

## Commentary

### ***TPMT* gene polymorphisms: On the doorstep of personalized medicine**

Since the initial human genome sequence has become available in 2001, sequence variability among individual genomes came into focus of the research in the field of human genetics. More than 99 per cent of the DNA sequence is identical among individuals. The remaining DNA is responsible for genetic diversity<sup>1</sup>. Polymorphisms are common genetic variations in the human genome representing sequence variations that occur in >1 per cent of gene alleles in a given population. The most studied polymorphisms, SNPs (single nucleotide polymorphisms) were discovered thirty years ago. These are distributed over the whole genome. The number of SNPs is estimated to range from 0.5 to 1 SNP per 100 base pairs (bp). Besides SNPs, there are other important classes of polymorphisms, such as VNTRs (variable number of tandem repeats, polymorphic sequence containing 20-50 copies of 6-100 bp repeats), STRs (short tandem repeats, a subclass of VNTR in which repeat unit consists of 2-7 bp) and copy number polymorphisms.

Despite extensive studies of polymorphisms, among a myriad of “promising” genetic markers, only a few have been shown to be valid ones. There is no doubt that the thiopurine S-methyltransferase (*TPMT*) gene polymorphism is one of them. Moreover it is already successfully applied at the bedside as a powerful pharmacogenetic marker.

*TPMT* gene polymorphisms are pharmacogenetic markers which enable the individualization of thiopurine drug therapy. Thiopurine drugs [6-mercaptopurine, (6-MP), thioguanine (TG) and azathioprine] are widely used in the treatment of many diseases, such as acute leukaemia, different types of inflammatory and autoimmune diseases and in transplantation medicine. Thiopurine S-methyltransferase (*TPMT*) is a cytosolic enzyme which catalyzes the S-methylation, and consequent partial inactivation of thiopurine

drugs<sup>2,3</sup>. Some patients treated with standard doses of thiopurine drugs accumulate high levels of TG nucleotides, usually leading to severe haematopoietic toxicity<sup>4</sup>. Mostly it is a consequence of inherited *TPMT* deficiency. Approximately 90 per cent of individuals inherit both functional *TPMT* alleles resulting in high *TPMT* activity. Intermediate *TPMT* activity is seen in carriers of one nonfunctional, polymorphism affected, *TPMT* allele, representing 10 per cent of population. Low or undetectable *TPMT* activity is reported in 0.3 per cent individuals who inherit two nonfunctional *TPMT* alleles<sup>5</sup>. Patients with low *TPMT* activity are at high risk of severe, eventually fatal, haematologic toxicity. Consequently, thiopurine drug dose reduction is necessary. Patients with intermediate *TPMT* activity also require dose reduction to avoid toxicity<sup>6</sup>. Therefore, the level of *TPMT* enzyme activity is essential for balance of therapeutic and toxic effects of thiopurine drug dose.

*TPMT* gene exhibits significant genetic polymorphism. At present, a total of 25 *TPMT* genetic polymorphisms, mostly SNPs, have been identified<sup>7</sup>. *TPMT* SNPs are, or may be associated with decreased levels of *TPMT* enzyme activity and thiopurine drug-induced toxicity. Among these, the most common are: c.238G>C, c.460G>A and c.719A>G. There are several *TPMT* variant alleles comprising one or more SNPs. On the basis of population studies, three alleles account for more than 95 per cent of the clinically relevant *TPMT* variants: *TPMT*\*3A, *TPMT*\*3C and *TPMT*\*2, with the last of them contributing to a lesser extent<sup>8</sup>. Wild type has been designated as *TPMT*\*1. *TPMT*\*2 allele contains single c.238G>C polymorphism, *TPMT*\*3A allele has two polymorphisms c.460G>A and c.719A>G, while *TPMT*\*3C has only c.719A>G polymorphism.

It is important to emphasize that the distribution of clinically relevant alleles is population specific<sup>9-12</sup>.

*TPMT*\*3A allele is the most common variant allele in Caucasians (frequency approximately 5%), while *TPMT*\*3C is predominant in subjects with Asian or African ancestry (frequencies of 0.3-3% and 5.5-7.6% respectively).

Since inherited decrease of *TPMT* activity results in potentially life-threatening clinical consequences for patients treated with thiopurine drugs, a need for measurement of *TPMT* enzyme activity emerged. *TPMT* genotyping is highly sensitive and specific alternative to expensive *TPMT* enzyme activity determination. More than 98 per cent concordance exists between *TPMT* genotype and phenotype. Therefore, *TPMT* genotyping is a reliable method for guiding thiopurine therapy. It is of great importance that *TPMT* genotyping becomes a part of standard diagnostic protocols for diseases treated with thiopurine drugs. Prior to that, it was necessary to determine which *TPMT* polymorphisms should be tested in a certain population. In this issue, an application of a new technique (SNaPshot) for analysis of *TPMT* gene polymorphisms is presented<sup>13</sup>. Kapoor and colleagues have developed a new approach (SNaPshot technique) for detection of three most common *TPMT* polymorphisms (c.238G>C, c.460G>A and c.719A>G) and applied it in Indian population.

Widely used methods for *TPMT* genotyping are ARMS (amplification refractory mutation system) and PCR-RFLP (polymerase chain reaction- restriction fragment length polymorphism)<sup>14</sup>. Kapoor *et al*<sup>13</sup> chose the SNaPshot method, a non-time consuming, half-automated method which enables testing of *TPMT* SNPs in a multiplex reaction. The method is based on a dideoxy single-base (ddNTP) extension of primers complementary to the sequences of three most common *TPMT* polymorphisms. The addition of one of four ddNTPs labeled with different fluorescent dyes at the position of the SNP is followed by electrophoresis and analysis of data.

The authors have designed a similar method in which cDNA is used as a template. Thiopurines are used in all phases of current therapy for childhood acute lymphoblastic leukaemia (ALL)<sup>15, 16</sup> and *TPMT* genotyping is necessary for appropriate adjustment of thiopurine drug doses. Since detection of several chimeric transcripts is a standard diagnostic procedure in childhood ALL, cDNA of each patient is available. Therefore, SNaPshot method for *TPMT* genotyping

using cDNA template is especially interesting and useful.

The authors suggest that the main advantage of the SNaPshot approach is its up-scalability, as a high throughput platform for *TPMT* SNPs analysis with possible automation. An actual contribution of this specific diagnostic approach will be tested and confirmed in clinical laboratory practice, especially its cost-effectiveness. The new methodology was used to determine the frequency of three most common *TPMT* polymorphisms in the population of India. The overall frequency of *TPMT* polymorphisms was 4.9 per cent<sup>13</sup>. The most common variant allele was *TPMT*\*3C reaching the frequency of 4.1 per cent. Kham *et al*<sup>17</sup> have previously reported the frequency of 0.8 per cent for *TPMT*\*3C among Indian migrant population in Singapore. However, this study is the first one reporting *TPMT* genotypes among resident (non-migrant) Indians<sup>13</sup>. Since *TPMT* genotyping is recommended in clinical routine before administrating thiopurine therapy, the application of SNaPshot technique for analysis of *TPMT* gene polymorphisms represents an important progress towards introducing a rapid method of *TPMT* polymorphism detection in well equipped laboratories.

*TPMT* pharmacogenetics has been studied extensively because of its clinical significance. Many patients got benefited from the knowledge of *TPMT* genotype-phenotype correlation. However, further research is needed to elucidate the influence of *TPMT* polymorphism on drug metabolism (pharmacokinetics) and drug targets (pharmacodynamics)<sup>18</sup>. Characterization of new *TPMT* polymorphisms and their effect on the level of enzyme activity will be a subject of future studies.

*TPMT* pharmacogenetics represents a great promise that personalized medicine, a dream of scientists and medical practitioners, will enter everyday medical practice in the near future.

#### Acknowledgment

SP was supported by grant 143 051 from the Ministry of Science and Technological Development, Serbia.

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