Pediatric RESEARCH

www.nature.com/pr

CLINICAL RESEARCH ARTICLE

Childhood acute lymphoblastic leukemia mercaptopurine intolerance is associated with *NUDT15* variants

Der-Shiun Wang[1,2], Chih-Hsiang Yu[3], Chien-Yu Lin[4], Ya-Hsuan Chang[4], Kai-Hsin Lin[5,6], Dong-Tsamn Lin[5,6,7], Shiann-Tarng Jou[5,6], Meng-Yao Lu[5,6], Hsiu-Hao Chang[5,6], Shu-Wha Lin[3], Hsuan-Yu Chen[4] and Yung-Li Yang[5,6,7]

BACKGROUND: Mercaptopurine-induced neutropenia can interrupt chemotherapy and expose patients to infection during childhood acute lymphoblastic leukemia (ALL) treatment. Previously, six candidate gene variants associated with mercaptopurine intolerance were reported. Herein, we investigated the association between the mean tolerable dose of mercaptopurine and these genetic variants in Taiwanese patients.

METHODS: In total, 294 children with ALL were treated at the National Taiwan University Hospital from April 1997 to December 2017. Germline variants were analyzed for *NUDT15*, *SUCLA2*, *TPMT*, *ITPA*, *PACSIN2*, and *MRP4*. Mean daily tolerable doses of mercaptopurine in the continuation phase of treatment were correlated with these genetic variants.

RESULTS: Mercaptopurine intolerance was significantly associated with polymorphisms in *NUDT15* (P value < 0.0001). Patients with *SUCLA2* variants received lower mercaptopurine doses (P value = 0.0119). The mean mercaptopurine doses did not differ among patients with *TPMT*, *ITPA*, *MRP4*, and *PACSIN2* polymorphisms (P value = 0.9461, 0.5818, and 0.7951, respectively). After multivariable linear regression analysis, only *NUDT15* variants retained their clinically significant correlation with mercaptopurine intolerance (P value < 0.0001).

CONCLUSION: In this cohort, the major genetic determinant of mercaptopurine intolerance was *NUDT15* in Taiwanese patients.

Pediatric Research (2021) 89:217–222; https://doi.org/10.1038/s41390-020-0868-8

IMPACT:

- *NUDT15* causes mercaptopurine intolerance in children with ALL.
- The *NUDT15* variant is a stronger predictor of mercaptopurine intolerance than *TPMT* in a Taiwanese cohort. This finding is similar with studies performed on Asian populations rather than Caucasians.
- Pre-emptive genotyping of the patients' *NUDT15* before administering mercaptopurine may be more helpful than genotyping *TPMT* in Asians.

INTRODUCTION

Thiopurines (e.g., 6-mercaptopurine (6MP), 6-thioguanine, and azathioprine) are important antimetabolites with different clinical indications. In childhood acute lymphoblastic leukemia (ALL), 6MP-based maintenance therapy is one of the most vital treatments, serving as the backbone of current clinical trials of pediatric ALL. Genetic variations in the genes responsible for thiopurine metabolism can directly influence drug toxicity and anti-leukemic efficacy. For example, genetic polymorphisms in thiopurine methyltransferase (*TPMT*) have been linked to the susceptibility of thiopurine-induced hematopoietic toxicity in patients, and pre-emptive *TPMT* genotype-guided dosing is a major example of genetics-based precision medicine in cancer treatment.[1] *TPMT* variants are rare in Asian populations, with most studies indicating that there are no associations between *TPMT* variants and the tolerable dose of 6MP.[2] Genome-wide association studies focusing on Asian populations have identified several new single-nucleotide polymorphisms (SNPs) associated with leukopenia after 6MP administration, including SNPs in inosine triphosphatase (*ITPA*)[3] and multidrug resistance protein 4 (an ATP-binding cassette subfamily C member 4) (*MRP4*).[4,5] However, these genes have not been subjected to thorough validations and have displayed controversial results.[6,7]

A genome-wide association study described a missense variant in the *NUDT15* gene (rs116855232, referred to as c.415C > T or p.Arg139Cys) that is strongly associated with thiopurine-related myelosuppression in patients with inflammatory bowel disease in the Korean population.[8] Jun Yang and co-workers[2] also identified this variant in association with 6MP intolerance in childhood ALL in a genome-wide association study. Moriyama et al.[9] identified

[1]Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan; [2]Department of Pediatrics, Tri-service General Hospital, National Defense Medical Center, Taipei, Taiwan; [3]Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei, Taiwan; [4]Institute of Statistical Science, Academia Sinica, Taipei, Taiwan; [5]Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan; [6]Department of Pediatrics, College of Medicine, National Taiwan University, Taipei, Taiwan and [7]Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan
Correspondence: Yung-Li Yang (yangyl92@ntu.edu.tw)
These authors contributed equally: Der-Shiun Wang, Chih-Hsiang Yu

Received: 21 October 2019 Revised: 2 March 2020 Accepted: 4 March 2020
Published online: 27 March 2020

four coding variants, c.415C > T, c.416G > A, c.52G > A, and c.36_37insGGAGTC that caused changes in the amino acid sequence of the *NUDT15* protein. Additionally, three novel genetic variants of *NUDT15* (p.R34T, p.K35E, and p.G17_V18del) have been identified and observed to account for 6MP intolerance.[10] Subsequent studies have also validated its clinical significance in childhood ALL. *NUDT15* encodes a nucleotide diphosphatase that inactivates thioguanosine triphosphate (TGTP) via conversion to thioguanosine monophosphate. Thus, *NUDT15* functions as a negative regulator of intracellular TGTP, with dysfunctional *NUDT15* variants leading to the accumulation of DNA-TG resulting in increased cytotoxicity.[11] The *NUDT15* variants have been subjected to intensive validation and confirmed to be a major genetic determinant of 6MP intolerance in the Asian population.[9]

In this study, we genotyped the SNPs associated with 6MP intolerance in a Taiwanese cohort treated with the TPOG-ALL (Taiwan Pediatric Oncology Group Acute lymphoblastic leukemia) protocol. The association of these genetic variants with the tolerable dose of 6MP was correlated. With this retrospective study, we hope to identify the most important genetic determination of 6MP intolerance in the Taiwanese cohort. This result can guide the pre-emptive genetic diagnosis before 6MP administration to avoid unwanted neutropenia in future ALL clinical trials.

METHODS

Participants and protocols

Participants younger than 18 years of age and diagnosed with ALL were selected at the National Taiwan University Hospital, Taipei, Taiwan. In total, 283 participants were enrolled in this study and treated with the TPOG-ALL protocol between April 1997 and December 2017. This study was approved (No: 201510016RIND) by the Institutional Review Board of the National Taiwan University Hospital. Informed consent was obtained from the parents or legal guardians of the patients, and, in total, 294 patients were enrolled at the study's initiation. Participants were divided into three groups: very high risk (VHR), high risk (HR), and standard risk (SR), according to the TPOG-ALL-93,[12,13] TPOG-ALL-2002,[13–15] and TPOG-ALL-2013 protocols. Risk classification using the TPOG-ALL protocols was based on the age of onset, initial white blood cell (WBC) count, and cytogenetic alterations at diagnosis. Participants were prospectively assigned to each group (SR, HR, or VHR) based on clinical features and the biological features of their leukemic cells. However, the risk classification of the TPOG-ALL-2013 protocol was additionally determined by the level of minimal residual disease at the end of induction therapy, in addition to the above-mentioned clinical parameters. Maintenance therapy with methotrexate (MTX) was administered at 40 mg/m^2 per week and 6MP was initially administered at a dosage of 50–75, 60, 50, or 40 mg/m^2 per night depending on the protocol. Dosages were titrated to maintain a WBC count between 1800 and 3000/mm^3, absolute neutrophil count (ANC) between 500 and 1200/mm^3, and platelet count ≥50,000/mm^3. If the counts were low, 6MP was the first to be reduced in dosage by a 25% decrement. If WBC or ANC failed to double at 1 week following dexamethasone pulse therapy, the 6MP and MTX dosages were reduced by 50% referring to reduction from the initial dose given. If WBC or ANC remained the same or decreased, 6MP and MTX doses were halted because the participant would be at a high risk of infection. Blood counts after 3–4 days were used to determine whether the 6MP therapy could be resumed.[15]

The genetic types of *NUDT15*, *PACSIN2*, *SUCLA2*, *ITPA*, *MRP4*, and *TPMT*

Germline DNA was extracted from remission peripheral blood or bone marrow using the phenol–chloroform method, and DNA concentrations were measured using a NanoDrop 1000 instrument (Thermo Fisher Scientific, Waltham, MA, USA). Three coding sequences of *NUDT15* were amplified by polymerase chain reaction (PCR) and evaluated using Sanger sequencing. SNPs in *PACSIN2*, *SUCLA2*, *ITPA*, *MRP4*, and *TPMT* were evaluated via TaqMan quantitative PCR (qPCR) assays. Supplementary Table S1 contains the six genes and their polymorphisms. Primer sequences and SNPs are listed in Supplementary Table S2.

For PCR, 5 ng genomic DNA was mixed with 0.5 μM of each primer and 1× Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific). PCR was performed via heating to 98 °C for 60 s, followed by 38 cycles at 98 °C for 10 s, maintenance at the annealing temperature for 30 s, and then 72 °C for 20 s; the final extension was performed at 72 °C for 5 min. PCR products were separated using gel electrophoresis, and bands were excised and extracted using a FavorPrep GEL purification kit (FavorGen, Ping-Tung, Taiwan).

Wild-type genotypes were considered to be two alleles that were the same as the reference genotype. Mono-allelic variants had an allele with a polymorphism and an allele with the wild-type genotype. Bi-allelic variants had two alleles that differed from the reference genotype. The *NUDT15* genotype was based on a study conducted by Moriyama et al.[9,10] and Yang et al.[16] Patients with *NUDT15* *1/*2 and *3/*6 showed the same sequence result. To determine the diplotype of these patients, we sequenced their parents' *NUDT15* genotype (data not shown).

6MP dosage collection

The mean doses of 6MP for children with ALL treated with the TPOG-ALL protocols in the continuation phase were collected by reviewing their medical records. The 6MP dosage was obtained from the continuation phase to the end of the therapy, completion of trial, or date of relapse or death.

Statistical analysis

Genotype and allelic frequencies for all polymorphisms were estimated by direct counting. The difference in mean daily 6MP dose from the diplotype of each gene was determined by one-way analysis of variance and subsequent *P* values. P values < 0.05 after Tukey's method for multiple testing were considered statistically significant. Exploratory analyses to assess *NUDT15* and *TPMT* were based on tests for trends. Cohen κ coefficient was employed to ascertain inter-gene agreement between *NUDT15* and *SUCLA2*. Cohen κ coefficient (κ, ranging from 0 to 1) was interpreted as almost perfect ($\kappa > 0.80$), substantial ($0.61 \le \kappa \le 0.80$), moderate ($0.41 \le \kappa \le 0.60$), fair ($0.21 \le \kappa \le 0.40$), slight ($0.00 \le \kappa \le 0.20$), and poor ($\kappa < 0.00$). A multivariable linear regression model was used to assess the mean daily 6MP dose of the different diplotypes among *NUDT15*, *TPMT*, *SUCLA2*, *ITPA*, *MRP4*, and *PACSIN2*. All analyses were performed using the SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Genotypes of *NUDT15*, *TPMT*, *SUCLA2*, *ITPA*, *MRP4*, and *PACSIN2*

In the present study, nine SNPs in five genes and all coding regions of *NUDT15* were analyzed in 294 children diagnosed with ALL. Three patients (1.1%) had bi-allelic variants in *NUDT15*, 67 (24%) had mono-allelic variants, and 213 patients (75%) had wild-type *NUDT15* (Table 1). No patient had bi-allelic variants in *TPMT*; 11 (4.1%) had mono-allelic variants; and 256 patients (96%) had wild-type *TPMT*. The minor allele frequency of *TPMT* was 0.02% in this cohort. *ITPA* (94C > A) polymorphisms were observed in 101 (34%) cases, 91 of which were mono-allelic and 10 of which displayed homozygosity. Fifteen patients (13%) had *PACSIN2* polymorphisms, all of which were heterozygous. Ninety-eight patients had *SUCLA2* polymorphisms, 88 of which were heterozygous, with 10 displaying homozygosity. *MRP4* polymorphisms were detected in 234 (34%) cases, 134 of which were mono-allelic,

Table 1. Diplotype and minor allele frequency of *NUDT15*, *SUCLA2*, *TPMT*, *ITPA*, *MRP4*, and *PACSIN2* in this cohort.

	NUDT15	*TPMT*	*SUCLA2*	*ITPA*	*MRP4*	*PACSIN2*
Wild type, *N* (%)	213 (75%)	256 (96%)	172 (64%)	170 (63%)	99 (37%)	235 (87%)
Mono-allelic variant, *N* (%)	67 (24%)	11 (4.1%)	88 (33%)	91 (34%)	134 (50%)	35 (13%)
Bi-allelic variant, *N* (%)	3 (1.1%)	0 0	10 (3.7%)	10 (3.7%)	36 (13%)	0 0
Minor allele frequency (%)	13	2.1	19	19	38	6.5

N number, *TPMT* thiopurine *S*-methyltransferase, *MRP4* multidrug resistance protein 4, *ITPA* inosine triphosphatase, *NUDT15* nudix hydrolase 15, *SUCLA2* succinate-CoA ligase ADP-forming β-subunit, *PACSIN2* protein kinase C and casein kinase substrate in neurons 2.

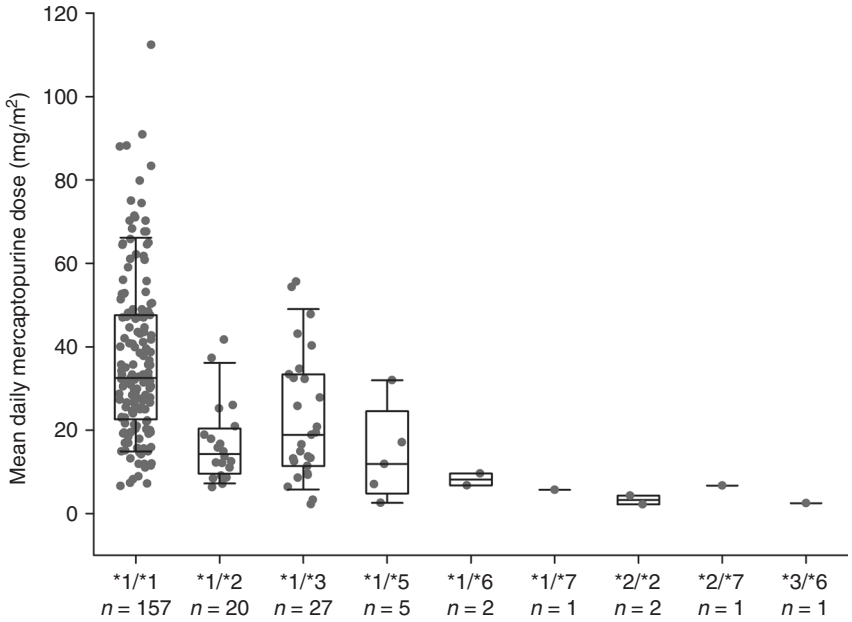

Fig. 1 Box plot for the mean daily mercaptopurine dose among affected individuals with standard and high risk defined by the *NUDT15* diplotype. Data points are represented by a dot. The lower and upper boundaries of the box correspond to the first and third quartiles, respectively. The line within the box represents the median. The upper whisker extends from the upper boundary of the box to the largest value, no further than 1.5× the interquartile range (IQR). The lower whisker extends from the lower boundary of the box to the lowest value, at most no further than 1.5× the IQR. The mean daily mercaptopurine dose was collected by reviewing the medical records. Median values and IQRs are provided to facilitate interpretation of mean daily mercaptopurine dose. Compared to the leftmost group, Kruskal–Wallis *P* values for the differences in mean daily mercaptopurine dose were <0.0001. Star alleles were assigned by the PharmGKB haplotype set translational table (https://www.pharmgkb.org/).

with 36 displaying homozygosity. The diplotype and minor allele frequency of these genes are listed in Table 1.

Mean dose of 6MP according to the genotypes of *NUDT15* and *TPMT*

In the analysis of the mean tolerable dose of 6MP, 70 patients with very high-risk ALL were excluded as 6MP and MTX constituted only one of the four pairs of drugs administered in rotation every 4 weeks and 6MP was scheduled to be administered for only 7 days every 4 weeks.

The mean daily doses of 6MP in the *NUDT15* wild-type (*1/*1), mono-allelic variant (*1/*2, *1/*3, *1/*5, *1/*6, *1/*7), and bi-allelic variant groups (*2/*2, *2/*7, *3/*6) were 36.8 ± 19.9 (mean ± s.d.), 19.0 ± 13.3, and 4.4 ± 2.3 mg/m^2, respectively ($P < 0.0001$). Patients with the bi-allelic variant *NUDT15* tolerated a lower 6MP dose than those with mono-allelic variants and wild-type *NUDT15* (Fig. 1).

The mean doses of 6MP in the *TPMT* (rs1142345, rs1800460, and rs1800462) wild-type and mono-allelic variant groups were 31.7 ± 20.3 and 17.7 ± 1.3 mg/m^2, respectively ($P = 0.4754$). No patient in this cohort had double bi-allelic variants of *NUDT15* and *TPMT*. There were two groups—wild-type *TPMT* with mono-allelic *NUDT15* group and mono-allelic *TPMT* with mono-allelic *NUDT15* group. The corresponding daily dose in the two groups were 19.6 ± 13.7 and 13.0 ± 0.9 mg/m^2 per day, respectively. Supplementary Figure S1 contains the 6MP dose and the variant alleles of *NUDT15* and *TPMT*. *NUDT15* variants remained as an independent genetic determinant of 6MP intolerance in this cohort, irrespective of the *TPMT* variants. A trend was also found, where the intermediate metabolizers of these compounds were found to tolerate a lower dose of 6MP than the *NUDT15* or *TPMT* variants alone (Supplementary Fig. S1).

Mean dose of 6MP according to genotypes of *SUCLA2*, *ITPA*, *MRP4*, and *PACSIN2* individually

Patients with *SUCLA2* mono-allelic and bi-allelic variants tolerated less 6MP than those with wild-type *SUCLA2* (Table 2, $P = 0.0119$). However, the mean 6MP dose did not differ among patients with *ITPA*, *MRP4*, and *PACSIN2* polymorphisms ($P = 0.9416$, 0.5818, 0.7951, respectively, Table 2).

Inter-gene agreement and multivariable linear regression model

We examined the correlation coefficient among these patients and the association between *NUDT15* and *SUCLA2* by the Cohen κ method. The level of *NUDT15* and *SUCLA2* agreement was

Table 2. Mean ± standard deviation of different diplotypes for the daily 6MP dose of the *NUDT15*, *SUCLA2*, *TPMT*, *ITPA*, *MRP4*, and *PACSIN2* variants in the standard and high-risk groups.

Gene	Wild type	Mono-allelic variant	Bi-allelic variant	*P* value
NUDT15	36.8 ± 19.9 *n* = 158	19.0 ± 13.3 *n* = 55	4.4 ± 2.3 *n* = 3	<0.0001**
TPMT	31.7 ± 20.3 *n* = 193	17.7 ± 1.3 *n* = 9	*n* = 0	0.4754
SUCLA2	34.0 ± 20.7 *n* = 132	27.8 ± 18.0 *n* = 64	16.3 ± 15.7 *n* = 8	0.0119*
ITPA	31.5 ± 20.1 *n* = 127	31.1 ± 19.6 *n* = 70	33.6 ± 17.9 *n* = 8	0.9461
MRP4	31.4 ± 19.6 *n* = 73	32.4 ± 20.1 *n* = 97	28.2 ± 21.5 *n* = 33	0.5818
PACSIN2	31.1 ± 18.8 *n* = 172	32.1 ± 25.3 *n* = 32	*n* = 0	0.7951

n number, *6MP* mercaptopurine.
**P* value < 0.05.
***P* value < 0.001.

Table 3. Multivariable linear regression of *NUDT15*, *TPMT*, *SUCLA2*, *PACSIN2*, *ITPA*, and *MRP4* to mercaptopurine dosage.

Gene	Coefficient	Standard error	*P* value
NUDT15	−18.43	3.31	<0.0001
TPMT	−4.11	6.32	0.5161
SUCLA2	1.79	2.87	0.5325
ITPA	0.11	2.31	0.9623
MRP4	−2.59	1.89	0.1724
PACSIN2	3.67	3.71	0.3234

moderate ($K = 0.5001$, 95% confident interval was 0.3971–0.6031, $P < 0.0001$, Supplementary Table S3). By examining *NUDT15*, *TPMT*, *SUCLA2*, *ITPA*, *MRP4*, and *PACSIN2* using a multivariable linear regression model, we found that only *NUDT15* exhibited significance relative to the mean daily 6MP dose (Table 3). *SUCLA2* completely lost its significance as revealed by the multivariable linear regression model after adjusting for *NUDT15*.

DISCUSSION

This retrospective study in Taiwan revealed that *NUDT15* variants are the most significant genetic determinants of 6MP intolerance. Patient with *TPMT* variants and the mono-allelic variant *NUDT15* tolerated less 6MP than patients with the mono-allelic variant of *NUDT15*, but without the *TPMT* variant. The study also reveals an additional effect of *TPMT* variants on the mono-allelic *NUDT15* variants. Although patients with *SUCLA2* variants tolerated less 6MP ($P = 0.0119$) than those with wild-type *SUCLA2*, this was identified as a genetic linkage effect by statistical analysis. Other reported candidate gene polymorphisms, including *ITPA*, *MRP4*, *SUCLA2*, and *PACSIN2*, had no effect on 6MP tolerance in this cohort.

Aligning with previous reports, especially among the Asian population, *NUDT15* variants are the most significant in the genetic determination of 6MP intolerance. In fact, *TPMT* variants were rare, with only 2.1% minor allele frequency; patients with the mono-allelic variant of *NUDT15* were found to be less tolerant to 6MP than those with wild-type *NUDT15*. Since their identification, *NUDT15* variants have proven to be a common factor associated with 6MP intolerance in a variety of diseases, especially in Asia. The frequency of *NUDT15* mutants was 12% in this study. Liang et al.[17] reported the association between 6MP intolerance and *NUDT15* exon 3; however, these authors did not sequence *NUDT15* in its entirety. In theory, they identified patients with (*1/*2) and (*1/*3), but missed an estimated 3% of patients with *NUDT15* variants. We performed Sanger sequencing for all three exons of *NUDT15*, the products of which were comprehensive genetic variants of *NUDT15* in the Taiwanese cohort. The allele frequency of *NUDT15* c.415C > T was 16% and 13% in Thailand,[18,19] 0.37% in Lebanon,[20] 11% and 26% in Japan,[21,22] 16% in China,[7] 7.2% in Korea,[23] 8.3% in Guatemala, and 12% in Singapore.[9] Our report of allele frequency is similar to other regional findings.[7,9,18,19,21] The importance of *NUDT15* variants on 6MP intolerance has received increasing attention in the Caucasian population as validated by several studies.[24] These data provide a compelling rationale for additional pre-emptive testing of *NUDT15* genetics, not only in Asians but also in Europeans.[25]

In contrast to *NUDT15*, the overall allele frequency of *TPMT* polymorphism was low in our Taiwanese population, only 2.1% in this cohort, whereas previously, Liang et al.[17] demonstrated an allelic frequency of 0.6% in 249 Taiwanese individuals. *TPMT* demonstrates a minor contribution to the poor 6MP tolerance according to most reports in Asian populations, including one report from Taiwan.[17] Only nine patients with mono-allelic mutations of *TPMT* and none with bi-allelic variants were identified in this cohort. However, a trend was observed, the compound intermediate metabolizers also tolerated a lower dose of 6MP than *NUDT15* or *TPMT* variants alone. A recent study by Zhu et al.[26] reported that a case with a combination of bi-allelic *NUDT15* and mono-allelic *TPMT* genotype suffered more severe leukopenia than patient with the *NUDT15* or *TPMT* variants alone. Another study in Asia failed to accurately discern the correlation between *TPMT* variants and 6MP tolerance.[27] Three patients had both *NUDT15* and *TPMT* mono-allelic variant, and these patients showed a low 6MP tolerability, similar to that in patients with the *NUDT15* mono-allelic variant. The effect of inheriting both *NUDT15* and *TPMT* variants has been reported in ALL patients.[2,7]

The relationship between *SUCLA2* polymorphisms and 6MP intolerance was first reported in Crohn's disease by Park et al.[28] However, a previous study showed that its significance was completely nullified by relying on the linkage effect with *NUDT15* (p.Arg139Cys), this is because both genes are located on the same chromosome. The *SUCLA2* variants were associated with low mean tolerable doses in the initial analysis ($P = 0.0119$). However, after correlation coefficient and multivariable linear regression analysis, the association between *SUCLA2* and the mean tolerable dose of 6MP was correlated with the status of *NUDT15* and not that of *SUCLA2* (Table 3).

Several studies have shown that *ITPA* genotypes significantly influence the metabolism of 6MP;[29–33] however, studies from China[7,34] have not confirmed this significance. A Japanese study revealed that polymorphisms in *MRP4* affect the dose of 6MP in childhood ALL.[4,35] Stocco et al.[36] identified that *PACSIN2* polymorphisms affected *TPMT* activity and 6MP-induced adverse effects in children with ALL. Smid et al.[37] also identified that the *PACSIN2* polymorphism was a significant risk factor for 6MP-induced toxicity in wild-type *TPMT* patients in Slovenia. These genetic polymorphisms in *TPMT*, *ITPA*, *MRP4*, and *PACSIN2* did not affect the 6MP dosage in our cohort. This non-consistent data might suggest that these genetic variants may not be the major genetic determinants of 6MP intolerance in the Taiwanese populations.

The *NUDT15* and *TPMT* variants are of absolute importance to the genetic determination of 6MP intolerance in childhood ALL; thus, pre-emptive genetic diagnosis before the administration of 6MP is suggested. In contrast to the higher *TPMT* allele frequency in Caucasian populations, *NUDT15* has a higher allele frequency in Asian populations. However, we have come across increasing reports of

NUDT15 with variants from Europe. In Taiwan, the pre-emptive diagnosis of *NUDT15* is likely due to its high prevalence rate. *TPMT*, although low in frequency, leads to the development of 6MP intolerance when combined with *NUDT15*, similar to that observed in patients with bi-allelic *NUDT15* variants. There have been reports of patients with an intermediate metabolizer status for both *TPMT* and *NUDT15* (i.e., compound intermediate metabolizers), and there is a trend revealing lower thiopurine tolerance in these individuals than in those with intermediate metabolizer status, with *TPMT* or *NUDT15* alone.[1] Although there were only three compound intermediate metabolizers for the *NUDT15* and *TPMT* variants in this cohort, the trend of lower 6MP tolerance in these patients seemed significant (Supplementary Fig. S1, trend $P < 0.0001$). Thus, to address its power due to the low frequency of *TPMT* variants in Asian patients with childhood ALL, this trend may require a larger clinical trial where more patients with *TPMT* variants are enrolled.

In conclusion, the most important genetic determinant of 6MP intolerance in this cohort was the *NUDT15* variant. This finding is compatible with reports from other East-Asian countries. Although rare in Asia, *TPMT* polymorphisms might further reduce the mean tolerable dose for patients with *NUDT15* variants. However, the impact of *TPMT* variants on 6MP intolerance may be overlooked in Taiwan and other Asian countries due to its low frequency. The clinical issue of the compound intermediate metabolizer status for both *NUDT15* and *TPMT* variants might be worthy of further investigation.

ACKNOWLEDGEMENTS

We thank all the patients who participated in this study and their parents. We also acknowledge TPOG and the Childhood Cancer Foundation in Taiwan for their assistance in data collection and management. This work was supported by the Ministry of Science and Technology (No. 106-2314-B-002-199-) and the National Taiwan University Hospital (107-S3890). We gratefully acknowledge the financial support of the National Science Council of Taiwan, ROC.

AUTHOR CONTRIBUTIONS

D.-S.W.: writing—original draft preparation, visualization, investigation, and data curation. C.-H.Y.: writing—original draft preparation, investigation, and data curation. Y.-H.C.: formal analysis, investigation, and data curation. C.-Y.L.: formal analysis. K.-H.L.: resources. S.-T.J.: resources. M.-Y.L.: resources. H.-H.C.: resources. S.-W.L.: supervision, writing—review and editing. D.-T.L.: Resources. H.-Y.C.: methodology, formal analysis. Y.-L.Y.: project administration, supervision, writing—review and editing, resources, conceptualization.

ADDITIONAL INFORMATION

The online version of this article (https://doi.org/10.1038/s41390-020-0868-8) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

Informed consent: Informed consent was obtained from the parents or legal guardians of the patients, and, in total, 294 patients were enrolled at the study's initiation.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Relling, M. V. et al. Clinical pharmacogenetics implementation consortium guideline for thiopurine dosing based on *TPMT* and *NUDT15* genotypes: 2018 update. *Clin. Pharmacol. Ther.* **105**, 1095–1105 (2019).
2. Sutiman, N. et al. Predictive role of *NUDT15* variants on thiopurine-induced myelotoxicity in Asian inflammatory bowel disease patients. *Pharmacogenom. J.* **19**, 31–43 (2018).
3. Gerbek, T. et al. Role of *TPMT* and *ITPA* variants in mercaptopurine disposition. *Cancer Chemother. Pharmacol.* **81**, 579–586 (2018).
4. Tanaka, Y. et al. Interaction between *NUDT15* and *ABCC4* variants enhances intolerability of 6-mercaptopurine in Japanese patients with childhood acute lymphoblastic leukemia. *Pharmacogenom. J.* **18**, 275–280 (2018).
5. Liu, C. et al. Differential effects of thiopurine methyltransferase (*TPMT*) and multidrug resistance-associated protein gene 4 (*MRP4*) on mercaptopurine toxicity. *Cancer Chemother. Pharmacol.* **80**, 287–293 (2017).
6. Choi, R. et al. Pathway genes and metabolites in thiopurine therapy in Korean children with acute lymphoblastic leukaemia. *Br. J. Clin. Pharmacol.* **85**, 1585–1597 (2019).
7. Zhou, H. et al. Optimal predictor for 6-mercaptopurine intolerance in Chinese children with acute lymphoblastic leukemia: *NUDT15*, *TPMT*, or *ITPA* genetic variants? *BMC Cancer* **18**, 516 (2018).
8. Yang, S. K. et al. common missense variant in *NUDT15* confers susceptibility to thiopurine-induced leukopenia. *Nat. Genet.* **46**, 1017–1020 (2014).
9. Moriyama, T. et al. *NUDT15* polymorphisms alter thiopurine metabolism and hematopoietic toxicity. *Nat. Genet.* **48**, 367–373 (2016).
10. Moriyama, T. et al. Novel variants in NUDT15 and thiopurine intolerance in children with acute lymphoblastic leukemia from diverse ancestry. *Blood* **130**, 1209–1212 (2017).
11. Lee, S. H. R. & Yang, J. J. Pharmacogenomics in acute lymphoblastic leukemia. *Best. Pract. Res. Clin. Haematol.* **30**, 229–236 (2017).
12. Liang, D. C. Unexpected mortality from the use of *E. coli* L-asparaginase during remission induction therapy for childhood acute lymphoblastic leukemia: a report from the Taiwan Pediatric Oncology Group. *Leukemia* **13**, 155–160 (1999).
13. Lin, W. Y., Liu, H. C., Yeh, T. C., Wang, L. Y. & Liang, D. C. Triple intrathecal therapy without cranial irradiation for central nervous system preventive therapy in childhood acute lymphoblastic leukemia. *Pediatr. Blood Cancer* **50**, 523–527 (2008).
14. Li, M. J. et al. Treatment for childhood acute lymphoblastic leukemia in Taiwan: Taiwan Pediatric Oncology Group ALL-2002 study emphasizing optimal reinduction therapy and central nervous system preventive therapy without cranial radiation. *Pediatr. Blood Cancer* **64**, 234–241 (2017).
15. Liang, D. C. et al. Long-term results of Taiwan Pediatric Oncology Group studies 1997 and 2002 for childhood acute lymphoblastic leukemia. *Leukemia* **24**, 397–405 (2010).
16. Yang, J. J. et al. Pharmacogene Variation Consortium Gene Introduction: *NUDT15*. *Clin. Pharmacol. Ther.* **105**, 1091–1094 (2019).
17. Liang, D. C. et al. *NUDT15* gene polymorphism related to mercaptopurine intolerance in Taiwan Chinese children with acute lymphoblastic leukemia. *Pharmacogenom. J.* **16**, 536–539 (2016).
18. Chiengthong, K. et al. *NUDT15* c.415C>T increases risk of 6-mercaptopurine induced myelosuppression during maintenance therapy in children with acute lymphoblastic leukemia. *Haematologica* **101**, e24–e26 (2016).
19. Buaboonnam. J. et al. Effect of *NUDT15* on incidence of neutropenia in children with acute lymphoblastic leukemia. *Pediatr. Int.* **61**, 754–758 (2019).
20. Zgheib, N. K. et al. *NUDT15* and *TPMT* genetic polymorphisms are related to 6-mercaptopurine intolerance in children treated for acute lymphoblastic leukemia at the Children's Cancer Center of Lebanon. *Pediatr. Blood Cancer* **64**, 146–150 (2017).
21. Tanaka. Y. et al. Susceptibility to 6-MP toxicity conferred by a *NUDT15* variant in Japanese children with acute lymphoblastic leukaemia. *Br. J. Haematol.* **171**, 109–115 (2015).
22. Suzuki, H. et al. Genotyping *NUDT15* can predict the dose reduction of 6-MP for children with acute lymphoblastic leukemia especially at a preschool age. *J. Hum. Genet.* **61**, 797–801 (2016).
23. Park, Y. et al. Star allele-based haplotyping versus gene-wise variant burden scoring for predicting 6-mercaptopurine intolerance in pediatric acute lymphoblastic leukemia patients. *Front. Pharmacol.* **10**, 654 (2019).
24. Yang, J. J. et al. Inherited *NUDT15* variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. *J. Clin. Oncol.* **33**, 1235–1242 (2015).
25. Schaeffeler, E. et al. Impact of *NUDT15* genetics on severe thiopurine-related hematotoxicity in patients with European ancestry. *Genet. Med.* **21**, 2145–2150 (2019).
26. Zhu, Y. et al. Combination of common and novel rare *NUDT15* variants improves predictive sensitivity of thiopurine-induced leukopenia in children with acute lymphoblastic leukemia. *Haematologica* **103**, e293–e295 (2018).
27. Kim, J. H. et al. Influences of thiopurine methyltransferase genotype and activity on thiopurine-induced leukopenia in Korean patients with inflammatory bowel disease: a retrospective cohort study. *J. Clin. Gastroenterol.* **44**, e242–e248 (2010).
28. Park, S. K. et al. Influences of *XDH* genotype by gene-gene interactions with *SUCLA2* for thiopurine-induced leukopenia in Korean patients with Crohn's disease. *Scand. J. Gastroenterol.* **51**, 684–691 (2016).
29. Tanaka, Y. et al. The activity of the inosine triphosphate pyrophosphatase affects toxicity of 6-mercaptopurine during maintenance therapy for acute lymphoblastic leukemia in Japanese children. *Leuk. Res.* **36**, 560–564 (2012).

30. Wan Rosalina, W. R. et al. Polymorphism of ITPA 94C>A and risk of adverse effects among patients with acute lymphoblastic leukaemia treated with 6-mercaptopurine. *J. Clin. Pharm. Ther.* **37**, 237–241 (2012).
31. Farfan, M. J. et al. Prevalence of *TPMT* and *ITPA* gene polymorphisms and effect on mercaptopurine dosage in Chilean children with acute lymphoblastic leukemia. *BMC Cancer* **14**, 299 (2014).
32. Khera, S. et al. Prevalence of *TPMT*, *ITPA* and *NUDT 15* genetic polymorphisms and their relation to 6MP toxicity in north Indian children with acute lymphoblastic leukemia. *Cancer Chemother. Pharmacol.* **83**, 341–348 (2019).
33. Moradveisi, B. et al. *ITPA*, *TPMT*, and *NUDT15* genetic polymorphisms predict 6-mercaptopurine toxicity in Middle Eastern children with acute lymphoblastic leukemia. *Front. Pharm.* **10**, 916 (2019).
34. Ma, X. et al. Inosine triphosphate pyrophosphohydrolase (*ITPA*) polymorphic sequence variants in Chinese ALL children and possible association with mercaptopurine related toxicity. *Int. J. Clin. Exp. Pathol.* **7**, 4552–4556 (2014).
35. Tanaka, Y. et al. Multidrug resistance protein 4 (*MRP4*) polymorphisms impact the 6-mercaptopurine dose tolerance during maintenance therapy in Japanese childhood acute lymphoblastic leukemia. *Pharmacogenom. J.* **15**, 380–384 (2015).
36. Stocco, G. et al. *PACSIN2* polymorphism influences *TPMT* activity and mercaptopurine-related gastrointestinal toxicity. *Hum. Mol. Genet* **21**, 4793–4804 (2012).
37. Smid, A. et al. *PACSIN2* polymorphism is associated with thiopurine-induced hematological toxicity in children with acute lymphoblastic leukaemia undergoing maintenance therapy. *Sci. Rep.* **6**, 30244 (2016).