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TPMT Genetic Variations in Populations of the Russian Federation

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Abstract

Background—Polymorphisms that reduce the activity of thiopurine S-methyltransferase (TPMT) cause adverse reactions to conventional doses of thiopurines, routinely used for antileukemic and immunosuppressive treatment. There are more than 20 variant alleles of TPMT that cause decreased enzymatic activity. We studied the most common variant alleles of TPMT and their frequency distribution in a large cohort of multiracial residents in the Russian Federation and compared their frequencies in children with and without malignancy to determine whether TPMT gene abnormality is associated with hematologic malignancy.

Procedure—The TPMT biochip was used to detect 6 TPMT single nucleotide polymorphisms (SNPs) corresponding to 7 TPMT-deficiency alleles (TPMT*2, TPMT*3A, TPMT*3B, TPMT*3C, TPMT*3D, TPMT*7, and TPMT*8). We analyzed allele frequencies in the whole cohort, the childhood cancer group, and the non-cancer group. We also characterized disease features and outcome according to the presence of TPMT SNPs in children with acute lymphoblastic leukemia (ALL).

Results—Fifty-five (5.5%) study participants overall had heterozygous TPMT genotypes (1 variant and 1 wild-type allele): TPMT*1/*3A (n=45; 4.5%), TPMT*1/*3C (n=8; 0.8%), and TPMT*1/*2 (n=2; 0.2%). TPMT SNPs were more frequent in children with hematologic malignancy than in other participants (7.5% vs. 4.0%, $P = 0.02$). We found no significant association between TPMT SNPs and ALL treatment outcome (median follow-up, 31.3 months).

Conclusions—TPMT*3A is the most prevalent variant allele in the Russian Federation. The estimated frequency of variant alleles in the study cohort (5.5%) was similar to that observed in the White populations in the U.S. and Eastern Europe.

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Keywords

genetic polymorphism; thiopurines; thiopurine S-methyltransferase (TPMT); hematologic malignancy; toxicity

Introduction

Variant alleles in genes encoding several dozen drug-metabolizing enzymes cause polymorphisms that affect enzymatic activity [1–3], in turn altering the optimal dosage and scheduling of medications for affected patients [4]. These polymorphisms tend to be expressed stably within a given population and are typically inherited as autosomal recessive or autosomal codominant traits [5]. Such polymorphisms that reduce the activity of thiopurine S-methyltransferase (TPMT) are an important contributor to adverse reactions to conventional doses of thiopurines, which are a mainstay of antileukemic and immunosuppressive treatment [2,5,6]. Prolonged maintenance therapy (1.5–2 years) with the thiopurines, 6-mercaptopurine (6MP) or 6-thioguanine, is a core component of treatment of acute lymphoblastic leukemia (ALL) [7–9]. Another thiopurine, azathioprine, is also used in some immunosuppressive regimens administered after solid organ transplantation.

6-Mercaptopurine, 6-thioguanine, and azathioprine are inactive prodrugs that must be metabolized to thioguanosine phosphates to exert their cytotoxic effects, which occur via incorporation of thioguanine nucleotides into DNA, RNA, or both. One of the major catabolic pathways is S-methylation catalyzed by the cytoplasmic enzyme TPMT [10–12]. Approximately 25 years ago, researchers found that variability in TPMT activity is due mainly to genetic polymorphism. They found that approximately 11% of 298 randomly selected American study participants had reduced TPMT activity (5–10 U/mL RBC) and that 1 in 300 had an inherited TPMT deficiency (<5 U/mL RBC) [13]. Low TPMT activity can cause toxic concentration of thioguanine nucleotides in hematopoietic cells. The greatest risk of hematopoietic toxicity is associated with homozygous *TPMT* variants; patients with heterozygous *TPMT* genotypes are at intermediate risk [2,14]. However, patients known to be TPMT-deficient can be successfully treated with reduced dosages of thiopurines [15,16].

The molecular mechanism of *TPMT* deficiency was described in 1995, when the first variant allele, *TPMT**2, was discovered [17], followed shortly by discovery of the other common variant alleles [18,19]. To date, more than 20 variant alleles associated with reduced enzymatic activity have been reported. Most contain single nucleotide polymorphisms (SNPs) leading to amino acid substitutions; the “wild-type” allele is designated *TPMT**1, and the variants are designated *TPMT**2, *3A, *3B, *3C, *5, *6, etc. [17]. The variant alleles most frequently identified in human populations are *TPMT**2, *TPMT**3A, and *TPMT**3C [15,20]. Current genotyping methods (allele-specific PCR) allow the detection of variant *TPMT* genotypes with more than 95% accuracy and can be used for the molecular diagnosis of TPMT deficiency or heterozygosity [21]. The reliability of such testing has been improved by the development of DNA arrays (“DNA biochips”) designed to detect the most common variations in the *TPMT* gene [22].

Studies of European, African, Chinese, Japanese, and Southwest Asian populations have demonstrated significant ethnic differences in the distribution of *TPMT* alleles [23–31]. Until recently, however, there was no information about the frequency of *TPMT* alleles among populations of the Russian Federation (RF). With the sponsorship of the International Outreach Program of St. Jude Children’s Research Hospital (Memphis, Tennessee), we reported a novel molecular approach for detecting SNPs in the *TPMT* gene and the results of genotyping of 700 individuals in the RF [22]. We also recently reported our comparison of

6-MP toxicity in children with ALL who had variant versus wild-type *TPMT* [32]. Here we describe the most common variant *TPMT* alleles and their frequency distribution in a large cohort of multiracial residents of the RF. We also compared the frequency of variant *TPMT* alleles in groups of children with and without malignancy to determine whether *TPMT* gene abnormality is associated with hematologic malignancy.

Materials and Methods

This study was approved by the institutional review board of the Institute for Pediatric Hematology of Russia (known since 2005 as the Federal Research Center for Pediatric Hematology, Oncology and Immunology of the RF). The study was conducted in accordance with the Helsinki Declaration on human experimentation. Informed consent was obtained from participants or their parents.

Study Population

We collected blood samples from all consenting pediatric patients admitted for treatment to the hematology-oncology, pediatric, and surgery departments of the Russian Children's Clinical Hospital (RCCH), Moscow ($n = 572$); the hematology department of the Morozovskaya Children's Clinical Hospital, Moscow ($n = 127$); the pediatric department of the Oncological Center of Moscow Region, Balashikha ($n = 45$); and the hematology-oncology department of the Regional Children's Clinical Hospital, Chelyabinsk ($n = 18$) between October 2001 and June 2005. We chose the RCCH as the main site for collection of data because it provides treatment to children from all regions of the RF. The cohort comprised 446 children with malignant diseases (329 with ALL, 76 with AML, 35 with NHL, and 6 with solid tumors) and a control group of 549 children and adults without malignant disease. The control group comprised all consenting children with nonmalignant chronic disease ($n = 251$) and acute infectious diseases or trauma ($n=65$), and all consenting adult donors at the RCCH blood bank ($n = 233$). All consenting subjects for whom adequate samples were available were included in analyses. Samples from 22 of 1017 individuals were excluded from analysis due to poor quality; 995 samples were available for analysis. The study subjects represented different ethnic groups and came from different areas of the RF. Wherever possible, we also gathered information regarding ethnicity and place of birth of participants and their parents.

The hematologic malignancies ALL and AML were diagnosed according to standard criteria by cytomorphologic, cytochemical, and immunophenotypic studies of bone marrow cells and by molecular genetic testing. The molecular genetic abnormalities most frequently associated with ALL [t(1;19)E2A/PBX, t(9;22)BCR/ABLp190, t(4;11)MLL/AF4, and t(12;21)TEL/AML1] were detected by microarray methods [33]. The diagnosis of non-Hodgkin lymphoma was based on standard morphologic and immunohistologic analysis of tumor cells in accordance with the WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues [34]. We compared identically treated patients with ALL who did and did not have *TPMT* variant alleles to determine whether disease characteristics (immunophenotype and molecular genetics of tumor cells), survival indices or the frequency of relapse differed between these groups.

Treatment protocols

Patients with ALL received treatment on protocol ALL BFM-90 [35,36] or ALL MB-91/2002. The latter protocol was proposed by investigators of the Research Institute of Pediatric Hematology, Moscow, and the Department of Pediatric Oncology/Hematology, Charite Universitätsmedizin, Berlin, Germany [37]. Event-free survival (EFS) and overall survival (OS) estimates on the two protocols were similar [38]. On both protocols, during

maintenance therapy patients received daily doses of 6MP (50 mg/m² po) and weekly doses of methotrexate (30 mg/m² im for MB 91 and 20 mg/m² po for BFM-90). Dosages were adjusted to keep the WBC count between 2 and 3 × 10⁹/L, in strict adherence to the ALL BFM-90 protocol [35]. The dose of 6MP was not adjusted on the basis of *TPMT* genotype.

DNA extraction

We collected peripheral blood samples (5–7 mL) in tubes containing sodium EDTA from study participants whose WBC count was >2 × 10⁹/L. Samples were assigned a unique accession number in the database and labeled with this unique number. DNA was extracted from leukocytes by using the QIAmp DNA blood kit (Qiagen Inc., Valencia, CA, or Qiagen GmbH, Germany), diluted in distilled water to a concentration of 1.25 ng/μL, and stored at –20°C in polypropylene tubes until use.

TPMT genotyping

A new molecular approach was used to detect 6 common inactivating *TPMT* SNPs (G238C, G292T, G460A, G644A, T681G, and A719G) corresponding to 7 *TPMT*-deficiency alleles (*TPMT**2, *TPMT**3A, *TPMT**3B, *TPMT**3C, *TPMT**3D, *TPMT**7, and *TPMT**8). The *TPMT* biochip was designed and prepared at the Engelhardt Institute of Molecular Biology, Academy of Science, Moscow, Russia [22]. A fluorescently labeled amplified DNA is hybridized with oligonucleotide DNA probes immobilized on gel pads of the biochip. This specially designed *TPMT* biochip can recognize clinically relevant inactivating *TPMT* alleles with a high degree of accuracy and reliability.

Data analysis

All data were entered into an electronic database and analyzed by the χ^2 test. GraphPad Prism version 3.0 (GraphPad Software, San Diego, CA) and Statistica 6.0 for Windows (StatSoft, Tulsa, OK) were used for all calculations. *P* values ≤0.05 were considered statistically significant.

Results

Of the 995 study participants, 55 (5.5%) had *TPMT* variant alleles and 940 (94.5%) had the wild-type genotype *TPMT**1/*1. No differences were detected in the frequency of *TPMT* variant alleles on the basis of sex or race (Table I). Forty-five (4.5%) participants had genotype *TPMT**1/*3A; eight (0.8%) had *TPMT**1/*3C; and two (0.2%) had *TPMT**1/*2. Accordingly, the frequencies of the variant alleles were 2.3% for *TPMT**3A, 0.4% for *TPMT**3C, and 0.1% for *TPMT**2 (Table II). We found no homozygous variant genotypes and no *TPMT**3B, *TPMT**3D, *TPMT**7, or *TPMT**8 alleles. Carriers of variant alleles were significantly more frequent in the malignancy group (33/446, 7.4%) than in the control group (22/549, 4.0%) (*P* = 0.0199). None of the *TPMT* variant alleles was identified in the 6 patients with solid tumors. The percentage distribution of the variant alleles in the malignancy and control groups was similar (Table III). The *3A variant was prevalent (81.8% of variant alleles) in both groups; the variant *3C accounted for 14.6% and the variant *2 for 3.6% of all variant alleles. The *TPMT**2 variant was identified only in 2 children, both with malignant disease.

We identified no significant difference in the frequency of *TPMT* variant alleles among children with AML (5.3%), ALL (7.6%), and NHL (11.4%) (*P* = 0.478). Of 241 patients with ALL for whom molecular analysis of blast cells was conducted, 18 had *TPMT* variant alleles. Blast cells of only 5 of these 18 patients had ALL-associated molecular genetic abnormalities: 4 patients had the t(12;21) and one had the t(9;22). We separately compared selected disease characteristics and prognostic indicators between patients with ALL who

did (23 patients) and did not (249 patients) have variant *TPMT* alleles; the groups did not differ in age, sex, or the morphology or immunophenotype of blast cells. The results of treatment did not differ significantly between patients who did and did not have *TPMT* gene variations: as shown in Fig. 1, the EFS estimate was 90% for patients with variations and 83% for patients without SNPs at a median follow-up of 31.3 months (range, 4–163 months) ($P = 0.562$).

Patients admitted to the RCCH represented a broad cross-section of ethnic groups and geographic areas of the RF. Most (880 of 995) were white (we use the term “white” rather than “Caucasian,” which also refers to a region in the northern Caucasus). People with parents of different races were considered to be of mixed race. The ethnic origin of one or both parents of 36 study participants was unknown (Table I). We observed no significant difference between white and Asian subjects in the frequency of carriers of variant alleles. The *3A variant was observed predominantly in white subjects; among Asians we observed only the *3C variant. In our study population, the *3C variant occurred most often among inhabitants of the southern regions of the RF.

Discussion

TPMT genetic polymorphism has been investigated intensively over the past decade. The frequency and types of variant *TPMT* alleles identified in the populations of different countries are described in Table IV. More than 80% of variant alleles in white populations in the United States and Northern Europe are *TPMT**3A. *TPMT**3C is the main allele (92% of variant alleles) in the Saami population of Norway, and it is the only variant allele found in Japanese, Chinese, Korean, and East African populations [23–31]. However, the frequency of variant *TPMT* alleles has not previously been evaluated in populations of the RF. In 995 inhabitants of different regions of the RF, we identified only 3 inactivating variants. Forty-five study participants (4.6%) had the *TPMT* genotype *1/*3A; 8 (0.8%) had the *1/*3C genotype; and 2 (0.2%) had the *1/*2 genotype. We identified no homozygous genotypes or variant *3B, *3D, *7, or *8 alleles.

The estimated frequency of the wild-type allele and of variant alleles was similar to those observed in the white populations of the U.S. and Eastern Europe (Table IV). Most residents of the RF who carried the *1/*3A genotype were white (Slavic). The genotype *1/*3C was found in one person of an Asian race (Buryat) and in residents of the North Caucasus and Ural regions. We conclude that the overall frequency of carriers of variant *TPMT* genotypes in the RF is 5.53%. Assuming a 2.8% frequency of variant *TPMT* alleles, the Hardy-Weinberg equilibrium predicts that approximately 1 in every 1300 persons in the Russian population is a *TPMT*-variant homozygote.

The difference between the frequencies of carriers of variant alleles in the malignancy group (7.4%) and the control group (4.0%) in this study cohort was statistically significant ($P = 0.0199$). Although these results are not grounds for concluding that there is an association or relationship between malignancy development and the presence of *TPMT* SNPs, they should be the basis for further investigation.

ALL patients with *TPMT* deficiency who are treated with standard doses of thiopurines may experience profound, long-lasting cytopenia, infectious complications, prolonged interruption of maintenance treatment, and (as a result) risk of therapy failure. It has previously been shown that patients in other populations who have inactivating *TPMT* variations can be given a reduced dosage of thiopurines without decreasing the cytostatic effect or reducing the likelihood of survival [16]. We previously found that Russian patients with a heterozygous *TPMT* genotype required a lower weekly dose of 6MP, but the results

of their treatment did not differ from those of patients who did not have *TPMT* SNPs [32]. The present study also found no significant difference in outcome, probably because the thiopurine dose was adjusted according to the WBC count. Most patients with ALL who had *TPMT* variations had characteristics associated with better prognosis; relapse was less frequent in this group, although a statistically significant difference was not shown. Patients treated with 6MP who have repeated episodes of prolonged cytopenia should be assessed for *TPMT* SNPs, and the dosage of thiopurines should be adjusted accordingly.

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Fig. 1.
EFS of ALL patients with (---) and without (____) *TPMT* variant alleles ($p=0.56$).

Table 1

Demographic data and frequency of TPMT variant alleles

Group	N	Sex		Race			
		Male	Female	White	Asian	Ural*	Other# NA¶
Pediatric Cancer Group	446	268	178	413	6	3	21 3
Non-Cancer Group							
Chronic Diseases	251	142	109	196	8	8	8 31
Healthy Individuals	298	188	110	271	8	4	13 2
Children	65	35	30	65	0	0	0 0
Adults	233	153	80	206	8	4	13 2
Total Non-Cancer	549	330	219	467	16	12	21 33
TPMT Variation (%)	55 (5.5)	34 (5.7)	21 (5.3)	52 (5.8)	1(4.6)	0	2 (4.8) 0

* The Ural (or Ugor) minor race comprises people living in the Ural region, which is on the border between Europe and Asia [30];

Mixed races; NA: Not available.

Table II

Distribution of *TPMT* alleles in the investigated groups

Groups	N	Total	Alleles, N (%), (95% CI)			
			*1	*2	*3A	*3C
Malignancies (children)	446	892 (100)	859 (96.3) (95.1–97.5)	2 (0.22) (0–0.5)	27 (3.03) (1.9–4.2)	4 (0.5) (0.01–0.9)
Others (children and adults)	549	1098 (100)	1076 (98.0) (97.2–98.8)	-	18 (1.6) (0.9–2.4)	4 (0.4) (0.01–0.7)

Note: $P = 0.0757$ for all variations (distribution of variations did not differ in the 2 groups).

Table III

Percentage distribution of variant alleles

Groups	N	Variant Alleles N (%)			P value
		*2	*3A	*3C	
Malignancies (children)	33	2 (6.1)	27 (81.8)	4 (12.1)	0.4346
Others (children and adults)	22	-	18 (81.8)	4 (18.2)	

Table IV

TPMT allele frequency in different countries and populations

Country	N	Allele Frequency (%)					Variant Allele(s) carriers (%)	Var/var (%)	Reference
		*1	*2	*3A	*3C	Other			
Great Britain	199	94.7	0.5	4.5	0.3	-	10.1	0.5	24
Germany	1214	91.9	0.25	4.5	0.37	0.2	10.1	0.6	21
Norway									
Saami	194	96.6	-	-	3.1	0.3	6.7	-	28
Whites	66	91.7	-	7.6	0.8	-	16.7	-	
Poland	358	96.8	0.4	2.7	0.1	-	6.4	-	31
Japan	522	98.4	-	-	1.6	-	2.9	0.4	27
Singapore									
Chinese	271	97	-	0.2	2.8	-	5.9	-	30
Malays	217	97.5	-	-	2.3	0.2	5.15	-	
Ghana	217	92.4	-	-	7.6	-	14.7	0.5	24
USA									
Whites	282	94.5	0.5	4.6	0.4	-	9.6	2.1 [#]	15,26
Blacks	248	95.4	0.4	0.8	2.4	1	9.3	-	
Russia	995	97.2	0.1	2.3	0.4	-	5.5	-	Current data

[#] Five of six individuals homozygous for variants in the *TPMT* gene were referred to investigators because they had low *TPMT* activity.