



OPEN

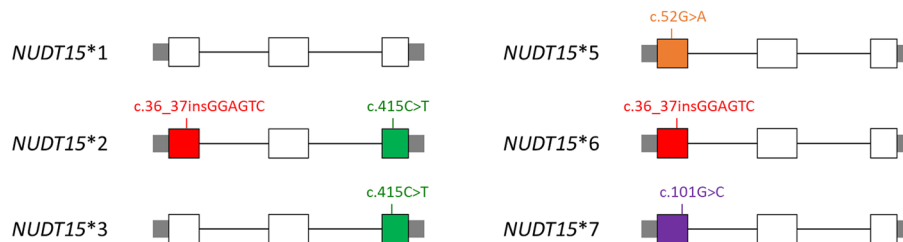
# Determination of *NUDT15* variants by targeted sequencing can identify compound heterozygosity in pediatric acute lymphoblastic leukemia patients

Chih-Hsiang Yu<sup>1</sup>, Ya-Hsuan Chang<sup>2</sup>, Der-Shiun Wang<sup>3,4</sup>, Shiann-Tarng Jou<sup>5</sup>, Chien-Yu Lin<sup>2</sup>, Kai-Hsin Lin<sup>5</sup>, Meng-Yao Lu<sup>5</sup>, Lovely Raghav<sup>2</sup>, Hsiu-Hao Chang<sup>5</sup>, Kang-Hsi Wu<sup>6</sup>, Shu-Wei Chou<sup>5</sup>, Yu-Ling Ni<sup>7</sup>, Dong-Tsamn Lin<sup>5,7</sup>, Shu-Wha Lin<sup>1</sup>, Hsuan-Yu Chen<sup>2</sup> & Yung-Li Yang<sup>5,7</sup>✉

Mercaptopurine intolerance is an adverse effect of mercaptopurine administration in pediatric acute lymphoblastic leukemia. Recently, *NUDT15* variants were identified as a major determinant of mercaptopurine intolerance. Two *NUDT15* variants, c.36\_37insGGAGTC and c.415C>T, are located on exons 1 and 3, respectively. Patients with heterozygous c.36\_37insGGAGTC and c.415C>T can be either compound heterozygous with two variants on different alleles or heterozygous with both variants on the same allele. Because patients with biallelic *NUDT15* variants are extremely sensitive to mercaptopurine, clinical identification of *NUDT15* diplotype would be advantageous. A cohort of 37 patients with c.36\_37insGGAGTC and c.415C>T *NUDT15* variants were selected for haplotyping by targeted sequencing. *NUDT15* complementary DNA was amplified and sequenced by 300-bp paired-end sequencing on Illumina MiSeq. Of the 37 patients carrying *NUDT15* variants, 35 had heterozygous *NUDT15*\*1/\*2 variants and two had compound heterozygous *NUDT15*\*3/\*6 and *NUDT15*\*2/\*7 variants. These two patients with compound heterozygous variants could only tolerate low doses of mercaptopurine, similar to patients with homozygous *NUDT15* variants. Targeted sequencing of *NUDT15* cDNA can be used to determine *NUDT15* diplotype and identify patients with compound heterozygous *NUDT15* variants.

Thiopurines (e.g., 6-mercaptopurine [6-MP], 6-thioguanine [6-TG], and azathioprine [AZA]) are important antimetabolite drugs that are used for different clinical indications. In pediatric acute lymphoblastic leukemia (ALL), 6-MP is widely used in the induction, consolidation (with high-dose methotrexate), and maintenance phases. Indeed, 6-MP-based maintenance therapy is one of the most critical components in the treatment regimen for pediatric ALL<sup>1-7</sup>. Variants of genes responsible for thiopurine metabolism can directly influence drug toxicity and antileukemic efficacy<sup>8-13</sup>. For example, polymorphisms in the thiopurine methyltransferase (*TPMT*) gene have been linked to susceptibility to thiopurine-induced marrow suppression in patients, and preemptive *TPMT* genotype-guided dosing represents a successful example of genetics-based precision medicine in cancer treatment<sup>14,15</sup>.

<sup>1</sup>Departments of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei, Taiwan. <sup>2</sup>Institute of Statistical Science Academia Sinica, Taipei, Taiwan. <sup>3</sup>Department of Pediatrics, Tri-Service General Hospital, Taipei, Taiwan. <sup>4</sup>Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan. <sup>5</sup>Department of Pediatrics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan. <sup>6</sup>Division of Pediatric Hematology and Oncology, China Medical University Children's Hospital, Taichung, Taiwan. <sup>7</sup>Department of Laboratory Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, No. 7, Chung Shan S. Rd, Zhongzheng Dist, Taipei City 10041, Taiwan. ✉email: yangyl92@ntu.edu.tw



**Figure 1.** Four coding variants in *NUDT15* were identified, representing six haplotypes (labeled as *NUDT15\*1–3* and *NUDT15\*5–7*).

Recent genome-wide association studies have reported a missense variant in *NUDT15* (*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 a Korean population<sup>16</sup>. In a genome-wide association study, Yang et al. identified this variant in association with mercaptopurine intolerance in childhood ALL<sup>17</sup>. Several *NUDT15* variants associated with decreased diphosphatase activity have been reported, and haplotypes with different combinations of the variant(s) have been assigned star allele numbers, which were proposed by Moriyama et al. (Fig. 1)<sup>18,19</sup>. All haplotypes except for *NUDT15\*2* have been reported to carry a single coding variant, including *NUDT15\*3* (*rs116855232*, c.415C>T), *NUDT15\*5* (*rs186364861*, c.52G>A), *NUDT15\*6* (*rs869320766*, c.36\_37insGGAGTC), and *NUDT15\*7* (*rs766023281*, c.101G>C). *NUDT15\*2* carries both c.415C>T and c.36\_37insGGAGTC, which are also present in *NUDT15\*3* and *NUDT15\*6*, respectively. Numerous studies have established that *NUDT15* variants are associated with mercaptopurine intolerance in pediatric ALL in different populations, highlighting the importance of preemptive genetic typing in these patients<sup>20–30</sup>.

Because of the strong correlation between the number of *NUDT15* risk alleles and median 6-MP dosage, accurate determination of diplotypes might allow preemptive dosing<sup>31</sup>. For example, patients with heterozygous c.415C>T and c.36\_37insGGAGTC can be determined to be either heterozygous (*NUDT15\*1/\*2*) or compound heterozygous (*NUDT15\*3/\*6*), and the tolerated 6-MP dosage by patients with the two diplotypes might be significantly different. In this study, we elucidated the *NUDT15* diplotypes in patients with multiple heterozygous variants by next-generation sequencing (NGS) of *NUDT15* complementary deoxyribonucleic acid (cDNA). Thus, this study will expand our knowledge of *NUDT15* variants in patients with pediatric ALL in Taiwan.

## Methods

**Patients.** We selected pediatric patients with ALL younger than 18 years of age treated in the National Taiwan University Hospital, Taipei, between April 1997 and December 2019. This study was approved by the Institutional Review Board of the National Taiwan University Hospital. Informed consent was obtained from the parents or legal guardians of the patients. This cohort was previously published<sup>30</sup>, and we selected a subset of patients for this study. There were 22 patients with heterozygous c.36\_37insGGAGTC and c.415C>T variants, and 10 patients with known *NUDT15* diplotypes (4 with *NUDT15\*1/\*1*, 2 with *NUDT15\*1/\*3*, 1 with *NUDT15\*1/\*5*, 1 with *NUDT15\*1/\*6*, and 2 with *NUDT15\*2/\*2*) were enrolled in the previous study<sup>30</sup>. In this study, another 15 patients with heterozygous c.36\_37insGGAGTC and c.415C>T variants, 1 patient with *NUDT15\*1/\*5*, and 1 patient with *NUDT15\*2/\*3* were enrolled.

**Targeted sequencing-based haplotyping.** Forty-nine patient samples were selected for haplotyping by targeted sequencing, including 37 patients with heterozygous c.36\_37insGGAGTC and c.415C>T variants. Samples from 12 patients with known *NUDT15* diplotypes (*NUDT15\*1/\*1*, *NUDT15\*1/\*3*, *NUDT15\*1/\*5*, *NUDT15\*1/\*6*, *NUDT15\*2/\*2*, and *NUDT15\*2/\*3*) were included as experimental controls. Total RNA was extracted using TRIzol reagent according to the manufacturer's instructions (Invitrogen, Waltham, MA, USA). We synthesized cDNA from germline total RNA using the Maxima First-Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). *NUDT15* cDNA was amplified using the Phusion Hot Start II High-Fidelity PCR Master Mix (Thermo Fisher Scientific) with barcoded primers (Tables S1 and S2). The barcoded amplicons were purified using AMPure XP beads (Beckman Coulter, Brea, CA, USA) and quantified using Qubit reagent (Thermo Fisher Scientific). Equimolar amounts of each amplicon were pooled and sequenced by 300-bp paired-end sequencing using the MiSeq Reagent Kit v3 (Illumina, San Diego, CA, USA). Raw sequencing data were further analyzed to discriminate the *NUDT15* haplotypes. The barcoded primers used for the targeted sequencing of cDNA are listed in Supplementary Table 1.

FastQC (v0.11.7) was performed to evaluate the quality and adaptor contamination of the sequencing reads. Adaptor sequences and low-quality bases were trimmed (average quality score of a four-base wide sliding window < 20) using Trimmomatic (v0.33). The trimmed reads were further aligned to *NUDT15* cDNA (NM\_018283) using bowtie2 (v2.3.3.1). The average insert length of the reads was 562 bp. Reads with specific variants were extracted from the total aligned reads and used to determine the two alleles of *NUDT15*. Finally, IGV tool (v2.7.2) was used to check the read alignment results and the two alleles of the samples.

**Cloning of *NUDT15* cDNA.** The *NUDT15* cDNA amplicons were purified using the FavorPrep GEL/PCR Purification Kit (Favorgen Biotech, Ping-Tung, Taiwan), cloned into a vector using the Zero Blunt TOPO PCR

Cloning Kit (Thermo Fisher Scientific), and transformed into TOP10 competent cells (Thermo Fisher Scientific). Single colonies were cultured in LB broth (50 µg/mL kanamycin) at 37 °C for 16 h, and plasmids were extracted using the FavorPrep Plasmid DNA Extraction Mini Kit (Favorgen Biotech). Sanger sequencing was performed using the SP6 or T7 primer (Supplementary Table 1). At least 20 plasmids from a single colony were sequenced to determine the *NUDT15* diplotypes.

**Statistical analyses.** Mann–Whitney U test was used to evaluate differences in median 6-MP dosages between patients with a heterozygous variant (*NUDT15*\*1/\*2) and homozygous/compound heterozygous variant (*NUDT15*\*2/\*2, *NUDT15*\*2/\*3, *NUDT15*\*2/\*7, and *NUDT15*\*3/\*6).

## Results

**Next-generation sequencing of cDNA.** To resolve the diplotypes of patients with multiple heterozygous variants, we sequenced *NUDT15* cDNA by paired-end sequencing. Thirty-seven patients had multiple heterozygous variants. We also included the cDNA of 12 patients with known diplotypes (4 with *NUDT15*\*1/\*1, 2 with *NUDT15*\*1/\*3, 2 with *NUDT15*\*1/\*5, 1 with *NUDT15*\*1/\*6, 2 with *NUDT15*\*2/\*2, and 1 with *NUDT15*\*2/\*3) as experimental controls<sup>30</sup>. The reads were phased, and the results showed that all but two samples could be classified as *NUDT15*\*1/\*2 (Fig. 2a). The two samples with different allele combinations were classified as *NUDT15*\*3/\*6 (Fig. 2b) and *NUDT15*\*2/\*7 (Fig. 2c).

**cDNA sequencing of the *NUDT15*\*3/\*6 patient.** To validate the targeted sequencing result for the patient with *NUDT15*\*3/\*6, the *NUDT15* cDNA was cloned into a vector, and the haplotypes were resolved by Sanger sequencing of the vector. The results showed that the c.36\_37insGGAGTC and c.415C>T alleles were located *in trans*; thus, the genome of this patient was determined to harbor a compound heterozygous *NUDT15*\*3/\*6 variant (Figure S1). We also genotyped the biological parents of this patient and found that the genomes of the parents harbored *NUDT15*\*1/\*6 and *NUDT15*\*1/\*3. The genotyping of the biological parents of patients provides an additional line of evidence to determine the diplotypes (Fig. 3).

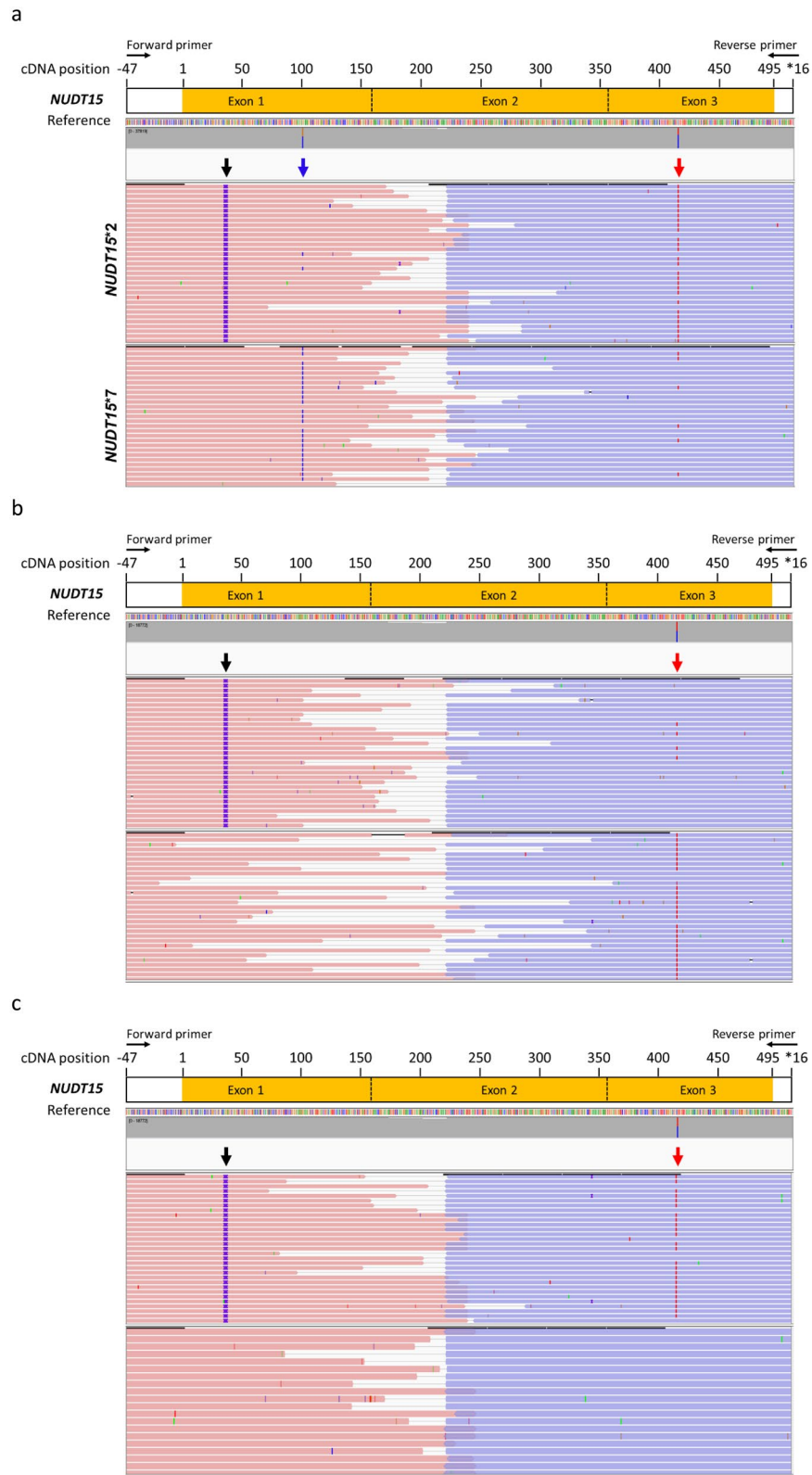
**cDNA sequencing of the *NUDT15*\*2/\*7 patient.** We also validated the result of the patient with *NUDT15*\*2/\*7 by cDNA cloning and showed that c.101G>C was located *in trans* with c.36\_37insGGAGTC and c.415C>T (Figure S2). The biological parents of the patients were genotyped, and we found that the father was homozygous for c.36\_37insGGAGTC and c.415C>T (*NUDT15*\*2/\*2), whereas the mother was heterozygous for the c.36\_37insGGAGTC, c.415C>T, and c.101G>C variants, which matched the genotype of the patient. Because the patient must have had an *NUDT15*\*2 allele from the father, the c.101G>C allele should have been inherited from the mother; we can posit that the diplotype of the mother is *NUDT15*\*2/\*7.

**Tolerated doses of 6-MP among patients with the *NUDT15* variant.** We evaluated the median tolerated 6-MP dose in patients with the *NUDT15* variants. The median tolerated 6-MP dose among all 35 patients with the *NUDT15*\*1/\*2 genotype was 12.5 mg/m<sup>2</sup> (5.0–42.5 mg/m<sup>2</sup>), and the doses were extremely low for the patients with *NUDT15*\*2/\*2 or *NUDT15*\*2/\*3 (1, 2.2, and 4.3 mg/m<sup>2</sup>). The two patients with compound heterozygous variants, *NUDT15*\*3/\*6 and *NUDT15*\*2/\*7, tolerated only 2.5 and 6.7 mg/m<sup>2</sup>/day mercaptopurine, respectively, and this was similar to the patients with homozygous variants. We observed that patients with homozygous and compound heterozygous *NUDT15* variants tolerated a significantly lower median 6-MP dose than patients with heterozygous variants (Fig. 4).

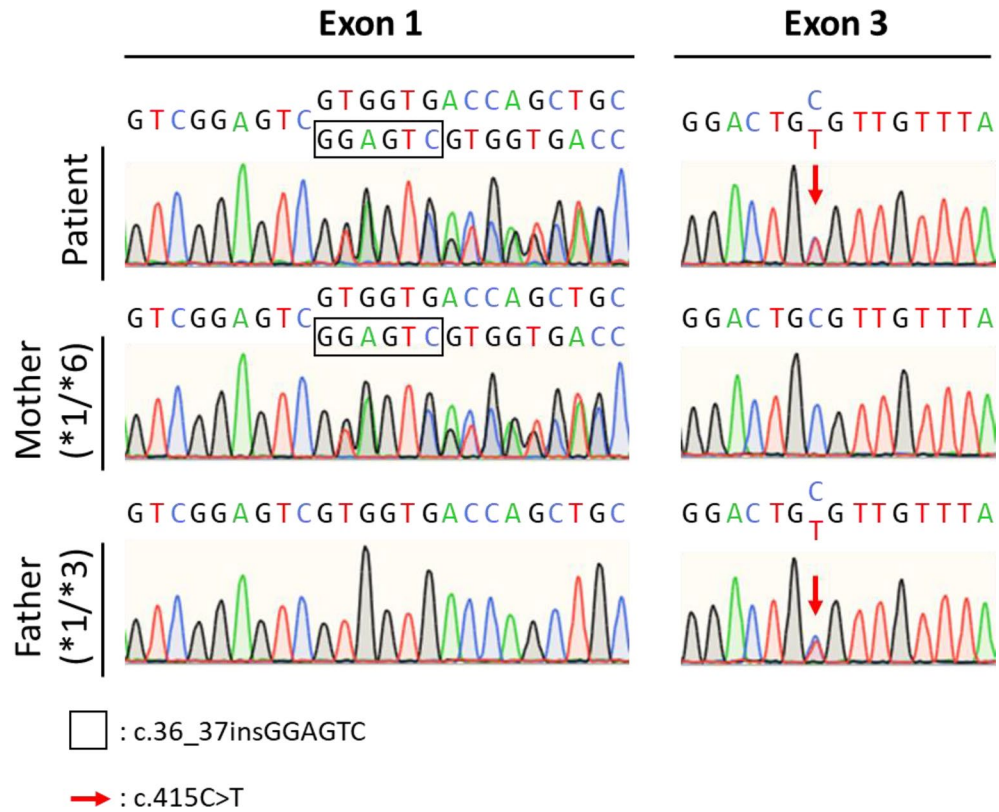
## Discussion

Our study and that of Tsujimoto et al.<sup>32</sup> showed that most patients with the *NUDT15* c.36\_37insGGAGTC and c.415C>T variants harbored heterozygous *NUDT15*\*1/\*2. Tsujimoto et al. defined the diplotypes of 14 patients carrying the two variants (i.e., c.415C>T and c.36\_37insGGAGTC) by droplet digital PCR (ddPCR) and restriction enzyme-PCR (RE-PCR)<sup>32</sup>. We developed another method using *NUDT15* cDNA-targeted sequencing. The *NUDT15* cDNA was sequenced by paired-end sequencing using Illumina MiSeq, and our results were confirmed by cloning. Compared with ddPCR and RE-PCR, targeted sequencing of *NUDT15* cDNA can help determine the diplotypes without genotyping information of a patient.

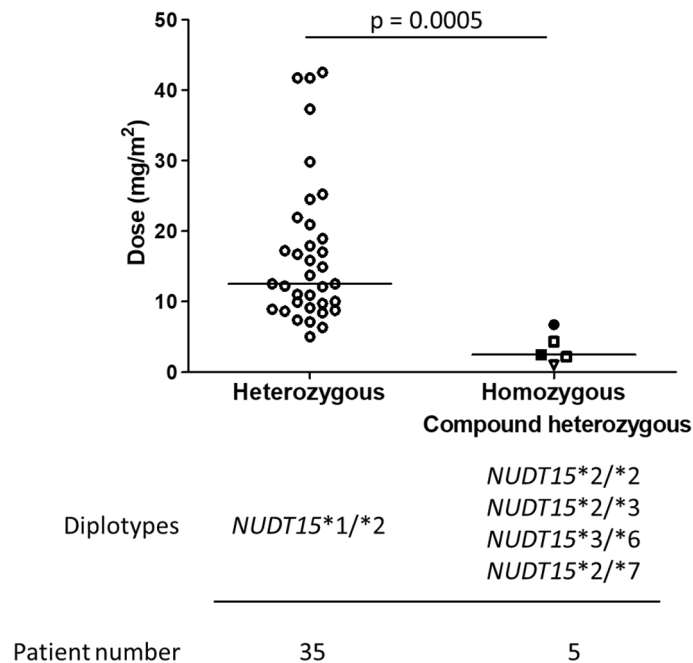
Currently, *NUDT15* genotyping is indicated before mercaptopurine administration for pediatric ALL<sup>31</sup>. Poor metabolizers are defined as individuals carrying two nonfunctioning alleles (*NUDT15*\*2/\*2, *NUDT15*\*2/\*3, and *NUDT15*\*3/\*3). Potential intermediate metabolizers are individuals carrying one allele of uncertain function plus one nonfunctioning allele, such as *NUDT15*\*2/\*5 and *NUDT15*\*3/\*6<sup>31</sup>. Our patient with the *NUDT15*\*3/\*6 combination tolerated only 2.5 mg/m<sup>2</sup>/day mercaptopurine, and this was similar to the dose for poor metabolizers. Another patient with *NUDT15*\*2/\*7 could also tolerate very low daily doses of mercaptopurine. The c.101G>C variant of *NUDT15* is also very rare in Asians, and its function was confirmed by Moriyama et al.<sup>19</sup>. From our experience with these two patients, any combination of two mutant alleles with known functional roles might be classified as compound heterozygous, and the mercaptopurine dose should be the same as that administered to homozygous patients. Because mercaptopurine is widely administered for other diseases, such as inflammatory bowel disease<sup>33,34</sup>, its clinical significance has also been validated in this subgroup of patients. The identification of these two compound heterozygous patients suggests that testing laboratories should be cautious while interpreting the c.36\_37insGGAGTC and c.415C>T variants of *NUDT15* in genetic testing of pediatric patients with ALL taking mercaptopurine or other related drugs, rather than only considering these genotypes.



**Figure 2.** Phased variants of *NUDT15*. (a) Patients (NTUCH07) with *NUDT15*\*1/\*2, (b) patients (NTUCH27) with *NUDT15*\*3/\*6, and (c) patients (NTUCH37) with *NUDT15*\*2/\*7 are shown. Reads were mapped to *NUDT15* mRNA reference sequence (NM\_018283), and cDNA position relative to gene structure is presented. The *NUDT15* variants are indicated with arrows, and reads were grouped by haplotype for these variants. Black arrow: c.36\_37insGGAGTC; blue arrow: c.101G>C; red arrow: c.415C>T. The paired-end reads are represented, and the reads are colored red for forward strand and blue for reverse strand.



**Figure 3.** Patient with heterozygous c.36\_37insGAGTCG and c.415C>T variants. We performed Sanger sequencing of the biological parents of the patient. The mother had a heterozygous c.36\_37insGAGTCG variant (*NUDT15*\*1/\*6) and father had a heterozygous c.415C>T variant (*NUDT15*\*1/\*3).



**Figure 4.** Distribution of tolerated 6-MP doses according to *NUDT15* diplotypes. Heterozygous (open circles: *NUDT15*\*1/\*2), homozygous (open triangle: *NUDT15*\*2/\*2, open square: *NUDT15*\*2/\*3), or compound heterozygous (closed square: *NUDT15*\*3/\*6, closed circle: *NUDT15*\*2/\*7) and patient number are represented. The P-value was calculated using Mann–Whitney U test.

There was a major limitation to this study. From a cost and benefit perspective, it might be difficult to use cDNA NGS to determine the diplotype of *NUDT15* in clinical practice; moreover, the incidence of compound heterozygous mutations, such as *NUDT15*\*3/\*6, is very rare<sup>18</sup>. Sanger sequencing remains the most convenient and cost-effective method for the analysis of genetic variants of *NUDT15*<sup>17,18,20,25,27,28,31,32,35</sup>. However, to improve clinical outcomes and reduce adverse effects in pediatric patients with ALL, appropriate preemptive diagnosis of pharmacogenetic variants might be warranted<sup>31,36,37</sup>. There were other genetic polymorphisms related to some common adverse effects of chemotherapy, such as vincristine neuropathy, asparaginase hypersensitivity, hypertension, and osteonecrosis due to steroid use<sup>37–45</sup>. NGS cDNA targeted therapy might have some advantages, including sequencing multiple genes simultaneously and identifying the diplotype of *NUDT15* variants if complex variants are present. If multiple genetic variants can be sequenced simultaneously, the cost might be reduced for individual SNPs and avoid tedious multiple Sanger sequencing. Although *NUDT15* variants are very rare, identifying compound homozygous patients before the administration of mercaptopurine might largely reduce the initial dose of mercaptopurine to avoid profound marrow suppression. Thus, the infectious adverse effect might be decreased in this small subset of patients.

In conclusion, most c.36\_37insGGAGTC and c.415C>T variants in *NUDT15* are located on the same allele and can be classified as monoallelic variants. Although very rare, there are patients with compound heterozygous *NUDT15* variants. Patients with any combination of functionally verified *NUDT15* alleles might be considered to be poor mercaptopurine metabolizers, and these patients should be administered a relatively low dose of this drug. An appropriate definition of compound heterozygous variants in *NUDT15* might require genotyping of the biological parents of the patient or other molecular methods, such as targeted sequencing, ddPCR, RE-PCR, and cloning.

**Ethics declaration.** This study was conducted in accordance with the Declaration of Helsinki guidelines. Written informed consent was obtained from all study participants or their guardians. The study was approved by the Institutional Research Board of National Taiwan University Hospital (NTUH IRB number 201510016RIND).

**Consent to participate.** None.

### Data availability

Data of cDNA sequencing of *NUDT15* have been deposited at <https://www.ncbi.nlm.nih.gov/sra/PRJNA655987>.

Received: 4 January 2020; Accepted: 17 August 2020

Published online: 01 September 2020

### References

- Vora, A. *et al.* Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. *Lancet Oncol.* **14**, 199–209 (2013).
- Alexander, T. B. *et al.* The genetic basis and cell of origin of mixed phenotype acute leukaemia. *Nature* **562**, 373–379 (2018).
- Pui, C. H. & Evans, W. E. Treatment of acute lymphoblastic leukemia. *N. Engl. J. Med.* **354**, 166–178 (2006).
- Pui, C.-H. *et al.* Childhood acute lymphoblastic leukemia: progress through collaboration. *J. Clin. Oncol.* **33**, 2938–2948 (2015).
- Maltzman, J. S. & Koretzky, G. A. Azathioprine: old drug, new actions. *J. Clin. Invest.* **111**, 1122–1124 (2003).
- Karran, P. & Attard, N. Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer. *Nat. Rev.* **8**, 24–36 (2008).
- Elion, G. The purine path to chemotherapy. *Science* **244**, 41–47 (1989).
- Kunz, J. B. *et al.* Pediatric T-cell lymphoblastic leukemia evolves into relapse by clonal selection, acquisition of mutations and promoter hypomethylation. *Haematologica* **100**, 1442–1450 (2015).
- Meyer, J. A. *et al.* Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia. *Nat. Genet.* **45**, 290–294 (2013).
- Tzoneva, G. *et al.* Clonal evolution mechanisms in NT5C2 mutant-relapsed acute lymphoblastic leukaemia. *Nature* **553**, 511–514 (2018).
- Tzoneva, G. *et al.* Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. *Nat. Med.* **19**, 368–371 (2013).
- Li, B. *et al.* Negative feedback-defective PRPS1 mutants drive thiopurine resistance in relapsed childhood ALL. *Nat. Med.* **21**, 563–571 (2015).
- Relling, M. V. *et al.* Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *JNCI J. Natl. Cancer Inst.* **91**, 2001–2008 (1999).
- Krynetski, E. Y. *et al.* A single point mutation leading to loss of catalytic activity in human thiopurine S-methyltransferase. *Proc. Natl. Acad. Sci. USA* **92**, 949–953 (1995).
- Relling, M. V. *et al.* Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. *Clin. Pharmacol. Ther.* **93**, 324–325 (2013).
- Yang, S. K. *et al.* A common missense variant in *NUDT15* confers susceptibility to thiopurine-induced leukopenia. *Nat. Genet.* **46**, 1017–1020 (2014).
- 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).
- Moriyama, T. *et al.* *NUDT15* polymorphisms alter thiopurine metabolism and hematopoietic toxicity. *Nat. Genet.* **48**, 367–373 (2016).
- 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).
- Buaboonnam, J. *et al.* Effect of *NUDT15* on incidence of neutropenia in children with acute lymphoblastic leukemia. *Pediatr. Int.* **61**, 754–758 (2019).
- 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).
- Khera, S. *et al.* Prevalence of TPMT, ITPA and *NUDT15* genetic polymorphisms and their relation to 6MP toxicity in north Indian children with acute lymphoblastic leukemia. *Cancer Chemother. Pharmacol.* **83**, 341–348 (2019).

23. 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).
24. Moradveisi, B. *et al.* ITPA, TPMT, and NUDT15 genetic polymorphisms predict 6-mercaptopurine toxicity in middle eastern children with acute lymphoblastic leukemia. *Frontiers Pharmacol.* <https://doi.org/10.3389/fphar.2019.00916> (2019).
25. Schaeffeler, E. *et al.* Impact of NUDT15 genetics on severe thiopurine-related hematotoxicity in patients with European ancestry. *Genet. Med.* <https://doi.org/10.1038/s41436-019-0448-7> (2019).
26. Soler, A. M. *et al.* TPMT and NUDT15 genes are both related to mercaptopurine intolerance in acute lymphoblastic leukaemia patients from Uruguay. *Br. J. Haematol.* **181**, 252–255 (2018).
27. 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).
28. 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).
29. Wahlund, M. *et al.* The role of TPMT, ITPA, and NUDT15 variants during mercaptopurine treatment of swedish pediatric patients with acute lymphoblastic leukemia. *J. Pediatr.* **216**, 150–157 (2020).
30. Wang, D.-S. *et al.* Childhood acute lymphoblastic leukemia mercaptopurine intolerance is associated with NUDT15 variants. *Pediatr. Res.* <https://doi.org/10.1038/s41390-020-0868-8> (2020).
31. 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).
32. Tsujimoto, S. *et al.* Diploidy analysis of NUDT15 variants and 6-mercaptopurine sensitivity in pediatric lymphoid neoplasms. *Leukemia* **32**, 2710–2714 (2018).
33. Akiyama, S. *et al.* Long-term effect of NUDT15 R139C on hematologic indices in inflammatory bowel disease patients treated with thiopurine. *J. Gastroenterol. Hepatol.* <https://doi.org/10.1111/jgh.14693> (2019).
34. Walker, G. J. *et al.* Association of genetic variants in NUDT15 with thiopurine-induced myelosuppression in patients with inflammatory bowel disease genetic variants associated with thiopurine-induced myelosuppression in IBD. *JAMA* **321**, 773–785 (2019).
35. Tanaka, Y. *et al.* Interaction between NUDT15 and ABCC4 variants enhances intolerance of 6-mercaptopurine in Japanese patients with childhood acute lymphoblastic leukemia. *Pharmacogenomics J* **18**, 275–280 (2017).
36. Moriyama, T., Relling, M. V. & Yang, J. J. Inherited genetic variation in childhood acute lymphoblastic leukemia. *Blood* **125**, 3988–3995 (2015).
37. Pui, C. H., Nichols, K. E. & Yang, J. J. Somatic and germline genomics in paediatric acute lymphoblastic leukaemia. *Nat. Rev. Clin. Oncol.* **16**, 227–240 (2019).
38. Diouf, B. *et al.* Association of an inherited genetic variant with vincristine-related peripheral neuropathy in children with acute lymphoblastic leukemia. *JAMA* **313**, 815–823 (2015).
39. Kamdem, L. K. *et al.* Genetic predictors of glucocorticoid-induced hypertension in children with acute lymphoblastic leukemia. *Pharmacogenet. Genom.* **18**, 507–514 (2008).
40. Karol, S. E. *et al.* Genetics of glucocorticoid-associated osteonecrosis in children with acute lymphoblastic leukemia. *Blood* **126**, 1770–1776 (2015).
41. French, D. *et al.* A PAI-1 (SERPINE1) polymorphism predicts osteonecrosis in children with acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Blood* **111**, 4496–4499 (2008).
42. Karol, S. E. *et al.* Genetic risk factors for the development of osteonecrosis in children under age 10 treated for acute lymphoblastic leukemia. *Blood* **127**, 558–564 (2016).
43. Fernandez, C. A. *et al.* HLA-DRB1\*07:01 is associated with a higher risk of asparaginase allergies. *Blood* **124**, 1266–1276 (2014).
44. Fernandez, C. A. *et al.* Genome-wide analysis links NFATC2 with asparaginase hypersensitivity. *Blood* **126**, 69–75 (2015).
45. Witt, H. *et al.* Variants in CPA1 are strongly associated with early onset chronic pancreatitis. *Nat. Genet.* **45**, 1216–1220 (2013).

## Acknowledgements

The authors express their gratitude to all patients who participated in this study and their parents. The authors also acknowledge the efforts of the TPOG and the Childhood Cancer Foundation in Taiwan.

## Author contributions

C.-H.Y. and Y.-L.Y. designed the study, analyzed data, and wrote the manuscript. C.-H.Y., D.-S.W., Y.-L.N. and S.-W.L. acquired and processed patient specimens and performed experiments. C.-Y.L., Y.-H.C. L.R. and H.-Y.C. performed the analysis. K.-H.L., S.-T.J., M.-Y.L., K.-H.W., H.-H.C., S.-W.C. and D.-T.L. provided clinical samples and data. The manuscript was written by C.-H.Y. and Y.-L.Y. and was reviewed and edited by all authors.

## Funding

This work was supported by grants from the Ministry of Science and Technology, Taiwan (MOST-103-2314-B-002-199- and MOST-107-2314-B-002-173-MY2 to YLY) and from the National Taiwan University Hospital (NTUH.105-N03 to YLY).

## Competing interests

The authors declare no competing interests.

## Additional information

**Supplementary information** is available for this paper at <https://doi.org/10.1038/s41598-020-71468-y>.

**Correspondence** and requests for materials should be addressed to Y.-L.Y.

**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).

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



**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2020