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ARTICLE

Rapid genotyping of common deficient thiopurine *S*-methyltransferase alleles using the DNA-microchip technique

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Thiopurine drugs are metabolized, in part, by *S*-methylation catalyzed by thiopurine *S*-methyltransferase (TPMT). Patients with very low or undetectable TPMT activity are at high risk of severe, potentially fatal hematopoietic toxicity when they are treated with standard doses of thiopurines. As human TPMT activity is controlled by a common genetic polymorphism, it is an excellent candidate for the clinical application of pharmacogenetics. Here, we report a new molecular approach developed to detect point mutations in the *TPMT* gene that cause the loss of TPMT activity. A fluorescently labeled amplified DNA is hybridized with oligonucleotide DNA probes immobilized in gel pads on a biochip. The specially designed *TPMT* biochip can recognize six point mutations in the *TPMT* gene and seven corresponding alleles associated with TPMT deficiency: *TPMT*2*; *TPMT*3A*, *TPMT*3B*, *TPMT*3C*, *TPMT*3D*, *TPMT*7*, and *TPMT*8*. The effectiveness of the protocol was tested by genotyping 58 samples of known genotype. The results showed 100% concordance between the biochip-based approach and the established PCR protocol. The genotyping procedure is fast, reliable and can be used for rapid screening of inactivating mutations in the *TPMT* gene. The study also provides the first data on the frequency of common *TPMT* variant alleles in the Russian population, based on a biochip analysis of 700 samples. *TPMT* gene mutations were identified in 44 subjects; genotype **1/*3A* was most frequent.

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Introduction

Thiopurine *S*-methyltransferase (TPMT, EC 2.1.1.67) is a cytosolic enzyme that catalyzes *S*-methylation of thiopurines such as 6-mercaptopurine (6-MP) and 6-thioguanine.[1,2] These drugs are used in the treatment of acute lymphoblastic leukemia, rheumatoid arthritis and autoimmune hepatitis, as well as in organ transplantation,[3–6] and most of their nucleotide metabolites undergo

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S-methylation by TPMT.[1,6] Approximately one in 300 persons (0.3%) have low or undetectable TPMT enzyme activity, and TPMT activity is intermediate in about 10% of the population.[2] Patients with very low or undetectable TPMT activity are at high risk of severe, potentially fatal hematopoietic toxicity when they are treated with standard doses of thiopurines.[5–6]

Recent studies have established that TPMT enzyme activity is altered by variations in the *TPMT* gene.[5,7–8] *TPMT* resides on chromosome band 6p22.3 and has 10 exons, eight of which encode the 28-kDa TPMT protein.[9] Approximately 20 variant human alleles are associated with decreased TPMT catalytic activity.[8] These variants result from point mutations in the *TPMT* open-reading frame or at intron/exon splice sites. The wild-type allele, *TPMT*1*, encodes active TPMT. *TPMT*2* (238G>C), *TPMT*3A* (460G>A, 719A>G) and *TPMT*3C* (719A>G) are the most prevalent (80–95%) of the polymorphic alleles that cause significantly reduced enzyme activity.[10] *TPMT*3B* (460G>A) is much more rare, whereas the remaining TPMT variants can be considered family-specific ('private') mutations found in individuals of various ethnic groups.[11–14] The TPMT phenotype is determined by measuring erythrocyte TPMT enzyme activity, a procedure that is a standard clinical test in some centers.[2,15] However, erythrocyte TPMT activity assays may be inaccurate if the patient has received, for example, a blood transfusion from a donor with normal TPMT activity within the previous 3 months. Genotyping has been proposed as a more reliable method for detecting TPMT deficiency, as exemplified by recent publication.[16] First, following transfusion of erythrocytes, blood contains predominantly the host's DNA; alternatively, DNA extraction from other tissues (eg buccal epithelium cells) overcomes the problem of erroneous classification of samples.

The *TPMT*-gene variations can be detected by using allele-specific (AS)-PCR, PCR–restriction fragment length polymorphism (PCR-RFLP),[10,17] or denaturing high-performance liquid chromatography.[8] The most common targets for such analyses are well-studied *TPMT* alleles: **2*, **3A*, and **3C*. Because PCR-based genotyping is usually performed as several individual reactions, it is too laborious for use as a standard clinical test.

To standardize *TPMT* genotyping and make the procedure more suitable for clinical testing, we used the gel-based DNA-microchip technological platform.[18] One of our goals was to develop and validate a rapid oligonucleotide microarray-based *TPMT* genotyping method. Another goal arose from the significant interethnic differences in the distribution of *TPMT* alleles worldwide. The very heterogeneous Russian population has not yet been studied for the presence of most clinically significant *TPMT* polymorphic alleles, whereas the clinical relevance of *TPMT* genotype can be assessed only in previously characterized populations. We therefore determined the frequencies of four common clinically significant *TPMT* alleles in the Russian population while validating the *TPMT* biochip by analyzing 700 clinical samples.

Materials and methods

Subjects

Fifty-eight control samples of DNA were obtained from St Jude Children's Research Hospital, Memphis, TN, USA. Seven hundred peripheral blood samples were collected in the Research Institute of Pediatric Hematology (RIPH), Moscow, Russia, from unrelated individuals: 280 patients with lymphoproliferative disorders and 420 healthy volunteers or patients with various gastroenteric diseases. Most subjects were less than 18 years of age. The study had been approved by the Ethics Committee of the Research Institute of Pediatric Hematology (RIPH) in Moscow. Written informed consent was obtained from patients or their parents for the collection and use of samples.

DNA extraction

Genomic DNA was extracted from leukocytes of EDTA-treated peripheral blood by using the QIAmp DNA blood kit (Qiagen, USA), diluted in distilled water to a concentration of 1.25 ng/μl, and stored at -20°C until use.

Target DNA

The target DNA was prepared by two-round nested multiplex PCR. The first round of PCR amplification was performed in 25 μl of multiplex mixture containing 1.5 U *Taq* polymerase (Sileks, Russia), 0.2 mM each of dNTPs, a mixture of primers (10 pmol of each primer), 11 mM Tris-HCl, pH 8.3, 55 mM KCl, 1.5 mM $MgCl_2$, and 1 μl of DNA template. PCR was carried out as follows: 5 min at 95°C; 35 cycles of 30 s at 95°C; 30 s at 62°C; 1 min at 72°C; and extension at 72°C for 5 min. The three pairs of primers (Table 1) in one tube amplified a 265-bp fragment (exon 5), a 371-bp fragment (exon 10) and a 694-bp fragment (exon 7) of the *TPMT* gene. The second round, an asymmetric PCR with fluorescently labeled primers, yielded a fluorescently labeled single-stranded target DNA. This reaction was performed in 25 μl of multiplex mixture containing 1.5 U *Taq* polymerase, 0.2 mM each of dNTPs, a mixture of primers (0.2 pmol of unlabeled forward primer and 10 pmol of labeled reverse primer), 11 mM Tris-HCl, pH 8.3, 55 mM KCl, 1.5 mM $MgCl_2$ and 2 μl of the first-round product. PCR was carried out as follows: 5 min at 95°C, 30 cycles of 30 s at 95°C, 30 s at 63°C, 1 min at 72°C, and extension at 72°C for 5 min. During the second round, the three pairs of primers (Table 1) amplified a 160-bp fragment (exon 5), a 176-bp fragment (exon 10), and a 218-bp fragment (exon 7) of the *TPMT* gene.

DNA oligonucleotide probes

Oligonucleotide probes were designed by using the program Oligo 5 (Molecular Biology Insights, Cascade, CO, USA). Oligonucleotides were synthesized in an ABI TM 394 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA, USA) by the standard phosphoramidite method. All oligonucleotides were synthesized with an amino group at the 3′- end by using 3′-Amino-Modifier C7 CPG (Glen Research, Sterling, VA, USA).

Manufacture of biochips

The gel-based microchips were formed by using photo-induced copolymerization as described.[19] Oligonucleotide probes were immobilized in hemispherical hydrogel pads 150 μm in diameter fixed to the hydrophobic surface of a microscope slide.[18] Briefly, a solution of copolymerization mixture and modified oligonucleotide was transferred to each well of a 384-well microplate (Genetix, New Milton, UK) and spotted by a robot pin (QArray, Genetix, New Milton, UK) on the surface of the slide. The gel arrays were polymerized under UV light for 20 min at 20°C in dry nitrogen, then washed in double-distilled water and dried. For the additional information about analytical capabilities of the method, biochip preparation and potential applications go to the web site www.biochip.ru.

Table 1 Primers used in two-round PCR

Abbreviation	*Sequence 5′ – 3′*	*Conc. (pmol/μl)*
F238(1)	AGTGTAAATGTATGATTTTATGCAGG	10
R238(1)	TAAATAGGAACCATCGGACACAT	10
F460(1)	CAAGCCTTATAGCCTTACACCCAGG	10
R460(1)	AGGCAGCTAGGGAAAAAGAAAGGTG	10
F719(1)	GAGACAGAGTTTCACCATCTTGG	10
R719(1)	CAGGCTTTAGCATAATTTTCAATTCCTC	10
F238(2)	AGTGTAAATGTATGATTTTATGCAGG	10
*R238(2)	Cy3-ACAAGCCTTAAATACTTTGGTTCCAGG	50
F460(2)	CTCTTTCTGGTAGGACAAATATTGGC	10
*R460(2)	Cy3-AAGTCTAAGCTGATTTTCTAGAACCCA	50
F719(2)	GAATCCCTGATGTCATTCTTCATAGT	10
*R719(2)	Cy3-CAATTCCTCAAAAACATGTCAGTGTG	50

Design of the *TPMT* biochip

The *TPMT* biochip contains a set of 12 oligonucleotide probes (Table 2) arrayed in six columns, each of which corresponds to a single-nucleotide substitution in a variable position. Each probe is presented in duplicate (eg, rows A and A′ and B and B′ contain identical sets of probes) (Figure 1). The biochip can simultaneously detect six *TPMT* mutations that lead to the loss of TPMT catalytic activity (G238C, G292T, G460A, G644A, T681G and A719G) and indicate the seven corresponding mutant alleles *TPMT*2*, *TPMT*3A*, *TPMT*3B*, *TPMT*3C*, *TMPT*3D*, *TPMT*7*, and *TPMT*8*.

Hybridization

Hybridization mixture was prepared by adding 17 μl of the second-round PCR amplificate to 10 μl of formamide and 13 μl of 20 × SSPE (0.2 M phosphate buffer, pH 7.4, 2.98 M NaCl, 0.02 M EDTA). The total volume of 40 μl usually containing 1–3 μg of DNA was completely denatured at 94°C before hybridization, briefly cooled on ice, then applied to a microchip in a hybridization chamber (Cover Well™, Bioworld, Dublin, USA) and left overnight at 37°C. The microchip was washed for 20 min with 40 ml of 1 × SSPE at room temperature and dried.

Image analysis

Fluorescence was measured with a portable chip analyzer equipped with CCD camera.[20] The dedicated software Imageware v1.1 developed at the Engelhardt Institute of Molecular Biology was used for data processing and image analysis.

	1	2	3	4	5	6
A	G238	G292	G460	G644	T681	A719
A′	G238	G292	G460	G644	T681	A719
B	238C	292T	460A	644A	681G	719G
B′	238C	292T	460A	644A	681G	719G

Figure 1 Scheme of biochip. Each column from one to six represents a set of probes for one variable position. Rows A and A′ contain the wild-type sequences; B and B′ contain the mutant sequences. Rows A and A′ and rows B and B′ are identical.

Table 2 Oligonucleotide probe sequences (+ strand) immobilized on the *TPMT* biochip

Exon	*SNP*	*Allele*	*Wild-type probe 5′-3′*	*Mutation probe 5′-3′*
5	G238C	*2	gcaggttt**g**cagaccgg	gcaggttt**c**cagaccgg
4	G292T	*3D	gggatacaa**g**aatttttac	gggatacaa**t**aatttttac
7	G460A	*3B, *3A, *3D	gatagagga**g**cattagttgc	gatagagga**a**cattagttgc
10	G644T	*8	tgcaatatac**g**ttgtcttgag	tgcaatatac**t**ttgtcttgag
10	T681G	*7	agaacgaca**t**aaaagttgg	gaacgaca**g**aaaagttgg
10	A719G	*3C, *3A, *3D	gaaaagttat**a**tctacttacag	gaaaagttat**g**tctacttaca

Bold characters mark the variable nucleotides.

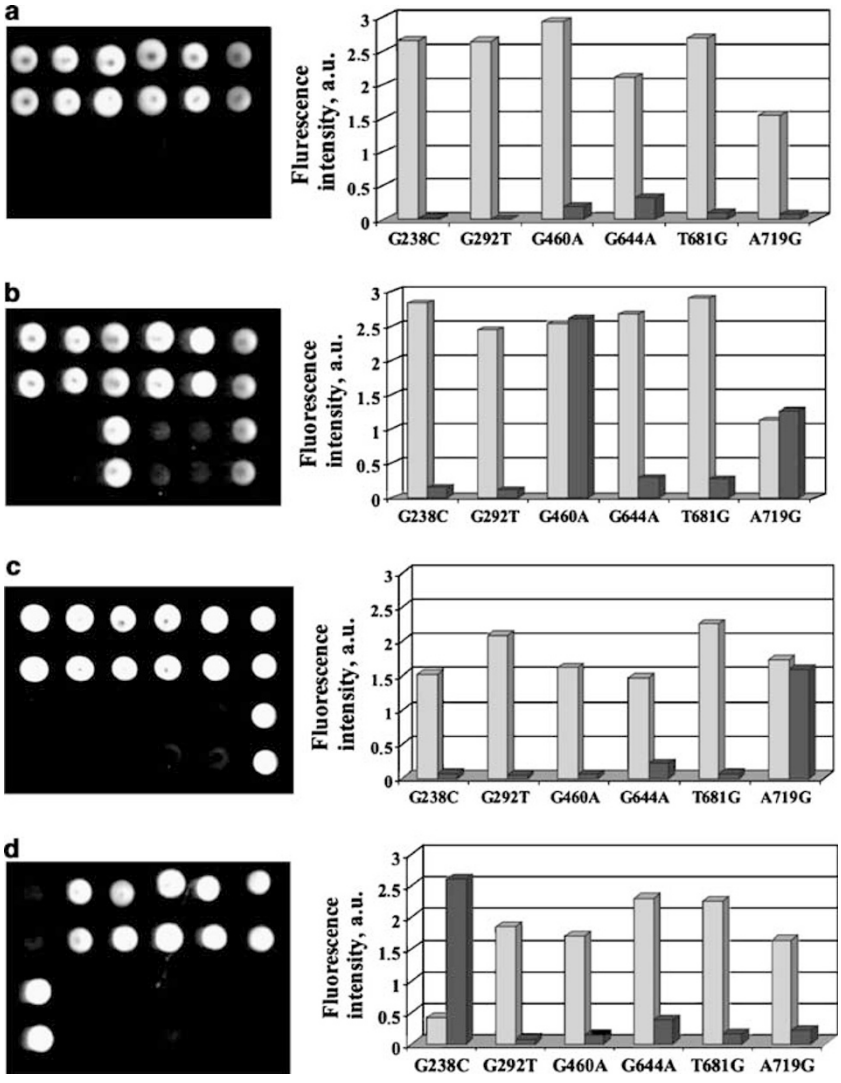

Figure 2 Biochip *TPMT* analysis of patients with different genotypes. Hybridization patterns (on the left) and histograms (on the right): (**a**) **1/*1*, (**b**) **1/*3A*, (**c**) **1/*3C*, and (D) **2/*2*. The bright spots in the hybridization patterns correspond to outstanding peaks on the histograms. The signals from identical probes (rows A–A′ and B–B′) were averaged in the histograms.

Data analysis and genotyping

The method of analysis is based on the difference in signal intensity between perfect and imperfect duplexes formed by fluorescently labeled DNA sample and the probes immobilized in gel pads. The brightest fluorescent signal corresponded to the perfectly matched duplex formed by oligonucleotide and hybridized DNA target. Oligonucleotides in the upper gel pads in each column (A1–A6) match the wild-type sequence, that is, they form perfect duplexes with the wild-type target DNA. Oligonucleotides in the B1–B6 rows form perfect duplexes with mutant alleles of *TPMT*. Figure 2 shows examples of hybridization patterns for different genotypes. Genotype assignment can be performed visually or by using the automated image analysis. The automated program compares the fluorescence intensity of the gel pads that contain wild-type sequence-specific probes to that of the pads that contain mutant sequence-specific probes. An example of the dialog window is shown in Figure 3.

The input data for the analysis were obtained from measurements of fluorescence intensity $J_m = (I_m - I_0)/(B_m - I_0)$. Here, I_m was the intensity per unit area in the internal region of a pad, B was the counterpart background intensity, I_0 was leakage current of registering device, and m enumerated the pads on the chip ($m = 1, \ldots, 24$). The signal intensities of the duplicate probes were compared, and the samples in which the duplicates differed more than 20% were reanalyzed. The J_m values were sorted and the reference gel pad was defined as the one that preceded a sharp increase in signal. The threshold values were

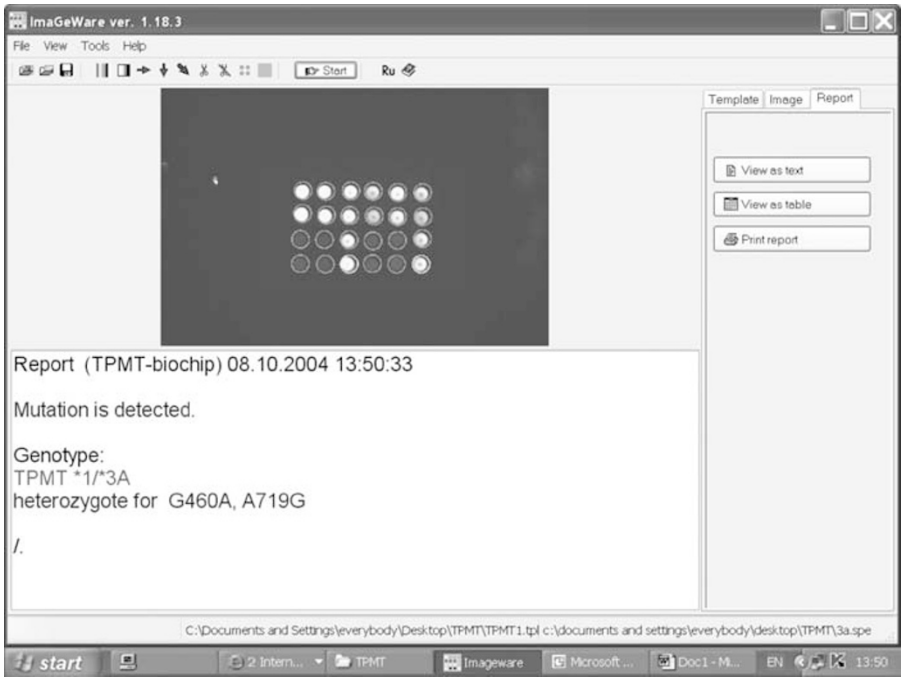

Figure 3 Example of automated analysis using Imageware™ software. The data were processed by using the algorithm described in the text, and the genotypes were assigned by automated image analysis.

Table 3 Comparative results of *TPMT* genotyping by PCR analysis and *TPMT* biochip technology

Genotype	*Allele*	*SNP*	*Method*			*Concordance*
			PCR	*Biochip first operator*	*Biochip second operator*	
Wild-type	*1/*1	—	43	43	43	100
Heterozygote	*1/*2	G238C	1	1	1	100
	*1/*3A	G460A, A719G	12	12	12	100
	*1/*3C	G460A	2	2	2	100

established statistically for each pair 'perfect probe–mismatch probe' after approximately 50 hybridization patterns had been processed. A gel pad was considered to have a positive signal if its fluorescence intensity exceeded the value of the reference pad signal intensity × threshold value $J_p \geq J_m{*}Trh_{gr}$, where Thr_{gr} was the threshold value for each pair 'perfect probe–mismatch probe'. If the signals in both pads (with normal and mutant probes) exceeded the threshold and the signals differed by a factor less than two, both signals were considered positive and the heterozygous genotype was assigned. If none of the signals in the column was positive, PCR amplification of the corresponding exon was assumed to have failed, and the sample was re-amplified. The main cause of PCR failure was the poor quality of isolated DNA, and in these cases the patient's DNA was re-isolated.

Results

TPMT genotyping of control samples

In blind experiments, we tested 58 DNA samples of known genotypes to assess the reliability and accuracy of the genotyping protocol. Reproducibility of the method was evaluated by two different operators performing the *TPMT*-biochip test in two independent series of experiments. All samples showed full agreement between the biochip-based approach and the PCR method (Table 3). The operator-to-operator reproducibility using control samples was 100%. The 95% confidence interval of the probability that the two methods disagree is (0–0.057), on the basis of the binomial distribution.

Population screening

Seven hundred blood samples were collected as part of a project to characterize *TPMT* genetic polymorphisms in the

Russian Federation: 280 from patients with acute lymphoblastic leukemia, acute myeloid leukemia, or non-Hodgkin's lymphoma and 420 from healthy donors or patients with gastroenteric diseases. The patients were from different parts of Russia, and most of them were of the Eastern European ethnic group. Six inactivating mutations of the *TPMT* gene (G238C, G292T, G460A, G644A, T681G, A719G) were identified by using *TPMT* biochips. The 700 samples were analyzed by three operators. The first 400 samples were divided equally between two operators who performed the *TPMT*-biochip analyses independently. Among these 400 samples, the program failed to determine genotype and asked to repeat the analysis in 16 cases. For 11 of these 16 cases, the DNA was re-isolated from the frozen cells. The repeated analysis was then performed successfully. All samples with mutant genotypes determined by one operator were blindly analyzed by another operator together with several samples of wild-type genotype. Seventy samples required such crossgenotyping, and discrepancy between two operators was found in one case. After additional examination, the correct genotype was determined. Thus, the operator-to-operator reproducibility in population screening was about 99%.

The last 300 samples were analyzed by the third operator. In nine of these 300 samples, the program failed to determine the genotype the first time and asked to repeat the analysis. In seven of these nine cases, the DNA was re-isolated from the frozen cells. After the genotyping was complete, a total of 55 samples (all samples with mutant genotypes together with a number of wild-type samples) had been analyzed repeatedly by the same operator. All previously defined genotypes were confirmed in this repeat testing. Thus, the repeatability of the analysis can be considered to be 100%.

As a result of testing, 37 individuals (5.3%) were found to have genotype **1/*3A*, five (0.7%) had **1/*3C* and two (0.3%) had **1/*2*. The remaining 656 individuals (93.7%) had the wild-type genotype **1/*1* (Table 4). No homozygous **2*, **3A*, or **3C* genotypes were found, and no mutant genotypes **3B*, **3D*, **7*, or **8* were found. The estimated allelic frequency was 96.8% (95% CI, 95.8–97.7) for wild-type allele *TPMT*1* (1356/1400) and 3.2% (95% CI, 2.3–4.2) for all variant alleles (44/1400). The frequency of specific variant alleles was 2.6% for *TPMT*3A* (37/1400), 0.36% for *TPMT *3C* (4/1400), and 0.14% for *TPMT *2* (2/1400) (see also Table 5).

Table 4 Genotype frequencies in the Russian population

TPMT genotype	*Number*	*Genotype frequency (%) (95% CI)*
**1/*1*	656	93.7 (91.7–95.4)
**1/*3A*	37	5.3 (3.7–7.2)
**1/*3C*	5	0.7 (0.2–1.6)
**1/*2*	2	0.3 (0–1.0)

Discussion

We have established a rapid DNA-microchip genotyping method that identifies clinically relevant inactivating *TPMT* alleles with high accuracy and reliability.

Determination of TPMT activity before the administration of thiopurine drugs is currently advocated, and recent studies have defined starting doses for 6-MP and its analogs on the basis of *TPMT* genotype.[5] Although TPMT deficiency can be diagnosed by assays of erythrocyte TPMT activity, different factors, including patient age, renal function, receiving of RBC transfusion and thiopurine administration, may alter these results.[15,16,21] Molecular genetic (PCR-based) methods are not affected by extraneous factors and offer a promising alternative to measurement of RBC TPMT activity. PCR-based methods require less than 1 μg of DNA, which is the amount contained in approximately 100 μl of whole blood. However, because each variant position of the *TPMT* gene is analyzed by using an individual pair of primers in AS-PCR, which is followed by additional enzyme treatment in the PCR-RFLP method, analysis for all significant TPMT polymorphisms requires many individual PCR reactions and is time- and resource-intensive.

The real-time PCR assay for the detection of *TPMT*2*,**3*, **4*, **5*, and **6* has been described.[22] However, the reagent and equipment costs for real-time PCR still limit its introduction into standard clinical laboratories. The pyrosequencing method has been proposed recently for genotyping of 10 single-nucleotide polymorphisms for *TPMT*1S*, **2*, **3A*, **3B*, **3C*, **3D*, **4*, **5*, **6*, **7*, and **8*.[23] Pyrosequencing potentially has a wide use in the large-scale identification of individual genotypes, but the cost of equipment will confine its application to large research centers.

The DNA-microchip technique described here helps to overcome certain drawbacks of PCR-based genotyping techniques. This approach for TPMT genotyping includes one multiplex PCR for all six mutations in one tube, followed by multiplex hybridization on a biochip. Taking only 1 μl of patient DNA solution, it is possible to identify *TPMT* genotype. Even in real-time assay, proposed by Schütz *et al.*,[24] four capillaries per one patient were used. So, our procedure is obviously patient material saving. The hybridization was carried out overnight, because it was convenient in the parallel analysis of many samples. However, principally, the time of hybridization can be reduced up to 3 h without worsening of discrimination ratio between perfect and mismatch duplexes. More noticeably, the time-saving aspect of biochip-based analysis is revealed in high-throughput genotyping. Conventional PCR methods usually require post-PCR analysis, like restriction and electrophoresis or sequencing. In real-time PCR assay, the reaction had a run time of about 1 h, but the genotype assignment was carried out after manual estimation of melting curves.[22] The biochip analysis using

Table 5 Variant *TPMT* allele frequencies in the Russian Federation compared with other populations

Population	*Total number of alleles*	*Allele frequency in % (95% CI)*			*Reference*
		*TPMT*3A*	*TPMT*3C*	*TPMT*2*	
Russian Federation	1400	2.6 (1.9–3.6)	0.36 (0.11–0.83)	0.14 (0–0.5)	Present study
Polish	716	2.7 (1.5–3.9)	0.1 (0–0.3)	0.4 (0–0.9)	Kurzawski *et al*[25]
British	398	4.5 (2.4–6.6)	0.3 (0–1.9)	0.4 (0–1.2)	McLeod *et al*[26]
American white	564	3.2 (1.7–4.6)	0.17 (0–0.5)	0.17 (0–0.5)	Hon *et al*[14]
African American	496	0.8 (0–1.6)	2.4 (1.1–3.8)	0.4 (0–1.0)	Hon *et al*[14]
Saami	388	0	3.3 (1.5–5.1)	0	Loennechen *et al*[27]
Kenyan	202	0	5.4 (2.3–8.5)	0	McLeod *et al*[26]
Ghanaian	434	0	7.6 (5.0–10.0)	0	Ameyaw *et al*[28]
Taiwanese	498	0	0.6 (0–1.3)	0	Chang *et al*[29]
Chinese	384	0	2.3 (0.8–3.8)	0	Collie-Duguid *et al*[30]

portable biochip analyzer takes about 1 min per one biochip, including fully automatic image analysis and genotype assignment. Tens of samples can be analyzed simultaneously in our assay under highly standardized conditions.

In three-dimensional gel pads of microchips, as compared to two-dimensional spots, the local concentration of each oligonucleotide probe is higher because of the greater immobilization capacity of a gel.[18] This leads to an increased fluorescence signal; as a result, the hybridization patterns can be visualized by using not only high-resolution devices but also a portable chip-reader.[20] The simplicity of the analysis and the relatively low cost of equipment offer some advantages over real-time PCR and pyrosequencing. The prime cost of small diagnostic biochips manufactured by gel-based biochip technology is around $1–2. The quantity of each fluorescent primer used in one reaction is 10 pmol, so if they are supplied in a commercial kit, the price for one genotyping for all six mutations would be reasonable and undoubtedly available for small clinical laboratories.

The recognition of mutations occurs during allelic-specific hybridization of target DNA with complementary oligonucleotide probes. Three-dimensional biochips allow performing the analysis of melting curves, as well as hybridization kinetics analysis at chosen temperature.[24] Usually such analysis is used as a preliminary step to optimize the hybridization conditions for a definite set of probes to provide the best discrimination ratio between perfect and mismatch duplexes. It is considered that the correct recognition of positive spots using microarrays is possible when the fluorescence signal exceeds more than 1.5 to two-fold the background signal. As we can see on diagrams (Figure 2), the discrimination ratio between positive and negative spots for each pair of wild-type mutation probes is ten folds or more. Thus, the conditions of hybridization were optimized to provide the highest reliability of the analysis. The accuracy and reliability of the method was estimated as 100%: we observed complete agreement between the results obtained with biochips and with standard methods analyzing 58 control samples. It was also proved in cross-analyzing of clinical samples, where the operator-to-operator reproducibility and repeatability of the analysis was approximately 99%.

Many (potentially all) pharmacologically important polymorphisms can be analyzed simultaneously by the DNA-microchip technique. The addition of new mutations does not significantly increase the cost of analysis. The reason we did not include all known variants in our assay was that most of these variations are unique and some are of a completely different ethnic origin. We found no individuals who had the rare **3D*, **7* or **8* alleles included in the assay. Previous analysis of TPMT genotype–phenotype relationship showed that the overall concordance rates of different population studies vary between 76 and 89%. At the same time, it was found that testing for very rare mutant alleles does not significantly enhance the reliability of *TPMT* genotyping for intermediate methylators.[8] More important could be the search for new mutations, which will be stimulated by introduction of the *TPMT*-biochip test. Retrospective analysis of clinical intolerance of 6-MP in patients with leukemias, together with *TPMT* genotyping of common deficient alleles, will help us to identify individuals who have none of these polymorphisms but are poor 6-MP metabolizers and who therefore may be carriers of new mutations.

The *TPMT*3B* allele was not detected among 1400 studied alleles and therefore is rare, if present at all, in the Russian population. For that reason, it is unlikely that any of the 37 subjects genotyped as **1/*3A* were actually **3B/*3C* compound heterozygotes; in our study, we assumed that subjects heterozygous for both the G460A and A719G mutations had genotype **1/*3A*.

The frequency of *TPMT* variant alleles has been reported in a variety of ethnic groups[25–30] (see Table 5). We found 6.3% of the Russian Federation study group to have *TPMT*-deficient genotypes, and the total frequency of deficiency alleles was 3.2%. *TPMT*3A* was the prevalent mutant allele

in Russia, as in European and white American populations, and composed 86% of all variant alleles, but the population frequency of *TPMT*3A* in Russia was noticeably lower (2.6%) than in the British population (4.5%)[25] or among white Americans (3.2%).[13] The frequency seems to be similar to that in other Slavic populations, such as the Polish (2.7%).[25]

These results show the feasibility of patient screening for the most prevalent TPMT-deficiency alleles by new biochip technology so that those who require a substantial reduction in thiopurine dosage can be identified.

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