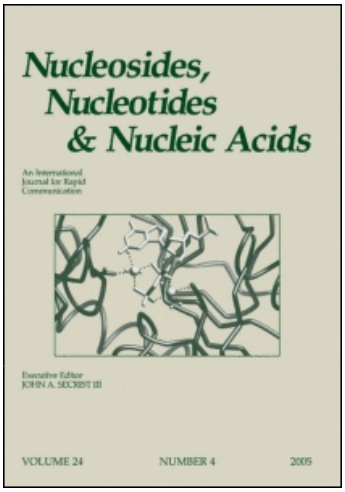

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Frequency of Thiopurine S-Methyltransferase (TPMT) Alleles in Southeast Iranian Population

Ali Bahari[a]; Mohammad Hashemi[bc]; Zohreh Bari[a]; Abdolkarim Moazeni-Roodi[d]; Mahmoud-Ali Kaykhaei[a]; Behzad Narouie[e]
[a] Department of Internal Medicine, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran [b] Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran [c] Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran [d] Department of Biology, Sistan and Baluchistan University, Zahedan, Iran [e] Clinical Research Development Center (CRDC), Ali-e-ebne-abitaleb Hospital, Zahedan University of Medical Sciences, Zahedan, Iran

Online publication date: 19 April 2010

To cite this Article Bahari, Ali , Hashemi, Mohammad , Bari, Zohreh , Moazeni-Roodi, Abdolkarim , Kaykhaei, Mahmoud-Ali and Narouie, Behzad(2010) 'Frequency of Thiopurine S-Methyltransferase (TPMT) Alleles in Southeast Iranian Population', Nucleosides, Nucleotides and Nucleic Acids, 29: 3, 237 — 244
To link to this Article: DOI: 10.1080/15257771003720418
URL: http://dx.doi.org/10.1080/15257771003720418

Nucleosides, Nucleotides and Nucleic Acids, 29:237–244, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257771003720418

Taylor & Francis
Taylor & Francis Group

FREQUENCY OF THIOPURINE S-METHYLTRANSFERASE (TPMT) ALLELES IN SOUTHEAST IRANIAN POPULATION

Ali Bahari,[1] Mohammad Hashemi,[2,3] Zohreh Bari,[1] Abdolkarim Moazeni-Roodi,[4] Mahmoud-Ali Kaykhaei,[1] and Behzad Narouie[5]

[1]*Department of Internal Medicine, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*
[2]*Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*
[3]*Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran*
[4]*Department of Biology, Sistan and Baluchistan University, Zahedan, Iran*
[5]*Clinical Research Development Center (CRDC), Ali-e-ebne-abitaleb Hospital, Zahedan University of Medical Sciences, Zahedan, Iran*

□ *Thiopurine S-methyltransferase (TPMT, EC 2.1.1.67) plays a key role in the metabolism of thioprine drugs. Subjects with intermediate or no TPMT activity are at risk of azathioprines toxicity treated with conventional dosages of thiopurine drugs. While TPMT polymorphisms have been extensively studied in many countries, there is insufficient data in Iranian populations. In the present study, we aimed to identify the common functional TPMT alleles in southeast Iranian population. The TPMT allele frequencies were determined by multiplexed allele-specific polymerase chain reaction.*

*Among 832 samples of Iranian population, the frequency for the TPMT*2, TPMT*3A, TPMT*3B and TPMT*3C, were 2.16%, 1.68%, 1.62%, and 0.54%, respectively. The distribution of the TPMT genotypes were 87.98% for TPMT*1/*1, 4.33% for TPMT*1/*2, 3.36% for TPMT*1/*3A, 3.24% for TPMT*1/*3B, and 1.08% for TPMT*1/*3C. This functional analysis of common TPMT alleles in an Iranian population could provide useful information for thioprine drugs therapy.*

Keywords Thiopurine S-methyltransferase; polymorphism; genetic polymorphism; Iranian

Received 10 January 2010; accepted 22 February 2010.
This project was financially supported by Zahedan University of Medical Sciences. The authors would like to thank all subjects who willingly participated in the study.
Address correspondence to Mohammad Hashemi, Department of Clinical Biochemistry, School of Medicine, Zahedan University of Medical Sciences, Zahedan, I.R. Iran. E-mail: mhd.hashemi@gmail.com; hashemim@zdmu.ac.ir

INTRODUCTION

Human thiopurine S-methyltransferase (TPMT, EC 2.1.1.67) is a cytosolic enzyme that catalyses the S-methylation of thiopurine drugs such as 6-mercaptopurine (6-MP), 6-thioguanine (6-TG) and azathioprine (AZA). These drugs are used as immunosuppressant, anticancer agents, and for treatment of various diseases, such as inflammatory bowel diseases and rheumatic diseases.[1,2]The TPMT gene is genetically polymorphic and the inverse relationship between TPMT activity and the risk of developing severe hematopoietic toxicity is well known.[3] Individuals show extensive variations in TPMT activity.[4–6] It has been reported that 89% of the population have wildtype TPMT (TPMT*1/*1), 11% are heterozygous (low enzyme activity) and 0.3% are homozygous for TPMT mutant.[7–9] Individuals with TPMT-deficient or decreased TPMT activity are vulnerable to the side effects of standard doses of the thioprine drugs.[10–15] It has been reported that the presence of TPMT*3A and *3B result in a virtual lack of TPMT enzyme activity. While TPMT*2 and TPMT*3C do not result in such striking decreases in levels of enzyme protein as do *3A and *3B, but they are also associated with significant decreases in quantity of TPMT protein.

TPMT*3A is the most common variant allele in Caucasians and TPMT*3C is the most common functionally significant variant allele in East Asia.[16] TPMT allele frequencies and types frequently vary greatly among different ethnic groups (refer to Table 4). Because of the clinical importance of TPMT polymorphism for patients receiving thiopurine drugs, all patients should be screened for TPMT genotyping before thiopurine therapy. TPMT polymorphisms are well known across in many different countries, but to the best of our knowledge there is little information regarding TPMT polymorphisms in the Iranian population.

The aim of the present study was to investigate the most common TPMT allele's frequencies in samples of southeast Iranian population.

MATERIAL AND METHODS

This population based cross-sectional study was performed in 832 apparently healthy subjects in Zahedan, southeastern Iran. The study includes 41.1% male and 58.9% female with mean age of 36.3 ± 14.1 years.

This investigation was approved by the local ethics committee of Zahedan University of Medical Sciences and written informed consent was taken from all subjects. Two milliliter of venous blood drawn from each subjects and genomic DNA was extracted from peripheral blood by rapid genomic DNA extraction procedure and stored at −20°C as described previously.[17]

The genotype of each individual at the TPMT*2, TPMT*3A, TPMT*3B, and TPMT*3C alleles was determined using previously described

TABLE 1 The primers used in the TPMT ARMS assay

Primer	Sequence (5′ to 3′)	Reaction
β2Mf	TGTAAACACTTGGTGCCTGATATAGCTTGA	ARMS1 and ARMS2
β2Mr	CATCAGTATCTCAGCAGGTGCCACTAATCT	ARMS1 and ARMS2
TPMT2C[c]	ATCTGCTTTCCTGCATGTTCTTTGAAACCC	ARMS1 and ARMS2
TPMT2WT[d]	CACACCAACTACACTGTGTCCCCGGTCTCC	ARMS1
TPMT2MU[e]	CACACCAACTACACTGTGTCCCCGGTCTCG	ARMS2
TPMT460C[c]	AGGTCTCTGTAGTCAAATCCTATACT	ARMS1 and ARMS2
TPMT460WT[d]	ATTTGACATGATTTGGGATAGAGGTG	ARMS1
TPMT460MU[e]	ATTTGACATGATTTGGGATAGAGGTA	ARMS2
TPMT719C[c]	ATTTTTAGTAGAGACAGAGTTTCACCATCT	ARMS1 and ARMS2
TPMT719WT[d]	TATGTCTCATTTACTTTTCTGTAAGTAGTT	ARMS1
TPMT719MU[e]	TATGTCTCATTTACTTTTCTGTAAGTAGTC	ARMS2

[c]Common primers.
[d]Wildtype specific primers.
[e]Mutant-type specific primers.

multiplexed allele-specific polymerase chain reaction.[18] The allele specific primers used are shown in Table 1. Two tubes (ARMS1, ARMS2) were used for determination of TPMT genotypes (Figure 1).

Each reaction consisted of a total volume of 25 μl containing 250 μM dNTPs, 0.5 μM of each primer, 4 mM $MgCl_2$, 1 U of Taq DNA polymerase (Roche Molecular Biochemicals, USA), and ~100 ng of genomic DNA. Polymerase chain reaction cycling conditions were as follows: 5 minutes at 94°C; 30 cycles of 30 seconds at 94°C, 30 seconds at 65°C, and 30 seconds at 72°C; 10 minutes at 72°C (Corbett Research, Australia). Each reaction was verified on a 2% agarose gel.

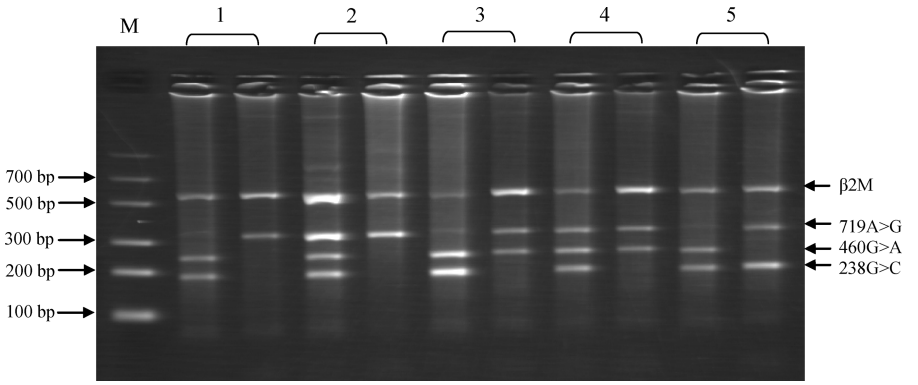

FIGURE 1 Multiplexed ARMS assay was used for the detection of TPMT*2, TPMT*3A, TPMT*3B, and TPMT*3C alleles. Each pair of lanes represents one patient sample. Five microliters of ARMS 1 (left lane of each pair) and ARMS2 (right lane of each pair) reaction products was separated on 2% agarose gels. Samples: (1) TPMT*1/*1; (2) TPMT*1/*3C; (3) TPMT*1/*3B; (4) TPMT*1/*3A; (5) TPMT*1/*2; (M) 1 kb DNA marker.

TABLE 2 TPMT allele frequencies in a sample of 832 healthy subjects of Iranian population

Allele	SNP position	Amino acidsubstitution	N	%
TPMT*1	Wildtype		1564/1664	93.99
TPMT*2	238G > C	Ala80Pro	36/1664	2.16
TPMT*3A	460G > A and 719A > G	Ala154Thr and Tyr240Cyc	28/1664	1.68
TPMT*3B	460G > A	Ala154Thr	27/1664	1.62
TPMT*3C	719A > G	Tyr240Cyc	9/1664	0.54

RESULTS

We analyzed three TPMT polymorphisms, 238G > C, 460G > A, and 719A > G, indicating the TPMT*2 (238G > C), TPMT*3A (460G > A and 719A > G), TPMT*3B (460G > A), and TPMT*3C (719A > G). As shown in Table 2, TPMT*1, TPMT*2, TPMT*3A, TPMT*3B, and TPMT*3C allele frequencies were 93.99% (1564/1664), 2.16% (36/1664), 1.68% (28/1664), 1.62% (27/1664), and 0.54% (9/1664), respectively.

TPMT genotypes were determined for all individuals. The frequency of the TPMT genotypes were 87.98% (737/832) for TPMT*1/*1, 4.33% (36/832) for TPMT*1/*2, 3.36% (28/832) for TPMT*1/*3A, 3.24% (27/832) for TPMT*1/*3B, and 1.08% (9/832) for TPMT*1/*3C (Table 3).

DISCUSSION

Thiopurine S-methyltransferase (TPMT) catalyzes the methylation of thiopurine drugs, which are used in cancer chemotherapy and as immunosuppressive agents. Using these drugs is limited due to severe adverse effects. Frequency and types of common TPMT alleles have been investigated generally in different parts of the world (Table 4). Genetic polymorphisms that affect the enzymatic activity are contributed to interindividual variations to the sensitivity and toxicity of thioprine drugs.[15,19] The activity of this cytosolic enzyme is characterized by interindividual and interethnic variability caused by the genetic polymorphism of the TPMT gene, which was discovered by the existence of three major phenotypes, high activity, intermediate activity and undetectable activity.[19]

TABLE 3 Genotype of TPMT in a sample of 832 Iranian populations

Genotype	% (n)
TPMT*1/*1	87.98% (732/832)
TPMT*1/*2	4.33% (36/832)
TPMT*1/*3A	3.36% (28/832)
TPMT*1/*3B	3.24% (27/832)
TPMT*1/*3C	1.08% (9/832)

TABLE 4 Comparison of the allele frequencies (%) of TPMT in various populations

Population	N[a]	TPMT*2	TPMT*3A	TPMT*3B	TPMT*3C	Ref.
Iranian	1664	2.16	1.68	1.62	0.54	Present study
Iranian	254	3.93	0.87	0	1.57	37
Korean	800	0	0	0	0.88	28
Taiwanese	498	0	0	0	0.60	24
Japanese	302	0	0	0	0.30	29
Japanese	384	0	0	0	0.8	25
West Asia	198	0	1	0	0	27
Chinese	450	0	0	0	1.33	38
German	2428	0.2	4.4	0	0.4	31
Swedish	1600	0.06	3.7	0.13	0.44	36
Slovenian	388	0	4.1	0.3	0.5	39
Czech	1392	0.1	4.3	0.1	0.4	40
Sardinians	518	1.74	0.58	0.39	0.77	33
Polish	716	0.4	2.7	0	0.14	32
Mexican	294	1	4.4	1.7	1.7	34
Brazilian	408	2.2	1.5	0.2	1	35
American Caucasian	564	0.2	0.3	0	0.2	30
African American	496	0.4	0.8	0	2.4	30
Egyptian	400	0	0.3	0	1.30	41

[a]N = number of alleles.

To predict drug toxicity, TPMT genotyping has been performed prior to the initiation of thiopurine treatment in several clinics.[20] In the present study, the allele frequencies of TPMT*2, TPMT*3A, TPMT*3B, and TPMT*3C were 2.16%, 1.68%, 1.62%, and 0.54%, respectively. Moreover the TPMT genotypes were 87.98% for TPMT*1/*1, 4.33% for TPMT*1/*2, 3.36% for TPMT*1/*3A, 3.24% for TPMT*1/*3B, and 1.08% for TPMT*1/*3C. The TPMT genotypes satisfied the Hardy-Weinberg equilibrium. We genotyped only 4 common TPMT mutant alleles since they are the most common alleles.[21] The samples in which these mutant alleles were not detected, were named as wild-type allele, TPMT*1. Until now, at least 23 genetic variants of the TPMT gene have been identified and associated with low enzyme activity.[22,23] Our results are in disagreement with studies that have found neither TPMT*2 nor TPMT*3B.[24–29] The absence of TPMT*3B has been reported in several studies[30–32] and our results are not consistent with these findings. Our data are corresponding with the studies having shown the presence of the common 4 TPMT polymorphism.[33–36] To the best of our knowledge there is only one report regarding the common polymorphism of TPMT in Iran which was performed on 127 subjects in Shariati Hospital in Tehran, the capital of Iran. It indicated that the allele frequencies of TPMT*2, TPMT*3A, TPMT*3B, and TPMT*3C were 3.93%, 0.87%, 0%, and 1.57%, respectively.[37] Our results are inconsistent with this study due to absence of TPMT*3B. Iran is an ethnically and culturally

diverse country. Hereby, it seems that this discrepancy might come from the different ethnicity of subjects participated in these studies.

In conclusion, the present study provides population-based information of the functional common TPMT alleles in an Iranian population.

REFERENCES

1. Coulthard, S.; Hogarth, L. The thiopurines: an update. *Invest. New Drugs*, **2005**, 23(6), 523–532.
2. Sahasranaman, S.; Howard, D.; Roy, S. Clinical pharmacology and pharmacogenetics of thiopurines. *Eur. J. Clin. Pharmacol.* **2008**, 64(8), 753–767.
3. Xin, H.W.; Xiong, H.; Wu, X.C.; Li, Q.; Xiong, L.; Yu, A.R. Relationships between thiopurine S-methyltransferase polymorphism and azathioprine-related adverse drug reactions in Chinese renal transplant recipients. *Eur. J. Clin. Pharmacol.* **2009**, 65(3), 249–255.
4. Tai, H.L.; Krynetski, E.Y.; Yates, C.R.; Loennechen, T.; Fessing, M.Y.; Krynetskaia, N.F.; Evans, W.E. Thiopurine S-methyltransferase deficiency: two nucleotide transitions define the most prevalent mutant allele associated with loss of catalytic activity in Caucasians. *Am. J. Hum. Genet.* **1996**, 58(4), 694–702.
5. Yates, C.R.; Krynetski, E.Y.; Loennechen, T.; Fessing, M.Y.; Tai, H.L.; Pui, C.H.; Relling, M.V.; Evans, W.E. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. *Ann. Intern. Med.* **1997**, 126(8), 608–614.
6. Otterness, D.; Szumlanski, C.; Lennard, L.; Klemetsdal, B.; Aarbakke, J.; Park-Hah, J.O.; Iven, H.; Schmiegelow, K.; Branum, E.; O'Brien, J.; et al. Human thiopurine methyltransferase pharmacogenetics: gene sequence polymorphisms. *Clin. Pharmacol. Ther.* **1997**, 62(1), 60–73.
7. Lennard, L.; Gibson, B.E.; Nicole, T.; Lilleyman, J.S. Congenital thiopurine methyltransferase deficiency and 6-mercaptopurine toxicity during treatment for acute lymphoblastic leukaemia. *Arch. Dis. Child.* **1993**, 69(5), 577–579.
8. Lennard, L.; Van Loon, J.A.; Weinshilboum, R.M. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. *Clin. Pharmacol. Ther.* **1989**, 46(2), 149–154.
9. Gardiner, S.J.; Gearry, R.B.; Begg, E.J.; Zhang, M.; Barclay, M.L. Thiopurine dose in intermediate and normal metabolizers of thiopurine methyltransferase may differ three-fold. *Clin. Gastroenterol. Hepatol.* **2008**, 6(6), 654–660; quiz 604.
10. Tassaneeyakul, W.; Srimarthpirom, S.; Reungjui, S.; Chansung, K.; Romphruk, A. Azathioprine-induced fatal myelosuppression in a renal-transplant recipient who carried heterozygous TPMT*1/*3C. *Transplantation* **2003**, 76(1), 265–266.
11. Slanar, O.; Chalupna, P.; Novotny, A.; Bortlik, M.; Krska, Z.; Lukas, M. Fatal myelotoxicity after azathioprine treatment. *Nucleosides Nucleotides Nucleic Acids* **2008**, 27(6), 661–665.
12. Relling, M.V.; Hancock, M.L.; Rivera, G.K.; Sandlund, J.T.; Ribeiro, R.C.; Krynetski, E.Y.; Pui, C.H.; Evans, W.E. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *J. Natl. Cancer Inst.* **1999**, 91(23), 2001–2008.
13. Evans, W.E.; Hon, Y.Y.; Bomgaars, L.; Coutre, S.; Holdsworth, M.; Janco, R.; Kalwinsky, D.; Keller, F.; Khatib, Z.; Margolin, J.; et al. Preponderance of thiopurine S-methyltransferase deficiency and heterozygosity among patients intolerant to mercaptopurine or azathioprine. *J. Clin. Oncol.* **2001**, 19(8), 2293–2301.
14. Black, A.J.; McLeod, H.L.; Capell, H.A.; Powrie, R.H.; Matowe, L.K.; Pritchard, S.C.; Collie-Duguid, E.S.; Reid, D.M. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. *Ann. Intern. Med.* **1998**, 129(9), 716–718.
15. Krynetski, E.Y.; Evans, W.E. Genetic polymorphism of thiopurine S-methyltransferase: molecular mechanisms and clinical importance. *Pharmacology* **2000**, 61(3), 136–146.
16. Wang, L.; Weinshilboum, R. Thiopurine S-methyltransferase pharmacogenetics: insights, challenges and future directions. *Oncogene* **2006**, 25(11), 1629–1638.
17. Hashemi, M.; Moazeni-Roodi, K.; Fazaeli, A.; Sandoughi, M.; Bardestani, G.; Kordi-Tamandani, D.M.; Ghavami, S. Lack of association between paraoxonasae-1 Q192R polymorphism and rheumatoid arthritis in Southeast Iran. *Gent. Mol. Res.* **2010**, 9(1), 333–339.

18. Roberts, R.L.; Barclay, M.L.; Gearry, R.B.; Kennedy, M.A. A multiplexed allele-specific polymerase chain reaction assay for the detection of common thiopurine S-methyltransferase (TPMT) mutations. *Clin. Chim. Acta* **2004**, 341(1–2), 49–53.
19. Weinshilboum, R.M.; Sladek, S.L. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am. J. Hum. Genet.* **1980**, 32(5), 651–662.
20. Fargher, E.A.; Tricker, K.; Newman, W.; Elliott, R.; Roberts, S.A.; Shaffer, J.L.; Bruce, I.; Payne, K. Current use of pharmacogenetic testing: a national survey of thiopurine methyltransferase testing prior to azathioprine prescription. *J. Clin. Pharm. Ther.* **2007**, 32(2), 187–195.
21. Rutherford, K.; Daggett, V. Four human thiopurine s-methyltransferase alleles severely affect protein structure and dynamics. *J. Mol. Biol.* **2008**, 379(4), 803–814.
22. Lindqvist, M.; Skoglund, K.; Karlgren, A.; Soderkvist, P.; Peterson, C.; Kidhall, I.; Almer, S. Explaining TPMT genotype/phenotype discrepancy by haplotyping of TPMT*3A and identification of a novel sequence variant, TPMT*23. *Pharmacogenet. Genomics* **2007**, 17(10), 891–895.
23. Schaeffeler, E.; Eichelbaum, M.; Reinisch, W.; Zanger, U.M.; Schwab, M. Three novel thiopurine S-methyltransferase allelic variants (TPMT*20, *21, *22)—association with decreased enzyme function. *Hum. Mutat.* **2006**, 27(9), 976.
24. Chang, J.G.; Lee, L.S.; Chen, C.M.; Shih, M.C.; Wu, M.C.; Tsai, F.J.; Liang, D.C. Molecular analysis of thiopurine S-methyltransferase alleles in South-east Asian populations. *Pharmacogenetics* **2002**, 12(3), 191–195.
25. Hiratsuka, M.; Inoue, T.; Omori, F.; Agatsuma, Y.; Mizugaki, M. Genetic analysis of thiopurine methyltransferase polymorphism in a Japanese population. *Mutat. Res.* **2000**, 448(1), 91–95.
26. Kham, S.K.; Tan, P.L.; Tay, A.H.; Heng, C.K.; Yeoh, A.E.; Quah, T.C. Thiopurine methyltransferase polymorphisms in a multiracial asian population and children with acute lymphoblastic leukemia. *J. Pediatr. Hematol. Oncol.* **2002**, 24(5), 353–359.
27. Collie-Duguid, E.S.; Pritchard, S.C.; Powrie, R.H.; Sludden, J.; Collier, D.A.; Li, T.; McLeod, H.L. The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations. *Pharmacogenetics* **1999**, 9(1), 37–42.
28. Lee, S.S.; Kim, W.Y.; Jang, Y.J.; Shin, J.G. Duplex pyrosequencing of the TPMT*3C and TPMT*6 alleles in Korean and Vietnamese populations. *Clin. Chim. Acta* **2008**, 398(1–2), 82–85.
29. Kubota, T.; Chiba, K. Frequencies of thiopurine S-methyltransferase mutant alleles (TPMT*2, *3A, *3B and *3C) in 151 healthy Japanese subjects and the inheritance of TPMT*3C in the family of a propositus. *Br. J. Clin. Pharmacol.* **2001**, 51(5), 475–477.
30. Hon, Y.Y.; Fessing, M.Y.; Pui, C.H.; Relling, M.V.; Krynetski, E.Y.; Evans, W.E. Polymorphism of the thiopurine S-methyltransferase gene in African-Americans. *Hum. Mol. Genet.* **1999**, 8(2), 371–376.
31. Schaeffeler, E.; Fischer, C.; Brockmeier, D.; Wernet, D.; Moerike, K.; Eichelbaum, M.; Zanger, U.M.; Schwab, M. 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(7), 407–417.
32. Kurzawski, M.; Gawronska-Szklarz, B.; Drozdzik, M. Frequency distribution of thiopurine S-methyltransferase alleles in a polish population. *Ther. Drug. Monit.* **2004**, 26(5), 541–545.
33. Rossino, R.; Vincis, C.; Alves, S.; Prata, M.J.; Macis, M.D.; Nucaro, A.L.; Schirru, E.; Congia, M. Frequency of the thiopurine S-methyltransferase alleles in the ancient genetic population isolate of Sardinia. *J. Clin. Pharm. Ther.* **2006**, 31(3), 283–287.
34. Taja-Chayeb, L.; Vidal-Millan, S.; Gutierrez, O.; Ostrosky-Wegman, P.; Duenas-Gonzalez, A.; Candelaria, M. Thiopurine S-methyltransferase gene (TMPT) polymorphisms in a Mexican population of healthy individuals and leukemic patients. *Med. Oncol.* **2008**, 25(1), 56–62.
35. Boson, W.L.; Romano-Silva, M.A.; Correa, H.; Falcao, R.P.; Teixeira-Vidigal, P.V.; De Marco, L. Thiopurine methyltransferase polymorphisms in a Brazilian population. *Pharmacogenomics J.* **2003**, 3(3), 178–182.
36. Haglund, S.; Lindqvist, 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(2), 288–295.
37. Azad, M.; Kaviani, S.; Soleimani, M.; Noruzinia, M.; Hajfathali, A. Common Polymorphism's Analysis of Thiopurine S-Methyltransferase (TPMT) in Iranian Population. *Yakhteh* **2009**, 11(3), 311–316.
38. Zhang, J.P.; Guan, Y.Y.; Wu, J.H.; Jiang, W.Q.; Huang, M. [Genetic polymorphism of the thiopurine S-methyltransferase of healthy Han Chinese]. *Ai Zheng* **2003**, 22(4), 385–388.

39. 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), 105–114.
40. Slanar, O.; Bortlik, M.; Buzkova, H.; Donoval, R.; Pechandova, K.; Sebesta, I.; Lukas, M.; Perlik, F. Polymorphisms of the TPMT gene in the Czech healthy population and patients with inflammatory bowel disease. *Nucleosides Nucleotides Nucleic Acids* **2008**, 27(6), 835–838.
41. Hamdy, S.I.; Hiratsuka, M.; Narahara, K.; Endo, N.; El-Enany, M.; Moursi, N.; Ahmed, M.S.; Mizugaki, M. Genotype and allele frequencies of TPMT, NAT2, GST, SULT1A1 and MDR-1 in the Egyptian population. *Br. J. Clin. Pharmacol.* **2003**, 55(6), 560–569.