

Homozygous mutation in NUDT15 in childhood acute lymphoblastic leukemia with increased susceptibility to mercaptopurine toxicity: A case report

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Abstract. As an essential component of consolidation and maintenance therapy for acute lymphoblastic leukemia (ALL), mercaptopurine (6-MP) causes critical myelosuppression. The current study aimed to clarify the reasons for severe myelosuppression and significant hyperpigmentation in a patient with ALL that received consolidation therapy. The present study performed patient NUDT15 testing with fluorescence *in situ* hybridization and whole-exome sequencing. The results revealed that the patient was a homozygous carrier (415C>T, TT) for rs116855232 (NUDT15). The dose of 6-MP was adjusted down from 30%, with the patient receiving maintenance therapy at 8% of the recommended dose. The homozygous mutant (TT genotype) of NUDT15 may cause hematopoietic toxicity with low doses of 6-MP. NUDT15 genotyping should therefore be performed prior to the administration of thiopurine, the dosage of which requires adjustment.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in childhood, accounting for 25-30% of all childhood malignancies (1,2). Pediatric patients with ALL are classified into standard-, intermediate- or high-risk categories based on age, white blood cell count, existing central nervous system leukemia or testicular leukemia, immunophenotype, cytogenetic and molecular characteristics, prednisone response, morphological remission at the end

of induction therapy, and the different expression of minimal residual disease (MRD) (3,4). Newly diagnosed patients with ALL require a rigorous, standardized and long-course chemotherapy treatment strategy, which includes induction, consolidation, intensification and maintenance therapy (5). Additionally, stratification of treatment intensity is based on the risk stratification of leukemic blasts identified in ALL (4). The disease-free survival and the curative rate have greatly improved with improved diagnosis and treatment regimens (6). However, treatment interruption or discontinuation due to hematopoietic toxicity is a common adverse event and results in a higher risk of relapse (7).

As an anticancer and immunosuppressive agent, 6-mercaptopurine (6-MP) is commonly used as part of the treatment strategy in patients with ALL, including consolidation, intensification and maintenance therapy (8). 6-MP is specifically important for maintenance therapy, the longest phase lasting ~2-3 years (9). 6-MP-induced life-threatening myelotoxicity is commonly associated with polymorphisms in genes encoding thiopurine methyltransferase (TPMT), inosine triphosphatase (ITPA) and nudix hydrolase 15 (NUDT15) (8-12). Given the low prevalence of polymorphisms in TPMT in the Asian population, polymorphisms in ITPA and NUDT15 are considered to be the main causes of severe myelotoxicity in the Asian population (13). Previous studies determined that adult patients with inflammatory bowel disease and pediatric patients with ALL homozygous for the NUDT15 variant were extremely sensitive to 6-MP (14,15), which suggests that the NUDT15 variant may be a potential factor associated with 6-MP-induced myelotoxicity.

To the best of our knowledge, the present report is the first to present the case of a Chinese pediatric patient with ALL who experienced 6-MP-induced life-threatening myelotoxicity and agranulocytosis due to the homozygous mutant (TT genotype) for rs116855232 (NUDT15).

Case report

A 5-year-old male presenting with intermittent fever, anorexia, increasing fatigue and a cough that had persisted for 15 days

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was hospitalized in January 2017 at the Department of Hematology of The First Hospital of Lanzhou University. The patient had a normal medical history with no known drug allergies, no travel to epidemic areas and no known exposure to toxins. There was no family history of malignancy. On physical examination, the skin-mucous membrane of the patient was pale and no petechiae or edema was identified on either of the lower limbs. There was no involvement of the superficial lymph nodes and rales were not detected. The patient had a normal cardiac rhythm without murmurs. In addition, the patient's abdomen was soft and the liver was not palpable. No splenic tenderness or costovertebral angle tenderness was observed. The patient's white blood cell (WBC) count was $97.72 \times 10^9/l$ (normal range, $4-10 \times 10^9/l$), with a platelet count of $13 \times 10^9/l$ (normal range, $100-300 \times 10^9/l$), and a hemoglobin count of 48 g/l (normal range, 110-150 g/l). Bone marrow aspiration revealed bone marrow characteristics typically observed in ALL. Immunophenotypic analysis of the blast population revealed that CD34, HLA-DR, CD10 and CD19 were expressed on all cells, while CD33 was partially expressed. The patient demonstrated an absence of blasts in the cerebrospinal fluid. Cytogenetic analysis of bone marrow cells from this patient demonstrated 46, XY of the 20 metaphases examined. Detection of fusion genes identified no fusion gene expression in the patient. In addition, molecular studies did not identify any genetic mutations. These findings were consistent with a diagnosis of acute B-cell lymphoblastic leukemia with immediate-risk. The patient was tested for prednisone sensitivity and treated according to the CCLG-2008 protocol (16) developed at Beijing Children's Hospital.

The patient received induction chemotherapy, achieved morphological remission and was negative for MRD four weeks post-therapy. However, during early consolidation therapy with cyclophosphamide (1 g/m^2 on day 1), cytarabine (75 mg/m^2 on days 1-3 and 10-13) and 6-MP (60 mg/m^2 for 14 days), the patient was admitted to the pediatric intensive care unit with a severe lung infection due to long-term and serious myelosuppression and agranulocytosis. Subsequently, the patient developed respiratory failure and required ventilator support for several days. In addition, clear hyperpigmentation appeared on the patient's hands and feet, which was pronounced at the interphalangeal joints and in the subungual region (Fig. 1). The dosage of 6-MP was not decreased as the patient's TPMT enzyme activity was within the normal range, and no genetic mutations were previously identified. However, consolidation therapy could not be performed according to the schedule outlined in the protocol (due to myelosuppression and agranulocytosis). Three months after the patient's initial presentation, examination of the patient's bone marrow cells confirmed an increased proportion of lymphoblast-like cells, although it had not increased sufficiently to be classified as a relapse. Due to the severe myelotoxicity and significant hyperpigmentation observed, NUDT15 testing including fluorescence *in situ* hybridization and whole exome sequencing was performed. The results demonstrated that the patient was a homozygous carrier (415C>T, TT) for rs116855232 (NUDT15) (Fig. 2). During follow-up consolidation treatment, as the 6-thioguanine nucleotide plasma concentration could not be detected, the dose of 6-MP was adjusted from 30 to 10% of the total recommended dose after examining previous

hematological toxicity records. The dosage adjustment of 6-MP alleviated the clinical symptoms of myelotoxicity and hyperpigmentation observed in the patient. Four months after the patient's initial presentation, the patient is having regular follow-ups and is continuing maintenance therapy with 8% of the recommended dose of 6-MP.

Discussion

A major component of the standardized chemotherapy treatment strategy used to treat patients with childhood ALL, involves daily exposure to 6-MP for 2-3 years (17). As a prodrug, 6-MP is enzymatically converted into TGTP, through multiple sequential reactions, and TGTP is further reduced into deoxy-thioguanosine triphosphate (TdGTP). TdGTP can be incorporated into double-strand DNA during cell replication, to trigger ineffective mismatch repair and eventually apoptosis, causing cell cytotoxicity (18). Myelosuppression is a serious complication, which can occur during treatment of ALL, and is associated with 6-MP therapy (19). Myelosuppression results in treatment disruption leading to an increased risk of relapse, an increased risk of life-threatening infections and the need for more extensive treatment (19,20). 6-MP-induced myelosuppression is largely associated with polymorphisms in TPMT and ITPA genes (21). The four major mutant alleles of TPMT, TPMT*2 (238G>C), *3A (460G>A, 719A>G), *3B (460G>A) and *3C (719A>G), account for the majority of TPMT deficiency; deletions of exons and copy number variations can also account for the variability in TPMT efficacy (22). The TPMT*3A and *3C variants are significantly associated with dose intensity (19). There is a high prevalence of all major TPMT polymorphisms in African and European populations, however there is a low prevalence in the East Asian population (13).

ITPA genetic polymorphisms exhibited no significant differences between genotypes, and 6-MP-induced myelosuppression can occur at any time point during maintenance therapy (12). ITPA-associated risks of myelosuppression induced by 6-MP have been less convincingly demonstrated, therefore testing for those variants may not be clinically warranted (15). A meta-analysis study demonstrated that genetic polymorphisms in NUDT15 were strongly associated with adverse drug reaction of thiopurines, and therefore NUDT15 may be considered as a highly credible pharmacogenetic indicator for the use of thiopurines, especially in the Asian population (23). In 2015, a genome-wide association study of childhood ALL revealed a significant association between a germline NUDT15 variant (rs116855232) and 6-MP dose intensity (13). Out of 657 patients, 31 patients were heterozygous (CT) and only 2 were homozygous (TT) for the NUDT15 variant. Patients with the TT genotype were sensitive to 6-MP and required an average dose intensity of 8.3% of the planned dose (13). The NUDT15 variant was most frequent in East Asians and Hispanics, while rare in Europeans and not observed in Africans, thereby contributing to ancestry-associated differences in 6-MP tolerance (13,24). In a study of 404 Taiwan Chinese patients with ALL, 5 patients were homozygous (TT) for the NUDT15 variant and the maximal tolerable daily



Figure 1. Hyperpigmentation clinical presentation in hands. (A) Normal control. (B) Two weeks post-early intensification chemotherapy with 6-MP (60 mg/m²/day). (C) Two weeks post-consolidation and delayed intensification chemotherapy with 6-MP (6 mg/m²/day). 6-MP, mercaptopurine.

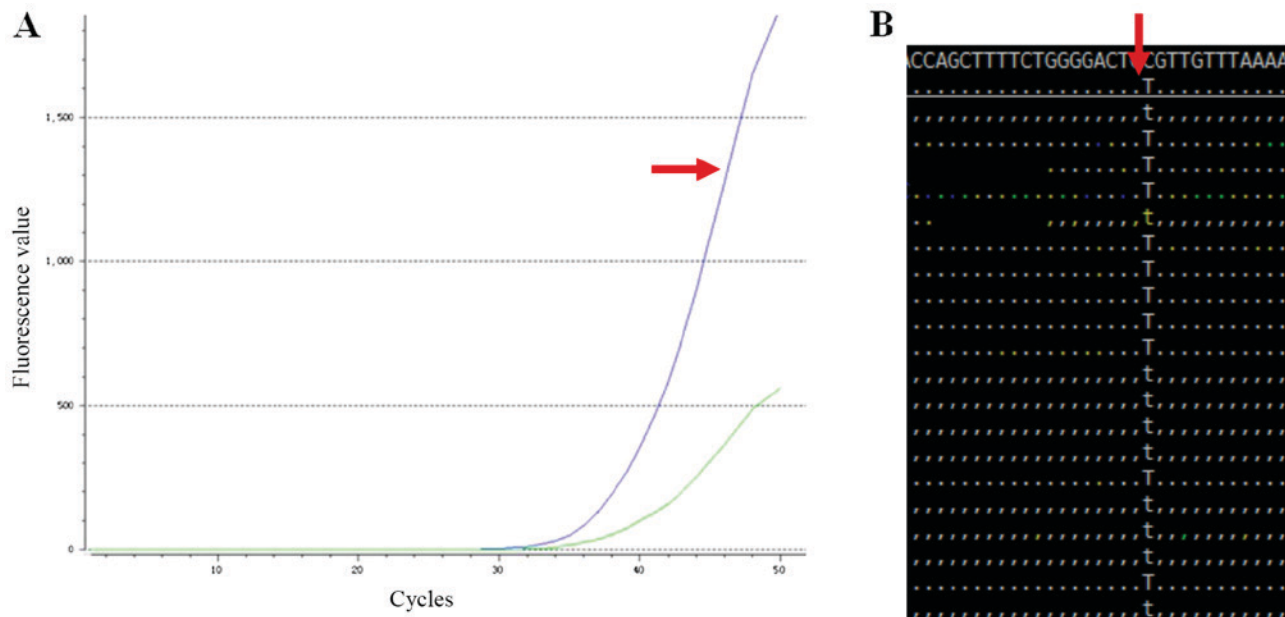


Figure 2. NUDT15 mutation analysis results demonstrated that the patient was a homozygous carrier (415C>T, TT) for rs116855232. (A) NUDT15 gene amplification testing by fluorescence *in situ* hybridization. (B) Whole exome sequencing was used to identify the NUDT15 variant was chr13: 48619855 NUDT15: 415C>T. NUDT15, nudix hydrolase 15; chr, chromosome.

dose of 6-MP in this group was 9.4 ± 3.7 mg/m²/day (24). A previous study demonstrated that 5 out of 182 Korean pediatric patients with ALL were homozygous for the NUDT15 variant and the lowest dose of 6-MP administered was 7.5 mg/m²/day (25). Previous studies suggest that a comprehensive pharmacogenetic model incorporating the specific genetic variation of NUDT15 may allow for further personalized thiopurine therapy (13,23-26). Although genetic testing prior to treatment revealed that the patient possessed the TPMT wild-type allele, the patient suffered from severe myelosuppression, in particular, neutropenia and hyperpigmentation, all of which led to a lung infection and treatment delay. The patient's blood sample was subsequently sent for genetic testing for the NUDT15 and ITPA variant, and it was revealed that the patient possessed the ITPA wild-type allele, but was homozygous (TT) for rs116855232 (NUDT15), which resulted in the observed adverse reaction to 6-MP.

In conclusion, to the best of our knowledge, the present report is the first to present the case of a Chinese pediatric patient with ALL who experienced 6-MP-induced life-threatening myelosuppression due to the homozygous mutant (TT genotype) for rs116855232 (NUDT15). The

present report demonstrates the importance of NUDT15 pharmacogenetics and early detection of genetic polymorphisms in children with ALL prior to treatment with 6-MP. Further studies are required to better understand the implications associated with NUDT15 variants in the treatment of childhood ALL, in order to determine the association between the necessary dosage of and length of treatment with 6-MP needed to achieve desirable therapeutic effects and avoid myelosuppression.

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Availability of data and materials

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JC contributed to conception of the study, interpretation of data and preparation of the manuscript. HZ performed the data analysis and contributed to the discussion. HZM and JL were responsible for data acquisition. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from the patient's parents prior to participation in the present report.

Patient consent for publication

The patient's parents provided their consent for the publication of the associated data of the patient.

Competing interests

The authors declare that they have no competing interests.

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