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Thiopurine S-methyltransferase polymorphisms and the relationship between the mutant alleles and the adverse effects in systemic lupus erythematosus patients taking azathioprine

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ABSTRACT

Objective. *The present study sought to elucidate the genetic basis of thiopurine methyltransferase (TPMT) polymorphism and subsequently to investigate the relationship between mutant TPMT and an adverse response observed in Korean patients with systemic lupus erythematosus (SLE) taking azathioprine (AZA).*

Methods. *The TPMT genotype of 342 patients with SLE was determined by MALDI-TOF mass spectrometry and correlated with the effects of clinical exposure to AZA.*

Results. *TPMT polymorphism was detected in 17 of the 342 study subjects (5.0%), 12 heterozygous for the TPMT*3C allele and 5 heterozygous for the TPMT*6 allele.*

*Numerous patients taking AZA demonstrated adverse drug responses. Severe nausea occurred in 4 patients with the TPMT*3C allele, while 1 patient with the TPMT*6 allele suffered severe bone marrow toxicity. Leucopenia (n = 17), nausea (n = 4), and abnormal liver function (n = 1) were suspected in 23 of the 94 lupus patients taking AZA. AZA was relatively well tolerated by the remainder of the patients.*

*The heterozygous genotype for the TPMT*3C and *6 alleles was frequently detected in Korean SLE patients.*

Conclusion. *Contrary to previous hypotheses, this study identified no statistical correlation between TPMT genotype and AZA toxicity. We thus conclude that TMPT genotyping cannot replace regular blood monitoring in SLE patients receiving AZA treatment.*

Introduction

Various pharmacogenetic studies have investigated pretreatment screening strategies for predicting fatal adverse events to azathioprine (AZA) treatment of rheumatologic diseases. These diseases include rheumatoid arthritis (RA) (1), systemic lupus erythematosus (SLE) (2), and such gastroenterologic diseases as autoimmune hepatitis (AIH) (3) and inflammatory bowel disease (IBD) (4). Thiopurine methyltransferase (TPMT) status has recently been implicated in adverse responses to AZA treatment of rheumatic diseases as well (5). While a correlation is theoretically possible, TPMT polymorphisms have not been reported to predict adverse drug reactions in SLE, AIH, or IBD.

In a recent pharmaco-economic analysis, we reported that a genotypic dosing strategy via PCR-based TPMT polymorphism screening would be less costly yet more effective than the conventional weight-based dosing strategy currently employed in Korea. Such genotype-based dosing was associated with a marked reduction in the number of serious adverse events in a hypothetical cohort (6). The present study could not be based on TPMT polymorphism frequency in the Korean population, however, because the frequency analysis itself has yet to be performed. We therefore investigated TPMT polymorphism through PCR screening of Korean patients with SLE who were or were not receiving AZA in order to confirm that our pharmaco-economic analytical model was clinically relevant and accurately predicted adverse drug responses in the Korean population.

Materials and methods

Patients

A retrospective analysis was conducted of 342 patients (326 females, 16 males) with SLE who fulfilled the 1982 revised criteria of the American College of Rheumatology for the classification of SLE (7) and its 1997 updated revision (8). The mean ages of female and male patients were 32.8 ± 11.6 years (range 6 to 70) and 29.4 ± 15.7 years (range 14 to 63), respectively. Among the 342 patients included in the analysis, 94 (27.5%; 91 females, 3 males) were treated with AZA (65.2 22.1 mg, 94.9 85.7 weeks).

Direct sequencing of the TPMT gene was performed for severe leucopenic patients with RA (n = 8) and SLE (n = 5) who had received AZA in order to evaluate the effects of TPMT polymorphism and to identify rare mutant alleles. If TPMT polymorphism was indeed associated with adverse response to AZA, these severely leucopenic patients would be expected to harbor mutant TPMT alleles.

Table I. Oligonucleotide sequence for each variation.

Variation	Primer	Sequence
TPMT*2	Forward	5`- ACGTTGGATGTGCATGTTCTTTGAAACCCTA
G238C	Reverse	5`- ACGTTGGATGCTACACTGTGTCCCCGGTCT
(A80P)	Extension	5`- CTACACTGTGTCCCCGGTCTG
TPMT*3AC	Forward	5`- ACGTTGGATGTGATGCTTTTGAAGAACGACAT
A719G	Reverse	5`- ACGTTGGATGCCTCAAAAACATGTCAGTGTGA
(Y240C)	Extension	5`- TGTCTCATTTACTTTTCTGTAAGTAGA
TPMT*3ABD	Forward	5`- ACGTTGGATGTGAAGTACCAGCATGCACCA
G460A	Reverse	5`- ACGTTGGATGTTACCATTTGCGATCACCTG
(A154T)	Extension	5`- CCTGGATTGATGGCAACTAATG
TPMT*6	Forward	5`- ACGTTGGATGCTTTTGTCCCTCCAGCCTTT
A539T	Reverse	5`- ACGTTGGATGCCCAACAACTTTACCTGGATG
(Y180F)	Extension	5`- CCTCCTGGGAAAGAAGTTTCAGT

Table II. Results of the TPMT genotyping.

Genotype	SLE patients (n = 342)
Wild type, TPMT*1/1	325 (95.0 %)
Mutant type	17 (5.0 %)
TPMT*1/2	0
TPMT*1/3A	0
TPMT*1/3B	0
TPMT*1/3C	12 (3.5 %)
TPMT*1/6	5 (1.5 %)

Genotyping

PCR primers were designed using the MIT Primer 3 program (http://www.genome.wi.mit.edu/cgi-bin/primer/ primer[3] www.cgi) and are listed in Table I. The detection of TPMT variations was based on analysis of the primer extension products geneated from previously-amplified genomic DNA using a chip-based MALDI-TOF platform with a 384-well SpectroCHIP and a SpectroJET mass spectrometer (Sequenom, Inc., CA). The manufacturer's standard protocol was followed with only slight modifications. After automatic overall measurement, assays displaying irregular peaks were re-checked manually.

Statistical analysis

The chi-square test was used to compare the prevalence of mutant alleles in SLE patients who did and did not take AZA. The odds ratio with a 95% confidence interval for adverse reactions between those with and without variant alleles was also calculated. SPSS system software (Version 10.0, SPSS, Chicago, Illinois) was used, with $p < 0.05$ considered statistically significant.

Results

We determined TPMT genetic polymorphism status in Korean SLE patients so as to investigate various drug responses related to AZA treatment. Among 342 patients studied, 17 (5.0%) carried mutant alleles (Table II), although none of these 17 patients was homozygous for the mutant allele. The mutant alleles detected were TPMT*1/3C (12 patients, 3.5%) and TPMT*1/6 (5 patients, 1.5%). Neither TPMT*2 nor 3A nor 3B was detected in this patient population. Table III summarizes the various clinical effects of AZA in the 17 patients carrying mutant TPMT alleles. Seven out of 17 patients were treated with AZA. Treatment with AZA was discontinued in only two patients because of adverse events, one each of severe nausea and leucopenia.

Ninety-four of the total patients received AZA, and 23 of these suffered adverse responses. Leucopenia (WBC < 4,000/mm^3 and < 75% before administration) was seen in 17 cases, gastrointestinal intolerance in four, abnormal liver function (≥ two-fold elevation of upper normal limit) in one, and skin rash in one. AZA was withdrawn for all but five patients with leucopenia and one patient with a skin rash. The frequency of adverse events did not differ for patients with wild type (21/86, 24.4%) or mutant alleles (2/8, 25.0%) (Table IV).

Direct sequencing of the entire TPMT gene was performed in cases of severe leucopenia following AZA treatment.

Table III. Clinical effects of AZA in SLE patients with mutant TPMT alleles (n = 17).

TPMT	sex	age	Dose (mg)	Duration (weeks)	Clinical effects
*1/3C	F	30	0	0	AZA (-)
*1/3C	F	21	0	0	AZA (-)
*1/3C	F	37	0	0	AZA (-)
*1/3C	F	21	0	0	AZA (-)
*1/3C	F	30	0	0	AZA (-)
*1/3C	F	41	0	0	AZA (-)
*1/3C	F	17	0	0	AZA (-)
*1/3C	F	28	75	45	No adverse event
*1/3C	F	29	50	20	No adverse event
*1/3C	F	25	75	252	No adverse event
*1/3C	F	37	75	76	No adverse event
*1/3C	F	38	25	2	Stop: nausea
*1/6	F	55	0	0	AZA (-)
*1/6	F	21	0	0	AZA (-)
*1/6	F	51	0	0	AZA (-)
*1/6	F	22	25	5	Stop: leucopenia
*1/6	F	25	100	194	No adverse event

AZA (-): never received AZA.

Of 13 such patients (8 RA and 5 SLE), only one patient with SLE was found to carry a mutant allele (TPMT*1/3C). Unexpectedly, we found several instances of a T→C change at nucleotide 474; three RA patients were heterozygotic and one SLE patient homozygotic for this change.

Table IV. Relationship between genotyping and adverse events.

Genotype of patients with AZA (n = 94)	Adverse event (+)	Adverse event (-)
Wild type, TPMT*1/1 (n = 86)	21 (24.4%)	65 (75.6%)
Mutant type (n = 8)	2 (25.0%)	6 (75.0%)
TPMT*1/3C (n = 6)	1	5
TPMT*1/6 (n = 2)	1	1

p-value = 0.9.

Discussion

AZA has most often been prescribed as an alternative to cyclophosphamide and MTX in SLE and RA, respectively. It is a kind of pro-drug that is only activated after serial metabolism by multiple enzymes, including TPMT (5). The enzymatic activity of TPMT is thus a factor in the efficacy and toxicity profiles of AZA and is known to be correlated with the polymorphic status of TPMT genotypes (9). Clinical interest exists, therefore, in genotypic screening of TPMT polymorphism prior to AZA prescription.

Our group has previously reported that a genotypic dosing strategy for AZA based on PCR screening of TPMT polymorphism is less costly and more effective than the conventional weight-based dosing strategy currently used in Korea (6). The present study is of significant value because it tests the previous study's hypothetical findings in a practical setting while maintaining the same ethnic population and study institute.

From this retrospective analysis, we conclude that pretreatment genotyping of TPMT polymorphism cannot predict severe drug response to need to discontinue treatment with AZA. We found that genotype was not correlated with severe leucopenia, gastrointestinal intolerance, or abnormal liver function in SLE patients taking AZA. Unfortunately at present we do not know exactly what causes the debating conclusion on the role of pretreatment genotyping of TPMT in terms of prediction of AZA-related toxicities, but it might be due to the differences of the ethnic group studied and the prevalence of TPMT mutant alleles in each population, the number(s) of TPMT mutant alleles genotyped in each studies, the kinds of adverse events included (just happened and/or needed to withdraw the drug), AZA dosage used, and the disease entity itself. It must be also noted that the presumptive clinical setting and polymorphic alleles investigated were a little bit different between our hypothetical and genotypic studies. Together with unpublished data by Bae, *et al*., which showed one TPMT*3C heterozygote male and one TPMT*6 heterozygote male in 200 healthy controls (138 males, 62 females; age 30.0 7.0 years, range 18 to 61), we found that TPMT*3C is the most common polymorphism in the Korean population. This result agrees with previous reports for other Asian groups, especially Chinese (10) and Japanese (11). We also identified TPMT*6 as a potentially unique mutant allele within the Korean population. While TPMT*6 has been reported in one Malay cord blood sample (12) and was previously found in Korean children (13), it was detected in relatively high frequency (1.5%) in this study. To the best of our knowledge, there have been no other reports of the TPMT*6 allele, even in such genetically-close populations as Chinese and Japanese. Finally, TPMT*1S, T474C, was detected in direct sequencing and found to be a silent single nucleotide polymorphism that neither alters enzymatic activity (14) nor relates to AZA toxicity in representative individuals (11).

In contrast to our previous pharmacoeconomic study that supported a pretreatment genotyping strategy, the present analysis did not demonstrate significant correlation between TPMT polymorphism and adverse events. However, pretreatment genotyping may still have value in order to avoid fatal myelotoxicity and to expect a favorable therapeutic effect to low-dose AZA when prescribing AZA in variant allele carriers, although the numbers are very small, according to our and previous studies (2, 4). Therefore this issue needs to be further clarified and justified in prospective studies.

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