**ITPA Polymorphisms and the Incidence of Toxicities in Children with Acute Lymphoblastic Leukemia**

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**Competing interests:** The authors declare no conflict of interest.

**Abstract.** Background: 6-Mercaptopurine (6-MP), a thiorouine agent, is a essential medication for treating pediatric acute lymphoblastic leukemia (ALL). However, its side effects of neutropenia and hepatotoxicity might interrupt treatment, resulting in poor outcomes. Inosine triphosphate pyrophosphatase (ITPA), an enzyme in the thiopurine pathway, may prevent the accumulation of toxic thiopurine metabolites. Studies on ITPA and thiopurine-associated toxicities are scarce.

Methods: This study retrospectively investigated 1- to 15-year-old children with ALL who received 6-MP during the maintenance phase of treatment between 2000 and 2020. Toxicity during the first year of maintenance therapy and the mean dose of 6-MP were analyzed.

Results: The 209 patients had a median age of 4.8 (0.3-14.8) years. Of these, 124 patients (59.3%) had wild-type ITPA, 73 patients (34.9%) had heterozygous ITPA 94C>A (hetITPA), and 12 patients (5.7%) had homozygous ITPA 94C>A (homITPA), with an allele frequency of 0.23. The incidence of neutropenia among ITPA polymorphisms did not significantly differ ($P = 0.813$). In patients harboring homITPA, transaminitis was more frequent than other polymorphisms but without a significant difference ($P = 0.063$). The mean dose of 6-MP for patients with homITPA was significantly lower than that for patients with hetITPA or wild-type ITPA ($P = 0.016$).

Conclusions: HomITPA had a higher incidence of transaminitis and required a significantly larger dose reduction of 6-MP than wild-type ITPA. Further study is warranted to elucidate the effects of ITPA polymorphisms on toxicity in patients with ALL treated with 6-MP.

**Keywords:** Inosine triphosphate pyrophosphatase; Leukemia; Mercaptopurine; Neutropenia; Transaminitis.


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**Introduction.** Acute lymphoblastic leukemia (ALL) is children's most common hematologic malignancy. With advances in treatment, its event-free survival is approximately 90%. 1-6-Mercaptopurine (6-MP) is one of...
the key medications used during maintenance therapy, with the most extended phase of treatment being at least two years. Nevertheless, 6-MP's side effects of neutropenia and hepatotoxicity may interrupt treatment. Two well-known thiopurine enzymatic genetic polymorphisms have demonstrated clinical significance for patients with 6-MP. They are thiopurine methyltransferase (TPMT), prevalent in European populations, and nudix (nucleoside diphosphate linked moiety X) type motif 15 (NUDT15), prevalent in Asian populations. Inosine triphosphate pyrophosphatase (ITPA), another enzyme in the multistep thiopurine pathway, prevents the accumulation of methylthioinosine triphosphate, a toxic thiopurine metabolite, thereby catalyzing inosine triphosphate to inosine monophosphate.\(^5\)\(^,\)\(^6\) \(ITPA\) 94 C\(\rightarrow\)A genotypes were found to have markedly decreased \(ITPA\) activity. However, the clinical relevance between toxicities and such polymorphisms still needs to be well elucidated, and clinical recommendations for dose modifications according to the \(ITPA\) polymorphism need to be developed. Such studies on thiopurine enzymatic genetic polymorphisms apart from \(TPMT\) and \(NUDT15\) may better guide physicians in prescribing doses of thiopurine drugs, especially 6-MP, to prevent toxicity. The present investigation aimed to determine the effects of \(ITPA\) polymorphisms on (1) neutropenia and hepatotoxicity, (2) the modification of 6-MP dosage, and (3) survival for childhood ALL.

Patients and Methods. This retrospective study included patients aged 1 to 15 years diagnosed with ALL between January 2000 and December 2020. The patients were classified into risk groups according to the National Cancer Institute classifications and Thai Pediatric Oncology Group classification.\(^8\)\(^,\)\(^9\) They were treated according to the national protocol of the Thai Pediatric Oncology Group.\(^8\)

Treatment during maintenance therapy consisted of monthly pulse intravenous vincristine and 5-day oral prednisolone, weekly oral methotrexate (20 mg/m\(^2\)), and daily oral 6-MP (50 mg/m\(^2\)). Patients who experienced prolonged neutropenia or severe infection during previous therapy with 6-MP may have been administered a decreased 6-MP dosage, depending on the attending physician's assessment. Complete blood counts and liver function tests were performed monthly and trimonthly, respectively. The dose of 6-MP was adjusted to maintain an absolute neutrophil count between 500 and 1500 cells/mm\(^3\). Treatment was temporarily interrupted if a patient developed cytopenia (an absolute neutrophil count < 500 cell/mm\(^3\) or a platelet count < 50,000/mm\(^3\)) or hepatic dysfunction (direct hyperbilirubinemia or an elevated transaminase > 20 times the upper normal value). Patients with elevated transaminase > 5 times but < 20 times the upper normal value may have been prescribed a decreased dose of 6-MP at the attending physician's discretion. The mean dose of 6-MP at each interval was calculated using the sum of a 28-day dose (mg) divided by the product of 28 and the body surface area.

Neutropenia was defined as an absolute neutrophil count of fewer than 500 cells/mm\(^3\). Transaminitis was defined as serum alanine aminotransferase more than five times the upper normal value.

As a retrospective study, the remaining blood specimens of patients during the diagnosis procedure were collected and subsequently evaluated for \(ITPA\) 94C\(\rightarrow\)A polymorphisms using an allele-specific polymerase chain reaction method. The wildtype primer was 5' CGTTCAGATTCTAGGAGATAAGTTCA-3'. The mutant forward primer was 5' CGTTCAGATTCTAGGAGATAAGTTCA-3'. For internal controls, 5'GCTTAGCACAAGCAGACCTGACG-3' and 5'TTCACAGAACATGTGTAATGCAGC-3' were used. Patients harboring \(TPMT\) or \(NUDT15\) polymorphisms were excluded from this cohort.

Before this research began, its protocol was approved by the Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand (Si-479/2021).

Statistical analysis. Demographic data were determined using descriptive statistics. Categorical variables are presented as a number and percentage, and continuous variables are given as a mean ± standard deviation (SD) or a median (range), as appropriate. The associations between \(ITPA\) polymorphisms, neutropenia, and transaminitis were determined using Pearson's chi-squared test. Simple and multiple binary logistic regression analyses assessed the associations between \(ITPA\) polymorphisms, 6-MP doses, and transaminitis. The odds ratio (OR) with a corresponding 95% confidence interval (95% CI) was used to evaluate the strength and directions of associations. Differences in 6-MP doses among the heterozygous and homozygous \(ITPA\) polymorphisms and wild-type \(ITPA\) at baseline and each follow-up were evaluated using 1-way ANOVA, followed by Bonferroni corrections for multiple post hoc comparisons of means. The Kaplan-Meier method was used to estimate the 3-year overall survival (OS) and event-free survival rates. A log-rank test was constructed to compare the 3-year OS and event-free survival rates. IBM SPSS Statistics for Windows, version 23 (IBM Corp, Armonk, NY, USA) was used for the data analyses. Probability (\(P\)) values of less than 0.05 were considered statistically significant.

Results. The study cohort consisted of 209 patients with ALL. There were 112 boys and 97 girls; their median age was 4.8 (0.3-14.8) years. The median duration of follow-
up was 36 (16–36) months. As for risk, 114 patients were classified as standard risk, 69 patients were high risk, and 26 patients were very high risk.

In terms of genotype, 124 patients (59.3%) had wild-type ITPA 94C>A, 73 patients (34.9%) showed heterozygous ITPA 94C>A (hetITPA), and 12 patients (5.7%) showed homozygous ITPA 94C>A (homITPA). The allele frequency of ITPA 94C>A was 23%. The percentage of standard risk, high risk, and very high risk in wild-type ITPA are 55.6%, 30.6%, and 13.7%, respectively. Those in hetITPA are 53.4%, 37%, and 9.6%, respectively, while those in homITPA are 50%, 33.3%, and 16.7%, respectively.

Univariable analysis was undertaken for ITPA 94C>A, the 1-year mean dose of 6-MP, and side effects. No association was revealed between ITPA 94C>A and neutropenia during one year of maintenance therapy ($P = 0.813$; Table 1). The incidences of neutropenia during one year of maintenance therapy in patients harboring wild-type ITPA, hetITPA, and homITPA were 33.9%, 37.0%, and 41.7%, respectively. The association between ITPA 94C>A and transaminitis during one year of maintenance therapy ($P = 0.063$) is demonstrated in Table 2. Pairwise comparisons using Bonferroni correction showed a significant difference between patients with and without transaminitis in homITPA (12.2% vs. 3.8%; $P = 0.036$). The incidences of transaminitis during one year of maintenance therapy in patients harboring wild-type ITPA, hetITPA, and homITPA were 20.2%, 24.7%, and 50.0%, respectively.

The 1-year mean doses of 6-MP for wild-type ITPA, hetITPA, and homITPA were 39.11 ± 14.19 mg/m², 39.40 ± 14.21 mg/m², and 27.08 ± 12.58 mg/m², respectively ($P = 0.016$). The Bonferroni post hoc test revealed a significantly lower 1-year mean dose of 6-MP for homITPA than for the wild-type and hetITPA groups ($P < 0.05$).

Two factors were significantly associated with transaminitis in univariable binary logistic regression analysis of factors and transaminitis. They were ITPA 94C>A and a 1-year mean dose of 6-MP. Patients with hetITPA and homITPA developed transaminitis more frequently than those harboring wild-type ITPA (hetITPA: OR, 1.296; 95% CI, 1.650–2.583; $P = 0.461$; homITPA: OR, 3.960; 95% CI, 1.177–13.328; $P = 0.026$). Higher doses of 6-MP were associated with no incidence of transaminitis (OR, 0.943; 95% CI, 0.919–0.968; $P < 0.001$). In multivariable binary logistic regression analysis, there was no statistically significant association between ITPA 94C>A and transaminitis.

Table 1. Comparison of homozygous ITPA, heterozygous ITPA, and wildtype ITPA for cases with and without neutropenia in Thai pediatric acute lymphoblastic leukemia patients.

<table>
<thead>
<tr>
<th>Events</th>
<th>All patients $n = 209$</th>
<th>Neutropenia $\text{ANC} &lt; 500$ $n = 74$</th>
<th>No neutropenia $\text{ANC} \geq 500$ $n = 125$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITPA 94C&gt;A</td>
<td>$\text{Wildtype}$</td>
<td>124 $\text{59.3(}$</td>
<td>42 $\text{56.8(}$</td>
<td>82 $\text{60.7(}$</td>
</tr>
<tr>
<td></td>
<td>$\text{Heterozygous}$</td>
<td>73 $\text{33.4(}$</td>
<td>27 $\text{36.5(}$</td>
<td>46 $\text{34.1(}$</td>
</tr>
<tr>
<td></td>
<td>$\text{Homzygous}$</td>
<td>12 $\text{5.7(}$</td>
<td>5 $\text{6.8(}$</td>
<td>7 $\text{5.2(}$</td>
</tr>
</tbody>
</table>

ANC, absolute neutrophil count; ITPA, inosine triphosphate pyrophosphatase.

Table 2. Comparison of homozygous ITPA, heterozygous ITPA, and wildtype ITPA for cases with and without transaminitis in Thai pediatric acute lymphoblastic leukemia patients.

<table>
<thead>
<tr>
<th>Events</th>
<th>All patients $n = 209$</th>
<th>Transaminitis $n = 49$</th>
<th>No transaminitis $n = 160$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITPA 94C&gt;A</td>
<td>$\text{Wildtype}$</td>
<td>124 $\text{59.3(}$</td>
<td>25 $\text{51.0(}$</td>
<td>99 $\text{61.9(}$</td>
</tr>
<tr>
<td></td>
<td>$\text{Heterozygous}$</td>
<td>73 $\text{34.9(}$</td>
<td>18 $\text{36.7(}$</td>
<td>55 $\text{34.4(}$</td>
</tr>
<tr>
<td></td>
<td>$\text{Homzygous}$</td>
<td>12 $\text{5.7(}$</td>
<td>6 $\text{12.2(}$</td>
<td>6 $\text{3.8(}$</td>
</tr>
</tbody>
</table>

ITPA, inosine triphosphate pyrophosphatase.

Table 3. Univariable and multivariable binary logistic regression analysis of factors and transaminitis.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Crude OR $95%$ CI</th>
<th>$P$ value</th>
<th>Adjusted OR $95%$ CI</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITPA 94C&gt;A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wildtype</td>
<td>Reference</td>
<td></td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Heterozygous</td>
<td>1.296 (0.650–2.583)</td>
<td>0.461</td>
<td>1.357 (0.657–2.804)</td>
<td>0.410</td>
</tr>
<tr>
<td>Homozygous</td>
<td>3.960 (1.177–13.328)</td>
<td>0.026</td>
<td>2.376 (0.655–8.627)</td>
<td>0.188</td>
</tr>
<tr>
<td>1-year dose of 6-MP</td>
<td>0.943 (0.919–0.968)</td>
<td>&lt; 0.001</td>
<td>0.946 (0.921–0.971)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

6-MP, 6-mercaptopurine; ITPA, inosine triphosphate pyrophosphatase.
metabolites. The increased accumulation might account for the varied responses and toxicities among patients receiving thiopurine drugs such as 6-MP. The polymorphisms vary among ethnicities. ITPA is a catalytic enzyme that hydrolyzes inosine triphosphate, a toxic noncanonical nucleotide, to less toxic metabolites.\textsuperscript{10} The allele frequency of 2 well-known polymorphisms, TPMT and NUDT15, varies among ethnicities, with the latter prevalent in Asian populations.\textsuperscript{11} As for ITPA, its allele frequency in this study was 0.23, consistent with other studies on Asian populations.\textsuperscript{4,12}

Neutropenia is among the most common complications for those treated with thiopurine agents. The polymorphisms of the genes involved in thiopurine metabolism account for the neutropenia of patients treated with such agents. Currently, thiopurine doses are based on the TPMT and NUDT15 polymorphisms.\textsuperscript{2} However, the recommended thiopurine doses for patients harboring ITPA polymorphisms need to be better established. Additionally, research findings on the association of ITPA and neutropenia in patients treated with thiopurine are controversial. Several studies have demonstrated a significant risk of neutropenia or neutropenic fever with decreased doses of thiopurine\textsuperscript{13,14} in patients with ITPA polymorphisms. Conversely, some studies did not identify any association between neutropenia and ITPA polymorphisms.\textsuperscript{15,16} In the present study’s cohort, the incidences of neutropenia in patients harboring ITPA polymorphisms were not significantly different from those with wild-type ITPA. The heterogeneity of the results might be due to the different 6-MP doses used by studies (range: 50-75 mg/m\textsuperscript{2}) and pharmacogenomic variations related to different ethnic or racial groups.

Transaminitis during treatment with combined 6-MP and MTX is common in pediatric ALL.\textsuperscript{17} The methylated metabolites of 6-MP account for hepatotoxicity. Therefore, the genetic polymorphisms leading to excessive methylated metabolites are considered plausible causes of hepatotoxicity.\textsuperscript{18} The correlation between ITPA polymorphisms and elevated transaminase is uncertain.\textsuperscript{4,6,14} In the univariate analysis of the current investigation, the incidence of transaminitis in patients with homITPA was significantly higher than in those with hetITPA and wild-type ITPA. Fortunately, this cohort did not exhibit severe transaminitis (> 20 times) or severe hepatic dysfunction, including coagulopathy. Careful monitoring of liver function tests may be necessary for individuals harboring homITPA.

The dose of 6-MP in patients harboring homITPA was significantly lower than that for patients with hetITPA or wild-type ITPA in this cohort. This finding may signify a clinically meaningful effect of ITPA polymorphisms, even though the incidences of neutropenia were not significantly different. Furthermore, dose reduction of 6-

Figure 1. Comparison of 3-year overall survival rates in Thai children with acute lymphoblastic leukemia by inosine triphosphate pyrophosphatase (ITPA) polymorphism status.

Figure 2. Comparison of 3-year event-free survival rates in Thai children with acute lymphoblastic leukemia by inosine triphosphate pyrophosphatase (ITPA) polymorphism status.

However, there was a significant association between the 1-year mean dose of 6-MP and no incidence of transaminitis (adjusted OR = 0.958; 95% CI, 0.930–0.987; \textit{P} = 0.005; \textbf{Table 3}).

All patients' estimated 3-year OS rate was 95.1%, while the estimated 3-year event-free survival rate was 81.1%. The 3-year OS rates of patients harboring wild-type ITPA, hetITPA, and homITPA were 90.8%, 92.8%, and 83.3%, respectively (\textit{P} = 0.370; \textbf{Figure 1}). The 3-year event-free survival rates for wild-type ITPA, hetITPA, and homITPA were 76.9%, 87.7%, and 82.5%, respectively (\textit{P} = 0.375; \textbf{Figure 2}).

\textbf{Discussion}. Genetic polymorphisms in thiopurine metabolism pathways increase the accumulation of toxic metabolites. The increased accumulation might account for the varied responses and toxicities among patients receiving thiopurine drugs such as 6-MP. The polymorphisms vary among ethnicities. ITPA is a catalytic enzyme that hydrolyzes inosine triphosphate, a toxic noncanonical nucleotide, to less toxic metabolites.\textsuperscript{10} The allele frequency of 2 well-known polymorphisms, TPMT and NUDT15, varies among ethnicities, with the latter prevalent in Asian populations.\textsuperscript{11} As for ITPA, its allele frequency in this study was 0.23, consistent with other studies on Asian populations.\textsuperscript{4,12}

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The dose of 6-MP in patients harboring homITPA was significantly lower than that for patients with hetITPA or wild-type ITPA in this cohort. This finding may signify a clinically meaningful effect of ITPA polymorphisms, even though the incidences of neutropenia were not significantly different. Furthermore, dose reduction of 6-

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MP did not appear to worsen ALL treatment outcomes, given that the survival rates of patients with *ITPA* and wild-type *ITPA* were not significantly different. However, further study with a larger cohort is warranted to determine whether dose reduction and *ITPA* do not affect such patients' relapse risk.

Some limitations need to be mentioned. First, as a retrospective study, some data might be missing or incomplete. Second, the number of cases with hom*ITPA* was low, which might impact the analysis of the outcomes of these patients. Third, the data were restricted to a single center. Consequently, the generalizability of our data and findings might be limited.

**Conclusions.** Patients harboring *ITPA*, especially homozygous *ITPA*, seemed to have transaminitis and significantly required a greater dose reduction of 6-MP than patients with wild-type *ITPA*. Nevertheless, the neutrophil count did not appear to be affected. Further study is needed to elucidate the effects of *ITPA* polymorphisms on toxicity in patients with ALL treated with 6-MP.

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