NUDT15 c.415C>T increases risk of 6-mercaptopurine induced myelosuppression during maintenance therapy in children with acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is the most common hematologic malignancy in children[1]. Prolongation of therapy by incorporating a maintenance phase, containing 6-mercaptopurine (6-MP) as the backbone, has improved treatment outcomes in pediatric ALL[2-4]. However, 6-MP can cause critical side effects such as myelosuppression and abnormalities in liver function. This may lead to treatment interruptions, and subsequently contribute to an increased incidence of late relapse[4,5]. The pharmacogenetics of 6-MP has been studied extensively. Polymorphisms in the *thiopurine methyltransferase (TPMT)* gene have a well-established effect on myelosuppression from 6-MP. A clinical guideline for dose adjustment of 6-MP according to types of *TPMT* polymorphisms has been developed and is being used in most modern pediatric ALL protocols[6]. However, the frequencies of *TPMT* variants associated with thiopurine-induced myelosuppression in most Asian populations, including Thai, are low[7-9]. Determination of *TPMT* polymorphisms, therefore, has limited clinical benefits to children with ALL in most Asian countries. More recently, single nucleotide polymorphisms (SNPs) of *inosine triphosphate pyrophosphohydrolase (ITPA)* and *nucleoside diphophate–linked moiety X-type motif 15 (NUDT15)* have been shown to be associated with myelosuppression induced by 6-MP or other drugs in the thiopurine group, in both benign and malignant conditions[10-14].

We present data on the association between the *ITPA* c.94C>A (rs1127354) and *NUDT15* c.415C>T (rs116855232) and 6-MP induced myelosuppression in our cohort of pediatric ALL children. Patients aged between 1 and 15 years old, who received maintenance therapy and were followed-up at King Chulalongkorn Memorial Hospital between January 2014 and January 2015, were recruited. All patients were treated according to the Thai Pediatric Oncology Group (Thai-POG) protocol for childhood ALL[15]. The Thai-POG protocol is based on the modified Berlin-Frankfurt-Munster (BFM) protocol for childhood ALL. Patients were initially categorized into different risk groups: standard-risk, high-risk and very high-risk, using age at diagnosis and initial white blood cell count according to the National Cancer Institute (NCI)/Rome criteria for pediatric ALL risk classification, with the addition of cytogenetic information. In brief, the Thai-POG treatment regimen consists of five phases of therapy including remission induction, consolidation, interim maintenance, delayed intensification and maintenance phase. The total duration of therapy is two and a half years for girls and three and a half years for boys.

Essentially, medications given during the maintenance phase included monthly intravenous vincristine, a monthly pulse of prednisolone, weekly oral methotrexate, daily oral 6-MP and intrathecal methotrexate once every three months. The initial 6-MP dose was 50 mg/m^2. A complete blood count (CBC) was performed at a 4-week interval. 6-MP was either increased by 25% of the previous dose or discontinued, to keep an absolute neutrophil count (ANC) of between 500-1,500 cells/μL.[15] The *ITPA* c.94C>A and *NUDT15* c.415C>T were evaluated by pyrosequencing using 5'-GACCAGCTTTTCTGGGGACTG-3'and biotinylated 5'-GGCTGAAAGAGTGGGGGATAC-3', and 5'-GACCAGCTTTTCTGGGGACTG-3'and biotinylated 5'-GGCTGAAAGAGTGGGGGATAC-3', respectively.

We retrospectively analyzed the myelosuppressive effect of 6-MP at months 2, 4 and 6 of the maintenance phase between the SNP groups in addition to comparing the median cumulative dose of 6-MP at each time point.

Out of 82 patients, the median age at diagnosis was 5.4 years old. The female to male ratio was 1.5 to 1. 50% of

Table 1. Associations between myelosuppression at the second, fourth and sixth month following the initiation of 6-MP during maintenance therapy in children with ALL and SNPs of the *ITPA* and *NUDT15*.

	2nd Month				4th Month				6th Month			
SNP	ANC >500 (%)	ANC < 500 (%)	OR (95% CI)	*P*	ANC > 500 (%)	ANC <500 (%)	OR (95% (CI)	*P*	ANC > 500 (%)	ANC <500 (%)	OR (95% CI)	*P*
ITPA												
CC	42 (91.3)	4 (8.7)		0.59	35 (76.1)	11 (23.9)		0.69	32 (69.6)	14 (30.4)		0.42
CA	31 (93.9)	2 (6.1)	0.62 (0.11-3.57)		23 (69.6)	10 (30.4)	1.22 (0.45-3.31)		19 (57.6)	14 (42.4)	1.45 (0.58-3.64)	
AA	0 (0)	3 (100)			0 (0)	3 (100)			0 (0)	3 (100)		
NUDT15												
CC	67 (95.7)	3 (4.3)			58 (82.9)	12 (17.1)			52 (74.3)	18 (25.7)		<0.001
CT	8 (80)	2 (20)	7.44 (1.3 - 42.63)	0.01	2 (20)	8 (80)	14.5 (3.41 -61.63)	<0.001	1 (10)	9 (90)	14.44 (2.89-72.26)	
TT	1 (50)	1 (50)			1 (50)	1 (50)			1 (50)	1 (50)		

SNP indicates single nucleotide polymorphism; ANC indicates absolute neutrophil count; OR indicates odds ratio; CI indicates confidence interval.

Table 2. Comparison of the median cumulative 6-MP doses (mg/m²) of 2, 4, and 6 months' duration following 6-MP initiation among ALL patients with different genotypes of *ITPA* c.94C>A and *NUDT15* c.415C>T.

Month	Median cumulative 6-MP dose (mg/m²)					
	ITPA-CC (95% CI)	*ITPA*-CA/AA (95% CI)	*P*	*NUDT15*-CC (95% CI)	*NUDT15*-CT/TT (95% CI)	*P*
2	2,492 (2,055 – 2,873)	2,628 (2,092 – 2,919)	0.55	2,600 (2,323 – 2,912)	2,089 (1,137 – 2,490)	0.03
4	4,637 (3,853 – 5,691)	5,069 (3,075 – 5,815)	0.81	5,067 (3,965 – 5,833)	3,117 (2,209 – 4,386)	0.001
6	6,833 (5,887 – 8,576)	7,512 (4,685 – 8,463)	0.78	7,616 (5,943 – 8,788)	4,650 (3,481 – 6,094)	0.001

patients were standard-risk, whereas 30% and 20% of patients were high-risk and very high-risk, respectively. The median duration of follow-up in this study was 8 years (1 to 20 years) from diagnosis. Genotypic determination of the ITPA c.94C>A showed that 46 (56.1%), 33 (40.2%), and 3 (3.7%) patients were CC, CA, and AA, respectively. Of the NUDT15 c.415C>T, 70 (85.4%), 10 (12.2%), and 2 (2.4%) patients were CC, CT, and TT, respectively. These were in the Hardy-Weinberg equilibrium.

Genetic polymorphism of *ITPA* showed no significant difference between genotypes, and 6-MP induced myelosuppression at any time point during maintenance (Table 1). However, the *NUDT15* c.415C>T was strongly associated with 6-MP induced early myelosuppression in the second, fourth, and sixth month. Patients with *NUDT15* CT or TT had a significantly increased risk of neutropenia as early as two months after 6-MP administration with OR of 7.4 (95% CI 1.3-42.6). Longer follow-up duration at the fourth and sixth month showed even higher risks, with OR of 14.5 (95% CI 3.4-61.6) and 14.4 (95% CI 2.9-72.3), respectively (Table 1).

According to the Thai-POG ALL protocol, we adjusted 6-MP dosage according to ANC levels, which were determined every 4 weeks. Our patients with different *ITPA* polymorphisms had no significant difference in the median cumulative 6-MP dose at any time point. On the contrary, the median cumulative doses of 6-MP of 2, 4, and 6 months' duration in the CT or TT groups of *NUDT15* were 80.3%, 61.5%, and 61.1%, respectively, of those with the CC genotype (Table 2). For the 6-month duration of 6-MP administration, our patients with *NUDT15* CT or TT received between 45.7% (3481/7616) and 80% (6094/7616) of the median cumulative 6-MP dose of those with the CC genotype.

Moreover, at the sixth month following 6-MP initiation, our patients with *NUDT15* CT and TT were given a median 6-MP dose of 28 mg/m²/day (range: 6-52 mg/m²/day), which was only 56% of the standard 6-MP initial dose of 50 mg/m²/day in the Thai population. Notably, our two patients with the homozygous TT genotype received 6 and 36 mg/m²/day of 6-MP at the sixth month following 6-MP initiation, which were 12% and 72% of the standard 6-MP initial dose in the Thai ALL protocol. 6-MP was continued at 36 mg/m²/day in the latter patient throughout the treatment period without any additional episode of severe neutropenia. Interestingly, the homozygous TT patient who received 6 mg/m²/day of 6-MP was heterozygous for ITPA c.94C>A, while the other carried the wild-type CC genotype. The discrepancy of final 6-MP dosage between these two patients with homozygous TT genotype could be caused by multifactorial elements, including epistatic effects from other modifier genes such as *ITPA*. In addition, we found that a total of six patients had a history of relapse. Two out of these six patients demonstrated a heterozygous mutation of the *NUDT15* c.415C>T. Due to the limited sample size, however, our study did not have enough resources to show a statistically significant correlation between *NUDT15* polymorphism and the relapse rate.

Our study shows that *NUDT15* c.415C>T has a strong effect on early myelosuppression from 6-MP in Asian children with ALL. The results were consistent with a recent study in Korean patients with inflammatory bowel disease, which showed that *NUDT15* c.415C>T was associated with 5-azathioprine-induced leukopenia within 8 weeks of the initiation with an OR of 35.6.[12] The 6-MP dose in the variant group of *NUDT15* c.415C>T was significantly lower compared to that in the wild-type group, especially in the homozygous group. Recently, a genome-wide association study (GWAS) in children with ALL also showed that the *NUDT15* c.415C>T was a determinant for 6-MP leukopenia. Their patients with the homozygous *NUDT15* TT variant could tolerate only 8.3% of their standard 6-MP dose, which was 75 mg/m²/day.[13] Moreover, a recent study in Japanese children with ALL showed significant 6-MP dose reductions of 42% and 82% in patients with the CT and TT genotypes, respectively.[14]

In conclusion, more studies on the role of *NUDT15* c.415C>T in 6-MP metabolism, the proper dose adjustment in patients with heterozygous and homozygous variants, 6-MP pharmacogenetics in different ethnicities, as well as a systematic evaluation of the cost-effectiveness of *NUDT15* screening are warranted.

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