

## CROSS-SECTIONAL 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 the particular inflammatory bowel disease (IBD) population.

*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 on 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 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, only one patient was homozygous for TPMT\*3A allele (0.3 %). The most prevalent mutant allele was TPMT\*3A (3.2 %). The distribution of the most common allelic variants of TPMT gene among Slovak IBD patients were in accordance with previously reported prevalence in Caucasian populations.

*Conclusion:* This study shows the prevalence of TPMT genetic polymorphisms in the Slovak IBD patient's population. As in other Caucasian populations, the most common mutant allelic variant is TPMT\*3A, and the prevalence of homozygosity is relatively low (Tab. 3, Ref. 16). Full Text in PDF [www.elis.sk](http://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 inducing and maintaining the remission of inflammatory bowel disease (IBD) (14). Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that plays an important role in the metabolism of thiopurines. It catalyses the S-methylation of thiopurine drugs (3, 12, 16). The final products of metabolism of thiopurines are 6-thioguanine nucleotides (6-TGN) responsible for clinical

benefit as well as for side effects of thiopurines (4). TPMT enzyme activity is under the control of genetic polymorphisms that have been extensively studied in the past (15). 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 (2, 16). To date, at least 24 mutant alleles are known that have been reported in association with reduced TPMT enzyme activity (2). The most common mutant alleles such as TPMT\*3A, TPMT\*2, TPMT\*3C and TPMT\*3B are detected in 80–95 % of Caucasian population (16). The molecular defect in TPMT\*3A allele is caused by 2 nucleotide transition mutations (G460→A and A719→G); the defect in TPMT\*2 contains the mutation G238→C, while TPMT\*3C and TPMT\*3B alleles carry A719→G and G460→A mutations, respectively. Ethnic differences in distribution of TPMT mutant alleles have been described (9, 11, 16). 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.

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**Tab. 1. Demographic characteristic of the 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		
TPMT *2/*2	0	0
TPMT *3A/*3A	1	0.3
TPMT *3B/*3B	0	0
TPMT *3C/*3C	0	0

## Material and methods

A total of 330 IBD patients treated in Slovakia between the year 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 (Tab. 1–3). The mode of therapy was not taken into consideration. The most common TPMT variant alleles TPMT\*2, TPMT\*3A, TPMT\*3B, and 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 PCRs were used for preamplification of fragments containing polymorphisms 460G>A, 719A>G and 238G>C, respectively. Amplifications were performed in 10 µl of 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 reactions with the BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). Electrophoresis was carried out on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA) and the 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, range 17–75 years, 180 (55 %) patients were males, 150 (45 %) were females. Ninety three percent of patients were homo-

**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

zygous 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 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 the 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.

## Discussion

TPMT genotyping is the way of how to determine the group of IBD patients treated with thiopurines at high risk of serious side effects such as bone marrow toxicity (13). 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 (10). In our cohort, 93 % of patients were homozygous for wild type TPMT, 6 % had a heterozygous genotype of any of the studied polymorphisms, and only one patient had a homozygous genotype for mutant variant TPMT\*3A. There are big ethnic differences and also ethnic heterogeneity in the distribution of TPMT mutant variants. It is important for each country to determine their own prevalence by reason of estimating the role of TPMT mutant alleles-related thiopurines toxicity in each particular IBD population. This is the 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 populations. 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 (7), Korean (9) and African populations, TPMT\*3C is the most prevalent mutant allele (5, 8), while in Latin-American population it was not detected et al (6). On the other hand the TPMT\*2 allele is present in 9.4 % of mutant alleles in British population, while in Slovak IBD patients it is very rare, and was not present in any of Ghanaian subjects (1). The TPMT\*3B was not detected in Slovak IBD population at all. The mutant allele TPMT\*7 has been recently found in European Caucasian population, TPMT\*8 in African Americans, and TPMT\*6 in Korean population (8). They seem to be rare in 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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