Multiple Thiopurine S-Methyltransferase Variation Detection: A Step toward Personalized Medicine

Genetic polymorphisms play an important role in the variability of drug responses among individuals (1). For thiopurine medications, the enzymatic activity of thiopurine S-methyltransferase (TPMT)\(^1\) inactivates thiopurine drugs and is influenced by polymorphisms within the TPMT\(^2\) gene (2). Currently, more than 20 single-nucleotide polymorphisms (SNPs) have been associated with decreased TPMT activity (2). Patients inheriting 2 nonfunctional alleles have undetectable TPMT activity, and standard doses of thiopurines may lead to life-threatening drug toxicities in such patients. To avoid toxicity, investigators have recommended TPMT genotyping to individually tailor the starting drug dosage (2).

In this issue of Clinical Chemistry, Schaeffeler et al. describe the development of a high-throughput genotyping method that can simultaneously detect 22 TPMT polymorphisms affecting TPMT activity (3). Their method consists of 16-plex and 7-plex SNP assays with single-base primer extension and MALDI-TOF mass spectrometric analysis to achieve a high level of multiplexing. All genotypes were correctly detected in control DNA samples and with synthetic templates of known genotypes. In addition, 586 clinical samples were genotyped, and the results were fully concordant with those obtained with denaturing HPLC (3, 4).

The comprehensive genotyping assay of Schaeffeler et al. provides a powerful tool for enhancing TPMT genotype prediction. Although many studies have shown a high correlation between TPMT genotype and phenotype, such concordance has not yet reached 100%, and the degree of correlation varies among different studies (4). These prior results have raised the question of whether TPMT phenotyping or genotyping provides better data for clinically adjusting thiopurine dosage (5). In fact, only the most polymorphic SNPs were targeted in these genotype-phenotype correlation studies, and the studies differed slightly in the numbers and types of SNPs investigated (4). The method of Schaeffeler et al. has the potential to advance the field by allowing a practical means of genotyping all known and clinically relevant TPMT SNPs in clinical samples. Their assay could minimize incorrect predictions due to rare SNP alleles.

Schaeffeler et al. also screened different ethnic populations (3) and, consistent with the previous findings, found different TPMT allelic distributions for Ghanaian, Korean, and German populations. The TPMT*8 and TPMT*6 alleles, which were rare in German populations, were the second most common alleles in the Ghanaian and Korean populations, respectively (3). The presence of such striking differences in allelic distributions among populations highlights the issue of considering ethnic variation in designing drug therapy. The high-throughput assay described by the authors provides a tool for large-scale application, enabling therapeutic strategies to be tailored for each ethnic population.

The mass spectrometric platform used by Schaeffeler et al. is a well-established genotyping system that possesses the advantages of correctness, automation, and throughput that are essential for clinical implementation (6). Several precautions have to be taken, however. First, the experimental procedures may be susceptible to contamination by amplicon carryover, because there are at least 3 procedures downstream of the PCR that involve exposing amplified templates to the laboratory environment or robotics. Therefore, negative controls are important for each experiment. Second, our experience has shown that excessive salt in samples and incomplete deoxyribonucleotide removal may occasionally lead to false-positive peaks in the resulting mass spectra. Thus, caution should be taken when interpreting mass spectra of low quality.

One well-known difficulty of TPMT genotyping not examined by these authors is distinguishing individuals carrying a wild-type allele and a TPMT*3A allele (*1/*3A), who have intermediate TPMT activities, from individuals possessing a TPMT*3B allele and a TPMT*3C allele (*3B/*3C), who have very low TPMT activities (2). The problem lies in the fact that the *3B and *3C alleles involve SNPs 460G>A and 719A>G, respectively, whereas the *3A allele contains both SNPs on the same chromosome. The authors genotyped the 2 SNPs independently, but information regarding the phase of the 2 SNPs was lacking (3). Such a problem can be overcome by haplotyping, which simultaneously genotypes several SNPs on the same DNA mol-

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\(^1\) Nonstandard abbreviations: TPMT, thiopurine S-methyltransferase; SNP, single-nucleotide polymorphism.

\(^2\) Human genes: TPMT, thiopurine S-methyltransferase; MTHFR, 5,10-methylene-tetrahydrofolate reductase (NADPH).
ecule. In fact, the genotyping system of Schaeffeler et al. could readily be adapted to a haplotyping format (7). For example, genomic DNA could first be diluted to approximately 1 DNA molecule per reaction. The multiplex genotyping reactions would then be carried out to detect the SNP alleles on each DNA molecule (7). Besides distinguishing the *1/*3A and *3B/*3C individuals, such haplotyping may provide additional genetic information for clinical use. Haplotypes consist of specific combinations of SNP alleles that interact with each other and are suggested to have greater power than individual SNPs to predict phenotypic outcomes (8). With regard to TPMT activity, a genomewide survey of 1.1 million SNPs in the HapMap Project identified numerous haplotypes associated with the TPMT activity phenotype (9). Further investigation of these candidate haplotypes would be valuable.

Although the complete lack of activity of 2 nonfunctional alleles is easily identified, the distribution in TPMT activity for wild-type and heterozygous carriers is continuous, and cutoff values are difficult to establish (2, 4). The existence of such overlapping activities highlights the possibility of unknown genetic polymorphisms that may influence TPMT activity. The variable number of tandem repeats (VNTR) polymorphism in the 5' untranslated region of the TPMT gene has been studied, but its effects on TPMT activity are moderate (10). Apart from TPMT, an SNP in the MTHFR gene [5,10-methylenetetrahydrofolate reductase (NADPH)] is associated with TPMT activity (11). These variations, along with the haplotype polymorphism mentioned above, are worthy of further study.

In conclusion, the multiplex mass spectrometry–based genotyping assays described by Schaeffeler et al. (3) provide a comprehensive yet simple tool for studying polymorphisms in the TPMT gene. Large-scale clinical evaluations for individualized therapy may follow.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures of Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

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DOI: 10.1373/clinchem.2008.112896