzymes involved in the degradation of thiopurines. ²⁷ It was observed in 2 individual patients with IBD who had intolerance to thiopurines in a cohort of 100 patients. ²⁷ Studies performed in kidney transplant recipients receiving azathioprine showed no association between this polymorphism and azathioprine dose requirements. ²⁸	Unknown
NUDT15	rs116855232	C	T	0.119	Thiopurine metabolism	T allele: ↑leukopenia CC genotype (wild): Treatment interruption? ^a	Well established in Asian populations	Comparable	Comparable

(Continues)

TABLE 5 (Continued)

Gene	rsID	Maj	Min	MAF in this study	Related drug pathway	Results of this study (identified associations)	Results of previous studies in paediatric ALL patients	Comments (studies in other diseases)	Comparable with previous study?
NUDT15	rs186364861	G	A	0.014	Thiopurine metabolism	GG genotype (wild): ↑6-TGN levels compared to GA genotype	Well established in Asian populations		Comparable
TPMT		*1	Other	0.022	Thiopurine metabolism	Variant allele: ↑6-TGN/6-MP dose ratio	Well established in various populations		Comparable

Abbreviations: ALL, acute lymphoblastic leukaemia; AOX1, aldehyde oxidase 1; IBD, inflammatory bowel disease; MAF, minor allele frequency; Maj, major allele; Min, minor allele; XDH, xanthine dehydrogenase.

^aPossible trend toward significance with $P < 0.10$ corrected with a false-discovery rate.

^bThis polymorphism is rarely investigated in paediatric ALL patients.

through future studies. The polymorphism rs3744900 in the MOCOS gene has rarely been investigated in paediatric ALL patients. Molybdenum cofactor sulfurase is encoded by the MOCOS gene, which sulfurates the molybdenum cofactor in xanthine dehydrogenase and aldehyde oxidase 1, key enzymes involved in the degradation of thiopurines.²⁸ Two patients with inflammatory bowel disease were reported to exhibit intolerance to thiopurines.²⁷ However, in kidney transplant recipients receiving azathioprine, there was no association between this polymorphism and azathioprine dose requirements.²⁸ Although the mechanisms of these SNPs have not been fully explained by previously published studies, these genetic polymorphisms in candidate genes with potential effects on the pharmacokinetics of thiopurine metabolism should be considered and validated with further studies.¹⁰

Although the relatively small number of patients included in this study might be a limitation, those SNPs have not previously been examined to determine if they are associated with thiopurine-related toxicity in Korean paediatric ALL patients. Dosing compliance might be another limitation that could affect thiopurine-related toxicity.

The value of the present study lies primarily in the emphasis placed on the genetic variants in the Korean paediatric ALL patient population which has not been assessed previously. Validation of the results presented here requires confirmatory clinical or biochemical studies, especially because functional data are not yet available for some genetic polymorphisms. Considering that the frequency of genetic polymorphisms is different among different populations and pharmacokinetics in association with pharmacogenetic differences among different populations, future studies in different ethnic populations are needed to confirm the relevance of our data.

In conclusion, this is the first study to explore various genetic polymorphisms related to thiopurine treatment in Korean paediatric ALL patients. This study provides basic knowledge for personalized thiopurine treatment in paediatric ALL patients. Further studies to enable more comprehensive pharmacogenetic-based thiopurine dose adjustments across diverse populations are needed.

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COMPETING INTERESTS

There are no competing interests to declare.

CONTRIBUTORS

R.C. conceptualized and designed the study, collected data, carried out the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript. J.W.L., Y. M. and E.S.Y. designed the data collection instruments and collected data, and reviewed and revised the manuscript. I.S. and M-J.K. carried out the initial analyses, and reviewed and revised the manuscript. H.I.W., H.H.K. and S-Y.L. conceptualized and designed the study, coordinated and supervised data

collection, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. The authors would like to express their gratitude to the patients and their families for their participation in the study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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