The impact of non-genetic factors on drug metabolism: towards better phenotype predictions

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Chapter 1

Introduction and thesis outline

Drug metabolism: a key determinant of pharmacokinetics

The response to drug treatments varies significantly among individuals, with 20–75% of patients failing to achieve the desired outcomes due to adverse drug reactions (ADRs) or inadequate therapeutic responses (1). ADRs are a significant cause of hospital admissions, accounting for approximately 5% of cases in the Netherlands (2), and about 15% of hospitalized patients experience ADRs during their stay (3). These high incidences highlight the need to address the underlying causes of variability in treatment outcomes. A fundamental determinant of drug efficacy and safety is the concentration of the drug in both blood and tissue, which is determined by its absorption, distribution, metabolism and excretion (ADME) (4). These physiological processes collectively shape the pharmacokinetic (PK) profile of a drug, influencing both its therapeutic effectiveness and toxic potential. A deeper understanding of the factors contributing to ADME variability is necessary to mitigate ADRs and enhance the efficacy of drug treatments.

Among the ADME processes, drug metabolism is a key factor that influences PK parameters, as it dictates the rate at which drugs are biotransformed and eliminated from the body. Drug metabolism primarily involves the enzymatic conversion of lipophilic drugs into more hydrophilic metabolites, which facilitates their excretion (5). This transformation predominantly occurs in the liver, though other tissues, such as the kidneys and gastrointestinal tract may also contribute to drug metabolism (6). Enzymatic transformation occurs by mechanism categorized as either phase I or phase II reactions (7). Phase I enzymes typically catalyze either oxidation, reduction or hydrolysis reactions, whereas most phase II enzymes catalyze conjugation reactions. Drugs are often metabolized through sequential reactions involving both phase I and phase II drug metabolizing enzymes (DMEs).

Cytochrome P450 enzymes (CYPs) are a key family of phase I enzymes responsible for the metabolism of ~75% of clinically administered drugs. These enzymes belong to a diverse superfamily of heme-containing proteins, systematically classified into families and subfamilies based on similarities in their amino acid sequences (8). Each enzyme is identified by a family number (e.g., CYP2), a subfamily letter (e.g., CYP2C), and an unique isoform identifier (e.g., CYP2C19). Among these, five key isoforms – CYP3A4, CYP2D6, CYP2C9, CYP2C19 and CYP1A2 – are primarily responsible for catalyzing the

biotransformation of most drugs. Of the 100 most prescribed drugs in European countries, 43 are mainly metabolized by CYP3A4, followed by 23 for CYP2D6, 23 for CYP2C9, 22 for CYP2C19 and 14 for CYP1A2 (9). Table 1 provides examples of sensitive drug substrates for these main DMEs, along with probe substrates used to quantify their activity in vitro and in vivo. Other important phase I enzymes that catalyze oxidations include the flavin-containing monooxygenases (FMOs) and the alcohol dehydrogenases (ADHs) (10). Beyond phase I, phase II enzymes also play a crucial role in drug metabolism. Notably, it is estimated that approximately 25% of the top 200 most prescribed small molecule drugs approved by the FDA rely predominantly on non-CYP enzymes for their clearance (11). Of these, 45% of biotransformation is executed by the phase II enzymes UDP-glucuronosyltransferases (UGTs), 10% by sulfotransferases (SULTs) and 7% by carboxylesterases (CESs). The activity of DMEs is a significant determinant of drug clearance, half-life and plasma concentrations, thereby influencing drug exposure and subsequent therapeutic efficacy or toxicity. As such, understanding the factors that govern drug metabolism is crucial for predicting and managing drug PK and ensuring both safe and effective treatment.

Table 1 Examples of commonly used drug substrates for the main DMEs, and in vitro and in vivo probes used to quantify their activity

	Drug substrates	In vitro probes (12)	In vivo probes (13)
CYP3A4	Carbamazepine, cyclosporine, imatinib, ketoconazole, midazolam, nifedipine, sildenafil, simvastatin, tacrolimus	Midazolam, testosterone	Midazolam
CYP2D6	Codeine, haloperidol, metoprolol oxycodone, paroxetine, tamoxifen	Bufuralol, dextromethorphan	Dextromethorphan, metoprolol
CYP2C9	Diclofenac, glimepiride, phenytoin, valproic acid warfarin	Diclofenac, tolbutamide	Diclofenac, flurbiprofen, losartan, s-warfarin, tolbutamide
CYP2C19	Citalopram, clopidogrel, escitalopram, fluvoxamine, omeprazole, pantoprazole, sertraline, voriconazole	S-mephenytoin	Omeprazole
CYP1A2	Clozapine, duloxetine, theophylline	Phenacetin	Caffeine

Interindividual variability in drug metabolism

One of the major challenges in the drug metabolism field is the significant interindividual variability that can lead to differences in systemic drug exposure between patients upon administration of a fixed dose. These interindividual differences in drug metabolism can stem from both genetic and non-genetic factors.

Pharmacogenetics

Pharmacogenetics (PGx) studies how inheritance impacts the individual variation in drug response. Over the past two decades, considerable attention has been devoted to genetic polymorphisms in metabolic enzymes as a key factor to explain interindividual variability in drug metabolism. Genetic polymorphisms are thought to explain ~30% of this variability (14). Importantly, these polymorphisms are generally considered to impact the treatment efficacy or safety of approximately 20-25% of all drugs (14). Currently, there are over 400 polymorphic CYP variants reported in the PharmVar repository that impact metabolic function (15). Variants can include loss-of-function alterations that result in lower or absence of protein activity, or gain-of-function alterations that cause increased protein expression and/or enhanced functional activity. To enable their use in clinical practice, identified variants are translated into haplotypes and corresponding predicted drug metabolizing phenotypes. For most CYP enzymes, four predicted phenotypes categories are recognized: poor, intermediate, normal and ultrarapid metabolizers. These phenotypes are incorporated into dosing recommendations provided by the Dutch Pharmacogenetic Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC), aiding clinicians in adjusting patient therapy based on the individual's genetic profile (16,17). Currently, guidelines are available for over 300 drug-gene pairs, with CYP2D6, CYP2C19 and CYP2C9 most extensively covered (18). Various randomized controlled trials have demonstrated that individualizing drug dosing based upon the pharmacogenetic profile results in better outcomes for specific drug-gene combinations (19,20). More recently, a large multicenter study has proven that genotype-guided treatment using a pre-emptive 12-gene pharmacogenetic panel approach significantly reduces the incidence of clinically relevant adverse reactions among patients with actionable genotypes (21).

While the implementation of PGx has significantly advanced the shift from a one-size-fits-all approach to a more individualized strategy, challenges remain that have to be addressed. PGx-guided drug dosing doesn't account for the impact of non-genetic factors on drug response, such as age, diet, sex, environmental factors, concomitant medication use or underlying disease conditions (22). Subsequently, in clinical practice we often see a mismatch between the phenotype we would predict based on the genetic testing and the actual observed phenotype, a phenomenon known as phenoconversion (23,24) (Figure 1).

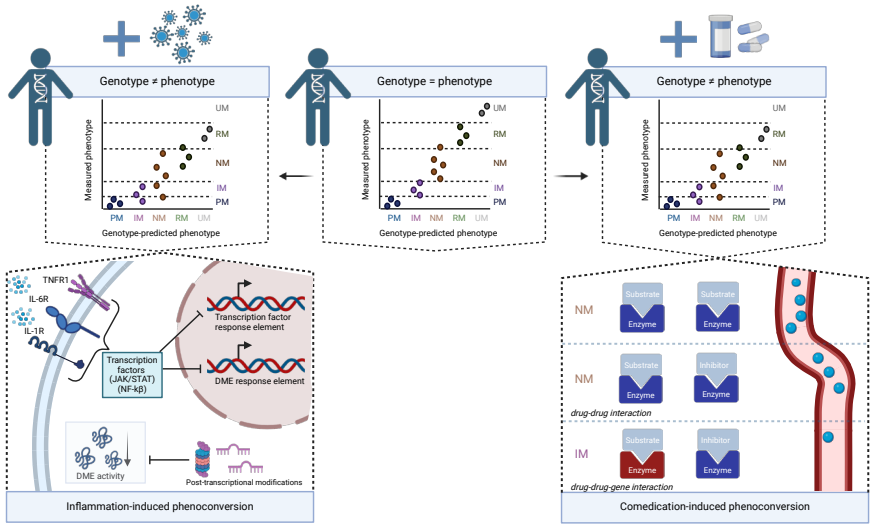

Figure 1 Overview of how inflammation and concomitant medication use alters drug metabolism, leading to discrepancies between genotype-predicted and measured DME phenotypes. Top panels depict baseline genotype-predicted phenotype relationships under normal conditions (middle) and phenoconversion scenarios induced by inflammation (left) and concomitant medication (right).

Various clinical studies have indeed highlighted that concomitant medication use, or patient/disease-specific factors impact the activity of key CYP enzymes, resulting in a shift in phenotype that could not have been predicted based on genotype alone (24). As an example, in CYP2C19-genotyped patients, escitalopram serum concentrations showed considerable overlap across all phenotype categories, illustrating that genotype alone does not always accurately predict metabolic capacity (25). Experimental studies using large cohorts of biobank liver

samples allow for a more controlled evaluation of metabolism-related variability, considering clinical features like adherence or variability introduced by differences in absorption or distribution are not confounding factors. These studied showed that significant variability in CYP activity persists within a single genotype group or among individual with similar gene activity scores (26–29). This highlights the need to incorporate the impact of non-genetic factors into drug metabolizer phenotype prediction in order to better reflect real-time metabolic capacity in patients.

Drug-drug-gene interactions as contributors to interindividual variability and phenoconversion

Similar to how genetically inherited variants can alter DME activity, administering concomitant drugs that inhibit or induce a DME can shift metabolic capacity, leading to a drug-drug interaction (DDI). Decades of experience have led to the establishment of standardized protocols for the clinical management of DDIs, including explicit warnings in drug labeling and clinical decision support systems. However, current approaches largely overlook the combined effects of DDIs with genetic variation, which can influence the likelihood or clinical significance of these interactions (30). For instance, individuals with one nonfunctional CYP2D6 allele are at increased risk of phenoconversion to a poor metabolizer (PM) status when exposed to a CYP2D6 inhibitor as compared to individuals with normal functioning alleles (31). These so-called drug–drug–gene interactions (DDGIs) thus occurs when the patient‘s genotype and another drug in the patient‘s regimen affect the individual‘s ability to clear a drug. Notably, DDGIs account for up to 20% of significant drug interactions, making them a substantial clinical concern (32–34).

Phenoconversion resulting from concomitant medication can thus compromise the accuracy of PGx-based drug dosing for specific drug-gene pairs. While studies have examined the impact of DDGIs through changes in drug exposure or clearance (30), this information is challenging to translate into clinical-decision making. A more practical approach would involve determining the switch in drug metabolizer phenotype when specific drug-gene pairs are combined with inhibitory or inducing concomitant medication, and subsequently add this information to existing drug-gene guidelines. In order to achieve this, more data is needed to quantify how PGx-based phenotype predictions are impacted by inhibitory or

inducing concomitant medication use affecting the same DME, and to determine whether this switch is genotype specific.

Inflammation as a contributor to interindividual variability and phenoconversion

Inflammation has emerged as another critical factor contributing to variability in drug metabolism. Inflammation is a critical component of the immune response to harmful stimuli, including pathogens, cellular injury and toxins (35). It involves a complex network of immune cells, signaling molecules and inflammatory mediators like cytokines and chemokines, which coordinate the body's defense and initiate tissue repair. Inflammatory mediators are central in initiating acute-phase responses and sustaining chronic inflammation. Mounting non-clinical and clinical evidence shows that elevated production of cytokines during inflammation, such as IL-1β, IL-6 and TNF-α, can significantly affect the expression and activity of certain DMEs (36–39) as well as drug transporters (40). These inflammation-driven changes in metabolism can result in an increased variability in drug exposure and may cause a transient and/or acute shift away from the genotype-predicted phenotype, resulting in phenoconversion. Considering the high prevalence of both acute and chronic inflammatory conditions, it is essential to consider how inflammation impacts hepatic metabolism for both new and existing drugs.

Clinical studies have demonstrated alterations in drug PK of CYP substrates in individuals with chronic inflammatory conditions and during episodes of acute inflammation or infection, presumable attributed to inflammation-induced modifications in drug metabolism (41). This is of specific relevance to drugs with a narrow therapeutic window, which are routinely subject to therapeutic drug monitoring (TDM). PK alterations during acute inflammatory episodes have been demonstrated for various drug classes, including antipsychotics (e.g., clozapine), antidepressants (e.g., citalopram), sedatives (e.g., midazolam), immunosuppressants (e.g., tacrolimus and cyclosporine) and antifungals (e.g., voriconazole) (41). Decreased CYP-mediated drug metabolism is also reported in several chronic inflammatory conditions including rheumatoid arthritis (42) and Crohn's disease (43), but also in metabolic diseases such as non-alcoholic fatty liver disease (NAFLD) (44) and type II diabetes (45), although it is unclear to what extent the inflammatory component of these latter diseases is responsible

for the alterations in drug PK. Less evidence is available for the potential impact of pro-inflammatory cytokines on non-CYP enzyme families, such as the UGTs, SULTs, FMOs and CESs, and the resulting alterations of non-CYP mediated drug PK during inflammation.

A few studies have attempted to quantify the phenotypic shift caused by inflammation, combing genotype data with alterations in DME activity (24). Generally, a shift towards a lower drug metabolizing phenotype is observed, where the shift depends on both the degree of inflammation/infection and the initial genotype. As such, inflammation adds an extra layer of variability to drug metabolism, which may necessitate adjustments in drug dosage regimens for patients with acute or chronic inflammatory conditions.

The use of immunomodulating therapeutics to battle conditions where excessive or chronic inflammation plays a role is on the rise (46). These include monoclonal antibodies (mAbs) that target cytokine (receptors) or modalities aimed at inhibiting the signaling pathways induced by inflammation. These anti-inflammatory treatments may, through the resolution of inflammation, restore CYP metabolic capacity resulting in a disease-drug-drug interaction (DDDI) which further introduces PK variability. As an example, treatment with the anti-IL6 receptor mAb tocilizumab in RA patient resulted in a 57% lower exposure of simvastatin as compared to treatment with simvastatin alone, mechanistically explained by restored CYP3A4 activity (42). Regulatory agencies have now installed guidelines to investigate the risk for such DDDIs with therapeutic proteins (47,48). Despite the recognized potential for DDDIs in patients receiving anti-inflammatory treatments, there is a lack of clarity regarding which patient population and medications carry the highest risk for these interactions. Furthermore, the potential effects of these interactions on therapeutic outcomes remain poorly understood.

Methodological strategies for studying drug metabolism and phenoconversion

Considering the numerous intrinsic and extrinsic factors that can influence drug metabolism, there is a need for tools to evaluate an individual's drug metabolizing phenotype.

In vitro or ex vivo models

In vitro models or ex vivo biopsy samples can be instrumental in quantifying how PGx impact drug metabolism. The functional relevance and substrate specificity of rare variants in e.g. CYP enzymes is often hard to tackle in clinical trials considering their low frequency. Thus, in vitro systems, such as liver microsomes, cell-based expression systems, ex vivo primary samples or purified variant proteins can be used to characterize the impact of rare variants. Large screens have been conducted to systematically characterize a wide range of rare variants on DME functionality in vitro, for example by utilizing deep mutational scanning methods to study the functional implications of missense variants in CYP2C9 and CYP2C19 (49), providing a first step towards evidence for potential clinically actionable variants.

Furthermore, cellular models such as hepatocyte cultures allow for the examination of drug metabolism under various experimental conditions, including the presence of inflammatory cytokines or DDIs. These models facilitate the assessment of specific quantitative parameters of e.g. enzyme kinetics, but can also yield mechanistic insights into the underlying molecular pathways. A fundamental prerequisite for these studies is the sustained and robust expression of DMEs. Primary human hepatocytes have long been considered the golden standard for drug metabolism studies, but their utility is significantly constrained by a rapid decline in DME activity when cultured in 2D and marked inter-donor variability (50,51). To overcome these limitations, advanced culture techniques such as 3D spheroids or liver-on-a-chip models have been developed to recreate a more physiologically relevant microenvironment for studying drug metabolism (52,53). Additionally, the HepaRG cell line has emerged as a robust alternative due to its capacity to maintain consistent metabolic activity over prolonged culture periods, making it a valuable tool for studying both baseline metabolism and the effects of non-genetic factors (54).

Modeling approaches

Physiologically-based pharmacokinetic (PBPK) models have been effectively employed to predict and understand the determinants of interindividual variability in drug PK. These models distinguish drug-specific and system-specific parameters and allow for simulation of concentration-time profiles under a range of clinical conditions. Over the past decade, this approach has gained substantial prominence

in drug development and has been increasingly endorsed by regulatory agencies (55). The interindividual variability in PK can be simulated in PBPK modeling by accounting for variations in key system parameters, such as changes in drug metabolism caused by genetic polymorphisms, inflammation or DDIs.

Multiple efforts have been made to apply PBPK modeling to predict the clinical impact of disease-drug or disease-drug-drug interactions in, for example, patients with rheumatoid arthritis, leukemia or surgical traumas (56–60). Additionally, PBPK modeling has shown useful in predicting the extent and clinical impact of drug-gene or drug-drug-gene interactions (61–65). A key advantage of bottom-up PBPK approaches is their ability to predict drug PK across various scenarios, leveraging systemic parameters and in vitro data to make quantitative predictions without requiring clinical data for every drug. This underscores the importance of robust in vitro data as a foundation for these models. As such, the integration of disease parameters or other non-genetic factors which impact ADME into PBPK models appears to be a promising method to approach personalized treatments by predicting individuals phenotypes.

Clinical approaches

The phenotyping cocktail approach is the most commonly employed method to assess real-time enzyme activity in patients (13). This method involves the simultaneous administration of probe substrates, each selective for a specific CYP isoform, followed by measurements of either the probe clearance or metabolite-to-parent drug ratio in plasma or urine. It operates on the assumption that the observed changes in probe drug clearance or metabolite-to-parent ratios are solely driven by alterations in CYP enzyme activity, and results are thus used to quantify how the factor studied impacts CYP activity. The phenotyping approach has long been a valuable tool in traditional pharmacokinetic studies, particularly for investigating drug-drug and drug-gene interactions (66,67). In recent years, its application has expanded to include the evaluation of how various (patho) physiological conditions – such as inflammation, obesity and pregnancy – affect in vivo enzyme activity (68–70). As such, the phenotyping cocktail approach is an important tool to study the impact of genetic and non-genetic factors on drug metabolism, and can be effectively utilized to predict drug metabolizer phenotypes in patient populations.

Aim and outline of this thesis

The aim of this thesis is to investigate how non-genetic factors, such as inflammation and concomitant medication, impact hepatic drug metabolism and subsequent drug metabolizing phenotype predictions. This work is grounded in the hypothesis that these factors significantly affect drug metabolism and, therefore, should be incorporated into PGx-based phenotype predictions. To address this, section one focusses on the impact of concomitant medication on drug metabolizing phenotype predictions based on PGx. Section two provides novel insights into the impact of inflammation on hepatic drug metabolism and its underlying mechanisms, as well as the potential of immunomodulating therapies to reverse these inflammation-induced alterations in drug metabolism. Finally, section three evaluates in vivo tools that are used to study alterations in drug metabolism under (inflammatory) disease conditions.

Section I – Impact of concomitant medication on drug metabolizer phenotype predictions

In **chapter 2**, we quantify the phenoconversion in various CYP2C19 genotype groups following administration of CYP2C19 inhibitors in a cohort of microsomal liver fractions from 40 patients. Additionally, clinical features will be matched to measured CYP2C19 activity to find the source of the discrepancy between genotype-predicted phenotype and actual measured phenotype in the cohort.

Section II – (Pre)clinical evaluation of inflammation-induced alterations in drug metabolism

In **chapter 3**, we summarize evidence assembled through human in vitro liver models on the effect of inflammatory mediators on expression and metabolizing capacity of clinically relevant CYP isoforms. Furthermore, we examine the distinct mechanistic pathways by which inflammation can modulate drug metabolism in hepatocytes. Subsequently, in **chapter 4**, we utilize the HepaRG in vitro model to study how non-CYP DME family members are affected by inflammatory mediators, and set out to establish a hierarchy of their sensitivity towards inflammation as compared to the CYPs. In the last part of this section, we focus on reversal of the impact of inflammation by immunomodulating therapeutics, which might result

in DDDIs. As such, **chapter 5** systematically summarizes the clinical and non-clinical evidence for reversion of inflammation-driven alterations in metabolic capacity of CYP enzymes upon treatment with immunomodulating therapeutics. It subsequently compares the available evidence for DDDIs to the risks that are described in the drug labeling information of both the FDA and the EMA.

Section III – In vivo tools to study alterations in drug metabolism during (inflammatory) disease

In **chapter 6** we investigate whether the CYP phenotyping cocktail approach accurately reflects alterations in enzyme activity under inflammatory and other (patho)physiological conditions. Using a PBPK workflow, we aim to investigate the sensitivity and specificity of plasma clearance of CYP probe drugs as a surrogate marker of enzyme activity in vivo.

Finally, in **chapter 7**, the results of this thesis will be summarized and discussed alongside the prospects for the implementation of inflammatory status and concomitant medication use into drug metabolizing phenotype predictions to enhance a more personalized medicine approach.

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