The future of thiopurine pharmacogenomics

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Since their invention [1], thiopurines (azathioprine, 6-mercaptopurine [6MP], and 6-thioguanine [6TG]) have significantly advanced the treatment of acute lymphoblastic leukemia, organ transplantation, inflammatory bowel disease (IBD) and various autoimmune diseases. These drugs are often regarded as the best model for a pharmacogenomic approach to drug dosing, which has led to a plethora of candidate genes being examined for their pharmacogenomic potential in modifying the prescription of thiopurines. These genes affect thiopurine response by acting on the metabolism, transport and receptor/effecter functions of the drugs, and are likely to remain the focus of investigations in the foreseeable future.

Thiopurine metabolic genes

- **TPMT**

Thiopurines have provided a prototype of how pharmacogenomics could improve clinical outcomes, and early studies related specifically to thiopurine methyltransferase (TPMT), encoded by the *TPMT* gene, which methylates thiopurine metabolites. Since the polymorphism affecting TPMT activity was first identified, more than 30 mutant alleles of the gene have now been identified [MAPPEL ET AL. NOMENCLATURE FOR ALLELES OF THE THIOPURINE METHYLTRANSFERASE (*TPMT*) GENE (2012), SUBMITTED], although only three functional mutations are common. Carriers of TPMT deficiency are at risk for toxicity with normal thiopurine doses but tolerate lowered doses. Patients with loss-of-function variants for both alleles are now usually prescribed very low doses, or not at all [2].

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However, the utility of the *TPMT* genetic-toxicity model in clinical practice remains a matter of ongoing debate, as *TPMT* mutant carriers account for only a third of leukopenia arising during thiopurine therapy [3]. This weakness of TPMT as a predictive tool for toxicity has focused attention on the clinical relevance of phenotyping. The enzyme assay itself is far from being physiological, and is further limited by its measurement in erythrocytes as a surrogate for the liver, the primary metabolic tissue. As a result, sole reliance on TPMT as a means of dose prediction has not proven to be clinically practicable. However, while TPMT is not always predictive for dosing of patients with normal activity, carriers and zero TPMT patients have benefited from reduced doses and lower toxicity risks: there can be no doubt that TPMT screening has prevented many drug-induced injuries and deaths.

- **MTHFR**

Deficiency alleles for this highly polymorphic gene, which regulates folate metabolism, are usually associated with homocysteinemia and risk of vascular pathologies. However, MTHFR also affects synthesis of *S*-adenosylmethionine, the essential cofactor for TPMT. Evidence has demonstrated that *MTHFR* polymorphisms may limit *S*-adenosylmethionine availability and thus TPMT activity *in vivo* [4], but follow-up studies are needed on MTHFR and other key steps in folate regulation.

- **ITPA**

Polymorphic deficiency of ITPA, with resultant accumulation of thioinosine-triphosphate, was proposed as causing a variety of adverse drug reactions to thiopurines [5]. The first study was unadjusted for TPMT status, and was followed by a flurry of conflicting publications. More recently, ITPA deficiency was convincingly shown to be associated with febrile neutropenia...
in young leukemia patients on 6MP, when adjusted for TPMT, as well as accumulation of methylated thiometabolites [6].

■ XDH, AOX1 & MOCOS

6MP is a substrate for XDH, which catabolizes the drug to 6-thioxanthine then 6-thiouric acid. Large individual differences in 6-thiouric acid excretion occur, although the genetics of this variation have not been fully dissected [7]. AOX1 can catalyze breakdown of both 6MP and azathioprine [8]. XDH and AOX1 rely upon availability of molybdenum cofactor, synthesized by the sulfurase MOCOS. A weak protective effect against adverse reactions to azathioprine has been associated with deleted XDH and MOCOS, and this effect was more significant where they coincided [8]. By contrast, an AOX1 SNP was shown to predict poor azathioprine response. When the analysis used the AOX1 SNP combined with TPMT, response became highly predictable, making these loci attractive targets for further research.

■ IMPDH1

IMP dehydrogenase is the key enzyme that diverts 6MP away from methylation and towards thioguanine nucleotides (TGNs), and seems an obvious candidate gene for clinical response. Recent work has shown IMPDH1 activity to be inversely proportional to 6MP methylation in mononuclear cells, but affirmed that erythrocyte metabolites are a poor guide for the status of the target immune cells [9].

■ Other candidate metabolic genes

There remain numerous metabolic steps in thiopurine pathways that have yet to be studied. ‘Blind spots’ in thiopurine pharmacogenomics include GDA, which catalyses the breakdown of 6TG to thioxanthine, and MTAP; cancers with deleted MTAP have increased sensitivity to thiopurines [10]. Low activity of NT5C2, which breaks down TGNs, has been associated with thiopurine toxicity [11], while numerous purine kinases remain unstudied.

Transporters & effectors

This area of thiopurine genomics has been extensively reviewed by Coulthard [101]. Thiopurines form two principal types of intracellular metabolites: myelotoxic TGNs and hepatotoxic methylated-6MP metabolites (MMPs, produced by TPMT). Resistance of T-lymphoblastic cell lines to thiopurines can be predicted to depend upon uptake transporters of the parent drugs (6MP/6TG), and export transporters of cytotoxic TGNs and MMPs. Uptake has been associated with two genes, SLC29A2 and SLC28A3, while genes significantly associated with systemic TGNs accumulation after 6MP therapy for acute lymphoblastic leukemia include the transporter SLC29A1. On the other hand, drug efflux from cells has been linked to members of the ABCC gene complex, in particular ABCG5, with overexpression being associated with thiopurine resistance.

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Chief among the effectors or molecular targets for TGNs is the Rac/Vav mechanism in activated leukocytes, which is controlled by Rac1 [12]. Other putative targets for TGNs include TNF ligands and receptors, and α4-integrin. On the other hand, 6MP metabolite methyl-thiolIMP is a well-documented inhibitor of purine de novo synthesis but there are conflicting in vitro data on the effect of this metabolite on leukemic cells. In vivo, methylated thiopurines, arising from TPMT shunting of 6MP, appear to cause hepatotoxicity [13]. Finally, DNA methylation is known to be altered by thiopurine therapy. For all of these transporters and putative effectors, the pharmacogenomics remain to be assessed.

Pharmacokinetics

The concentrations of TGNs and MMPs in erythrocytes are used to predict dosage, explain toxicity, recognise noncompliance and identify methylation shunters. Therapeutic ranges for these two metabolites, or their ratio, have been widely adopted. Alternately, TPMT activity has been combined with levels of TGNs to guide dosing [14]. However, these metabolites correlate poorly with weight-corrected dose, TPMT activity and clinical response. Significantly, TGNs and MMPs are not measured directly but as acid derivatives, and erythrocytes are poor surrogates for the target leukocytes [15]. Thiopurine pharmacokinetics thus remain unsophisticated. A priori prediction of 6MP shunting is not currently possible but is desirable. In the future, advanced technologies such as mass spectroscopy may directly measure thiometabolites with sufficient sensitivity for detection in leukocytes [16]. Improved technologies are needed to improve links between thiopurine pharmacokinetics and pharmacogenomics, which are presently lacking.
Conclusion & future perspective

Thiopurines are undergoing a revival, driven by the tightening of national medical budgets [10] combined with the improvements in safety afforded by pharmacogenetic testing. It remains good practice to use TPMT combined with metabolite concentrations to assess compliance or refine doses. Even then, there will always be limitations to the accuracy of pharmacogenomic guidance for prescribing: cotherapies such as aminosalicylates, cotrimoxazole and diuretics, as well as intercurrent viral infections, have all been implicated in thiopurine-induced leukopenia. Routine blood counts remain essential for thiopurine monitoring; however, two major advances in thiopurine prescribing have arisen from pharmacogenomic knowledge. Importantly, both of these therapies involve ‘bypassing’ known genetic impediments.

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However, often-quoted studies demonstrating 6TG toxicity have used doses that retrospectively appear to be high. A prospective IBD study has shown that prolonged 6TG therapy with lower dosing was well-tolerated and safe for IBD treatment, with a high response rate [18]. Recent animal data suggest that veno-occlusive disease can be abolished with dose splitting to lower the peak drug exposure for liver endothelium [19]. Like allopurinol cotherapy, 6TG bypasses known pharmacogenomic obstacles – TPMT, XDH and ITPA – and holds promise for faster and more effective response rates [10].

Next-generation sequencing is becoming a reality in areas of medicine such as cancer, to aid in diagnosis. In time it may become the modus operandi, especially as the costs of tests decrease. Combined with systems biology, this could facilitate better targeting of drug profiling, including thiopurines, by identifying pharmacologically-active mutations. The real questions for the future of pharmacogenomics are straightforward: which SNPs to genotype, and more importantly, how to guide the prescriber? Thiopurines may continue providing a working pharmacogenomic model to provide answers. In science and medicine, crystal-ball gazing is fraught with dangers, nonetheless the future for thiopurines and their pharmacogenomics looks promising.

Financial & competing interests disclosure

JA Duley is a coapplicant of a US patent on ITPA related to thiopurine side effects in inflammatory bowel disease. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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