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Conflicts of interest
The authors disclose the following: Max Nieuwdorp is supported by a ZONMW-VIDI grant 2013 (016.146.327). Silvia Melgar and the APC Microbiome institute receive the financial support of Science Foundation Ireland (SFI) under Grant Number SFI/12/RC/2273.

Most current article

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2015.08.039

TPMT Testing Before Starting Azathioprine or Mercaptopurine: Surely Just Do It?

See "Identification of patients with variants in *TPMT* and dose reduction reduces hematological events during thiopurine treatment of inflammatory bowel disease," by Coenen MJH, de Jong DJ, van Marrewijk CJ, et al, on page 907.

Across every field of clinical medicine, there are now multiple examples of how genetic variation between individuals can have an important influence on the response—adverse or favorable—to a specific class of drug. This concept is known as pharmacogenomics, a field which has expanded massively over the last 20 years. Indeed, >150 drugs approved by the US Food and Drug Administration have a recommendation regarding pharmacogenetic variation included in the official drug labeling.[1] Despite this, the uptake of pretreatment pharmacogenetic testing into routine clinical practice remains remarkably low.[2] In some cases, this results from a positive predictive value that is genuinely too low. In others, the clinical utility is far from clear. In some, however, a highly positive predictive value and clearly defined clinical utility make debate regarding incorporation of a test into clinical practice hard to understand. Such is the case for the role of genetic variation in the activity of thiopurine-S-methyl transferase (TPMT) in predicting important toxicity to the thiopurine analogues, mercaptopurine and its prodrug, azathioprine. Hence, the article in this issue of *Gastroenterology* by Coenen et al[3] addressing the value of pretreatment TPMT testing in patients with inflammatory bowel disease (IBD) receiving azathioprine exemplifies this debate perfectly.

TPMT is a methylating enzyme present in the body for the detoxification of rogue purines that might damage nucleic acid integrity if incorporated into DNA or RNA. Along with xanthine dehydrogenase (which is only very rarely completely deficient), TPMT is the main competing influence on the passage of thiopurines along the purine salvage pathway toward thioguanine nucleotides (TGN), considered the active metabolites responsible for the immunosuppressive effects of these drugs. Approximately 1 in 11 individuals of a Caucasian population are

Table 1. Selected Pharmacogenetic Biomarkers of Proven or Potential Clinical Value Related to Drugs Used for Inflammatory Bowel Disease

Pharmacogenetic biomarker	Drug class influenced	Associated change in clinical outcome	Likelihood of adoption into clinical practice
TPMT mutations	Thiopurines	Increased risk of early myelosuppression and other side effects	Accepted
ITPase c.94C>A	Thiopurines	Flu-like illness, myelosuppression	Possible
NUDT15	Thiopurines	Myelotoxicity	Possible
HLA-DQA1-HLA-DRB1 haplotype	Thiopurines	Thiopurine induced pancreatitis	Possible
CYP3A polymorphism	Calcineurin inhibitors	Reduced drug levels in fast metabolizers	Possible
ACBC1 (formerly MDR-1) polymorphism	Calcineurin inhibitors	Reduced drug levels related to membrane efflux	Possible
MTHFR C677T mutation	Methotrexate	Adverse effects and clinical response	Unlikely
TNF gene and TNF cell surface receptor gene polymorphism Fas L gene polymorphism	Anti–TNF-α antibody agents	Altered clinical response	Unlikely

TNF, tumor necrosis factor; TPMT, thiopurine-S-methyl transferase.

heterozygous for common mutations in the TPMT gene (usually *TPMT *3A* and *TPMT *3C*). In these individuals, unstable enzyme decay leads to around 50% enzyme activity. Approximately 1 in 300 are either homozygous or have compound heterozygosity and both have almost zero TPMT enzyme activity. In both situations, exposure to standard doses of azathioprine or mercaptopurine leads to excessive TGN production and hence a high likelihood of myelosuppression. In those with zero enzyme activity, the excessive TGN production is sufficient to cause severe pancytopenia and a significant risk of death.[4] Interestingly, although patients with heterozygous TPMT deficiency exposed to standard doses of azathioprine or mercaptopurine frequently have to withdraw treatment, they do so more commonly because of nausea than myelotoxicity, presumably because this occurs earlier than the influence on bone marrow.[5] The mechanism of this effect is unknown.

In those with a wild-type TPMT genotype, there is a broad range of TPMT enzyme activity that largely follows a normal distribution but with a skewed tail of a few individuals with very high TPMT activity. It is a frequently held misconception that TPMT in the wild-type range provides information predictive of outcome, either adverse effects or response. However, although there is a weak relationship between TPMT activity and efficacy of thiopurine treatment, TPMT activity variation across this range does not inform dosing and, somewhat surprisingly, does not predict those at risk of thiopurine hypermethylation, one of the main reasons for resistance to thiopurine treatment. Approximately 15% of patients demonstrate hypermethylation on standard regimens of azathioprine or mercaptopurine and risk side effects (primarily hepatotoxicity) or nonresponse. In these individuals, pretreatment TPMT activity cannot predict the situation but thiopurine metabolite monitoring by measurement or red cell TGN levels detects this after 4-12 weeks of therapy. In this group of patients, a combination of low dose thiopurine (initially 25% of usual target dose) and allopurinol is now a proven effective alternative to restore the chance of a full drug response.[5]

So, we know absolutely that complete or heterozygous TPMT deficiency is associated with a high chance of withdrawal from azathioprine or mercaptopurine due to adverse effects. In heterozygotes, this risk is 2- to 3-fold greater compared with those with wild-type TPMT.[6] When you compare this with the risk of adverse outcome of other established pharmacogenetic markers, this influence is certainly among the most significant. For this reason, the influence of TPMT deficiency on thiopurine therapy is considered the classic model of pharmacogenetics. Nevertheless, pretreatment testing for TPMT is by no means universal. Despite recommendations regarding testing in information contained within drug packaging and a recommendation to test included in many disease-specific consensus guidelines, there remains a view among some prescribers that the value of testing is not greater enough to justify the cost of the test. For some, this comes from the misconception that if a test only explains a relatively small proportion of side effects related to a particular drug, it is not worth doing, despite the critical impact on those patients unfortunate enough to have TPMT deficiency. For others, it is more genuinely about demonstrating the absolute benefit of the test in terms of improved outcomes across cohorts of patients receiving azathioprine or mercaptopurine.

The study by Coenen et al attempted to address this issue by enrolling patients receiving thiopurines for IBD into 1 of 2 arms: one receiving standard treatment and the other receiving treatment adjusted according to pretreatment TPMT testing such that patients with zero activity received 0%-10% and those with heterozygous activity 50% of the standard thiopurine dose. The study was unblinded, permitting physician-directed changes in thiopurine dosing in each group according to broad guidance. An impressive 783 patients were recruited across 30 Dutch centers. The primary outcome measures were the occurrence of hematologic adverse drug effects (ADR; white cell count $<3.0 \times 10^9$ or platelet count $<100 \times 10^9$) and treatment efficacy as defined by change