

# The prevalence of polymorphisms of Thiopurine Smethyltransferase gene In Iranian Alopecia areata patients

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# ABSTRACT

Azathioprine therapy was recently used to treat dermatologic conditions such as Alopecia areata (AA). Previous reports showed that Thiopurine S-methyltransferase (TPMT) activities in human red blood cell are associated with a polymorphism in this gene. Therefore, patients carrying mutant allele of *TPMT* may show severe myelosuppression when they are treated with standard doses of Azathioprine drugs. This study aimed to evaluate the *TPMT* gene amongst Alopecia areata patients and healthy adult in Iranian populations. *TPMT* gene polymorphisms were investigated in 1285 Iranian healthy adult blood donors and 632 patients with Alopecia Areata Universalis (AU). Tetra Arms PCR, Real-Time PCR and Sequencing were used to evaluate the presence of allele-specific polymorphisms of *TPMT* gene (TPMT \*2(c.238 GC,), TPMT \*3A (c.460 GA and c.719 AG), TPMT \*3B (c.460 GA), and TPMT \*3C (c.719 AG). Results were shown that the TPMT\*2 allele is associated with a low enzymatic activity that was detected in 22.51% (863 in 1917) of Iranian individuals. Heterozygous genotypes were in 827 (43.14%) subjects (232 AA and 595 healthy), and homozygous genotypes were in 18 (0.94%) individuals (3 AA and 15 healthy). The normal allele (wild-type) was found in 55.92% of the studied individuals (20.70% AA and 35.21% healthy). According to a higher frequency of TPMT genotype in may have the clinical benefit to thiopurine dosage selection and treat patients as well.

**Keywords:** Thiopurine S-methyltransferase (TPMT), Alopecia Areata (AA), rs1142345, rs1800460, rs1800462.

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# INTRODUCTION

Alopecia areata was known as hair loss of all or part of the body and classified as an autoimmune disease (Sadeghi et al., 2015; Golnoosh Taghiabadi et al., 2018). Unfortunately, several therapeutic ways which have been presented to treat alopecia areata were not curative or preventive. Also, many of therapeutic agents may have a risky efficacy of result for patients in the long-term (Alsantali, 2011). Thiopurine therapy is used to treat dermatologic conditions, rheumatic disease, inflammatory disease, autoimmune disease such as alopecia areata, organ transplant rejection, certain types of cancer such as acute lymphoblastic leukaemia (Lee et al., 2017; Murugesan et al., 2010; Caufield and Tom, 2013; Broekman et al., 2017; Liu et al., 2015). It has been proven thiopurines, 6-mercaptopurine, 6-thioguanine and azathioprine have a toxic effect on hematopoietic cells that may cause red blood cell macrocytosis, leukopenia, selective ervthroid hypoplasia, severe anaemia and rarely pancytopenia (Lee et al., 2017). Thiopurines a purine analogue that intervenes the DNA synthesis of leukocytes and results in DNA damage and inhibits the cell proliferation and leads to death and myelosuppression (DiPiero and Hicks, 2015; Farfan et al., 2014) and thus decreases proliferation of cells, especially T and B lymphocytes (Alsantali, 2011; Liu et al., 2015). Metabolism of azathioprine is based on several pathways which are oxidation catalysed by xanthine oxidase (XO) and S-methylation catalysed by thiopurine methyltransferase (TPMT). This inactivates 6mercaptopurine through methylation to 6-methyl mercaptopurine (Murugesan et al., 2010; Moran et al., 2015). In alopecia areata and other skin autoimmune diseases, Azathioprine is responsible for the reduction of the number of Langerhans cells and other antigenpresenting cells in the skin (Alsantali, 2011). TPMT gene has 27kb length and is located on chromosome 6p22.3 with ten exons (Murugesan et al., 2010; Oro, 2008).

It is previously reported that TPMT activity plays a significant role in human tissue, and controlled by genetic polymorphism. *TPMT* polymorphism has been recognised as a risk factor in pharmacogenetic studies and the best example for prediction of individual response to specific medicine with the use of genetic information. *TPMT* gene locus variation exhibit an incidence of life-threatening myelosuppression, severe toxicity due to thiopurine drugs intake (Murugesan et al., 2010). Several studies express that TPMT activity in human RBC is trimodal:

- high activity, inheritance of two functional copies (90%)

- intermediate activity, heterozygotes for deficient alleles (10%)

- low or no detectable activity, inheritance of two nonfunctional copies (0.3%) (Murugesan et al., 2010; Caufield and Tom, 2013).

In the last group, thiopurine therapy accumulates

excessive levels of active 6-mercaptopurine that turned to cytotoxic 6-thioguanine nucleotide analogues (TGNA) which may lead to hematologic toxicity, whereas heterozygous patients in risk of toxicity, too (Meijer et al., 2017; Lee et al., 2017). Extensive studies have shown that single nucleotide polymorphisms (SNPs) placed in enzymes that metabolise thiopurine drugs universally prescribed for autoimmune disease such as alopecia are prognostic factors for investigation of the toxicity and activity of TPMT level in patients with alopecia (Jimenez-Morales, 2016; Oro, 2008).

There have been at least 37 variant alleles of TPMT that three of them incorporate the majority of TPMT deficiency, which named TPMT 3A, - 3C and - 2 and have considered in this article (Ujiie et al., 2008; Oliveira et al., 2007; Jimenez-Morales, 2016). TPMT\*3A is exclusively restricted to Caucasian people (5% frequency) that contains two non-synonymous variations in the coding region. These variants may lead to the amino acid substitution in exon 7 Ala154Thr (c.G460A, rs1800460) and exon 10 Tyr240Cys (c.A719G, rs1142345), while allele TPMT\*3C is the most frequent among Asians that including exon 10 variant (2% frequency) and lead to the amino acid substitution Tyr240Cys (c.A719G, rs1142345) (Otterness et al., 1997), allele TPMT\*2 (c.G238C, rs1800462) is infrequent no synonymous variant with low catalytic activity and is basically confined to Caucasians, it leads to the amino acid substitution Ala80Pro (Krynetski et al., 1995; Oliveira et al., 2007), such variants like TPMT\*3B is scarcely occurred and leads to the amino acid substitution in exon 7 Ala154Thr (c.G460A, rs1800460) (Otterness et al., 1997; Krynetski et al., 1995) and TPMT\*8 rarely occur and is more frequent in Africans (2% frequency) (Oliveira et al., 2007; Kim, 2015; Relling et al., 1999). The present study aimed to investigate rs1142345, rs1800460, rs1800462 SNPs of the TPMT gene among patients with AA and healthy subjects.

# METHODS AND MATERIALS

# Subject selection and sampling

Subjects were included 632 patients with AA and 1285 healthy adult subjects. The age range of subjects was 40 to 50 years old and had not any history of autoimmune or allergic diseases or D3 vitamin deficiency. Also, there was no cousin marriage up to three previous generations. Subjects recruited from the different areas of Iran. K2 EDTA tube (VACUETTE® EDTA) was used to collect 2cc of the peripheral blood sample. All participants have signed a written informed consent.

# DNA extraction and primer

Genomic DNA was extracted from blood samples according to protocol DNA extraction of Cinna Pure DNA (PR881612-EX6001) kit. Extracted DNA's quality was measured by both 1.5% agarose gel electrophoresis and Denovix Nanodrop device (Model Ds-11). The concentration of extracted DNA was about 250 mg/ml. Specific primers were designed using oligo 7 software and synthesised by

Dezhgir et al. 69

SinaClon Company (Table1).

Three area of known TPMT gene mutations being responsible for TPMT deficiency (c.238 G/C, c.460 G/A, and c.719A/G) were determined according to the three methods Tetra Arms PCR, Real-Time PCR and Sequencing.

In brief, allele-specific PCR amplification was used to detect the c.238 G<sub>2</sub>C transversion in exon 5, c.460 G<sub>2</sub>A and c.719 A<sub>2</sub>G mutations in exon 7 and 10, respectively.

#### Tetra arms PCR

The final volume for all PCR reactions was 25 ml in which genomic

DNA (200 to 250 ng) was amplified according to the protocol in Table 2. The reaction was used Taq DNA Polymerase Master Mix RED 2X-MgCl<sub>2</sub>; 1.5 mM ampliqon kit (#180301-50). Also, an advanced biometric thermocycler was used.

The protocol of PCR amplification for three mention polymorphism included of an initial denaturation step at 95°C for 5 min followed by 32 cycles of denaturation at 95°C for 30 s, annealing at 61.7°C (c.238 G<sub>2</sub>C), 58.3°C (c.460 G<sub>2</sub>A), 55°C (c.719A<sub>2</sub>G) for 30 s and extension at 72°C for 30 s. The final extension step was 72°C for 5 min.

Electrophoresis analysed PCR products in 1.5% agarose gel and Thermo Scientific Gene Ruler 100 bp DNA Ladder. Explanation of DNA fragments was present in Figure 1.

**Table 1.** Sequence of primers used, product size.

SNP	Primer sequence $5 \rightarrow 3$	Product size	Annealing temp. °C
rs1800462 (TPMT*2) c.G238C	Forward inner primer: AGT GTA AAT GTA TGA TTT TAT GCA GGT ATG Reverse inner primer: ACT ACA CTG TGT CCC CGG TCA GG Forward outer primer: ATA GAT CTG CTT TCC TGC ATG TTC TTT Reverse outer primer: AAT ACT CAC ACT GAG AAA AAC TTT TGT GG	Control fragment: 354 bp G allele: 225 bp C allele: 181 bp	61.7
rs1800460 (TPMT*3B) c.G460A	Forward inner primer: AATTTG ACA TGA TTT GGG ATA GAG TAG Reverse inner primer: CTG GAT TGA TGG CAA CTA AGG T Forward outer primer: TTC ATG TCC CCA AAT CAT ACC A Reverse outer primer: GCC TTA CAC CCA GGT CTC TGT A	Control fragment: 507 bp G allele: 257 bp A allele: 298 bp	58.3
rs1142345 (TPMT*3C) c.A719G	Forward inner primer: TGA CTG TCT TTT TGA AAA GTT CTG Reverse inner primer: ATG TCT CAT TTA CTT TTC TGT AAG TAT AT Forward outer primer: TGT CAT TCT TCA TAG TAT TTT AAC ATG Reverse outer primer: TTT AGA AAA AGT AAA TGG CTT TAC TAA	Control fragment: 323 bp A allele: 168 bp G allele: 207 bp	55

 Table 2. Tetra arms PCR reaction protocol.

	Primer				DNA (200 250 pg)	Master mix BCB	
SNP	Forward inner (µl)	Reverse inner (µl)	Forward outer (µl)	Reverse outer (μl)	μl)	μl)	water (µl)
rs1800462 (TPMT*2)	1.5	1.5	1	1	1	12	7
rs1800460 (TPMT*3B)	1.5	1.5	1	1	1	10	9
rs1142345 (TPMT*3C)	1.5	1.5	2.5	2.5	1	14	2

<sup>1</sup>Diethyl pyrocarbonate.



**Figure 1.** Agarose gel electrophoresis (1.5%) of PCR product of tetraprimer ARMS-PCR. (a) Lanes 1, 2, 4 and 5 show heterozygous TPMT\*2 genotype (length of control fragment: 354 bp, length of G allele: 225 bp and C allele: 181 bp). (b) Lanes 1, 3, 4 and 5 show wild type TPMT\*2 genotype (length of control fragment: 354 bp, length of G allele: 225 bp). (c) Lanes 1, 2 and 4 show wild-type TPMT\*3B genotype (length of control fragment: 507 bp, length of G allele: 257 bp). (d) Lanes 1, 2 and 4 show wild-type TPMT\*3C genotype (length of control fragment: 323 bp, length of A allele: 168 bp). Lane 6 shows negative control. Ladder 100 bp was used as the molecular weight marker.

#### Sequencing

PCR was carried out by using an Outer primer (reverse and forward). Then PCR product was sent to SinaClon Company to sequence PCR production in both forward and reverse directions. The purpose of that confirmation of tetra arms PCR was. Sequencing results were analysed by Finch TV (Figures 2 and 3).

#### **Real-time PCR**

Polymorphisms above (*TPMT*\*2, *TPMT*\*3C, *TPMT*\*3B, *TPMT*\*3A) were assessed by real-time PCR using Corbett machine (Corbett rg6000, Australia). First, one µl of DNA template (20 ng/µl) and ten µl of a real-time PCR master mix (2xRealTime PCR Master Mix Green-no Rax Kit (#A323402-25)), as shown in Table 3, were used to perform real-time PCR. Three real-time PCR reactions should detect one sample. The tube I detected the mutation of the *TPMT* gene, tube II detected the wild type of *TPMT* gene and the tube III detected negative control.

Real-time PCR was performed using the following conditions:  $95^{\circ}$ C for 5 min, followed by 45 cycles of  $95^{\circ}$ C for 30 s and  $61.7^{\circ}$ C (c.238 G<sub>2</sub>C), 58.3°C (c.460 G<sub>2</sub>A), 55°C (c.719A<sub>2</sub>G) for 30 s and 72°C for 20 s. All standards and samples were performed in triplicate. Real-time PCR results were analysed by gene runner

6000 software (Figure 4).

#### Statistical analysis

The data were presented as percentages and also were analysed by using SPSS version 24. 95% confidence intervals (95% CI) were estimated for all diagnostic tests. Proportions assessed categorical variables, and differences amongst the groups were compared using Fisher's exact chi-square analysis. P-values less than 0.05 were considered as statistically significant.

# RESULTS

In the present study, Tetra Arms PCR and Real-Time PCR methods were used to study the polymorphisms: *TPMT*\*2, *TPMT*\*3C, *TPMT*\*3B, *TPMT*\*3A. Then DNA sequencing was carried out to ensure the accuracy of previous results. Outcomes of all used techniques were the same.

The *TPMT* genotype (*TPMT*\*2, TPMT\*3A, *TPMT*\*3B, and *TPMT*\*3C mutations) were studied in 1285 healthy



Figure 2. The sequencing results of three genotypes of the TPMT gene. The genotypes scored from the tetra arms PCR were in 100% accordance with results of sequencing.

adult and 632 patients with AA in the Iranian population. There were 1069 males (55.7%) (725 healthy adult and 344 AA patients) and 788 females (44.3%) (560 healthy adult and 228 AA patients). The age range of all healthy subjects participating in the study was 40 to 50 years old, and up to three generations were reviewed to ensure health. *TPMT*\*3C (carrying c.719 A)G polymorphism), *TPMT*\*3B (carrying single nucleotide c.460 G)A polymorphism) and *TPMT*\*3A (carrying nucleotide c.460 G)A and c.719 A)G polymorphisms) were not detected in any of the Iranian individuals.

The *TPMT*\*2 heterozygous allele was detected in 827 subjects, with a frequency of 43.14% (595 controls (46.30%) and 232 patients with AA (36.70%)). Also, *TPMT*\*2 homozygous (carrying c.238 G>C polymorphism) was found in 18 blood donor (0.93%) that was included 15 healthy individuals (1.16%) and three patients

# (0.47%).

Among the 1917 Iranian participants (1285 healthy adults and 632 patients with AA), 78 were from area in the western north of Iran (4.06%) (28 AA patients and 50 controls with frequency of 35.90 and 64.10%, respectively), 88 were from the Lurs population (4.59%) (60 controls (68.18%) and 28 AA patient (31.81%)), 166 were from the Kurd population with a frequency of 8.66% (56 AA patients (33.73%) and 110 healthy adults (66.26%)), 324 (16.90%) subjects were from central and near the southern area of Iran included 213 of Esfahan, 87 of Shiraz and 24 of Kerman (220 healthy adult (67.90%) and 104 patient with AA (32.10%)), 28 were from the northern area of Iran with a frequency of 1.46% (8 AA patients (28.57%) and 20 healthy adults (71.43%)), 246 were from the southern area of Iran (12.83%) (76 AA patients (30.89%) and 170 healthy adults (69.11%)),



**Figure 3.** The sequencing results of new genotypes of the TPMT gene in exon 5 and 7. (a) A18143812G or 18143812 A>G and 18143811-10 del GA in non codin area. (b) 18143870 del T. (c) C 18138983 T: ATT  $\rightarrow$  ATC (isoleucine ====>> isoluecine).

Table 3. Real-time PCR reaction protocol.

CND	Pri	mer	DNA	Master mix RT-	DEPC <sup>1</sup>	
SNP	Forward outer (µI)	Reverse outer (µI)	(200-250 ng) (µl)	PCR (µI)	water (µl)	
rs1800462 (TPMT*2)	1	1	1	12	10	
rs1800460 (TPMT*3B)	1	1	1	10	12	
rs1142345 (TPMT*3C)	2.5	2.	1	14	5	



**Figure 4.** Examples of real-time PCR results. Each curve shows all of the interpreted results for the TPMT\*2.

216 were from the eastern north of area of Iran (11.27%) (76 AA patient and 140 controls with a frequency of 35.18% and 64.81% respectively) and 771 subjects were from capital of Iran and another area close to it (589 of Tehran, 74 of Karaj, 84 of Arak, 24 of Kashan) with a frequency 40.22% (515 controls (66.80%) and 256 AA patient (33.20%)) (Figure 5).

The frequency of the TPMT\*2 in the populations of the different regions of Iran are showed in Figure 6 and Table 4c.

Differences in the *TPMT* alleles, in the population of healthy adult and patient with alopecia areata, no significant relationship were found between them (P-values > 0.05) (Table 4a).

We found four new mutations not reported their relation with genetic susceptibility polymorphisms for taking Thiopurine drugs. Three of which were located in exon 5, and one other is located in exon 7. The first identified mutation was 1814381 A >G, 18143811-10 deletion GA in the non-coding area. Both of them were detected in the same subject. the other identified mutation, were 18143870 del T in exon 5 and C 18138983 T: ATT  $\rightarrow$ ATC(isoleucine ====>> isoleucine) in exon 7 (Figure 3).

#### DISCUSSION

One of the conventional drugs prescribed for the

treatment of autoimmune patients, including alopecia areata, is mercaptopurine family medicine such as azathioprine. TPMT-deficient patients are not able to treat with standard doses of mercaptopurine (6MP), and even treatment can be fatal in these patients (Lee et al., 1995; Schutz et al., 1993). Heterozygous patients have common activity, and homozygous patients have low or no activity (Rossi et al., 2001; McLeod et al., 1993; McLeod et al., 2000; Jimenez-Morales et al., 2016) (4, 34, 13 and 35).

TPMT activity in most races worldwide is known. The present study is the first assessment of *TPMT* allele's frequency in Iranian patients with AA population. The current research has conveyed that 43.14% of the Iranian population carring*TPMT2*\* allele heterozygous and 0.94% carring*TPMT2*\* allele homozygous with an overall allele frequency of *TPMT* allele was 22.51% in the Iranian population. That is a relatively high repartition for this polymorphic gene in Iranian compared with an array of the ethnic groups such as European(Jimenez-Morales et al., 2016), American, Latin American (Zeglam et al., 2015), Caucasians (Collie-Duguid et al., 1999) and other Asian nations (Kham et al., 2002; Lu et al., 2005; Srimartpirom et al., 2004).

In contrast to those previous reports in other national populations, *TPMT*\*3C and *TPMT*\*3A alleles were the most popular mutant alleles in the other studied population (Zeglam et al., 2015), they were not detected



**Figure 5.** Categorizing the regions of Iran to study the frequency of the TPMT allele. The frequency of volunteers participating in the research indicated on the map of Iran. a: Area in the western north of Iran. b: capital of Iran and another area close to it. c: the northern region of Iran. d: Lur population. e: Kurd people. f: southern area of Iran. g: central area and near the south of the district of Iran. h: Area in the eastern north of Iran. k: south-east region of Iran.



**Figure 6.** Comparison of allele frequency of Mutant allele of TPMT\*2 with the frequency of volunteers in the different zone of Iran. This graph clearly states that the frequency of participants in each region of Iran corresponds to the distribution of TPMT\*2 Mutant allele frequency. 1: Western north is of Iran. 2: The capital of Iran and another area close to it. 3: Northern region of Iran. 4: Lurs population. 5: Kurd people. 6: Southern area of Iran. 7: Central area and near the south of the

distribution.

district of Iran. 8: Area in the eastern north of Iran. 9: South-east region of Iran. Although there is a

Table 4. Statistical analysis of TPMT\*2 mutant alleles. (a) Comparison in Iranian society regardless of sexuality. (b) Comparison of the frequency of this allele in the population of female and male. (c) Frequency of the TPMT\*2 allele in the populations of the different regions of Iran.

Table a							
Name	Number	Genotype frequency	Allelic frequency	Chi-square	p-value	Odd ratio	
TPMT*2 wild-type allele (patient)	397	20.70944	81.17088	0 7418	0 200	1 24	
TPMT*2 wild-type allele (healthy)	675	35.21127	75.68093	0.741	0.369	1.34	
TPMT*2 heterozygous allele (patient)	232	12.10224	18.35443	0 767 <sup>a</sup>	0 291	0 725	
TPMT*2 heterozygous allele (healthy)	595	31.03808	23.15175	0.707	0.301	0.735	
TPMT*2 mutant homozygous allele (patient)	3	0.156495	0.474683	1 005 <sup>a</sup>	0.216	1.01	
TPMT*2 mutant homozygous allele (healthy)	15	0.782473	1.167315	1.005	0.316	1.01	
Overall TPMT*2 mutant allele (patient)	235	12.25874	18.82911	0 7448	0.000	0.740	
Overall TPMT*2 mutant allele (healthy)	610	31.82055	24.31906	0.741	0.389	0.743	
Overall TPMT*2 wild-type allele (patient)	397	20.70944	81.17088	0 741 <sup>a</sup>	0.290	1.24	
Overall TPMT*2 wild-type allele (healthy)	675	35.21127	75.68093	0.741	0.369	1.54	
a= 0 cells (0.0%) have expected count less that	in 5. The mir	limum expected	a count is 21.50	).			
Table b							
TPMT*2 wild-type allele (female)	558	29.107981	82.7830188	0.035 <sup>a</sup>	0.852	1 072	
TPMT*2 wild-type allele (male)	709	36.984872	82.4134705	0.000	0.002	1.072	
TPMT*2 heterozygous allele (female)	288	15.023474	16.9811320	0.0268	0.840	1 075	
TPMT*2 heterozygous allele (male)	344	17.944705	16.0898035	0.030	0.049	1.075	
TPMT*2 mutant homozygous allele (female)	2	0.1043296	0.23584905	1 005 <sup>a</sup>	0.316	1 01	
TPMT*2 mutant homozygous allele (male)	16	0.8346374	1.49672591	1.000	0.010	1.01	
Overall TPMT*2 mutant allele (female)	300	15.649452	17.8066037	0 000 <sup>a</sup>	1	1	
Overall TPMT*2 mutant allele (male)	360	18.779342	17.5865294	0.000	I	I	
Overall TPMT*2 wild-type allele (female)	558	29.107981	82.7830188	0 035 <sup>a</sup>	0 852	1 072	
Overall TPMT*2 wild-type allele (male)	709	36.984872	82.4134705	0.000	0.002	1.072	
a: 0cells (0.0%) have expected count less than 5. The minimum expected count is 34.00.							

Table c

Deputation of different regions of tran	G			
Population of different regions of fram	G/G (%)	G/C (%)	C/C (%)	Allenc frequency (%)
Western north area of Iran	0	19.23	80.77	0.50
Lurs	0	45.45	54.55	1.46
Kurd	0	47.59	52.41	2.06
Central and near the southern area of Iran	0	12.34	87.66	1.04
The northern area of the Iran	0	50	50.00	0.36
The southern area of the Iran	0	13	87.00	0.83
The eastern north of area of the Iran	0	48.15	51.85	2.61
The capital of Iran and other area close to it	2.64	62.64	34.72	12.60

in Iranian population. On the other hand, TPMT\*2 was found to be the most prevalent mutation among Brazilians (Boson et al., 2003), Turks (Zeglam et al., 2015) and Iranians (Zeglam et al., 2015), and also in the present study, it was detected with the most common frequency. The remarkable point is that, although *TPMT*\*2 is a rare mutant allele found in Asian populations, it was the majority proportion of our subjects carried it and this is the amount was much higher than previous studies related to Iranian population as well.

This indicates the importance of TPMT testing before prescribing drug. About half of the population of Iran is susceptible to the typical dose of that drug in another hand it is toxic and would be fatal for them (44.08%). Although this study was not designed to distinguish differences in allele frequency between the different races of Iranian populations, it was determined by differences in allele frequency between the various areas of Iran.

As aforementioned, there was no significant difference between the patient and healthy subjects (P-values > 0.05). The highest frequency of mutated  $TPMT^*2$  alleles was found in the population of the capital of Iran and another area close to it (483 of 771 individuals), then the northeastern regions of Iran (104 of 216 individuals) with the frequencies 26.13 and 5.43%, respectively. It has also illustrated that the overall frequency of  $TPMT^*2$ alleles in the areas were 13.56 and 2.71% in Iranian population than 55.97% of its proportion belongs to the population of the capital of Iran and its near cities (Table 4).

In our study, the northwestern cities and southern area of Iran have the lowest share of the *TPMT2*\* allele frequency (19 of 78 subjects with an allele frequency of 0.50% and 32 of 246 individuals with an allele frequency of 0.83%, respectively).

Via carefully examining three generations of family descent of participants in the project, it became conspicuous that most of the people were living in Tehran returned to Azeri, Tabari, Geake and Fars ethnicities. This seems to be expected owing to the high diversity of ethnic groups in the Tehran population. Given the limited access to ethnic and racial information in the project, only the ethnicities being available were examined. The results of this study showed that among the various Iranian ethnicities (Azeri, Kurd, Lurs, Baluch, Lak, Gilak, Tabari, Turkmen, Arab, Jewish, Armenian and Fars) only Azeri (223 of 378 subjects), Fars (220 Of 725 subjects), Arabs (32 of 193 individuals), Tabari (49 of 78 individuals), Gilak (53 of 85 individuals), Kurds (49 of 78 individuals) and Lurs (56 of 88 individuals) ethnicities were identifiable with a frequency of TPMT2\* allele 6.51, 6.42, 0.93, 1.43, 1.55, 2.30 and 1.63%, respectively.

In this study, the frequency of allele was different among females and the male population. Although, equal populations were selected in both groups (848 females (44.24%) and 1069 males (55.76%)), the frequency of the TPMT2\* heterozygous allele mutant in the male population (patients group and healthy group) was significantly higher than female groups (29.37% male and 13.77% female with a frequency allele of 14.68 and 6.88%, respectively) (CI = 95% odd ratio = 1). However, the overall frequency of TPMT \*2 mutant alleles of the female and male group in Iranian population were 7.88% and 9.80% respectively, and it was not significant statistically (p-values < 0.05). This demonstrates more importance to study of the TPMT gene in the both was equal (Table 4b).

Homozygous and heterozygous state of the *TPMT*\*2 mutation leads to a reduction in catalytic activity and immunoreactive protein alike (Thiesen et al., 2017; Bhavsar et al., 2017; El-Rashedy et al., 2015; Schaeffeler et al., 2001). Consistent with the previous finding, the *TPMT* activity of individuals with heterozygous *TPMT*\*2 was almost 50% lower than that of the homozygous wild-type (Liu et al., 2016; Tamm et al., 2017; Park-Hah et al., 1996; Krynetski and Evans, 2000).

Results from our study revealed that TPMT\*2 was the most frequent mutant allele among Iranian population with remarkably superior frequency than other national populations and this offer that 22.50% of Iranian population might have an increased hazard of thiopurineinduced toxicity. Giving this fact that Xanthine oxidase enzyme (XO) in hematopoietic tissues is absence, TPMT seems to be only the critical enzyme causing to allay thiopurine drugs in these tissues, and thus consumption of that drug by the patients with TPMT deficiency might rapidly cause potential myelotoxicity (Zeglam et al., 2015; Srimartpirom et al., 2004). Hence determination of TPMT genotype in advance of prescribing any thiopurine dosage might have clinical benefit to treating Iranian patients as well. However, it is highly possible that other rare mutants alleles may be detected in some of the wildtype samples. Therefore, investigation of other variations in the TPMT gene would be useful.

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