Thiopurine S-Methyltransferase Phenotype and Genotype in Pediatric Patients with Inflammatory Bowel Disease; Implication for Azathioprine Treatment

Maciej Jankowski1,2, Piotr Landowski1, Robert Kowalski1, Ewelina Kreft1, Irena Audzeyenka2, Małgorzata Kasztan1, Barbara Kamińska1, and Mirosława Szczepańska-Konkel1

1 Department of Therapy Monitoring and Pharmacogenetics, Medical University of Gdańsk, Poland
2 Laboratory of Cellular and Molecular Nephrology, Mossakowski Medical Research Centre PAS, Warsaw, Poland

Abstract

Inflammatory Bowel Disease (IBD) is more prevalent in children than adults, and the incidence is increasing. IBD is treated with thiopurines, metabolized by thiopurine S-methyltransferase (TPMT) and inter-individual variability in activity of TPMT affecting therapy efficiency and drug toxicity arises from genetic polymorphisms, mainly TPMT*2, *3A, and *3C. The aim was to investigate the frequency distribution of TPMT activity, determine the penetration rate of TPMT*2, *3A, and *3C alleles in children, and compare TPMT activity in children and adults with IBD. The study included 85 children, 45% with Crohn’s disease (CD) and 55% with ulcerative colitis (UC), and 31 adults with IBD. TPMT activity was measured with radiochemistry. TPMT*2, *3A, and *3C alleles were investigated with PCR and restriction fragment length polymorphism analyses. Children showed median TPMT activities of 13.12 and 13.19 U/ml RBC in CD and UC, respectively, with 4.8-fold variability (range, 4.74 - 22.56 U/ml RBC). TPMT activity was similar in children and adults; ranges: 5.56-21.34 vs. 9.61-17.84 U/ml RBC, respectively, in CD; and 4.74-22.56 vs. 5.19-21.98 U/ml RBC, respectively, in UC. Patients with CD and UC treated with azathioprine displayed similar TPMT activities, similar adverse event frequencies, and similar numbers of non-responders. One out of 85 patients (1.18%) was heterozygous with TPMT*1/TPMT*2 (TPMT activity: 5.19 ± 0.05 U/ml RBC). Individuals with low-intermediate TPMT activity (≤8 U/ml RBC) did not carry mutant alleles *3A or *3C. TPMT phenotypes were similar in children and adults with inflammatory bowel disease.

Keywords: Azathioprine; Children; Thiopurine S-Methyltransferase; Inflammatory Bowel Disease

Abbreviations: CD: Crohn’s Disease; IBD: Inflammatory Bowel Disease; RBC: Red Blood cells; TPMT: Thiopurine S-Methyltransferase; UC: Ulcerative Colitis

Introduction

Epidemiological studies from Europe have shown an increasing incidence of pediatric inflammatory bowel disease (IBD). Childhood-onset IBD accounts for nearly 30% of all cases and over 25% of patients are diagnosed under the age of 16. Furthermore, IBD is more extensive and severe in children and adolescents than in adults [1,2]. Purine analogues, like azathioprine, and its active metabolite, 6-mercaptopurine, are considered first-line steroid-sparing immunosuppressive therapies for treating IBDs, including Crohn’s disease (CD) and ulcerative colitis (UC). Additionally, thiopurines have demonstrated broad inter-individual variability in terms of response; 15-28% of patients experienced adverse drug reactions (e.g., bone marrow depression, hepatic and pancreatic dysfunctions) and 9% of patients were resistant to thiopurines [3,4]. It has been established that adverse clinical conditions mainly result from thiopurine metabolism, involving S-methylation catalyzed by thiopurine S-methyltransferase (TPMT). TPMT is a cytosolic enzyme expressed ubiquitously in humans; e.g., in liver, intestine, kidney, lymphocytes, and red blood cells, RBCs [5]. TPMT expression/activity in the liver, the major body compartment for deactivating thiopurines, was correlated with TPMT activity measured in RBCs [6]. Therefore, in clinical practice, TPMT activity is measured in RBC lysates. The susceptibility of patients to adverse drug reactions is due to inter-individual variations in TPMT activity, a consequence of genetic polymorphisms. TPMT activity varies over a large range, but about 0.3% of Caucasians exhibit complete deficiency, and 11% exhibit intermediate TPMT activity. These subjects are at elevated risk of life-threatening thiopurine-induced adverse drug reactions, and they should be considered for alternative forms of therapy, or they should receive low doses of thiopurines [7]. To date, 30 variant alleles (TPMT*2-28) have been identified that are predictive of decreased TPMT activity compared to the wild-type allele (TPMT*1). Importantly, the variant alleles TPMT*2, *3A, and *3C account for over 95% of inherited TPMT deficiency cases in Caucasian subjects [8,9]. This has led to the general assumption that TPMT phenotype/genotype should be determined in pediatric patients with IBD before treating with thiopurines [10]. The primary objective of the current study was to investigate the TPMT activity frequency distribution and the penetration rates of TPMT*2, *3A, and *3C alleles in children with CD and UC. The secondary objective was to compare TPMT activities in children and adults. Understanding how these variables occur in pediatric patients with IBD is critical for optimizing the management of IBD in children.

*Corresponding author: Maciej Jankowski, Department of Therapy Monitoring and Pharmacogenetics, Medical University of Gdańsk, Poland, Tel: (+4858) 3492776; Fax: (+4858) 3492784; E-mail: majank@gumed.edu.pl

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Materials and Methods

Study patients

The experiments were conducted in accordance with the protocol approved by the Regional Bioethical Commission at the Medical University of Gdansk (NKEBN/2/2009). Patients were included in the study after they or their parents had been fully informed and had signed the study consent form. In addition, verbal assent was obtained from older children. The cutoff age was ≤ 16 years for children, due to physical development and the completion of puberty. Diagnosis of CD or UC was established by standard clinical, radiological, histological, and endoscopic criteria. One hundred-sixteen patients (85 children and 31 adults) with IBD were included in the study; all were Caucasian. For assessments of CD and UC activities, we used the Crohn’s disease activity index (CDAI) and the Truelove-Witts score, respectively.

TPMT phenotyping

Blood samples were drawn by venipuncture and collected into heparinized vacutainer tubes. After measuring the hematocrit, washed RBC lysates were prepared, as describe elsewhere, and stored at -80°C. In this condition, the TPMT enzyme remains stable for several weeks [11]. TPMT activity in RBC lysates was measured with a radiochemical method based on the conversion of 6-mercaptopurine into 6-methylmercaptopurine in the presence of [14C-methyl]-S-adenosyl-L-methionine (PerkinElmer, MA, USA), as the methyl donor [6]. Briefly, 50 μl of RBC lysate was incubated with 156 mM K2HPO4/ KH2PO4, pH 7.5 and 7.5 mM 6-mercaptopurine for 3 min at 37°C. The enzyme reaction was initiated by the addition of the following reagents: 12.5 μM [14C-methyl]-S-adenosyl-L-methionine (specific activity 1.81 GBq/mmol), 12.5 μM S-adenosyl-L-methionine, 1 mM dithiothreitol (DTT), and 50 μM allopurinol. The reaction tubes were incubated for 1 h at 37°C, and then, the reaction was stopped with the addition 0.5 M borate buffer (pH 10). The 6-methylmercaptopurine product was extracted into the organic phase of 20% isoamyl alcohol in toluene and measured in a 1409 Wallec liquid scintillation counter. TPMT activity was expressed as the amount of 6-methylmercaptopurine formed (nmol) per ml of RBC per hour (nmol/ml RBC/h=U/ml RBC).

TPMT genotyping

Total genomic DNA was isolated from whole blood samples with a standard procedure (Blood Mini, Heliconius A & A Biotechnology). PCR amplification was performed to detect the G238C transversion in the TPMT*2 allele and the transition mutations, G460A and A719G, in the TPMT*3A and TPMT*3C alleles, respectively [12]. To detect the G238C transversion, two pairs of primers were used. The first pair (F1 5’-GTATGATTATTATGCAGTTTG-3’ and R 5’-TAAATAGGGACCATCGGACA-3’) detected the wild type allele, and the second pair (F2 5’-GTATGATTTATGCAGGTTC-3’ and R 5’-TAAATAGGGACCATCGGACA-3’) detected the G238C mutation. A DNA fragment was amplified with F1 and R primers when G238 (wild-type) was present, whereas a DNA fragment was amplified with F2 and R primers when C238 (mutant) was present (Figure 1A).

PCR was performed in duplicate in a total volume of 50 μl containing: 30 ng genomic DNA, 0.5 μM of each sense and antisense primer, 0.2 mM dNTPs, 2.5 mM MgCl2, and 1.25 U of GoTaq® Flexi DNA polymerase. The conditions for PCR were as follows: 1 min denaturation at 94°C, 2 min annealing at 57°C, 1 min extension at 72°C for 30 cycles, and a final 7 min extension at 72°C. The same PCR protocol was used to detect the G460A and A719G transitions with the following primers:

\[
\begin{align*}
\text{G460A:} & \quad \text{F: 5’-ATAACACAGATGGGAGGCTGC-3’ and} \\
\text{R: 5’-CTAGAACCAGAAAAAGTATAG-3’} \\
\text{A719G:} & \quad \text{F: 5’-TGTTGGGATTACAGGTTGAGCCAC-3’ and} \\
\text{R: 5’-CAGGCCTTTAGCATAATTTCCAATTCCTC-3’}.
\end{align*}
\]

The amplification products were purified on microcolumns (GenElute PCR Clean-Up Kit, Sigma) and digested with the appropriate restriction enzyme (Mwo I or Acc I, New England Biolabs, UK). Mwo I entirely digested the wild-type DNA into two shorter fragments, but no digestion occurred in the presence of a G460A mutation. Conversely, Acc I did not digest wild-type DNA, but the A719G mutation introduced an Acc I restriction site in the sequence. The Mwo I and Acc I digestions were performed with 1 μg of product DNA at 60°C for 1 h and at 37°C for 1.5 h, respectively. Digested products were separated on a 2% agarose gel, stained with ethidium bromide, and visualized with UV irradiation (Figures 1B and 1C).

Statistical methods

Statistical analysis was performed with Sigma Plot computer software version 11. The Mann-Whitney U test was used to compare TPMT enzyme activity between the groups.

Results

Eighty-five pediatric patients with IBD were included in the study; their characteristics are displayed in Table 1. These patients included 45% with CD and 55% with UC; both groups comprised about 58% males. The median patient ages in the CD and UC groups were 13 and 14 years, respectively, with similar ranges (1-16 years). The distribution frequency of disease activity was similar in CD and UC groups. Most of the patients in both groups (58% CD and 66% UC) displayed mild disease activity. Only 3 patients with UC (6%) displayed severe disease activity, based on the Truelove-Witts score. The percent of patients treated with}{
Patterns of Adverse Drug Reactions for Cancer Drugs

Thiopurine S-methyltransferase (TPMT) enzyme activity in pediatric and adult patients with inflammatory bowel disease. Values represent the median (range) of TPMT activity. Adults ages were (median [range] years): Crohn’s disease: 19 (17-25); ulcerative colitis: 18 (17-23).

| Therapeutic group | TPMT activity [U/ml RBC] | P |
|-------------------|--------------------------|---|
| Crohn’s disease    |                          |   |
| Children           | 13.12 (5.56-21.34)       |   |
| Adults             | 13.67 (9.61-17.84)       | 0.0415 |
| Ulcerative colitis |                          |   |
| Children           | 13.19 (4.74-22.56)       |   |
| Adults             | 13.15 (5.19-21.98)       | 0.607 |

Table 2: Thiopurine S-methyltransferase (TPMT) enzyme activity in pediatric and adult patients with inflammatory bowel disease. Values represent the median (range) of TPMT activity. Adults ages were (median [range] years): Crohn’s disease: 19 (17-25); ulcerative colitis: 18 (17-23).

| Therapeutic group | TPMT activity [U/ml RBC] | P |
|-------------------|--------------------------|---|
| AZA-treated       |                          |   |
| Children           | 12.96 (7.85-21.34)       | 0.021 |
| Adults             | 12.89 (4.74-22.56)       | 0.472 |
| Adverse events     |                          |   |
| Children           | 13.20 (9.64-12.34)       | 0.471 |
| Adults             | 9.64 (6.95-12.34)        | 0.667 |
| No response        |                          |   |
| Children           | 13.20 (7.85-21.34)       | 0.343 |
| Adults             | 13.20 (9.64-12.34)       | 0.343 |

AZA: azathioprine

Values represent medians and ranges of TPMT activity.

Table 3: Thiopurine S-methyltransferase (TPMT) enzyme activity in pediatric patients with inflammatory bowel disease.

AZA in the CD group was about twice that in the UC group, but the percentage of adverse events (myelosuppression, hepatotoxicity and pancreatitis) was similar between groups (i.e., 3-4%). The median thiopurine S-methyltransferase (TPMT) activity in children was 13.12 U/ml RBC in CD and 13.19 U/ml RBC in UC groups. The range of TPMT activity was not significantly different in children and adults with IBD (Table 2); 5.56-21.34 vs. 9.61-17.84 U/ml RBC, respectively, for CD and 4.74-22.56 vs. 5.19-21.98 U/ml RBC, respectively, for UC. The distribution of TPMT activity in the whole study population (85 individuals) is graphically represented in Figure 2. TPMT activity levels showed a 4.8-fold variability, ranging from 4.74 up to 22.56 U/ml RBC. The TPMT activities in different pediatric therapeutic groups are presented in Table 3. The TPMT activity was similar between CD and UC groups for individuals treated with azathioprine, individuals that experienced adverse events, or individuals that failed to respond to azathioprine treatment. The latter two therapeutic response groups tended to have higher TPMT activities in the CD compared to the UC group, but the differences were not statistically significant.

Genotyping revealed that only one out of 85 patients (1.18%) carried the G238C mutation; TPMT allele *2. That individual displayed a heterozygous genotype of TPMT*1/*TPMT*2, which was accompanied by a phenotype of low-intermediate TPMT activity (5.19 ± 0.05 U/ml RBC; measured in two independent venipuncture blood samples). Next, we genotyped individuals that displayed low-intermediate TPMT activity (i.e., <8 U/ml RBC); these included two patients with activity <5 U/ml RBC and six patients with activity of 5-8 U/ml RBC. We screened these patient samples for the G460A and A719G mutations (TPMT alleles *3A, and *3A*3C, respectively). No TPMT*3A or TPMT*3C polymorphisms were detected in this study group.

Discussion

Thiopurines including azathioprine are rapidly metabolized in competing pathways. One of these pathways is regulated by thiopurine S-methyltransferase (TPMT). Variation in TPMT activity is partly responsible for the variability of the clinical response to thiopurines, including adverse reactions. We investigated the impacts of inflammatory bowel disease (IBD) and age on TPMT activity. In our study group, a significant percentage of children with IBD (i.e., 32%) had received azathioprine at some time during the course of the disease. This was consistent with other studies, which reported that 29-32% of children with IBD were treated with azathioprine [13,14].

We found a significant male predilection for both CD and UC in children; both groups showed a male:female ratio of approximately 1.35, in contrast adult IBD cohorts showed a mild female predominance [15]. The male predominance may suggest that the cohort was mainly sporadic [16], however, significant numbers of patients or their parents were unaware of family history, and therefore it was difficult to confirm
patterns of adverse drug reactions for cancer drugs

reported in previous studies [26].

individuals with wild-type genotype and deficient activity has been i.e, or 3A, in children with IBD was mostly not associated with TPMT alleles i.e., 0.2-0.5% [24,25]. Importantly, low-intermediate TPMT activity allele in our population of children with The frequency of the TPMT polymorphisms. Further genetic analysis of patients with TPMT activity at the low end of C alleles. /TPMT.*2.*1 (1.18%) that carried a heterozygous mutation of TPMT revealed only one patient that observed among healthy Northern Americans -11.7% [9,23]. 9.41% than observed among healthy Europeans -16.7%, but similar to included a smaller percentage with low-intermediate TPMT activity - [8.15 U/ml RBC], the nadir of the frequency distribution histogram for samples intermediate and high activity samples; this value is close to 13.7 U/ml RBC. We used the clinical laboratory “cutoff” value of 15 U/ml RBC to separate cases classified as low TPMT activity (0-2.5 U/ml RBC). We used activity (>15 U/ml RBC). Our study population did not include any normal activity (8-15 U/ml RBC), and 22.35% displayed very high displayed intermediate activity (3-8 U/ml RBC), 68.24% displayed the mean increase in TPMT activity was only 30-35% [20,21].

Our frequency distribution analysis of TPMT activity indicated a trimodal distribution. According to a previously published classification scale for TPMT activity [22], in our population, 9.41% displayed intermediate activity (3-8 U/ml RBC), 68.24% displayed normal activity (8-15 U/ml RBC), and 22.35% displayed very high activity (>15 U/ml RBC). Our study population did not include any cases classified as low TPMT activity (0-2.5 U/ml RBC). We used the clinical laboratory “cutoff” value of 15 U/ml RBC to separate intermediate and high activity samples; this value is close to 13.7 U/ml RBC, the nadir of the frequency distribution histogram for samples from healthy subjects [9]. We considered TPMT activities <8 U/ml RBC to be low-intermediate values. Our cohort of children with IBD included a smaller percentage with low-intermediate TPMT activity - 9.41% than observed among healthy Europeans -16.7%, but similar to that observed among healthy Northern Americans -11.7% [9,23].

Inter-individual variability in TPMT activity is partly caused by the presence of single nucleotide polymorphisms in the TPMT gene. Genotyping individuals for TPMT allele ‘2 revealed only one patient (1.18%) that carried a heterozygous mutation of TPMT*1/TPMT*2. Moreover, this patient did not carry the TPMT*3A or TPMT*3C alleles. The mutated genotype was accompanied by reduced TPMT activity. Further genetic analysis of patients with TPMT activity at the low end of the spectrum did not identify TPMT*3A or TPMT*3C polymorphisms. The frequency of the TPMT*2 allele in our population of children with IBD was similar to that found in adults from a European population i.e., 0.2-0.5% [24,25]. Importantly, low-intermediate TPMT activity in children with IBD was mostly not associated with TPMT alleles 2A, 3A, or 3C, which typically show the highest population penetration i.e., 60-95%. This discordance between genotype and phenotype among individuals with wild-type genotype and deficient activity has been reported in previous studies [26].

In conclusion, the similar activity of thiopurine S-methyltransferase in children and adults with inflammatory bowel disease suggests that thiopurines may be metabolized in the similar swiftness in theses groups of patients.

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References

1. de Mesquita MB, Civitelli F, Levine A (2008) Epidemiology, genes and inflammatory bowel diseases in childhood. Dig Liver Dis 40: 3-11.
2. Rueemme DL (2010) Pediatric inflammatory bowel diseases: coming of age. Curr Opin Gastroenterol 26: 332-336.
3. Sahasranaman S, Howard D, Roy S (2008) Clinical pharmacology and pharmacogenomics of thiopurines. Eur J Clin Pharmacol 64: 753-767.
4. Duley JA, Florin TH (2005) Thiopurine therapies: problems, complexities, and progress with monitoring thioguanine nucleotides. Ther Drug Monit 27: 647-659.
5. Karas-Kuzelicki N, Milnaric-Rascan I (2009) Individualization of thiopurine therapy: thiopurine S-methyltransferase activity and beyond. Pharmacogenomics 10: 1309-1322.
6. Szumlanski CL, Honchel R, Scott MC, Weinsilboum RM (1992) Human liver thiopurine methyltransferase pharmacogenomics: biochemical properties, liver-erythrocyte correlation and presence of isozymes. Pharmacogenomics 1: 148-159.
7. Weinshilboum RM, Sladek SL (1980) Mercaptopurine pharmacogenomics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. Am J Hum Genet 32: 651-662.
8. Garat A, Caufieze C, Renault N, Lo-Guide MC, Allorge D, et al. (2008) Characterisation of novel defective thiopurine S-methyltransferase allelic variants. Biochem Pharmacol 76: 404-415.
9. Otterness D, Szumlanski C, Lennard N, Klemetsdal B, Aarbakke J, et al. (1997) Human thiopurine methyltransferase pharmacogenomics: gene sequence polymorphisms. Clin Pharmacol Ther 62: 69-73.
10. Benkov K, Lu Y, Patel A, Rahhal R, Russell G, et al. (2013) Role of thiopurine metabolite testing and thiopurine methyltransferase determination in pediatric IBD. J Pediatr Gastroenterol Nutr 56: 333-340.
11. Weinshilboum RM, Raymond FA, Pazmiño PA (1978) Human erythrocyte thiopurine methyltransferase: radiochemical microassay and biochemical properties. Clin Chim Acta 85: 323-333.
12. Yates CR, Krynetsi EY, Loennechen T, Fessing MY, Tai HL, et al. (1997) Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med 126: 608-614.
13. Pozler O, Maly J, Bonova O, Dedeck P, Frühnap P, et al. (2006) Incidence of Crohn disease in the Czech Republic in the years 1990 to 2001 and assessment of pediatric population with inflammatory bowel disease. J Pediatr Gastroenterol Nutr 42: 186-189.
14. Turonen P, Kolho KL, Auvinen A, Illsten S, Huhtala H, et al. (2006) Incidence of inflammatory bowel disease in Finnish children, 1987-2003. Inflamm Bowel Dis 12: 677-683.
15. Sauer CG, Kugathasan S (2009) Pediatric inflammatory bowel disease: highlighting pediatric differences in IBD. Gastroenterol Clin North Am 38: 611-628.
16. Peeters M, Cortot A, Vermeire S, Colombel JF (2000) Familial and sporadic inflammatory bowel disease: different entities? Inflamm Bowel Dis 6: 314-320.
17. Chouchana L, Nwaroz C, Beaune P, Loriot MA, Robin X (2012) Review article: the benefits of pharmacogenetics for improving thiopurine therapy in inflammatory bowel disease. Aliment Pharmacol Ther 35: 15-36.
18. Petterson B, Almer S, Albertoni F, Söderhälf S, Peterson C (2002) Differences between children and adults in thiopurine methyltransferase activity and metabolite formation during thiopurine therapy: possible role of concomitant methotrexate. Ther Drug Monit 24: 351-358.
19. Ganiere-Monteil C, Medard Y, Lejus C, Bruneau B, Pineau A, et al. (2004) Phenotype and genotype for thiopurine methyltransferase activity in the French Caucasian population: impact of age. Eur J Clin Pharmacol 60: 89-96.
20. Keuzenkamp-Jansen CW, Leegwater PA, De Abreu RA, Lambooy MA, Bokkerink JP, et al. (1996) Thiopurine methyltransferase: a review and a clinical pilot study. J Chromatogr B Biomed Appl 678: 15-22.
21. Lennard L, Lilleyman JS, Van Loon J, Weinshilboum RM (1990) Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukaemia. Lancet 336: 225-229.
22. Ansari A, Hassan C, Duley J, Marinaki A, Shobowale-Bakre EM, et al. (2002) Thiopurine methyltransferase activity and the use of azathioprine in inflammatory bowel disease. Aliment Pharmacol Ther 16: 1743-1750.
23. Spire-Vayron de la Moureyre C, Debuysere H, Mastain B, Vinner E, Marez D, et al. (1998) Genotypic and phenotypic analysis of the polymorphic thiopurine S-methyltransferase gene (TPMT) in a European population. Br J Pharmacol 125: 879-887.
24. Schaeffeler E, Fischer C, Brockmeier D, Wernet D, Moerike K, et al. (2004) Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. Pharmacogenetics 14: 407-417.
25. Booth RA, Ansari MT, Loit E, Tricco AC, Weeks L, et al. (2011) Assessment of thiopurine S-methyltransferase activity in patients prescribed thiopurines: a systematic review. Ann Intern Med 154: 814-823, W-295-8.
26. Donnan JR, Ungar WJ, Mathews M, Rahman P (2011) Systematic review of thiopurine methyltransferase genotype and enzymatic testing strategies. Ther Drug Monit 33: 192-199.