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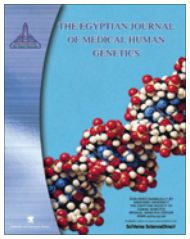

ORIGINAL ARTICLE

Thiopurine *S*-methyltransferase genetic polymorphism in the Tunisian population

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Abstract *Background:* Determine the incidence of four thiopurine *S*-methyltransferase (*TPMT*) mutant alleles, *TPMT*2*, **3A*, **3B* and **3C* in the Tunisian population involved in adverse drug reactions.

Genomic DNAs were isolated from peripheral blood leucocytes of 119 healthy Tunisian volunteers. The frequencies of four allelic variants of the *TPMT* gene, *TPMT*2*, *3A, *3B, *3C were determined using allele specific polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism technique.

Results: Of the 119 Tunisian subjects participating in this study, 117 subjects (98.3%) were homozygous for *TPMT*1* and only two subjects (1.68%) were heterozygous for *TPMT*1/*3A*. The frequency of *TPMT*3A* mutant allele was 0.009.

Conclusions: Our study provides the first data on the frequency of common TPMT variants in the Tunisian population. TPMT*3A, which causes the largest decrease in enzyme activity, seemed to be a unique variant allele found in this our population.

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1. Introduction

Thiopurine *S*-methyltransferase (*TPMT*) is a very important cytoplasmic transmethylase enzyme that preferentially catalyzes *S*-methylation of thiopurine drugs, activating or detoxifying the immunosuppressant azathioprine and the anticancer agents, 6-mercaptopurine and 6-thioguanine [1]. These thiopurine drugs are used in the treatment of inflammatory diseases, such as ulcerative colitis, dermatitis, and rheumatoid arthritis, and following organ transplantation. *TPMT* was shown to be present in most tissues, e.g., blood cells, heart, pancreas, placenta, and intestine [2].

The activity of the human TPMT varies greatly between individuals because of genetic polymorphism. *TPMT* activity is inherited as an autosomal codominant trait and was shown to be highly variable, with 90% of individuals having high/normal activity, 10% with intermediate activity and 0.3% with low/absent activity [3]. The TPMT gene variant was originally described in 1980 [4]. To date, more than 20 variant alleles associated with reduced enzymatic activity have been reported [5].

The prevalent alleles that have been associated with low or absent enzyme activity, are *TPMT*2* (G238C), *TPMT*3A* (G460A and A719G), *TPMT*3B* (only G460A) and *TPMT*3C* (only A719G). *TPMT* deficiency is associated with severe hematopoietic toxicity when conventional dosages of thiopurine drugs are used. In a recent meta-analysis Higgs et al. suggests that individuals with both intermediate and absent *TPMT* activity have an increased risk of developing thiopurine-induced myelosuppression, compared with individuals with normal activity [6].

The greatest risk of hematopoietic toxicity seems to be associated with homozygous *TPMT* variants while; patients with heterozygous *TPMT* genotypes are at intermediate risk [2,7,8]. However, *TPMT*-deficient patients can be successfully treated with reduced dosages of thiopurines [9,10]. Therefore, the level of *TPMT* enzyme activity is essential to prevent thiopurine toxicity and to optimize therapeutic drug treatment. The identification and frequencies distributions of variant *TPMT* alleles have been reported in many ethnic groups but there is no study on the *TPMT* polymorphism in Tunisians population.

The aim of this study is to characterize the most common *TPMT* alleles in the Tunisian population.

2. Material and methods

Hundred and nineteen Tunisian volunteers (67 females, 52 males, aged 35.4 ± 10.7 years) were included in this study. Subjects were healthy as defined by medical history and physical examination.

Informed written consent was obtained from all the subjects and the protocol was accepted by the Ethical Committee of the university Hospital (CHU) Sahloul of Sousse(Tunisia).

Genomic DNA was isolated from blood samples using the Wizard Genomic DNA Purification Kit (cat. # A1120) (Promega. Madison, USA) according to the instructions of the manufacturer.

The genotypes of each individual at the principal *TPMT* mutations G238C (*TPMT*2*), G460A and A719G (*TPMT*3A*), G460A (*TPMT*3B*) and A719G (*TPMT*3C*) were determined using previously described Polymerase Chain Reaction (PCR)-based assays with minor modification. An allele-specific PCR method was used to analyze the G238C mutation in exon 5, while PCR amplification and restriction enzyme digestion were used to detect the G460A and A719G mutation, respectively. The resultant PCR products of exon 7 and 10 were digested with restriction enzyme *Mwol and AccI* (New England Biolabs, Hertfordshire, UK), respectively.

The PCR reactions were performed in a volume of 25 µl with 30 cycles denaturation for *TPMT*3C* and *TPMT*3B* and 29 cycles for *TPMT*2*. PCR was carried out using 100 ng of DNA in a final volume 25 µl, with 25 mM dNTP, 10 µM of each primer, 25 mM $MgCl_2$ Boehringer and 0.1 µl of Taq polymerase 5 U/µl (Boehringer Mannheim GmbH). PCR amplification consisted of an initial denaturation step at 94 °C for 1 min followed by 29 cycles or 30 cycles of denaturation at 94 °C for 2 min, annealing at 60 °C (*TPMT*2*) or 58 °C (*TPMT*3C* and *TPMT*3B*) and extension at 72 °C for 1.5 min. The final extension step was performed at 72 °C for 7 min.

The statistical significance in allele or genotype frequency between different populations was evaluated using a Chi-square test or χ^2 test.

3. Results and discussion

The results of the genotype analysis are summarized in Table 1. A total of 117 subjects (98.32%; 95% CI 94.06–99.79) was homozygous for the wild-type allele (*TPMT*1*), i.e. they did not carry any of the tested mutations. Two subjects (1.68%; 95% CI 0.2–5.93) were heterozygous for *TPMT*1/*3A* giving allele frequencies of 0.009.

The genotype analysis of the Tunisian population revealed that the frequency of total *TPMT* variant alleles was low (1.6%). The low overall frequency of variant alleles in this study may be due to limited size of the population studied (238 alleles) and could be due to the peculiarity of the Tunisian population. The *TPMT*3A*, which causes the largest decrease in enzyme activity, is the only variant allele detected in this Tunisian population.

The frequency of this variant found in the present study (0.009) is similar ($P > 0.05$) to the frequency reported for Egyptians [11] and South West Asians [12], but significantly lower than the frequencies reported for Caucasian populations, including Americans [13], British [14], French [14], Italians [15], and Polish [16] (Table 2). It is also lower than the frequencies reported for Latin-Americans populations [17–20] (Table 2). *TPMT*3A* is the most prevalent nonfunctional variant allele of *TPMT* in Caucasians, Latin-Americans and Turkishs populations [27–28]. Subjects with heterozygous genotype **1/*3A* have one active *TPMT* allele, and their risk of thiopurine hematopoietic toxicity is greater than that of patients who have a homozygous wild-type *TPMT* genotype (35% versus 7%), but not as great a risk as those who have 2 nonfunctional *TPMT* alleles (100%) [21]. *TPMT*3A* has not been detected in most of the Asian populations [22–25].

*TPMT*3C* was detected in all Africans except our Tunisian population (Table 2). In Africans such as Egyptians, this mutant allele represents 86% of the *TPMT* variant allele [12]. *TPMT*3C* accounts also for 100% in the Chinese, Japanese and Ghanaian [12,22,26], 70% of African-Americans [13], 5% of Caucasians [14] while it has not been detected in Argentinean and Colombian populations [17,18].The results from the

Table 1 Observed and expected genotype frequencies of TPMT in Tunisian subjects.

TPMT tested gene	N	% Observed frequency (95% CI)
*1/*1	117	98.32 (94.06–99.79)
*1/*2	0	0
*1/*3A	2	1.68 (0.2–5.93)
*1/*3B	0	0
*1/*3C	0	0

Observed genotype frequency was expressed in percentage with 95% confidence interval (CI) shown in brackets.

Table 2 Comparative allele frequencies of thiopurine *S*-methyltransferase in Tunisian compared with populations reported in other studies.

Populations	Allele frequency				
	N	*1	*2	*3A	*3C
Africans					
Tunisian (Present study)	238	0.991	0	0.009	0
Kenyan [14]	202	0.946**	0	0	0.054**
Ghanaian [26]	434	0.924**	0	0	0.076**
Egyptian [11]	400	0.985	0	0.003	0.013
Asians					
Japanese [22]	1044	0.984	0	0	0.016
Japanese [23]	142	0.979	0	0	0.014
Thai [22]	400	0.950**	0	0	0.050**
Chinese [14]	384	0.97	0	0	0.023*
Taiwanese [25]	498	0.994	0	0	0.006
South-West [12]	198	0.99	0	0.01	0
Caucasians					
American [13]	564	0.964*	0.002	0.032*	0.002
British [14]	398	0.947**	0.005	0.045*	0.003
French [14]	382	0.93**	0.005	0.057**	0.008
Italian [15]	412	0.946**	0.005	0.039*	0.010
Polish [16]	716	0.939**	0.008	0.05**	0.003
Latin-Americans					
Argentinean [17]	294	0.972	0.007	0.021	0
Colombian [18]	280	0.96*	0.004	0.036*	0
Chilean [19]	420	0.9619*	0.0024	0.0286	0.0071
Brazilian [20]	408	0.951**	0.022	0.015	0.01
Turkish [27]	212	0.981	0	0.0094	0.0094
Turkish [28]	116	0.914	0	0.034	0.009

N: number of alleles detected. Significant differences from Tunisian population:
* $P < 0.05$.
** $P < 0.005$.

present study show that the general pattern of *TPMT* allele frequency in the Tunisian population is similar to those determined for South West Asians populations including people from India, Pakistan, Sri Lanka and Nepal [12]. The variants allele *TPMT*2* and *TPMT*3B* have also not been detected in these populations. *TPMT*2* and *TPMT*3B* were not detected among 238 studied alleles suggesting that it may be absent or rare in Tunisian population.

4. Conclusions

To our knowledge, the current study is the first one that presents data for TPMT polymorphisms in Tunisian population.

The results from the present study revealed that *TPMT*3A*, variant enzyme with negligible activity compared to the wild type, was the only mutant allele among the Tunisian population of this study, suggesting that among Tunisians patients treated by thiopurine drugs a risk of severe hematopoietic toxicity is present. Several previous studies have shown that low *TPMT* activity is associated with bone marrow toxicity and adverse drug reactions but the efficacy of the strategy of screening the *TPMT* gene mutation in all patients prior to initiating treatment with thiopurine drugs is a matter of debate. This study support the assessment of *TPMT* genotype prior to thiopurine drugs therapy as a supplement to other routine and still essential measure for ensuring safe thiopurine drugs use, including monitoring of blood cell counts.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors contributed to the development of the project, the realization, the critical analysis and interpretation of the results. All authors reviewed and approved the manuscript.

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