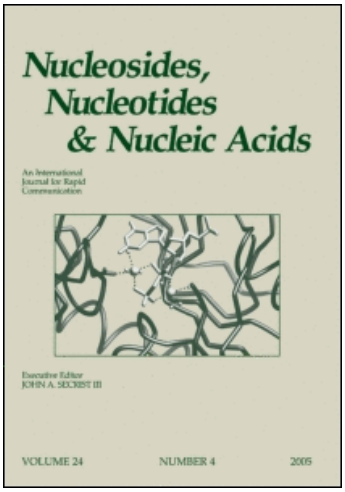

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POLYMORPHISMS OF THE *TPMT* GENE IN THE CZECH HEALTHY POPULATION AND PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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□ *Genetic variation in thiopurine S-methyltransferase (TPMT) is a major factors for wide variation in the metabolism and safety of thiopurine drugs. We investigated the frequency of functional gene polymorphisms in 396 patients with inflammatory bowel disease and 300 healthy subjects. Frequencies of functionally deficient alleles* TPMT ∗ 2, TPMT ∗ 3A, TPMT ∗ 3B, and*TPMT ∗ 3B in the patient group were 0.1%, 4.3%, 0.1%, and 0.4%, respectively, and were similar to those of healthy subjects in the Czech population. Our results provide necessary information for pharmacoeconomic studies in the Czech Republic.*

Keywords Thiopurine S-methyltransferase; polymorphism; azathioprine; pharmacogenetics

INTRODUCTION

The thiopurine drug azathioprine is metabolised to 6-mercaptopurine which acts as a purine antimetabolite, inhibiting purine biosynthesis by both de novo and salvage pathways. Metabolically, 6-mercaptopurine is converted by hypoxanthine phosphoribosyltransferase to activated 6-thioguanine nucleosides. These latter intermediates are ultimately incorporated into DNA as false bases.[1] Inactivation of 6-mercaptopurine and 6-mercaptopurine nucleosides is mediated by methylation with thiopurine S-methyltransferase (TPMT) to inactive metabolites methylmercaptopurine or methylmercaptopurine nucleosides, respectively. TPMT is a well characterized cytosolic

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enzyme, with several functionally important genetic polymorphisms leading to decreased enzyme activity.

There is substantial evidence, which shows that individual activity of TPMT is one of the major factors for wide variation in the metabolism, the drug efficacy, and mainly severe toxicity of thiopurine drugs.[2] Patients who have low or no detectable enzyme activity suffer more frequently from myelotoxicity when treated with standard doses of azathioprine. On the other hand, subjects with high activity of the enzyme could receive a reduced clinical effect of the treatment. The genetic basis for such a variation in TPMT activity is known only for deficient states while no corresponding factors for ultra-rapid type of TPMT metabolism have been found so far. More than 20 variant alleles leading to deficient methylation phenotype have been described.[3] Four of them *TPMT*2, TPMT*3A, TPMT*3B,* and *TPMT*3C* account for 80–95% of low activity alleles in various populations.[4]

The aim of our study was to compare the prevalence of the most common dysfunctional TPMT alleles in healthy volunteers and patients with inflammatory bowel disease (IBD), who are the most common recipients of azathioprine treatment.

MATERIALS AND METHODS

Three hundred young healthy volunteers and 396 IBD patients were included in the study after obtaining their written informed consent. All subjects participating in the study were unrelated subjects of Czech nationality. The study was approved by the Ethics Committee of the General Teaching Hospital in Prague.

Peripheral blood samples were collected from all volunteers in 7 ml collecting tubes with ethylenediaminetetraacetic acid (EDTA). Genomic DNA was subsequently isolated by a standard phenol-chlorophorm method and stored at 4°C until analysis.

PCR amplifications were done in a MyCycler (Bio-Rad, USA) using primers as described previously.[5] Subsequent RFLP analysis was applied for *TPMT*3B*, and *TPMT*3C*; allele specific PCR was performed for *TPMT*2*. The fragments were separated in 3% agarose gel and visualized by staining with ethidium bromide. The primers were ordered from Sigma-Aldrich (USA); all other components of the PCR reaction mix and Top Vision agarose were purchased from Fermentas (Lithuania).

Expected genotype frequencies were calculated using Hardy-Weinberg equilibrium from the observed allelic frequencies. Prevalence was compared by the chi-square test. Microsoft Excel 8.0 (Microsoft, USA) and Statgraphics Plus 3.1 (StatPoint, Inc., USA) were used for data handling.

TABLE 1 Numbers of observed genotypes and allelic frequencies in groups of patients and control subjects

	Observed genotype (n)			Allelic frequency (%)	
Allele	W/W	W/V	V/V	w	v
Healthy subjects					
*TPMT*2*	299	1	0	99.84	0.16
*TPMT*3A*	273	26	1	95.33	4.67
*TPMT*3B*	300	0	0	100.00	0.00
*TPMT*3C*	297	3	0	99.50	0.50
IBD patients					
*TPMT*2*	395	1	0	99.87	0.13
*TPMT*3A*	363	32	1	95.71	4.29
*TPMT*3B*	395	1	0	99.87	0.13
*TPMT*3C*	393	9	0	99.62	0.38

RESULTS AND DISCUSSION

Frequencies of all variant alleles were very similar in both studied groups: 5.33% in the healthy subjects versus 4.93% in the IBD patients.Table 1 shows the distribution of genotypes and specific variant alleles in the study populations. The most frequent variant allele in our study was *TPMT*3A*, which accounted for approximately 85% of all variant alleles. There was one homozygous carrier of a variant allele in each of the groups, both carriers of the most prevalent *TPMT*3A* allele. In total, 30 (10.0%) healthy volunteers and 37 (9.3%) IBD patients were heterozygous for any of the variants. The distribution of variant alleles complied with Hardy-Weinberg equilibrium in both groups. There was no significant difference in allelic or genotype frequencies between IBD patients and healthy subjects.

We have screened the subjects only for the most common variant alleles in other Caucasian populations.[6–9] Therefore, the unrecognized presence of other rare deficient allele could lead to underestimation of the frequency of homozygous poor metabolizers and heterozygotes based on this genotype screen.[10] However, the expected frequency of other functionally deficient variants is very low in Caucasians and, thus, it is unlikely that more thorough genotyping would bring substantially more information regarding the predicted phenotype in our groups, while the costs of the genotyping process would dramatically increase. Our data illustrate that the frequencies of the most common alleles in the healthy Czech subjects are similar to other Caucasian populations, in which the frequency of the most common variant allele *TPMT*3A* ranges between 3% and 8%.[6–11]

CONCLUSION

We found a similar distribution of the most frequent variant *TPMT* alleles in the Czech healthy populations and patients with IBD. The allelic frequencies were also comparable to other Caucasian populations. Therefore the negative consequences of azathioprine therapy arising from TPMT deficiency can be expected to be similar to that described in some other European countries. These results are of importance for future pharmacogenetic and pharmacoeconomic studies in the Czech population.

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