Pretreatment determination of TPMT – state of the art in clinical practice

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In this issue of the *European Journal of Clinical Pharmacology*, the impact of thiopurine S-methyltransferase (TPMT) polymorphisms on thiopurine use in clinical practice is presented.

Xin et al. investigated the relationship between TPMT phenotype and genotype (*TPMT**2, *3A, *3B, and *3C) and adverse drug reactions (ADR) in 150 Chinese kidney transplant patients under azathioprine treatment [1]. Twenty-four patients showed intermediate TPMT activity, and 126 had a normal or high activity. TPMT deficiency was not observed. Genetically, seven patients were heterozygous carriers for the *TPMT**3C allele (allele frequency of 2.3%), the most frequent allele in Asians. Other *TPMT* variants (*TPMT**2, *3A, *3B) were not detected. Twelve patients experienced hematological ADR and another 18 patients experienced hepatological ADR followed by azathioprine treatment cessation or dose reductions. Generally, patients with hematological ADR, but not those with hepatological ADR, had a significantly lower TPMT activity than patients without ADR ($P<0.05$). Four of the seven patients with heterozygosity developed ADR, two of them hematological and another two with hepatic

side effects. The authors concluded that patients with a heterozygous genotype are at risk for developing hematotoxicity under treatment with azathioprine using standard dosage.

Efrati et al. determined the *TPMT* allelic variants *TPMT**2, *3A, *3B, and *3C in 881 unrelated healthy Israeli volunteers of Jewish ($n=531$), Moslem ($n=194$) or Druze ($n=156$) descent [2]. The frequency of *TPMT* variants differed significantly among the three populations, with the highest frequency of heterozygous/homozygous variants in subjects of Druze origin (7.1%), and the lowest frequency in those of Jewish origin (1.5%). In Jews, only *TPMT**3A was found (allele frequency 0.73%), whereas in Druze *TPMT**3A (3.19%) and *TPMT**3C (0.75%), and in Moslems *TPMT**3C (1.05%) and *TPMT**3A (0.79%) were determined. *TPMT**2 and *3B alleles were not detected. The authors concluded that the Israeli population is composed of three major diverse subpopulations, which may be important for genetic testing.

TPMT serves as a model for pharmacogenetic research. Large inherited variations in TPMT activity with a trimodal frequency distribution were described as early as 1980 by Weinshilboum et al. [3]. In a large population of more than 1,200 Caucasians, very low TPMT activity was determined in 0.6%, and about 10 and 90% were found to be intermediate and normal/high methylators, respectively [4]. The most common variant alleles showing decreased activity are *TPMT**3A (4.5%) and *TPMT**3C (0.4%); all other mutations are rare (*TPMT**2 0.17%) or have been found only in single cases [4, 5].

In other ethnic groups, the frequency distribution differs significantly from that in Caucasians as summarized recently [6]. For instance, in subjects with African or Asian ancestry, *TPMT**3C is the predominant allele with frequencies of 5.4–7.6% and 0.3–3%, respectively [6, 7]. Most

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recently, Schaeffeler et al. investigated 24 functionally relevant *TPMT* alleles in 586 Germans, 116 Ghanians, and 118 Koreans by MALDI-TOF mass spectrometry [8]. As expected, in the Ghanian and Korean populations, *TPMT*3C* was the most frequent variant allele. However, eight Ghanians were found to be heterozygous carriers for *TPMT*8* (allele frequency 3.4%), and three Koreans showed heterozygosity for *TPMT*6* (allele frequency 1.3%). Both alleles appeared to be absent in Caucasians of German origin. This study demonstrates that a genotyping strategy can be recommended to replace measurement of *TPMT* activity only if either a complete genetic analysis of all currently known functionally relevant *TPMT* alleles is conducted or if the selection of all frequently known alleles in a certain ethnic population is performed.

Based on this information, it is of interest that the phenotype-genotype correlation in the work by Xin et al. is rather poor. Twenty-four patients with renal transplantation had an intermediate *TPMT* activity, but only seven patients had a heterozygous genotype. Thus, 17 patients with intermediate activity had a *TPMT* wild-type. The authors explained this discrepancy by concomitant drug treatment, red blood cell age, or blood transfusion, but as another important explanation, heterozygous carriers for *TPMT*6* could not be excluded. The same holds true for the paper by Efrati et al. [2]. Definite conclusions on the frequency distribution of *TPMT* activity or *TPMT* genotype cannot be drawn for populations of Middle Eastern origin until comprehensive phenotype-genotype correlations are studied.

A clinically important question is how predictive testing for *TPMT* may be to avoid dose-related ADR under treatment with thiopurines. In the present paper by Xin et al., a combination of azathioprine, prednisone, and cyclosporine A was used for immunosuppression of patients after renal transplantation [1]. Nine of the twelve patients with leucopenia, thrombopenia, or anemia showed intermediate *TPMT* activity, i.e., 75% of the hematologic ADR could be explained by *TPMT*. Severe myelotoxicity did not occur, which fits well with the absence of *TPMT* deficiency in the study population by Xin et al.

Patients with *TPMT* deficiency treated with standard doses of thiopurines are at approximately 100% at risk of developing severe and sometimes even fatal myelosuppression [9–12]. Because azathioprine and 6-mercaptopurine are metabolized by *TPMT* in hematopoietic cells, patients with *TPMT* deficiency accumulate excessive concentrations of the active compounds of azathioprine and 6-mercaptopurine. Pretreatment determination and pharmacogenetically guided thiopurine therapy have therefore been suggested for different diseases such as acute lymphoblastic leukemia (ALL) [13] and inflammatory bowel diseases (IBD) [14, 15] and should be performed routinely. An initial dose reduction to 10–15% of the standard thiopurine

dose is a reliable approach for treatment of *TPMT*-deficient patients [16, 17]. Nevertheless, it is important to note that leucopenia or myelosuppression can also be caused by other factors such as viral infections or drug-drug interactions (e.g., allopurinol) [18].

Hepatotoxicity was not associated with decreased *TPMT* activity in the manuscript by Xin et al. [1]. The mean prevalence of thiopurine-induced liver injury was 3.4% in a recently published review [19]. Dubinsky et al. postulated for the first time that methylation capacity via *TPMT* leading to preferential 6-methyl-mercaptopurine (6-MMP) levels is associated with hepatotoxicity [20]. Children and adolescents with IBD and 6-MMP levels above 5,700 pmol/ 8×10^8 RBC had a threefold increased risk of hepatotoxicity compared to those with lower levels. Later, in a dose-escalation approach in 6-MP nonresponders, the same investigators showed that higher doses of 6-MP led to preferential production of 6-MMP in a subset of patients, resulting in hepatotoxicity without initiating a therapeutic response [21]. The relationship was confirmed in children with ALL who were treated with 6-MP, revealing a significant correlation between elevated 6-MMP concentrations and aminotransferase levels [22]. Accordingly, Gardiner et al. recently presented three patients in whom thiopurine dose escalation based on clinical status and low to normal 6-thioguanine nucleotide levels resulted in severe hepatotoxicity, including liver failure in two cases, associated with very high 6-MMP levels of 26,000–40,000 pmol/ 8×10^8 RBC [23].

In conclusion, pretreatment *TPMT* determination is reasonable in clinical routine before commencing thiopurine therapy with subsequent pharmacogenetically guided dosing and is recommended to avoid hematotoxicity in patients treated with thiopurines. Genotyping is an attractive and highly sensitive and specific alternative to replace measurement of *TPMT* activity. Because the prevalence of *TPMT* alleles is ethnically different and ethnic heterogeneity is increasing, particularly in industrial countries, systematic genetic analysis of all currently known functionally relevant *TPMT* alleles is necessary. Since nongenetic aspects clearly predispose patients to ADR independent of *TPMT*, repeated laboratory controls have to be performed during thiopurine therapy.

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