Correlation of Genotypes for Thiopurine Methyltransferase and Inosine Triphosphate Pyrophosphatase with Long Term Clinical Outcomes in Korean Patients with Inflammatory Bowel Diseases During Treatment with Thiopurine Drugs

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Directed by Professor Jae Hee Cheon

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Yoon Suk Jung

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Background and Aims: There is a lack of research describing associations between thiopurine methyltransferase (TPMT)/inosine triphosphate pyrophosphatase (ITPA) genotypes or phenotypes and long-term clinical outcomes after thiopurine treatment. We investigated whether TPMT/ITPA genotypes and TPMT activity would predict long-term clinical response in Korean patients with inflammatory bowel diseases (IBD) undergoing thiopurine treatment.

Methods: A total of 113 patients with IBD in whom thiopurine treatment was indicated were enrolled and categorized by TPMT and ITPA genotypes and TPMT enzyme activity. Long-term follow-up clinical data for these patients were analyzed with specific focus on disease relapse.

Results: Seventy-eight of 113 patients (69.0%) using thiopurines achieved remission and were included in the analysis. There were no significant differences in disease relapse-free survival between wild and mutant types of TPMT (p=0.690) or ITPA (p=0.403) according to the results of log rank analysis. The mean TPMT activity in the 'non-relapsers' (n=27) was significantly higher than in 'relapsers' (n=51) (p = 0.001).

Conclusions: Our study suggests that TPMT and ITPA genotypes or TPMT activity may not affect rates of disease relapse in Korean IBD patients treated with thiopurines. Further studies are indicated to appropriately guide clinicians formulating individualized treatments for IBD patients requiring thiopurine therapy.

Key words: azathioprine, inflammatory bowel disease, relapse, thiopurine S-methyl transferase, inosine triphosphase pyrophosphatase

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I. INTRODUCTION

The thiopurine drugs, 6-Mercaptopurine (6-MP) and its prodrug azathioprine (AZA), are effective for the induction and maintenance of remission in patients with inflammatory bowel disease (IBD).[1-3] However, adverse drug reactions to these agents occur in 15~38% of patients and often necessitate dose reduction or discontinuation of the drugs.[4-6] There has been a great deal of interest regarding thiopurine metabolism as a means to identify ways in which individual therapy might maximize clinical response and minimize adverse effects.

Two enzymes appear to be important in AZA metabolism, thiopurine S-methyl transferase (TPMT) and inosine triphosphase pyrophosphatase (ITPA). Genetic polymorphisms in the TPMT gene have been associated with

decreased TPMT activity and the development of myelotoxicity due to high thioguanine metabolite concentrations.[7-9] Moreover, ITPA deficiency has recently been associated with AZA toxicity due to the accumulation of 6-thioinosine-triphosphate (6-TITP).[10-12] Many studies have described an association between TPMT/ITPA genotypes or TPMT enzyme activity and adverse effects from AZA or 6-MP.[7-12] However, studies regarding a possible association between TPMT/ITPA genotypes or TPMT activity and long-term clinical response to thiopurines are lacking. There have been a few studies addressing the impact of TPMT activity and genotype on the clinical response of IBD patients treated with AZA/6-MP, which have suggested that measurement of TPMT activity might predict clinical response to thiopurines.[9,13,14] However, these investigations have been performed exclusively in Western countries, and it is unclear whether the results can be applied to Asian populations with different environmental and genetic backgrounds. For example, Korean and Japanese patients taking AZA/6-MP seem to experience myelotoxicity more frequently than patients of European descent.[15,16] Moreover, the impact of the ITPA genotype on the long-term clinical response of IBD patients treated with AZA/6-MP has not yet been examined in any study population. In this study, we investigated whether TPMT/ ITPA genotypes or TPMT activity could affect long-term clinical outcomes as measured by disease relapse in Korean patients with IBD undergoing AZA or 6-MP treatment.

II. METERIALS AND METHODS

Study subjects

A total of 113 patients with IBD (62 patients with Crohn's disease, 30 with ulcerative colitis, and 21 with intestinal Behcet's disease) were initiated on AZA treatment during the study period from June 1999 to November 2008 at Severance Hospital, Seoul, Korea. The protocol for AZA use in IBD patients was as follows. Patients were initially administered AZA at a fixed dose of 50 mg daily. If the drug was well tolerated without neutropenia or hepatotoxicity after two weeks, the dose was increased to 75 mg daily. The dose of AZA might be further increased to 2~3.5 mg/kg every two weeks if no obvious therapeutic response was seen, provided that the patient continued to tolerate therapy and white blood cell (WBC) counts remained greater than 3 X 10^3/L and serum alanine transaminase levels were less than twice the upper limit of normal (50 IU/L). Indications for AZA therapy included steroid dependency, steroid refractoriness, or enterocutanoeus fistulae including perianal fistulae. If the patient could not tolerate AZA due to adverse effects such as severe gastrointestinal symptoms, 6-MP was substituted for AZA. 6-MP was initially started at 25 mg daily. If the medication was well tolerated after two weeks, the dose of 6-MP was increased to 50 mg daily. Likewise, the dose was further increased to 1~1.5 mg/kg every two weeks if no adverse effect such as neutropenia and hepatotoxicity were observed.

Pretreatment and posttreatment clinical data were recorded. Clinical characteristics collected in this study included age, sex, height, body weight, type and duration of symptoms, comorbid conditions, physical examination findings, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, reason for thiopurine treatment, concomitant drugs, complications, and disease recurrence. The investigators evaluating patients' clinical data were blinded to the results of TPMT activity, TPMT genotype, and ITPA genotype. Ethical approval for this study was obtained from the Human Research Review Board of Severance Hospital.

Clinical response measurement

AZA and 6-MP are both slow-acting drugs, and therapeutic effect can typically be expected after 2-3 months of treatment. Thus, only those patients treated with AZA or 6-MP for a minimum of 3 months were included in the analysis of clinical response. Disease activity was evaluated using the CDAI (Crohn's disease activity index) for Crohn's disease and the partial Mayo score for ulcerative colitis. Remission was defined as a CDAI < 150 for Crohn's disease and a partial Mayo score < 2.5 for ulcerative colitis. Relapse was defined as a CDAI ≥150 for Crohn's disease and a partial Mayo score ≥2.5 for ulcerative colitis.[17,18] Patients with intestinal Behcet's disease were considered to have achieved remission if gastrointestinal symptoms had resolved and either no lesion or only an ulcer scar was found on colonoscopic

or radiological examinations. Relapse was defined as a recurrence of clinical symptoms, reappearance of ulcers at the sites of ulcer scars, or the development of new ulcers during the follow-up period.[19]

TPMT and ITPA genotyping

Blood samples for TPMT activity, TPMT genotyping, and ITPA genotyping were taken from patients at the initial clinic visit. Genomic DNA was extracted from the blood after informed consent was obtained. Single nucleotide polymorphisms (SNP) in the TPMT (exon 5, 7, 10) and ITPA (exon 2) genes flanking their exon-intron junctions were identified using denaturing high-performance liquid chromatography (DHPLC) and subsequent direct sequencing as previously described with some modifications.[20,21] Identical amplification conditions were used in a total volume of 25 μl containing 200 μM dNTPs, 50 ng of template DNA, 10 pmol of each primer, and 0.5 U I-StarmaxTM II DNA Taq DNA Polymerase (Intron biotechnology, Seoul, Korea) in 1X reaction buffer. Polymerase chain reaction (PCR) amplification was performed using a PTC-100 Peltier Thermal Cycler (MJ Research, Watertown, MA, USA) by means of an initial denaturation step at 94°C for 5 min, 35 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 45 sec, followed by 10 min of final extension at 72°C. Unpurified PCR products were mixed 3:1 with a sequence-confirmed wild-type reference or samples before being subjected to a 5 min 95 °C

denaturing step followed by gradual cooling to 65°C with a temperature change of 1°C/min before DHPLC analysis. The addition of wild-type DNA to the sample denaturation step enabled reliable detection of homozygous alteration in order to identify homozygous sequence variations. Five microliters of each mixture was loaded onto a DNASep-HT column (Transgenomic, Omaha, Neb., USA) and the amplicons were eluted in 0.1 M triethylammonium acetate (pH 7) with a linear acetonitrile gradient at a flow rate of 1.5 ml/min. Heteroduplex mismatches were recognized by the appearance of aberrant patterns in the elution profiles under appropriate temperature conditions, which were calculated by the Navigator Software of the Wave Nucleic Acid Fragment Analysis system device (Transgenomic). The most important criteria for assigning the presence of a sequence alteration in each DHPLC fragment were the numbers and the shape of the elution peaks compared to a sequence-confirmed wild-type control subject elution profile that was used as reference.

To test the specificity of the DHPLC method for the detection of a sequence variant, we compared DHPLC results with direct sequencing. Fragments in which DHPLC produced aberrant elution patterns were subjected to bi-direct sequencing from PCR products using ABI Prism BigDye chemistry (Applied Biosystems, Foster City, CA, USA)

TPMT activity assay

TPMT activity was measured as described previously by a tandem mass spectrometry method.[22] Venous blood from the experimental individuals was collected in EDTA tubes and centrifuged immediately at room temperature for 5 min at 1000 x g. The plasma was separated and the remaining cells were washed twice in sterile isotonic saline (same volume as discarded). The packed red blood cells (RBC) were diluted with water (1:2:5), for complete hemolysis and stored at -70°C. After thawing, the RBC lysates were centrifuged for 4 min at 13,000 × g to remove debris. The supernatants were then used to measure TPMT activity. In theory, the S-methylation of 6-MP and 6-TG is catalysed by TPMT, using S-adenosyl-L-methionine (SAM) as methyl donor, resulting in the products 6-MMP and 6-MTG, respectively. Quadruplicate measurements at a final volume of 500 µl were performed after an incubation period of 60 min at 37°C using a dry-heating-block. Twenty microliters of HCl (0.1 M) were diluted with 315 µl phosphate buffer (0.067 M, pH 7.4) in microfuge tubes and then 20 µl of the stock solution of 6-MP or 6-TG and of SAM were added, resulting in final concentrations of 600 µM (6-MP/6-TG) and 120 µM (SAM). The enzymatic reaction was started by the addition of 100 µl of the prepared RBC lysate. Following incubation, the reaction was stopped by the addition of 25 µl of 60% HClO4 with vortex mixing. The remnant was centrifuged for 4 min at 13,000 × g after a protein precipitation of 3 min. Finally the clear supernatant was analyzed by LC/MS/MS API4000 system (Applied Biosystems) without further

pretreatment.

Statistical analysis

All collected variables were subjected to descriptive analysis. Results were expressed as mean or median (±standard deviation or range) for numerical variables and as percentages for qualitative variables. A Student's t-test or Mann-Whitney U-test was used to compare numerical variables between groups and the chi-square test or Fisher's exact test was used to compare categorical variables. Relapse-free survival (time to first relapse) was compared by Kaplan-Meier curves using log rank analysis. Multivariate regression analysis was performed using Cox's proportional hazards modeling. P-values < 0.05 were accepted as statistically significant. The software program SPSS (version 12) was used for statistical analysis.

III. RESULTS

Patient baseline characteristics including TPMT/ITPA genotypes

Table 1 shows the baseline characteristics of the 113 patients enrolled in the study. The subjects included 62 patients with Crohn's disease, 30 with ulcerative colitis, and 21 with intestinal Behcet's disease. All patients were initially treated with AZA. However, 6 patients were unable to tolerate AZA due to adverse effects such as severe gastrointestinal trouble and were

eventually treated with 6-MP. Sixty-three patients were male and 50 were female with a median age of 34 years (range, 15~70 years). Indications for AZA or 6-MP treatment were steroid dependency in 86 patients, steroid refractoriness in 2 patients, and enterocutanoeus fistula in 25 patients. The median TPMT enzyme activity was 4.2 U/mL (range, 0.0~19.0).

Table 1. Characteristics of patients enrolled in the study.

characteristic	No.
Total IBD Patients	113
Azathioprine/6-mercaptopurine (%)	107/6 (94.7/5.3)
Sex (M/F) (%)	63/50 (55.8/44.2)
Age in years (median, range)	34 (15~70)
Type of disease	
Crohn's disease (%)	62 (54.9)
Ulcerative colitis (%)	30 (26.5)
Intestinal Behcet's disease (%)	21 (18.6)
Thiopurine indication	
Steroid dependent (%)	86 (76.1)
Steroid refractory (%)	2 (1.8)
Fistula (%)	25 (22.1)
Starting azathioprine dose in mg/kg (median, range)	0.96 (0.27~3.05)
Maximum azathioprine dose in mg/kg (median, range)	1.96 (0.63~3.26)

Treatment length in months (median, range)	35.5 (0.4~150.4)
TPMT activity in U/mL (median, range)	4.2 (0.0~19.0)
Oral 5-aminosalicylic acid use (%)	103 (91.2)

TPMT, thiopurine methyltransferase

TPMT and ITPA genotypes were assessed in all 113 patients (Table 2 & 3). Of the 113 patients genotyped for TPMT, 107 (94.7%) were homozygous wild type (*1/1), and 6 (5.3%) were heterozygous mutant (*1/3C). No patients were found to be homozygous mutant. With regard to ITPA, 80 patients (70.8%) were wild type for ITPA 94C>A (C/C), 31 (27.4%) were heterozygous for 94C>A (C/A), and 2 (1.8%) were homozygous for the 94C>A missense mutation (A/A). The mean TPMT activity of patients with the wild type was 5.3U/mL and 4.9U/mL in those with the mutant type, however, this was not statistically significant (Table 4) (p= 0.759).

Table 2. Genotype frequencies of TPMT by disease.

	No. (%)			
Genotype	total	Crohn's disease	Ulcerative colitis	Behcet's disease
	n=113	n=62	n=30	n=21
*1/1	107 (94.7)	60 (96.8)	27 (90.0)	20 (95.2)
*1/2	0	0	0	0

*1/3A	0	0	0	0
*1/3B	0	0	0	0
*1/3C	6 (5.3)	2 (3.2)	3 (10.0)	1 (4.8)

TPMT, thiopurine methyltransferase

Table 3. Genotype frequencies of ITPA by disease.

Genotype	No. (%)			
	total	Crohn's disease	Ulcerative colitis	Behcet's disease
	n=113	n= 62	n=30	n=21
Wild	80 (70.8)	43 (69.4)	22 (73.3)	15 (71.4)
Heterozygote 94 C>A	31 (27.4)	19 (30.6)	7 (23.3)	5 (23.8)
Homozygote 94 C>A	2 (1.8)	0	1 (3.3)	1 (4.8)
IVS2+21 A>C	0	0	0	0

ITPA, inosine triphosphate pyrophosphatase

Table 4. Association between TPMT genotype and activity.

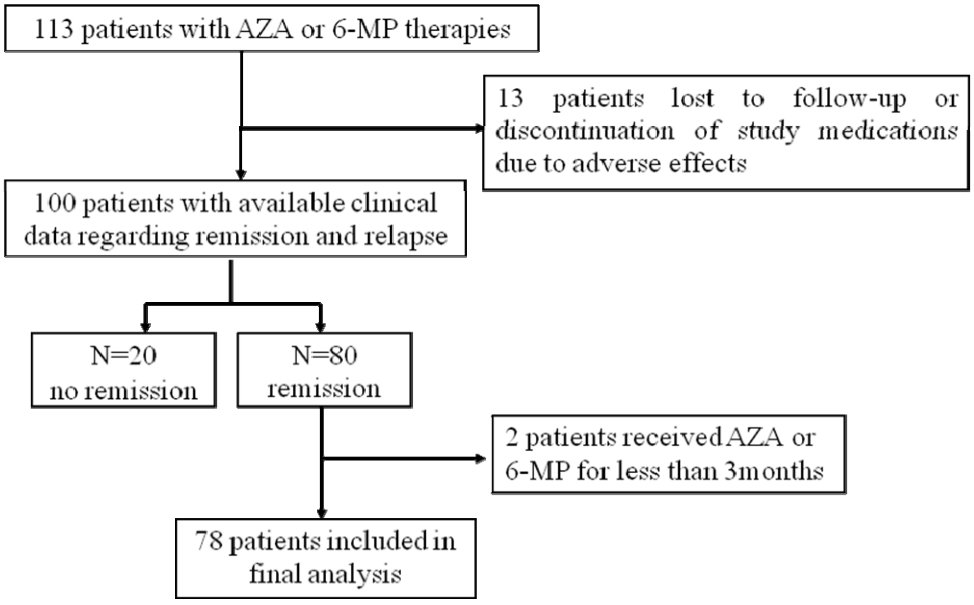
	Wild type (n=107)	Mutant type* (n=6)	p value
	mean±SD	mean±SD	
TPMT activity	5.3±3.3	4.9±2.4	0.759

Mutant type* = *1/3C; TPMT, thiopurine methyltransferase

Clinical course of the patients

The median duration for AZA or 6-MP therapy was 7.2 months (range, 0.4~150.4 months) with a median AZA starting dose of 0.94mg/kg (range, 0.27~3.05) and a median maximum AZA dose of 1.94mg/kg (range, 0.63~3.26). Of the 113 patients studied, 13 were lost to follow up or discontinued study medications due to side effects. Of the 100 remaining patients who had available medical records regarding clinical response, 80 achieved clinical remission. Seventy-eight of the 80 patients that achieved a remission had received AZA or 6-MP for 3 months or more and were thus included in the final analysis of clinical outcomes in terms of relapse (Figure 1). Twenty-seven of 78 patients maintained remission and 51 patients relapsed more than once during the follow-up period. The cumulative relapse-free rate after 1 and 2 years of thiopurine treatment was 62.6% and 50.0%, respectively.

Figure 1. Flow diagram of enrolled patients.



Correlations of TPMT and ITPA genotypes with disease relapse

Starting AZA dose, maximum AZA dose, and duration of AZA use were similar between the 'non-relapsers' group and the 'relapsers' group (Table 5). To compare clinical outcomes between wild type and mutant type TPMT and ITPA, Kaplan-Meier survival curves using log rank analysis were constructed based on the time to first relapse in the IBD patients who had received AZA or 6-MP for 3 months or more (Figure 2A & B). There was no significant difference in disease relapse-free survival between wild types (n=74) and mutant types (n=4) of TPMT by log rank analysis (p=0.690). In the same manner, there was no significant difference in disease relapse-free survival between wild types (n=56) and mutant types (n=22) of ITPA by log rank

analysis (p=0.403). With respect to ITPA genotype, further analyses were performed on each disease entity, which revealed that there was no significant difference in disease relapse-free survival in any of the three groups (for Crohn's disease: n=44, p=0.928; for ulcerative colitis: n=25, p=0.153; for intestinal Behcet's disease: n=9, p=0.542).

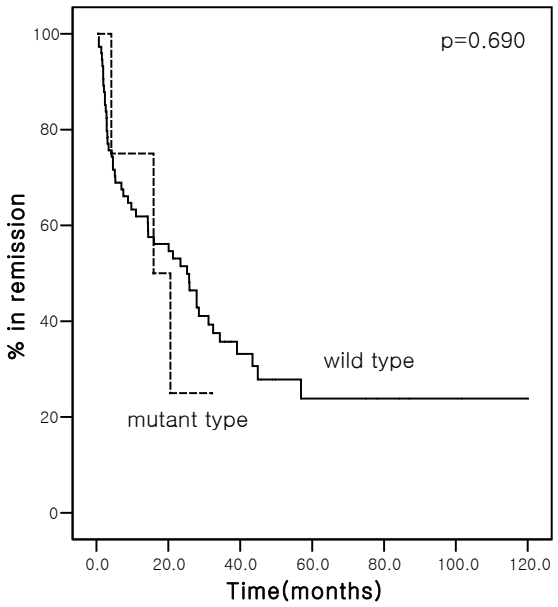
Table 5. Characteristics of non-relapsers and relapsers.

	Non-relapsers (n=27)	Relapsers (n=51)	p value
	mean±SD or N	mean±SD or N	
TPMT activity (U/mL)	7.0±4.0	4.5±2.8	0.001
Starting AZA dose (mg/kg)	1.19±0.55	1.09±0.35	0.367
Maximum AZA dose (mg/kg)	1.93±0.63	2.96±6.59	0.420
AZA duration (months)	50.7±34.9	49.9±30.4	0.916
TPMT genotype (wild/mutant)	26/1	48/3	1.000
ITPA genotype (wild/mutant)	20/7	36/15	0.745

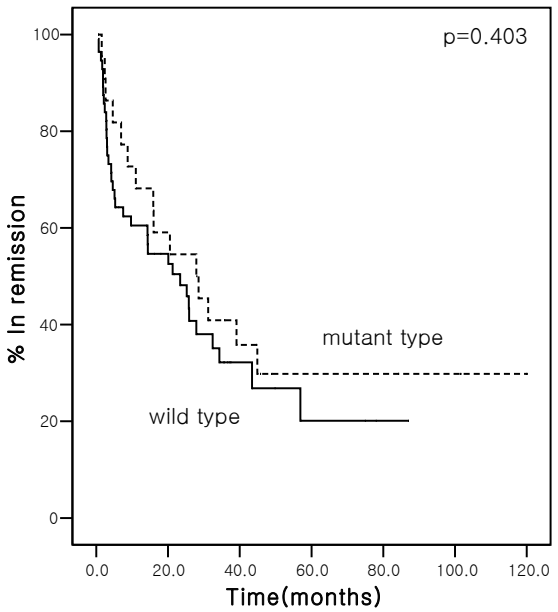
TPMT, thiopurine methyltransferase; ITPA, inosine triphosphate pyrophosphatase; AZA, azathioprine or equivalent 6-mercaptopurine

Figure 2. Time to first relapse in IBD patients treated AZA or 6-MP according to TPMT (A) or ITPA (B) genotype.

(A)


p=0.690
% in remission
100
80
60
40
20
0
wild type
mutant type
0.0
20.0
40.0
60.0
80.0
100.0
120.0
Time(months)

(B)


p=0.403
% In remission
100
80
60
40
20
0
mutant type
wild type
0.0
20.0
40.0
60.0
80.0
100.0
120.0
Time(months)

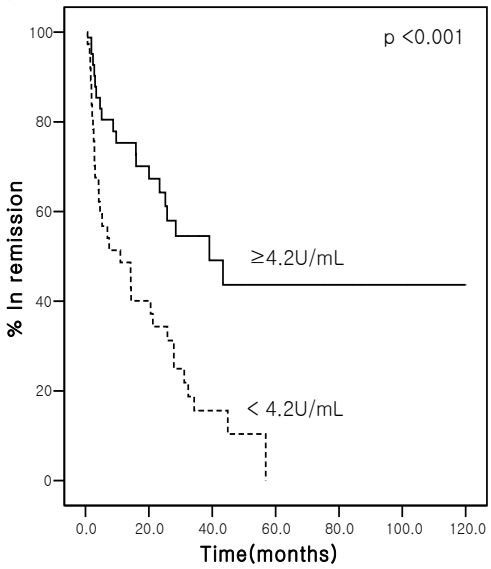
TPMT activity and disease relapse

The mean TPMT activity of the 'non-relapser' group (n=27) was 7.5 U/mL, which was significantly higher than 4.5 U/mL in the 'relapser' group (n=51) (p = 0.001) (Table 5). The patients were divided into those with below-median (<4.2U/mL) and those with above-median (≥4.2U/mL) TPMT activity and survival curves were constructed based on time to first relapse (Figure 3). Log rank analysis showed a significantly shorter time to relapse in patients with a TPMT activity <4.2U/mL (p<0.001). Further analyses were performed on each disease entity. Log rank analysis revealed a significantly shorter time to relapse in ulcerative colitis patients with a TPMT activity <4.2U/mL (n=25, p=0.001) but not in Crohn's disease or Behcet's disease patients with a TPMT activity <4.2U/mL (Crohn's disease: n=44, p=0.116; Behcet's disease: n=9, p=0.373).

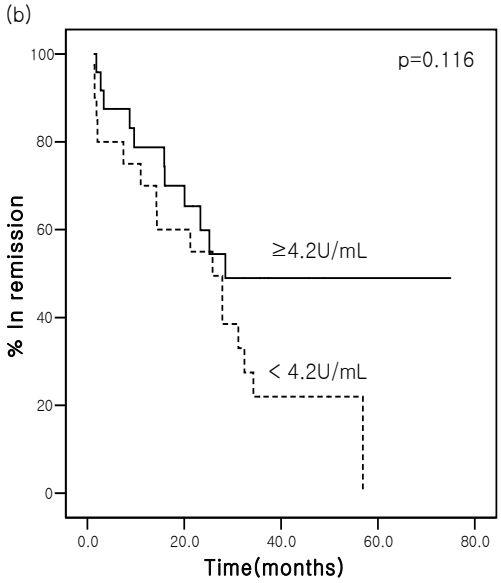
In multivariate analysis using Cox's proportional hazards model, TPMT genotype, ITPA genotype, AZA dose, age, and sex did not affect relapse-free survival. Only the TPMT activity was a prognostic factor for relapse-free survival (TPMT activity <4.2U/mL, hazard ratio=2.878, p<0.001) (date not shown).

Figure 3. Time to first relapse in IBD patients treated AZA or 6-MP according to TPMT activity: (a) all IBD patients (n=78); (b) Crohn's disease (n=44); (c) Ulcerative colitis (n=25); (d) intestinal Behcet's disease (n=9).

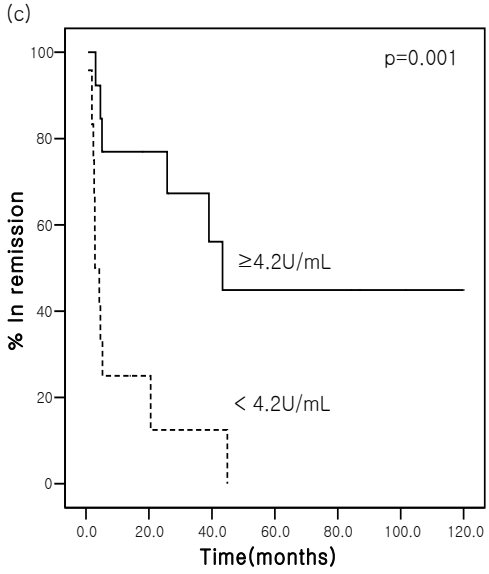
(a)


p <0.001
% In remission
≥4.2U/mL
< 4.2U/mL
Time(months)
0
20
40
60
80
100
0.0
20.0
40.0
60.0
80.0
100.0
120.0

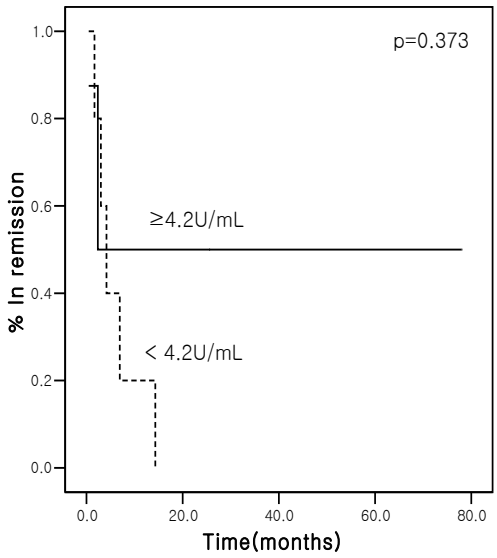
(b)


p=0.116
% In remission
≥4.2U/mL
< 4.2U/mL
Time(months)
0
20
40
60
80
100
0.0
20.0
40.0
60.0
80.0

(c)


p=0.001
% In remission
≥4.2U/mL
< 4.2U/mL
Time(months)

(d)


p=0.373
% In remission
≥4.2U/mL
< 4.2U/mL
Time(months)

IV. DISCUSSION

6-MP and its prodrug AZA have emerged as important therapeutic agents for induction and maintenance of remission in patients with IBD.[1-3] Many studies have shown that patients with mutant TPMT alleles tend to have decreased TPMT activity and are more likely to develop myelotoxicity compared to wild type patients, suggesting that TPMT genotype and activity may predict the development of myelosuppression.[7-9] Therefore, some authors suggest that identifying TPMT genotypes or activity in all patients prior to initiating treatment with thiopurines would help minimize the risk of myelotoxicity.[13,23] Theoretically, low TPMT activity might augment the therapeutic efficacy of thiopurines and lead to better long-term clinical outcomes. However, there have been few studies to address this concern. To confirm the usefulness of the TPMT/ITPA genotype or TPMT activity in clinical prediction, research regarding the relevance of measurement for prevention of both adverse effects and clinical outcomes are necessary. In this study, we sought to investigate whether TPMT/ITPA genotypes and TPMT activity could affect long-term clinical outcomes in terms of relapse in Korean patients with IBD undergoing AZA or 6-MP treatment.

We found that neither TPMT nor ITPA genotype influenced disease relapse in IBD patients treated with thiopurines. These results are consistent with results from several other studies concerning adverse effects which showed no or very poor correlation between TPMT status and thiopurine

adverse effects. In a study of 41 Crohn's disease patients who developed myelosuppression during treatment with AZA, only 27% of the patients had mutant alleles of the TPMT gene associated with enzyme deficiency.[24] Another study with 56 patients showed a slight trend for more frequent TPMT mutations in the patients with adverse reactions, however, these results did not achieve statistical significance.[25] A possible explanation for these results is that myelosuppression may more often be caused by other factors, such as viral infection or drugs interfering with AZA metabolism (sulfasalazine, 5-aminosalicylates, or allopurinol) than TPMT genotype.[26-30] In Asian populations, including Koreans, the frequencies of TPMT mutation are lower than those reported for Western countries, but myelotoxicity develops more frequently.[15,16,31,32] Therefore, determination of TPMT genotype prior to the initiation of treatment does not appear to be of significant use for predicting adverse effects with AZA in Asian populations. Several studies in Western samples have addressed the impact of TPMT activity and genotype on the clinical response of IBD patients treated with AZA/6-MP and suggested that measurement of TPMT activity might predict clinical response to AZA/6-MP. In a retrospective study with 106 IBD patients, high TPMT activity (≥14U/mL) was found to predict treatment failure.[13] In a prospective study with 207 IBD patients given 2 mg/kg of AZA, a baseline TPMT activity below 35 pmol/h/mg/Hb was associated with a greater chance of clinical response compared with a TPMT above 35 pmol/h/mg/Hb.[14] A study of 34

IBD patients given low-dose AZA (<2.0 mg/kg) demonstrated that low TPMT activity (<20 nmol/mL red blood cells/h) appeared to be predictive of a favorable response to treatment.[33] Currently, the reasons for discrepancies among these studies are unclear. Further research is warranted to reveal the true impact of TPMT on the clinical efficacy of thiopurines.

Several studies have described an association between ITPA genotypes and AZA toxicity. Recently, however, two other studies have failed to demonstrate a correlation between ITPA genotypes and AZA adverse effects. In a study by Gearry and colleagues, no significant association between the ITPA94C>A genotype and adverse effects, flu-like symptoms, rash or pancreatitis were found.[34] In a study by Allorge *et al*., ITPA genotyping also failed to improve detection of Crohn's disease patients at risk of AZA/6-MP-induced myelosuppression.[35] However, the impact of ITPA genotype on the long-term clinical response of IBD patients treated with AZA/6-MP has not yet been studied in any population. To our knowledge, this is the first study evaluating the relationship between ITPA genotypes and clinical outcomes during thiopurine treatment. In our study, the allele frequency of the ITPA 94C>A mutation in Korean IBD patients was 0.155, higher than that found in Europeans (0.060-0.070) and similar to the frequencies found in Japanese (0.135) and Chinese (0.15) populations.[36,37] However, we determined that ITPA genotype did not influence clinical outcomes in IBD patients treated with thiopurines. Therefore, the ITPA

genotyping test may not be useful for predicting relapse in Asian populations. Further research is necessary to explain why ITPA genotypes are not associated with clinical outcomes in Asian samples.

An interesting and unexpected finding is that lower TPMT activity was associated with shorter relapse-free survival, the reason for which is still unclear. One possible explanation is that methods of TPMT activity assay are not standardized among laboratories. In our study, the median TPMT activity was 4.2 U/mL, much lower than that previously reported in a population of 360 unrelated healthy Korean subjects (12.0 nmol ml-1 packed RBC h-1).[38] Moreover, several studies have demonstrated 5-ASA compounds to be potent TPMT inhibitors.[30,39,40] Of the 113 patients studied, 105 (91.2%) used 5-ASA and therefore one of the reasons that the median TPMT activity is low may have been that most patients used 5-ASA. However, the lack of standardized TPMT activity assays was attenuated in this study, as all of the patients in our sample were compared using the same method. Several other mechanisms can affect AZA and 6-MP metabolism and might be involved in determination of clinical response. One of the other genetic factors affecting thiopurine metabolism is glutathione-S-transferase. A significant association between AZA-related adverse effects and polymorphism of the glutathione-S-transferase-M1 gene was identified in a recent study.[41] Moreover, it has been suggested that factors other than the subject's genotype might influence TPMT activity expression; for example, the concomitant use

of salfasalazine[42], 5-aminosalisylic acid (5-AZA)[43], furosemide[42], allopurinol[42], infliximab[42], blood transfusion[44] and smoking.[45]

As AZA dose might affect clinical response, we looked for relationships between AZA dosing and TPTM activity. However, the mean AZA dose was not related to significant differences in TPMT activity <4.2U/mL and ≥ 4.2U/mL. Multivariate regression analysis using Cox's proportional hazards modeling demonstrated that TPMT genotype, ITPA genotype, AZA dose, age and sex did not affect relapse-free survival, but that TPMT activity was a prognostic factor for relapse-free survival. At this time, the mechanism underlying this result is unclear. However, our finding suggests that low TPMT activity could not predict favorable clinical outcomes in patients taking thiopurines. Further studies are warranted to elucidate this mechanism.

Our study has several limitations. First, our study is limited by sample size. Second, we did not measure 6-TGN, the active metabolite of the thiopurine drugs, or study its relationship to ITPA activity. Therefore, the relationships between 6-TGN concentration and clinical efficacy or ITPA activity and clinical efficacy could not be investigated. Third, the median TPMT activity was 4.2 U/mL, much lower than that reported in previous studies, suggesting that the TPMT activity assay may be unreliable.

V. CONCLUSION

In conclusion, our study suggests that TPMT and ITPA genotypes do not

predict relapse in IBD patients using thiopurine treatment. Patients with lower TPMT activity appear to have a shorter time to relapse. This study should serve as a cautionary note that adoption of TPMT genotype, TPMT activity, and ITPA genotype into routine clinical practice may be premature, particularly in Korea or in other East Asian populations. Further large-scale studies should be performed to validate the usefulness of TPMT genotype, TPMT activity and ITPA genotype for guiding clinicians formulating individualized treatments for patients requiring thiopurine therapy for the treatment of IBD.

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< ABSTRACT(IN KOREAN)>

Thiopurine 약물치료를 받고 있는 한국의 염증성 장질환 환자들의 Thiopurine Methyltransferase와 Inosine Triphosphate Pyrophosphatase의 유전자형과 장기간 치료 반응과의 상관관계

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<배경 및 목적> Thiopurine 치료를 받은 환자들의 thiopurine methyltransferase(TPMT)와 inosine triphosphate pyrophosphatase (ITPA) 유전자형이나 표현형과 장기간의 임상 결과와의 관련성을 조사한 연구들은 많지 않다. 따라서 본 연구는 thiopurine 치료를 받고 있는 한국의 염증성 장질환 환자를 대상으로 TPMT, ITPA 유전자형과 TPMT activity 가 장기간의 임상 반응을 예측할 수 있는지 조사하고자 하였다.

<대상 및 방법> Thiopurine 치료를 받고 있는 총 113명의 염증성 장질환환자가 본 연구에 참여하였고, 환자들을 TPMT, ITPA 유전자형과 TPMT activity 에 따라 분류하여 재발에 초점을 맞추어서 장기간의 임상 자료를 분석하였다.

<결과> Thiopurine 치료를 받고 있는 113명중 78명(69.0%)이 관해에 도달하였고 관해에 도달한 78명을 대상으로 분석하였다. log rank 분석을 이용한 결과 TPMT (p=0.690)와 ITPA (p=0.403) 모두 wild type 과 mutant type 사이에 relapse-free survival 의 차이가 없었다. TPMT activity 평균값은 재발하지 않은 군(27명)이 재발한 군(51명)보다 통계학적으로 유의하게 더 높았다 (p = 0.001).

<결론> 본 연구를 통해 thiopurine 치료를 받고 있는 한국의 염증성 장질환 환자들에 있어서는 TPMT, ITPA 유전자형과 TPMT activity가 relapse-free survival, 즉 장기간의 임상결과 영향을 끼치지 않음을 알 수 있었다. 향후 임상 의사들이 thiopurine 치료가 필요한 염증성 장질환 환자에게 맞춤형 치료를 위한 가이드라인을 제시하기 위해서는 더 많은 연구들이 이루어져야 할 것이다.

핵심되는 말 : azathioprine, 염증성 장질환, 재발, thiopurine S-methyl transferase, inosine triphosphase pyrophosphatase