The Application of Clinical Genetics

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ORIGINAL RESEARCH

ITPA:c.94C>A and *NUDT15:c.415C>T* Polymorphisms and Their Relation to Mercaptopurine-Related Myelotoxicity in Childhood Leukemia in Thailand

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Background: Mercaptopurine is a key agent in childhood leukemia treatment. Genetic polymorphism in the genes involving thiopurine metabolisms is related to 6-MP related toxicity.

Objective: This study aimed to determine the prevalence of *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms among Thai children diagnosed with leukemia and their association with mercaptopurine-related myelotoxicity.

Methods: Patients and survivors with a diagnosis of leukemia treated with mercaptopurine-containing chemotherapy regimens were enrolled. Clinical data and laboratory parameters during treatment as well as *ITPA:c.94C>A* and *NUDT15:c.415C>T* genotypes were analyzed.

Results: In all, 99 patients with acute leukemia or survivors were enrolled in the study. The prevalences of *ITPA:c.94C>A*, *NUDT15:c.415C>T*, and co-occurrence of *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms were 34, 17, and 4%, respectively. Numbers of absolute neutrophil count (ANC) and platelet count significantly decreased among patients carrying *NUDT15:c.415C>T* compared with *NUDT15* wild type patients with p-values<0.001 and 0.019, respectively. The differences were not observed among patients carrying *ITPA:c.94C>A* compared with *ITPA* wild type patients. According to multivariate GEE, *NUDT15:c.415C>T* and co-occurrence of *ITPA:c.94C>A* and *NUDT15:c.415C>T* had a significant negative effect on ANC during treatment (coefficient: −463.81; CI: −778.53, −149.09; p-value=0.004 and coefficient: −527.56; CI: −1045.65, −9.48; p-value=0.046). No significant effect of *ITPA:c.94C>A* on ANC during treatment was observed.

Conclusion: *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms are common among Thai children with leukemia. A strong association with mercaptopurine-related myelotoxicity was observed among patients carrying either *NUDT15:c.415C>T* alone or combined with *ITPA:c.94C>A*.

Keywords: *NUDT15*, *ITPA*, 6-MP, myelotoxicity, pediatric oncology

Background

The survival rate of childhood cancer has dramatically improved over the past decades.[1] The key elements attributing to this successful achievement include early recognition and initial management of cancer as well as its complications, newly developed cancer treatment strategies and, most importantly, improved supportive care throughout the length of cancer treatment. Pediatric hematologic malignancies,

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including leukemia and lymphoma, are considered as prototypes of curative cancer.[2–5] Despite the favorable outcomes, significant numbers of patients still encounter treatment-related toxicities. Systemic chemotherapy incorporated in most pediatric cancer therapeutic regimens contains cytotoxic effects involving not only cancerous cells, but also normal growing cells resulting in suppression of hematopoiesis and eventually leading to anemia, hemorrhage, and a higher risk of serious infection from decreased red blood cell, platelet, and white blood cell production, respectively.[6,7]

Mercaptopurine (also referred as 6-mercaptopurine or 6-MP) is a prodrug of a purine analog functioning as an antagonist to endogenous purines required for DNA synthesis and replication during the S-phase of the eukaryotic cell cycle as well as inhibiting RNA and protein synthesis.[8,9] The drug has been implemented as a key agent in childhood acute lymphoblastic leukemia (ALL) treatment regimens and also been used in other therapeutic regimens for childhood lymphoblastic lymphoma and acute promyelocytic leukemia (APL). Related studies reported that the tolerance to 6-MP varies among each individual patient, in which the most common adverse effect of the drug is myelosuppression with the onset at 7–10 days, nadir at 14 days, and recovery occurring by 21 days.[10,11] Pathogenesis of this discrepancy of the drug-related toxicity seems to be related to genetic polymorphisms in the genes involved in thiopurine metabolisms.[12]

Genetic polymorphisms in the gene encoding thiopurine methyltransferase (TPMT) are widely accepted to be involved in thiopurine metabolisms resulting in 6-MP related myelotoxicity; however, geographic variation of frequencies and distributions of variant *TPMT* alleles have been reported in which the allelic frequencies were much lower in Asian compared with Caucasian populations.[13–15] On the other hand, genetic polymorphisms in other genes encoding enzymes involved in thiopurine metabolisms including inosine triphosphate pyrophosphohydrolase (*ITPA*) and nucleoside diphosphate linked moiety X-type motif 15 (*NUDT15*) has been reported to be more evident in Asian compared with Caucasian populations.[16–18] ITPase is an enzyme involved in the hydrolysis of thioinosine triphosphate (TITP) to thioinosine monophosphate (TIMP) in which the deficiency of this enzyme among patients receiving 6-MP could result in toxic accumulation of TITP.[16,19] However, the clinical correlation between *ITPA* genotypes and 6-MP related toxicity remains controversial and is not routinely applied to clinical practice. The NUDT15 protein is an enzyme involved in thiopurine metabolisms by catalyzing the conversion of toxic thioguanine triphosphate to less toxic thioguanine monophosphate, resulting in a reduced cytotoxic effect of 6-MP.[16,19] Therefore, genetic polymorphisms of *ITPA* and *NUDT15* could potentially lead to 6-MP related cytotoxicity effects.

Herein, we conducted a prospective cross-sectional observational study to determine the prevalence of *NUDT15* and *ITPA* polymorphisms among Thai children diagnosed with acute leukemia and their association with 6-MP related myelotoxicity.

Methods

Patient Selection

Pediatric oncology patients undergoing chemotherapy at the Division of Hematology and Oncology, Department of Pediatrics, Phramongkutklao Hospital from January 1, 2018 to January 31, 2020 were enrolled in this study. Written informed consent and assent forms to participate in the study were obtained from all participants including the children themselves as well as their parents or legal guardians before engaging in the study. This prospective study was approved by the Institutional Review Board, Royal Thai Army Medical Department according to the ethics principles of the Declaration of Helsinki (1975) and its revision (reference number: IRBRTA 799/2562). The study was also registered and approved by the Thai Clinical Trials Registry (TCTR20190828003). The study's inclusion criteria included patients aged less than 18 years receiving a diagnosis of acute leukemia and undergoing 6-MP containing chemotherapeutic regimens or pediatric leukemia survivors who had a history of receiving the treatment regimens. The study's exclusion criteria consisted of patients with a history of allergy or contra-indication to 6-MP, unanalyzable medical information due to poor documented medical records, or those who denied having blood samples for *ITPA* and *NUDT15* polymorphisms obtained.

Outcome Measurement

The study adhered to the Preferred Reporting items for Observational studies in Endodontics (PROBE) guidelines. The study schema is shown in the flow diagram in Figure 1. One hundred and three patients were initially recruited to the study; however, four were excluded and 99 consented and enrolled in the study. The primary outcome of this study was to describe the prevalence of *ITPA* and *NUDT15* polymorphisms among pediatric leukemia patients treated with 6-MP containing chemotherapeutic regimens. The secondary outcomes were to determine the

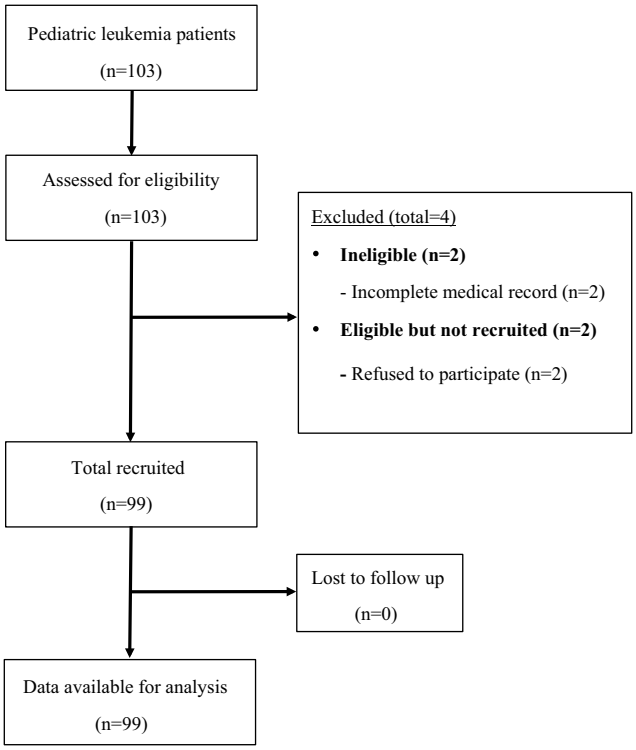

Figure 1 Study flow diagram.

association between *ITPA* and *NUDT15* polymorphisms and 6-MP related myelotoxicity. The study defined neutropenia as an absolute neutrophil count (ANC) of less than 1,500 cells/mm^3 and myelotoxicity as an adverse effect of cancer treatment in which bone marrow activity is decreased resulting in decreased numbers of red blood cells, white blood cells, and platelet count.

6-MP Containing Chemotherapy Regimens

According to the national protocols for childhood cancers conducted by the Thai Pediatric Oncology Group (ThaiPOG), 6-MP was administered at the dose of 50 mg/m^2/dose orally once daily during the maintenance phase of acute lymphoblastic leukemia protocols for a total duration of 20 months among females and 32 months among males. The 6-MP dose of 50 mg/m^2/dose was also administered to patients receiving a diagnosis of APL during the maintenance phase of APL protocol for a total duration of 24 months. All patients were followed up at the Oncology Clinic and had complete blood count (CBC) measured monthly throughout the duration of treatment. 6-MP dosing would then be adjusted on each individual patient based on the results of ANC and platelet count.

Identification of *ITPA:c.94C>A* and *NUDT15:c.415C>T* Polymorphisms

After informed consent was obtained from the patients and their parents, genomic DNA was extracted from peripheral blood leukocytes using commercial kits following manufacturer instructions. The *ITPA:c.94C>A* and *NUDT15:c.415C>T* fragments were amplified by PCR using the primers as previously described.[20,21] Each 50 µL PCR mixture contained 1.5 mM MgCl2, 200 µM of each dNTP, 0.2 µM of each primer, 100–200 ng of genomic DNA and 1 units Taq DNA polymerase. PCR amplification started with initial denaturation at 95°C for 5 minutes, and continued with 30 cycles of 95°C for 20 seconds, 55°C for 20 seconds, and 72°C for 30 seconds. Final extension was performed at 72°C for 5 minutes.

A 328-bp PCR product for *ITPA*:c.94C>A and 269-bp product for *NUDT15*:c.415C>T were digested with *Nsp*I and *Taa*I restriction enzymes, respectively. The *ITPA*:c.94C>A allele yielded two fragments of 238 bp and 90 bp; whereas, the *NUDT15*:c.415C>T allele yielded 142 bp and 127 bp after digestion. The PCR products were resolved in 2% agarose gels, as shown in Figure 2. Sanger sequencing was also performed on several random samples which were positive and negative for restriction enzyme digestion, and the results were 100% concordance.

Statistical Analysis

Baseline values of selected variables were analyzed and presented as mean with standard deviation (SD) or median (range) for continuous variables and calculated using frequency and percentage for categorical variables. Comparisons between two independent data sets were analyzed using Fisher's exact test for categorical data and independent sample *t*-test or Mann–Whitney *U*-test for continuous data. Longitudinal data from time-dependent variables were analyzed using a multivariate generalized estimating equation (GEE) to examine the associations between *ITPA* and *NUDT15* polymorphisms as well as 6-MP administrative dosing and ANC measured monthly during the maintenance phase of acute leukemia protocols. With GEE, the relationships between the variables of the model at different time-points were analyzed simultaneously. STATA/MP, Version 12 Software (STATA Corp., TX, USA) was used and a p-value<0.05 was considered statistically significant.

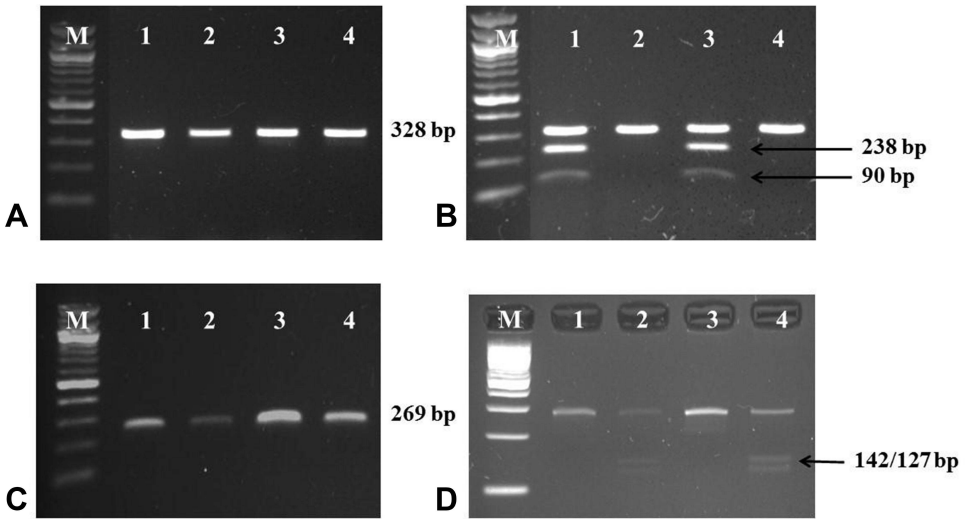

Figure 2 Gel electrophoresis of 328-bp PCR products of *ITPA*:c.94C>A variant (**A**) before and (**B**) after *Nsp*I restriction enzyme digestion and 269-bp PCR products of *NUDT15*:c.415C>T variant (**C**) before and (**D**) after *Taa*I restriction enzyme digestion, respectively (lane 1–4, M-100 bp marker). The 328-bp PCR products of *ITPA*:c.94C>A variant were digested by *Nsp*I and yielded 238-bp and 90-bp fragments as shown in lane 1 and 3; (**B**) suggesting the heterozygous for *ITPA*:c.94C>A variant. The 269 bp-PCR products of *NUDT15*:c.415C>T variant were digested by *Taa*I and yielded 142-bp and 127-bp fragments as shown in lane 2 and 4; (**D**) indicating the heterozygous for *NUDT15*:c.415C>T variant.

Results

Patient Characteristics

Patient characteristics including age, sex, leukemia type, central nervous system (CNS) status, ploidy from conventional cytogenetic analysis, and risk stratification for ALL and disease status at enrollment are summarized in Table 1. Most participating patients were younger children of preschool age. Males were more predominant than females at a ratio of 1.3:1. Patients with various subtypes of acute leukemia were enrolled in this study. The most common leukemia subtype was pre-B ALL followed by T-ALL resembling a typical leukemia distribution among pediatric patients. Both APL and mixed phenotype acute leukemia (MPAL) were less common and rare subtypes of pediatric acute leukemia, respectively, and only one patient with APL and one with MPAL were enrolled in this study. Most patients had a normal karyotype with no CNS involvement at initial diagnosis and successfully achieved complete remission at the time of enrollment to this study. In addition, most ALL patients were stratified as standard and high risks; however, the 6-MP dose during the maintenance phase of standard and high risk ALL protocols was similar to the dose in a very high-risk ALL protocol.

Prevalence of *ITPA:c.94C>A* and *NUDT15:c.415C>T* Polymorphisms

PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) performed on the genomic DNA obtained from 99 enrolled patients with acute leukemia was used to identify *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms and the results are summarized in Table 2. Interestingly, 47 patients (47%) were found to be heterogeneous for either one of these two polymorphisms. The prevalences of *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms among pediatric patients with leukemia in this study were 34% and 17%, respectively, and 4% of the patients were found to have co-occurrence of *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms.

Patient Characteristics According to *ITPA* and *NUDT15* Genotypes

As shown in Table 3, various demographic characteristics including age, sex, leukemia type, CNS status, ploidy from conventional cytogenetic analysis, risk stratification for ALL and disease status at enrollment were described among participating patients with and without *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms. No differences were found regarding clinical information between patients carrying *ITPA* and *NUDT15* wild types versus those carrying *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms.

Association Between *ITPA:c.94C>A* and *NUDT15:c.415C>T* Polymorphisms and 6-MP Related Myelotoxicity

The effects of *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms on bone marrow toxicity were determined

Table 1 Patient Demographic Data

Patients (n=99)	N (%)
Age at diagnosis (years)	
Mean±SD	5.1±4.4
Median (min–max)	4.5 (0.3–18.0)
Gender	
Male	55 (56)
Female	44 (44)
Diagnosis	
Pre-B ALL	87 (88)
T-ALL	10 (10)
MPAL	1 (1)
APL	1 (1)
CNS status	
CNS-1	91 (92)
CNS-2	6 (6)
CNS-3	2 (2)
Ploidy	
Normal	95 (96)
Hyperdiploidy	4 (4)
Risk stratification for ALL (n=97)	
Standard	47 (48)
High	42 (43)
Very high	8 (8)
Disease status	
Remission	83 (84)
Relapse	16 (16)

Note: Data are presented as mean±SD and median (range) for continuous variables and number (%) for categorical variables.
Abbreviations: ALL, acute lymphoblastic leukemia; ANC, absolute neutrophil count; APL, acute promyelocytic leukemia; CNS, central nervous system; MPAL, mixed phenotype acute leukemia.

Table 2 Prevalence of *ITPA:c.94C>A* and *NUDT15:c.415C>T* Polymorphisms

Genetic Polymorphisms	N (%)
ITPA:c.94C>A	
Wild type	65 (66)
Heterozygous genotype	34 (34)
NUDT15:c.415C>T	
Wild type	82 (83)
Heterozygous genotype	17 (17)
Both *ITPA:c.94C>A* and *NUDT15:c.415C>T* heterozygous genotypes	
Yes	4 (4)
No	95 (96)

Note: Data are presented as number (%) for categorical variables.
Abbreviations: *ITPA*, inosine triphosphate pyrophosphohydrolase; *NUDT15*, nucleoside diphosphate linked moiety X-type motif 15.

using the mean numbers of ANC and platelet counts as well as hemoglobin levels measured on each cycle of chemotherapy during the maintenance phase of leukemia treatment protocols, as shown in Figure 3. Interestingly, no differences were observed concerning ANC, platelet count, and hemoglobin levels between patients carrying *ITPA:c.94C>A* polymorphism and those carrying *ITPA* wild type (Figure 3A–C). However, the numbers of ANC and platelet counts were significantly decreased among patients carrying *NUDT15:c.415C>T* polymorphism compared with those carrying *NUDT15* wild type with p-values<0.001 and 0.019, respectively (Figure 3E and F). In addition, 6-MP administrative dosing among patients carrying *NUDT15:c.415C>T* polymorphism was also significantly less compared with the dose among wild type patients with a p-value<0.001 (Figure 3H). In contrast, the significant decrease of daily 6-MP administrative dosing among patients carrying *ITPA* wild type (p-value=0.007) could be from patients presenting the *NUDT15:c.415C>T* being included in this group (Figure 3D).

Moreover, multivariate GEE was used to identify the relationship between *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms as well as 6-MP administrative dosing and ANC measured monthly during the maintenance phase of acute leukemia protocols as described in Table 4. Interestingly, *ITPA:c.94C>A* polymorphism and 6-MP administrative dosing among patients presenting this specific genetic polymorphism did not have a significant effect on monthly ANC during treatment with p-values of 0.948 and 0.062, respectively (Table 4, Model 1). On the other hand, both *NUDT15:c.415C>T* polymorphism and 6MP administrative dosing among patients presenting this specific genetic polymorphism were found to have a significantly negative effect on monthly ANC during treatment (coefficient: −463.81; CI: −778.53, −149.09 and coefficient: −9.29; CI: −18.05, −0.52) with p-values of 0.004 and 0.038, respectively (Table 4, Model 2). Moreover, co-occurrence of *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms was also found to have a significant negative impact on monthly ANC (coefficient: −527.56; CI: −1045.65, −9.48) with a p-value of 0.046. However, no significant association was observed between 6MP administrative dosing among patients presenting co-occurrence *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms and

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Table 3 Characteristics of Patients with Acute Leukemia According to *ITPA:c.94C>A* and *NUDT15:c.415C>T* Genotypes

	ITPA:c.94C>A					*NUDT15:c.415C>T*					*ITPA:c.94C>A* and *NUDT15:c.415C>T*				
	WT		GT		*p*-value	WT		GT		*p*-value	WT		GT		
	N	%	N	%		N	%	N	%		N	%	N	%	
Age at diagnosis (years)	6.4±4.3		5.3±4.1		0.241	6.2±4.3		5.4±3.8		0.437	6.1±4.3		5.0±2.9		
Gender															
Male	32	58	23	42	0.080	49	89	6	11	0.065	54	98	1	2	
Female	33	75	11	25		33	75	11	25		41	93	3	7	
Diagnosis															
Pre-B ALL	56	64	31	36	0.659	71	82	16	18	0.900	83	95	4	5	
T-ALL	7	70	3	30		9	90	1	10		10	100	0	0	
MPAL	1	100	0	0		1	100	0	0		1	100	0	0	
APL	1	100	0	0		1	100	0	0		1	100	0	0	
CNS status															
CNS-1	59	65	32	35	0.569	75	82	16	18	0.801	87	96	4	4	
CNS-2	5	83	1	17		5	83	1	17		6	100	0	0	
CNS-3	1	50	1	50		2	100	0	0		2	100	0	0	
Ploidy															
Normal	62	65	33	35	0.688	79	83	16	17	0.672	91	96	4	4	
Hyperdiploidy	3	75	1	25		3	75	1	25		4	100	0	0	
Risk stratification for ALL (N=97)															
Standard	28	60	19	40	0.286	38	81	9	19	0.360	44	94	3	6	
High	31	74	11	26/50		37	88	5	12		42	100	0	0	
Very high	4	50	4			5	63	3	37		7	88	1	12	
Disease status															
Remission	54	65	29	35	0.776	68	82	15	18	0.588	79	95	4	5	
Relapse	11	69	5	31		14	88	2	12		16	100	0	0	

Notes: Data are presented as mean±SD for continuous variables and number (%) for categorical variables; comparison between two independent data sets was analyzed using Fisher's exact test for categorical data and Mann–Whitney *U*-test for continuous data; *p*-value<0.05 was considered as statistically significant.

Abbreviations: ALL, acute lymphoblastic leukemia; ANC, absolute neutrophil count; APL, acute promyelocytic leukemia; CNS, central nervous system; GT, genetic polymorphism; *ITPA*, inosine triphosphate pyrophosphohydrolase; *NUDT15*, nucleoside diphosphate linked moiety X-type motif 15; MPAL, mixed phenotype acute leukemia; WT, wild type.

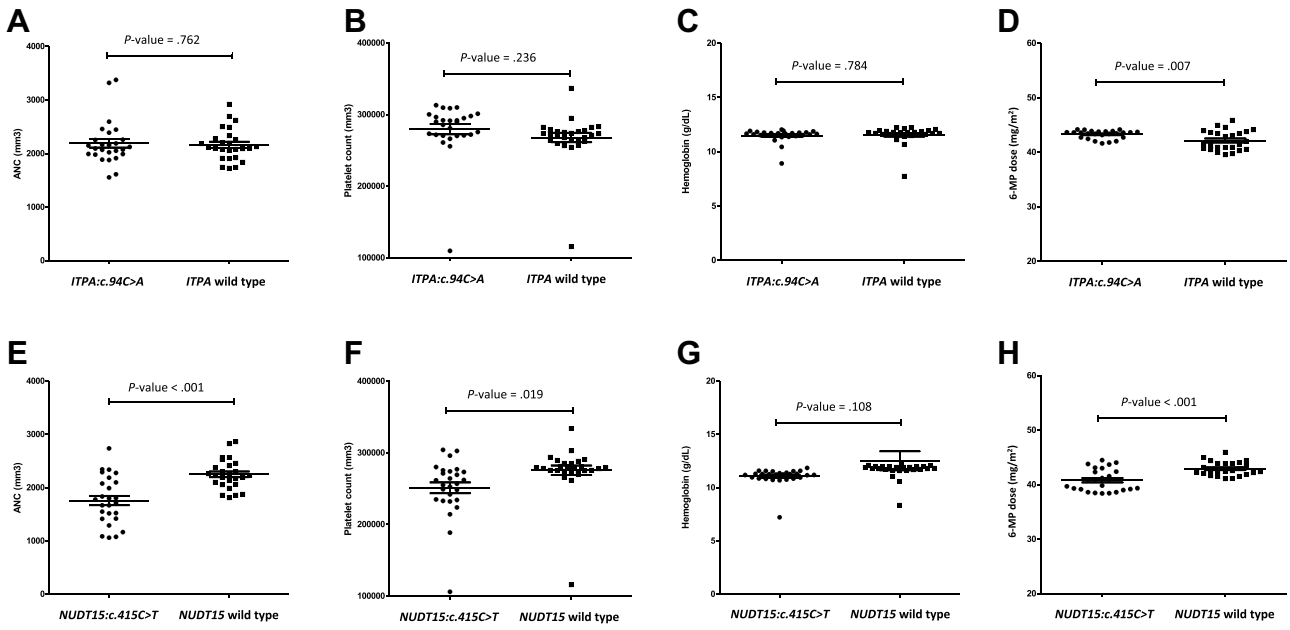

Figure 3 Associations between genetic variants and various blood counts as well as 6-MP administrative dosing among patients with acute leukemia undergoing 6-MP containing regimens. (**A**) ANC, (**B**) platelet count, (**C**) hemoglobin, and (**D**) daily 6-MP administrative dosing among patients with *ITPA:c.94C>A* polymorphism versus those with *ITPA* wide type. (**E**) ANC, (**F**) platelet count, (**G**) hemoglobin, and (**H**) daily 6-MP administrative dosing among patients with *NUDT15:c.415C>T* polymorphism versus those with *NUDT15* wide type.

Notes: Data in the graph are shown as dot plots. One dot plot represents a mean blood count or daily 6-MP administrative dosing on each cycle of treatment during the maintenance phase of leukemia treatment protocols. The two independent data sets were analyzed using independent sample *t*-test and Mann–Whitney *U*-test; *p*-value<0.05 was considered as statistically significant.

Abbreviations: ANC, absolute neutrophil count; *ITPA*, inosine triphosphate pyrophosphohydrolase; *NUDT15*, nucleoside diphosphate linked moiety X-type motif 15; 6-MP, mercaptopurine.

monthly ANC during treatment (*p*-value=0.061) (Table 4, Model 3). Overall frequency of myelotoxicity (neutropenia, anemia, and thrombocytopenia) from 1,635 episodes of CBC obtained on each monthly cycle of chemotherapy from participating patients was described in Table 5.

Table 4 Effect of *ITPA:c.94C>A* and *NUDT15:c.415C>T* Polymorphisms and 6-MP Dosing on ANC

ANC	Coefficient	*p*-value	95% CI	
			Lower	Upper
Model 1				
ITPA:c.94C>A	11.90	0.948	−343.11	366.92
6-MP dose	−8.40	0.062	−17.22	0.42
Model 2				
NUDT15:c.415C>T	−463.81	0.004*	−778.53	−149.09
6-MP dose	−9.29	0.038*	−18.05	−0.52
Model 3				
ITPA:c.94C>A and *NUDT15:c.415C>T*	−527.56	0.046*	−1045.65	−9.48
6-MP dose	−8.41	0.061	−17.19	0.38

Notes: Longitudinal data from time-dependent variables were analyzed using multivariate GEE to examine the associations between *ITPA* and *NUDT15* polymorphisms as well as 6-MP administrative dosing and ANC measured monthly during the maintenance phase of acute leukemia protocols; *p-value<0.05 was considered as statistically significant.

Abbreviations: ANC, absolute neutrophil count; *ITPA*, inosine triphosphate pyrophosphohydrolase; *NUDT15*, nucleoside diphosphate linked moiety X-type motif 15; 6-MP, mercaptopurine.

Table 5 Overall Frequency of Myelotoxicity

CBC (N=1635)	Genetic Polymorphisms					
	ITPA:c.94C>A		*NUDT15:c.415C>T*		*ITPA:c.94C>A* and *NUDT15:c.415C>T*	
	Heterozygous	Wild type	Heterozygous	Wild type	Yes	No
	N=549	N=1086	N=261	N=1374	N=59	N=1576
ANC (cells/mm^3)						
<500	23	65	25	63	3	85
500–999	90	146	47	189	14	222
1,000–1,500	101	207	62	246	14	294
Hemoglobin (g/dL)						
<8.0	4	21	5	11	1	15
8.0–9.9	60	111	34	137	4	167
10–12	285	484	149	620	38	731
Platelets (cells/mm^3)						
<25,000	3	3	0	6	0	6
25,000–49,999	2	8	3	7	1	9
50,000–74,999	3	8	0	11	0	11
75,000–150,000	42	100	19	123	7	135

Abbreviations: ANC, absolute neutrophil count; CBC, complete blood count; *ITPA*, inosine triphosphate pyrophosphohydrolase; *NUDT15*, nucleoside diphosphate linked moiety X-type motif 15.

Discussion

Pharmacogenetics has become one of the key elements in individualized cancer therapy over the past decades.[22,23] Genetic variability of each individual patient could greatly affect drug metabolisms, therapeutic sensitivity, and risk for developing adverse effects. One of the common pharmacogenetic approaches to drug therapy is to identify single nucleotide polymorphisms or SNP and its impact on individual patient's phenotype as well as subsequent clinical consequences.[24] The ultimate goal of this approach is to minimize treatment-related toxicity while preserving efficacy, while 6-MP is considered as the backbone of the maintenance phase of the acute lymphoblastic leukemia protocol. Several genetic polymorphisms of *TPMT*, *ITPA*, and *NUDT15* have been identified and reported to be associated with drug metabolism, resulting in increasing drug-related myelotoxicity.[25–28] Given geographic variation, *TPMT* polymorphisms were less reported in Asian populations. Conversely, *NUDT15* and *ITPA* polymorphisms were found more in this specific population.[13–18]

Herein, we conducted a prospective cross-sectional observational study to evaluate the prevalence of *NUDT15* and *ITPA* polymorphisms among Thai children with acute leukemia and further explore their association with 6-MP related myelotoxicity. Ninety-nine children receiving a diagnosis of acute leukemia as well as pediatric leukemia survivors were recruited and enrolled in this study. All patients participated and were involved to the end of the study with no loss of follow-up cases. The most common leukemia subtype diagnosis among participating patients in this study was pre-B ALL, which was in keeping with the common subtype prevalence among pediatric patients. Despite the diversity of acute leukemia subtypes, the recommended 6-MP administrative dosing on all treatment regimens was 50 mg/m^2/day. Interestingly, our PCR-RFLP method could identify *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms among up to one half of the patients although all carried heterozygous genotypes. In addition, the prevalence of *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms among patients in this study was 34 and 17%, respectively, in which 4% of the patients were found to have co-occurrence of these two genetic polymorphisms. These results affirm the commonness of *ITPA* and *NUDT15* polymorphisms in Asian compared with Caucasian populations[16–18,29,30] although a higher incidence of *ITPA* polymorphism was observed in our study compared with 5–7% in Caucasian and up to 15% in Asian populations.[31] We then further investigated potential associated factors including patient demographic data, disease information and treatment response among patients with and without *ITPA:c.94C>A* and *NUDT15:c.415C>T*

polymorphisms and found no differences in the demographic information between patients with and without polymorphisms. However, *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms, involved in 6-MP metabolisms, potentially increase the risk of developing drug-related myelotoxicity resulting in delayed cycles of chemotherapy. Additionally, they possibly increase the risk of disease relapse. The insignificant difference of treatment response between the two groups of patients in our study could be from careful monitoring of CBC measured monthly during the maintenance phase of treatment and adequate adjustment of 6-MP dosing based on ANC instead of holding off on treatment.

Associations between *ITPA* and *NUDT15* genotypes and 6-MP related myelotoxicity were further explored. Interestingly, suppression of granulopoiesis and megakaryopoiesis was remarkable among patients presenting the *NUDT15:c.415C>T* polymorphism, but not among those carrying the *ITPA:c.94C>A* polymorphism. This result was confirmed by significantly decreased 6-MP administrative dosing among patients presenting the *NUDT15:c.415C>T* polymorphism. However, the significant decrease of 6-MP administrative dosing among patients carrying the *ITPA* wild type could be from patients presenting *NUDT15:c.415C>T* being included in this group. Multivariate GEE was used to evaluate the effect of *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms as well as 6-MP administrative dosing among patients carrying these specific polymorphisms on monthly ANC longitudinally measured during the maintenance phase of acute leukemia treatment protocols. The result of our study affirmed the significant negative relationship between *NUDT15:c.415C>T* polymorphism and neutropenia (p-value=0.004) in which patients carrying this specific polymorphism would have ANC 463 cells/mm^3 lower than those carrying the *NUDT15* wild type. In addition, ANC among patients carrying the *NUDT15:c.415C>T* polymorphism would decrease by a decrement of 9 cells/mm^3 on every 1 mg increased 6-MP dose (p-value=0.038). Interestingly, the effect of *ITPA:c.94C>A* polymorphism and 6-MP administrative dosing among patients carrying this specific polymorphism were not significantly evident in our study. These findings were consistent with recent studies among Japanese and Chinese children in which no differences were found between 6-MP dose administrative dosing and *ITPA* polymorphisms.[16,32] Moreover, we found a significant negative relationship between co-occurrence of *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms and neutropenia (p-value=0.046) in which patients having co-occurrence of these two polymorphisms would have ANC 527 cells/mm^3 lower than that of wild type patients. However, the effect of 6-MP administrative dosing was insignificant which might have stemmed from this dilutional effect of the *ITPA* polymorphism group. According to Table 5, there were certain numbers of patients who did not carry *ITPA:c.94C>A* and *NUDT15:c.415C>T* polymorphisms and developed myelotoxicity. The development of myelotoxicity among those patients could be from several factors such as *TPMT* polymorphisms which were not identified in this study, a direct myelosuppressive effect from 6-MP itself or other chemotherapy co-administered with 6-MP in the treatment regimen, less bone marrow reserve from previous treatment prior to entering the maintenance phase or recent infection-associated bone marrow suppression.

The limitations of this study included the small sample size of participating patients, which might have contributed to insignificant differences of some results. In addition, data obtained from the unique and specific populations in this study might not be generally applicable to all patients at different age ranges and ethnic groups. Moreover, the correlation between genetic polymorphisms and treatment interruption as well as total duration of neutropenia not examined in our study needs to be further explored. Since *TPMT* polymorphisms were less reported in Asian populations, we did not include evaluation of *TPMT* polymorphisms in our study.

Conclusion

Although the incidence of *ITPA:c.94C>A* polymorphism among Thai children with acute leukemia in our study was higher than that reported from other Asian countries, *ITPA* genotyping could not be used as a predictor for 6-MP induced myelotoxicity. In contrast, the incidence *NUDT15:c.415C>T* polymorphism in our study was comparable to other Asian countries and a strong association was also observed between either *NUDT15* polymorphism alone or combined with *ITPA* polymorphism and developing 6-MP induced myelotoxicity.

Abbreviations

ALL, acute lymphoblastic leukemia; ANC, absolute neutrophil count; APL, acute promyelocytic leukemia; CBC, complete blood count; CI, confidence interval; CNS, central nervous system; GEE, generalized estimating equation; ITPA, inosine triphosphate pyrophosphohydrolase; MPAL, mixed phenotype acute leukemia; NUDT15, nucleoside diphosphate linked moiety X-type motif 15; PCR-RFLP, PCR-Restriction Fragment Length

Polymorphism; PROBE, Preferred Reporting items for OBservational studies in Endodontics; SD, standard deviation; ThaiPOG, Thai Pediatric Oncology Group; TPMT, thiopurine methyltransferase; 6-MP, 6-mercaptopurine.

Data Sharing Statement

The data that support the findings of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Author Contributions

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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