

# NUDT15 R139C variation increases the risk of azathioprine-induced toxicity in Chinese subjects

## Case report and literature review

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### Abstract

**Introduction:** Azathioprine (AZA) is widely used as an immunosuppressive agent, and its efficacy has been recommended by many clinical studies. However, leukopenia, the most common toxicity, still restricts its clinical applications. Recent studies found that *NUDT15 R139C* polymorphism is strongly associated with AZA-induced leukopenia in Koreans. However, the follow-up studies available are all limited to inflammatory bowel disease (IBD). Here, we report a case of a Chinese patient with Sjögren syndrome (SS) with wild-type *TPMT\*3C* who was diagnosed with AZA-induced severe toxicity due to *NUDT15* mutation based on clinical and laboratory characteristics.

**Case presentation:** A 22-year-old Chinese woman with SS developed severe leukopenia after AZA administration for 21 days. Detection of 6-thioguanine nucleotides (6-TGN) showed that the erythrocyte concentration had beyond the monitoring range, indicating that severe leukopenia might be caused by AZA. Furthermore, gene sequencing showed that *NUDT15 R139C* (poor metabolizer) homozygosity might explain this adverse event. Based on the evidence, AZA administration was immediately stopped and supportive treatments provided, and the patient eventually recovered.

**Conclusion:** In this report, we first provide detailed clinical and laboratory characteristics of AZA-induced leukopenia in a patient with SS with a mutant *NUDT15 R139C* genotype (TT allele) and normal *TPMT* activity. This case indicates that *NUDT15 R139C* and *TPMT\*3C* genotypes, and more importantly, 6-TGN levels, should be routinely monitored for those administered with AZA to predict and prevent AZA-induced toxicity.

**Abbreviations:** 6-MP = 6-mercaptopurine, 6-TGDP = 6-thioguanine-diphosphate, 6-TGN = 6-thioguanine nucleotides, 6-TGTP = 6-thioguanine-triphosphate, AZA = azathioprine, FDA = the US Food and Drug Administration, FK506 = tacrolimus, IBD = inflammatory bowel disease, SNPs = single nucleotide polymorphisms, SS = Sjögren's syndrome, *TPMT* = thiopurine S-methyltransferase.

**Keywords:** azathioprine, Chinese, *NUDT15 R139C*, severe leukopenia, Sjögren syndrome

## 1. Introduction

Azathioprine (AZA) is a thiopurine prodrug commonly used as an immunosuppressive agents in the treatment of autoimmune

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The authors have no conflicts of interest to disclose.

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diseases, such as inflammatory bowel disease (IBD), Sjögren syndrome (SS), and other autoimmune diseases.<sup>[1]</sup> AZA is first nonenzymatically broken down to 6-mercaptopurine (6-MP), which is then converted to the predominant active metabolites 6-thioguanine nucleotides (6-TGN) by a key enzyme, thiopurine S-methyltransferase (*TPMT*).<sup>[2]</sup> Myelosuppression is the main toxicity induced by AZA, which presents as leukopenia, anemia, thrombocytopenia, and pancytopenia.<sup>[3]</sup> The leukopenia of AZA has been reported to be correlated with the erythrocyte level of 6-TGN.<sup>[4–5]</sup> In addition, the association between AZA-induced leukopenia and *TPMT* mutation is well established in Western patients, and a *TPMT* gene test before AZA exposure is recommended by the US Food and Drug Administration (FDA).<sup>[6]</sup> However, an increasing number of studies have found that the frequency of *TPMT* variation is approximately 0.9% in Chinese patients,<sup>[7]</sup> which is considerably lower than that found in European populations, but the incidence of AZA-induced leukopenia in the Chinese patient population is still high (27%–41.3%).<sup>[8]</sup> A recent study suggested that *NUDT15 R139C* (rs116855232), a gene that mediates the hydrolysis of some nucleoside diphosphate derivatives, was strongly associated with AZA-induced leukopenia in Koreans. Additionally, Zhu et al<sup>[9]</sup> reported an association between *NUDT15 R139C* and early leukopenia in Chinese patients with IBD.

SS is a long-term multisystem autoimmune disease that mainly affects the exocrine glands, with dryness of the main mucosal

**Table 1**  
**Characteristics of the patient.**

Characteristics	
Age, years	22
Gender	Female
Weight, kg	55.0
Disease	SS
AZA dose, mg/kg/d	0.91
Date at onset of azathioprine treatment, month/day/year	10/26/2016
Duration of azathioprine treatment, days	21
Date at end of azathioprine treatment, month/day/year	11/16/2016
6-TGN concentration, pmol/8 × 10 <sup>8</sup> RBCs	
11/19/2016	347.5
11/23/2016	600.1
<i>TPMT*3C</i> (rs1142345)	TT (wild type) (normal metabolizer)
<i>NUDT15</i> (rs116855232)	TT (homozygous) (poor metabolizer)

SS = Sjögren's syndrome.

surfaces as the key symptom, and other symptoms including dry skin, chronic cough, and joint pains.<sup>[10]</sup> A regimen of corticosteroids combined with immunosuppressive drugs (e.g., cyclophosphamide, tacrolimus and AZA) was used to treat SS, and the guidelines recommend biologic therapy for SS patients with internal organ or systemic involvement.<sup>[11]</sup> This case reports a Chinese patient with SS with normal *TPMT* activity who developed AZA-induced severe leukopenia due to *NUDT15* R139C variation.

**2. Case presentation**

A 22-year-old Chinese female patient was diagnosed with SS based on the American College of Rheumatology Classification Criteria (2012)<sup>[12]</sup> on January 27, 2015. Patient characteristics

are listed in Table 1. She took 5mg prednisone and 2mg tacrolimus (FK506) every other day to treat SS before she revisited her physician on October 26, 2016. At that time, her proteinuria was still relatively high, indicating her disease was not controlled well by the previous medication. Given that the patient was in the childbearing period, the physician prescribed 50mg/day AZA instead of FK506 because her genotype *TPMT\*3C* (rs1142345) was wild type (normal metabolizer). Unfortunately, severe leukopenia occurred after taking AZA for 21 days. All drugs were immediately stopped, and she was admitted to the rheumatology department on November 19, 2016. During her hospitalization, recombinant human granulocyte colony-stimulating factor (30 μg/day), red blood cells (2U/day), and other supportive treatments were administered. Eventually, the patient recovered on day 10 and physician chose the biologic therapy as follow-up treatment. The results of her blood test during this period are shown in Table 2. Informed consent was obtained from the patient for publication of the case report.

Bone marrow aspiration showed hypoplasia of granulocyte RBC and megakaryocytic series, indicating acute arrest of hemopoiesis. We suspected that severe leukopenia may be induced by AZA. On November 19, 2 mL of venous blood samples (EDTA anticoagulation) were obtained, and the 6-TGN concentration (347.5 pmol/8 × 10<sup>8</sup> RBCs) in the erythrocytes was measured using high performance liquid chromatography as previously described.<sup>[13]</sup> When 6-TGN was measured again on November 23, it was up to 600.1 pmol/8 × 10<sup>8</sup> RBCs. Furthermore, genotyping for *NUDT15* R139C and *TPMT\*3C* was performed using Custom TaqManSNP genotyping assays in accordance with manufacturer's information. The *NUDT15* R139C sequencing indicated that the patient was homozygous (TT), which is a poor metabolizer genotype of this enzyme (Fig. 1). Combined with the patient's clinical and laboratory characteristics, we were convinced that AZA contributed to her severe leukopenia.

**Table 2**  
**Routine blood counts during hospitalisation.**

Date, month/day/year	White blood cells (3.5–9.5 × 10 <sup>9</sup> /L)	Neutrophils (1.8–6.3 × 10 <sup>9</sup> /L)	Red blood cells (3.8–5.1 × 10 <sup>12</sup> /L)	Platelets (125–325 × 10 <sup>9</sup> /L)	Hemoglobin (110–150 g/L)
11/16/2016	1.3	0.5	NA	68	93
11/17/2016	1.6	0.6	NA	65	88
11/19/2016	1.4	0.3	2.33	67	74
11/20/2016	1.3	0.3	2.37	70	75
11/21/2016	1.5	0.1	2.21	53	70
11/23/2016	1.7	0.1	2.03	40	56
11/24/2016	2.2	0.1	2.18	39	57
11/26/2016	2.7	0.6	2.21	34	72
11/29/2016	8.0	4.3	2.28	29	72
12/02/2016	4.7	1.5	2.37	52	75
12/05/2016	4.9	2.0	2.81	111	87

NA = not available.



**Figure 1.** The patient's genotype of *NUDT15* is homozygous (TT allele).

### 3. Literature review and discussion

To the best of our knowledge, one report has been published on primary biliary cirrhosis and autoimmune hepatitis in a patient with the *NUDT15 R139C* variant who developed severe leukopenia following AZA treatment.<sup>[14]</sup> In this report, we first provide detailed clinical and laboratory characteristics of AZA-induced leukopenia in a patient with SS with mutant (TT allele) *NUDT15 R139C* genotype and normal *TPMT* activity. Compared with *TPMT\*3C*, *NUDT15 R139C* maybe a promising biomarker that can be used to predict AZA-induced myelotoxicity.

*TPMT* plays a key role in AZA metabolism. Mutations in the enzyme cause abnormal metabolism and lead to accumulation of excess 6-TGN,<sup>[15]</sup> which can increase the risk of AZA-induced adverse reactions, particularly leukopenia.<sup>[16,17]</sup> Paradoxically, AZA-induced leukopenia is more common in Asians, and the mutant frequency is lower than the 10% prevalence of *TPMT\*3C* reported in the European population.<sup>[18]</sup> Single nucleotide polymorphisms (SNPs) of not only *TPMT\*3C*, but also *NUDT15 R139C* could account for thiopurine-induced toxicity in a variety of diseases.

A literature review showed that *NUDT15 R139C* gene was initially found in 2014 to have a strong association with AZA-induced leukopenia (odds ratio=35.6;  $P=4.8810^{-94}$ ).<sup>[19]</sup> Of 978, Korean patients with IBD, 346 (35.4%) developed leukopenia at a median AZA dose of 1.70 mg/kg/day, and all patients (14/14) homozygous for the *NUDT15 R139C* variant developed early leukopenia (occurrence of leukopenia before 8 weeks). In addition, 25.6% (45/176) and 50% (88/176) of patients who were heterozygous for the *NUDT15 R139C* variant developed early and late leukopenia (occurrence of leukopenia after 8 weeks), respectively. The association of *NUDT15 R139C* with AZA-induced leukopenia has subsequently been demonstrated by other IBD studies.<sup>[20–23]</sup> Besides, other studies in children with acute lymphoblastic leukemia (ALL) have recognized that the *NUDT15 R139C* variant increased the risk of developing thiopurine-induced toxicity ( $P < .00001$ ) and identified a variant in *NUDT15 R139C* gene to be associated with intolerance of thiopurine dose.<sup>[17,24–26]</sup> Suzuki et al<sup>[27]</sup> found that *NUDT15 R139C* genotyping could be beneficial in estimating the tolerated dose of 6-MP for Japanese patients with childhood ALL, particularly during the preschool age (younger than 7 years) ( $P=.04$ ). Furthermore, the variant frequency of *NUDT15 R139C* showed ethnic variability: 9.8% in East Asians, 4.1% in Hispanics, 0.2% in Europeans, and 0.0% in Africans.<sup>[24]</sup> Therefore, the *NUDT15 R139C* variant is common in East Asians and Hispanics and rare in Europeans and Africans.

*NUDT15* is a member of the nudix hydrolase enzyme family, which consists mainly of pyrophosphohydrolases that act on nucleoside diphosphates linked to other moieties, X.<sup>[28]</sup> Moriyama et al<sup>[25]</sup> suggested that *NUDT15* may prevent the incorporation of TGTP and TdGTP into the deoxyribonucleic acid by dephosphorylating the thiopurine-active metabolites TGTP and TdGTP and thus negatively affecting the desired cytotoxic effects of thiopurine in vivo. In vitro studies showed a higher percentage of apoptosis and necrosis in cells transfected with the *NUDT15 R139C* construct compared with cells with the wild type construct.<sup>[19]</sup> However, the specific role of *NUDT15 R139C* in toxicity induced by thiopurine remains unclear.

Additionally, increasing number of studies suggested that the 6-TGN concentration is correlated with clinical responses and adverse drug reactions. 6-TGN blood concentrations not only

help physicians determine the patient's compliance to medication, but can also be used to timely detect AZA-induced adverse effects. Studies have recommended that the reasonable 6-TGN concentration monitoring range is approximately 235 to 450 pmol/8 × 10<sup>8</sup> RBCs for patients with IBD.<sup>[29]</sup> The patient's 6-TGN concentration was 347.5 pmol/8 × 10<sup>8</sup> RBCs when she stopped taking AZA for 3 days. However, after AZA was stopped for 7 days, the level increased up to 600.1 pmol/8 × 10<sup>8</sup> RBCs. The steady-state concentration of AZA is usually achieved after 6 to 8 weeks. The patient had been administered with AZA for 3 weeks; hence, the 6-TGN concentration was still accumulating at that time. Monitoring 6-TGN routinely is important and essential in detecting AZA-induced toxicity in time. However, we found that 6-TGN concentration in autoimmune diseases (such as systemic lupus erythematosus, SS, and others) is significantly lower than the monitoring range in IBD based on our studies (unpublished). The appropriate 6-TGN range for patients with autoimmune disease needs to be established in future. In contrast, Moriyama et al. suggested that *NUDT15* may dephosphorylate the AZA-active metabolites TGTP and TdGTP rather than 6-TGN.<sup>[25]</sup> The new therapeutic monitoring method that detects the metabolites TGTP and TdGTP may have better clinical value than the one that detects 6-TGN.

### 4. Conclusion

We report a case of a Chinese patient with SS with normal metabolizer *TPMT* who developed AZA-induced severe toxicity due to *NUDT15 R139C* homozygosity (TT allele). Previous reports along with this study support the hypothesis that *NUDT15 R139C* variant is a factor for thiopurine toxicity. *NUDT15 R139C* and *TPMT\*3C* genotypes are recommended to be tested. Moreover, 6-TGN level should be monitored routinely to predict and prevent AZA-induced hematotoxicity.

### Author contributions

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