Meta-analysis of the impact of thiopurine S-methyltransferase polymorphisms on the tolerable 6-mercaptopurine dose considering initial dose and ethnic difference

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Abstract: A meta-analysis was conducted to decide whether to reduce an initial 6-mercaptopurine (6-MP) dose in TPMT heterozygote in the case of an initial 6-MP dose of $<75$ mg/m$^2$/d and to compare the tolerable 6-MP dose among different ethnic groups. The study was undertaken according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. The differences in mean values of the tolerable 6-MP dose were calculated by using Comprehensive Meta-Analysis version 3. The results of the meta-analysis indicated that the tolerable 6-MP dose was significantly lower in the TPMT heterozygote group ($\text{difference in mean values} = 11.729$, 95% confidence interval $= 7.617–15.842$, $P < 0.001$) even when the initial 6-MP dose was $<75$ mg/m$^2$/d. The TPMT*3C allele-dominant ethnic group (Asian) needed less reduction in mean 6-MP dose in comparison to the TPMT*3A allele-dominant ethnic group (Caucasian, Mediterranean, South American) ($\text{difference in mean values} = 8.884$ vs 15.324). In conclusion, the initial 6-MP dose needs to be reduced in TPMT heterozygote when compared to the wild-type, and ethnic difference might influence the tolerable 6-MP dose in TPMT heterozygotes.

Keywords: 6-mercaptopurine, thiopurine S-methyltransferase, polymorphism, meta-analysis

Introduction

6-Mercaptopurine (6-MP) with methotrexate constitutes the mainstream of the maintenance therapy for acute lymphocytic leukemia (ALL), which is a prevalent hematologic disease seen in those under 20 years of age.¹ To exhibit its cytotoxicity, 6-MP must be converted to its active metabolite, 6-thioguanine nucleotide, by multiple enzymes such as hypoxanthine–guanine phosphoribosyltransferase, inosine-5′-monophosphate dehydrogenase, and guanine monophosphate synthetase. The drug is inactivated into 6-methylmercaptopurine through S-methylation by a polymorphic enzyme named thiopurine S-methyltransferase (TPMT). Thus, TPMT enzyme deficiency results in increased 6-thioguanine nucleotide and hematologic toxicity.

Presently, more than 20 mutant alleles have been reported, and it is known that TPMT*, *3A, and *3C account for approximately 90% of the total mutant alleles.² The Clinical Pharmacogenetics Implementation Consortium (CPIC) proposed a dosing recommendation according to TPMT genotypes. According to the recommendation, a homozygous wild-type starts 6-MP with a normal dose, the heterozygote starts as 30%–70% of the full dose (50 mg/m$^2$/d), and the homozygous variant starts with an extremely reduced dose.³,⁴ However, in most of Europe and Asia, the initial dose of...
6-MP is 50 mg/m^2/d. Under this condition, it is questionable whether a 6-MP dose should further be reduced.

A reduction in 6-MP dose is recommended for TPMT heterozygotes because an increased risk of hematologic toxicity was observed in comparison to those with wild-type TPMT in many American and European studies. However, TPMT enzyme activity differs between mutant alleles. The TPMT*3A (460G>A, 719A>G) allele, which is a major mutant allele in Caucasian, Mediterranean, South American, Middle Eastern, and Mexican origins, results in negligible activity, while the TPMT*3C (719A>G) allele, which is a major allele in Asian and African origins, causes moderate activity compared to the wild-type one. Furthermore, Asians have another important variant, NUDT15, which is strongly associated with tolerable 6-MP dose according to a state-of-the-art genome-wide association study. Thus, 6-MP maintenance therapy according to TPMT genotype, which reflects ethnic allele distribution, needs to be evaluated. Although a number of Asian studies have evaluated the relationship between TPMT genotypes and tolerable 6-MP dose, the results are still controversial.

For example, heterozygotes received less 6-MP dose in comparison to those with wild-type allele (32.1 vs 46.2 mg/m^2/d, P=0.05). One Indian study concluded that the identification of TPMT genotype might be important. On the other hand, the heterozygotes received a similar 6-MP dose (41.5 vs 42.92 mg/m^2/d) in another Indian study, and the study concluded that the identification of TPMT genotype might not be important.

Evidence for individual study was weak because the number of TPMT heterozygotes was ≤12, whereas a meta-analysis of these studies could increase the number of TPMT heterozygotes and strengthen the evidence.

The primary objective of this study was to decide whether to reduce an initial 6-MP dose in TPMT heterozygote when the maintenance regimen included a 6-MP dose of <75 mg/m^2/d. The secondary objective was to compare the tolerable 6-MP dose between TPMT heterozygotes and those with wild-type allele among different ethnic groups.

Materials and methods

Search strategy

The meta-analysis process was predetermined prior to the study and conducted according to the checklist in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. For this study, we searched PubMed, Embase, and Cochrane Library Databases for studies that were conducted on childhood ALL patients using 6-MP so as to identify the impact of TPMT polymorphism on tolerable 6-MP dose. The latest search was updated on January 20, 2016. The following keywords were employed: (leukemia) AND (TPMT OR thiopurine methyltransferase OR thiopurine s methyltransferase OR thiopurine s-methyltransferase) AND (6-MP OR 6-mercaptopurine OR mercaptopurine) AND (polymorphism OR variant OR variation OR mutant OR mutation OR genotype OR haplotype). There were no limitations in the search process. To reduce publication bias, unpublished trials were searched via conference abstracts and trial registries such as the World Health Organization’s International Clinical Trials Registry Platform Search Portal (www.who.int/trialsearch/) and ClinicalTrials.gov.

Study selection

Two authors of this study independently evaluated all papers for eligibility. The criteria for eligibility for this study should 1) involve childhood ALL patients treated with 6-MP as a part of a maintenance therapy, 2) have an initial 6-MP dose of <75 mg/m^2/d, 3) analyze TPMT genotypes in the patients, and 4) compare tolerable 6-MP dose information according to the status of TPMT genotypes.

Articles were excluded if the research 1) was not carried on human subjects, 2) just evaluated the effects of TPMT phenotypes, and 3) did not report tolerable doses (pharmacokinetic outcomes or clinical outcomes). Reviews, guidelines, letters, comments, and case reports were also excluded.

EndNote X7.2 (Thomson Reuters, New York, NY, USA; 2014) software was used in the citation process. Cohen’s κ coefficient was calculated to confirm that the consensus between the researchers is in the range of excellent agreement. Disagreements were resolved by discussion between two researchers, and a third party was consulted for unresolved disagreements.

Quality assessment and data extraction

A quality assessment was independently performed by two authors according to the Newcastle–Ottawa Scale. Reporting, external and internal validities, confounding bias, selection bias, and study power of each study were assessed. A study was regarded as “high quality” if it scored five or more A’s out of eight.

Some data were requested by the corresponding author if the included study had no obvious data (eg, only a graph without an exact number). Data were extracted by a standardized data extraction sheet that was prepared in advance. Data included authors, citations, countries or ethnic groups, initial 6-MP dose, 6-MP dose adjustment criteria, TPMT genotypes, number of patients according to genotype, and tolerable 6-MP dose information. Statistical measures such
as mean ± standard deviations (SDs), median with the range of tolerable 6-MP dose, and P-values were extracted.

Statistical analysis
The ethnic groups were categorized as TPMT*3A allele-dominant group or *3C allele-dominant group. Weekly 6-MP doses were converted into daily doses, and dose intensities were converted into exact doses by multiplying the intensity percentage by the 6-MP start dose.

All meta-analyses were carried out by using either the Mantel–Haenszel fixed-effects model or the DerSimonian–Laird random-effects model, which depended on the heterogeneity. The degree of heterogeneity was assessed by I^2 measures (an I^2>50% was considered to have substantial heterogeneity). Potential publication bias was detected by reviewing the funnel plot, and a sensitivity analysis was conducted.

An analysis was undertaken by using differences in mean values because tolerable 6-MP dose is continuous data. The mean and SD values from the included studies were used directly, or a substituted median for mean and range/4 for SD in the case that median and range values were presented.

The analysis was conducted using Comprehensive Meta-Analysis version 3 (CMA 3; Biostat Inc., Englewood, NJ, USA). All statistical tests were two-sided, and the results are presented as forest plots.

Results
Included studies
A total of 397 potentially relevant articles were obtained and reviewed from a computerized search. Among them, 253 articles were excluded on the basis of publication type. Another 137 articles were excluded as they did not fulfill the inclusion criteria (Figure 1). A total of seven studies, comprising 3,018 wild-type and 53 mutant allele pediatric ALL patients, were included (Table S1). All studies showed no risk of bias when assessing for quality using the Newcastle–Ottawa Scale. All of the completed trials satisfying our inclusion and exclusion criteria have been published, and there were no unpublished trials. Table 1 describes the basic information of the included studies.

Three studies from Europe (France), South America (Brazil), and the Middle East (Turkey) were categorized as the TPMT*3A allele-dominant group, and four studies from Asia (India: 2, South Korea: 1, and Taiwan: 1) were categorized as the TPMT*3C allele-dominant group. In a study, one patient with a homozygous mutant allele was included in the heterozygote group because it could not be separated. The *2/*2 mutant allele carrier included in the study by Kim et al was excluded from this meta-analysis.

Tolerable 6-MP dose of TPMT heterozygote in the case of an initial 6-MP dose <75 mg/m^2/d
Before conducting a pooled meta-analysis, it was observed that three of seven studies showed an insignificant difference in tolerable 6-MP dose between TPMT wild-type and heterozygote. The value of the observed F was 30.7%, and the heterogeneity of the included studies might not be important. Thus, the Mantel–Haenszel fixed-effects model was used to estimate the effect of TPMT genotype on the tolerable 6-MP dose. The meta-analysis results indicated that the tolerable 6-MP dose was significantly lower in the TPMT heterozygote
Table 1  Characteristic of included studies

| References       | Country | TPMT allele | Initial 6-MP dose | Dose adjustment |
|------------------|---------|-------------|-------------------|-----------------|
| Dervieux et al24  | France  | TPMT*3A dominant | 50 mg/m²/d       | Target: WBC 2,000–3,000/μL |
|                  |         |             |                   | Adjustment: increase by 25% if two consecutive WBC >4,000/μL, reduce by 25% if WBC <1,500/μL |
| Silva et al41     | Brazil  | TPMT*3A dominant | 50 mg/m²/d       | Target: WBC 2,000–3,000/μL, ANC >500/μL or WBC 1,500–3,000/μL, ANC >300/μL according to maintenance regimen |
| Kapoor et al15    | India   | TPMT*3C dominant | 50 mg/m²/d       | Target: WBC 2,500–3,500/μL, ANC 1,000–2,000/μL |
| Albayrak et al29  | Turkey  | TPMT*3A dominant | 50 mg/m²/d       | Target: WBC 2,000–3,000/μL, ANC <1,000/μL |
| Kim et al25       | Korea   | TPMT*3C dominant | 50 mg/m²/d       | Adjustment: reduce by 50% if WBC <1,000/μL |
| Linga et al16     | India   | TPMT*3C dominant | 56.25 mg/m²/d    | Not mentioned |
| Liang et al25     | Taiwan  | TPMT*3C dominant | 60 mg/m²/d       | Target: WBC 1,800–3,000/μL, ANC 500–1,200/μL, PLT $\geq$50,000/μL |

| References       | Country | TPMT allele | Initial 6-MP dose | Dose adjustment |
|------------------|---------|-------------|-------------------|-----------------|
| Liang et al25     | Taiwan  | TPMT*3C dominant | 60 mg/m²/d       | Adjustment: reduce by 25% if counts are low |

Abbreviations: ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; MP, mercaptopurine; PLT, platelet; TPMT, thiopurine S-methyltransferase; Uln, upper limit of normal; WBC, white blood cell.

The present meta-analysis showed a difference in tolerable 6-MP dose between different ethnic groups. The meta-analysis revealed that the 6-MP dose should further be reduced in TPMT heterozygote even with an initial 6-MP dose of <75 mg/m²/d. An initial 6-MP dose of 50–60 mg/m²/d still had severe hematologic toxicity and induced intolerance in pediatric ALL patients with the TPMT heterozygous allele. Some studies indicated that liver toxicity also partly influenced the dose reduction, although a meta-analysis of inflammatory bowel disease suggested that the TPMT polymorphisms were not associated with hepatotoxicity. The present meta-analysis supports the CPIC recommendation even in the case of an initial 6-MP dose of 50–60 mg/m²/d.

This meta-analysis showed a difference in tolerable 6-MP dose between TPMT heterozygotes and wild-type among different ethnic groups. The TPMT*3A-dominant ethnic group had a more reduced tolerable 6-MP dose in comparison to the TPMT*3C-dominant ethnic group when they had a heterozygous allele. In a functional study of 23 allelic variants of the TPMT gene, which were TPMT*2 to *24, the expression levels of TPMT*2, *3A, *5, *12, *14, and *22 were significantly lower than that of TPMT*1 (P<0.005), while TPMT*3B and *3C and other variants (except TPMT*8 and *18) showed a nonsignificant difference with TPMT*1. Thus, the TPMT

Discussion
This is the first meta-analysis that compares maximum tolerable 6-MP dose between pediatric ALL patients with TPMT wild-type and heterozygous allele when considering ethnicity and receiving an initial 6-MP dose of <75 mg/m²/d. Although the CPIC guidelines gave an example of an initial 6-MP dose of 75 mg/m²/d for wild-type patients, currently most countries use <75 mg/m²/d because of hematologic toxicity. We found that studies conducted in the USA, the UK, Northern Europe (Denmark, Finland, Iceland, Norway, and Sweden), and Lebanon (only for lower risk patients) used a 6-MP dose of 75 mg/m²/d. These particular studies were excluded from the meta-analysis.

Three studies from the TPMT*3A allele-dominant countries showed controversial results. The included studies had heterogeneity in their features with an I² value of 70.2%. The DerSimonian–Laird random-effects model revealed that the TPMT heterozygote received a lower 6-MP dose than the wild-type (difference in mean values =11.729, 95% confidence interval [CI] =7.617–15.842, P<0.001) (Figure 2A).

Among the four studies conducted in Asia, two showed a significant difference in 6-MP dose. The value of I² was 35.2%, and the Mantel–Haenszel fixed-effects model was used. The meta-analysis resulted in a significant difference in 6-MP dose between TPMT heterozygote and wild-type (difference in mean values =8.884, 95% CI =2.917–14.850, P=0.004) (Figure 2B).

This meta-analysis showed a difference in tolerable 6-MP dose between TPMT heterozygotes and wild-type among different ethnic groups. The TPMT*3A-dominant ethnic group had a more reduced tolerable 6-MP dose in comparison to the TPMT*3C-dominant ethnic group when they had a heterozygous allele. In a functional study of 23 allelic variants of the TPMT gene, which were TPMT*2 to *24, the expression levels of TPMT*2, *3A, *5, *12, *14, and *22 were significantly lower than that of TPMT*1 (P<0.005), while TPMT*3B and *3C and other variants (except TPMT*8 and *18) showed a nonsignificant difference with TPMT*1. Thus, the TPMT
enzyme encoded by TPMT*3A had no activity, whereas the enzyme encoded by TPMT*3C had similar $V_{\text{max}}$ in comparison with the wild-type enzyme, which means decreased enzymatic activity (112±9 vs 111±6 nmol/mg protein/min and 156±12 vs 80±8 µmol/L, respectively).\(^{31}\) Alloenzyme activities of TPMT*3A and *3C were 1.6±0.6% and 17%±1.3% of the wild-type, respectively.\(^{32}\)

In spite of our efforts to perform a complete and thorough analysis, there are several limitations to this meta-analysis. First, differences in the characteristics of the studies might lead to heterogeneity in the results. Especially, disparate countries in the TPMT*3A allele-dominant groups (France, Brazil, and Turkey) might increase the $F$ (degree of heterogeneity) over 70%. The DerSimonian–Laird random-effects model was applied to the analysis, which resulted in a decrease in the $F$ value. However, a sensitivity analysis could not be carried out because of the small number of studies. Second, treatment-related advantages, such as continuous response and event-free survival of the TPMT genotype-based dosing in pediatric ALL patients, could not be evaluated because of the limited number of studies. Only one study showed that a reduced 6-MP initial dose for TPMT heterozygote might reduce secondary malignancy risk, but increase relapse risk, which is comparable to TPMT wild-type.\(^{33}\) Further studies are warranted to build a consensus on treatment-related advantages of TPMT genotype-based dosing. Third, a direct comparison between TPMT*1/*3A and *1/*3C was not possible because no studies have analyzed these particular TPMT combinations. Despite the limitations, our study can offer inspiration for a subset analysis according to the ethnicity or haplotypes.

**Conclusion**

The initial 6-MP dose needs to be reduced in TPMT heterozygote in comparison to wild-type, and ethnic differences might influence the tolerable 6-MP dose of TPMT heterozygote.
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Disclosure
The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 Demographics of studied population

| References          | Sex (male/female) | Age in years | Haplotype distribution (n) |
|---------------------|-------------------|--------------|----------------------------|
| Dervieux et al, 2001| 47/31             | 5.5±3.1 (mean ± SD) | *1/*1 (70), *1/*2 (2), *1/*3A (4), *1/*3C (1), *3C/*3C (1) |
| Silva et al, 2008   | 58/58             | <1 yr: n=1, 1–9.9 yr: n=95, ≥10 yr: n=20 | *1/*1 (104), *1/*2 (1), *1/*3A (9), *1/*3C (2) |
| Kapoor et al, 2010  | 49/15             | ≤9 yr: n=43, >9 yr: n=21 | *1/*1 (64) |
| Wild-types (n=64)   | 6/1               | ≤9 yr: n=7, >9 yr: n=0 | *1/*2 (3), *1/*3A (1), *1/*3C (3) |
| Albayrak et al, 2011| 31/16             | 7.2±4.7 (mean ± SD) | *1/*1 (47) |
| Wild-types (n=47)   | 2/2               | 8.2±4.6 (mean ± SD) | *1/*3A (3), *1/*3C (1) |
| Variants (n=7)      |                   |              |                           |
| Kapoor et al, 2012  | 57/43             | 5.2 (1.4–16) (median [range]) | *1/*1 (93), *2/*2 (1), *1/*3A (1), *1/*3C (5) |
| Linga et al, 2014   | 57/15             | 9 (2–18) (median [range]) | *1/*1 (69), *1/*2 (1), *1/*3A (1), *1/*3C (1) |
| Liang et al, 2015   | NA                | NA           | *1/*1 (298), 1/*3A or *3C (12) |

Abbreviations: NA, not available; SD, standard deviation; yr, years.

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