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Research Article

NUDT15 Haplotypes and Diplotypes Predict Thiopurine-Induced Leukopenia and the Influence of Prolonged Exposure to Azathioprine on Hematologic Indices in Patients with Inflammatory Bowel Diseases

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Background. NUDT15 gene polymorphisms have been identified to predispose Asian patients with an inflammatory bowel disease (IBD) to thiopurine-induced leukopenia. This study predicted the influence of NUDT15 haplotypes and diplotypes on azathioprine (AZA)-induced leukopenia as well as the long-term influence of AZA on hematologic parameters in IBD. *Methods*. 194 IBD patients were tested for NUDT15 genotypes. We collected clinical data of 80 patients with AZA treatment including adverse events, dosage, white blood cell (WBC) count, platelet (PLT) count, and mean corpuscular volume (MCV) after AZA initiation. Patients without adverse events and drug withdrawal were followed up for at least one year. The relationship between NUDT15 haplotypes and diplotypes and leukopenia was analyzed. *Results*. The haplotypes NUDT15 c.415C > T and c.36_37insGGAGTC as well as the diplotypes NUDT15 *1/*2, *3/*3, and *3/*5 were significantly associated with AZA-induced leukopenia. Only one patient with NUDT15 c.52G > A experienced leukopenia. NUDT15 *1/*3 was not associated with leukopenia. After AZA initiation, the WBC count showed a downward trend in both wild types and mutants. The mean of WBC count in the mutant group at 1st month after AZA initiation was lower than that in the wild-type group ($P = 0.006$). The MCV increased gradually in mutant cases ($P = 0.039$), and the differences were obvious at 6th and 12th months compared with the baseline ($P = 0.014$ and $P = 0.042$, respectively). The PLT count showed a decreasing trend in the mutant group, but there was no difference until 11 months after initiating treatment ($P = 0.023$). The final dose of AZA in the NUDT15 mutant group was significantly lower than that in the wild-type group ($P = 0.006$). *Conclusion*. NUDT15 polymorphisms may be an appropriate predictor of AZA abnormal hematologic indices in IBD patients. It is necessary for IBD patients to monitor hematological indices and optimize AZA therapy.

1. Introduction

Thiopurine are widely applied to maintain remission in patients with inflammatory bowel disease (IBD), especially steroid-dependent and steroid-resistant cases [1–3]. In addition, thiopurine can prevent the development of antidrug antibodies in those receiving antitumor necrosis factor (TNF)-α antibody [4, 5]. In patients with Crohn's disease (CD), it can also be used to prevent relapse after enterectomy [6]. Nevertheless, drug-induced adverse events often interfere with the application of thiopurine in clinical practice. Up to 15%–30% of IBD patients discontinue thiopurine therapy due to the intolerable adverse effects [7]. Myelosuppression, especially leukopenia, is the most common adverse event which might increase the risk of infection and mortality [8–10].

Thiopurine-induced leukopenia was thought to be related with genetic variants of the thiopurine S-methyltransferase (TPMT) gene which encodes a thiopurine-metabolizing enzyme in Europeans; however, the frequency of the TPMT polymorphisms was considerably lower in Asians (about 1%–3%) [11]. The incidence of thiopurine-induced leukopenia is higher in Asians [9, 12, 13]. A study in 2014 first reported a strong association between NUDT15 c.415C > T and early thiopurine-induced leukopenia in Korean Crohn's disease patients, but not with TPMT gene mutation [14]. This result was subsequently confirmed in Japan, Singapore, and China [13, 15, 16]. Moriyama et al. have identified four NUDT15 coding variants: c.415C > T (p.Arg139Cys), c.36_37insGGAGTC (p.Val18_Val19insGlyVal), c.52G > A (p.Val18Ile), and c.416G > A (p.Arg139His), which resulted in thiopurine-induced leukopenia in patients with acute lymphoblastic leukemia (ALL). Six haplotypes as well as diplotypes composed of different haplotypes with different enzyme activities were also defined [17]. Subsequently, a retrospective study in China confirmed that NUDT15 c.415C > T, c.52G > A, and c.36_37insGGAGTC were associated with thiopurine-induced leukopenia [18]. Recently, a study confirmed that a novel NUDT15 variant (p.Gly17_Val18del) was associated with thiopurine-induced myelosuppression in IBD patients of European ancestry [19].

However, it remains unknown whether the diplotypes to predict thiopurine-induced leukopenia in IBD patients is superior to the haplotypes. In addition, there is still a lack of studies on the long-term effects of thiopurine on hematological indices in Chinese IBD patients. Therefore, we evaluated the associations between NUDT15 polymorphisms and azathioprine (AZA)-induced leukopenia by testing the haplotypes and diplotypes. Meanwhile, we also evaluated the influence of prolonged exposure to AZA on hematologic parameters in Chinese patients with IBD by NUDT15 polymorphisms.

2. Materials and Methods

2.1. Enrollment of Patients and Data Collection. We enrolled 194 Chinese IBD patients who were treated at the First Affiliated Hospital of Nanjing Medical University from September 2018 to June 2020 and tested for NUDT15 genotypes; 80 of 194 patients (73 cases with CD and 7 cases with UC) received AZA treatment and were further analyzed. The self-reported ethnicity of all patients was Chinese Han. The study inclusion criteria were as follows: (1) diagnosis based on the Consensus on Diagnosis and Treatment of Inflammatory Bowel Disease (2018, Beijing) [20] and (2) complete clinical data. Exclusion criteria were as follows: (1) recent blood transfusion; (2) administration of other immunosuppressants such as ciclosporin or methotrexate (MTX); (3) treatments potentially interfering with AZA metabolism, including allopurinol, febuxostat, and angiotensin-converting enzyme inhibitor (ACEI); (4) severe cardiac, hepatic, and/or renal insufficiency; (5) active infection; and (6) pregnancy or lactation.

The initial dose of AZA was 0.5–1.0 mg/kg daily [20]. Patients treated with AZA underwent routine blood tests once a week during the first month, then twice a month for two months, followed by every month, as well as liver function tested every month. AZA dose was increased to 2 mg/kg daily as the target maintenance dosage if patients had no adverse effects [20].

2.2. Genotyping Methods and Hardy–Weinberg Equilibrium (HWE). Two milliliters of peripheral blood samples (EDTA anticoagulation) were obtained for NUDT15 genotype analysis. Genomic DNA was extracted by the TIANamp Blood Spots DNA Kit (Tiangen Biotech (Beijing) Co., Ltd.). DNA samples were amplified by polymerase chain reaction with GoTaq Hot Start Polymerase (Promega, Madison, WI, USA) and purified by TIANquick Mini Purification Kit (Tiangen Biotech (Beijing) Co., Ltd.). Then, DNA samples were genotyped for NUDT15 c.415C > T, c.416G > A, c.52G > A, and c.36_37insGGAGTC by the Sanger chain termination method with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

HWE analysis of the research subjects was carried out by comparing the detected distribution of allele frequencies with the theoretical distribution estimated based on the SNP allelic frequencies. $P > 0.05$ (chi-squared statistics) was considered to indicate equilibrium.

2.3. Data Collection and Follow-Up. The following data were collected: age, sex, weight, type of IBD, Montreal classification, dosage and duration of AZA, concomitant medications, NUDT15 genotypes, white blood cell (WBC) count, platelet (PLT) count, mean corpuscular volume (MCV), and adverse events. The primary endpoint was leukopenia. The secondary endpoint was drug withdrawal for other adverse events or ineffectiveness. Patients without adverse events and drug withdrawal were followed up for at least one year. The tolerance dose of AZA was calculated from the final dose of the patients who could continue AZA for more than six months [18].

Leukopenia was defined as WBC $< 3.5 * 10^9$/L [13, 21], which was graded by common toxicity criteria as follows: grade 1, WBC $3.0–3.5 * 10^9$/L; grade 2, WBC $2.0–3.0 * 10^9$/L; grade 3, WBC $1.0–2.0 * 10^9$/L; and grade 4, WBC $< 1.0 * 10^9$/L. The occurrence of leukopenia within 8 weeks, 8 to 24 weeks, and more than 24 weeks was defined as early, middle, and late leukopenia, respectively [22].

The Ethics Committee of the First Affiliated Hospital of Nanjing Medical University approved this study (2020-SR-132). Written informed consent was obtained from every patient included in the study. The study conforms to the Declaration of Helsinki.

2.4. Statistical Analysis. Student's *t*-test or one-way analysis of variance (ANOVA) was used for normally distributed variables. The Mann–Whitney *U*-test was utilized for non-normally distributed variables. Probability (*P*) values were adjusted by Bonferroni correction when conducting the

pairwise comparison. Differences between categorical variables were analyzed using chi-squared or Fisher's exact test. Logistic regression analyses were performed to identify the associations of leukopenia with candidate variant and the relevant factors. All statistical analyses were carried out using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 8 (GraphPad software, La Jolla, CA). P values less than 0.05 were considered statistically significant.

3. Results

3.1. Clinical Characteristics of IBD Patients. A total of 194 patients with IBD were tested for NUDT15 genotypes. 80 of 194 received AZA treatment. The baseline characteristics of 80 patients treated with AZA are summarized in Table 1. There was no difference in other clinical characteristics between the two groups (Table 1).

3.2. The Adverse Events and NUDT15 Polymorphisms of Patients with IBD. The incidence of adverse events in patients with NUDT15 mutants was significantly higher than that in NUDT15 wild types (68.2% vs. 27.6%, $P = 0.002$). Leukopenia accounted for 68% of all adverse events. The difference in the incidence of leukopenia between patients with NUDT15 wild types and NUDT15 mutants was significant (15.5% vs. 54.5%, $P = 4 \times 10^{-4}$). In addition, AZA final dosage of NUDT15 mutant cases was lower than the dosage of NUDT15 wild-type cases ($P = 0.013$).

NUDT15 gene mutations were detected in 29.4% (57/194) of all patients and 27.5% (22/80) of patients with AZA. No deviation from the Hardy–Weinberg equilibrium (HWE) was detected ($P > 0.05$). Among patients who received AZA therapy, 23.75% (19/80) patients carried NUDT15 c.415C > T heterozygotes (CT) and 1.25% (1/80) patients carried NUDT15 c.415C > T homozygotes (TT). 13.75% (7/80) and 1.25% (1/80) patients carried NUDT15 c.36_37insGGAGTC or c.52G > A heterozygotes, respectively. Homozygotes of NUDT15 c.36_37insGGAGTC or c.52G > A and patients with NUDT15 c.416G > A were not observed (Table 2).

3.3. Associations of NUDT15 Haplotypes with Leukopenia. The mutant allelic frequencies of NUDT15 c.415C > T, c.36_37insGGAGTC, and c.52G > A were 13.1%, 6.9%, and 0.6%, respectively (Table 2). There were significant associations of NUDT15 c.415C > T and c.36_37insGGAGTC with AZA-induced leukopenia ($P = 8 \times 10^{-5}$ and $P = 0.006$, respectively). We observed only one patient with NUDT15 c.52G > A-experienced leukopenia.

Furthermore, we analyzed the incidence of different grades and phases of leukopenia among all NUDT15 genotypes (Table 3). Patients with NUDT15 c.415C > T heterozygotes were more likely to experience grade 2 leukopenia early ($P = 0.002$ and $P = 8 \times 10^{-4}$, respectively). The only one patient carrying NUDT15 c.415C > T homozygote developed grade 3 leukopenia early, while NUDT15 c.36_37insGGAGTC was associated with grade 1 and late leukopenia ($P = 0.003$ and $P = 0.016$, respectively). The only one NUDT15 c.52G > A suffered grade 2 and early leukopenia.

3.4. Associations of NUDT15 Diplotypes with Leukopenia. According to a previous study [17], NUDT15 c.415C > T, c.52G > A, and c.36_37insGGAGTC were inferred as haplotypes *3, *5, and *6. Haplotype *2 was defined as the variants with both NUDT15 c.415C > T and c.36_37insGGAGTC. We found nine diplotypes (*1/*1, *1/*2, *1/*3, *1/*5, *1/*6, *2/*3, *2/*5, *3/*3, and *3/*5) including a new type *2/*5 as compared with previous research in China [18]. Nine patients (15.5%) carrying NUDT15 *1/*1 and 12 patients (54.5%) with NUDT15 mutations (*1/*2, *1/*3, *3/*3, and *3/*5) developed leukopenia (Table 4). NUDT15 *1/*2, *3/*3, and *3/*5 were significantly associated with AZA-induced leukopenia ($P = 4 \times 10^{-4}$ and $P = 0.031$, respectively). However, NUDT15 *1/*3 was not associated with leukopenia ($P = 0.345$). The two patients carried NUDT15 *1/*6 did not develop leukopenia. Individuals carrying NUDT15 *1/*2 were more likely to develop grade 1 leukopenia early and late ($P = 0.001$, $P = 0.012$, and $P = 0.005$, respectively). Early leukopenia was observed not only in the patients with compound-heterozygous genotype *3/*5 but also in the patients with homozygous-variant genotype *3/*3, they experienced grade 3 and grade 2 leukopenia, respectively.

3.5. Univariate and Multivariate Analyses of AZA-Induced Leukopenia. Univariate analysis showed that NUDT15 c.415C > T and c.36_37insGGAGTC significantly contributed to AZA-induced leukopenia. In contrast, sex, age, type of IBD, AZA final dosage, AZA duration, and concomitant therapy were not associated with the risk of leukopenia (Table 5). Further multivariate logistic regression analysis confirmed that NUDT15 c.415C > T was the only independent factor increasing the risk of developing AZA-induced leukopenia (Table 5).

3.6. Influence of Prolonged Exposure to AZA on Hematologic Indices in Different Genotypes of NUDT15. We investigated the alterations of PLT, MCV, and WBC counts after initial AZA treatment (Figure 1). At the baseline, there was no significant difference in theses hematologic indices between NUDT15 mutant group and NUDT15 wild-type group. The WBC count showed a downward trend in both wild types and mutants after AZA therapy, but there was no statistical difference. The mean of WBC count in the mutant group at 1st month after AZA initiation was significantly lower than that in the wild-type group ($P = 0.006$) (Figure 1(a)). We found that MCV in mutant cases increased gradually ($P = 0.039$) (Figure 1(b)). The differences were obvious at 6th and 12th months after initial therapy compared with the baseline ($P = 0.014$ and $P = 0.042$, respectively). The PLT

Table 1: Clinical characteristics and adverse events of patients.

Characteristics	NUDT15 wild type ($n = 58$)	NUDT15 mutant type ($n = 22$)	Total ($n = 80$)	P value
Sex (male/female)	40/18	15/7	55/25	0.946
Age (yr), M(R)	31.5 (17–78)	30.5 (18–58)	31 (17–78)	0.385
Disease type				
CD/UC	52/6	21/1	73/7	0.667
AZA final dosage ($mg \cdot kg^{-1} \cdot d^{-1}$), M(R)	1.36 (0.56–2.5)	1.02 (0.35–2.0)	1.23 (0.35–2.5)	**0.013**
AZA duration (wk), M(R)	48 (4–288)	46 (1–336)	48 (1–336)	0.159
Concomitant therapy	46	14	60	0.148
5-ASA	13	1	14	0.097
Steroids	28	8	36	0.339
IFX	7	6	13	0.171
Thalidomide	10	1	11	0.274
Montreal classification of CD	52	21	73	0.667
Age range at diagnosis (A1/A2/A3)	0/38/14	0/17/4	0/55/18	0.48
Disease location (L1/L2/L3/L4)	21/3/28/7	4/1/16/2	25/4/44/9	0.305
Disease behavior (B1/B2/B3/P)	26/22/6/28	14/5/2/9	40/27/8/37	0.485
Disease extension of UC (E1/E2/E3)	0/0/6	0/0/1	0/0/7	
Adverse events (%)	16 (27.6)	15 (68.1)	31 (38.8)	**0.002**
Leukopenia	9 (15.5)	12 (54.6)	21 (26.2)	$\mathbf{4 \times 10^{-4}}$
Hepatotoxicity	1 (1.7)	0 (0)	1 (1.3)	1
Acute pancreatitis	1 (1.7)	1 (4.5)	2 (2.5)	0.477
Rash	3 (5.1)	1 (4.5)	4 (5.0)	1
Gastrointestinal side effects	2 (3.4)	0 (0)	2 (2.5)	1
Alopecia	0 (0)	1 (4.5)	1 (1.3)	0.275

The P values in bold in the table are less than 0.05.

Table 2: NUDT15 haplotypes and AZA-induced leukopenia.

Genotype		Total patients	Patients with AZA (%)	Prevalence of leukopenia (%)	P	Allele	Allele frequency (%)	P	Odds ratios (95% CI)
c.415C > T	CC	143 (73.7)	60 (75)	9 (15)	$\mathbf{8 \times 10^{-5}}$	C	86.9	$\mathbf{7 \times 10^{-5}}$	6.16 (2.33–16.28)
	CT	45 (23.2)	19 (23.75)	11 (57.9)		T	13.1		
	TT	6 (3.1)	1 (1.25)	1 (100)					
c.36_37ins GGAGTC	–/–	168 (86.6)	69 (86.25)	14 (20.3)	**0.006**	—	93.1	**0.008**	5.70 (1.58–20.62)
	–/ins	26 (13.4)	11 (13.75)	7 (63.7)		Ins	6.9		
c.52G > A	GG	187 (96.4)	79 (98.75)	20 (25.3)	0.262	G	99.4	0.262	3.88 (2.98–5.05)
	GA	7 (3.6)	1 (1.25)	1 (100)		A	0.6		

The P values in bold in the table are less than 0.05.

Table 3: Associations of NUDT15 haplotypes with grades and phases of leukopenia.

Group	NUDT15 c.415C > T, n			P	NUDT15 c.36_37 insGGAGTC, n		P	NUDT15 c.52G > A, n		P
	CC	CT	TT		–/–	–/ins		GG	GA	
Total, n	60	19	1	$\mathbf{8 \times 10^{-5}}$	69	11	**0.006**	79	1	0.262
Normal, n	51	8	0		55	4		59	0	
Leukopenia grade, n	9	11	1	$\mathbf{2 \times 10^{-4}}$	14	ccc7	**0.006**	20	1	0.125
Grade 1	6	5	0	0.025	6	5	**0.003**	11	0	
Grade 2	3	6	0	**0.002**	7	2	0.177	8	1	
Grade 3	0	0	1	0.15	1	0	1	1	0	
Leukopenia phase, n	9	11	1	$\mathbf{5 \times 10^{-4}}$	14	7	**0.015**	20	c1	0.123
Early	1	4	1	$\mathbf{8 \times 10^{-4}}$	4	2	0.09	5	1	
Middle	2	2	0	0.115	3	1	0.288	4	0	
Late	6	5	0	0.025	7	4	**0.016**	11	0	

P values were adjusted by Bonferroni correction. The P values in bold in the table are the most statistically significant in the subgroup analysis.

Table 4: Associations of NUDT15 diplotypes with grades and phases of leukopenia.

Group	*1/*1	*1/*2	*P*	*1/*3	*P*	*3/*3 + *3/*5	*P*
Total, *n*	58	9		9		2	
Normal, *n*	49	2	**0.0004**	6	0.345	0	**0.031**
Leukopenia grades, *n*	9	7	**0.0007**	3	0.056	2	**0.0019**
Grade 1	6	5	**0.001**	0		0	
Grade 2	3	2	0.036	3		1[†]	0.075
Grade 3	0	0		0		1[‡]	**0.02**
Leukopenia phases, *n*	9	7	**0.0005**				**0.0026**
Early	1	2	**0.012**	1		2	**0.039**
Middle	2	1	0.16	1		0	
Late	6	4	**0.005**	1		0	

[†]NUDT15 *3/*5; [‡]NUDT15 *3/*3; *P* values were adjusted by Bonferroni correction. The *P* values in bold in the table are the most statistically significant in the subgroup analysis.

count decreased gradually in the mutant group, but the decreasing trend was not significant ($P > 0.05$). The changing trend of PLT count was also not apparent in the wild-type group. Although there was no statistical difference in PLT count between the two groups at 1st, 3rd, 6th, and 9th months, the PLT count was lower in mutant cases than wild-type cases at 12th month ($P = 0.023$) (Figure 1(c)).

3.7. AZA Final and Tolerance Dosage among Patients with Different NUDT15 Genotypes. We analyzed the final dose of AZA among individuals at the last visit (Figures 2(a) and 2(b)). The dosage of AZA in patients carrying NUDT15 mutant alleles was lower than the dosage in patients with NUDT15 wild types (1.02 (0.35–2.0) $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ vs. 1.36 (0.56–2.5) $mg{\cdot}kg^{-1}{\cdot}d^{-1}$, $P = 0.013$). There was no significant difference in final dose among diplotypes except for *1/*1 and *3/*5 (1.36 (0.56–2.50) $mg{\cdot}kg^{-1}{\cdot}d^{-1}$ vs. 0.35 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$, $P = 0.034$). There were no significant differences in the tolerance dose between individuals with wild types and mutations (Figures 2(c) and 2(d)). The tolerance dose could not be evaluated because of early discontinuation of AZA in both *3/*3 and *3/*5 cases.

4. Discussion

Azathioprine is currently the first-line therapy for IBD patients to maintain long-term remission [1–3]. However, drug-related adverse events often affect its application in clinical practice, among which myelosuppression is most common. In our study, we found that 26.3% of IBD patients experienced leukopenia, which was similar to previous reports [14, 15, 18]. It is urgent to explore the predictors of AZA-induced leukopenia to guide the rational regimen of AZA therapy.

The nucleoside diphosphate-linked moiety X-type motif 15 (NUDT15, also known as MTH2) gene encodes a purine-specific Nudix hydrolase which hydrolyzes nucleoside diphosphate. NUDT15 downregulates the level of thioguanosine triphosphate (TGTP), the ratio of TGTP/monophosphate thioguanosine nucleotide (TGMP), and the percentage of TGTP in total 6-thioguanine nucleotides (6-TGN), which weakens the cytotoxicity of thiopurine and finally reduces the efficacy [17]. Four NUDT15 coding variants (c.415C > T, c.416G > A, c.52G > A, and c.36_37 insGGAGTC) result in 74.4–100% loss of nucleotide diphosphatase activity [17]. The association of NUDT15 c.415C > T with thiopurine-induced leukopenia has been confirmed in Asian IBD [13–16].

In this study, we verified again that NUDT15 c.415C > T, c.52G > A, and c.36_37insGGAGTC were associated with thiopurine-induced leukopenia. Our results suggested that NUDT15 polymorphisms can predict AZA-induced leukopenia, and the monitoring of hematologic indices in IBD patients with AZA therapy should be long-term and careful. Logistic regression analysis confirmed that NUDT15 polymorphism was the only factor increasing the risk of developing AZA-induced leukopenia. There were no differences in sex, AZA dosage, and corticosteroid usage in patients with or without leukopenia. Combined with previous results, we inferred that corticosteroid might have no protective effect on AZA-induced leukopenia in IBD patients [13, 18, 21], and the myelosuppression caused by NUDT15 polymorphisms may be dose independent [8]. The association between sex with leukopenia is still controversial [13, 18, 23]. More studies are needed to prove whether there is a gender difference in AZA-induced leukopenia in the IBD population.

This study analyzed the relationship between NUDT15 diplotypes and AZA-induced leukopenia. Early leukopenia was found in the carriers of low-activity genotype *3/*3 and *3/*5, and they experienced grade 3 and grade 2 leukopenia, respectively. NUDT15 *1/*3 was not associated with leukopenia. The two patients carried NUDT15 *1/*6 did not develop leukopenia. Individuals carrying *1/*2 were more likely to develop grade 1 leukopenia early and late. Haplotype *2 is composed of NUDT15 c.415C > T and c.36_37insGGAGTC which were in strong gene linkage disequilibrium, so we infer that the enzyme activity of NUDT15 *1/*2 may be lower than that of NUDT15 *1/*3 and *1/*6. By testing NUDT15 c.36_37insGGAGTC, we can distinguish the diplotype *1/*2 from the diplotype *1/*3 and *1/*6, which may be helpful to guide the administration of AZA.

In this study, we evaluated the influence of prolonged exposure to AZA on hematologic indices in Chinese IBD population by NUDT15 polymorphisms, including WBC count, MCV, and PLT count. WBC count showed a downward trend after beginning AZA therapy. The mean of WBC count in the mutant group at 1st month after AZA initiation

Table 5: Univariate and multivariate analyses of AZA-induced leukopenia.

Characteristics	With leukopenia	Without leukopenia	Univariate analysis			Multivariate analysis		
			P	OR	95% CI	*P*	OR	95% CI
No. of subjects, *n*	21	59						
Female number (%)	7 (30)	18 (30.5)	0.81					
Age (years), M(R)	32 (20–58)	29 (17–78)	0.491					
Group of age (yr)			0.39					
17–40	14	45						
>40	7	14						
NUDT15 (%)								
c.415C > T	12 (57.1)	8 (13.6)	**7.5×10^{-5}**	**8.5**	**2.72–26.61**	**0.02**	**4.90**	**1.28–18.69**
c.36_37insGGAGTC	7 (30)	4 (6.8)	**0.006**	**6.88**	**1.76–26.82**	0.193	2.39	0.87–16.21
c.52G > A	1 (4.8)	0 (0)	0.262	3.95	2.71–5.77			
Type of disease, *n*			0.669					
CD	20	53						
UC	1	6						
AZA final dosage ($mg \cdot kg^{-1} \cdot d^{-1}$), mean ± SD	1.23 ± 0.52	1.30 ± 0.44	0.549					
AZA duration (wk), M(R)	48 (2–336)	48 (1–228)	0.248					
Concomitant therapy, *n*	13	40	0.624					
5-ASA	2	15	0.213					
Steroids	8	27	0.459					
IFX	2	5	1					
Thalidomide	3	5	0.426					

The *P* values in bold in the table are less than 0.05.

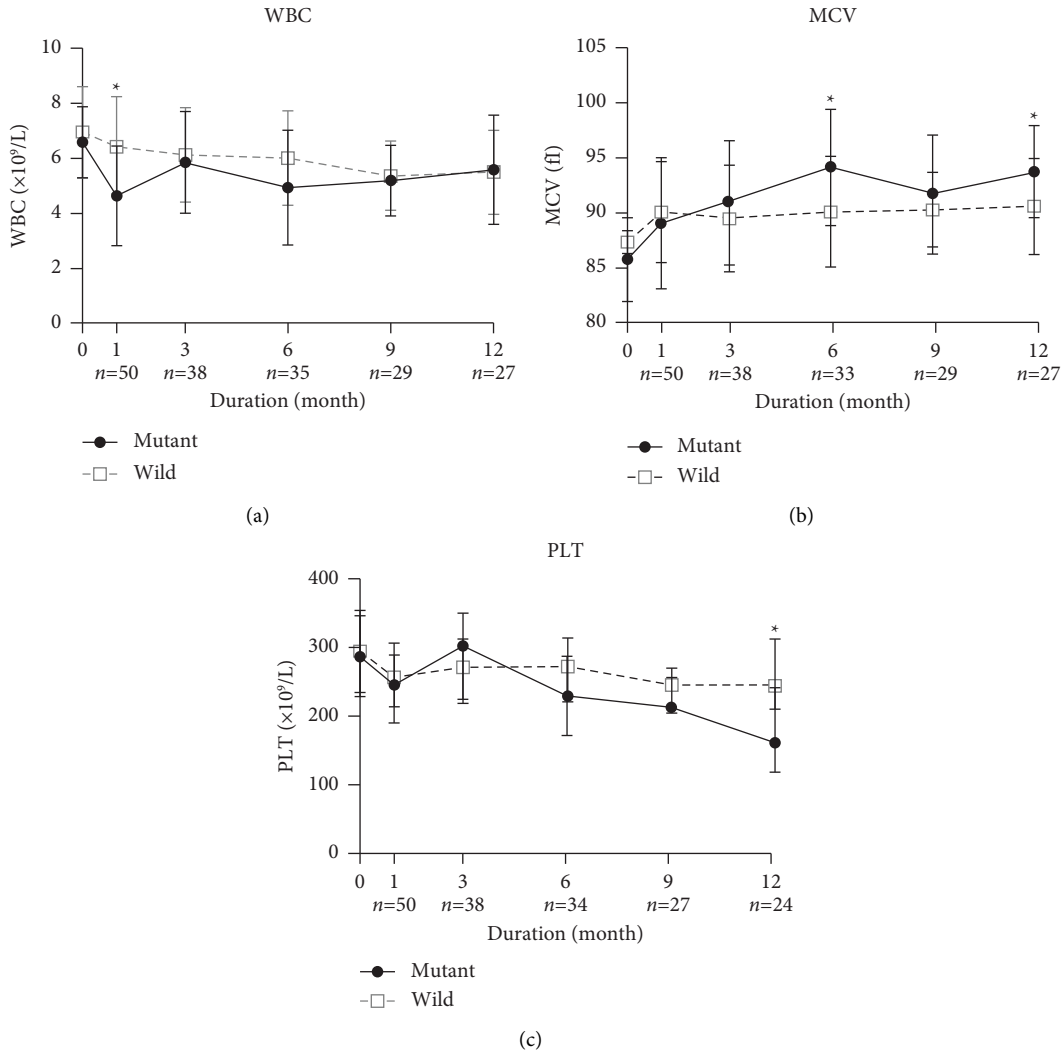

Figure 1: Influence of prolonged exposure to AZA on hematologic indices in different NUDT15 genotypes: (a) changes of WBC count in the wild-type group and the mutant group. *Significant difference between the mutant group and wild-type group at 1st month ($P = 0.006$, Student's t-test). (b) Changes of MCV in the wild-type group and mutant group. *Significant differences between the baseline and 6th and 12th months. ($P = 0.014$ and $P = 0.042$, respectively, ANOVA and Bonferroni correction adjusted P values). (c) Changes of PLT count in the wild-type group and mutant group. *Significant difference between the mutant group and wild-type group at 12th month ($P = 0.023$, Mann–Whitney U-test).

was significantly lower than that in the wild-type group. The results were consistent with our previous conclusion that patients with mutant diplotypes were prone to early leukopenia, which was similar to a previous study in Japanese IBD population [24]. In addition, we also found that the differences in the WBC count between two groups at 9th and 12th month were slight. This phenomenon might be due to the fact that the patients who could not tolerate AZA already discontinued the therapy, while the WBC count of remaining patients who could tolerate AZA might be similar to that of patients with NUDT15 wild-types. Therefore, we should monitor the WBC count more closely, especially in the first month.

Several studies demonstrated that thiopurine-mediated inhibition of DNA synthesis in bone marrow precursor cells leads to megaloblastic erythropoiesis, which is characterized by an increase in the MCV value [25–28]. MCV elevation above 7 fl is also reported to be an indicator of optimal 6-TGN levels [26]. Measurement of MCV may be a simple and inexpensive alternative to measurement of 6-TGN concentrations in patients treated with azathioprine or 6-mercaptopurine [26]. We did not measure the concentrations of folate and vitamin B12, but the MCV of all patients were in the normal range before AZA initiation. We observed that the MCV increased gradually in mutant cases, which indicated that the best MCV value for predicting the optimal 6-TGN levels should be adjusted according to NUDT15 genotypes. It also suggested that the red blood cells of patients with mutations were more sensitive to AZA than those with wild-types. These conclusions were similar to a Japanese study reporting that the MCV was higher in the NUDT15 c.415C > T C/T group than in the C/C group [24].

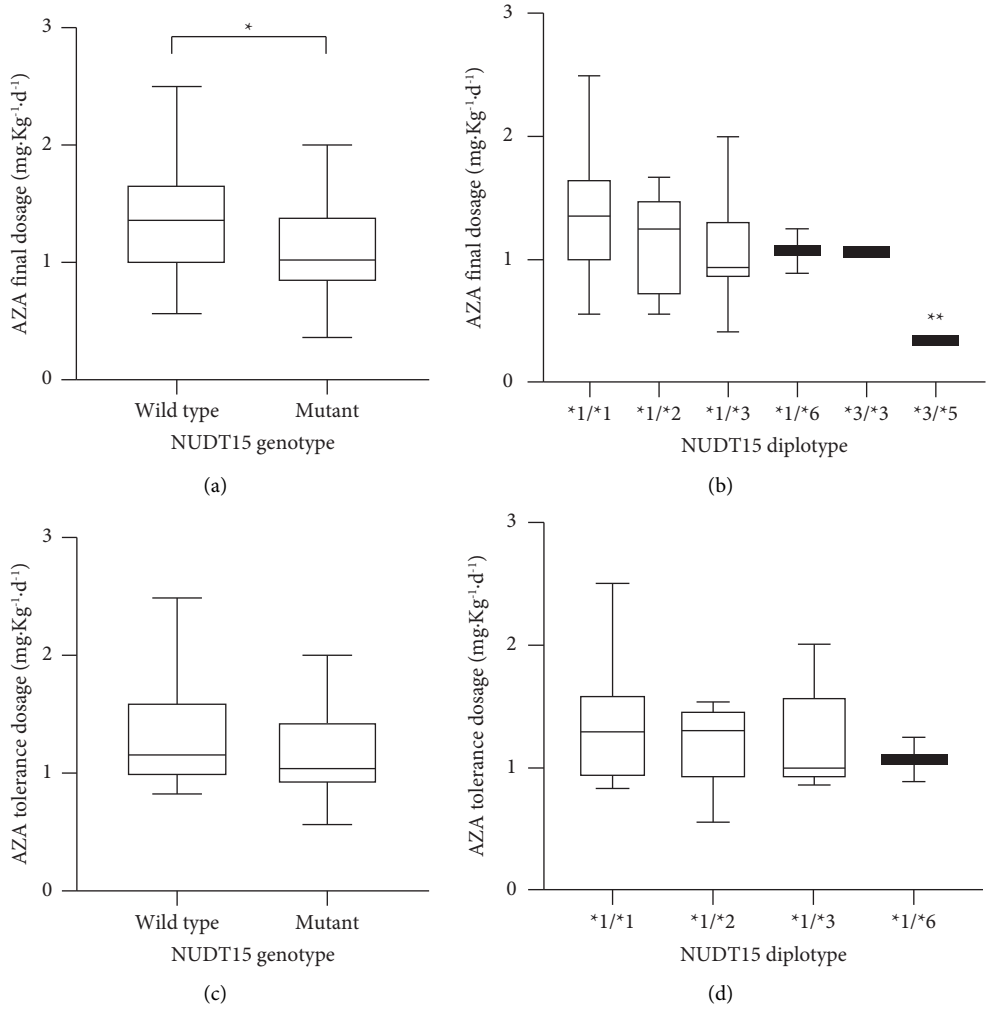

Figure 2: AZA final and tolerance dosage among patients with different NUDT15 genotypes: (a) final dosage between NUDT15 wild-type cases and mutant cases. *Significant difference between the mutant group and wild-type group (1.02 (0.35–2.0) mg·kg^{-1}·d^{-1} vs. 1.36 (0.56–2.5) mg·kg^{-1}·d^{-1}, $P = 0.006$, Mann–Whitney U-test). (b) Final dosage among individuals with different diplotypes. *1/*1 (1.36 (0.56–2.50) mg·kg^{-1}·d^{-1}), *1/*2 (1.25 (0.56–1.67) mg·kg^{-1}·d^{-1}), *1/*3 (0.94 (0.41–2.0) mg·kg^{-1}·d^{-1}), *1/*6 (1.07 (0.89–1.25) mg·kg^{-1}·d^{-1}), *3/*3 (1.06 mg·kg^{-1}·d^{-1}), and *3/*5 (0.35 mg·kg^{-1}·d^{-1}). **Significant difference between the NUDT15 *1/*1 group and NUDT15 *3/*5 group ($P = 0.034$). (c) Tolerance dosage between wild-type cases and mutant cases (1.15 (0.83–2.50) mg·kg^{-1}·d^{-1} vs. 1.04 (0.56–2.0) mg·kg^{-1}·d^{-1}, $P > 0.05$). (d) Tolerance dosage among individuals with different diplotypes ($P > 0.05$). *1/*1 (1.15 (0.83–2.50) mg·kg^{-1}·d^{-1}), *1/*2 (1.31 (0.56–1.54) mg·kg^{-1}·d^{-1}), *1/*3 (1.00 (0.86–2.00) mg·kg^{-1}·d^{-1}), and *1/*6 (1.07 (0.89–1.25) mg·kg^{-1}·d^{-1}).

It is controversial whether there exists a difference in the PLT count between the NUDT15 wild-type group and mutant group after AZA initiation [21, 24]. Our study revealed that the PLT count decreased gradually in the mutant group but not statistically different. However, the PLT count in the mutant group was significantly lower at the 12th month after initiation of AZA. These results suggested that it is necessary for IBD patients to carefully monitor hematological indices for a long time and optimize the AZA dose.

Finally, we compared the final dose of AZA in all patients and the tolerance dose in patients with different diplotypes. The final dose of AZA in the NUDT15 mutant group is lower than that in the wild-type group. There was no significance in the tolerance dose among these diplotypes. Patients with both *3/*3 and *3/*5 diplotypes were unable to tolerate AZA and discontinued AZA therapy at an early stage. There might be type 2 errors in the statistical analysis due to the small sample size. However, we cannot rule out the possibility that there was no difference in the tolerance dose between patients with *1/*1 and patients, who did not develop leukopenia after six months of AZA therapy, with mutations. This result may support the view that the myelosuppression caused by NUDT15 polymorphisms may be dose independent [8] and suggest that other factors besides NUDT15 polymorphisms may contribute to AZA intolerance in Chinese IBD patients.

This study also has several limitations. First, it was a small sample study in a single center. Second, because of the various causes of anemia in IBD patients, we did not analyze the hemoglobin count of IBD patients. Third, other genes that may affect AZA metabolism were not detected.

In conclusion, despite these limitations, NUDT15 polymorphism is indeed an appropriate predictor of AZA intolerance in IBD patients. Our study compared the differences in grades and phases of leukopenia caused by AZA among different diplotypes. We found that testing diplotypes to predict thiopurine-induced leukopenia in IBD patients may be superior to the haplotypes. In addition, this study had evaluated the effects of NUDT15 polymorphisms on the prediction of prolonged exposure to AZA on hematological indices of Chinese IBD population, which provides more suggestions for rational regimen of AZA therapy in patients with IBD.

Data Availability

The data used to support the findings of this study are available from the corresponding author on reasonable request.

Disclosure

This study has been presented as AGA Abstracts previously.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

Wenyu Jiang and Shasha Wu contributed to this work equally.

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