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Alimentary Tract

TPMT genotype and the use of thiopurines in paediatric inflammatory bowel disease

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Abstract

Background. Thiopurines are used in the treatment of inflammatory bowel disease. They are metabolised via methylation by thiopurine-S-methyltransferase (TPMT), which displays a genetically determined polymorphic activity. Subjects with reduced TPMT activity have a higher concentration of active thiopurine metabolites and may be at increased risk of bone-marrow suppression.

Aims. To evaluate the relevance of *TPMT* genotyping in the management of thiopurines therapy in inflammatory bowel disease patients.

Patients and methods. Adverse effects and clinical response were determined retrospectively and correlated with TPMT genotype in 70 paediatric inflammatory bowel disease patients.

Results. Nineteen patients (27.1%) developed adverse effects; of the 51 who did not, 34 (66.7%) responded to treatment. Five patients (7.1%) were heterozygous for a variant *TPMT* allele; two of these (40%) were intolerant to thiopurines, compared to 17 of the 65 patients (26.2%) with a wild type gene (O.R. 1.88, 95% CI 0.29–12.2, $p = 0.61$); among the 34 responders, the median dosage of the drug required to obtain remission was lower for mutated than for wild type patients (1.6 mg kg^{-1} day^{-1} versus 2.0 mg kg^{-1} day^{-1}, $p = 0.043$).

Conclusions. There was no significant association between adverse effects of thiopurines and *TPMT* heterozygous genotype, but *TPMT* genotyping could be useful in establishing the most appropriate dose of thiopurines to start treatment.

Keywords: Genotyping; Inflammatory bowel disease; Thiopurines (azathioprine, 6-mercaptopurine); Thiopurine-S-methyl transferase

1. Introduction

Azathioprine (AZA) and 6-mercaptopurine (6MP) are immunosuppressive drugs used in the treatment of inflammatory bowel disease (IBD); however, in 15–30% of patients, adverse effects such as leucopoenia, hepatotoxicity and pancreatitis occur [1–6]. AZA is a pro-drug requiring in vivo conversion to an active form [7]: the first step in its biotransformation involves conjugation with sulphide groups and the formation of 6MP, which has no intrinsic activity. The cytotoxic activity of 6MP is mainly exerted after enzymatic conversion to monophosphate nucleotides (6TGN) catalysed by the enzyme hypoxanthine phosphoribosyltransferase (HPRT). These thionucleotides cause cytotoxicity by interfering with de novo purine biosynthesis

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and modification of DNA structure after their incorporation in the nucleic acid, which produces an alteration of the function of DNA-processing enzymes [8,9]. In addition, 6TGN are structural analogues of ATP and GTP nucleotides, which are essential for intracellular messaging and energy carrying processes, and 6TGN may compete with these endogenous counterparts along many biochemical pathways, resulting in impeded growth and proliferation of T and B lymphocytes, and thereby determining the suppression of the overactive immune defence mechanism in IBD patients [10]. It has recently been demonstrated that AZA-induced apoptosis in T lymphocytes is mediated by specific blockade of intracellular anti-apoptotic pathways by 6TGN [11].

In vivo biotransformation of thiopurines also leads to their oxidation to thiouric acid as catalysed by xanthine oxidase (XO), or by methylation of the thiol moiety of the molecule by thiopurine-S-methyltransferase (TPMT) to 6-methylmercaptopurine (6MMP); this compound is thought to be inactive, although some authors have shown that its nucleotide derivatives might play a role in drug toxicity [12]. The occurrence of bone marrow toxicity has been ascribed to a genetically determined deficiency of the TPMT enzyme [13–15], which exhibits genetic polymorphism in all large ethnic groups studied to date [16–19]; approximately 1 individual in 300 inherits two mutant *TPMT* alleles and is thus TPMT-deficient, and about 10% of the population is heterozygous at the *TPMT* gene locus and has intermediate enzyme activity. More than 10 non-functional mutant alleles for *TPMT* have been reported, and reliable polymerase chain reaction (PCR)-based assays exist for detecting the three most prevalent mutant alleles *TPMT**2, *TPMT**3A and *TPMT**3C [20]. While variant alleles other than *TPMT**2, *TPMT**3A and *TPMT**3C may lead to reduced enzyme activity, their frequency is very low; indeed, genotyping for these mutant alleles yielded a 95% concordance between genotype and phenotype in different populations [20–23]. It has been reported that subjects with inherited TPMT deficiency treated with standard doses of thiopurines present higher levels of the active metabolites 6TGNs and have an increased risk of adverse events. Unless patients with two defective alleles are treated with 10–15-fold lower doses of these medications, they develop a pronounced, potentially fatal, haematopoietic toxicity which requires immediate suspension of treatment [13,14,19,24,25]. It is still highly controversial whether patients heterozygous for a *TPMT* mutation are more likely to develop bone marrow suppression compared to those with normal *TPMT* and more studies are needed to establish recommendations to vary treatment, based upon an individual presents wild type (full activity) or heterozygous (intermediate activity) TPMT genotype [26].

The aim of this study was to investigate the relationship between adverse effects of treatment with thiopurines in IBD patients and the genetic polymorphisms of *TPMT*. Moreover, among patients who responded to therapy, we examined the correlation between dose-effectiveness and genetic polymorphism.

2. Materials and methods

2.1. Patients

Patients with IBD who presented between July 2002 and March 2004 at the Gastroenterology Units of the Children's Hospitals of Florence, Genoa, Milan and Trieste were consecutively enrolled. The study included all patients who had been taking a thiopurine for at least 3 months or who had experienced adverse effects during the treatment with this drug. Clinical data were obtained from the records available, and reviewed blindly from the *TPMT* genotyping results. Blood samples were sent to the Department of Biomedical Sciences for *TPMT* genotyping and measurement of TPMT enzyme activity, which were performed as described below.

2.2. Blood sample preparation

Blood samples of the patients were collected in Vacutainer Tubes, using EDTA as an anticoagulant. Total genomic DNA was isolated using a commercial kit (Talent, Trieste, Italy) in accordance with the suppliers' instructions. Erythrocyte lysates were prepared from blood samples to measure TPMT activity following a procedure described by Anglicheau et al. [27].

2.3. TPMT enzyme assay

TPMT activity was measured by HPLC assay based on in vitro conversion of 6MP to 6MMP, using S-adenosyl-L-methionine (SAM) as the methyl donor [27]. Because of the necessity of starting from a fresh sample for the measurement of TPMT activity, it was assayed only in samples coming from Trieste; moreover, the clinician did not always allow blood to be collected for this assay because of the patient's clinical condition.

2.4. TPMT genotyping

The genotype of each individual at the *TPMT**2, *TPMT**3A, *TPMT**3B and *TPMT**3C alleles was determined using previously described polymerase chain reaction (PCR)-based assays [20,28,29].

2.5. Evaluation of adverse effects

Bone marrow suppression was defined as leucopoenia (WBC $< 3000\,mm^{-3}$) and/or thrombocytopenia (platelets $< 100{,}000\,mm^{-3}$); liver toxicity was ALAT, GGT or alkaline phosphatase more than twice their normal levels; pancreatitis was defined as severe abdominal pain, accompanied by a serum amylase level of greater than twice their normal levels [22,30].

2.6. Evaluation of response to therapy

In keeping with previous reports [1,22], treatment with thiopurines was considered successful if the drug was discontinued due to prolonged disease remission, or, when therapy was ongoing at the end of the study, if it prompted discontinuation of corticosteroids or reduced the need for steroid therapy to a daily dose of prednisolone of 5 mg or less. Treatment failure was assumed if the drug was discontinued for clinically judged non-response or surgery.

2.7. Statistical analysis

The differences of the distribution of continuous variables like TPMT activity and the dose of drugs administered to patients with different genotypes were evaluated using the non-parametric two-sided Wilcoxon rank sum test. The association between adverse effects and TPMT polymorphisms was tested using two-sided Fisher's exact test, odds ratios and 95% confidence intervals.

3. Results

Table 1 contains a summary of the characteristics of the 70 patients enrolled in the study. Of these, 63 (90.0%) were taking aminosalicylates in association with the thiopurine.

3.1. TPMT genotype and TPMT activity

Among the 70 patients enrolled in the study, 5 (7.1%) were heterozygous mutated (4 for the allele *TPMT**3A and 1 for the allele *TPMT**2); no subjects were homozygous for a mutated *TPMT* gene. Fig. 1 shows TPMT activity measured in erythrocytes; this was done in 28 patients. The median TPMT activity in subjects with a wild type genotype was 9.7 nmol h^{-1} ml^{-1} erythrocytes (range 8.1–19.5 nmol h^{-1} ml^{-1}). In subjects with a heterozygous genotype, the activity was 5.2 (range 4.2–7.6). The difference between the two values is statistically significant (Wilcoxon test, $p = 0.0060$).

3.2. TPMT genotype and AZA adverse effects

AZA was withdrawn or reduced due to adverse effects in 19 patients (27.1%). Adverse effects comprised bone marrow suppression, liver toxicity, pancreatic toxicity, neuropathy or arthralgy (Table 2). Three cases of bone marrow toxicity and three cases of hepatotoxicity were resolved with dose reduction; all others adverse effects were resolved with drug withdrawal. Two of the five patients (40%) with heterozygous *TPMT* genotype were intolerant to thiopurines, compared with 17 of the 65 (26.2%) with a wild type *TPMT* gene (O.R. 1.88, 95% CI 0.29–12.2, $p = 0.61$). None of the subjects who developed bone marrow suppression was heterozygous for a mutated *TPMT* genotype; of the two subjects with a mutated *TPMT* genotype who developed adverse effects one had neuropathy and the other arthralgia. Twelve patients (17.1%) developed lymphopoenia (lymphocytes < 1000 cell/mm^3), which has been considered a direct effect of the treatment and

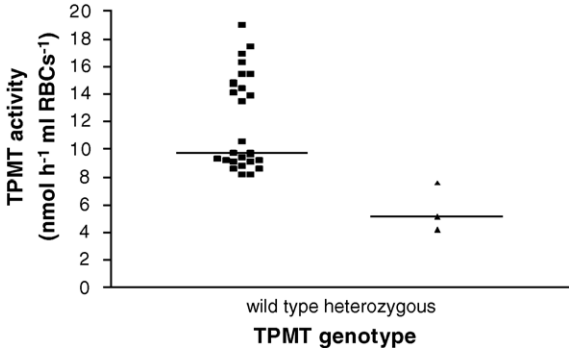

Fig. 1. TPMT genotype and enzymatic activity in patient's erythrocytes. The picture reports the values of the enzymatic activity measured in lysates of the erythrocytes of 28 patients with IBD; patients have been divided in two groups, according to *TPMT* genotype. TPMT activity is expressed as nmol of 6MMP produced by lysates during 1 h of incubation at 37 °C in the presence of 6MP; there is a significant difference in median values of the two groups ($p = 0.0060$, Wilcoxon test).

Table 2
Thiopurine adverse effects observed among the enrolled patients

	Total[a]	%
Bone marrow toxicity[b]	7 (3)	10.0
Hepatotoxicity	6 (3)	8.6
Pancreatic toxicity	4	5.7
Neuropathy	1	1.4
Arthralgia	1	1.4

[a] The number indicated is that of patients treated with AZA or 6MP; the number treated with 6MP is in parenthesis.
[b] Bone marrow toxicity manifesting as five cases of leucopoenia, one case of pancytopoenia and one case of thrombocytopenia.

Table 1
Description of the patients enrolled in the study

	Patients with adverse effect	Patients without adverse effects	All patients
Median age (years [range])	13.6 [4.0–37.0]	14.2 [0.8–38.8]	14.2 [0.8–38.8]
Sex (F/M)	10/9	26/25	36/34
IBD diagnosis (CD; UC; IC)[a]	8; 11; 0	30; 20; 1	38; 31; 1
Thiopurine used (AZA; 6MP)	13; 6	39; 12	52; 18
Median AZA dose (mg kg^{-1} day^{-1} [range])	1.8 [1.0–4.0]	2.0 [1.1–3.0]	2.0 [1.0–4.0]
Median length of treatment (months [range])	24.9 [0.5–85.0]	18.6 [3.0–76.6]	19.6 [0.5–85.0]

[a] CD: Crohn's disease; UC: ulcerative colitis; IC: indeterminate colitis.

not an adverse event; none of these patients had a mutated *TPMT* allele.

3.3. Response to AZA, dose required for the treatment and TPMT genotype

The dose of the drug was adjusted in order to obtain optimal clinical response: depending on the patient's clinical condition, administration was commenced at a median dose of 1.5 mg kg^{-1} day^{-1} of AZA (range 0.5–4.0 mg kg^{-1} day^{-1}). Due to differences in molecular weight, in order to present the data in this paper, the dose of 6MP was multiplied by 2 to calculate the equivalent dose of AZA. At subsequent follow-up examinations, the dose was increased so as to obtain optimal clinical response. In presence of adverse effects, the dose was reduced or the treatment suspended as required. After adjustment of drug dosage in each patient on the basis of clinical judgment, the median dose administered was of 2.0 mg kg^{-1} day^{-1} (range 1.0–4.0 mg kg^{-1} day^{-1}). Among the 51 patients who did not develop adverse effects, 34 (66.7%) responded to treatment while 17 did not (33.3%). All three patients heterozygous for *TPMT* mutations who did not develop adverse effects responded promptly to therapy; the median dose adopted was 1.6 mg kg^{-1} day^{-1} (range 1.2–1.6 mg kg^{-1} day^{-1}) and was significantly lower than the one used in the 31 responder patients with a wild type genotype (2.0 mg kg^{-1} day^{-1}, range 1.1–3.0 mg kg^{-1} day^{-1}, $p = 0.043$).

4. Discussion

Although thiopurine drugs are effective in the treatment of IBD, their use is limited by the occurrence of adverse effects that may be so severe as to require the interruption of therapy. Individual differences have been observed in susceptibility to thiopurines, and attributed to different intracellular concentrations of 6TGN, a cytotoxic metabolite of the drugs [31]. It is known that *TPMT* deficiency caused by a genetic polymorphism, can induce profound bone marrow suppression in patients, since it reduces methylation of 6MP, with consequent accumulation of 6TGN. Approximately 10% of Caucasians have an inherited mutant allele of the *TPMT* gene, and therefore have reduced TPMT activity, while about 1 in 300 is homozygous for the variant alleles of *TPMT* and has no measurable TPMT activity.

Among the 70 subjects examined in the present study, no subject homozygous for *TPMT* mutated gene was identified and 5 subjects (7.1%) were found to be heterozygous. The overall frequency of defective alleles obtained in the present study is comparable with that reported in the Italian population [31] and also in other populations of Caucasian origin [20,29,32]. As far as phenotype is concerned, TPMT activity was measured in 28 subjects and, as expected, the average level of enzymatic activity measured in patients with a mutated *TPMT* genotype was significantly lower than that of the remaining group not carrying the mutations considered [20–23].

There have been numerous studies on the relationship between TPMT-reduced enzymatic activity and the adverse effects of thiopurines, recently reviewed by Al Hadithy et al. [31]. Some of these studies show a significant increase in occurrence of bone marrow toxicity in subjects with a mutated *TPMT* gene, while other side effects (such as hepatotoxicity, pancreatitis, mild lymphopoenia and reduced platelet counts) appeared not to be related to *TPMT* mutations [23]. Other studies, on the other hand, show that thiopurine-induced bone marrow suppression can be caused by factors other than a mutated *TPMT* gene. In particular, in a group of 41 patients with Crohn's disease displaying significant myelotoxicity following treatment with thiopurines, only 10% were homozygous for the considered *TPMT* mutations and 17% were heterozygous for the same mutated alleles. Although these mutations were over-represented in this group as compared to the general population, in most patients the bone marrow toxicity was not associated with the genotype corresponding to low TPMT activity [33]. Another study performed on a group of 92 paediatric patients showed that just 1 of the 36 patients with drug-related toxicity was heterozygous for *TPMT* mutations [12]. Similar results were published in a further study, reporting the *TPMT* genotype of 50 patients with IBD treated with thiopurines who suffered adverse effects requiring suspension of drug administration: five patients (10%) were heterozygous for *TPMT* mutated alleles, and one (2%) was homozygous. Despite the fact that the single subject with the homozygous mutated genotype developed bone marrow suppression after thiopurine treatment, in most of the patients there was no relationship between toxicity of treatment and reduced TPMT activity [34].

In the present series of patients, too, there was no association between *TPMT* heterozygosity and adverse effects of thiopurines (bone marrow toxicity or other side effects). None of the seven patients who developed bone marrow suppression presented a mutated *TPMT* allele. These results are thus in agreement with those showing that the most common forms of myelosuppression during thiopurine administration to IBD patients are caused by factors other than mutations leading to reduced TPMT activity. We found no subject homozygous for *TPMT* mutated alleles among our sample; the relevant literature stresses that these patients have a significantly higher risk of developing severe adverse effects and should be treated with very low doses of thiopurines [23].

The adverse effects exhibited by the two subjects who were heterozygous for a *TPMT* mutation (neuropathy and arthralgia) might therefore be considered as idiosyncratic responses, more in keeping with type I hypersensitivity reactions than with direct drug toxicity, and are unlikely to be associated with *TPMT* mutated alleles [35].

Interestingly, among the 34 responders to therapy in absence of adverse events, the 3 subjects with a mutated *TPMT* genotype were treated with a lower dose of thiopurines

than patients with a normal gene. Although this finding needs to be confirmed in larger prospective studies, we suggest that subjects heterozygous for mutations of *TPMT* respond to the treatment with thiopurines at a lower dosage compared to patients with a normal *TPMT* genotype; this might be a result of the higher concentration of 6TGN metabolites in subjects with a mutated *TPMT* genotype, which many studies have linked to a better clinical response [26]. Our findings suggest, in agreement with a recent review article [31], that paediatricians could start the treatment of IBD patients with a normal genotype at full dose (2.0–2.5 mg kg^{-1} day^{-1}); subjects heterozygous for *TPMT* mutations should be treated with a lower dose of the drug (1.4–1.6 mg kg^{-1} day^{-1}) and, if there is no clinical response to this dosage, a different immunosuppressive agent should probably be used.

Drug toxicity is a multifactorial phenomenon involving numerous genetic and epigenetic factors, including drug interactions. Drugs such as aminosalycilates are commonly used to treat IBD in combination with AZA; they have been reported to influence the thiopurine metabolite levels, although the mechanism of this interaction remains to be fully elucidated [36–39]. Moreover, genetic factors other than *TPMT* mutated alleles, such as the polymorphism of genes whose transcripts are involved in detoxifying or metabolising thiopurines, such as inosine triphosphate pyrophosphatase (ITPase), might also influence their pharmacokinetics and consequently their clinical efficacy [40,41].

In conclusion, our retrospective study shows that *TPMT* genotyping can be useful to suggest the dose of thiopurines most appropriate to start the treatment of patients with IBD. However, clinicians should still monitor patients being treated with these toxic medications, by careful surveillance of WBC or whole blood cell count and liver and pancreatic function, so as to detect the common forms of toxicity unrelated to *TPMT* genotype. Further studies are needed to throw light on the genetic characteristics of subjects suffering adverse effects from thiopurine; such studies should be aimed at identifying additional genetic markers of toxicity which can guide the clinician in a more tailored treatment of patients needing these medications.

Conflict of interest statement

None declared.

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