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Thiopurine methyltransferase genotype and thiopurine S-methyltransferase activity in Greek children with inflammatory bowel disease

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Abstract

Background Azathioprine (AZA) and 6-mercaptopurine (6MP) are used in the treatment of pediatric inflammatory bowel disease (IBD). Genetic variations in thiopurine S-methyltranfarase (TPMT) gene have been correlated with enzyme activity and with the occurrence of adverse events to AZA and 6MP. The aim of the present study was to examine the sensitivity and specificity of *TPMT* genotyping for TPMT enzymatic activity, reducing harm from thiopurine by pretesting, and the association of thiopurine toxicity with TPMT status in children with IBD.

Methods TPMT red blood cell (RBC) activity was measured by using a radiochemical method and genotype was determined for the *TPMT alleles* *2, *3A, *3B and *3C in 108 thiopurine-treated pediatric IBD patients with a mean age of 11.3 years (range 3-16).

Results Significant TPMT activity differences between wild-type and heterozygous and homozygous mutated subjects were observed. We divided TPMT activity into three categories according to frequency distribution: low (16.67%), intermediate (25.92%) and high (57.41%). The whole population included a total of 77.78% of homozygous wild-type subjects, 15.74% heterozygous variants, 1.85% homozygous variants and five (4.63%) compound heterozygous variant *TPMT**3B/*3C. The overall concordance rate between *TPMT* genotypes and phenotypes was 88.2%. Seven carriers of at least one variant allele and low or intermediate TPMT activity developed adverse effects.

Conclusions Our findings suggest that carriers of at least one variant allele and both intermediate and absent TPMT activity have an increased risk of developing thiopurine-induced myelotoxicity compared with individuals with normal genotype and TPMT activity.

Keywords azathioprine, 6MP, pharmacogenetics, TPMT, inflammatory bowel disease

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Conflict of Interest: None

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Introduction

Azathioprine (AZA) and 6-mercaptopurine (6MP) are widely used as immunosuppressive drugs in the treatment of children with inflammatory bowel disease (IBD) [Crohn's disease (CD) and ulcerative colitis (UC)]. A number of questions remain regarding the need to optimize AZA metabolism in IBD patients treated with thiopurines. Thiopurines are metabolized by a complex multistep enzymatic pathway. After it is absorbed, AZA is metabolized to 6MP in the liver then during metabolism, hypoxanthine-guanine phosphoribosyltransferase (HGPRT) converts 6MP to cytotoxic 6-thioguanine nucleotide analogues, while thiopurine methyltransferase (TPMT) inactivates 6MP through methylation to form 6-methylmercaptopurine [1,2].

The metabolism and the production of active metabolites are mainly regulated by TPMT.

The *TPMT* gene has an autosomal codominant inheritance and the TPMT activity is largely influenced by polymorphisms, which results in a trimodal distribution; those patients heterozygous or homozygous for the "low activity" mutation gene might have an increased susceptibility for myelotoxicity with thiopurine therapy [3-6]. It has been reported that patients with inherited TPMT deficiency treated with standard doses of thiopurines present with higher levels of the thioguanine active metabolites and have an increased risk for adverse events. Unless patients with two defective alleles are treated with 10- to 15-fold lower doses of this medication, potentially fatal, hematopoietic toxicity, which requires immediate discontinuation of treatment, may follow [7-9].

TPMT genetic polymorphism was first described by Weinshilboum and Sladek [10]. In Caucasians, approximately 11% of the population harbour heterozygous and 0.3% homozygous *TPMT* mutations, leading to an intermediate or low *TPMT* activity, respectively. In these patients, thiopurine metabolism was shunted towards an increased production of active and toxic compounds. A high degree of concordance was demonstrated between *TPMT* genotype and phenotype in Caucasians [11,12]. Whether determining *TPMT* status prior to the start of thiopurine therapy, and adapting the dose accordingly, should be systematically performed in order to minimize the risk of myelotoxicity remains controversial [13-15]. In contrast to European [16] guidelines, American guidelines suggest the use of *TPMT* determination before thiopurine administration [17-19].

Therefore, in our study we aimed to examine the sensitivity and specificity of *TPMT* genotyping for TPMT enzymatic activity, reducing harm from thiopurine by pretesting, and the association of thiopurine toxicity with *TPMT* status in children with IBD.

Material and Methods

Patients with IBD who presented between February 2007 and August 2011 at the First Department of Pediatrics of the 'Aghia Sophia' Children's Hospital were consecutively enrolled. IBD was diagnosed based on clinical, endoscopic, radiological and histological criteria [20]. The study included patients who had been taking AZA or 6MP for at least 3 months or who had experienced adverse effects during treatment with these drugs. Thiopurine dosage had to be 0.3-2.5 mg/kg. Bone marrow suppression was defined as leukopenia (WBC <3000 /mm^3) and/or thrombocytopenia (platelets <100 000/mm^3), hepatotoxicity by serum alanine transaminase levels greater than twice the upper normal limit resolving after withdrawal of thiopurine drug, and pancreatitis by severe abdominal pain and hyperamylasemia resolving after withdrawal of thiopurine drug. The study was approved by the Ethics Committees of the participant centers.

After lysis of red blood cell (RBC), RBC TPMT activity was measured by a radiochemical method, as previously described [21].

For genotype analysis, venous blood samples (2 mL from each pediatric patient) were collected. Genomic screening was accomplished by a polymerase chain reaction (PCR) and restriction fragment length polymorphisms assay as previously described [15]. DNA of the patients was screened for TPMT*3A (both G460A and A719G mutation), TPMT*3B (only G460A mutation), TPMT*3C (only A719G mutation) and TPMT*2 (G238C mutation). Differences in allele frequencies were compared with the chi-square test (GRAPHPAD V. 3.00; GraphPad Software, San Diego, CA, USA). The statistical associations were tested using two-sided Fisher's exact test, and compared using the odds ratios and 95% confidence intervals. Strong association (significance) was assumed at $p < 0.05$.

Results

The clinical data of the 108 patients enrolled in the study are presented in Table 1. Seventy-seven were diagnosed with CD (71.29%), 20 with UC (18.52%) and 11 with indeterminate colitis (IC) (10.18%). All patients received standard recommended thiopurine doses for 6MP 1.0-1.5

Table 1 Demographic distribution of the population

	Patients with adverse effect (n= 3)	Patients without adverse effects (n=95)	All patients (n=108)
Median age [years (range)]	12.4 [2.5-14]	14 (3-17)	11.5 (2.5-17)
Gender (male/female)	5/8	43/52	48/60
IBD diagnosis (CD/ UC/ IC)	6/ 6/ 1	71/ 14/ 10	77/20/11
Thiopurine used (AZA/ 6MP)	11/ 2	89/ 6	100/ 8
Median thiopurine dose [mg/kg/day (range)]	1.13 [0.3-2]	1.41 (0.5-2)	1.38 (0.3-2)
Median length of treatment [months (range)]	31.9 [2-84]	20.24 (0.5-60)	22.3 (0.5-84)

CD, Crohn's disease; UC, ulcerative colitis; IC, indeterminate colitis; IBD, inflammatory bowel disease; AZA, azathioprine; 6MP, 6-mercaptopurine

mg/kg/day and for AZA 2.0-2.5 mg/kg/day. The patients were closely monitored through blood tests and regular medical visits in order to evaluate the clinical response and development of adverse events. According to toxicity, 13 children presented side effects to AZA and 6MP (Table 2).

The TPMT activity ranged from 0.36 up to 78 U/mL RBC. Among all patients included, 62 (57.41%) had normal to high TPMT activity (>15.6 U/mL RBC), 28 (28.92%) had intermediate (5.6 – 15.5 U/mL RBC) and 18 (16.67%) had low (<5.5 U/mL RBC) *TPMT* activity. Distribution of *TPMT* activity is represented in Fig. 1.

Among the 108 IBD children enrolled in the study a total of 77.78% were homozygous wild-type subjects, 15.74% heterozygous variants, 1.85% homozygous variants and 5 (4.63%) compound heterozygous variant TPMT*3B/*3C. The median TPMT activity in patients with a wild-type genotype was 27.27 U/mL RBC (range 15.8 - 78 U/mL RBC), in subjects with heterozygous phenotype 7.64 U/mL RBC (range 0.79 – 15.46 U/mL RBC) and in carriers homozygous mutated genotype 1.25 U/mL RBC (range 0.36 – 5.7 U/mL RBC). The difference between the values of patients with wild-type genotype and those of carriers of at least one *TPMT* variant is statistically significant (p <0.0001). The overall concordance rate between *TPMT* genotypes and phenotypes was 88.2 % (Fig. 2). Ten of the 46 carriers of at least one variant allele and/or with low or intermediate TPMT activity developed adverse effects, compared with 3 out of 62 with a wild-type *TPMT* gene and normal or high TPMT activity (O.R. 5.46, 95% CI 1.41 – 21.18, p = 0.014). More specifically, 2 patients with high TPMT activity and one with intermediate *TPMT* activity, all with wild-type genotype, developed hepatotoxicity. One patient with high TPMT activity, and 2 with intermediate *TPMT* activity, all with wild-type genotype; developed pancreatitis. The majority of the patients with low or intermediate TPMT activity developed bone marrow toxicity. All these patients' thiopurines (AZA or 6MP) were withdrawn or reduced due to adverse effects.

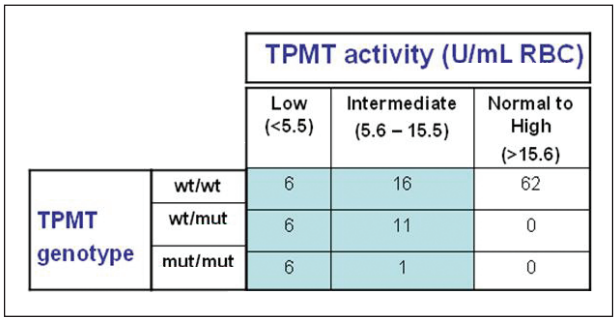

Figure 1 Thiopurine methyltransferase (TPMT) activity distribution

Discussion

Thiopurines (AZA and 6MP) are widely used drugs in IBD treatment and are especially effective for the maintenance of remission in IBD [22], even though their use is limited by the occurrence of adverse effects.

Several genetics variants in *TPMT* have been systematically related to the occurrence of toxicity in thiopurine treatment.

Table 2 Side effects observed in the patients enrolled

Type of adverse effect	n (%)
Pancreatitis	3 (2.77)
Myelosuppression/Leukopenia	8 (7.41)
Hepatotoxicity	2 (1.85)

More than 25 mutations are now indexed but the clinical relevance of some of them remains unclear [23]. TPMT*3A, TPMT*3C, and TPMT*2 represent the most prevalent mutant alleles in Caucasians and African-Americans and account for 80-95% of intermediate or deficient methylator phenotypes [24,25]. It is accepted that patients carrying *TPMT* alleles associated with low TPMT activity, especially homozygosis, show a significant increase in the risk of adverse effects as a result of excessive accumulation of 6-TGN (Q3. Please explain abbreviation) [26,27]. TPMT activity might be determined in erythrocytes prior to therapy; nevertheless *TPMT* activity might be decreased by the use of commonly used drugs like sulfalazine, salicylic acid and their derivatives [28]. Therefore, Yates et al [29] reported that the predictability of *TPMT* genotype on the patients TPMT phenotype resulted in a greater than 95% correlation.

In agreement with previous studies, among the 108 subjects examined in the present study, the average level of enzymatic activity measured in patients with a mutated *TPMT* genotype was significantly lower than that of the remaining group carrying a wild-type *TPMT* genotype [28-30]. There are numerous studies on the relationship between TPMT low enzymatic activity and the adverse effects of thiopurines. Some of these studies show a notable increase in adverse effects such as bone marrow toxicity in patients with a mutated *TPMT*

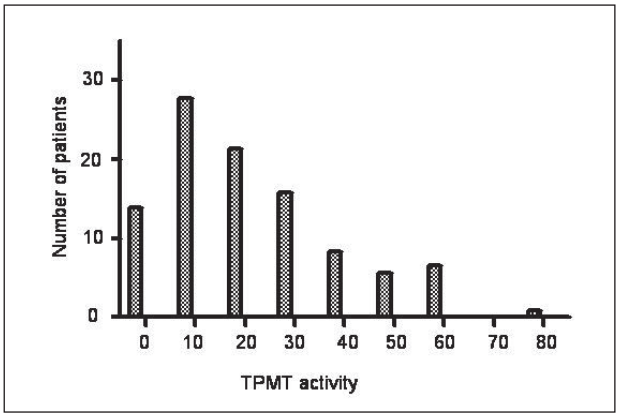

Figure 2 Concordance between *TPMT* genotype and *TPMT* phenotype in the entire cohort (n = 108)

gene [16,31]. On the other hand, other studies suggest that thiopurine-induced adverse effects can be caused by factors other than *TPMT* mutations [32]. Our results are not in agreement with studies suggested that myelosuppression during thiopurine therapy of IBD patients are caused by factors other than mutations leading to reduced *TPMT* activity [29].

Our concordance rate between genotype and phenotype assays is 88.2% and is comparable to that reported in previous studies [13,28]. It is possible that additional genetic and epigenetic factors can contribute to an explanation of the differences between genotype and phenotype [33].

Even though it seems clear that patients with severe deficiency should not receive thiopurines because of the high risk of adverse effects [34], it is also well known that TPMT deficiency only explains a portion of all thiopurine-related adverse effects [3]. Additional single nucleotide polymorphisms in genes encoding enzymes involved in thiopurine metabolism (i.e. *MDR1*, *ITPA*, *MTHFR* etc) may also be responsible for this inter-individual response variability [25]. Consequently, opinion is still divided as to whether *TMPT* activity and *TPMT* genotyping determination is needed prior to starting AZA or 6MP therapy [35]. On the other hand, some authors have reported that patients with high *TPMT* activity are at high risk of suffering from hepatotoxicity because they accumulate methylated metabolites [36]. In this study, two patients with high TPMT activity and wild-type genotype developed hepatotoxicity. Even though this study has important limitations such as the management of the patients including concomitant drugs, it is suggested that the practice of examining *TPMT* genotype and *TPMT* activity prior to starting therapy with thiopurines has improved our ability to manage these patients more effectively and safely than previously. The practice of monitoring thiopurine metabolism has been well established in several countries such as Sweden, UK, New Zealand [19,37,38], where the national guidelines for treatment of IBD state that *TPMT* genotype and/or phenotype determination should be considered in all patients prior to initiation of thiopurine treatment. Recently, Chouchana et al [18] proposed a therapeutic algorithm allowing a dosage individualization to optimize and secure the management of patients under thiopurines. Incorporating pharmacogenetic factors into therapeutic management is promising for ensuring maximal therapeutic response of thiopurines with a minimization of the risk for adverse effects in IBD patients. Further studies evaluating such factors as well as the potential effects on patients' safety are needed.

Summary Box

What is already known:

- The patients with inherited TPMT deficiency treated with standard doses of thiopurines present with higher levels of the thioguanine active metabolites and have an increased risk for adverse events
- In Caucasians, approximately 11% of the population harbour heterozygous and 0.3% homozygous *TPMT* mutations, leading to an intermediate or low *TPMT* activity, respectively
- More than 25 mutations are now indexed but the clinical relevance of some of them remains unclear
- TPMT*3A, TPMT*3C, and TPMT*2 represent the most prevalent mutant alleles in Caucasians and African-Americans and account for 80–95% of intermediate or deficient methylator phenotypes

What the new findings are:

- Significant TPMT activity differences between wild-type and heterozygous and homozygous mutated subjects were observed
- The carriers of at least one variant allele and with both intermediate and absent TPMT activity have an increased risk of developing thiopurine-induced myelotoxicity compared with individuals with normal genotype and TPMT activity
- A high degree of concordance was demonstrated between TPMT genotype and phenotype

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