Pharmacometabolomics may be the next stamp in the pharmacogenetic passport

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1. Pharmacogenetics testing improves drug efficacy and safety

The field of personalized medicine is currently witnessing the start of a new phase of the pharmacogenomics (PGx) revolution. Based on several large pragmatic trials, the added value of PGx testing is becoming increasingly clear, notably showing its potential to enhance drug efficacy and safety through personalizing drug choices and dosages based on individual genetic factors. Consequently, expert panels, such as the Dutch Pharmacogenetics Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC), have been developing guidelines to optimize drug dosing based on PGx testing, and routine application of these tests is increasing rapidly [1].

Current PGx tests mainly target variation in genes encoding the enzymes responsible for drug metabolism which causes altered conversion rates of drugs and their metabolites. Consequently, this type of variation may be associated with under- or overexposure to active pharmaceutical ingredients. PGx tests are thus most urgent for drugs with narrow therapeutic windows and when the consequences of an under- or overexposure can be life-threatening. Accordingly, most PGx guidelines focus on anticancer drugs, immunosuppressants, antithrombotic agents, and psychotropic medicines [1].

2. A principal pillar of pharmacogenetics-based personalized medicine seems unstable

PGx guidelines generally rest on three pillars: (1) knowledge about the enzymes involved in the metabolism of a drug and the metabolites formed; (2) insights into how genetic variation in the enzymes responsible for this metabolism affect drug efficacy and safety; and (3) the availability of analytical tests to (rapidly and reliably) determine a user's metabolizer status for these enzymes. In contemporary PGx research, attention is mostly paid to the latter two pillars, while information on drug metabolism is typically derived from small-scale, pre-registration trials conducted during commercial drug development. However, it is often disregarded that the generalizability of findings on metabolite patterns from these studies may be limited, notably because regulatory guidelines for drug metabolism studies recommend rather basic sets of experiments [2,3]. Specifically, regulations require studies on the identification of drug-metabolizing enzymes by *in-vitro* experiments targeting only the seven 'major' drug metabolizing (cytochrome P450) enzymes. Other enzymes only have to be studied if a drug candidate is not found to undergo significant metabolism by these seven enzymes [2]. Furthermore, *in-vivo* drug metabolite investigations are generally conducted in four to six young, healthy, male volunteers during early-phase clinical research in so-called 'mass balance' studies [3]. Therefore, we postulate that the metabolite patterns observed in these studies may be less heterogeneous than can be expected in individuals receiving the drug once it is approved.

3. Pharmacometabolomics confirms and complements knowledge of drug metabolism (and excretion)

To test the hypothesis that metabolite patterns found in pre-registration trials differ from those found in clinical drug users, we conducted so-called 'pharmacometabolomics' (PMx) experiments to profile drug metabolites in the real-world setting of liver and kidney transplantation [4,5]. We first applied this approach to the immunosuppressive drug azathioprine (AZA) which represents an early success story of PGx-driven personalized medicine. In addition, we studied mycophenolate mofetil (MMF) which has largely replaced AZA usage in the past decades. For both drugs, disagreements between metabolite patterns expected from clinical trials and patterns detected in clinical samples were substantial (see Fig. 1). In particular, we found more AZA and MMF metabolites in the urine samples of transplant recipients than could be expected based on prior knowledge of how these drugs are metabolized and excreted. Importantly, some of the identified metabolites are unknown or unreported thus confirming the abovementioned hypothesis.

The value of PMx studies can be illustrated further by taking AZA as an example. This prodrug is converted via a series of intermediate metabolites to thioguanine nucleotides which exert cytotoxic effects through incorporation in DNA and RNA. However, some of the intermediate metabolites are scavenged by the enzymes XDH, TPMT and NUDT15, which results in inactive metabolites that are eliminated from the body.

Based on the current PGx consensus understandings [1], our PMx data should indicate the presence of two known inactive AZA metabolites (*i.e.,* methylmercaptopurine, thiouric acid), which were both found.

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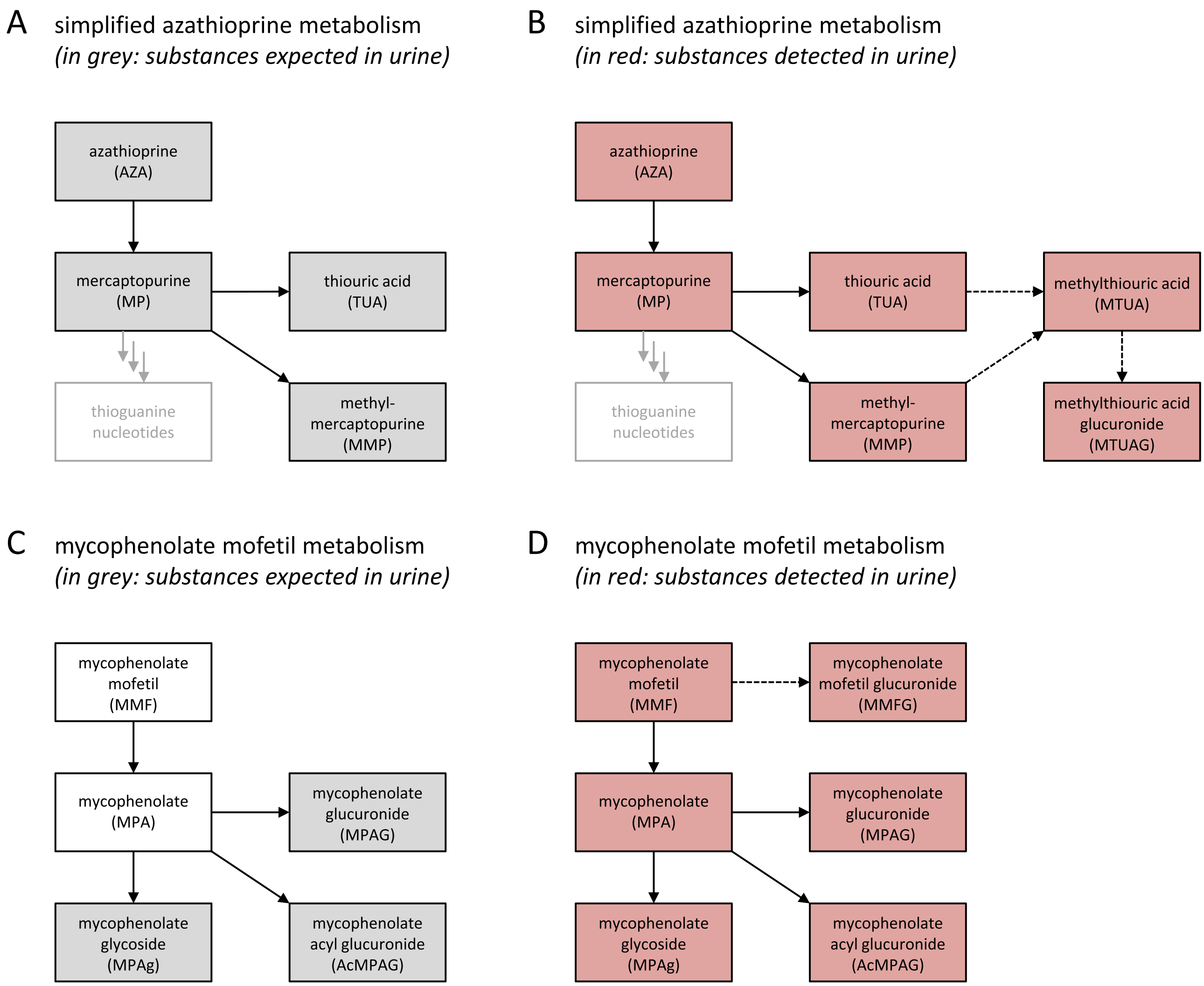

Fig. 1. Overview of (a,b) azathioprine and (c,d) mycophenolate mofetil metabolic pathways as (a,c) are expected in urine and as (b,d) were detected in urine of liver and kidney transplant recipients. Corresponding pharmacometabolomics data of exemplary azathioprine and mycophenolate mofetil users are presented in the Figs. S1 and S2, respectively.

In addition, azathioprine itself and the intermediate metabolite mercaptopurine are expected following previous bioanalytical findings, and both were detected. Importantly, however, we also identified two previously unknown/unreported urinary AZA-related signals, and computational calculations indicated that these signals both correspond to S-methylated thiouric acid (MTUA) species [4]. Recent insights furthermore uncovered that one reflects unconjugated MTUA and that the other originates from glucuronidated MTUA (see Fig. S1). Our findings thus indicate the presence of metabolic pathways other than those described in the current PGx guidelines, which seemingly paint an incomplete picture of AZA metabolism.

This example shows how PMx can inform PGx by unveiling previously unknown metabolic pathways which could be considered to realize more effective and safer use of this immunosuppressive drug. Clearly, it would be important to elucidate these mechanisms and provide insights into the activity/toxicity of the unknown metabolites. Subsequently, potential genetic variants of the associated enzymes that may lead to decreased or increased functions could be included in the pharmacogenetic passports of AZA users and thereby contribute to guiding drug dosing.

Besides informing, PMx can also complement PGx by providing insights into active and inactive drug fractions in biological matrices like blood and urine. The AZA example is revealing in this regard, because all AZA-related substances found in urine reflect a portion of the administered drug that has (presumably) never been active in the human body. Admittedly, a person's genetic makeup is an important determining factor herein by affecting the efficiency of metabolite scavenging through XDT, TPMT, and NUDT15. Drug metabolism is, however, also affected by non-genetic factors such as drug-drug interactions, co-exposure to other xenobiotics (*e.g.,* dietary, lifestyle, environmental), and non-inherited liver dysfunction. These factors are captured in PMx data and can thus provide a phenotypic view of drug metabolism. PMx may accordingly hold considerable clinical potential as a stand-alone tool, for example for the long-term monitoring of efficacy and safety profiles. In this regard, potential future applications of PMx should be designed taking into account existing analytical workflows for untargeted clinical metabolite profiling. Notably, this includes the urinary steroid profiling workflow which has been serving in clinical laboratories for decades as the primary test to detect and monitor disorders of steroid hormone synthesis based on relative metabolite abundances [6].

4. Pharmacometabolomics can readily be implemented on analytical instruments routinely used in many hospitals and clinical laboratories

Our PMx platform is a variant of the well-known metabolomics methodology, which is commonly used in biomedical research to profile small-molecule metabolites within biological systems. Additionally, it builds upon the pioneering work of Prof. Rima Kaddurah-Daouk, a key innovator of the PMx field, who has mostly focused on the effects of therapeutic drugs on the abundances of endogenous metabolites [7]. Our workflow, however, specifically targets the abundances of drugs and their (exogenous) metabolites, which can also be present in metabolomics datasets but are frequently filtered out during data processing to limit data complexity.

Moreover, our workflow was designed for analytical instruments that are used routinely for toxicological screening in clinical laboratories. This technique, called 'high-resolution mass spectrometry', is also commonplace in doping analysis and clinical chemistry for detection of (unknown) doping substances and profiling of endogenous steroids, respectively. Admittedly, employing novel applications on analytical instruments being used in a regulatory environment is not straightforward, and implementation of profiling workflows is arguably complex, while also their cost-effectiveness needs to be demonstrated. In this regard, we would mostly like to stress that our PMx platform does not depend on a complex technique that is only available in highly specialized academic institutions. Instead, it matches infrastructure present in various (ISO 15189) certified medical laboratories, which can expedite its potential clinical implementation in the future. Lastly, it is worth mentioning that more affordable high-resolution mass spectrometers are becoming increasingly available, and these instruments are not inherently less sensitive than many triple quadrupole mass spectrometers commonly used for therapeutic drug monitoring purposes. These instruments can simultaneously quantify pre-specified drugs and metabolites (using internal standards) and also generate untargeted profiles of other metabolites, thereby providing a phenotypic view of drug metabolism at the same time.

5. In conclusion

Our pharmacometabolomics platform and its application to studying drug metabolite patterns in a real-world setting can be used to inform pharmacogenetics research and clinical practice. Moreover, PMx can complement PGx-driven personalized medicine given that variation in detected metabolite patterns is not solely determined solely by genetic differences. Factors such as drug-drug interactions, co-exposure to other xenobiotics, kidney dysfunction, and liver dysfunction can also impact drug metabolism, and the corresponding variability is captured in PMx data. These data thus allow for studying drug metabolism at the phenotype level, hence PMx as a stand-alone tool may also be considered to form the basis of future clinical applications.

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CRediT authorship contribution statement

Marieke A.J. Hof: Writing – review & editing, Validation, Conceptualization. **Frank Klont:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jos G.W. Kosterink:** Writing – review & editing, Supervision, Conceptualization. **Eelko Hak:** Writing – review & editing, Supervision, Conceptualization. **Daan J. Touw:** Writing – review & editing, Conceptualization. **Fleur B. Nijdam:** Writing – review & editing, Conceptualization. **Gérard Hopfgartner:** Writing – review & editing, Resources, Conceptualization. **Stephan J.L. Bakker:** Writing – review & editing, Resources, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Frank Klont reports financial support was provided by the Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) and by the European Union's Horizon 2020 Research and Innovation Program. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data presented in this work are freely available via the respective papers discussed/referenced.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phrs.2024.107191.

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