Association of thiopurine *S*-methyltransferase and NUDT15 polymorphisms with azathioprine-induced myelotoxicity in Chinese patients with rheumatological disease

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To the Editor: Rheumatic diseases are disorders characterized by joint inflammation, but other organs are also affected.[1] Rheumatic diseases are common in China, and the clinical application of azathioprine (AZA), a purine analog immunosuppressant, for these diseases is attracting increasing attention. Although AZA is widely used in the treatment of rheumatic diseases, its clinical application is limited by adverse effects, especially leukopenia.

Thiopurine *S*-methyltransferase (TPMT) is a key enzyme in metabolism of thiopurines, including AZA and 6-mercaptopurine. Mutations in the TPMT gene result in changes in enzyme activity and significantly increase the risk of life-threatening myelosuppression.[2] Recent studies have found that the frequency of *TPMT* variation in the Chinese patient population is approximately 0.9%,[3] yet the incidence of AZA-induced leukopenia in these patients remains high (27.0–41.3%). Other genes are also likely to play a role in determining AZA toxicity.[4] A relationship between NUDT15 (rs116855232, c.415C>T) and AZA-induced leukopenia in patients with acute lymphoblastic leukemia and inflammatory bowel disease (IBD) has been reported. However, there is no published report on the correlation between this NUDT15 polymorphism and AZA-induced leukopenia in patients with rheumatological disease in China. Therefore, we investigated the risk allele frequencies of the TPMT and NUDT15 genes and observed their roles in predicting AZA-induced myelotoxicity in patients with rheumatological disease from Fujian Province in China.

This study was approved by the Ethics Committee of the First Hospital of Quanzhou Affiliated with Fujian Medical University, and written informed consent was received prior to examination.

In this study, 70 patients with rheumatological disease (49 females and 21 males), including systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), according to the criteria of the American College of Rheumatology were recruited. All of the patients visited the Quanzhou First Hospital Affiliated to Fujian Medical University, Fujian, China, from January 2017 to November 2018.

We collected 2 to 3 mL of peripheral blood from the enrolled patients and extracted total genomic DNA using EZBead blood DNA extraction kit (Xiamen, Fujian, China). Genotyping of TPMT p.Tyr240Cys (c.719A>G, rs1142345), NUDT15 p.Arg139Cys (c.415C>T, rs116855232), and NUDT15 p.Arg139His (c.416G>A, rs147390019) was performed via Sanger sequencing using a 3730xl genetic analyzer (Applied Biosystems) at Biomedical Port Medical and Health Co. Ltd. (Xiamen, Fujian, China). The sequences of the forward and reverse primers were 5′-GGTTGATGCTTTT-GAAGAACGAC-3′ and 5′-TGCAAGACACATAGGCA-TAATCT-3′ for rs1142345, respectively, and 5′-AGCTTA CCCAAATAAACACCCT-3′ and 5’-TCAAATCTTCT CGGCCACCT-3′ for rs116855232 (and rs147390019), respectively.

All of the patients received AZA (50 mg/d, orally) combined with hydroxychloroquine (0.2 g, twice a day, orally). Patients receiving AZA treatment underwent routine blood tests once a week for the first month, followed by once a month, and liver function tests monthly. If AZA has clinical efficacy without adverse reactions, patients should be treated with it for a long period of time. For patients who develop leukopenia (white blood cell count $<3.5 \times 10^9$/L) or thrombocytopenia (platelet count $<1.5 \times 10^9$/L) during AZA therapy, the dose should be reduced by 50%, or the drug should be discontinued. If a 50% reduction in dose is associated with cytopenia, AZA should generally be discontinued permanently.

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000000756

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Chinese Medical Journal 2020;133(8)

Received: 27-09-2019 **Edited by:** Li-Shao Guo

Table 1: Baseline characteristics and gene mutations results for all the 70 patients with rheumatological disease.

Variables	No myelotoxicity	Myelotoxicity	Total	*P* value*	OR	95% CI
Age (years)	43.4 (18.0–55.0)	42.1 (20.0–56.0)	42.9 (18.0–56.0)	0.610		
Sex (M/F)	12/30	9/19	21/49	0.749		
Body weight (kg)	53.54 ± 5.28	51.52 ± 3.81	52.73 ± 4.82	0.086		
BMI (kg/m^2)	21.46 ± 1.35	18.84 ± 1.27	20.41 ± 1.84	<0.001		
Diagnosis				0.855		
RA	28 (65.1)	15 (34.9)	43			
SLE	17 (62.96)	10 (37.04)	27			
TPMT genotype				0.973		
A/A	41	27	68			
A/G	1	1	2			
G/G	0	0	0			
NUDT15 genotype				0.005	5.191	1.654–16.291
C/C	36	15	51			
C/T	6	12	18			
T/T	0	1	1			

Data are presented as the number (%), mean ± standard deviation, or median (range). *No myelotoxicity *vs.* myelotoxicity. OR: Odds ratio; CI: Confidence interval; BMI: Body mass index; RA: Rheumatoid arthritis; SLE: Systemic lupus erythematosus; TPMT: Thiopurine *S*-methyltransferase.

We compared the baseline characteristics of the patients using the Chi-squared test or Student *t* tests. The odds ratio (OR) of myelotoxicity associated with NUDT15 and TPMT variant alleles was determined by logistic regression analysis. Statistically significant differences were considered at *P* values less than 0.05. SPSS version 22 (SPSS Inc., Chicago, IL, USA) was used for all the analyses.

The variant allele frequency of TPMT p.Tyr240Cys (c.719A>G, rs1142345) was 1% in this study. Only two patients (2.9%) were heterozygous for TPMT*3C, with none being homozygous carriers of the variant allele. Wild type, heterozygous, and homozygous variant NUDT15 p.Arg139Cys (c.415C>T, rs116855232) genotypes were carried by 51 (72.9%), 18 (25.7%), and 1 (1.4%) patients, respectively. The NUDT15 p.Arg139His (c.416G>A, rs147390019*)* variant was not observed in this study cohort. The TPMT mutation rate was 1%, and that of NUDT15 was 14% ($P < 0.001$).

The patients' characteristics at baseline are shown in Table 1. There were no significant differences in sex, age, body weight, or disease type. The average body mass index (BMI) of the patients with myelotoxicity was significantly lower than that of those who did not have myelotoxicity ($P < 0.001$). Of the 70 patients, 68 were homozygous for TPMT (A/A), and 27/68 (39.7%) experienced leukopenia after receiving AZA. Only two patients were heterozygous for TPMT (A/G), and 1/2 (50.0%) developed myelotoxicity. No significant relationship between the TPMT polymorphism and myelotoxicity was observed ($P = 0.973$). These data indicate that the TPMT polymorphism is not an effective predictor of AZA-induced myelotoxicity.

We also analyzed NUDT15 gene polymorphisms. After receiving AZA therapy, 12/18 (66.7%) patients with the NUDT15 C/T genotype had myelotoxicity, though only 15/51 (29.4%) with the NUDT15 C/C genotype developed myelotoxicity; 1/1 (100%) patient with the NUDT15 T/T genotype developed myelotoxicity. Logistic regression analyses showed that NUDT15 c.415C>T (rs116855232) was significantly associated with a 5.19-fold higher risk of myelotoxicity (OR: 5.19; 95% confidence interval: 1.65) [Table 1].

AZA is a purine-mimic anti-metabolite that is a prodrug. The activity of TPMT is closely related to the toxicity of AZA. In particular, insufficiency of this enzyme leads to the accumulation of thioguanine nucleotides, which appears to explain much of the hematologic toxicity related to AZA. However, the frequency of the most common TPMT allele (*3C) is low (1–3%) in Asians.[5] In this study, TPMT variant alleles only occurred at a low overall frequency of 1%. Therefore, TPMT polymorphisms cannot predict AZA-induced myelotoxicity in patients with rheumatological disease in China. NUDT15 c.415C>T is involved in the hydrolysis of some nucleoside diphosphate derivatives, and the c.415C>T polymorphism is closely related to AZA-induced myelotoxicity. Our findings confirm similar associations in Chinese patients with rheumatological disease.

In conclusion, the mutation frequency of NUDT15 c.415C>T was found to be markedly higher than that of TPMT *3C in Chinese patients with rheumatological disease, and the difference was statistically significant. NUDT15 c.415C>T is a better choice than TPMT *3C for predicting AZA-induced myelotoxicity. As the incidence of AZA-induced myelotoxicity is higher in patients with lower BMI, malnutrition may affect the incidence of myelotoxicity in patients treated with AZA. Further studies are needed to validate our findings.

Funding

This work was supported by grants from the Natural Science Foundation of Fujian Province, China (No. 2017J01351) and the Fujian Medical University Sailing Fund, China (No. 2016QH086).

Conflicts of interest

None.

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How to cite this article: Su SS, Lin YF, Zhou H. Association of thiopurine *S*-methyltransferase and NUDT15 polymorphisms with azathioprine-induced myelotoxicity in Chinese patients with rheumatological disease. Chin Med J 2020;133:1002–1004.' doi: 10.1097/CM9.0000000000000756