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Research Article

Impact of *NUDT15* polymorphisms on myelosuppression in Thai patients with autoimmune diseases undergoing azathioprine therapy

Kanyarat Khaeso[1], Chingching Foocharoen[2], Ajanee Mahakkanukrauh[2], Chinadol Wanitpongpun[2], Siraphop Suwannaroj[2], Nontaya Nakkam[1], Suda Vannaprasaht[1], Areerat Dornsena[1], Wichittra Tassaneeyakul[1]*

[1] Department of Pharmacology, Faculty of Medicine, Khon Kaen University, Thailand
[2] Department of Medicine, Faculty of Medicine, Khon Kaen University, Thailand

ABSTRACT

Azathioprine (AZA) is an immunosuppressive drug widely used to treat autoimmune diseases but poses a significant risk of myelosuppression. Genetic polymorphisms affecting AZA metabolism, particularly in nucleoside diphosphate-linked moiety X-type motif 15 (*NUDT15*), thiopurine S-methyltransferase (*TPMT*), inosine triphosphatase (*ITPA*), and ATP-binding cassette sub-family C member 4 (*ABCC4*), may influence this risk. This study aimed to evaluate the impact of *NUDT15*, *TPMT*, *ITPA*, *ABCC4* polymorphisms on AZA-induced myelosuppression in Thai patients with autoimmune diseases. A total of 125 Thai patients receiving AZA were enrolled. Genotyping of *NUDT15*, *TPMT*, *ITPA*, and *ABCC4* polymorphisms was performed, and phenotypes were inferred from genotype data. Clinical and laboratory parameters were monitored for one year following AZA initiation. Among 125 patients, 19 (15.2%) developed AZA-induced leukopenia. *NUDT15* polymorphisms showed the strongest association with leukopenia risk. Intermediate metabolizer (IM) and poor metabolizer (PM) phenotypes of *NUDT15* were significantly associated with increased leukopenia risk, with odds ratios of 8.57 (95% CI 2.37–31.07, P = 0.001) and 30.00 (95% CI 2.82–319.20, P = 0.005) compared with the normal metabolizer (NM). No significant associations were observed for *TPMT*, *ITPA*, or *ABCC4* polymorphisms. Patients with IM *NUDT15* exhibited significantly lower final white blood cell counts and required lower average AZA doses. Therefore, *NUDT15* polymorphisms are key determinants of AZA-induced leukopenia in Thai patients. Early identification of *NUDT15* polymorphism allows personalized dosing strategies, reducing the risk of AZA-induced myelosuppression.

Keywords:
Autoimmune diseases; Azathioprine; Leukopenia; Myelosuppression; *NUDT15*

1. INTRODUCTION

Azathioprine (AZA) is a thiopurine drug commonly used as immunosuppressive drug in the treatment of several autoimmune disorders including Crohn's disease, rheumatoid arthritis (RA), Systemic lupus erythematosus (SLE)[1]. AZA is a prodrug that undergoes extensive metabolism in the extracellular medium to 6-mercaptopurine (6-MP) through glutathione-S-transferase (GST) or non-enzymatic reactions[2]. The disposition and metabolic pathways of AZA is shown in Figure 1[3]. Once formed, 6-MP is transported into cells, where it is metabolized by various enzymes into its active metabolites. Initially, 6-MP is catalyzed by hypoxanthine phosphoribosyltransferase (HPRT) to form 6-thioinosine monophosphate (6-TIMP), which is further metabolized by inosine monophosphate dehydrogenase (IMPDH) and guanosine monophosphate synthetase (GMPS) into the active metabolites thioxantosine monophosphate (TXMP) and 6-thioguanine nucleotides (6-TG). These 6-TGN

***Corresponding author:**
* Wichittra Tassaneeyakul Email: wichitt@kku.ac.th

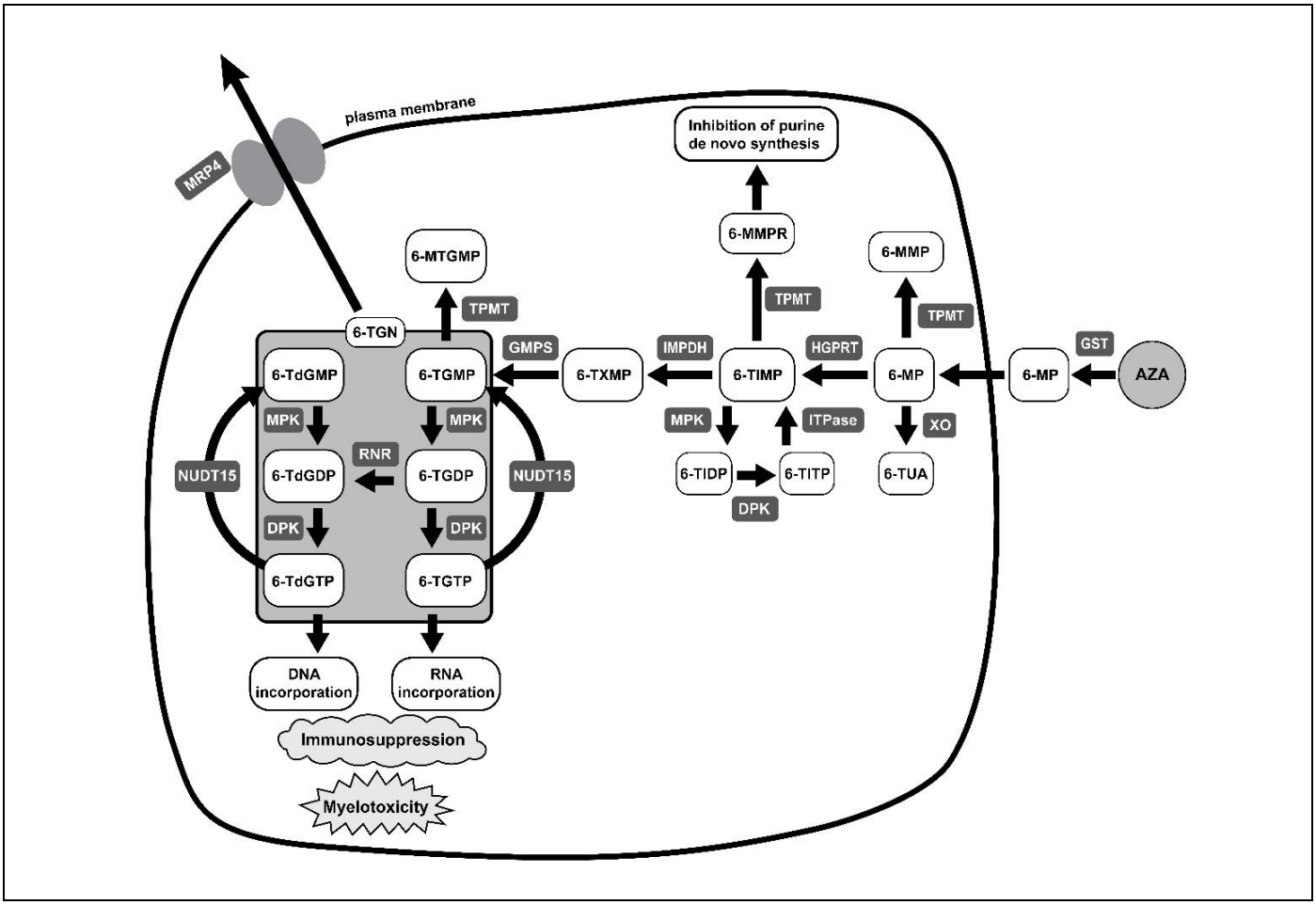

Figure 1. Azathioprine (AZA) metabolic and disposition pathways

Notes: Enzymes or transporters are shown in black boxes: DPK, diphosphate kinase; GMPS, guanosine monophosphate synthetase; GST, glutathione-S-transferase; HGPRT, hypoxanthine phosphoribosyl transferase; IMPDH, inosine monophosphate dehydrogenase; ITPase, inosine triphosphate pyrophosphatase; MPK, monophosphate kinase; MRP4 multidrug resistance associated protein; *TPMT* thiopurine S-methyl transferase; XO, xanthine oxidase.

Metabolites of AZA are shown in white boxes: 6-MMP, 6-Methylmercaptopurine; 6-MMPR, 6-methylmercaptopurine ribonucleotides; 6-MP 6-mercaptopurine; 6-MTGMP, 6-methylthioguanine monophosphate; 6-TdGMP, 6-thio-deoxyguanine monophosphate; 6-TdGDP, 6-thio-deoxyguanine Diphosphate; 6-TdGTP, 6-thio-deoxyguanine triphosphate; 6-TGDP, 6-thioguanine diphosphate; 6-TGMP, 6-thioguanine monophosphate; 6-TGN, 6-thioguanine nucleotides; 6-TGTP, 6-thioguanine triphosphate; 6-TIDP, 6-thioinosine diphosphate; 6-TIMP, 6-thioinosine monophosphate; 6-TITP, 6-thioinosine triphosphate; 6-TUA, 6-thiouric acid; 6-TXMP, 6-thioxanthosine monophosphate.

metabolites serve as precursors for incorporation into DNA and RNA, thereby inhibiting nucleotide and protein synthesis, leading to cytotoxic and immunosuppressive effects. Additionally, the dephosphorylation of 6-TGN, which hinders the incorporation of these metabolites into DNA, is mediated by nucleoside diphosphate-linked moiety X-type motif 15 (*NUDT15*) enzyme. Moreover, intracellular inactivation of 6-MP catalyzes by two major enzymes, thiopurine S-methyltransferase *(TPMT)* and xanthine oxidase (XO), which convert it into inactive methylated metabolites, 6-methylmercaptopurine, and 6-thiouric acid. These inactive metabolites are subsequently excreted via urine.

Although azathioprine (AZA) provides important immunosuppressive benefits, its use can be limited by adverse effects, most notably myelosuppression. This potentially life-threatening condition can cause profound neutropenia or agranulocytosis, predisposing patients to severe infections, sepsis, multi-organ failure, and, in rare cases, death. Even nonfatal episodes often require hospitalization, intravenous antibiotics, granulocyte colony-stimulating factor (G-CSF), blood transfusions, and prolonged follow-up. Patients who develop hematologic toxicity frequently need abrupt drug discontinuation or dose reductions, which can lead to loss of disease control and increased reliance on alternative therapies that may be more toxic or costly. The resulting healthcare and economic burden include inpatient care, additional outpatient visits and laboratory monitoring, and indirect costs from morbidity and lost productivity.

Genetic variations in the genes encoding enzymes responsible for the metabolism of this drug and its derivative, 6-MP have been proposed as key factors influencing individual susceptibility to AZA-induced myelosuppression. *TPMT* polymorphisms have been extensively studied[4-6]. The most common

variants associated with reduced *TPMT* activity include *TPMT**2 (c.238G>C, p. Ala80Pro), *TPMT* *3A (c.460G>A and c.719A>G), and *TPMT* *3C (c.719A>G)[6]. Ethnic differences in the polymorphism of *TMPT* gene are well recognized. The *TPMT*3C* allele is the most prevalent variant allele in Asian populations whereas *TPMT*3A*, and *TPMT*2* are the common variant alleles in Caucasians[6]. Homozygous carriers of these variants have no functional *TPMT* activity, while heterozygous individuals exhibit intermediate activity[6]. Several lines of evidence suggest that individuals who carry these variant alleles were at a higher risk of thiopurine-induced myelosuppression[5,7,8].

Unlike *TPMT*, which mainly affects detoxification of thiopurine drugs, *NUDT15* affects thiopurine activation. The common variant alleles are *NUDT15*2* (c.36_37insGGAGTC; c.415C > T), *NUDT15*3* (c.415C > T), *NUDT15*4* (c.416G > A), *NUDT15*5* (c.52G > A), and *NUDT15*6* (c.36_37insGGAGTC)[9]. The *NUDT13*3* is the most prevalent in in East Asian and Southeast Asian populations,[9-12] with up to 20% of individuals being carriers. Homozygous carriers of *NUDT15**3 have a markedly increased risk of thiopurine-induced myelosuppression[12-15]. Associations between thiopurine-induced myelosuppression and other less common variants, such as *NUDT15*5*, and *NUDT15*6* are less well-characterized[16-18].

In addition to *NUDT15* and *TPMT*, polymorphisms in *ITPA* gene and the ABC transporter gene, *ABCC4* have also been reported to be associated with thiopurine-induced myelosuppression[17-19]. More than ten single-nucleotide polymorphisms in *ITPA* have been described; however, only two variants c.94C>A (rs1127354) and g.IVS2+21A>C (rs7270101) have been most consistently associated with reduced enzyme activity and thiopurine toxicity[20,21]. The c.94C>A (rs1127354) coding variant is associated with markedly reduced ITPase activity (likely via altered protein stability and/or aberrant processing), with heterozygotes showing partially reduced activity and homozygotes exhibiting little or no detectable activity[21]. The intronic g.IVS2+21A>C variant leads to abnormal mRNA splicing and reduced *ITPA* expression; heterozygotes have been reported to retain roughly 60% residual activity[20]. The c.94C>A allele is relatively common in Asian populations but rare in Caucasians[22], whereas g.IVS2+21A>C has been reported more frequently in Caucasians and appears absent or rare in some Asian cohorts[20,23]. In a previous acute leukemia Thai cohort, the frequency of A allele of the c.94C>A was reported as 0.175 [95% CI 0.136-0.219][24], data on the frequency of g.IVS2+21A>C in Thais remain limited. Previous study has suggested that individual who exhibit lower ITPase activity may lead to accumulation of toxic 6-TITP and subsequently lead to higher thiopurine toxicity[11,25,26,27].

The *ABCC4* gene encodes the multidrug resistance-associated protein 4 (MRP4), an ATP-binding cassette transporter involved in the efflux of the active thiopurine metabolite 6-thioguanine nucleotide (6-TGN) (Figure 1). In a multi-ethnic survey of 270 healthy individuals (African American, Caucasian, Asian American, and Mexican American), more than 98 *ABCC4* variants were identified, but only three non-synonymous variants reached frequencies >5% in at least one ethnic group[28]. These variants c.559G>T (rs11568658), c.2269G>A (rs3765534), and c.912C>A (rs2274407) have been reported to reduce MRP4 function relative to the reference protein[28]. It has been hypothesized that reduced MRP4 activity can increase intracellular 6-TGN accumulation and thereby enhance thiopurine-related toxicity. Studies in MRP4-deficient mice demonstrated decreased efflux, subsequent 6-TGN accumulation and increased thiopurine toxicity[25]. In Japanese patients with inflammatory bowel disease, carriers of *ABCC4* c.2269G>A exhibited higher 6-TGN levels and a significant association with thiopurine-induced leukopenia[26]. However, the contribution of *ITPA* and *ABCC4* polymorphisms to AZA-induced myelosuppression remains controversial, as several studies have failed to find significant associations between these variants and thiopurine-related hematotoxicity[11,29,30].

To date, studies investigating genetic polymorphisms of these key enzymes involved in AZA disposition and AZA-induced myelosuppression within the same cohort remain limited. However, several studies have demonstrated that the impact of *NUDT15* polymorphism on thiopurine-induced hematopoietic toxicity appears to be more pronounced than that of *TPMT* in East Asian populations[31-33]. The reduced influence of *TPMT* polymorphism in East Asian populations may be partly explained by the lower frequency of *TPMT* variants in these populations. Interestingly, the frequency of *TPMT* polymorphism in the Thai population is nearly three times higher than in East Asian populations, while the prevalence of *NUDT15* polymorphism is comparable [24,30]. This study was conducted in Thai patients with autoimmune diseases to extensively characterize the association between *NUDT15, TPMT, ITPA c.94C>A* and *ABCC4 c.2269G>A* polymorphisms and the occurrence of myelosuppression during AZA treatment.

2. MATERIALS AND METHODS

2.1 Patients

One hundred twenty-five of Thai patients with autoimmune diseases treated with azathioprine (AZA) were retrospectively enrolled from the outpatient clinic at Srinagarind Hospital, Khon Kaen University, from

December 2018 to September 2020. Patients who received allopurinol or other drugs which inhibit XO enzymes were excluded in this study. Patients were treated with Aza from Written informed consent was obtained from patients after receiving information about experimental procedures and purposes of the study. Approval for this study was obtained from the Ethics Committee for Human Research of Khon Kaen University (HE611405).

2.2 Detection of *TPMT*, *NUDT15*, *ITPA* and *ABCC4* variants

Genomic DNA was purified from peripheral blood leucocytes and genotyping of *TPMT*3C* (Assay ID ANU7DH7), *ITPA* c.94C>A (Assay ID C__27465000_10), *ABCC4* c.2269G>A (Assay ID C__27478235_20) and *NUDT15*3* (Assay ID C_154823200_10), *NUDT15*5* (Assay ID C_181955856_10) were performed using TaqMan SNP genotyping assays on a QuantStudio 6 Flex Machine (Applied Biosystems, Waltham, MA) as previously described[30]. *NUDT15* variants in exon 1 including two common variants *(i.e. NUDT15*2* and *NUDT15*6*) and other nine rare variants *(i.e. NUDT15*7-*12*, *NUDT15*14*, *NUDT15*16* and *NUDT15*19*) were determined as previously described[9].

2.3 Data collection and statistical analysis

Clinical and laboratory data including complete blood counts, liver function and clinical chemistry as well as well as list of drugs at each hospital visit were obtained by retrospectively reviewing patients' medical records for about 1 year after AZA initiation. The white blood cell (WBC) count at the last dose of AZA that the patient received was record as the final WBC count while the average WBC count was the average value of WBC counts during the AZA treatment periods.

The information about the dose, adverse drug reactions/drug toxicity, history of discontinuous of AZA during the AZA treatment period were collected. Patients with AZA-induced leukopenia defined as the patients who had WBC <3,000 per µl after treatment of AZA. Early leukopenia was defined as the patient developed leukopenia within 8 weeks after initiation of AZA treatment while late leukopenia was defined as the patient developed leukopenia after 8 weeks of AZA initiation.

The phenotypes of *TPMT* and *NUDT15* were classified based on the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline of thiopurines dosing[34] in which the *TPMT*1/*3C* carriers are categorized as intermediate metabolizers (IM) and the *TPMT*3C/*3C* carriers are classified as poor metabolizers (PM) of *TPMT* whereas individuals who did not carry *TPMT*3C* are classified as normal metabolizers (NM) of *TPMT*. The *NUDT15*1/*1* carriers are classified as normal metabolizers (NM) whereas *NUDT15*1/*5*, *NUDT15*1/*6* carriers are classified as indeterminate metabolizer (IDM), *NUDT15*1/*2*, *NUDT15*1/*3* carriers are classified as intermediate metabolizer (IM), *NUDT15*2/*5*, *NUDT15*3/*6* carriers are classified as possible intermediate (possible IM) *NUDT15*2/*3, and NUDT15*3/*3* are classified as poor metabolizer (PM)[34].

Allele frequencies were calculated as the number of copies of the allele of interest divided by twice the number of individuals with non-missing genotype data and 95% confidence intervals (CIs) were reported. Departure from Hardy–Weinberg equilibrium was assessed by comparing observed and expected genotype frequencies using the chi-square ($\chi 2$) test. Associations between genetic polymorphisms and AZA-induced myelosuppression were evaluated with univariate logistic regression; odds ratios (ORs) and 95% CIs are reported. Haldane's modification of Woolf's formula was used for samples containing zero values[35]. The relationship between *TPMT*, *NUDT15*, *ITPA c.94C>A* and *ABCC4 c.2269G>A* genetic polymorphisms on dosage of AZA, amounts of WBC counts and duration of AZA treated were determined by using Kruskal-Wallis test with pairwise comparisons. A *P*-value of less than 0.05 was considered statistically significant. Data analyses were performed using SPSS software (IBM Corp., New York City, NY) or Stata software (StataCorp, College Station, TX).

3. RESULTS

3.1 Patient characteristics

A total of 125 autoimmune diseases patients consisting of 19 males (15.20%) and 106 females (84.80%). According to the type of diseases, most of them were diagnosed with SLE (87 patients, 69.60%), followed by RA (14 patients, 11.20%). The clinical demographic data of enrolled patients are shown in Table 1.

Among the *NUDT15* variants, *NUDT15*3* was the most common variant with an allele frequency of 0.076 [95% CI 0.046-0.116], followed by *NUDT15*6* (0.032, 95% CI 0.014-0.062), *NUDT15*2* (0.008, 95% CI 0.001-0.029] and *NUDT15*5* (0.004, 95% CI 0-0.022). Other variants and no novel variants in the exon 1 of the *NUDT15* gene were not detected in the study population The allele frequency of the *TPMT*3C* allele was 0.032 (95% CI 0.014-0.062) whereas those of the A allele of *ITPA c.94C>A* variant was 0.180 (95% CI 0.134-0.233) and *ABCC4 c.2269G>A* variant was 0.028 (95% CI 0.011-0.057). All variants of these genes were in Hardy–Weinberg Equilibrium.

Table 1. Characteristic of Thai patients with autoimmune diseases (N =125)

Characteristics	n (%)
Gender	
Male	19 (15.20)
Female	106 (84.80)
Age (years)	
Median [range]	42 [18-79]
Mean ± SD	41.63 ± 15.35
Diseases	
Arthritis	4 (3.20)
Behcet	4 (3.20)
Connective tissue disease	3 (2.40)
Myositis	6 (4.80)
Neuropathy	1 (0.80)
Pemphigus valgaris	1 (0.80)
Rheumatoid arthritis (RA)	14 (11.20)
Systemic sclerosis	2 (1.60)
Systemic lupus erythematosus (SLE)	87 (69.60)
Systemic vasculitis	3 (2.40)

Note: n= number of subjects. SD, standard deviation.

Among the 125 patients, 19 (15.2%) patients experienced leukopenia (Table 2), with 14 patients categorized as having early leukopenia and 5 patients as having late leukopenia. There were no significant differences in gender or age between patients with and without leukopenia (Table 2). Regarding the dosage of AZA administered, no significant differences were observed in the initial or final doses between the leukopenia and non-leukopenia groups (Table 2). However, the average AZA dose during the treatment period was significantly higher in patients without leukopenia compared to those with leukopenia (Table 2). Additionally, the final WBC counts and the duration of AZA treatment were significantly lower in the leukopenia group compared to the non-leukopenia group (Table 2).

3.2 The relationship between *TPMT*, *NUDT15*, *ITPA* and *ABCC4* genetic polymorphisms on amounts of WBC counts and risks of AZA-induced myelosuppression

Based on *NUDT15* phenotypes, the final WBC counts in IM and PM patients were approximately 1.5 to 1.9 times lower compared to NM patients, whereas the WBC counts were comparable between IDM and NM (Figure 2). Additionally, the average WBC counts during AZA treatment for IM and PM patients were noticeably lower compared to those with the NM patients, although these differences were not statistically significant (Figure 2).

Table 2. The clinical demographic of patients with and without leukopenia

Characteristics	Non-leukopenia [N= 106]	Leukopenia [N =19]
Gender		
Male	18	1
female	88	18
Age (years)	42.47 ± 15.09 [18-76]	36.95 ± 16.36 [19-79]
Initial AZA dose (mg/kg/d)	0.93 ± 0.43 [0.27-2.11]	0.88 ± 0.33 [0.38-1.76]
[a]Average AZA dose (mg/kg/d)	1.28 ± 0.50 [0.22-2.53]	0.65 ± 0.32 [0.20-1.26] *
Final AZA dose (mg/kg/d)	1.33 ± 0.63[0.14-3.13]	1.09 ± 0.70 [0.21-2.27]
Days of AZA treatment; Median days of AZA treatments	291 ± 92 [13-365] 329	107 ± 114 [5-342] * 49
Final WBC counts (cell/mm^3)	6,903 ± 2,507 [3,500-14,500]	2,777 ± 521* [940-3,300]

Note: Data was presented as mean ± SD [range]; [a]Average AZA dose represents an average dose of AZA during AZA therapy. **P-value* ≤ 0.001.

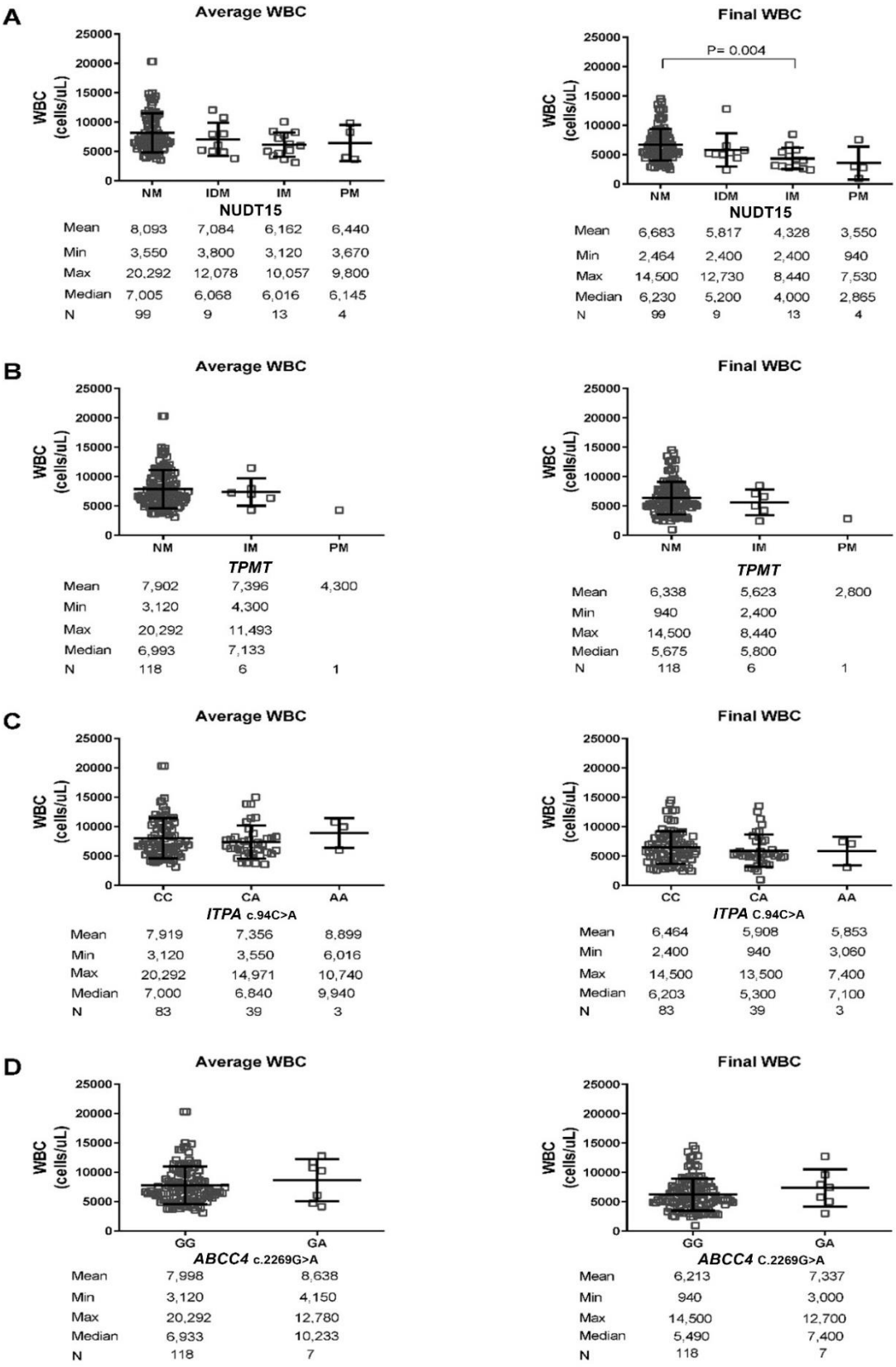

Figure 2. The relationships between genetic polymorphisms of *NUDT15 (A), TPMT (B), ITPA (C) and ABCC4 (D)* on average WBC, final WBC during treatment course.

Notes: *ABCC4*, ATP-binding cassette sub-family C member 4; *ITPA*, inosine triphosphatase; *NUDT15*, nucleoside diphosphate-linked moiety X-type motif 15; *TPMT*, thiopurine S-methyltransferase; NM, normal metabolizers; IDM, indeterminate metabolizers; IM, intermediate metabolizers; PM, poor metabolizers; P, P-value.

Table 3. The degree of association between *NUDT15* and *TPMT* phenotypes as well as *ITPA c.94C>A* and *ABCC4 c2269G>A* genotypes and myelosuppression during the first year of AZA treatment.

		Number of subject			
Phenotype/Genotype	**N**	**No leukopenia n (%)**	**Leukopenia n (%)**	**OR [95% CI]**	**P-value**
NUDT15					
NM (**1/*1*)	99	90 (90.9)	9 (9.1)	***Reference***	
IDM *(*1/*5, *1/*6)*	9	8 (88.9)	1 (11.1)	1.25 [0.14-11.16]	0.842
IM (**1/*2, *1/*3*)	13	7 (53.8)	6 (46.2)	**8.57 [2.37-31.07]**	**0.001**
PM (*3/*3, *2/*3)	4	1 (25)	3 (75)	**30.00 [2.82-319.20]**	**0.005**
IM and PM *(*1/*2,*1/*3, *3/*3,*2/*3)*	17	8 (47.1)	9 (52.9)	**11.25 [3.48-36.37]**	**<0.01**
TPMT					
NM *(*1/*1)*	118	101 (85.6)	17 (14.4)	***Reference***	
IM *(*1/*3C)*	6	5 (83.3)	1 (16.7)	1.58 [0.24-10.32]	0.610
***PM** (*3C/*3C)*	1	0 (0)	1 (100)	17.40 [0.68-444.54]	0.068
ITPA c.94C>A					
CC	83	71 (85.5)	12 (14.5)	***Reference***	
CA	39	33 (84.6)	6 (15.4)	1.08 [0.37-3.12]	0.893
AA	3	2 (66.7)	1 (33.3)	2.96 [0.25-35.23]	0.391
ABCC4 c2269G>A					
GG	118	100 (84.7)	18 (15.3)	***Reference***	
GA	7	6 (85.7)	1 (14.3)	0.93 [0.11-8.16]	0.945

Notes: N, total number of subject per phenotype/genotype; n, number of subjects observed. *ABCC4*, **ATP-binding cassette sub-family C member 4;** *ITPA*, inosine triphosphatase; 95% CI, 95% confidence interval; NM, normal metabolizers; IDM, indeterminate metabolizers; IM, intermediate metabolizers; PM, poor metabolizers

Regarding *TPMT* polymorphism, only one subject was homozygous for *TPMT*3C/3C* and was classified as a PM. The average and final WBC counts in this PM patient were lower than those of the NM patients. However, both values in the IM patient carrying *TPMT*1/*3C* were comparable to those of the NM phenotype (Figure 2). Furthermore, neither the average WBC count nor the final WBC count showed significant differences among the various *ITPA c.94C>A* and *ABCC4 c2269G>A* genotypes (Figure 2).

The degree of association between *NUDT15* and *TPMT* phenotypes as well as *ITPA* c.94C>A and *ABCC4* c2269G>A genotypes and myelosuppression during the first year of AZA treatment were analyzed and data are shown in Table 3. The risk of leukopenia the *NUDT15* PM patients was at 30-folds (95% CI 2.82-319.20) whereas that of the IM patients was at 8.57-folds (95% CI 2.37-31.07) higher risk of leukopenia than those of the NM. In contrast, the *NUDT15* IDM patients as well as patients who carried *TPMT, ITPA c.94C>A* and *ABCC4 c.2269G>A* variants showed no significant increase in the risk of leukopenia compare with the NM phenotype or wild-type genotypes.

3.3 The relationship between *TPMT, NUDT15, ITPA* and *ABCC4* genetic polymorphisms on dosage of AZA

Although the initial doses of AZA prescribed by the physician were not statistically significant different among patients with different *NUDT15* phenotypes, the average dose of AZA during AZA therapy in the IM group was significantly lower than that of the NM group (Figure 3). No significant differences in the final dose of AZA among patients with different *NUDT15* phenotypes. Moreover, there were no significant differences between the initial dose prescribed by the physician, the average dose, and the final dose of AZA among patients with different *TPMT, ITPA c.94C>A* and *ABCC4 c.2269G>A* variants (Figure 3).

The duration of AZA treatment in the *NUDT15* PM was notably shorter compared to the NM (Figure 4). It is important to note that among the *NUDT15* PM patients, 3 out of 4 developed leukopenia (2 patients with early onset and 1 patient with late onset), leading to the discontinuation of AZA therapy. One *NUDT15* PM patient did not develop leukopenia but experienced stomatitis, resulting in the discontinuation of AZA. Notably, *TPMT* IM appeared to have a longer duration

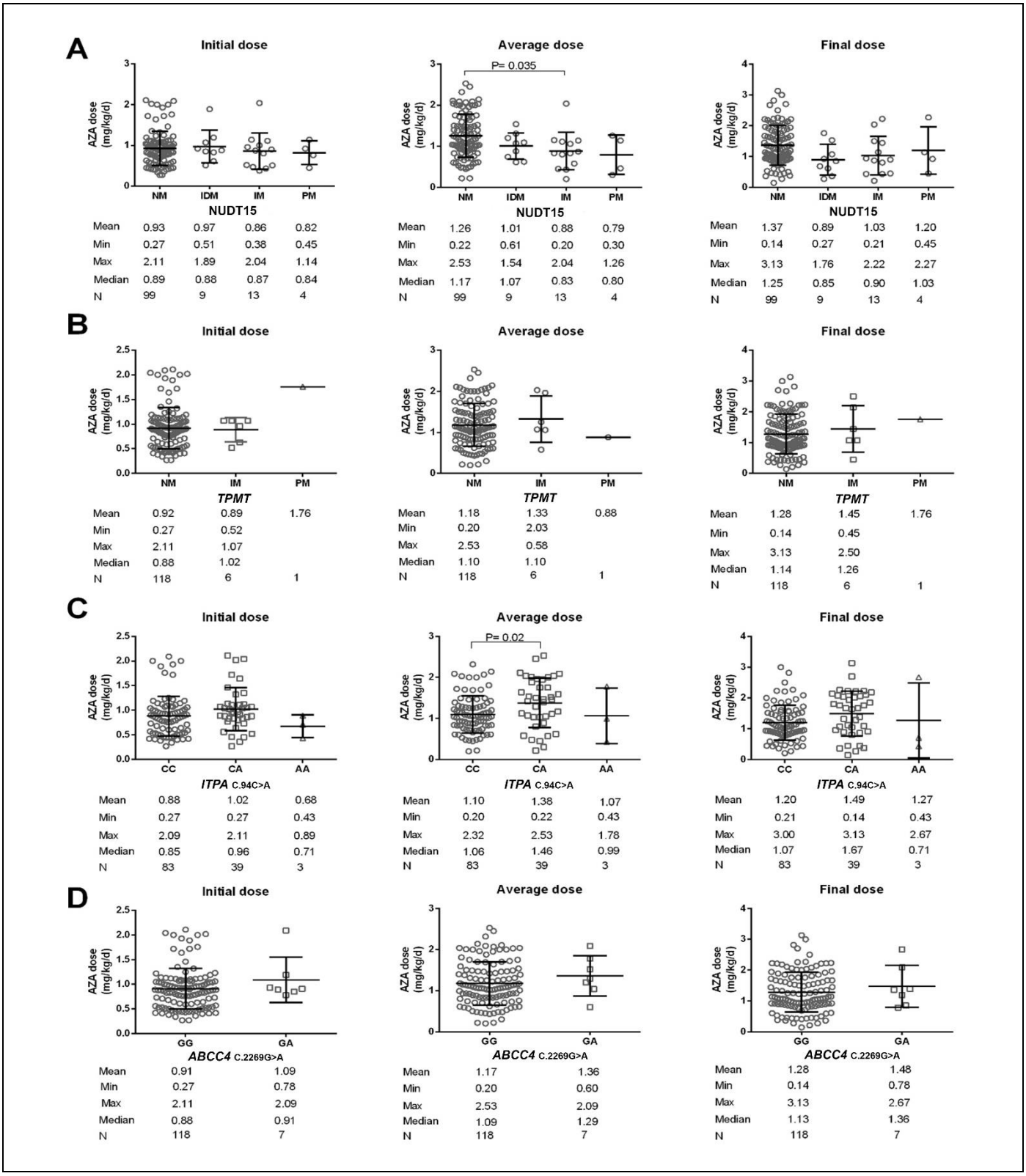

Figure 3. The relationships between genetic polymorphisms of *NUDT15 (A), TPMT (B), ITPA (C) and ABCC4 (D)* on initial, average and final doses of AZA during treatment course.

Notes: *ABCC4*, ATP-binding cassette sub-family C member 4; *ITPA*, inosine triphosphatase; *NUDT15*, nucleoside diphosphate-linked moiety X-type motif 15; *TPMT*, thiopurine S-methyltransferase; NM, normal metabolizers; IDM, indeterminate metabolizers; IM, intermediate metabolizers; PM, poor metabolizers, P, P-value.

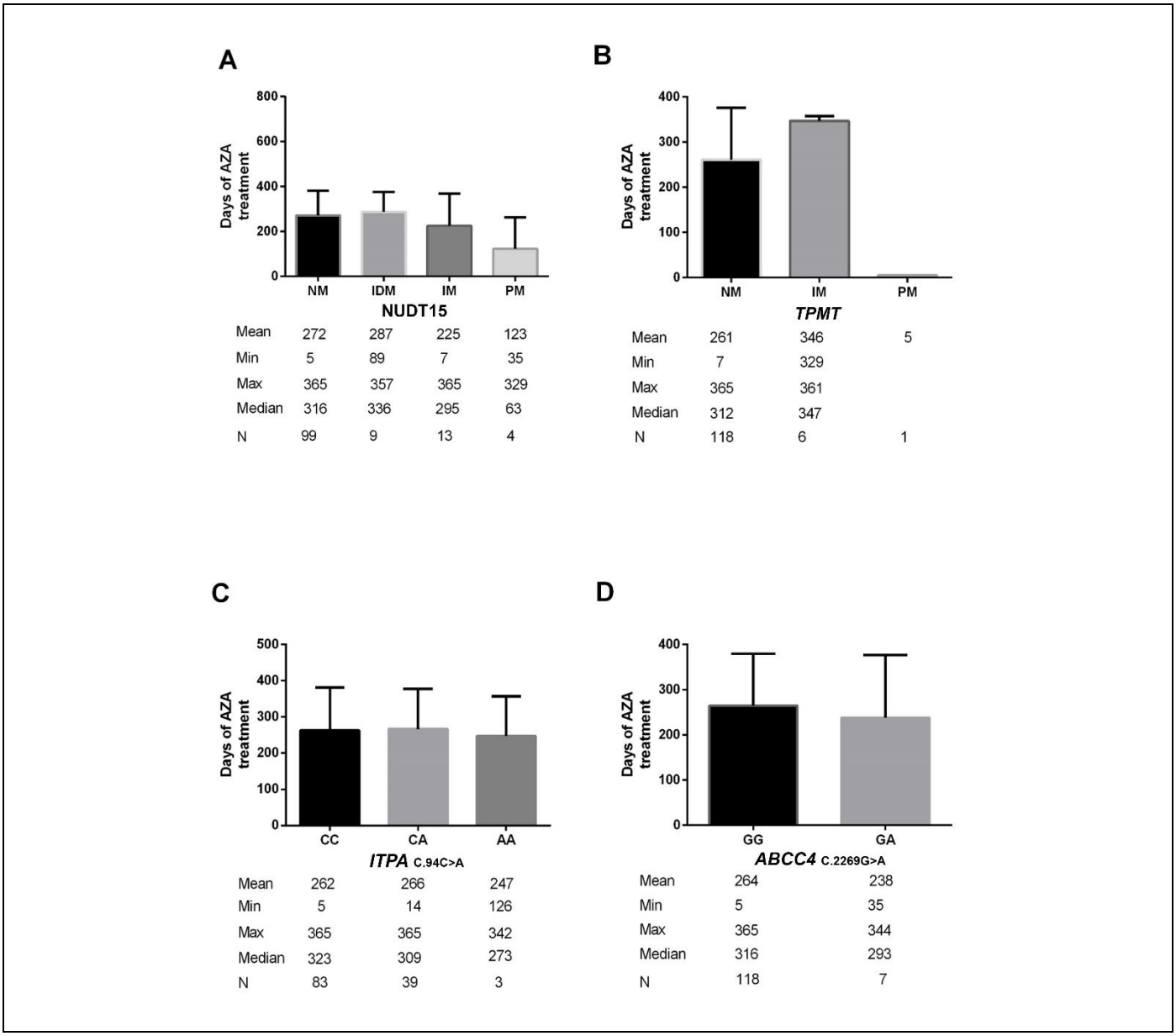

Figure 4. The relationships between genetic polymorphisms of *NUDT15*, *TPMT*, *ITPA* and *ABCC4* on days of treatment during treatment course.
Notes: *ABCC4*, ATP-binding cassette sub-family C member 4; *ITPA*, inosine triphosphatase; *NUDT15*, nucleoside diphosphate-linked moiety X-type motif 15; *TPMT*, thiopurine S-methyltransferase; NM, normal metabolizers; IDM, indeterminate metabolizers; IM, intermediate metabolizers; PM, poor metabolizers.

of AZA therapy compared to those with the NM group but not statistically significant differences (Figure 4). Only one subject was identified as a *TPMT* PM in this study, and leukopenia occurred after 5 days of treatment with 1.76 mg/kg/day of AZA, leading to the prompt discontinuation of AZA. Additionally, there was no significant association between clinical characteristics and the different genotypes of *ITPA c.94C>A* and *ABCC4 c.2269G>A* (Figure 4).

4. DISCUSSION

This study extensively investigated the associations between key genetic polymorphisms involved in the disposition of AZA including *TPMT*, *NUDT15*, *ITPA c.94C>A* and *ABCC4 c.2269G>A* with myelosuppression in Thai autoimmune diseases patients undergoing AZA therapy. The results demonstrated that the final WBC counts in patients with IM and PM phenotypes of *NUDT15* were significantly lower than those of the NM phenotype. In contrast, *TPMT*, *ITPA c.94C>A* and *ABCC4 c.2269G>A* variants showed no significant association with AZA-induced myelosuppression in this cohort. Only patients with the IM or PM phenotype of *NUDT15* were at significantly higher risk of AZA-induced myelosuppression compared to those with the NM phenotype.

It should be noted that the majority of the patients with autoimmune diseases recruited in the

present study were SLE and females (Table 1). These findings align with the previous national report showing that SLE is the most common autoimmune disease in Thailand and that females are more susceptible than males[36].

There was no significant difference in the initial dose of AZA prescribed between patients with and without leukopenia, however, the average WBC count at the end of AZA treatment in the non-leukopenia group was approximately three times higher than that of the leukopenia group (Table 2). It is important to note that the average AZA dose during the treatment period was lower in the leukopenia group compared to the non-leukopenia group, although no significant difference was observed in the final AZA dose between these groups. This finding may be attributed to the fact that the physicians adjusted the AZA dose of the patients based on their WBC counts during AZA therapy and they tend to increase the AZA dose as patients recover from leukopenia. Additionally, the physicians often decided to discontinue AZA once leukopenia is definitely confirmed rather than continue to use the reduced dose.

The association between *NUDT15* polymorphism and AZA-induced leukopenia was first been discovered through a genome-wide association study conducted in Korean patients with Crohn's disease[12]. In that study, *NUDT15*3* was found to be strongly associated with AZA-induced early leukopenia (OR 35.6, $P = 4.88\times10^{-94}$)[12]. Although associations between *NUDT15*3* and AZA-induced leukopenia in patients with autoimmune diseases, particularly inflammatory bowel disease (IBD) have been reported in several Asian populations including Japanese[37,38], Chinese[33] and Indian[39], this association has not previously been demonstrated in Thai patients.

The results of the present study revealed that among the genetic polymorphisms investigated in the present study, only the *NUDT15* polymorphism showed a significant association with AZA-induced leukopenia in Thai patients with autoimmune diseases (Table 3). Patients with homozygous *NUDT15*2* or *NUDT15*3* variants, classified as the PM had approximately 30-fold increased risk of leukopenia. Additionally, patients with heterozygous *NUDT15*2 or NUDT15*3* variants which classified as IM had an 8.57-fold increased risk compared with the NM (Table 3). In contrast, the risk of leukopenia did not significantly increase in the patients carrying *NUDT15*5* or *NUDT15*6* variants, classified as the IDM when compared with the NM (Table 3). These finding suggest that *NUDT15*2* and *NUDT15*3*, which attributed to the IM and PM of *NUDT15* are the key variants that determining the risk of leukopenia of AZA in Thai patients with autoimmune diseases.

The absence of significant associations between *TPMT*3C*, *ITPA c.94C>A* and *ABCC4 c.2269G>A* polymorphisms and AZA-induced leukopenia in our cohort (Table 3) indicates that *NUDT15* is the principal genetic determinant of AZA-induced leukopenia in Thai patients with autoimmune diseases. This observation aligns with reports from Japanese, Chinese, and Indian IBD cohorts that similarly found no link between these genetic polymorphism and AZA-induced leukopenia [33,37,39]. Together with prior evidence, these results suggest that *TPMT*3C*, *ITPA c.94C>A* and *ABCC4 c.2269G>A* polymorphism are unlikely to serve as reliable genetic predictor of thiopurines-related hematotoxicity in Asian populations [11,29-31].

The relatively high frequency of loss-of-function *NUDT15* variants, *NUDT15*2* and *NUDT15*3* in the Thai population which leads to an increased prevalence of PM and IM of NUDT15 underscores the importance of pre-treatment genetic screening for these *NUDT15* variants. Such screening is essential to reduce the risk of severe myelosuppression in Thai patients with autoimmune diseases who receive AZA therapy. It should also be noted that the *NUDT15*2* variant comprises two SNPs, a rs869320766 SNP (c.36_37insGGAGTC) and a rs116855232 SNP (c.415C>T) whereas the *NUDT15*3* variant contains only a rs116855232 SNP (c.415C>T). Since both variants share the same rs116855232 SNP (c.415C>T), we propose that the identification of the IM and PM of *NUDT15* can be effectively determined through the detection of a rs116855232 SNP (c.415C>T). However, other rare *NUDT15* alleles (such as **5*, **6*, and other loss-of-function alleles) are not detected by testing only rs116855232, so rs116855232 SNP genotyping may miss patients with reduced *NUDT15* activity for example, those with a possible IM phenotype such as *NUDT15*2/*5* or *NUDT15*3/*6* carriers.

This study has several limitations. The relatively small sample size may have reduced statistical power to detect associations with rarer variants, and the single-center, retrospective design may limit the generalizability of our findings to the broader Thai population. The study population was also enriched for female patients with SLE; larger, multi-center, prospective studies are needed to validate and extend these results. Despite these limitations, the study has important strengths. To our knowledge it is among the first in Southeast Asia to genotype *NUDT15*, *TPMT*, *ITPA*, and *ABCC4* concurrently in the same cohort, enabling direct comparison of their relative contributions to azathioprine therapy. Unlike most prior studies that focused on inflammatory bowel disease, our cohort predominantly comprises patients with SLE and other autoimmune conditions, demonstrating that the predictive value of genetic testing of *NUDT15* polymorphism extends beyond inflammatory bowel disease. Because allele frequencies and clinical penetrance of these pharmacogenetic variants vary across

Asian subpopulations, generating direct evidence from a Thai cohort is important for local clinical guidance.

5. CONCLUSIONS

This study highlights the importance of integrating genetic testing *NUDT15* polymorphism, particularly a rs116855232 SNP (c.415C>T) which can predict the status of NUDT activity into routine clinical practice for Thai patients with autoimmune diseases receiving AZA therapy. Given the markedly increased risk of myelosuppression in PM and IM of *NUDT15*, early identification of these autoimmune disease patients could enable personalized dosing strategies, thereby reducing the risk of AZA-induced myelosuppression.

In Thailand, targeted genotyping assays for rs116855232 are technically feasible in many tertiary centers and are relatively low cost compared with full sequencing. Formal cost-effectiveness analyses in the Thai health-care context are warranted before making broad policy recommendations.

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Author contributions

WT and KK: Conception and Study design; CF, AM, CW, SS and KK: Diagnosis and Subject enrollment; NN, SU, AD and KK: Genotyping; WT, NN, CF, AM, CW, SS and KK: Clinical data collection. WT, NN and KK: Performed data analysis; KK and WT: Manuscript drafting; All authors read and approved the final manuscript.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethics approval

Approval for this study was obtained from the Ethics Committee for Human Research of Khon Kaen University (HE611405)

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REFERENCES

1. Diaz-Villamarin X, Fernandez-Varon E, Rojas Romero MC, Callejas-Rubio JL, Cabeza-Barrera J, Rodriguez-Nogales A, et al. Azathioprine dose tailoring based on pharmacogenetic information: Insights of clinical implementation. Biomed Pharmacother. 2023;168:115706.
2. Zaza G, Cheok M, Krynetskaia N, Thorn C, Stocco G, Hebert JM, et al. Thiopurine pathway. Pharmacogenet enomics. 2010;20(9):573-4.
3. Kakuta Y, Kinouchi Y, Shimosegawa T. Pharmacogenetics of thiopurines for inflammatory bowel disease in East Asia: prospects for clinical application of *NUDT15* genotyping. J Gastroenterol. 2018;53(2):172-80.
4. Lennard L. The clinical pharmacology of 6-mercaptopurine. Eur J Clin Pharmacol. 1992;43(4):329-39.
5. Lennard L, Van Loon JA, Lilleyman JS, Weinshilboum RM. Thiopurine pharmacogenetics in leukemia: correlation of erythrocyte thiopurine methyltransferase activity and 6-thioguanine nucleotide concentrations. Clin Pharmacol Ther. 1987;41(1):18-25.
6. Weinshilboum RM. Pharmacogenomics: catechol O-methyltransferase to thiopurine S-methyltransferase. Cell Mol Neurobiol. 2006;26(4-6):539-61.
7. Ishioka S, Hiyama K, Sato H, Yamanishi Y, McLeod HL, Kumagai K, et al. Thiopurine methyltransferase genotype and the toxicity of azathioprine in Japanese. Intern Med. 1999;38(12):944-7.
8. Tassaneeyakul W, Srimarthpirom S, Reungjui S, Chansung K, Romphruk A, Tassaneeyakul W. Azathioprine-induced fatal myelosuppression in a renal-transplant recipient who carried heterozygous *TPMT**1/*3C. Transplantation. 2003;76(1):265-6.
9. Moriyama T, Nishii R, Perez-Andreu V, Yang W, Klussmann FA, Zhao X, et al. *NUDT15* polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet. 2016;48(4):367-73.
10. Kim HT, Choi R, Won HH, Choe YH, Kang B, Lee K, et al. NUDT15 genotype distributions in the Korean population. Pharmacogenet Genomics. 2017;27(5):197-200.
11. Yang JJ, Landier W, Yang W, Liu C, Hageman L, Cheng C, et al. Inherited *NUDT15* variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. J Clin Oncol. 2015;33(11):1235-42.
12. Yang SK, Hong M, Baek J, Choi H, Zhao W, Jung Y, et al. A common missense variant in *NUDT15* confers susceptibility to thiopurine-induced leukopenia. Nat Genet. 2014;46(9):1017-20.
13. Tanaka Y, Kato M, Hasegawa D, Urayama KY, Nakadate H, Kondoh K, et al. Susceptibility to 6-MP toxicity conferred by a *NUDT15* variant in Japanese children with acute lymphoblastic leukaemia. Br J Haematol. 2015;171(1):109-15.
14. Chao K, Wang X, Cao Q, Qian J, Wu K, Zhu X, et al. Combined detection of *NUDT15* variants could highly predict thiopurine-induced leukopenia in Chinese patients with inflammatory bowel disease: A multicenter analysis. Inflamm Bowel Dis. 2017;23(9):1592-9.
15. Chiengthong K, Ittiwut C, Muensri S, Sophonphan J, Sosothikul D, Seksan P, et al. *NUDT15* c.415C>T increases risk of 6-mercaptopurine induced myelosuppression during maintenance therapy in children with acute lymphoblastic leukemia. Haematologica. 2016;101(1):e24-6.
16. Marinaki AM, Ansari A, Duley JA, Arenas M, Sumi S, Lewis CM, et al. Adverse drug reactions to azathioprine therapy are associated with polymorphism in the gene encoding inosine triphosphate pyrophosphatase (ITPase). Pharmacogenetics. 2004;14(3):181-7.
17. Tanaka Y. [Susceptibility to 6-mercaptopurine toxicity related with *NUDT15* and *ABCC4* variants in Japanese childhood acute lymphoblastic leukemia]. Rinsho Ketsueki. 2017;58(8):950-6.

18. Tanaka Y, Nakadate H, Kondoh K, Nakamura K, Koh K, Manabe A. Interaction between *NUDT15* and *ABCC4* variants enhances intolerability of 6-mercaptopurine in Japanese patients with childhood acute lymphoblastic leukemia. Pharmacogenomics J. 2018;18(2):275-80.
19. Gerbek T, Ebbesen M, Nersting J, Frandsen TL, Appell ML, Schmiegelow K. Role of *TPMT* and *ITPA* variants in mercaptopurine disposition. Cancer Chemother Pharmacol. 2018;81(3):579-86.
20. Sumi S, Marinaki AM, Arenas M, Fairbanks L, Shobowale-Bakre M, Rees DC, et al. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. Hum Genet. 2002;111(4-5):360-7.
21. Simone PD, Pavlov YI, Borgstahl GEO. *ITPA* (inosine triphosphate pyrophosphatase): from surveillance of nucleotide pools to human disease and pharmacogenetics. Mutat Res. 2013;753(2):131-46.
22. Cao H, Hegele RA. DNA polymorphisms in *ITPA* including basis of inosine triphosphatase deficiency. J Hum Genet. 2002;47(11):620-2.
23. Maeda T, Sumi S, Ueta A, Ohkubo Y, Ito T, Marinaki AM, et al. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency in the Japanese population. Mol Genet Metab. 2005;85(4):271-9.
24. Khaeso K, Nakkam N, Komwilaisak P, Wongmast P, Chainansamit SO, Dornsena A, et al. Genetic polymorphisms of drug-metabolizing enzymes involved in 6-mercaptopurine-induced myelosuppression in Thai pediatric acute lymphoblastic leukemia patients. J Pediatr Genet. 2021;10(1):29-34.
25. Krishnamurthy P, Schwab M, Takenaka K, Nachagari D, Morgan J, Leslie M, et al. Transporter-mediated protection against thiopurine-induced hematopoietic toxicity. Cancer Res. 2008;68(13):4983-9.
26. Ban H, Andoh A, Imaeda H, Kobori A, Bamba S, Tsujikawa T, et al. The multidrug-resistance protein 4 polymorphism is a new factor accounting for thiopurine sensitivity in Japanese patients with inflammatory bowel disease. J Gastroenterol. 2010;45(10):1014-21.
27. Boonyawat B, Monsereenusorn C, Photia A, Lertvivatpong N, Kaewchaivijit V, Jindatanmanusan P, et al. *ITPA:*c.94C>A and *NUDT15*:c.415C>T polymorphisms and their relation to mercaptopurine-related myelotoxicity in childhood leukemia in Thailand. Appl Clin Genet. 2021;14:341-51.
28. Abla N, Chinn LW, Nakamura T, Liu L, Huang CC, Johns SJ, et al. The human multidrug resistance protein 4 (MRP4, *ABCC4*): functional analysis of a highly polymorphic gene. J Pharmacol Exp Ther. 2008;325(3):859-68.
29. Zhou H, Li L, Yang P, Yang L, Zheng JE, Zhou Y, et al. Optimal predictor for 6-mercaptopurine intolerance in Chinese children with acute lymphoblastic leukemia: *NUDT15*, *TPMT*, or *ITPA* genetic variants? BMC Cancer. 2018;18(1):516.
30. Khaeso K, Komvilaisak P, Chainansamit SO, Nakkam N, Suwannaying K, Kuwatjanakul P, et al. *NUDT15* is a key genetic factor for prediction of hematotoxicity in pediatric patients who received a standard low dosage regimen of 6-mercaptopurine. Drug Metab Pharmacokinet. 2022;43:100436.
31. Zhu X, Wang XD, Chao K, Zhi M, Zheng H, Ruan HL, et al. *NUDT15* polymorphisms are better than thiopurine S-methyltransferase as predictor of risk for thiopurine-induced leukopenia in Chinese patients with Crohn's disease. Aliment Pharmacol Ther. 2016;44(9):967-75.
32. Lee YJ, Hwang EH, Park JH, Shin JH, Kang B, Kim SY. *NUDT15* variant is the most common variant associated with thiopurine-induced early leukopenia and alopecia in Korean pediatric patients with Crohn's disease. Eur J Gastroenterol Hepatol. 2016;28(4):475-8.
33. Wang HH, He Y, Wang HX, Liao CL, Peng Y, Tao LJ, et al. Comparison of *TPMT* and *NUDT15* polymorphisms in Chinese patients with inflammatory bowel disease. World J Gastroenterol. 2018;24(8):941-8.
34. Relling MV, Schwab M, Whirl-Carrillo M, Suarez-Kurtz G, Pui CH, Stein CM, et al. Clinical pharmacogenetics implementation consortium guideline for thiopurine dosing based on *TPMT* and *NUDT15* genotypes: 2018 Update. Clin Pharmacol Ther. 2019;105(5):1095-105.
35. Haldane JB. The estimation and significance of the logarithm of a ratio of frequencies. Ann Hum Genet. 1956;20(4):309-11.
36. Pongkulkiat P, Foocharoen C, Onchan T, Suwannaroj S, Mahakkanukrauh A. Prevalence and incidence of systemic lupus erythematosus in Thailand based on national health data. Lupus Sci Med. 2025;12(2).
37. Kakuta Y, Kawai Y, Okamoto D, Takagawa T, Ikeya K, Sakuraba H, et al. *NUDT15* codon 139 is the best pharmacogenetic marker for predicting thiopurine-induced severe adverse events in Japanese patients with inflammatory bowel disease: a multicenter study. J Gastroenterol. 2018;53(9):1065-78.
38. Kishibe M, Nozaki H, Fujii M, Iinuma S, Ohtsubo S, Igawa S, et al. Severe thiopurine-induced leukocytopenia and hair loss in Japanese patients with defective *NUDT15* variant: Retrospective case-control study. J Dermatol. 2018;45(10):1160-5.
39. Banerjee R, Ravikanth VV, Pal P, Bale G, Avanthi US, Goren I, et al. *NUDT15* C415T variant compared with *TPMT* genotyping in predicting azathioprine-induced leucopenia: prospective analysis of 1014 inflammatory bowel disease patients in India. Aliment Pharmacol Ther. 2020;52(11-12):1683-94.