Case Report

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Successful Azathioprine Treatment with Metabolite Monitoring in a Pediatric Inflammatory Bowel Disease Patient Homozygous for *TPMT*3C*

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Thiopurine *S*-methyltransferase (TPMT) methylates purine analogues, showing TPMT activity in inverse relation to concentrations of active metabolites such as 6-thioguanine nucleotide (6-TGN). With conventional dosing of thiopurines, patients with homozygous variant *TPMT* alleles consistently suffer from severe myelosuppression. Here, we report a patient with *TPMT*3C/*3C* who managed successfully with monitoring of thiopurine metabolites. The patient was an 18-year-old male diagnosed with Crohn's disease. The standard dose of azathioprine (AZA) (1.8 mg/kg/day) with mesalazine (55.6 mg/kg/day) was prescribed. Two weeks after starting AZA treatment, the patient developed leukopenia. The DNA sequence analysis of *TPMT* identified a homozygous missense variation (NM_000367.2: c.719A>G; p.Tyr240Cys), *TPMT*3C/*3C*. He was treated with adjusted doses of azathioprine (0.1-0.2 mg/kg/day) and his metabolites were closely monitored. Leukopenia did not reoccur during the follow-up period of 24 months. To our knowledge, this is the first case of a patient homozygous for *TPMT*3C* successfully treated with azathioprine in Korea. While a *TPMT* genotyping test may be helpful to determine a safe starting dose, it may not completely prevent myelosuppression. Monitoring metabolites as well as routine laboratory tests can contribute to assessing drug metabolism and optimizing drug dosing with minimized drug-induced toxicity.

Key Words: Thiopurine methyltransferase, azathioprine, inflammatory bowel disease, metabolite levels

INTRODUCTION

Azathioprine (AZA) plays a pivotal role in the treatment of inflammatory bowel disease (IBD). AZA rapidly changes to 6-mercaptopurine (6-MP) and consequently turns into 6-thioguanine nucleotides (6-TGN) by hypoxanthine-guanine phosphoribosyltransferase to exercise its immunosuppressive action. Alternatively, AZA is converted to inactive metabolites, 6-methylmercaptopurine (6-MMPN), by thiopurine *S*-methyltransferase (TPMT).

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To date, about 30 allelic variants of *TPMT* with affecting protein stability or enzymatic activity have been identified.[1] In Whites and Asians, *TPMT*3A* and *TPMT*3C* are the most important variants with low enzyme activity, respectively.[2] *TPMT*3A* exhibits deficient activity, while *TPMT*3C* has moderate activity *in vitro*.[3,4] In patients with deficient TPMT, 6-TGN is rapidly accumulated, causing potentially fatal myelotoxicity.[2]

Adverse effects, including myelosuppression, are found in 9-34% of IBD patients.[5] Consequently, AZA should be decreased or stopped in up to 30% of patients.[6] Considering the chronic course of IBD, it is important to optimize AZA dosing before treatment failure.[6] In cases with deficient TPMT, laboratory tests are applicable to measure thiopurine metabolites. These tests can confirm a TPMT phenotype and optimize personalized dosing to prevent myelosuppression.[7] Here, we report the first case of a Korean IBD patient homozygous for *TPMT*3C*, successfully treated with AZA by monitoring metabolite levels.

CASE REPORT

An 18 year-old male was referred to our hospital for abdominal pain and loose stool. On physical examination, he had tenderness on the lower left quadrant of the abdomen. Routine laboratory tests, including complete blood cell counts (CBC) and liver function were all within normal limits except mild anemia and an elevated erythrocyte sediment rate of 82 mm/hr (reference interval, 0-22 mm/hr).

The patient was diagnosed with Crohn's disease according to standard clinical, endoscopic, and histologic criteria. Oral treatment with AZA (1.8 mg/kg/day) and mesalazine (55.6 mg/kg/day) was started. Two weeks later, the absolute neutrophil count (ANC) and white blood cell count (WBC) decreased from 5140/μL to 1010/μL, and 6270/μL to 2810/μL, respectively. The AZA dosage was reduced from 1.8 to 0.9 mg/kg/day. Three weeks later, ANC and WBC continued to decline further to 190/μL and 1910/μL, respectively. The AZA was discontinued. After leukopenia was recovered, the patient was restarted on AZA (0.8 mg/kg/day) with the discontinuation of mesalazine. The patient's daily dose of AZA was cautiously increased to 1.2 mg/kg while monitoring CBC levels.

Peripheral blood samples were taken from the patient for *TPMT* genotyping. After obtaining written informed consent, sequence analysis of all coding exons with their flanking intron regions of *TPMT* gene were performed, and we identified a homozygous variant (c.719A>G; p.Tyr240Cys), *TPMT*3C/*3C*.

Simultaneously, 6-TGN and 6-MMPN concentrations were measured by the Waters 2795 Alliance HPLC system and a Quatro Micro API tandem mass spectrometer (Waters, Manchester, UK).[8] The thresholds indicating increased likelihood efficacy (6-TGN >235 pmole/8×10^8 red blood cells, RBC), increased risk of leukopenia (6-TGN >450 pmole/8×10^8 RBC), and increased risk for hapatotoxicity (6-MMPN >5700 pmole/8×10^8 RBC) were suggested.[9,10] The patient's 6-TGN concentration (7206 pmole/8×10^8 RBC) corresponded to a higher risk of leukopenia, although the daily dose had already been reduced from 1.2 mg/kg to 0.8 mg/kg because of neutropenia. The daily dosage was readily decreased to 0.2 mg/kg, and 6-TGN declined to therapeutic levels (437 pmole/8×10^8 RBC). Daily AZA dose was reduced further to 0.1 mg/kg as 6-TGN concentration increased again to 745 pmole/8×10^8 RBC (Fig. 1). 6-MMPN concentrations were detected at less than the limit of quantitation. The patient's laboratory parameters, including CBC and liver function, were within normal limits during the follow-up period of 24 months.

DISCUSSION

The effect of extremely low or absent TPMT activity can be fatal due to severe myelosuppression. The most significant variants with low TPMT enzyme activity in Western and Asian countries are *TPMT*3A* and *TPMT*3C*. Compared with Whites, East Asians showed different allele frequencies of *TPMT*3A* (Whites vs. East Asians: 3.2-5.7% vs. 0.0%) and *TPMT*3C* (0.2-0.8% vs. 0.3-2.3%).[11] Although *TPMT*3C* is a predominant variant in Asian populations, this is the first case of an IBD patient homozygous for *TPMT*3C* in Korea.

Myelosuppression in IBD patients treated with AZA/6-MP was reported as more common in Koreans (31.0-56.4%)[12-14] than in Western countries (2.0-16.7%).[15-17] The mechanism contributing to a higher incidence of leukopenia in Koreans remains unclear. According to some reports, *TMPT* polymorphisms affected myelotoxicity in only a small portion of patients, in which large numbers of patients with leukopenia also had wild type *TPMT*.[13,18,19] As the *TPMT* genotype could not exclusively elucidate myelosuppression during AZA/6-MP therapy, one possible hy-

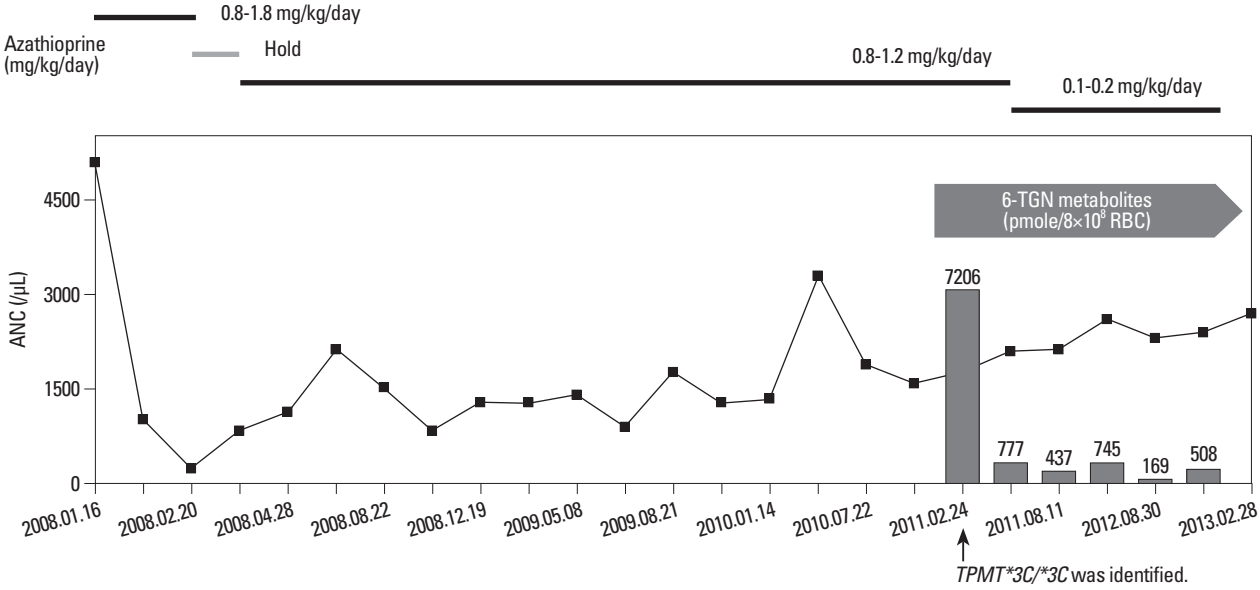

Fig. 1. Absolute neutrophil count (ANC) and erythrocyte 6-thioguanine nucleotides (6-TGN) concentrations according to dosages of azathioprine. Leukopenia was developed 2 weeks after starting azathioprine (AZA). AZA was reduced and discontinued. After leukopenia was recovered, AZA treatment was restarted. The patient continued to receive AZA treatment with optimized daily dose adjustments (0.1-0.2 mg/kg) and ANC was maintained within normal limits by monitoring thiopurine metabolites such as 6-TGN. RBC, red blood cells; TPMT, thiopurine S-methyltransferase.

pothesis is that genetic variants of genes other than *TPMT*, such as *ITPA* and *MRP4*, involved in thiopurine pharmacokinetics in Asians may also influence inter-individual variability in thiopurines toxicity.[20,21]

Because there is genetic variation in thiopurine metabolism, the optimal dose varies. AZA doses are typically reduced by 10-fold and given three times per week instead of daily in the homozygous variant,[22,23] while standard doses are given daily at 1.5-2.5 mg/kg in the wild type.[24] Currently empiric weight-based dosing adjustment is widely accepted: AZA should be started with low daily doses (0.5-1.5 mg/kg) in order to prevent severe adverse reaction, followed by gradual upward daily dose titration at a maximum of 2.5 mg/kg.[25]

In the present case, even after reducing the daily dose of AZA (0.8 mg/kg), the first thiopurine metabolite measurement showed an extremely high 6-TGN level (7206 pmole/8×10^8 RBC) and a 6-MMPN concentration less than the quantitation limit. TPMT with deficient activity cannot readily catabolize 6-MP to inactive metabolites, resulting in more 6-MP available for the anabolism of 6-TGN. The secondary metabolite of 6-MP, thioinosine monophosphate (TIMP), is also a substrate for TPMT. Deficient TPMT cannot methylate TIMP to methyl TIMP forming 6-MMPN, which explains why 6-MMPN levels in our patient were less than the quantitation limit. According to the metabolite quantitation results, we reduced the daily maintenance dose (0.1-0.2 mg/kg) by up to 5% of the daily starting dose (1.8 mg/kg). This suggests the importance of metabolite monitoring for individualized-dose adjustment.

To determine a safe starting dose, evaluation of the *TPMT* genotype or phenotype (TPMT activity) prior to beginning AZA treatment is recommended. Slow metabolizers typically respond to much lower doses of medication. Patients treated with AZA require periodic monitoring of CBC and liver function to prevent AZA-induced toxicities. The monitoring of thiopurine metabolites may be helpful in assessing drug metabolism and optimizing drug dosing, as well as drug-interaction, dosing compliance, and intraindividual variability of TPMT activity during AZA treatment, which cannot be explained exclusively by the *TPMT* genotype.[9] The onset of toxicity distinctively develops within one month.[26] In cases with a *TPMT* variant, long-term thiopurine therapy is likely to fail because of significant toxicity or an inadequate response during treatment. In this report, our patient had experienced myelosuppression only two weeks after starting AZA therapy. Since dose adjustment based on *TPMT* genotype followed by metabolites monitoring was applied, the patient's disease activity was successfully controlled without relapse of neutropenia.

In conclusion, this is the first reported case of an IBD patient homozygous for *TPMT*3C* in Korea. The patient received successful AZA treatment without recurrent leukopenia after dose optimization based on the presence of the *TPMT* genotype and metabolite monitoring. Our report sug-

gests that AZA dosage should be determined based on the presence of a *TPMT* genotype and with careful metabolite monitoring as this may provide safe and efficient dosing.

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REFERENCES

1. Appell ML, Berg J, Duley J, Evans WE, Kennedy MA, Lennard L, et al. Nomenclature for alleles of the thiopurine methyltransferase gene. Pharmacogenet Genomics 2013;23:242-8.
2. Krynetski EY, Tai HL, Yates CR, Fessing MY, Loennechen T, Schuetz JD, et al. Genetic polymorphism of thiopurine S-methyltransferase: clinical importance and molecular mechanisms. Pharmacogenetics 1996;6:279-90.
3. Tai HL, Krynetski EY, Schuetz EG, Yanishevski Y, Evans WE. Enhanced proteolysis of thiopurine S-methyltransferase (TPMT) encoded by mutant alleles in humans (TPMT*3A, TPMT*2): mechanisms for the genetic polymorphism of TPMT activity. Proc Natl Acad Sci U S A 1997;94:6444-9.
4. Tai HL, Krynetski EY, Yates CR, Loennechen T, Fessing MY, Krynetskaia NF, et al. Thiopurine S-methyltransferase deficiency: two nucleotide transitions define the most prevalent mutant allele associated with loss of catalytic activity in Caucasians. Am J Hum Genet 1996;58:694-702.
5. Hindorf U, Lindqvist M, Hildebrand H, Fagerberg U, Almer S. Adverse events leading to modification of therapy in a large cohort of patients with inflammatory bowel disease. Aliment Pharmacol Ther 2006;24:331-42.
6. Dewit O, Starkel P, Roblin X. Thiopurine metabolism monitoring: implications in inflammatory bowel diseases. Eur J Clin Invest 2010;40:1037-47.
7. Relling MV, Gardner EE, Sandborn WJ, Schmiegelow K, Pui CH, Yee SW, et al. Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. Clin Pharmacol Ther 2013;93:324-5.
8. Dervieux T, Meyer G, Barham R, Matsutani M, Barry M, Boulieu R, et al. Liquid chromatography-tandem mass spectrometry analysis of erythrocyte thiopurine nucleotides and effect of thiopurine methyltransferase gene variants on these metabolites in patients receiving azathioprine/6-mercaptopurine therapy. Clin Chem 2005;51:2074-84.
9. Dubinsky MC, Lamothe S, Yang HY, Targan SR, Sinnett D, Théorêt Y, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. Gastroenterology 2000;118:705-13.
10. Cuffari C, Dassopoulos T, Turnbough L, Thompson RE, Bayless TM. Thiopurine methyltransferase activity influences clinical response to azathioprine in inflammatory bowel disease. Clin Gastroenterol Hepatol 2004;2:410-7.
11. Kumagai K, Hiyama K, Ishioka S, Sato H, Yamanishi Y, McLeod HL, et al. Allelotype frequency of the thiopurine methyltransferase (TPMT) gene in Japanese. Pharmacogenetics 2001;11:275-8.
12. Kim JH, Cheon JH, Kim WH. [The frequency and the course of the adverse effects of azathioprine/6-mercaptopurine treatment in patients with inflammatory bowel disease]. Korean J Gastroenterol 2008;51:291-7.
13. Kim JH, Cheon JH, Hong SS, Eun CS, Byeon JS, Hong SY, et al. Influences of thiopurine methyltransferase genotype and activity on thiopurine-induced leukopenia in Korean patients with inflammatory bowel disease: a retrospective cohort study. J Clin Gastroenterol 2010;44:e242-8.
14. Lee HJ, Yang SK, Kim KJ, Choe JW, Yoon SM, Ye BD, et al. The safety and efficacy of azathioprine and 6-mercaptopurine in the treatment of Korean patients with Crohn's disease. Intest Res 2009;7:22-31.
15. Hindorf U, Lindqvist M, Peterson C, Söderkvist P, Ström M, Hjortswang H, et al. Pharmacogenetics during standardised initiation of thiopurine treatment in inflammatory bowel disease. Gut 2006;55:1423-31.
16. Gisbert JP, Niño P, Rodrigo L, Cara C, Guijarro LG. Thiopurine methyltransferase (TPMT) activity and adverse effects of azathioprine in inflammatory bowel disease: long-term follow-up study of 394 patients. Am J Gastroenterol 2006;101:2769-76.
17. Zelinkova Z, Derijks LJ, Stokkers PC, Vogels EW, van Kampen AH, Curvers WL, et al. Inosine triphosphate pyrophosphatase and thiopurine s-methyltransferase genotypes relationship to azathioprine-induced myelosuppression. Clin Gastroenterol Hepatol 2006;4:44-9.
18. Colombel JF, Ferrari N, Debuysere H, Marteau P, Gendre JP, Bonaz B, et al. Genotypic analysis of thiopurine S-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. Gastroenterology 2000;118:1025-30.
19. Takatsu N, Matsui T, Murakami Y, Ishihara H, Hisabe T, Nagahama T, et al. Adverse reactions to azathioprine cannot be predicted by thiopurine S-methyltransferase genotype in Japanese patients with inflammatory bowel disease. J Gastroenterol Hepatol 2009;24:1258-64.
20. 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;45:1014-21.
21. Uchiyama K, Nakamura M, Kubota T, Yamane T, Fujise K, Tajiri H. Thiopurine S-methyltransferase and inosine triphosphate pyrophosphohydrolase genes in Japanese patients with inflammatory bowel disease in whom adverse drug reactions were induced by azathioprine/6-mercaptopurine treatment. J Gastroenterol 2009;44:197-203.
22. Evans WE, Horner M, Chu YQ, Kalwinsky D, Roberts WM. Altered mercaptopurine metabolism, toxic effects, and dosage requirement in a thiopurine methyltransferase-deficient child with acute lymphocytic leukemia. J Pediatr 1991;119:985-9.
23. Kaskas BA, Louis E, Hindorf U, Schaeffeler E, Deflandre J, Graepler F, et al. Safe treatment of thiopurine S-methyltransferase deficient Crohn's disease patients with azathioprine. Gut 2003;52:140-2.
24. Grossman AB, Noble AJ, Mamula P, Baldassano RN. Increased dosing requirements for 6-mercaptopurine and azathioprine in inflammatory bowel disease patients six years and younger. Inflamm

Bowel Dis 2008;14:750-5.
25. Nielsen OH, Vainer B, Rask-Madsen J. Review article: the treatment of inflammatory bowel disease with 6-mercaptopurine or azathioprine. Aliment Pharmacol Ther 2001;15:1699-708.
26. Boonsrirat U, Angsuthum S, Vannaprasaht S, Kongpunvijit J, Hirankarn N, Tassaneeyakul W, et al. Azathioprine-induced fatal myelosuppression in systemic lupus erythematosus patient carrying TPMT*3C polymorphism. Lupus 2008;17:132-4.