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Short Communication

Phenotyping and Genotyping Studies of Thiopurine *S*-Methyltransferase in Kazaks

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Objective. This study was conducted to investigate the thiopurine *S*-methyltransferase (TPMT) activity distribution and gene mutations in Kazaks, and compared the results with those of other ethnic groups.
Methods. Erythrocyte TPMT activity was measured in Kazaks (n = 327) via a validated high-performance liquid chromatography assay. Polymerase chain reaction-based methods were used to analyze three commonly reporter-inactivating mutations: G238C, G460A, and A719G.
Results. Unimodal distribution of TPMT activity was found in Kazaks. Six *TPMT*3C* heterozygotes and two *TPMT*3A* heterozygotes were found in 327 Kazaks, with allele frequencies of 0.9 and 0.3%, respectively. The subjects with *TPMT*3A* and *TPMT*3C* heterogygotes had substantial TPMT activity over the range of 6.40–11.75 U/ml RBC.
Conclusion. Unlike in most Caucasians, *TPMT*3C* is a common mutant allele in Kazaks, whereas *TPMT*3A* is a rare mutant allele. Further studies are needed to explore the clinical impact of these *TPMT* mutants to thiopurine therapy in Kazak patients.

KEY WORDS: enzyme activity; mutation; polymorphism; thiopurine *S*-methyltransferase.

INTRODUCTION

Thiopurine *S*-methyltransferase (TPMT, EC2.1.1.67) is a cytoplasmic enzyme that preferentially catalyzes the *S*-methylation of aromatic and heterocyclic sulfhydryl compounds. This enzyme is responsible for the inactivation of thiopurine drugs such as 6-mercaptopurine (6-MP), 6-thioguanine, and azathioprine (1,2). These drugs have been widely used in the treatment of leukemia, autoimmune disorders, and organ transplants. Oral 6-MP is routinely used in maintenance treatment of acute lymphoblastic leukemia in children, which contributes to the high cure rates achieved (3–5). Azathioprine is widely used for the treatment of inflammatory bowel disease, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, dermatologic conditions, and organ transplantation (6,7). Individual difference in thiopurine drug metabolism, response, and toxicity in humans have been correlated with polymorphism of *TPMT* gene. TPMT is encoded by a 34-kb gene consisting of ten exons and nine introns with a cDNA of ~3,000 bp and an open reading frame of 735 bp that encodes for a 245-amino acid peptide with a molecular mass of ~35 kDa (8). *TPMT* gene is localized to chromosome 6p22.3. TPMT activity is inherited as an autosomal codominant trait with genetic polymorphism in all large populations studied to date (1,2).

From molecular genetic and familial studies, more was learned about the hereditary nature of TPMT deficiency in humans. Patients with intermediate activity are heterozygous at the *TPMT* gene locus and the TPMT-deficient subjects are homozygous for low activity alleles. Altered TPMT activity predominantly results from single nucleotide polymorphisms. Patients with inherited very low levels of TPMT activity are at greatly increased risk for thiopurine-induced toxicity such as myelosuppression when treated with standard doses of these drugs, whereas subjects with very high activity may be undertreated. The wild-type allele is designated as *TPMT*1* and to date, at least 18 variant alleles of *TPMT* gene have been reported. Based on the population phenotype–genotype studies performed to date, assays for the molecular diagnosis of TPMT deficiency have focused on *TPMT*2* (G238C) (9), *TPMT*3A* (G460A/A719G) (10), *TPMT*3B* (G460A), and *TPMT *3C* (A719G) (11). These four mutant alleles account for 80–95% of low activity alleles in all human populations. Like many other important genes, the pattern and frequency of mutant TPMT alleles is different among various ethnic populations (12). The most prevalent TPMT mutant allele in the Caucasian and Latin American population is *TPMT*3A* (13,14), whereas *TPMT*3C* is predominant in

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ABBREVIATIONS: 6-MP, 6-mercaptopurine; PCR, polymerase chain reaction; TPMT, thiopurine *S*-methyltransferase.

Chinese, Egyptians, and African-Americans (15,16). However, there have been no reports on TPMT activity distribution and genetic analysis in Kazaks who are Caucasian in ethnic origin. In the present study, we investigated the TPMT activity distribution and allele frequency of common alleles *TPMT*2*, *TPMT*3A*, *TPMT*3B*, and *TPMT*3C* in Kazaks and compared the results with those of other ethnic groups.

MATERIALS AND METHODS

Study Population

Blood samples were collected from healthy Kazaks (n = 327, consisting of 184 males and 143 females whose ages ranged from 15 to 60 years). The volunteers were from the People's Hospital and Xinhua Hospital of Xinjiang Kazakstan Autonomous Region, People's Republic of China. The ethnicity of all volunteers was confirmed by their social and culture characteristics and officially registered identity information. Ethical approval of this study was obtained from the Ethics Committee at Sun Yat Sen University, and written informed consent was obtained from all participants.

Determination of Erythrocyte TMPT Activity

Erythrocyte TPMT activity was measured in healthy Kazaks (n = 327) by a validated high-performance liquid chromatography (HPLC) assay as previously described (17). A Waters HPLC system was used, which was equipped with a Hypersil BDS C18 analytical column (5 μm, 250 × 4.6 mm I.D.). The mobile phase consisted of water/methanol/triethylamine (76.8:23:0.2, v/v), which was adjusted to pH 3.2 with phosphoric acid. The flow rate was 1.0 ml/min and the detection wavelength was 290 nm. The modality of the data was evaluated by the probit plot and the Kolmogorov–Smirnov test. One unit of TPMT activity represents the formation of 1 nmol of 6 MP per hour of incubation. TPMT activity was normalized per milliliter of packed erythrocytes.

Genotyping Study

Total genomic DNA was extracted from peripheral leukocytes using the phenol–chloroform extraction method as previously described (18). Three known deficient mutations, G238C, G460A, and A719G, were determined using previously described polymerase chain reaction (PCR)-based assays with minor modifications (17) (see Table I for all primers used). Restriction enzymes *MWo*I and *Acc*I (New England Biolabs, Hertfordshire, UK) were used to detect G460A and A719G mutations, respectively.

Table II. Activity of Erythrocyte TPMT in 327 Kazaks

Ethnic group gender	No. of subjects	TPMT activity		
		Range	Mean ± SD	Median
Kazaks	327	2.29−24.67	12.27 ± 3.42	12.33
Female	143	4.02−24.03	12.06 ± 4.02	11.89
Male	184	3.29−24.67	12.43 ± 3.29	12.53

Statistical Analysis

Data are presented as mean ± SD. Statistical significance of differences in mean erythrocyte TPMT activity values between gender groups and racial groups were tested with one-way ANOVA. Values of $P < 0.05$ were considered significant. Deviation from the normal distribution of erythrocyte TPMT activity was examined with the Shapiro–Wilk test.

RESULTS AND DISCUSSION

The mean erythrocyte TPMT activity in Kazak adults was 12.27 ± 3.42 U/ml RBC with a basically normal distribution. All subjects had detectable erythrocyte TPMT activity. There was no significant difference in TPMT activity between men and women in study population (Table II). Two *TPMT*3A* (G460A/A719G) heterozygote and six *TPMT*3C* (A719G) heterozygotes were found in 327 Kazakstan subjects (Table III). The allele frequencies of *TPMT*3A* and *TPMT*3C* were 0.3 and 0.9%, respectively, in Kazaks. These subjects with *TPMT*3A* and *TPMT*3C* heterozygotes had substantial TPMT activity over 6.40–11.75 U/ml RBC (Table IV).

The present study elucidated the genetic basis for TPMT deficiency in unimodal distributions of TPMT activity found in Kazaks. As in the case with other Asian populations including Chinese (16,19) and Japanese (20), *TPMT*3C* is a common mutant allele in Kazaks, whereas *TPMT*3A* is a rare mutant allele. However, the total frequency of mutant *TPMT* alleles in our study population (1.2%) is lower than that in Chinese (2.3–3.0%) reported by other groups (16,19), but compatible with that in Koreans (0.6%) (21) and Japanese (0.8%) (20). The low frequency of mutant *TPMT* alleles in Kazaks conformed to the normal distribution of TPMT activity in this population.

Table I. Primers Used to Detect Major Mutations in *TMPT* Gene

Exon	Primer	Sequence of primer	Length of product (bp)	Annealing temperature (°C)
5	P2W	5-GTATGATTTTATGCAGGTTTG-3	254	58
5	P2M	5-GTATGATTTTATGCAGGTTT-3	254	58
5	P2C	5-TAAATAGGAACCATCGGACAC-3	254	58
7	P460F	5-GGGACGCTGCTCATCTTCT-3	338	61
7	P460R	5-GCCTTACACCCAGGTCTCTG-3	338	61
10	P719F	5-AAGTGTTGGGATTACAGGTG-3	273	60
10	P719R	5-TCCTCAAAAACATGTCAGTGTG-3	273	60

Table III. Allelic Frequency of *TPMT* in Kazaks

Allele	No. of alleles	Frequency (%)
Total of alleles	654	100
*TPMT*1*	646	98.8
*TPMT*2*	0	0
*TPMT*3A*	2	0.3
*TPMT*3B*	0	0
*TPMT*3C*	6	0.9
Total mutant alleles	8	1.2

A comparison of the frequency of *TPMT*3A* and *TPMT*3C* variants in Kazaks with other ethnic groups indicates that *TPMT*3C* is the dominant mutants in Kazaks, which is close to most Asian populations (Table V). In contrast, *TPMT*3A* allele predominates in American and British Caucasians (13,15). Furthermore, the overall frequencies of mutant alleles in Kazaks (1.2%) are lower than those in American Caucasians (3.7%) (13,15) or British Caucasians (5.3%) (16). Thus, Kazaks have a distinct pattern and frequency of main defective TPMT alleles compared to other Caucasians. The reason for this is unknown, but may be associated with migration of Kazaks and other identified factors.

Both TPMT activity measurement and genotyping methods can be used to diagnose TPMT deficiency (22). A simple activity assay by HPLC or radiochemical methods would allow the identification of "rapid" or "slow" metabolizers. Accordingly, proper dose adjustment or alternative therapeutic agent is needed for "rapid" or "slow" metabolizers to prevent drug toxicity and therapeutic failure (23,24). However, the use of standard phenotype assay is limited by several factors. This method is not suitable for patients receiving blood transfusion (25). There is a large interindividual and interlaboratory variability in the activity of TPMT. On the other hand, genotyping methods can reliably detect the major and rare mutant allele at human *TPMT* locus, particularly when genetic polymorphism is highly likely to provide an explanation for TPMT deficiency in individuals (22). To date, it has become possible to detect TPMT inactivating mutations with more than 95% concordance between genotype and phenotype (25).

In this study, only four *TPMT* mutant alleles, *TPMT*2*, **3A*, **3B*, and **3C*, were genotyped in Kazaks. We inferred that the samples in which these mutant alleles were not detected had the wild-type *TPMT*1*. However, it remains a possibility that the presence of other rare mutant allele were not detected in this study. A number of rare mutant TPMT alleles (*TPMT*3D*, **4*, **5*, **6*, **7*, **8*, **10*, **11*, **12*, **13*, **14*, **15*, **16*, and **19*) have been identified (15,21,26–31). Their allele contribution to total variation in Kazaks has not yet been defined. Polymorphisms in the 5-flanking promoter region of *TMPT* gene have also been identified due to a variable number of tandem repeats (VNTR) with three kinds of motif (A, B, and C) differing by the length of the unit core and nucleotide sequence (2,32–38). However, a recent study demonstrated that the variable number tandem repeats (VNTR*3 to VNTR*9) in the promoter region of the *TPMT* gene had no significant impact on enzyme activity in British Asians and Caucasians (34). Further studies are needed to sequence the open reading frame and promoter region of *TPMT* gene for novel mutations in Kazaks.

Table V. A Comparison of Frequencies of *TPMT* Variant Alleles in Kazaks with Other Ethnic Groups

Ethnic group	No. of alleles	Frequency (%) *3A	Frequency (%) *3C	Ref.
Asians				
Chinese	400	0	3.0	(19)
Indian	400	0	2.3	(19)
Malay	400	0.5	0.8	(19)
Japanese	384	0	0.8	(20)
Chinese	384	0	2.3	(16)
Egyptian	400	0	0.3	(39)
Blacks				
African-American[a]	496	0.8	2.4	(15)
Ghanaian	434	0	7.6	(40)
Kenyan	202	0	5.4	(14)
Caucasians				
Caucasian-American[a]	564	3.2	0.2	(13,15)
British	398	4.5	0.3	(16)
French	382	5.7	0.8	(1)
Italian	412	3.9	1.0	(41)
Norwegian	132	3.4	0.3	(42)
Saami-Norwegian	388	0	3.3	(42)
Kazak	654	0.3	0.9	

[a] Calculated.

Table IV. Mutant *TPMT* Alleles in Kazaks

No.	Sex	Age (years)	Genotype of *TPMT*	TPMT activity (U/mL erothrocytes)
1	Female	28	*TPMT*3C* heterozygote	6.4
2	Male	20	*TPMT*3C* heterozygote	7.73
3	Male	47	*TPMT*3C* heterozygote	8.13
4	Female	21	*TPMT*3C* heterozygote	9.37
5	Male	21	*TPMT*3C* heterozygote	11.76
6	Male	39	*TPMT*3C* heterozygote	13.51
7	Female	20	*TPMT*3A* heterozygote	9.11
8	Female	20	*TPMT*3A* heterozygote	9.56

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