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Original Scientific Paper

AN EPIDEMIOLOGICAL STUDY OF THIOPURINE-METHYLTRANSFERASE VARIANTS IN A CROATIAN INFLAMMATORY BOWEL DISEASE PATIENT COHORT

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SUMMARY – Thiopurine S-methyltransferase (TPMT) is an enzyme that converts thiopurine drugs into inactive metabolites. Over 20 variant TPMT-encoding alleles, which cause reduced enzymatic activity, have been discovered so far. Our aim was to investigate the frequencies of variant alleles, i.e. genotypes in inflammatory bowel disease (IBD) patients and healthy individuals and to compare these frequencies with selected world populations. The most common variant alleles TPMT*2, TPMT*3A, TPMT*3B and TPMT*3C were analyzed with polymerase chain reaction-based assays and allele-specific polymerase chain reaction-based assays in 685 participants including 459 IBD patients and 226 healthy volunteers. Study results revealed 434/459 (94.55%) IBD patients and 213/226 (94.25%) healthy subjects to be homozygous for the wild-type allele (TPMT*1/*1). TPMT*1/*2 and TPMT *1/*3C genotypes were found in 4/459 (0.87%) and 7/459 (1.53%) IBD patients, respectively; in healthy volunteers they were not found. TPMT*1/*3A genotype was found in 14/459 (3.05%) IBD patients and 13/226 (5.75%) healthy subjects. Variant genotypes were statistically significantly more common in Crohn's disease subgroup than in ulcerative colitis subgroup. The prevalence of variant genotypes was 23/338 (6.80%) in Crohn's disease subgroup as compared with 2/121 (1.65%) in ulcerative colitis subgroup (χ^2=4.59; p=0.032). In conclusion, the most frequently occurring nonfunctional TPMT allele in Croatian population is TPMT*3A. The overall frequency of mutant alleles in our population is statistically nonsignificantly lower when compared with other populations of Caucasian origin. The Crohn's disease group had more mutant alleles than the ulcerative colitis group.

Key words: *Crohn's disease – genetics; Colitis, ulcerative – genetics; Thiopurine-S-methyltransferase deficiency; Polymorphism, genetic*

Introduction

Thiopurine drugs (azathioprine, 6-thioguanine and 6-mercaptopurine) are extensively used as steroid-sparing drugs in autoimmune disorders, hematologic malignancies, inflammatory bowel diseases (IBD) and in organ transplantation[1]. Although very useful, they have a narrow therapeutic index (the ratio between therapeutic efficacy and toxicity).

In humans, thiopurines are metabolized *via* three competing pathways: (a) oxidation by xanthine oxidase (XO) (product is an inactive metabolite 6-thiouric acid); (b) phosphorybosyl transfer by hypoxanthine guanine phosphoribosyltransferase (HGRPT) (products are 6-thioguanine nucleotides); and (c) methylation by thiopurine-methyltransferase (TPMT) (product is 6-methylmercaptopurine)[2]. The balance between

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these three pathways determines the amount of final product and henceforth the therapeutic efficacy/toxicity of thiopurine drugs.

During the last three decades, TPMT has been extensively studied. It is a cytosolic enzyme encoded by TPMT gene[3-5]. Up to now, more than 20 allelic variants of the gene have been found. The majority of them contain a single nucleotide polymorphism leading to amino acid substitution. The most frequent allelic variants are denoted TPMT*2, TPMT*3A, TPMT*3B and TPMT*3C[6]. Each of these variants codes TPMT protein that undergoes rapid proteolysis and results in TPMT deficiency[7].

Caucasians show a trimodal gene distribution: 89% of subjects are homozygous for wild type gene (denoted TPMT*1/*1); 6%-11% of subjects are heterozygous (one wild-type allele and one variant allele); and 0.33% of subjects are homozygous for some variant allele[8-9]. This finding has clinical implications: "wild-type" patients get full dose of the drug; "heterozygous" patients get reduced dose; and "variant homozygous" patients do not get the drug in order to avoid possible toxicity.

The frequency distribution of variant alleles varies among countries, in particular among continents. Out of the aforementioned variant alleles, TPMT*3C is found mostly in African and Asian inhabitants, TPMT*3A in European and Northern American inhabitants, and TPMT*2 in Latin American inhabitants.

A few reports on Slavic populations have revealed differences in the frequency of variant alleles among Slavic populations[10-15]. This is the first study on TPMT polymorphisms in a Croatian population of IBD patients. In addition, the study compared variant allele frequencies between healthy Croatian subjects and IBD cohort in order to determine whether there is an association between TPMT variants and IBD; and between Croatian population and selected world populations to determine interethnic differences.

Patients, Materials and Methods

Study population

A total of 685 Croatian subjects participated in the study, including 459 IBD patients (338 with Crohn's disease, (CD) group and 121 with ulcerative colitis, (UC) group) and 226 healthy adult volunteers (control group). The participants with IBD were recruited among in- and outpatients admitted to the Zagreb University Hospital Center and Osijek University Hospital Center. Control subjects were selected among hospital staff and other Zagreb citizens appointed for routine check-up.

Ethical approval for the study was obtained from the Ethics Committees of the Zagreb University Hospital Center and School of Medicine, University of Zagreb. All procedures were performed in accordance with ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration from 1975, as revised in 2008. A written informed consent was obtained from all subjects.

Blood samples were collected in Vacutainer tubes using EDTA as anticoagulant.

TPMT genotyping

Genomic DNA was extracted from peripheral lymphocytes using the salting out procedure[16]. Detection of the single nucleotide polymorphisms (SNPs) of TPMT was carried out using a validated Real-time PCR method, TaqMan® Drug Metabolism Genotyping Assay obtained from Applied Biosystems (Foster City, CA, USA); Assay ID: TPMT*2 (rs1800462), C_12091552_30; TPMT*3B (rs1800460), C_30634116_20; TPMT*3C (rs1142345), C_19567_20. The TaqMan® primer/probe set designed for each SNP allele was included in the kits.

Polymerase chain reactions (PCR) were performed in a 96-well microplate format in 15 μL reaction mixture containing TaqMan® universal PCR master mix and using amplification protocol of 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 92 °C for 15 s, then 60 °C for 1.5 min. The following allelic discrimination analysis was carried out using the 500 Real Time PCR Systems (Applied Biosystems, Foster City, CA, USA). TPMT genotyping analysis is included in external quality control schemes (DGKL, Germany).

The inflammatory bowel disease was defined according to the standard criteria[17,18].

Statistical analysis

Differences in TPMT genotype frequencies between the groups were analyzed using the χ^2-test. In

Table 1. TPMT genotype frequency in IBD patients and control group

	IBD			Control group			p-value*
	n	%	(95% CI)#	n	%	(95% CI)	
TPMT *1/*1	434	94.55	(92.48-96.63)	213	94.25	(91.21-97.28)	0.870
Mutant type							
TPMT *1/*2	4	0.87	(0.02-1.72)	0	0.0	-	0.308
TPMT *1/*3A	14	3.05	(1.48-4.62)	13	5.75	(2.72-8.79)	0.087
TPMT *1/*3C	7	1.53	(0.40-2.65)	0	0.0	-	0.102
Total	25	5.45	(3.37-7.52)	13	5.75	(2.72-8.79)	0.870

*significance level p<0.05; #95% CI = 95% confidence interval; TPMT = thiopurine-S-methyltransferase; IBD = inflammatory bowel diseases

cases of small expected frequencies (i.e. smaller than five), Fisher exact test was used. The frequency of each genotype was given with 95% confidence interval (CI). The level of significance of p=0.05 was considered statistically significant. The SPSS version 17 was used on data analysis. Test of departure of Hardy-Weinberg equilibrium was performed using Markov-chain method[19], implemented in Arlequin version 3.5.1.2[20].

Results

A total of 685 Croatian participants were enrolled in the study, 459/685 (67.0%) of them IBD patients and 226/685 (33.0%) healthy volunteers. In IBD group, there were 338 patients with Crohn's disease and 121 patients with ulcerative colitis.

No significant deviations from the expected Hardy-Weinberg proportions were observed in either control group (p>0.999) or IBD group (p>0.999).

None of the participants was homozygous for any of the investigated variant allele.

In IBD group, 434/459 (94.55%) subjects were homozygous for the wild-type allele (TPMT*1/*1) *versus* 213/226 (94.25%) control subjects. Difference in the prevalence of homozygous wild-type alleles between the control and IBD groups was not statistically significant (χ^2=0.03; p=0.870).

TPMT*1/*2 genotype was found in 4/459 (0.87%) and TPMT*1/*3C in 7/459 (1.53%) IBD patients. Neither of these variants was found in control group. Differences for these two genotypes were not statistically significant (p=0.308 and p=0.102, respectively) (Table 1).

TPMT*1/*3A genotype was found in 14/459 (3.05%) IBD patients and 13/226 (5.75%) control subjects. Difference between IBD and control groups did not reach statistical significance (p=0.087).

Analysis of CD and UC subgroups yielded statistically significant differences; mutant genotypes were more common in CD subgroup (23/338, 6.80%) as compared with UC subgroup (2/121, 1.65%) (χ^2=4.59; p=0.032) (Table 2).

Table 2. TPMT genotype frequency in Crohn's disease and ulcerative colitis patients

	Crohn's disease			Ulcerative colitis			p-value*
	n	%	(95% CI)#	n	%	(95% CI)	
TPMT *1/*1	315	93.20	(90.51-95.88)	119	98.35	(96.08-100.0)	**0.032**
Mutant type							
TPMT *1/*2	4	1.18	(0.03-2.34)	0	0.0	-	0.577
TPMT *1/*3A	12	3.55	(1.58-5.52)	2	1.65	(0.0-3.92)	0.373
TPMT *1/*3C	7	2.07	(0.55-3.59)	0	0.0	-	0.198
Total	23	6.80	(4.12-9.49)	2	1.65	(0.0-3.92)	**0.032**

*significance level p<0.05; # 95% CI= 95% confidence interval; TPMT = thiopurine-S-methyltransferase

Table 3. Comparison of TPMT allelic frequency in Croatia and selected countries

Population	N of alleles	% of mutant alleles	TPMT*1 (p-value*)	TPMT*2 (p-value)	TPMT*3A (p-value)	TPMT*3C (p-value)	Method	Ref.
Croatia	**1370**	**2.77**	**97.23**	**0.29**	**1.97**	**0.51**	**Realtime PCR**	**This study**
Poland	716	3.21	96.79 (0.572)	0.4 (0.697)	2.7 (0.313)	0.14 (0.277)	PCR-RFLP [a] AS-PCR[b]	15
Slovenia	388	4.9	95.1 **(0.037)**	0.0 (0.582)	4.1 **(0.015)**	0.5 (>0.999)	PCR-RFLP Realtime PCR for TPMT*2	12
Italy	206	5.34	94.66 **(0.048)**	0.49 (0.504)	3.88 (0.121)	0.97 (0.333)	PCR-RFLP AS-PCR	21
Serbia	400	4.0	96.0 (0.210)	0.2 (>0.999)	3.2 (0.130)	0.0 (0.361)	PRC-RFLP	14
France	936	4.2	95.8 (0.067)	0.7 (0.134)	3.0 (0.115)	0.4 (>0.999)	PCR-reverse dot blot	22
Sweden	1600	4.38	95.63 **(0.020)**	0.06 (0.188)	3.75 **(0.004)**	0.44 (0.771)	Pyrosequencing	23
United Kingdom	398	5.03	94.72 **(0.014)**	0.5 (0.622)	**4.52 (0.004)**	0.25 (0.692)	PCR-RFLP	24
Germany	2428	5.0	95.0 **(0.001)**	0.2 (0.731)	4.4 **(<0.001)**	0.4 (0.660)	PCR Sequencing	25
China	450	1.33	98.67 (0.084)	0 (0.578)	0 **(0.003)**	1.33 (0.100)	PCR-RFLP AS-PCR	26
Japan	384	0.8	99.2 **(0.022)**	0 (0.582)	0 **(0.006)**	0.8 (0.464)	PCR-RFLP AS-PCR	27
USA (Caucasians)	564	3.7	96.3(0.270)	0.2 (>0.999)	3.2 (0.106)	0.2 (0.450)	PCR-RFLP	28
USA (Afro-Americans)	496	4.6	95.4 **(0.046)**	0.4 (0.660)	0.8 (0.082)	2.4 **(<0.001)**	PCR-RFLP AS-PCR	29
Colombia	280	3.93	96.07 (0.300)	0.36 (>0.999)	3.57 (0.099)	0 (0.610)	PCR-RFLP AS-PCR	30
Brazil	408	4.9	95.1 **(0.034)**	2.2 **(<0.001)**	1.5 (0.511)	1.0 (0.288)	PCR-RFLP AS-PCR	31
Argentina	294	4.1	95.9 (0.233)	0.7 (0.287)	3.1 (0.244)	0 (0.614)	PCR-RFLP AS-PCR	32
Turkey	296	4.39	95.61	2.03	1.01	1.35	PCR-RFLP	33
Egypt	400	1.5	98.5 (0.150)	0.0 (0.580)	0.3 **(0.015)**	1.3 (0.157)	AS-PCR for TPMT*2 AS-Realtime PCR for *3C PCR RFLP for *3A	34
Ghana	434	7.6	92.4 **(<0.001)**	0 (0.578)	0.0 **(0.003)**	7.6 **(<0.001)**	PCR-RFLP	24

*significance level p<0.05; p = significance of difference in the distribution of polymorphic alleles compared to Croatians; TPMT = thiopurine-S-methyltransferase; [a] PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism; [b] AS-PCR = allele specific PCR

Comparison of TPMT allelic frequency between Croatia and other countries

We were interested in the historical background of TPMT variants in different parts of the world and therefore compared our results with selected countries for which data were available (Table 3).

TPMT*3A was the most prevalent variant allele in Croatia (27/1370, 1.97%), although with a significantly lower frequency when compared with Slovenia 16/388 (4.1%) (χ^2=5.38; p=0.015); Germany 197/2306 (4.4%) (χ^2=11.0; p=0.001); Sweden 60/1600 (3.75%) (χ^2=5.40; p=0.020); and United Kingdom 17/398 (4.27%) (χ^2=8.10; p=0.010)

TPMT*3C allele was found in 7/1370 (0.51%) of total Croatian alleles. It was significantly lower when compared with Ghana 33/434 (7.6%) (χ^2=76.47; p<0.001) and Afro-American population 12/496 (2.4%) (χ^2=13.16; p<0.001).

TPMT*2 allele was found in 4/1370 (0.29%) of Croatian alleles. It also showed a significantly lower prevalence when compared with Brazil 9/408 (2.2%) (p<0.001) and Turkey 6/296 (2.0%) (p=0.003).

Discussion

Our results showed the most common variant allele in Croatian population to be TPMT*3A (1.97% of mutant alleles). This is in concordance with the previous result reported on Croatian population[35], although with a smaller frequency. Some differences in the prevalence of TPMT alleles between Croats and neighboring countries (Slovenia), as well as other Caucasian populations could be discussed and explained considering genotyping methods that were applied. In earlier researches, PCR-RFLP methods were used, which were found to be less accurate than Real-time PCR methods that were applied in this and some other recently published investigations. The results for the Croatian and Slovenian samples do not differ for TPMT*2 variant allele, as the Real-time PCR was applied on both sides, but differ for other alleles where the methods were different[12]. Our data are in accordance with the data obtained for Serbian population[14]. Since our laboratory is taking part in the external quality control schemes (DGKL, Germany) for TPMT genotyping, we are rather confident for the data presented in this study. Another advantage of our study was a larger number of participants compared to other published data.

Another interesting and to us unexpected finding was that CD patients had a greater proportion of mutant alleles than UC patients. The frequency of variant alleles in the UC group was 1.65%, while in the CD group it was 6.80%. A limitation of this finding is that we had almost twice more participants in CD group than in UC group. We carried out this study in a tertiary referral center, where we had more patients with extensive Crohn's disease. This possibly presents a chance finding because of a selection bias. A similar Russian study found a statistically significant difference in the frequencies of carriers of variant alleles between the malignancy group and control group, with a greater frequency in the malignancy group[10]. Both these findings urge the question that mutant alleles somehow 'weaken' the genetic construction of a person, which could be a basis for new investigations.

Studies done so far speculated that TPMT*3C is the ancestral allele, originating from the northeastern Africa. This allele is present to some degree in all populations, but mostly in African and Asian. TPMT*3A and all other variant alleles were probably acquired over centuries through migrations and mixing of civilizations. Genetic analysis of modern Croatian population shows it to be a territory of an extraordinary genetic mixture[36]. However, there was no significant mixing of Croatians with African populations, which could be the reason why Croats exhibit mostly TPMT*3A as the most prevalent variant allele and not TPMT*3C, just as other Caucasian populations.

Conclusion

Our results confirm that TPMT*3A is the most prevalent allele in IBD population, as well as in general Croatian population. This is in concordance with other Caucasian populations. Variant alleles were more prevalent in CD than in UC patients, which could be the basis for new investigations.

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References

1. Paterson ARP, Tidd DM. 6-Thiopurines. In: Sartorelli AC, Johns DG, editors. Antineoplastic and Immunosuppressive Agents II. New York, Springer Verlag; 1975. p. 384-403.
2. Remy CN. Metabolism of thiopyrimidines and thiopurines: S-methylation with S-adenosylmethionine transmethylase and catabolism in mammalian tissues. J Biol Chem. 1963;238:1078-84.
3. Otterness DM, Szumlanski CL, Wood TC, Weinshilboum RM. Human thiopurine methyltransferase pharmacogenetics kindred with a terminal exon splice junction mutation that results in loss of activity. J Clin Invest. 1998 March;101(5):1036-44, http://dx.doi.org/10.1172/jci1004
4. Krynetski EY, Tai HL, Yates CR, Fessing MY, Loennechen T, Schuetz JD, *et al*. Genetic polymorphism of thiopurine methyltransferase: clinical importance and molecular mechanisms. Pharmacogenetics. 1996;6:279-90, http://dx.doi.org/10.1097/00008571-199608000-00001
5. Gearry RB, Barclay ML. Azathioprine and 6-mercaptopurine pharmacogenetics and metabolite monitoring in inflammatory bowel disease. J Gastroenterol Hepatol. 2005;20:1149-57, http://dx.doi.org/10.1111/j.1440-1746.2005.03832.x
6. Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. Am J Hum Genet. 1980;32:651-62.
7. Zhou S. Clinical pharmacogenomics of thiopurine S-methyltransferase. Curr Clin Pharmacol. 2006 Jan;1(1):119-28, http://dx.doi.org/10.2174/157488406784111627
8. Evans WE, Hon YY, Bomgaars L, Coutre S, Holdsworth M, Janco R, *et al*. Preponderance of thiopurine S-methyltransferase deficiency and heterozygosity among patients intolerant to mercaptopurine or azathioprine. J Clin Oncol. 2001 Apr 15;19(8):2293-301.
9. Tai HL, Fessing MY, Bonten EJ, Yanishevsky Y, d'Azzo A, Krynetski EY, *et al*. Enhanced proteasomal degradation of mutant human thiopurine S-methyltransferase (TPMT) in mammalian cells: mechanism for TPMT protein deficiency inherited by TPMT*2, TPMT*3A, TPMT*3B or TPMT*3C. Pharmacogenetics. 1999;9:641-50, http://dx.doi.org/10.1097/01213011-199910000-00011
10. Samochatova EV, Chupova NV, Rudneva A, Makarova O, Nasedkina TV, Fedorova OE, *et al*. TPMT genetic variations in populations of the Russian Federation. Pediatr Blood Cancer. 2009 Feb;52(2):203-8, http://dx.doi.org/10.1002/pbc.21837
11. Slanar O, Bortlík M, Buzková H, Donoval R, Pechandová K, Sebesta I, *et al*. Polymorphisms of the TPMT gene in the Czech healthy population and patients with inflammatory bowel disease. Nucleos Nucleot Nucl. 2008 Jun;27(6):835-8, http://dx.doi.org/10.1080/15257770802146478
12. Milek M, Murn J, Jaksic Z, Lukac Bajalo J, Jazbec J, Mlinaric Rascan I. Thiopurine S-methyltransferase pharmacogenetics: genotype to phenotype correlation in the Slovenian population. Pharmacology 2006;77(3):105-14, http://dx.doi.org/10.1159/000093278
13. Indjova D, Atanasova S, Shipkova M, Armstrong VW, Oellerich M, Svinarov D. Phenotypic and genotypic analysis of thiopurine S-methyltransferase polymorphism in the Bulgarian population. Ther Drug Monit. 2003 Oct;25(5):631-6, http://dx.doi.org/10.1097/00007691-200310000-00013
14. Dokmanovic L, Urosevic J, Janic D, Jovanovic N, Petrucev B, Tosic N, *et al*. Analysis of thiopurine S-methyltransferase polymorphism in the population of Serbia and Montenegro and mercaptopurine therapy tolerance in childhood acute lymphoblastic leukemia. Ther Drug Monit. 2006 Dec;28(6):800-6, http://dx.doi.org/10.1097/01.ftd.0000249947.17676.92
15. Kurzawski M, Gawronska-Szklarz B, Drozdzik M. Frequency distribution of thiopurine S-methyltransferase alleles in a Polish population. Ther Drug Monit. 2004 Oct;26(5):541-5, http://dx.doi.org/10.1097/00007691-200410000-00013
16. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cell. Nucl Acid Res. 1988;16:1215, http://dx.doi.org/10.1093/nar/16.3.1215
17. Gasche C, Scholmerich J, Brynskov J, D'Haens G, Hanauer S, Irvine E, *et al*. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. Inflamm Bowel Dis. 2000;6(1):8-15, http://dx.doi.org/10.1002/ibd.3780060103
18. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 2006 Jun;55(6):749-53, http://dx.doi.org/10.1136/gut.2005.082909
19. Guo SW, Thompson EA. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics. 1992 Jun;48(2):361-72, http://dx.doi.org/10.2307/2532296
20. Excoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010;10:564-7, http://dx.doi.org/10.1111/j.1755-0998.2010.02847.x
21. Rossi AM, Bianchi M, Guarnieri C, Barale R, Pacifici GM. Genotype-phenotype correlation for thiopurine-methyltransferase in healthy Italian subjects. Eur J Clin Pharmacol. 2001;57:51-4, http://dx.doi.org/10.1007/s002280000246
22. Ganiere-Monteil C, Medard Y, Lejus C, Bruneau B, Pineau A, Fenneteau O, *et al*. Phenotype and genotype for thiopurine methyltransferase activity in the French Caucasian population: impact of age. Eur J Clin Pharmacol. 2004;60:89-96, http://dx.doi.org/10.1007/s00228-004-0732-5
23. Haglund S, Lindquist M, Almer S, Peterson C, Taipalensuu J. Pyrosequencing of TPMT alleles in a general Swedish population and in patients with inflammatory bowel disease. Clin Chem. 2004;50:288-95, http://dx.doi.org/10.1373/clinchem.2003.023846

24. Ameway MM, Collie-Duguid ES, Powrie RH, Adjei-Ofori D, McLeod HL. Thiopurine methyltransferase alleles in British and Ghanaian populations. Hum Mol Genet. 1999;8(2):367-70, http://dx.doi.org/10.1093/hmg/8.2.367
25. Schaeffeler E, Fischer C, Brockmeier D, Wernet D, Moerike K, Eichelbaum M, *et al.* Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. Pharmacogenetics. 2004;14:407-17, http://dx.doi.org/10.1097/01.fpc.0000114745.08559.db
26. Zhang IP, Guan YY, Wu IH, Jiang WQ, Huang M. Genetic polymorphism of the thiopurine S-methyltransferase of healthy Han Chinese. Az' Zheng. (Chinese Journal of Cancer) 2003;22:385-8.
27. Hiratsuka M, Inoue T, Omori F, Agatsuma Y, Mizugaki M. Genetic analysis of thiopurine methyltransferase polymorphism in a Japanese population. Mutat Res. 2000;448:91-5, http://dx.doi.org/10.1016/s0027-5107(00)00004-x
28. Yates CR, Krynetski EY, Loennechen T, Fessing MY, Tai HL, Pui CH, *et al.* Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med. 1997 Apr 15;126(8):608-14, http://dx.doi.org/10.7326/0003-4819-126-8-199704150-00003
29. Hon YY, Fessing MY, Pui CH, Relling MV, Krynetski EY, Evans WE. Polymorphism of the thiopurine S-methyltransferase gene in African-Americans. Hum Mol Genet. 1999;8:371-6, http://dx.doi.org/10.1093/hmg/8.2.371
30. Isaza C, Henao J, Lopez AM, Cacabelos R. Allelic variants of the thiopurine methyltransferase (TPMT) gene in the Colombian population. Method Find Exp Clin Pharmacol. 2003;25:423-9, http://dx.doi.org/10.1358/mf.2003.25.6.769646
31. Boson WL, Romano-Silva MA, Correa H, Falcão RP, Teixeira-Vidigal PV, De Marco L. Thiopurine methyltransferase polymorphism in a Brazilian population. Pharmacogenomics J. 2003;3:178-82, http://dx.doi.org/10.1038/sj.tpj.6500175
32. Larovere LE, de Kremer RD, Lambooy LH, De Abreu RA. Genetic polymorphism of thiopurine S-methyltransferase in Argentina. Ann Clin Biochem. 2003;40:388-93, http://dx.doi.org/10.1258/000456303766477039
33. Sayitoglu MA, Yildiz I, Hatirnaz O, Ozbek U. Common cytochrome p4503A (CYP3A4 and CYP3A5) and thiopurine S-methyltransferase (TPMT) polymorphisms in Turkish population. Turk J Med Sci. 2006;36:11-5.
34. Hamdy SI, Hiratsuka M, Narahara K, Endo N, El-Enany M, Moursi N, *et al.* Genotype and allele frequencies of TPMT, NAT2, GST, SULT1A1 and MDR-1 in the Egyptian population. Br J Clin Pharmacol. 2003;55:560-9, http://dx.doi.org/10.1046/j.1365-2125.2003.01786.x
35. Kapitanovic S, Jokic M, Jurisic G. TPMT Gene polymorphisms in Croatian population. Abstracts of the European Human Genetics Conference 2006 in European Journal of Human Genetics. Amsterdam: Nature Publishing Group 2006;353-353.
36. Primorac D, Marjanović D, Rudan P, Villems R, Underhill PA. Croatian genetic heritage: Y-chromosome story. Croat Med J. 2011;52:225-34, http://dx.doi.org/10.3325/cmj.2011.52.225

Sažetak

EPIDEMIOLOŠKA STUDIJA VARIJANTI TIOPURIN-METILTRANSFERAZE U SKUPINI HRVATSKIH BOLESNIKA S UPALNIM BOLESTIMA CRIJEVA

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Tiopurin S-metiltransferaza (TPMT) je enzim koji sudjeluje u konverziji tiopurinskih lijekova u inaktivne metabolite. Dosad je otkriveno više od 20 varijanti TPMT-kodirajućih alela. Ovi aleli uzrokoju smanjenu enzimatsku aktivnost. Naš cilj je bio istražiti frekvenciju varijantnih alela odnosno genotipova u bolesnika oboljelih od upalnih bolesti crijeva i u zdravih osoba te usporediti dobivene frekvencije s frekvencijama odabranih svjetskih populacija. Najčešći varijantni aleli TPMT*2, TPMT*3A, TPMT*3B i TPMT*3C analizirani su metodama lančane reakcije polimeraze, odnosno alel-specifičnim metodama lančane reakcije polimeraze. U istraživanje je bilo uključeno 685 ispitanika; 459 ispitanika bili su bolesnici s upalnom bolesti crijeva, a 226 bili su zdravi dobrovoljci. Rezultati su pokazali da su 434/459 (94,55%) pacijenata s upalnom bolesti crijeva i 213/226 (94,25%) zdravih osoba homozigoti za divlji tip alela (TPMT*1/*1). Genotipovi TPMT*1/*2 i TPMT*1/*3C nađeni su u 4/459 (0,87%) odnosno 7/459 (1,53%) bolesnika; u zdravih dobrovoljaca nisu nađeni. Genotip TPMT*1/*3A nađen je u 14/459 (3,05%) bolesnika i 13/226 (5,75%) zdravih dobrovoljaca. Varijantni genotipovi bili su statistički značajno češći u podskupini bolesnika s Crohnovom bolešću, s učestalošću od 23/338 (6,80%) u odnosu na podskupinu bolesnika s ulceroznim kolitisom, gdje je učestalost varijantnih genotipova bila 2/121 (1,65%) (χ^2=4,46; p=0,035). U zaključku, najčešći nefunkcionalni TPMT alel u Hrvatskoj populaciji je TPMT*3A. Ukupna frekvencija varijantnih alela u našoj je populaciji statistički neznačajno niža u odnosu na druge populacije bjelačkog podrijetla. Bolesnici s Crohnovom bolešću imaju više varijantnih alela u odnosu na podskupinu bolesnika s ulceroznim kolitisom.

Ključne riječi: *Crohnova bolest – genetika; Kolitis, ulcerozni – genetika; Tiopurin-S-metiltransferaza, deficijencija; Polimorfizam, genetski*