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CLINICAL STUDY

Prevalence of mutations in thiopurine S-methyltransferase gene among Slovak IBD patients

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Abstract: *Background:* Thiopurine S-methyltransferase (TPMT) plays an important role in the metabolism of thiopurines. It has been suggested that TPMT genetic polymorphisms lead to dose-related hematopoietic toxicity. Since there are major ethnic differences in the prevalence of particular TPMT variants, it is important for each country to study their own prevalence in order to estimate the role of TPMT variants-related thiopurines toxicity in population suffering from particular inflammatory bowel disease (IBD).
Aims: The aim of this study was to determine the frequency of the four most common allelic variants of TPMT gene in the population of Slovak IBD patients.
Methods: TPMT genetic polymorphisms (TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C) were amplified using PCR and consequently genotyped with genetic analyzer. The allele frequencies of particular allelic variants were calculated and compared with other Caucasian populations reported so far.
Results: Three hundred and thirty IBD patients were included; 196/132/2 cases of Crohn´s disease/ulcerative colitis/unclassified colitis; 180 (55 %) males. Ninety-three percent of patients were homozygous for wild-type TPMT variant. Heterozygous genotype of any of the studied polymorphisms was present in 6 % of patients while only one patient was homozygous for TPMT*3A allele (0.3 %). The most prevalent mutant allele was that of TPMT*3A (3.2 %). The distribution of most common allelic variants of TPMT gene among Slovak IBD patients was in accordance with previously reported prevalence in Caucasian populations.
Conclusion: This study shows the prevalence of TPMT genetic polymorphisms in population of Slovak IBD patients. As in other Caucasian populations, the most common mutant allelic variant is that of TPMT*3A while the prevalence of homozygosity is relatively low *(Tab. 3, Ref. 22)*. Full Text in PDF *www.elis.sk.*
Key words: thiopurine S-methyltransferase, thiopurines toxicity, genetic polymorphisms.

Thiopurines such as azathioprine (AZA) and 6-mercaptopurine (6-MP) are immunosuppressive drugs effective in the induction and maintenance of remission of inflammatory bowel disease (IBD) (20). These antimetabolites are involved in the metabolism of nucleic acids. They downregulate their production and have an impact on the activity of lymphocytes. They decrease the proliferation of lymphocytes involved in the inflammation and directly inhibit their cytotoxic activity (15, 16). Thiopurines are used in the treatment of cancer, various autoimmune and chronic inflammatory diseases such as inflammatory bowel diseases, autoimmune hepatitis, autoimmune myasthenia gravis, sclerosis multiplex, psoriasis, systemic lupus erythematosus, primary biliary cirrhosis and rheumatoid arthritis (19, 21). The substance of 6-mercaptopurine was first synthesized in 1951 (15). Azathioprine, a derivate of 6-mercaptopurine, was developed in 1957 and has a longer biological half-life. In 1969 it was also used in the treatment of Crohn's disease (15). Thiopurines are inactive pro-drugs that require multi-enzyme activation after their entry into organism. The final products of metabolism of thiopurines are 6-thioguanine nucleotides (6-TGN) which are in addition to the clinical benefit responsible also for side effects of thiopurines (6). 6-TGN act as purine antagonists and downregulate the synthesis of nucleic acids and proteins. They compete with endogenous guanozine triphosphate (GTP) that is an essential part of signaling pathways and the source of energy for cells. In addition they influence the growth and proliferation of T and B lymphocytes and inhibit the activated immune system in IBD patients (1, 12). After oral ingestion and absorption, 90 % of azathioprine is converted to 6-mercaptopurine by nonenzymatic reaction with contribution of glutathione or cystein (19). 6-mercaptopurine is then metabolized by three competitive enzymes, xantin-oxidase, thiopurine S methyltransferase and

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hypoxantin-guanin phosphoribozyltransferase (5, 19). The drug response and also side effects vary among individuals. Age, gender, disease activity, co-morbidity but also inherited signs like single nucleotide polymorphisms (SNPs) are responsible for these differences. SNPs are simple nucleotide variations in DNA localized anywhere in the genome, and they are the most common cause of inter-individual differences in the reaction of organism to the drug.

Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that plays an important role in the metabolism of thiopurines. It catalyses the thiopurines S-methylation (4, 17, 22). TPMT enzyme activity is inherited as an autosomal codominant sign and is under the control of genetic polymorphisms that have been extensively studied in the past (21). It has been suggested that TPMT genetic polymorphisms are associated with reduced TPMT enzyme activity that can lead to dose-related hematopoietic toxicity in patients treated with thiopurines. TPMT enzyme is encoded by an approximately 34 kb gene located on chromosome 6 (6p22.3) and contains 10 exons and 9 introns (3, 22). To date, at least 24 mutant alleles are known and have been reported in association with reduced TPMT enzyme activity (3). The most common mutant alleles such as TPMT*3A, TPMT*2, TPMT*3C and TPMT*3B are detected in 80–95 % of the Caucasian population (22). The molecular defect in TPMT*3A allele is caused by two nucleotide transition mutations (G460 → A and A719 → G). The defect in TPMT*2 contains the mutation G238 → C. In TPMT*3C allele, it is the mutation A719 → G and in TPMT*3B, G460 → A. Patients homozygous for wild type TPMT genotype have the TPMT enzyme activity normal or high and are good metabolizers of thiopurines. Patients with both mutant alleles, homozygotes for mutant TPMT genotype have very low TPMT enzyme activity. They are poor metabolizers of thiopurines, which leads to high levels of 6-TGN in organism as well as increases the risk of myelotoxicity. Patients with heterozygous genotype have intermediate enzyme activity and intermediate drug level in organism. In patients with high TPMT enzyme activity, the risk of hepatotoxicity is high because of huge production of methylated mercaptopurine metabolites damaging the liver (1, 12). Ethnic differences between distribution of TPMT mutant alleles have been described (11, 14, 22). The activity in Caucasian population has a trimodal distribution with poor, intermediate and high methylators. Since there are major ethnic differences in the prevalence of particular TPMT variants, it is important for each country to study their own prevalence in order to estimate the role of TPMT variants-related thiopurines toxicity in the particular IBD population.

Material and methods

A total of 330 IBD patients treated in Slovakia between years 2007 and 2009 were included. The only inclusion criterion was the diagnosis of IBD (Crohn's disease, ulcerative colitis or unclassified colitis) established by endoscopic, histological and radiological findings. The mode of therapy was not taken into consideration. The most common TPMT variant alleles TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C were determined. The allele frequencies of particular allelic variants were calculated and compared with other Caucasian populations reported so far. For descriptive statistics and frequencies SPSS system version 15.0 was used. Genomic DNA was isolated from 1 milliliter of venous blood using the Puregene Blood Core kit (Qiagen, Hilden, Germany). Three different PCR were used for preamplification of fragments containing polymorphisms 460G>A, 719A>G and 238G>C, respectively. Amplifications were performed in a 10 µl reaction volume containing 50 ng of genomic DNA, 1x concentrated 5PRIME HotMasterMix (5PRIME, Hamburg, Germany) and 2.5 pmol of primers. After amplifications, PCR products were used as a template for sequencing the reactions with BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). Electrophoresis was carried out with ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA) and obtained sequences were compared with the reference sequences using BLAST sequence alignment software.

Results

One hundred and ninety six patients with Crohn's disease, 132 with ulcerative colitis and 2 with unclassified colitis were included in this study. The mean age of included patients was 37 years in range of 17–75 years while 180 (55 %) patients were males and 150 (45 %) were females. Ninety three percent of patients were homozygous for wild type TPMT variant (TPMT*1/*1). Heterozygous genotype of any of the studied polymorphisms was present in 6 % of patients, only one patient was homozygous for TPMT*3A allele (0.3 %). The most prevalent mutant allele was that of TPMT*3A (3.2 %). The frequency of mutant alleles TPMT*3C and TPMT*2 was 0.2 %. In this studied group, no TPMT*3B mutant allele was detected. The distribution of most common allelic variants of TPMT gene among Slovak IBD patients were in accordance with previously reported high prevalence of TPMT*3A variant and lower frequencies of TPMT*3C and TPMT*2 in Caucasian populations (Tabs 1–3).

Tab. 1. Demographic characteristic of Slovak IBD population.

Demographic Characteristic	Number of patients
Number of patients	330
Male/Female	180/150
Age (mean + range) in years	37 (17–75)
Crohn's disease/Ulcerative colitis/Unclassified colitis	196/132/2

Tab. 2. Prevalence of wild type homozygotes, mutant heterozygotes and mutant homozygotes among Slovak IBD patients.

	Number of patients	%
Number of patients	330	100
Wild type homozygotes (TPMT *1/*1)	308	93
Mutant heterozygotes		
TPMT *2/*1	1	0.3
TPMT *3A/*1	19	5.8
TPMT *3B/*1	0	0
TPMT *3C/*1	1	0.3
Mutant homozygotes	1	0.3
TPMT *2/*2	0	0
TPMT *3A/*3A	1	0.3
TPMT *3B/*3B	0	0
TPMT *3C/*3C	0	0

Tab. 3. Allelic variants of TPMT gene and their frequencies among Slovak IBD patients.

Allelic variants	Number of alleles	%
Number of alleles	660	100
TPMT *1	637	96.5
TPMT *2	1	0.2
TPMT *3A	21	3.2
TPMT *3B	0	0
TPMT *3C	1	0.2

Discussion

TMPT genotyping is a way of determining a group of IBD patients treated with thiopurines with high risk of serious side effects such as bone marrow toxicity (18). Patients heterozygous or homozygous for mutant TPMT allele (low TPMT activity) are at higher risk of developing severe hematopoietic toxicity while treated with standard dose of thiopurines (13). In our cohort, 93 % of patients were homozygous for wild type TPMT, 6 % had heterozygous genotype of any of the studied polymorphisms, and only one patient had homozygous genotype for mutant variant TPMT*3A. There are big ethnic differences and also an ethnic heterogeneity in distribution of TPMT mutant variants. It is important for each country to determine their own prevalence by reason of estimating the role of TMPT mutant alleles-related thiopurines toxicity in each particular IBD population. This is a TPMT genotype study that shows the distribution of TPMT mutant variants among Slovak IBD patients. The allele frequencies of TPMT mutant allelic variants are comparable with those in other Caucasian and Latin-American population. The most common allelic variant among Slovak IBD patients was TPMT*3A (3.2 %), while TPMT*2 and TPMT*3C were present only rarely (0.2 %). In Chinese, Japanese, Indian (9), Korean (11) and African populations, TMPT*3C is the most prevalent mutant allele (7, 10) while in Latin-American population it was not detected at all (8). On the other hand the TPMT*2 allele is present in 9.4 % of mutant alleles in British population, while being very rare in Slovak IBD patients and not present in any of Ghanaian subjects (2). The TMPT*3B was not detected in Slovak IBD population at all. The mutant allele TPMT*7 has been recently found in the European Caucasian population, TPMT*8 in African Americans, and TPMT*6 in the Korean population (10). They seem to be rare in the Caucasian population, but they have not been investigated in our cohort.

In conclusion, we have determined that the distribution of mutant TPMT variants in Slovak IBD patients are in accordance with previously reported distribution in other Caucasian populations.

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