

## The impact of non-genetic factors on drug metabolism: towards better phenotype predictions Iong. L.M. de

#### Citation

Jong, L. M. de. (2025, June 5). *The impact of non-genetic factors on drug metabolism: towards better phenotype predictions*. Retrieved from https://hdl.handle.net/1887/4249242

Version: Publisher's Version

Licence agreement concerning inclusion of doctoral

License: thesis in the Institutional Repository of the University

of Leiden

Downloaded from: <a href="https://hdl.handle.net/1887/4249242">https://hdl.handle.net/1887/4249242</a>

**Note:** To cite this publication please use the final published version (if applicable).

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

Laura Maria de Jong

**Cover** Robin Weijland

LayoutRenate Siebes | Proefschrift.nuPrinted byProefschriftmaken.nl | De Bilt

**ISBN** 978-94-6510-597-0

#### © Copyright, Laura Maria de Jong, 2025

All rights reserved. No part of this book may be reproduced in any form or by any means without permission of the author.

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

#### **Proefschrift**

ter verkrijging van de graad van doctor aan de Universiteit Leiden, op gezag van rector magnificus prof.dr.ir. H. Bijl, volgens besluit van het college voor promoties te verdedigen op donderdag 5 juni 2025 klokke 13.00 uur

door

Laura Maria de Jong

geboren te Den Haag in 1997

#### **Promotores:**

Prof.dr. R. Rissmann

Prof.dr. J.J. Swen

#### **Copromotor:**

Dr. M.L. Manson

#### Promotiecommissie:

Prof.dr. H. Irth

Prof.dr. E.C.M. de Lange

Prof.dr. S. de Wildt (Radboudumc)

Prof.dr. P. Olinga (Rijksuniversiteit Groningen)

Dr. C. Samer (Université de Genève)

#### **Table of contents**

7	CHAPTER 1 Introduction and thesis outline						
SECTION I	IMPACT OF CONCOMITANT MEDICATION ON DRUG METABOLIZER PHENOTYPE PREDICTIONS						
27	<b>CHAPTER 2</b> The impact of <i>CYP2C19</i> genotype on phenoconversion by concomitant medication <i>Published in Frontiers in Pharmacology (2023)</i>						
SECTION II	(PRE)CLINICAL EVALUATION OF INFLAMMATION-INDUCED ALTERATIONS IN DRUG METABOLISM						
65	CHAPTER 3 Distinct effects of inflammation on cytochrome P450 regulation and drug metabolism: Lessons from experimental models and a potential role for pharmacogenetics <i>Published in Genes (2020)</i>						
107	<b>CHAPTER 4</b> CYP and non-CYP drug metabolizing enzyme families exhibit differential sensitivities towards proinflammatory cytokine modulation <i>Published in Drug Metabolism and Disposition (2024)</i>						
139	<b>CHAPTER 5</b> A systematic review on disease-drug-drug interactions with immunomodulating drugs: A critical appraisal of risk assessment and drug labelling <i>Published in the British Journal of Clinical Pharmacology (2022)</i>						
SECTION III	IN VIVO TOOLS TO STUDY ALTERATIONS IN DRUG METABOLISM DURING (INFLAMMATORY) DISEASE						
175	CHAPTER 6 Changes in plasma clearance of CYP450 probe drugs may not be specific for altered in vivo enzyme activity under (patho)physiological conditions – how to interpret findings of probe cocktail studies  Published in Clinical Pharmacokinetics (2024)						
197	<b>CHAPTER 7</b> General discussion and perspectives						
APPENDICES 216 222 223	Nederlandse samenvatting Curriculum Vitae						
223	List of publications						



## 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 UDPglucuronosyltransferases (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

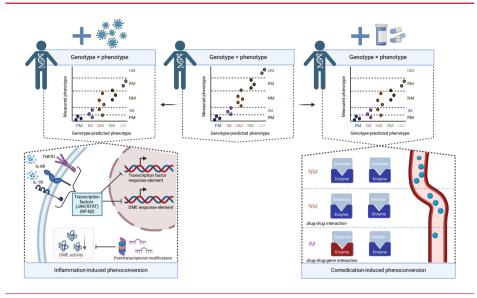
#### Interindividual variability in drug metabolism

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-1 $\beta$ , IL-6 and TNF- $\alpha$ , 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 metaboliteto-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 nonclinical 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.

#### References

- Spear BB, Heath-Chiozzi M, Huff J. Clinical application of pharmacogenetics. Trends Mol Med. 2001;7(5):201–4.
- Leendertse AJ, Egberts ACG, Stoker LJ, Van Den Bemt PMLA. Frequency of and risk factors for preventable medication-related hospital admissions in the Netherlands. Arch Intern Med. 2008 Sep 22:168(17):1890–6.
- 3. Davies EC, Green CF, Taylor S, Williamson PR, Mottram DR, Pirmohamed M. Adverse drug reactions in hospital in-patients: a prospective analysis of 3695 patient-episodes. PLoS One. 2009 Feb 11;4(2).
- 4. Ritter JM, Flower RJ. Rang & Dale's Pharmacology. 2023. 872 p.
- Rendic S, Guengerich FP. Survey of Human Oxidoreductases and Cytochrome P450 Enzymes Involved in the Metabolism of Xenobiotic and Natural Chemicals. Chem Res Toxicol. 2015 Jan 20:28(1):38–42.
- Danielson PB. The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Curr Drug Metab. 2002 Mar 25;3(6):561–97.
- Iyanagi T. Molecular mechanism of phase I and phase II drug-metabolizing enzymes: implications for detoxification. Int Rev Cytol. 2007;260:35–112.
- 8. Nelson DR. The cytochrome p450 homepage. Hum Genomics. 2009 Oct 1;4(1):59–65.
- 9. Iversen DB, Andersen NE, Dalgård Dunvald AC, Pottegård A, Stage TB. Drug metabolism and drug transport of the 100 most prescribed oral drugs. Basic Clin Pharmacol Toxicol. 2022 Nov 1;131(5):311–24.
- Pang X, Tang C, Guo R, Chen X. Noncytochrome P450 enzymes involved in the oxidative metabolism of xenobiotics: Focus on the regulation of gene expression and enzyme activity. Pharmacol Ther. 2022 May 1:233.
- 11. Saravanakumar A, Sadighi A, Ryu R, Akhlaghi F. Physicochemical Properties, Biotransformation, and Transport Pathways of Established and Newly Approved Medications: A Systematic Review of the Top 200 Most Prescribed Drugs vs. the FDA-Approved Drugs Between 2005 and 2016. Clin Pharmacokinet. 2019 Oct 1;58(10):1281–94.

- Spaggiari D, Geiser L, Daali Y, Rudaz S. A cocktail approach for assessing the in vitro activity of human cytochrome P450s: an overview of current methodologies. J Pharm Biomed Anal. 2014 May 1;101:221–37.
- Andrés F, LLerena A. Simultaneous
   Determination of Cytochrome P450
   Oxidation Capacity in Humans: A Review
   on the Phenotyping Cocktail Approach. Curr
   Pharm Biotechnol. 2016 Sep 28;17(13):1159–80
- 14. Ingelman-Sundberg M. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. Trends Pharmacol Sci. 2004 Apr;25(4):193–200.
- 15. PharmVar [Internet]. [cited 2024 Nov 29]. Available from: https://www.pharmvar.org/genes
- 16. Bank PCD, Caudle KE, Swen JJ, Gammal RS, Whirl-Carrillo M, Klein TE, et al. Comparison of the Guidelines of the Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group. Clin Pharmacol Ther. 2018 Apr 1;103(4):599–618.
- Swen JJ, Nijenhuis M, De Boer A, Grandia L, Maitland-Van Der Zee AH, Mulder H, et al. Pharmacogenetics: from bench to byte--an update of guidelines. Clin Pharmacol Ther. 2011 May;89(5):662–73.
- Genes-Drugs CPIC [Internet]. [cited 2023 Feb 26]. Available from: https://cpicpgx.org/ genes-drugs/
- 19. Claassens DMF, Vos GJA, Bergmeijer TO, Hermanides RS, van 't Hof AWJ, van der Harst P, et al. A Genotype-Guided Strategy for Oral P2Y12 Inhibitors in Primary PCI. N Engl J Med. 2019 Oct 24;381(17):1621–31.
- Pirmohamed M, Burnside G, Eriksson N, Jorgensen AL, Toh CH, Nicholson T, et al. A randomized trial of genotype-guided dosing of warfarin. N Engl J Med. 2013 Dec 12;369(24):2294–303.
- Swen JJ, van der Wouden CH, Manson LE, Abdullah-Koolmees H, Blagec K, Blagus T, et al. A 12-gene pharmacogenetic panel to prevent adverse drug reactions: an open-label, multicentre, controlled, clusterrandomised crossover implementation study. Lancet (London, England). 2023 Feb 4;401(10374):347–56.

- Zanger UM, Klein K, Thomas M, Rieger JK, Tremmel R, Kandel BA, et al. Genetics, epigenetics, and regulation of drugmetabolizing cytochrome P450 enzymes. Clin Pharmacol Ther. 2014 Mar;95(3):258–61.
- 23. Shah RR, Smith RL. Addressing phenoconversion: The Achilles' heel of personalized medicine. Br J Clin Pharmacol. 2015 Feb 1;79(2):222–40.
- Klomp SD, Manson ML, Guchelaar H-J, Swen JJ. Phenoconversion of Cytochrome P450 Metabolism: A Systematic Review. J Clin Med. 2020 Sep 7:9(9):2890.
- Jukić MM, Haslemo T, Molden E, Ingelman-Sundberg M. Impact of CYP2C19 Genotype on Escitalopram Exposure and Therapeutic Failure: A Retrospective Study Based on 2,087 Patients. Am J Psychiatry. 2018 May 1;175(5):463–70.
- 26. Shirasaka Y, Chaudhry AS, McDonald M, Prasad B, Wong T, Calamia JC, et al. Interindividual variability of CYP2C19-catalyzed drug metabolism due to differences in gene diplotypes and cytochrome P450 oxidoreductase content. Pharmacogenomics J. 2016 Aug 1;16(4):375–87.
- 27. Dalton R, Lee S been, Claw KG, Prasad B, Phillips BR, Shen DD, et al. Interrogation of CYP2D6 Structural Variant Alleles Improves the Correlation Between CYP2D6 Genotype and CYP2D6-Mediated Metabolic Activity. Clin Transl Sci. 2020 Jan 1;13(1):147–56.
- Kiss ÁF, Vaskó D, Déri MT, Tóth K, Monostory K. Combination of CYP2C19 genotype with non-genetic factors evoking phenoconversion improves phenotype prediction. Pharmacol Rep. 2018 Jun 1:70(3):525–32.
- 29. Tanner JA, Prasad B, Claw KG, Stapleton P, Chaudhry A, Schuetz EG, et al. Predictors of Variation in CYP2A6 mRNA, Protein, and Enzyme Activity in a Human Liver Bank: Influence of Genetic and Nongenetic Factors. J Pharmacol Exp Ther. 2017 Jan 1;360(1):129–39.
- Bahar MA, Setiawan D, Hak E, Wilffert B. Pharmacogenetics of drug-drug interaction and drug-drug-gene interaction: a systematic review on CYP2C9, CYP2C19 and CYP2D6. Pharmacogenomics. 2017 May 1;18(7):701– 39.

- 31. Storelli F, Matthey A, Lenglet S, Thomas A, Desmeules J, Daali Y. Impact of CYP2D6 Functional Allelic Variations on Phenoconversion and Drug-Drug Interactions. Clin Pharmacol Ther. 2018 Jul 1:104(1):148–57.
- 32. Hocum BT, White JR, Heck JW, Thirumaran RK, Moyer N, Newman R, et al. Cytochrome P-450 gene and drug interaction analysis in patients referred for pharmacogenetic testing. Am J Health Syst Pharm. 2016 Jan 15:73(2):61–7.
- 33. Turner RM, de Koning EM, Fontana V, Thompson A, Pirmohamed M. Multimorbidity, polypharmacy, and drugdrug-gene interactions following a non-ST elevation acute coronary syndrome: analysis of a multicentre observational study. BMC Med. 2020 Dec 1:18(1):367.
- 34. Verbeurgt P, Mamiya T, Oesterheld J. How common are drug and gene interactions? Prevalence in a sample of 1143 patients with CYP2C9, CYP2C19 and CYP2D6 genotyping. Pharmacogenomics. 2014;15(5):655–65.
- 35. Medzhitov R. Inflammation 2010: new adventures of an old flame. Cell. 2010;140(6):771–6.
- Aitken AE, Morgan ET. Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. Drug Metab Dispos. 2007 Sep;35(9):1687–93.
- Dickmann LJ, Patel SK, C. Wienkers L, Greg Slatter J. Effects of Interleukin 1β (IL-1β) and IL-1β/Interleukin 6 (IL-6) Combinations on Drug Metabolizing Enzymes in Human Hepatocyte Culture. Curr Drug Metab. 2012 Aug 2:13(7):930–7.
- 38. Klein M, Thomas M, Hofmann U, Seehofer D, Damm G, Zanger UM. A systematic comparison of the impact of inflammatory signaling on absorption, distribution, metabolism, and excretion gene expression and activity in primary human hepatocytes and HepaRG Cells. Drug Metab Dispos. 2015 Jan 1;43(2):273–83.
- 39. Tanner N, Kubik L, Luckert C, Thomas M, Hofmann U, Zanger UM, et al. Regulation of Drug Metabolism by the Interplay of Inflammatory Signaling, Steatosis, and Xeno-Sensing Receptors in HepaRG Cells. Drug Metab Dispos. 2018 Apr 1;46(4):326–35.

- Cressman AM, Petrovic V, Piquette-Miller M. Inflammation-mediated changes in drug transporter expression/activity: implications for therapeutic drug response. Expert Rev Clin Pharmacol. 2012 Jan;5(1):69–89.
- 41. Stanke-Labesque F, Gautier-Veyret E, Chhun S, Guilhaumou R. Inflammation is a major regulator of drug metabolizing enzymes and transporters: Consequences for the personalization of drug treatment. Pharmacol Ther. 2020 Nov 1;215.
- Schmitt C, Kuhn B, Zhang X, Kivitz AJ, Grange S. Disease–Drug–Drug Interaction Involving Tocilizumab and Simvastatin in Patients With Rheumatoid Arthritis. Clin Pharmacol Ther. 2011 May 23;89(5):735–40.
- Sanaee F, Clements JD, Waugh AWG, Fedorak RN, Lewanczuk R, Jamali F. Drugdisease interaction: Crohn's disease elevates verapamil plasma concentrations but reduces response to the drug proportional to disease activity. Br J Clin Pharmacol. 2011 Nov:72(5):787–97.
- 44. Fisher CD, Lickteig AJ, Augustine LM, Ranger-Moore J, Jackson JP, Ferguson SS, et al. Hepatic cytochrome P450 enzyme alterations in humans with progressive stages of nonalcoholic fatty liver disease. Drug Metab Dispos. 2009 Oct;37(10):2087–94.
- Gravel S, Chiasson JL, Turgeon J, Grangeon A, Michaud V. Modulation of CYP450 Activities in Patients With Type 2 Diabetes. Clin Pharmacol Ther. 2019 Dec 1:106(6):1280–9.
- 46. Strzelec M, Detka J, Mieszczak P, Sobocińska MK, Majka M. Immunomodulation—a general review of the current state-of-the-art and new therapeutic strategies for targeting the immune system. Front Immunol. 2023;14:1127704.
- 47. Committee for Medicinal Products for Human Use (CHMP). Guideline on the clinical investigation of the pharamcokinetics of therapeutic proteins. Chmp/ Ewp/89249/2004. 2007;(January).
- 48. U.S. Food and Drug Administration Center for Drugs Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER). Drug-Drug Interaction Assessment for Therapeutic Proteins. Guidance for Industry. FDA Guid. 2020;(August):1–11.

- Zhang L, Sarangi V, Moon I, Yu J, Liu D, Devarajan S, et al. CYP2C9 and CYP2C19: Deep Mutational Scanning and Functional Characterization of Genomic Missense Variants. Clin Transl Sci. 2020 Jul 1:13(4):727–42.
- 50. Kiamehr M, Heiskanen L, Laufer T, Düsterloh A, Kahraman M, Käkelä R, et al. Dedifferentiation of Primary Hepatocytes is Accompanied with Reorganization of Lipid Metabolism Indicated by Altered Molecular Lipid and miRNA Profiles. Int J Mol Sci. 2019 Jun 2;20(12).
- 51. Godoy P, Hewitt NJ, Albrecht U, Andersen ME, Ansari N, Bhattacharya S, et al. Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. Arch Toxicol. 2013 Aug;87(8):1315–530.
- 52. Docci L, Milani N, Ramp T, Romeo AA, Godoy P, Franyuti DO, et al. Exploration and application of a liver-on-a-chip device in combination with modelling and simulation for quantitative drug metabolism studies. Lab Chip. 2022 Jan 25;22(6):1187–205.
- 53. Klöditz K, Tewolde E, Nordling Å, Ingelman-Sundberg M. Mechanistic, Functional, and Clinical Aspects of Pro-inflammatory Cytokine Mediated Regulation of ADME Gene Expression in 3D Human Liver Spheroids. Clin Pharmacol Ther. 2023 Sep 1;114(3):673–85.
- 54. Guillouzo A, Corlu A, Aninat C, Glaise D, Morel F, Guguen-Guillouzo C. The human hepatoma HepaRG cells: a highly differentiated model for studies of liver metabolism and toxicity of xenobiotics. Chem Biol Interact. 2007 May 20;168(1):66–73.
- 55. Sager JE, Yu J, Ragueneau-Majlessi I, Isoherranen N. Physiologically Based Pharmacokinetic (PBPK) Modeling and Simulation Approaches: A Systematic Review of Published Models, Applications, and Model Verification. Drug Metab Dispos. 2015 Nov 1;43(11):1823–37.
- Machavaram KK, Almond LM, Rostami-Hodjegan A, Gardner I, Jamei M, Tay S, et al. A physiologically based pharmacokinetic modeling approach to predict disease-drug interactions: Suppression of CYP3A by IL-6. Clin Pharmacol Ther. 2013 Aug 1;94(2):260– 8.

- 57. Machavaram KK, Endo-Tsukude C, Terao K, Gill KL, Hatley OJ, Gardner I, et al. Simulating the Impact of Elevated Levels of Interleukin-6 on the Pharmacokinetics of Various CYP450 Substrates in Patients with Neuromyelitis Optica or Neuromyelitis Optica Spectrum Disorders in Different Ethnic Populations. AAPS J. 2019 May 1:21(3).
- 58. Xu Y, Hijazi Y, Wolf A, Wu B, Sun YN, Zhu M. Physiologically Based Pharmacokinetic Model to Assess the Influence of Blinatumomab-Mediated Cytokine Elevations on Cytochrome P450 Enzyme Activity. CPT pharmacometrics Syst Pharmacol. 2015 Sep 1:4(9):507–15.
- 59. Jiang X, Zhuang Y, Xu Z, Wang W, Zhou H. Development of a Physiologically Based Pharmacokinetic Model to Predict Disease-Mediated Therapeutic Protein-Drug Interactions: Modulation of Multiple Cytochrome P450 Enzymes by Interleukin-6. AAPS I. 2016 Mar 9:18(3):767-76.
- 60. Lenoir C, Niederer A, Rollason V, Desmeules JA, Daali Y, Samer CF. Prediction of cytochromes P450 3A and 2C19 modulation by both inflammation and drug interactions using physiologically based pharmacokinetics. CPT pharmacometrics Syst Pharmacol. 2022 Jan 1;11(1):30–43.
- 61. Kanacher T, Lindauer A, Mezzalana E, Michon I, Veau C, Mantilla JDG, et al. A Physiologically-Based Pharmacokinetic (PBPK) Model Network for the Prediction of CYP1A2 and CYP2C19 Drug-Drug-Gene Interactions with Fluvoxamine, Omeprazole, S-mephenytoin, Moclobemide, Tizanidine, Mexiletine, Ethinylestradiol, and Caffeine. Pharmaceutics. 2020 Dec 1;12(12):1–15.
- 62. Wojtyniak JG, Selzer D, Schwab M, Lehr T. Physiologically Based Precision Dosing Approach for Drug-Drug-Gene Interactions: A Simvastatin Network Analysis. Clin Pharmacol Ther. 2021 Jan 1;109(1):201–11.
- 63. Feick D, Rüdesheim S, Marok FZ, Selzer D, Loer HLH, Teutonico D, et al. Physiologically-based pharmacokinetic modeling of quinidine to establish a CYP3A4, P-gp, and CYP2D6 drug-drug-gene interaction network. CPT pharmacometrics Syst Pharmacol. 2023 Aug 1;12(8):1143–56.

- 64. Kovar C, Kovar L, Rüdesheim S, Selzer D,
  Ganchev B, Kröner P, et al. Prediction of
  Drug-Drug-Gene Interaction Scenarios of
  (E)-Clomiphene and Its Metabolites Using
  Physiologically Based Pharmacokinetic
  Modeling, Pharmaceutics, 2022 Dec 1:14(12).
- Storelli F, Desmeules J, Daali Y.
   Physiologically-Based Pharmacokinetic
   Modeling for the Prediction of CYP2D6 Mediated Gene-Drug-Drug Interactions.
   CPT pharmacometrics Syst Pharmacol.
   2019:8(8):567-76.
- 66. European Medicines Agency. Guideline on key aspects for the use of pharmacogenomic methodologies in the pharmacovigilance evaluation of medicinal products (Draft). Ema/281371/2013. 2014;44(January):1–18.
- 67. M12 Drug Interaction Studies | FDA [Internet]. [cited 2024 Nov 29]. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m12-drug-interaction-studies
- 68. Ghasim H, Rouini M, Safari S, Larti F, Khoshayand M, Gholami K, et al. Impact of Obesity and Bariatric Surgery on Metabolic Enzymes and P-Glycoprotein Activity Using the Geneva Cocktail Approach. J Pers Med. 2023 Jul 1;13(7).
- Tracy TS, Venkataramanan R, Glover DD, Caritis SN. Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. Am J Obstet Gynecol. 2005;192(2):633–9.
- Lenoir C, Daali Y, Rollason V, Curtin F, Gloor Y, Bosilkovska M, et al. Impact of Acute Inflammation on Cytochromes P450 Activity Assessed by the Geneva Cocktail. Clin Pharmacol Ther. 2021 Jun 1;109(6):1668–76.



#### Section I

# Impact of concomitant medication on drug metabolizer phenotype predictions



## Chapter 2

## The impact of *CYP2C19* genotype on phenoconversion by concomitant medication

Laura M. de Jong, Soukayna Boussallami, Elena Sánchez-López, Martin Giera, Maarten E. Tushuizen, Menno Hoekstra, Lukas J. A. C. Hawinkels, Robert Rissmann, Jesse J. Swen, Martijn L. Manson



#### **Abstract**

**INTRODUCTION:** Pharmacogenetics-informed drug prescribing is increasingly applied in clinical practice. Typically, drug metabolizing phenotypes are determined based on genetic test results, whereupon dosage or drugs are adjusted. Drug-drug-interactions (DDIs) caused by concomitant medication can however cause mismatches between predicted and observed phenotypes (phenoconversion). Here we investigated the impact of *CYP2C19* genotype on the outcome of CYP2C19-dependent DDIs in human liver microsomes.

**METHODS:** Liver samples from 40 patients were included, and genotyped for *CYP2C19\*2*, \*3 and \*17 variants. S-mephenytoin metabolism in microsomal fractions was used as proxy for CYP2C19 activity, and concordance between genotype-predicted and observed CYP2C19 phenotype was examined. Individual microsomes were subsequently co-exposed to fluvoxamine, voriconazole, omeprazole or pantoprazole to simulate DDIs.

**RESULTS:** Maximal CYP2C19 activity (V<sub>max</sub>) in genotype-predicted intermediate metabolizers (IMs; \*1/\*2 or \*2/\*17), rapid metabolizers (RMs; \*1/\*17) and ultrarapid metabolizers (UMs; \*17/\*17) was not different from  $V_{max}$  of predicted normal metabolizers (NMs; \*1/\*1). Conversely, CYP2C19\*2/\*2 genotyped-donors exhibited V rates ~9% of NMs, confirming the genotype-predicted poor metabolizer (PM) phenotype. Categorizing CYP2C19 activity, we found a 40% concordance between genetically-predicted CYP2C19 phenotypes and measured phenotypes, indicating substantial phenoconversion. Eight patients (20%) exhibited CYP2C19 IM/PM phenotypes that were not predicted by their CYP2C19 genotype, of which six could be linked to the presence of diabetes or liver disease. In subsequent DDI experiments, CYP2C19 activity was inhibited by omeprazole (-37% ± 8%), voriconazole (-59%  $\pm$  4%) and fluvoxamine (-85%  $\pm$  2%), but not by pantoprazole (-2 ± 4%). The strength of CYP2C19 inhibitors remained unaffected by CYP2C19 genotype, as similar percental declines in CYP2C19 activity and comparable metabolism-dependent inhibitory constants  $(K_{inact}/K_t)$  of omeprazole were observed between CYP2C19 genotypes. However, the consequences of CYP2C19 inhibitor-mediated phenoconversion were different between CYP2C19 genotypes. In example, voriconazole converted 50% of \*1/\*1 donors to a IM/PM phenotype, but only 14% of \*1/\*17 donors. Fluvoxamine converted all donors to phenotypic IMs/PMs, but \*1/\*17 (14%) were less likely to become PMs than \*1/\*1 (50%) or \*1/\*2 and \*2/\*17 (57%).

**CONCLUSION:** This study suggests that the differential outcome of CYP2C19-mediated DDIs between genotypes are primarily dictated by basal CYP2C19 activity, that may in part be predicted by *CYP2C19* genotype but likely also depends on disease-related factors.

28 SECTION I • CHAPTER 2

#### Introduction

Pharmacogenetics aims to increase patient safety and drug efficacy by tailoring drug treatment to an individual's genetic profile. Based on this genetic profile, patients can be categorized into drug metabolizing phenotypes which subsequently can be used for selecting the right drug and optimal dose. Therapeutic guidance for actionable drug-gene interactions (DGIs) have been developed by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetic Work Group (DPWG) for over 75 drugs (1,2). However, a common problem encountered using drug metabolizing phenotypes is that a patient's genetically-predicted phenotype can deviate from its actual metabolizer status – a phenomenon called phenoconversion (3,4).

Non-genetic factors that skew this genotype-based prediction include inflammatory or liver diseases as well as drug-drug interactions (DDIs) caused by concomitant medication use (3). The individual impact of genetic polymorphisms and DDIs on pharmacokinetics of drugs has been vastly investigated. However, the interplay between pharmacogenetics and DDIs that may result in drug-drug-gene interactions (DDGIs) is not yet taken into account in clinical practice. Importantly, DDGIs account for up to 20% of total major or substantial drug interactions and are thus a clinical concern (5,6).

Numerous studies demonstrate that a patient's genotype determines the clinical relevance of a DDGI (7). For example, Storelli *et al.* showed that the presence of one nonfunctional CYP2D6 allele increases the risk of phenoconversion to a poor metabolizer (PM) status in the presence of a CYP2D6 inhibitor (8). This suggests that the occurrence of DDIs in patients with reduced enzyme functionality at baseline creates a higher susceptibility for phenoconversion towards an actionable genotype. In contrast, PMs are not considered prone to DDIs involving the same enzyme, as these individuals already exhibit null enzymatic activity at baseline. Considering the importance of DDI-induced phenoconversion, CPIC guidelines suggest that the concomitant use of CYP2D6 inhibitors should be taken into account for calculating the genotype-based activity score (9).

The *CYP2C19* gene is highly polymorphic and responsible for metabolism of frequently prescribed proton-pump inhibitors (PPIs) and other commonly used drugs including clopidogrel and antidepressants. A large proportion of CYP2C19-related drugs acts as CYP2C19 inhibitors, for which concomitant use may result

in DDIs. As a consequence, concomitant medication use may commonly lead to phenoconversion of CYP2C19-mediated metabolism. For instance, when considering phenoconversion caused by DDGIs, the CYP2C19 PM phenotype was found 5-fold more frequently than expected based on genotype alone in a group of 2905 patients (10). Consequently, the predicted phenotype based on genotype solely could be erroneous when concomitant use of CYP2C19 inhibitors is not contemplated while predicting CYP2C19 phenotype. However, phenoconversion rates for CYP2C19-mediated drug metabolism following treatment with an inhibitor have not been determined due to sparse availability of data to help predict the drug metabolizing phenotype after inhibitor use.

To ultimately provide concise DDGI recommendations that combine knowledge on pharmacogenetics and concomitant medication use, it is important to gain a quantitative understanding of the phenoconversion that occurs after co-administration of an inhibitor of the same enzyme. To this end, we aimed to quantify to what extent *CYP2C19* polymorphisms can impact the outcome of a DDI with various CYP2C19 inhibitors in human liver microsomes. Firstly, we set out to assess the genotype-phenotype discordance in this cohort and link this to known phenoconversion risk factors. We then investigated whether the intrinsic inhibitory activity of the most prescribed PPI and CYP2C19 inhibitor omeprazole was affected by the *CYP2C19* genotype. Lastly, we quantified phenoconversion after co-administration of various clinically relevant CYP2C19 inhibitors.

#### Materials and methods

#### Human liver samples

Macroscopically healthy liver samples from 40 patients with colorectal cancer derived liver metastasis were retrieved from the gastroenterology biobank at the Leiden University Medical Center (LUMC, Leiden, Netherlands). Fresh tissue samples were obtained directly after surgery, and macroscopically healthy liver tissues distant from the metastasis (at tumor free resection margins) were collected, snap frozen end stored at -80°C until use. The collection and use of these samples was approved by the Medical Ethics Committee of Leiden Den Haag Delft, Netherlands through protocol B21.072 entitled "The modulating potential of CYP450 genetic variability on phenoconversion by concomitant medication."

30 SECTION I • CHAPTER 2

#### Genotyping

Genomic DNA from the human liver samples was extracted using the NucleoSpin Tissue mini kit from Macherey-Nagel (Hoerdt, France). The *CYP2C19* variant alleles *CYP2C19\*2* (NC\_000010.11: g.94781859G>A), *CYP2C19\*3* (NC\_000010.11: g.94780653G>A), and *CYP2C19\*17* (NC\_000010.11: g.94761900C>T) were analyzed using pre-designed TaqMan-based real-time polymerase chain reaction (PCR) assays, with probes obtained from ThermoFisher. The Quantstudio and ViiA7 systems were employed for analysis. All genotyping was conducted following standard protocols used in routine diagnostics, in an ISO-15189 certified laboratory. The variants were checked for Hardy-Weinberg equilibrium. Predicted phenotypes were assigned using conventional methods based on translation tables from CPIC and DPWG (11).

#### RNA preparation and real time-qPCR

Liver RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Concentration and purity of RNA was subsequently measured using a NanoDrop 3300 (Thermo Scientific, Wilmington, US). RNA was reverse-transcribed into cDNA using a RevertAid H Minus First Strand cDNA Synthesis kit (Thermo Scientific, Wilmington, US) according to the instructions provided. RT-qPCR analysis was performed using a QuantStudio™ 6 Flex System.

All PCR primers were designed in-house and subsequently checked for amplification efficiency through a serial dilution of cDNA where 90–110% efficiency was desired (Supplementary Table S1). A *CYP2C19* primer targeting exon 9 was designed to amplify total *CYP2C19* mRNA. As this primer does not distinguish between mRNA encoding for functional or non-functional CYP2C19 protein, an additional exon-spanning primer pair was designed that could predominantly detect functional mRNA. This was achieved through a reverse primer binding within the first 40 basepairs of exon 5, as this region is deleted in *CYP2C19\*2* carriers and the most commonly observed variant linked to the formation of non-functional CYP2C19 protein (12).

Relative mRNA levels were calculated using the comparative Ct method and normalized to the geometric mean of the housekeeping genes Ribosomal Protein Lateral Stalk Subunit P0 (RPLP0) and RNA Polymerase II, I and III Subunit

L (POLR2L), which were determined as the most stable endogenous controls through GNOrm software analysis (13).

#### Liver microsomal preparations

Human liver microsomes were prepared from obtained liver resections with the aid of a microsome isolation kit from Sigma-Aldrich (St. Louis, MO, United States). Total protein concentrations were determined in triplicate with the BCA protein assay (Pierce, Rockford, IL, United States). Aliquots of the final microsomal suspension were stored at -80°C. The microsomal protein per gram of liver (MPPGL, mg/g) was calculated by dividing the microsomal protein yield by the liver weight input and was on average  $7.4 \pm 2.0$  mg/g in this cohort. Individual microsomal preparations were used for all experiments except for the experiment in which inhibitory parameters of omeprazole were determined. In these omeprazole-related experiments, genotype-matched microsome pools where generated by pooling an equal amount of microsomal protein from either 8 (\*1/\*17), 16 (\*1/\*1) or 10 (\*1/\*2 or \*2/\*17) donors.

#### CYP2C19 activity assays in microsomes

#### Kinetic analysis of CYP2C19 dependent S-mephenytoin hydroxylation

Various concentrations of S-mephenytoin (1–400  $\mu$ M) were incubated with individual genotyped human liver microsomes (final protein concentration: 0.03 mg/mL) in 200  $\mu$ L incubation mixtures containing 0.05 mM potassium phosphate buffer (pH 7.4) with MgCl<sub>2</sub> (3 mM), EDTA (1 mM), NADP (1 mM), glucose-6-phosphate (5 mM) and glucose-6-phosphate dehydrogenase (1 unit/mL). Incubations were performed in duplicate in Protein LoBind\* Tubes (Eppendorf, Hamburg, Germany). After 30 min, reactions were terminated by the addition of equal volumes of ice-cold acetonitrile containing the internal standard 4'-hydroxymephenytoin-d<sub>3</sub> (20 ng/mL). Insoluble protein was precipitated by centrifugation (10,000 × g for 5 min at 4°C), and supernatant was diluted 2.5 times in LC-MS quality water before 4'-hydroxymephenytoin concentration measurements. A validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay was used to quantify 4'-hydroxymephenytoin (see "Quantification of 4'-hydroxymephenytoin by LC-MS/MS, Supplementary Material").

32 SECTION I • CHAPTER 2

#### Determination of kinetic parameters

Maximal velocity of S-mephenytoin 4'-hydroxylation (Vmax) and affinity (Km) values were obtained for each individual donor by fitting individual data to the Michaelis-Menten equation: V=Vmax[S]/Km[S] in Graphpad Prism 9 (Graphpad Software, San Diego, CA), where V represents the initial metabolism rate of S-mephenytoin (pmol/min/mg protein) and [S] represents the S-mephenytoin substrate concentration ( $\mu$ M). No Michaelis-Menten curve fitting was done for donors with non-saturable product formation kinetics. For these donors,  $V_{max}$  values were estimated by means of simple linear regression.  $K_{m}$  values were only determined when S-mephenytoin 4'-hydroxylation followed Michaelis-Menten kinetics. To analyze the kinetic parameters for S-mephenytoin 4'-hydroxylation across donors with the same genotype, non-linear least-squares analysis in Graphpad Prism was done without restrictions.

#### Determination of basal phenoconversion in cohort

CYP2C19 genotypes were first used to predict the drug metabolizing activity of donors classified into the phenotype categories: ultrarapid metabolizer (UM), rapid metabolizer (RM), normal metabolizer (NM), intermediate metabolizer (IM) and poor metabolizer (PM), according to CPIC guidelines (11). Secondly, cut-off values for the metabolic activity of phenotype groups were defined based on the study by Kiss *et al.*, in which S-mephenytoin hydroxylation at a saturating substrate concentration was determined in genotyped liver microsomes of 114 donors (14). Since Kiss *et al.* did not define a RM group, boundaries between NMs and RMs were determined using the same method and thus based on the median S-mephenytoin hydroxylation activity in 24 donors. Hence, cut-off values between the phenotypic groups PM/IMs, IMs/NMs, NMs/RMs and RMs/UMs were set in this study at 8, 23, 58, and 75 pmol/min/mg protein respectively.

The observed maximal S-mephenytoin hydroxylation activity in individual donors was then compared to the expected activity for these donors based on their genotype-predicted phenotype. Concordance/non-concordance between measured and genotype-predicted hydroxylation activity was determined for every individual donor to indicate basal phenoconversion.

#### Determination of inhibitor-induced phenoconversion

#### Inhibitor concentrations

To simulate the outcome of DDIs for different CYP2C19 genotypes, individual microsomal fractions were co-exposed to clinically relevant concentrations of the CYP2C19 inhibitors fluvoxamine, voriconazole, omeprazole or pantoprazole. Concentrations were based on the calculated unbound maximum hepatic inlet concentration in plasma ( $I_{in,max,u}$ ), which incorporates both the drug entering the liver from the systemic circulation as well as the drug entering the liver from the gut via the hepatic portal vein following the equation (15):

$$I_{in,max,u} = Fu_p \left( Plasma I_{max} + \frac{\left( \frac{Dose*Fa*Fg*Ka}{Qh} \right)}{Rb} \right)$$

where  $Fu_p$  is the fraction unbound in plasma, Plasma  $I_{max}$  represents the total systemic  $C_{max}$  in plasma, Dose is the oral dose, Fa\*Fg represent the fraction of drug absorbed from the gastrointestinal tract into the hepatic portal blood, Ka is the rate of absorption of drug from the intestine, Qh is the hepatic blood flow and Rb the drug concentration in blood to the drug concentration in plasma.

Input parameters were retrieved from literature and are described in Table 1, as well as the final calculated  $I_{\text{in,max,u}}$  used in this assay. The calculation of the  $I_{\text{in,max,u}}$  was based on the clinically standard starting dose for all inhibitors. The Qh was assumed to be 1.62 L/min (as used by all regulatory agencies). Input plasma  $I_{\text{max}}$  values are detailed in the Supplementary Material under "Calculating the unbound maximum hepatic inlet concentration".

**Table 1** Input parameters for calculating the unbound maximum hepatic inlet concentration in plasma  $(I_{in,max,u})$ . In the absence of experimentally determined values, the Ka was assumed to be 0.1 min<sup>-1</sup>, and the Fa<sup>\*</sup>Fg and Rb were assumed to be 1 (15).

	Dose (mg)	Dose (µmol)	Mean plasma I <sub>max</sub> (μΜ)*	Ka (min <sup>-1</sup> )	Refs Ka	Rb	Refs Rb	Fraction unbound in plasma (Fu <sub>p</sub> )**	I <sub>in,max,u</sub> (μM)
Fluvoxamine	100	314.0	0.3	0.020	(16)	1.0		0.25	1.0
Omeprazole	40	115.8	3.3	0.100	(17)	0.6	(17)	0.05	0.8
Voriconazole	200	572.6	7.3	0.012	(18)	2.1	(19)	0.42	3.9
Pantoprazole	40	104.3	6.5	0.018	(20)	1.0		0.02	0.2

<sup>\*</sup> References for mean plasma Imax levels can be found in the supplementary method.

34 SECTION I • CHAPTER 2

<sup>\*\*</sup> Fraction unbound was derived from the drug prescribing information.

#### Incubations with inhibitors

From the 40 donors, 10 donors had a maximum rate of formation lower than 10 pmol/min/mg protein in the absence of inhibitors, which corresponds to a PM phenotype. These donors were therefore excluded in subsequent experiments in which the consequences of the different CYP2C19 inhibitors were determined. To assess the direct inhibition of CYP2C19 by fluvoxamine, voriconazole and pantoprazole for the 30 individual donors, the selected concentrations of inhibitors were incubated with 30 µM of S-mephenytoin (frequently reported Km value), microsomes (0.03 mg/mL) and the NADPH generating system described above in 0.05 mM phosphate buffer (pH = 7.4) for 7 min. Incubations without inhibitor served as control. Omeprazole is a metabolism-dependent inhibitor (MDI) of CYP2C19, meaning that the formation of omeprazole metabolites increases the inhibitory potency of omeprazole over time (17). To simulate the MDI of CYP2C19 by omeprazole, omeprazole was pre-incubated at 37°C with NADPH-fortified microsomes for 40 min. After the pre-incubation, S-mephenytoin (30 µM, final) was supplemented and the incubation time was continued for 7 min to measure residual CYP2C19 activity. Incubations without omeprazole but with 40 min preincubation served as control.

#### Cut-off values phenotype groups

Published thresholds for defining CYP2C19 phenotype categories are only available at formation rates determined with maximal substrate stimulation (14). In order to investigate DDI-induced phenoconversion, the rate of formation for individual donors was determined at S-mephenytoin concentration of 30  $\mu M$ . A calculated scaling factor (activity at 400  $\mu M/activity$  at 30  $\mu M)$  was used to transform the phenotype cut-off thresholds used at maximum substate formation. Accordingly, thresholds between the phenotypic groups PM/IMs, IMs/NMs, NMs/RMs and RMs/UMs were 5, 14, 40 and 53 pmol/min/mg protein.

#### K<sub>I</sub> and K<sub>inact</sub> determinations for omeprazole

 $K_{\rm I}$  (inhibitor concentration that supports half the maximal rate of inactivation) and  $K_{\rm inact}$  (maximal rate of enzyme inactivation) parameters were determined as described by Ogilvie *et al.* (17), using the non-dilution method (21). In order to determine  $K_{\rm I}$  and  $K_{\rm inact}$  values for the inactivation of CYP2C19 by omeprazole, genotype-pooled microsomes were pre-incubated with various concentrations of

omeprazole (1–30  $\mu M)$  for 0–30 min at 37°C. After pre-incubation, S-mephenytoin (30  $\mu M)$  was added and residual CYP2C19 activity was determined as described under "Kinetic analysis of CYP2C19 dependent S-mephenytoin hydroxylation."  $K_{_{I}}$  and  $K_{_{inact}}$  parameters were determined using non-linear regression in Graphpad Prism 9

#### Chemicals and reagents

S-mephenytoin, 4'-hydroxymephenytoin, 4'-hydroxymephenytoin- $d_3$ , voriconazole and omeprazole were purchased from LGC (Wesel, Germany). Fluvoxamine maleate was purchased from Tocris (Bristol, United Kingdom). Pantoprazole sodium, nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate and glucose-6-phosphate dehydrogenase from baker's yeast (*S. cerevisiae*) were purchased from Sigma-Aldrich. Acetonitrile, methanol, water and formic acid of LC-MS grade were obtained from Merk (Darmstadt, Germany).

#### Statistical analysis

For data which showed no normal distribution based on the Shapiro-Wilk test of normality and QQ-plots, the Kruskal–Wallis test was performed followed by a Dunnett's multiple comparison test to compare genotype-groups. For normally distributed data, the one-way ANOVA followed by a Dunnett's multiple comparison test was used. Correlation analysis were performed with the non-parametric Spearman test. A p-value of < 0.05 was considered to be statistically significant.

#### Results

#### Patient characteristics

A total of 40 liver samples from 15 female, 23 male and 2 donors of unknown sex were included in the study. The patient characteristics are summarized in Table 2. Complete information on age, body mass index (BMI), comorbidities and concomitant medication use at the time of surgery was not always available from the medical records. Of the donors, 12.5% suffered from an additional liver disease, 17.5% from a chronic inflammatory disease, 12.5% patients had diabetes mellitus and 5% of patients used CYP2C19 inhibitors before surgery.

 Table 2
 Population characteristics of the cohort.

	Mean (N)	Range
Age (years)	62.6 (38)	42-87
BMI (kg/m²)	26.8 (28)	18-37
	N	%
Sex		
Female	15	37.5
Male	23	57.5
Unknown	2	5.0
Liver disease		
Cirrhosis	1	2.5
Cholangitis	2	5.0
Choledocholithiasis	1	2.5
Liver abscess	1	2.5
None	30	75.0
Unknown	5	12.5
Inflammatory disease		
Skin	2	5.0
Lung	4	10.0
Joins	1	2.5
None	29	72.5
Unknown	5	12.5
Diabetes mellitus		
Present	5	12.5
Not present	30	75.0
Unknown	5	12.5
Drug use before operation		
CYP2C19 inhibitor	2	5.0
CYP2C19 inducer	0	0.0
None	20	50.0
Unknown	18	45.0

#### Genotyping

Liver donors were genotyped for *CYP2C19* variants \*1, \*2, \*3, and \*17. All allele variants were consistent with Hardy-Weinberg equilibrium (\*2:  $x^2 = 3.2$ , p = 0.07, \*17:  $x^2 = 0.4$ , p = 0.54, \*1:  $x^2 = 2.05$ , p = 0.15). *CYP2C19*\*3 was not detected in the study samples. *CYP2C19* genotype frequencies and predicted phenotypes are summarized in Table 3. Expected genotype frequencies were in concordance with reported frequencies in the PharmGKB database for Europeans (11).

**Table 3** Genotype distribution and frequency in this study population and corresponding mean kinetic parameters  $(V_{max} \text{ and } K_m)$  for CYP2C19-catalyzed S-mephenytoin metabolism per CYP2C19 genotype. Kinetic parameters were obtained from the data presented in Figure 1A. \* p < 0.05, significantly different from kinetic parameter in CYP2C19\*1/\*1 donors.

CYP2C19 genotype	Observed frequency N (%)	Expected frequency* (%)	Genotype- predicted phenotype <sup>&amp;</sup>	V <sub>max</sub> (pmol/min/ mg protein) Mean ± SD	K <sub>m</sub> (μM) Mean ± SD
*1/*1	16 (40.0)	39.1	NM	50.2± 36.5	18.4±4.8
*1/*2	7 (17.5)	18.3	IM	32.3±28.1	21.2±5.5
*2/*17	3 (7.5)	6.3	IM	$42.2 \pm 37.5$	23.0±7.4
*2/*2	4 (10.0)	2.2	PM	4.3± 2.9*	-
*1/*17	8 (20.0)	26.7	RM	60.4± 32.2	18.8±3.9
*17/*17	2 (5.0)	4.6	UM	$28.1 \pm 6.1$	33.4±8.4
Total	40 (100)				

<sup>\*</sup> Based on genotype frequencies for Europeans in PharmGKB. \* Translation based on PharmGKB database (11). NM = normal metabolizer, IM = intermediate metabolizer, PM = poor metabolizer, RM = rapid metabolizer, UM = ultrarapid metabolizer.

#### Impact of genotype on CYP2C19-mediated metabolism of S-mephenytoin

CYP2C19 activity was measured in all genotyped liver microsomes using S-mephenytoin as a probe substrate. Formation of 4'-hydroxymephenytoin was saturable for all investigated genotypes, with the exception of the \*2/\*2 genotype (Figure 1A). Michaelis-Menten parameters were obtained from the kinetic analysis of individual donors (Table 3). Mean maximal velocity rates ( $V_{max}$ ) were comparable to S-mephenytoin 4'-hydroxylation activities in microsomes published by Shirasaka *et al.* (22). Compared with the *CYP2C19\*1/\*1* genotype, donors with the *CYP2C19\*2/\*2* genotype exhibited decreased  $V_{max}$  values (~9% of \*1/\*1, p = 0.04).  $V_{max}$  values of all other genotypes did not differ from that of \*1/\*1. CYP2C19 substrate affinities ( $K_{m}$ ) were, as expected, not different between genotype groups. Importantly,  $K_{m}$  values were comparable to published microsomal affinity values of S-mephenytoin for CYP2C19 (22).

To investigate basal phenoconversion, genotype-predicted drug metabolizing phenotypes (PM, IM, NM, RM or UM) were compared to the observed activities of individual donors (Figure 1B). All genetically-predicted PMs indeed showed a PM phenotype, indicative of a complete loss of functional CYP2C19 activity. However, the 4'-hydroxylation activity of six other donors also corresponded to a PM phenotype. In contrast, five donors showed an UM phenotype despite not having two increased function alleles (\*17). Altogether, a relatively low concordance (40%)

was observed between measured CYP2C19 metabolizing phenotype for the donors within this study and literature based genotype-predicted phenotypes, suggesting the occurrence of phenoconversion in absence of concomitant medication use.

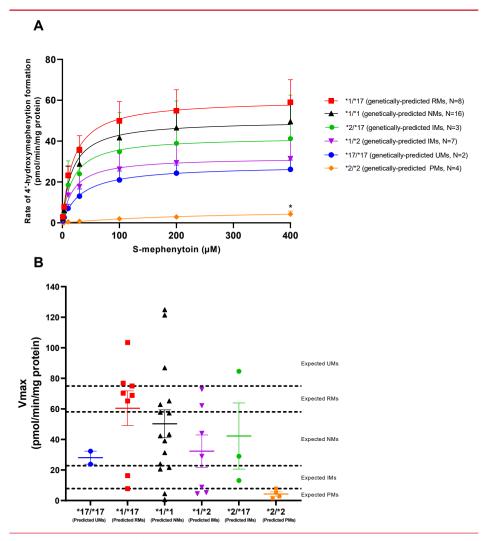


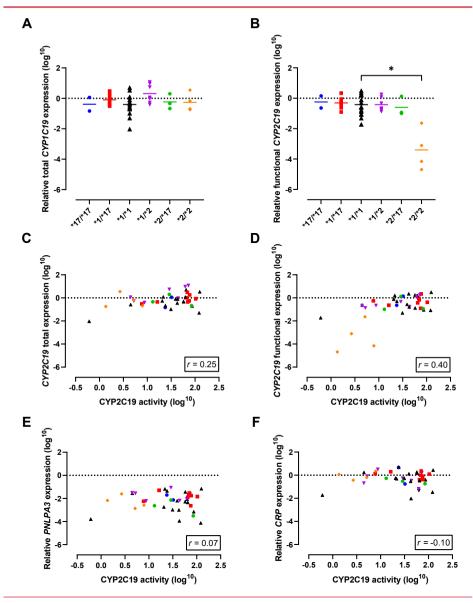
Figure 1 Kinetic analysis of CYP2C19-mediated S-mephenytoin metabolism in genotype-matched donors. (A) Mean velocities +SEM at each substrate concentration are shown. Between genotype-group comparisons of maximal 4'-hydroxymephenytoin formation was done using a Kruskal–Wallis test with a Dunn's multiple comparisons test to \*1/\*1. \* p < 0.05. (B) Maximal measured CYP2C19 activity (symbols) versus genetically-predicted maximal CYP2C19 activities from literature (dotted lines) in subjects with different *CYP2C19* genotypes. Cut-off values for CYP2C19 phenotype groups are based on Kiss *et al.* (14). Means per genotype + SEM are shown.

#### Correlation between CYP2C19 mRNA levels and metabolic activity

CYP2C19 enzyme activity is both affected by genetic polymorphisms as well as disease-related factors including inflammation and chronic liver disease (23). We therefore set out to assess the predictive relationship of *CYP2C19* mRNA expression levels for CYP2C19 activity, and link demographic variables from this cohort to metabolic activity to find explanations for the observed discrepancy between genotype-predicted activity and measured metabolizing phenotype.

First, total *CYP2C19* mRNA transcriptional levels for the different genotypes were examined. The different genotype groups did not exhibit differences in total *CYP2C19* mRNA expression levels (Figure 2A). One significant limitation of mRNA expression studies is that the functional consequences of the mRNA produced are often not considered. In the case of CYP2C19, the presence of the *CYP2C19\*2* allele is linked to splicing defects in mRNA production and the formation of inactive protein (12). To address this limitation, we utilized a primerpair that primarily detects functional mRNA rather than *CYP2C19\*2* mRNA. Indeed, functional *CYP2C19* expression levels were dramatically reduced in the \*2/\*2 genotype as compared to the \*1/\*1 genotype (p = 0.01, Figure 2B). Mean functional *CYP2C19* expression levels followed the rank order of \*17/\*17, \*1/\*17, \*1/\*17, \*1/\*2, \*2/\*17, and was lowest for \*2/\*2, as would be expected based on allele functionality.

Next, mRNA expression levels were correlated to measured CYP2C19 metabolizing activities to investigate a potential predictive relationship. Total *CYP2C19* expression levels did not correlate with CYP2C19 activity (r = 0.25, p = 0.12, Figure 2C). In contrast, the activity level of CYP2C19 was positively correlated with functional *CYP2C19* mRNA levels (r = 0.40, p = 0.01, Figure 2D), suggesting transcriptional regulation may in part explain the differences in enzyme activity between the genotype groups. It should however be noted that this increased positive correlation as compared to total mRNA levels was mainly driven by PM donors.



**Figure 2** Gene expression analysis in the cohort to investigate the observed discrepancy between genotype-predicted CYP2C19 activity and measured CYP2C19 activity. (**A**) Total *CYP2C19* mRNA expression stratified per genotype. Individual values + means per genotype are presented. (**B**) Levels of mRNA that lead to functional CYP2C19 protein stratified per genotype. Individual values + means are presented. (**C**) Correlation between *CYP2C19* mRNA and enzyme activity for total mRNA levels and (**D**) levels of mRNA that lead to functional CYP2C19 protein. (**E**) Correlation between CYP2C19 enzyme activity and known regulators of CYP2C19 activity: liver disease (PNPLA3) and (**F**) inflammation (CRP). Blue circles represent \*17/\*17 donors, red squares represent \*1/\*17 donors, black triangles represent \*1/\*1 donors, purple triangles represent \*1/\*2 donors, green circles represent \*2/\*17 donors and orange diamond represent \*2/\*2 donors. Spearman correlation (*r*) was calculated using GraphPad Prism 9.

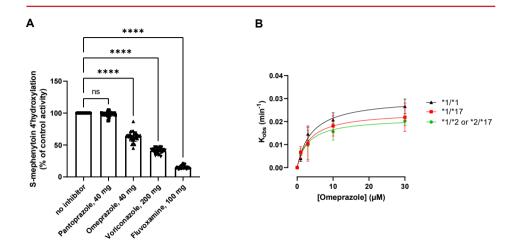
### Influence of disease-related factors and concomitant medication on CYP2C19 metabolic activity

Liver disease is a non-genetic factor shown to alter CYP450 activity (24,25). PNPLA3 is an established genetic marker of progressive liver disease (26), but PNPLA3 mRNA expression did not correlate to CYP2C19 activity in this cohort (r = 0.07, p = 0.68, Figure 2E). Among the five patients with confirmed liver disease, the presence of cirrhosis, cholangitis or liver abscess was associated with lower CYP2C19 activity compared to what's expected based on genotype. Importantly, this included two genetically-predicted RMs that phenoconverted to an IM or PM phenotype, and one \*1/\*1 donor that converted to a PM phenotype. Diabetes mellitus is recently identified as a modifying factor of CYP2C19 activity, with patients displaying mean reduced activity of ~50%. In our cohort, 5 patients suffered from diabetes mellitus of which one was genetically-predicted PM. For the other four donors, three of them showed phenoconversion to a PM phenotype. Inflammation is another non-genetic factors altering CYP2C19 activity (28). Overall, there was no correlation between mRNA levels of CRP, a measure of inflammation, and CYP2C19 activity (r = -0.10, p = 0.53, Figure 2F). In line, although 17.5% of patients in this cohort suffered from a (systemic) inflammatory disease, not all of them displayed phenoconversion.

The use of concurrent medication can also lead to phenoconversion, as this can result in induced expression or inhibition of drug metabolizing enzymes (4). Prior to surgery, two patients were on CYP2C19 inhibitor therapy. No phenoconversion was evident for the patient on pantoprazole, in line with its classification as a weak inhibitor. The second patient exhibited a PM phenotype despite their \*1/\*17 genotype. The underlying cause of this phenoconversion could be dual, as this patient was using esomeprazole before surgery and suffered from the comorbidity cholangitis. It is crucial to note that unlike CYP induction, the inhibition in liver microsomes caused by clinically administered CYP2C19 inhibitors is less probable to persist due to the necessary washing steps in the liver microsome isolation and the reversible nature of CYP inhibition.

#### Genotype-dependent impact of drug-drug interactions

The main objective of this study was to assess the occurrence of phenoconversion in various *CYP2C19* genotype groups following administration of either a strong (fluvoxamine), moderate (omeprazole or voriconazole) or weak (pantoprazole) inhibitor of CYP2C19, and thereby quantify to which phenotype they switch. On a group-level, CYP2C19 activity was inhibited (p < 0.0001) by omeprazole (-37%  $\pm$  8%), voriconazole (-59%  $\pm$  4%) and fluvoxamine (-85%  $\pm$  2%), but not by pantoprazole (-2%  $\pm$  4%) (Figure 3A). This percental decrease in activity was independent of *CYP2C19* genotype (Supplementary Figure S2), indicating that inhibitor strength is not affected by *CYP2C19* genotype.



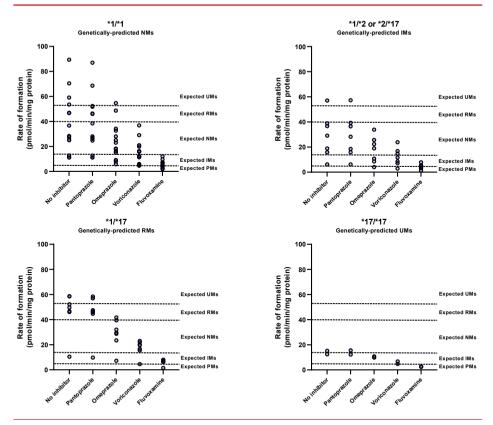
**Figure 3** Kinetic analysis of the impact of various CYP2C19 inhibitors on CYP2C19 activity and inactivation. (A) Impact of selected CYP2C19 inhibitors on CYP2C19 activity for all included donors. Donors that were phenotypically PMs at baseline were excluded for treatment with inhibitors. 4'-hydroxylation activity is shown as compared to control, where omeprazole is matched to its own time-dependent control. A one way ANOVA with matching was done to test the impact of the inhibitors; \*\*\*\* p < 0.0001. (B)  $K_{inact}$  and  $K_{i}$  determinations for the MDI of CYP2C19 by omeprazole for the various genotype groups. The values of the apparent inactivation rate constant ( $K_{obs}$ ) at each concentration of omeprazole are obtained from the slopes of the initial rates of inactivation (Supplementary Figure S1). Individual data points represent the average of three separate experiments  $\pm$  SD.

Omeprazole is a metabolism-dependent inhibitor (MDI) of CYP2C19, meaning that biotransformation of the substrate into its active metabolites contributes to the inhibitory potency of the drug. Since genotype impacts the degree of metabolite formation, we investigated whether the inhibitory potency of omeprazole would be affected by CYP2C19 genotype. The inhibitory constants  $K_{inact}$  (the first order rate

constant of CYP2C19 inactivation) and  $K_{_{\rm I}}$  (concentration of omeprazole supporting half-maximal rate of CYP2C19 inactivation) were determined in genotype-matched donor pools (Figure 3B). Genotype-matched donor pools were either a pool of donors with two wild type alleles (\*1), one non-functional allele (\*2) or one gain-of-function allele (\*17). \*17/\*17 donors were excluded due to their already low activities at baseline (basal phenoconversion). For the various genotypes, omeprazole inactivated CYP2C19 with similar KI values of either 3.01  $\pm$  0.83  $\mu$ M for RMs, 4.47  $\pm$  1.8 for NMs and 8.9  $\pm$  12.38  $\mu$ M for IMs. The mean maximal rate of inactivation ( $K_{inact}$ ) was 0.028  $\pm$  0.002 min $^{-1}$  for RMs, 0.031  $\pm$  0.004 min $^{-1}$  for NMs and 0.026  $\pm$  0.01 min $^{-1}$  for IMs, and not different between the genotype groups. Similar inactivation rate constants for CYP2C19 for omeprazole were reported by Shirasaka *et al.* in a microsome pool of 7 non-genotyped donors (29). Altogether this suggest that the intrinsic inhibitory potency of omeprazole is not affected by the *CYP2C19* genotype.

To investigate whether genotype impacts the outcome of DDIs with a CYP2C19 inhibitor, individual microsomes were co-exposed to inhibitors and the observed phenotypic switch was classified (Figure 4; Supplementary Table S1). The consequences of CYP2C19 inhibitor-mediated phenoconversion were different between CYP2C19 genotypes. In \*1/\*1 donors, voriconazole caused 50% of donors to exhibit residual activities representing IMs or lower, whereas only 14% of \*1/\*17 exhibited such activities. Of the genetically-predicted IMs, 5 out of 7 donors displayed NM activities at baseline. Subsequent voriconazole treatment resulted in 57% of genetically-predicted IMs to show a IM or PM phenotype. Likewise, although fluvoxamine converted all donors to phenotypic IMs or lower, predicted RMs (14%) were less likely to be converted to functional PMs than predicted NMs (50%) or IMs (57%). Treatment with omeprazole resulted in 43% of geneticallypredicted IMs to exhibit IM or PM activities, whereas this was 21% for \*1/\*1 and only 14% for \*1/\*17 donors. The two donors with a \*17/\*17 genotype converted to either IMs or PMs upon inhibitor treatment, but this phenoconversion may be an overprediction due to low basal activity in these donors. Pantoprazole did not result in phenoconversion in any of the genotypes.

These results suggest that the differential outcomes of CYP2C19-mediated DDIs between genotypes are not dictated by distinctive inhibitory strengths between genotypes but by the donors basal CYP2C19 activity, that may in part be predicted by *CYP2C19* genotype.



**Figure 4** CYP2C19 inhibitor-induced phenoconversion of CYP2C19 metabolism in various *CYP2C19* genotypes. Individual microsomal fractions were co-exposed to clinically relevant concentrations of inhibitors and residual CYP2C19 activity was measured. Concentrations resembled calculated unbound maximal hepatic inlet concentrations for either 100 mg fluvoxamine, 40 mg omeprazole, 200 mg voriconazole or 40 mg pantoprazole (standard dosing). Donors that were already phenotypically measured to be PM at baseline were excluded for treatment with inhibitors. Phenotype thresholds were based on Kiss *et al.* (14), after applying a scaling factor for S-mephenytoin substate concentration used in this experiment.

#### **Discussion**

In this study we aimed to quantify to what extent *CYP2C19* polymorphisms can impact the outcome of a DDI with various CYP2C19 inhibitors in human liver microsomes. In order to deliver recommendations for DDGIs it is imperative to acquire a quantitative comprehension of the phenoconversion that arises subsequent to the co-administration of an inhibitor targeting the same enzyme. Our results demonstrate that the outcome of a DDI is dictated by both inhibitor

strength and CYP2C19 activity, which is in turn dependent on genotype and non-genetic factors including comorbidities. This study provides a quantitative understanding of the magnitude of DDGIs, which can ultimately aid in tailoring drug therapy recommendations to an individual's needs.

Phenoconversion due to the use of concomitant medication can limit the accuracy of pharmacogenetic-based drug dosing. As such, considering concomitant medication use seems an integral part of CYP2C19 pharmacogenetic-based personalized therapy. Quantitative data is required to assess phenoconversion after concomitant medication use. Mostafa *et al.* used a conservative approach to predict the corrected phenotype following the use of concomitant moderate or strong CYP2C19 inhibitors (10). They estimated that carriers of one or two functional alleles (\*1) would convert to a PM, and carriers of one or two increased functional alleles (\*17) would convert to an IM phenotype. Our results on strong inhibition are in accordance with these predictions. Fluvoxamine, a strong inhibitor of CYP2C19, caused 86% of \*1/\*17 donors to become phenotypically IM, whereas most of genetically-predicted IMs were converted to a PM phenotype (57%). In accordance with unaltered CYP2C19 activity in patients with gastroesophageal reflux disease taking pantoprazole, weak inhibition by pantoprazole did not induce phenoconversion (30).

However, the outcomes of DDIs with moderate inhibitors (omeprazole/ voriconazole) matched less well to the proposed phenoconversion model by Mostafa et al., which predicted that NMs/IMs convert to a PM phenotype upon moderate inhibition of CYP2C19. In our study, voriconazole, which acts as a moderate CYP2C19 inhibitor, significantly reduced the drug metabolizing capabilities of CYP2C19 by approximately one level (i.e., from a phenotypic NM to a IM). As a result, 40% of the donors (12/30) were converted into IM or PM phenotypes by voriconazole. Though, none of the NMs were converted into PMs, except for one donor who already exhibited impaired CYP2C19 activity in the absence of voriconazole treatment (basal phenoconversion). For omeprazole, phenoconversion into IM or PM phenotypes was even less frequently seen, in only 10% of the donors (3/30). These findings are in contrast to a clinical study, in which the pantoprazole-<sup>13</sup>C breath test indicated that 96% of patients converted to a PM phenotype after treatment with omeprazole or esomeprazole (31). The underlying cause of these significant alterations in the phenotype upon PPI treatment observed in this study remains unclear. Especially since concomitant administration of

omeprazole generally results in changes in area under the curve (AUC) of low magnitude (< 2-fold), with little clinical importance (32). Moreover, a study on the effect of omeprazole on the pharmacokinetics of the CYP2C19 substrate moclobemide showed that the AUCs of NMs after omeprazole treatment did not reach the observed AUCs of PMs within the study, indicating phenoconversion to an IM rather than a PM phenotype (33). Altogether, our data suggest that CYP2C19 inhibition by moderate inhibitors can result in phenoconversion, but it seems unlikely to result into a PM phenotype for wild-type \*1/\*1 genotypes.

Omeprazole is considered to be a MDI indicating that part of its inhibitory activity of CYP2C19 is dependent on the biotransformation of omeprazole into its active metabolites. For this reason, we hypothesized that the inhibitory potency ( $K_I/K_{inact}$ ) of omeprazole could be affected by the *CYP2C19* genotype. Nonetheless, our data in *CYP2C19* genotype-matched donor pools showed no effect of *CYP2C19* genotype on the inhibitory potency of omeprazole. This is in accordance with results for paroxetine, a MDI of CYP2D6, for which the inhibitory parameters were also similar between different CYP2D6 genotypes in a microsomal assay (34). These two studies highlight that the type of inhibitor (direct vs. MDI) is presumably not a determinant in the outcome of DDI-induced phenoconversion in donors with different genotypes. Instead, our study reinforces that the outcome of a DDI and the conversion of a patient's phenotype depends on both the strength of the CYP2C19 inhibitor and the basal activity of CYP2C19. Therefore, both factors should be taken into account for phenotype predictions, as successfully demonstrated for CYP2D6 (35).

As mentioned, one primary factor in determining the outcome of a DDI is the initial enzyme activity, which is partly determined by an individual's genotype. However, our cohort also revealed discordance between genotype-based prediction of CYP2C19 activity and actual metabolizing capacity at baseline. These marked genotype-phenotype discrepancies for CYP2C19 metabolism are consistent with other studies. In a large PK study, Lorenzini *et al.* reported the concordance between *CYP2C19* genotype-predicted phenotypes and measures phenotypes and showed a low(er) concordance for genetically-predicted NMs (33%) and UM's (19%) in comparison to genetically predicted IMs (91%) (36). This CYP2C19 genotype-phenotype discrepancy is retained in different ethnic populations (37–39). In isolated microsomes, Kiss *et al.* reported, similarly to our own results, a 40% concordance (14). Importantly, we found a 2.5-fold increase in the occurrence

of PMs among our donors than what would be expected based on genotype data. This is in concordance with previous population studies which report that the prevalence of phenotypic PMs could be up to 5–10 fold higher than genetically-predicted (10,40). This could have important consequences, as drug interactions are typically pertinent when an individual has a poor or intermediate capacity in the primary metabolic pathway. Indeed, various clinical studies indicate that PMs are at risk of decreased responsiveness or toxicity during CYP2C19 substrate therapy (i.e., citalopram, omeprazole and clopidogrel) (41–43). It is therefore crucial to consider factors that could be responsible for phenotype-genotype discrepancies and thereby evoke phenoconversion and phenotypic poor metabolism despite the presence of functional alleles.

A recent clinical phenotyping study by Gloor *et al.* demonstrated that concomitant medication use could only explain 32% of the CYP2C19-related phenoconversion (40). This underscores the importance of non-genetic factors and presumably disease-related effects on CYP2C19 activity. In our cohort, the inclusion of disease-related information could provide an explanation why two RMs were phenotypically IMs/PMs, since even modest liver illness significantly affects CYP2C19's ability to metabolize drugs (44). Another co-morbidity that is increasingly connected to changes in drug metabolism is diabetes mellitus (45,46). In three of the four donors suffering from diabetes mellitus, a PM phenotype was observed despite the presence of one or two functional alleles. Importantly, the observed disease-related changes were not related to C-reactive protein (CRP) suggesting that metabolic rather than inflammatory mechanisms contribute to these disease-related changes in drug metabolism. Hence, similar to conclusions made by Kiss *et al.*, including disease-related factors could help to enhance the prediction of the CYP2C19 phenotype (14).

There is an increased interest in finding biomarkers to predict the rate of drug metabolism in the liver to facilitate phenotype predictions (47,48). We investigated whether mRNA expression in the liver itself can predict the hepatic metabolizing capacity of CYP2C19. As previously reported, total *CYP2C19* mRNA levels were not a good predictor of *CYP2C19* mRNA activity (49,50). One major limitation of expression studies is that the functional consequences of the produced mRNA are not taken into account when assessing the relationship between mRNA expression and activity. For example, with respect to *CYP2C19*, the *CYP2C19\*2* alleles are

linked to splicing defects of mRNA and hence formation of inactive protein (12). Therefore, to better examine the true relationship between mRNA expression and activity, we utilized a primer-pair that predominantly detects functional mRNA and not *CYP2C19\*2* mRNA. Examining functional *CYP2C19* mRNA indeed improved the correlation between expression and activity by ~2 fold, but a large proportion of the variance remained unexplained. Moreover, the moderate correlation that was observed was largely driven by the genetic PMs within our cohort. This reinforces that, in addition to genotyping, incorporation of hepatic mRNA expression provides limited complementary value for predicting the drug metabolizing capacity of individuals.

There are some limitations to address. First of all, the phenotype thresholds used to define phenoconversion are based on values reported in literature and might under- or overpredict the extent of phenoconversion. However, phenotype assessment is essential in order to ultimately create DDGI guidelines, since dosing adjustments are made based on phenotypes in clinical practice. Van der Lee et al. proposed that a patient's phenotype prediction can be improved by using a continuous scale for this prediction rather than a set threshold between two phenotype groups (51). Still, 21% of interindividual variability in CYP2D6 could not be explained by this approach, rendering it likely that non-genetic factors contribute to this variability. As such, the CYP450 genotype should be interpreted in the clinical context of the individual patient, considering all feasible contributors to CYP450 metabolic function. Borges et al. used a scoring system that incorporates both CYP2D6 genetic variation and CYP2D6 mediated DDIs, which showed to improve phenotype prediction as compared to genetic information alone (35). Such a scoring system lends itself well to be extended to other non-genetic factors, such as the presence of liver disease or other comorbidities. A scoring system tool that incorporates both CYP2C19 activity on a continuous scale, together with the inhibitory effect of DDIs and comorbidities (i.e., liver disease) will likely improve the pharmaco-genotype to phenotype translation.

Secondly, this study was conducted in liver biopsies that were genotyped for \*2, \*3 and \*17 variants, as these alleles are most prevalent among Europeans and recommended for clinical testing by the pharmacogenetics working group of the American association for molecular pathology (52). While disease-related factors may explain most of the observed phenoconversion into lower drug-metabolizing

phenotypes among our patients, it is important to consider that other (rare) genetic variants within CYP2C19 could also have influenced the mismatch between predicted and observed activities in our study (53). Furthermore, it is necessary to acknowledge that extrapolating our findings to non-European populations may be challenging due to differences in the genomic architecture of CYP2C19 across populations (54). Therefore, investigating phenoconversion in other populations, such as Asians or Africans, where alleles like \*3 or \*9 may contribute to basal activity and modulate DDIs for CYP2C19-dependent drugs, would be of great interest.

Another potential limitation relates to the selection of concentrations of the inhibitors in this study. Input parameters for calculating these concentrations were dependent on available literature. Still, the EMA and FDA support that the unbound maximum hepatic inlet concentration adequately mimics the clinical inhibition of hepatic P450 enzymes (15). Goutelle et al. utilized reported AUCs in NMs with and without CYP2C19 inhibitors, along with the contribution ratio of the substrate drug, to calculate inhibitory potencies of CYP2C19 inhibitors for predicting drug interactions in vivo (55). Their calculated AUC ratios for omeprazole 40 mg/day and voriconazole 400 mg/day were 43% and 66%, which are consistent with the inhibitory potency observed in our microsomal assay (37% and 59%, respectively). It should be noted that our chosen concentration of fluvoxamine may underestimate the phenoconversion to some extent since we report 85% inhibition, whereas Goutelle et al. reported 97%. Calculated unbound maximum hepatic inlet concentrations used in our assay are thus likely to represent the observed inhibitory potencies in vivo. A clinical trial investigating the risk of DDI-induced CYP2C19 phenoconversion in healthy volunteers is now ongoing, and will likely inform whether the magnitude of CYP2C19 inhibition observed in our *in vitro* system matches a clinical setting (NCT05264142).

In conclusion, this study suggests that the differential outcomes of CYP2C19-mediated DDIs are not determined by different inhibitory strengths between genotypes, but by the basal activity of CYP2C19. This activity can in part be predicted by *CYP2C19* genotype, but is also influenced by disease-related factors. This underlines the necessity to integrate both genetic data as well as comedication use and disease-related factors into a person's predicted phenotype.

#### Acknowledgements

We would like to thank Johan vd Reijden (Department of Gastroenterology-Hepatology, Leiden University Medical Center) for excellent technical support regarding sample collection. We would also like to thank Maaike van der Lee (Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center) for excellent help with the genotyping. We would like to thank Elke Krekels (Department of Systems Pharmacology and Pharmacy, Leiden Academic Centre for Drug Research) for excellent guidance with selecting and calculating relevant inhibitor concentrations.

#### References

- Genes-Drugs CPIC [Internet]. [cited 2023 Feb 26]. Available from: https://cpicpgx.org/ genes-drugs/
- Swen JJ, Nijenhuis M, De Boer A, Grandia L, Maitland-Van Der Zee AH, Mulder H, et al. Pharmacogenetics: from bench to byte--an update of guidelines. Clin Pharmacol Ther. 2011 May;89(5):662–73.
- Shah RR, Smith RL. Addressing phenoconversion: The Achilles' heel of personalized medicine. Br J Clin Pharmacol. 2015 Feb 1:79(2):222–40.
- Klomp SD, Manson ML, Guchelaar H-J, Swen JJ. Phenoconversion of Cytochrome P450 Metabolism: A Systematic Review. J Clin Med. 2020 Sep 7:9(9):2890.
- Verbeurgt P, Mamiya T, Oesterheld J. How common are drug and gene interactions?
   Prevalence in a sample of 1143 patients with CYP2C9, CYP2C19 and CYP2D6 genotyping. Pharmacogenomics. 2014;15(5):655–65.
- 6. Hocum BT, White JR, Heck JW, Thirumaran RK, Moyer N, Newman R, et al. Cytochrome P-450 gene and drug interaction analysis in patients referred for pharmacogenetic testing. Am J Health Syst Pharm. 2016 Jan 15;73(2):61–7.
- Bahar MA, Setiawan D, Hak E, Wilffert B. Pharmacogenetics of drug-drug interaction and drug-drug-gene interaction: a systematic review on CYP2C9, CYP2C19 and CYP2D6. Pharmacogenomics. 2017 May 1;18(7):701– 39.
- 8. Storelli F, Matthey A, Lenglet S, Thomas A, Desmeules J, Daali Y. Impact of CYP2D6 Functional Allelic Variations on Phenoconversion and Drug-Drug Interactions. Clin Pharmacol Ther. 2018 Jul 1;104(1):148–57.
- Crews KR, Monte AA, Huddart R, Caudle KE, Kharasch ED, Gaedigk A, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2D6, OPRM1, and COMT Genotypes and Select Opioid Therapy. Clin Pharmacol Ther. 2021 Oct 1;110(4):888–96.

- Mostafa S, Kirkpatrick CMJ, Byron K, Sheffield L. An analysis of allele, genotype and phenotype frequencies, actionable pharmacogenomic (PGx) variants and phenoconversion in 5408 Australian patients genotyped for CYP2D6, CYP2C19, CYP2C9 and VKORC1 genes. J Neural Transm. 2019 Jan 21:126(1):5–18.
- Gene-specific Information Tables for CYP2C19 [Internet]. [cited 2024 Dec 1]. Available from: https://www.pharmgkb.org/ page/cyp2c19RefMaterials
- Chaudhry AS, Prasad B, Shirasaka Y, Fohner A, Finkelstein D, Fan Y, et al. The CYP2C19 Intron 2 Branch Point SNP is the Ancestral Polymorphism Contributing to the Poor Metabolizer Phenotype in Livers with CYP2C19\*35 and CYP2C19\*2 Alleles. Drug Metab Dispos. 2015 Aug 1;43(8):1226–35.
- Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002;3(7).
- 14. Kiss ÁF, Vaskó D, Déri MT, Tóth K, Monostory K. Combination of CYP2C19 genotype with non-genetic factors evoking phenoconversion improves phenotype prediction. Pharmacol Rep. 2018 Jun 1;70(3):525–32.
- Parkinson A. Regulatory Recommendations for Calculating the Unbound Maximum Hepatic Inlet Concentration: A Complicated Story with a Surprising and Happy Ending. Drug Metab Dispos. 2019 Jul 1;47(7):779–84.
- Iga K. Use of Three-Compartment Physiologically Based Pharmacokinetic Modeling to Predict Hepatic Blood Levels of Fluvoxamine Relevant for Drug-Drug Interactions. J Pharm Sci. 2015 Apr 1;104(4):1478–91.
- 17. Ogilvie BW, Yerino P, Kazmi F, Buckley DB, Rostami-Hodjegan A, Paris BL, et al. The proton pump inhibitor, omeprazole, but not lansoprazole or pantoprazole, is a metabolism-dependent inhibitor of CYP2C19: Implications for coadministration with clopidogrel. Drug Metab Dispos. 2011 Nov;39(11):2020–33.

- Shi C, Xiao Y, Mao Y, Wu J, Lin N. Voriconazole: A Review of Population Pharmacokinetic Analyses. Clin Pharmacokinet. 2019 Jun 1;58(6):687–703.
- 19. Grensemann J, Pfaffendorf C, Wicha SG, König C, Roedl K, Jarczak D, et al. Voriconazole Pharmacokinetics Are Not Altered in Critically Ill Patients with Acute-on-Chronic Liver Failure and Continuous Renal Replacement Therapy: An Observational Study. Microorganisms. 2021 Oct 1:9(10).
- Gawrońska-Szklarz B, Adamiak-Giera U, Wyska E, Kurzawski M, Gornik W, Kaldonska M, et al. CYP2C19 polymorphism affects single-dose pharmacokinetics of oral pantoprazole in healthy volunteers. Eur J Clin Pharmacol. 2012 Sep;68(9):1267–74.
- Parkinson A, Kazmi F, Buckley DB, Yerino P, Paris BL, Holsapple J, et al. An evaluation of the dilution method for identifying metabolism-dependent inhibitors of cytochrome P450 enzymes. Drug Metab Dispos. 2011 Aug;39(8):1370–87.
- 22. Shirasaka Y, Chaudhry AS, McDonald M, Prasad B, Wong T, Calamia JC, et al. Interindividual variability of CYP2C19catalyzed drug metabolism due to differences in gene diplotypes and cytochrome P450 oxidoreductase content. Pharmacogenomics J. 2016 Aug 1;16(4):375–87.
- Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013 Apr;138(1):103–41.
- 24. Duthaler U, Bachmann F, Suenderhauf C, Grandinetti T, Pfefferkorn F, Haschke M, et al. Liver Cirrhosis Affects the Pharmacokinetics of the Six Substrates of the Basel Phenotyping Cocktail Differently. Clin Pharmacokinet. 2022 Jul 1;61(7):1039–55.
- Ohnishi A, Murakami S, Akizuki S, Mochizuki J, Echizen H, Takagi I. In vivo metabolic activity of CYP2C19 and CYP3A in relation to CYP2C19 genetic polymorphism in chronic liver disease. J Clin Pharmacol. 2005 Nov;45(11):1221-9.
- 26. Dubuquoy C, Burnol AF, Moldes M. PNPLA3, a genetic marker of progressive liver disease, still hiding its metabolic function? Clin Res Hepatol Gastroenterol. 2013 Feb;37(1):30–5.

- 27. Gravel S, Chiasson JL, Turgeon J, Grangeon A, Michaud V. Modulation of CYP450 Activities in Patients With Type 2 Diabetes. Clin Pharmacol Ther. 2019 Dec 1:106(6):1280–9.
- de Jong LM, Jiskoot W, Swen JJ, Manson ML. Distinct effects of inflammation on cytochrome P450 regulation and drug metabolism: Lessons from experimental models and a potential role for pharmacogenetics. Genes (Basel). 2020 Dec 16;11(12):1–24.
- Shirasaka Y, Sager JE, Lutz JD, Davis C, Isoherranen N. Inhibition of CYP2C19 and CYP3A4 by omeprazole metabolites and their contribution to drug-drug interactions. Drug Metab Dispos. 2013 Jul;41(7):1414–24.
- 30. Modak AS, Klyarytska I, Kriviy V, Tsapyak T, Rabotyagova Y. The effect of proton pump inhibitors on the CYP2C19 enzyme activity evaluated by the pantoprazole-13C breath test in GERD patients: clinical relevance for personalized medicine. J Breath Res. 2016 Dec 16;10(4).
- 31. Klieber M, Oberacher H, Hofstaetter S, Beer B, Neururer M, Amann A, et al. CYP2C19 Phenoconversion by Routinely Prescribed Proton Pump Inhibitors Omeprazole and Esomeprazole: Clinical Implications for Personalized Medicine. J Pharmacol Exp Ther. 2015 Sep 1;354(3):426–30.
- 32. Ogawa R, Echizen H. Drug-drug interaction profiles of proton pump inhibitors. Clin Pharmacokinet. 2010;49(8):509–33.
- 33. Yu KS, Yim DS, Cho JY, Park SS, Park JY, Lee KH, et al. Effect of omeprazole on the pharmacokinetics of moclobemide according to the genetic polymorphism of CYP2C19. Clin Pharmacol Ther. 2001;69(4):266–73.
- 34. Storelli F, Desmeules J, Daali Y. Genotypesensitive reversible and time-dependent CYP2D6 inhibition in human liver microsomes. Basic Clin Pharmacol Toxicol. 2019 Feb 1;124(2):170–80.
- Borges S, Desta Z, Jin Y, Faouzi A, Robarge JD, Philips S, et al. Composite functional genetic and comedication CYP2D6 activity score in predicting tamoxifen drug exposure among breast cancer patients. J Clin Pharmacol. 2010;50(4):450–8.

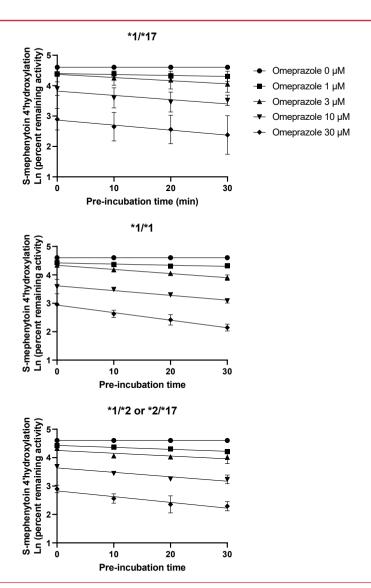
- Ing Lorenzini K, Desmeules J, Rollason V, Bertin S, Besson M, Daali Y, et al. CYP450 Genotype—Phenotype Concordance Using the Geneva Micrococktail in a Clinical Setting. Front Pharmacol. 2021 Aug 26:12:730637.
- 37. de Andrés F, Altamirano-Tinoco C, Ramírez-Roa R, Montes-Mondragón CF, Dorado P, Peñas-Lledó EM, et al. Relationships between CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 metabolic phenotypes and genotypes in a Nicaraguan Mestizo population. Pharmacogenomics J. 2021 Apr 1;21(2):140–51.
- de Andrés F, Sosa-Macías M, Ramos BPL, Naranjo MEG, LLerena A. CYP450 Genotype/Phenotype Concordance in Mexican Amerindian Indigenous Populations-Where to from Here for Global Precision Medicine? OMICS. 2017 Sep 1;21(9):509–19.
- De Andrés F, Terán S, Hernández F, Terán E, Llerena A. To Genotype or Phenotype for Personalized Medicine? CYP450 Drug Metabolizing Enzyme Genotype-Phenotype Concordance and Discordance in the Ecuadorian Population. OMICS. 2016 Dec 1:20(12):699–710.
- 40. Gloor Y, Lloret-Linares C, Bosilkovska M, Perroud N, Richard-Lepouriel H, Aubry JM, et al. Drug metabolic enzyme genotype-phenotype discrepancy: High phenoconversion rate in patients treated with antidepressants. Biomed Pharmacother. 2022 Aug 1:152.
- 41. Hicks J, Bishop J, Sangkuhl K, Ji Y, Leckband S, Leeder J, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and CYP2C19 Genotypes and Dosing of Selective Serotonin Reuptake Inhibitors.
- 42. Lima JJ, Thomas CD, Barbarino J, Desta Z, Van Driest SL, Rouby N El, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C19 and Proton Pump Inhibitor Dosing. Clin Pharmacol Ther |.
- 43. Lee CR, Luzum JA, Sangkuhl K, Gammal RS, Sabatine MS, Stein CM, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2C19 Genotype and Clopidogrel Therapy: 2022 Update. Clin Pharmacol Ther. 2022 Nov 1;112(5):959–67.

- 44. Frye RF, Zgheib NK, Matzke GR, Chaves-Gnecco D, Rabinovitz M, Shaikh OS, et al. Liver disease selectively modulates cytochrome P450-mediated metabolism. Clin Pharmacol Ther. 2006 Sep;80(3):235–45.
- 45. Darakjian L, Deodhar M, Turgeon J, Michaud V. Chronic Inflammatory Status Observed in Patients with Type 2 Diabetes Induces Modulation of Cytochrome P450 Expression and Activity. Int J Mol Sci. 2021 May 1;22(9).
- 46. Neyshaburinezhad N, Shirzad N, Rouini M, Namazi S, Khoshayand M, Esteghamati A, et al. Evaluation of important human CYP450 isoforms and P-glycoprotein phenotype changes and genotype in type 2 diabetic patients, before and after intensifying treatment regimen, by using Geneva cocktail. Basic Clin Pharmacol Toxicol. 2023 Feb 3;
- 47. Achour B, Gosselin P, Terrier J, Gloor Y, Al-Majdoub ZM, Polasek TM, et al. Liquid Biopsy for Patient Characterization in Cardiovascular Disease: Verification against Markers of Cytochrome P450 and P-Glycoprotein Activities. Clin Pharmacol Ther. 2022 Jun 1;111(6):1268–77.
- 48. Rowland A, Ruanglertboon W, van Dyk M, Wijayakumara D, Wood LS, Meech R, et al. Plasma extracellular nanovesicle (exosome)-derived biomarkers for drug metabolism pathways: a novel approach to characterize variability in drug exposure. Br J Clin Pharmacol. 2019 Jan 1;85(1):216–26.
- Rodríguez-Antona C, Donato MT, Pareja E, Gómez-Lechón MJ, Castell J V. Cytochrome p-450 mRNA expression in human liver and its relationship with enzyme activity. Arch Biochem Biophys. 2001 Sep 15;393(2):308– 15.
- Pridgeon CS, Johansson I, Ingelman-Sundberg M. Liquid Biopsies or Therapeutic Drug Monitoring for CYP Activity Profile Determination. Clin Pharmacol Ther. 2022 Nov 1;112(5):1000–3.
- 51. Van der Lee M, Allard WG, Vossen RHAM, Baak-Pablo RF, Menafra R, Deiman BALM, et al. Toward predicting CYP2D6-mediated variable drug response from CYP2D6 gene sequencing data. Sci Transl Med. 2021 Jul 21;13(603).
- 52. Pratt VM, Del Tredici AL, Hachad H, Ji Y, Kalman L V., Scott SA, et al. Recommendations for Clinical CYP2C19 Genotyping Allele Selection: A Report of the Association for Molecular Pathology. J Mol Diagn. 2018 May 1;20(3):269–76.

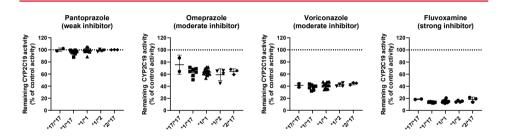
- 53. Ingelman-Sundberg M, Mkrtchian S, Zhou Y, Lauschke VM. Integrating rare genetic variants into pharmacogenetic drug response predictions. Hum Genomics. 2018 Jan 26;12(1).
- 54. Zhou Y, Ingelman-Sundberg M, Lauschke VM. Worldwide Distribution of Cytochrome P450 Alleles: A Meta-analysis of Populationscale Sequencing Projects. Clin Pharmacol Ther. 2017 Oct 1:102(4):688–700.
- 55. Goutelle S, Bourguignon L, Bleyzac N, Berry J, Clavel-Grabit F, Tod M. In vivo quantitative prediction of the effect of gene polymorphisms and drug interactions on drug exposure for CYP2C19 substrates. AAPS J. 2013 Apr;15(2):415–26.

#### Supplemental information

#### Supplemental figures



Supplementary Figure S1 Time dependent inhibition of CYP2C19 at various concentration of omeprazole. Omeprazole was pre-incubated for 0–30 minutes at concentrations 0-30  $\mu$ M and residual CYP2C19 activity was measured, see materials & methods "Kinetic analysis of CYP2C19 dependent S-mephenytoin hydroxylation". The slope of each line is the value of the observed rate constant ( $K_{obs}$ ) for the inactivation of CYP2C19 by omeprazole at a given concentration. Individual points represent the average of triplicate determinations  $\pm$  SD.



**Supplementary Figure S2** Decreased activity of CYP2C19 following inhibitor treatment is independent of genotype. For every inhibitor and genotype, S-mephenytoin 4'-hydroxylation activity is shown as compared to control (no inhibitor, 100%). A one-way ANOVA with Dunnett's post hoc test was done to test whether the percentual decrease was different between genotypes.

#### Supplemental tables

Supplementary Table S1 Remaining phenotype after treatment with various CYP2C19 inhibitors for different genotype groups.

	No inhibitor (n = 40*)	Pantoprazole (n = 30)	Omeprazole (n = 30)	Voriconazole (n = 30)	Fluvoxamine (n = 30)
*17/*17 (genetically predicted UMs)	1× NM 1× IM	1× NM (50%) 1× IM (50%)	2× IM (100%)	1× IM (50%) 1× PM (50%)	2× PM (100%)
*1/*17 (genetically predicted RMs)	2× UM 4× RM 1× IM 	2× UM (29%) 4× RM (57%) 1× IM (14%)	1× RM (14%) 5× NM (71%) 1× IM (14%)	6× NM (86%) 1× PM (14%)	6× IM (86%) 1× PM (14%)
*1/*1 (genetically predicted NMs)	4× UM 2× RM 5× NM 3× IM 	2× UM (14%) 4× RM (29%) 5× NM (35%) 3× IM (21%)	1× UM (7%) 1× RM (7%) 9× NM (64%) 3× IM (21%)	7× NM (50%) 6× IM (43%) 1× PM (7%)	7× IM (50%) 7× PM (50%)
*1/*2 or *2/*17 (genetically predicted IMs)	1× UM 5× NM 1× IM 3× PM	1× UM (14%) 5× NM (71%) 1× IM (14%)	4× NM (57%) 2× IM (29%) 1× PM (14%)	3× NM (43%) 3× IM (43%) 1× PM (14%)	3× IM (43%) 4× PM (57%)
*2/*2 (genetically predicted PMs)	4× PM				

<sup>\*</sup> Donors (indicated in *italics*, n = 10) that were phenotypically measured to be PM at baseline were excluded for treatment with inhibitors. Percentages indicate phenoconverted individuals per genotype group.

#### Supplemental materials & methods

#### Primer sequences and amplification efficiencies

**Supplementary Table S2** Primer sequences and amplification efficiencies. Amplification efficiency (%) was calculated using the formula:  $\left(10^{\frac{-1}{010pe}}-1\right)*100$ .

	Sequence	Amplification efficiency
CYP2C19 functional	For 5'-AAAACCAAGGCTTCACCCTGTGATCC-3' Rev 5'-CCGGGAAATAATCAATGATAGTGGGAAA-3'	98.7%
CYP2C19 total	For 5'-GCTCTCTTTCCTCTGGTCCAAATTTCAC-3' Rev 5'- GCACAGTGAAACTTTTTTAATGGAGGCTG-3'	99.2%
CRP	For 5'-CTCTCTCATGCTTTTGGCCAGACAG-3' Rev 5'-AAGAATTCACAGCCCCACAAGGTTC-3'	96.3%
PNLPA3	For 5'-TCACTCGAGTGCTGATGTGTCTGC-3' Rev 5'-CCTCTGCTTTGGTCTCTGCTGGAC-3'	97.8%

#### Quantification of 4'hydroxymephenytoin by LC-MS/MS

Quantification of 4'hydroxymephentoin in the microsomal incubations was done using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system consisting of a Nexera LC-40 high-performance liquid chromatography (HPLC) system equipped with a DGU-403 degassing unit, two LC-40D pumps, a SIL-40C autosampler, and a CTO-40S column oven (Shimadzu, 's-Hertogenbosch, the Netherlands). A Kinetex C18 column (1.7 µM, 50 x 2.1 mm) (Phenomenex, Utrecht, The Netherlands) with a SecurityGuard Ultra C8, 2.7  $\mu$ m, 5 × 2.1 mm cartridge (Phenomenex, Utrecht, The Netherlands) as guard column were used to separate 4'hydroxymephenytoin from other analytes present in the sample matrix. Mobile phases consisted of water (A) and methanol (B) both containing 0.1% formic acid. The gradient, with a flow rate of 0.4 ml/min, started at 5% B and increased to 100% B in 4 min, maintaining 100% B for 2 min, and then returned to initial conditions for another 2 min. The column was kept at 50°C and the injection volume was 20 μL. The HPLC was coupled to a Sciex QTRAP 6500+ mass spectrometer (AB Sciex Netherlands B.V., Nieuwerkerk aan den IJssel, The Netherlands) operating in positive electrospray mode (ESI+).

The MS conditions were as follows: curtain gas 20 psi, collision gas "medium", ion source gas 1 40 psi, ion source gas 2 40 psi, ion spray voltage 5500 V and temperature 550°C. The MS was operated in the multiple reaction monitoring (MRM) mode

and was optimized by direct infusion of the standards individually. The optimized MRM transitions, retention time, declustering potential (DP), collision energy (CE) and cell exit potential (CXP) for 4'hydroxymephenytoin and internal standard 4'hydroxymephenytoin-d, are summarized in Supplementary Table S3.

Supplementary Table S3 MRM parameters and retention time for the quantified analytes by the LC-MS/MS method.

Analyte	Q1 mass	Q3 mass	Retention time	DP	CE	CXP
	(Da)	(Da)	(min)	(V)	(V)	(V)
4'-hydroxymephenytoin	235.1	150.1	2.7	51	25	10
4'-hydroxymephenytoin-d <sub>3</sub>	238.1	150.1	2.7	41	25	14

Assay accuracy and precision were determined by analyzing quintuplicates of quality controls at five concentration levels quality controls that were prepared like the microsomal samples. Within – and between runs coefficients of variation (CV) were  $\leq 2\%$  (n = 3). The mean bias was in the range of -4% to 7% across all concentration levels (n = 3). Analyst software version 1.4 (AB Sciex Netherlands B.V., Nieuwerkerk aan den IJssel, The Netherlands) was used for data analysis.

#### Calculating the unbound maximum hepatic inlet concentration

The unbound maximum hepatic inlet concentration in plasma incorporates the sum of two concentrations, namely the maximum concentration of drug in plasma (Plasma  $I_{max}$ ) and the maximum concentration of drug that was absorbed from the gut into the hepatic portal system (Total portal  $C_{max}$  in plasma), and is predicted to adequately mimic the clinical inhibition of hepatic P450 enzymes (1).

The mean maximum concentration of inhibitors in plasma after dosing to steady state (Plasma  $I_{max}$ ) with the chosen clinical dose was retrieved from literature (Supplementary Table S4).

Supplementary Table S4 Retrieved mean total systemic  $I_{max}$  values in plasma for clinically relevant dosages of CYP2C19 inhibitors.

	Dose (mg)	Mean plasma I <sub>max</sub> (μΜ)	References
Fluvoxamine	100	0.3	Summarized from references within (2)
Omeprazole	40	3.3	(3-6)
Voriconazole	200	7.3	Summarized from references within (7)
Pantoprazole	40	6.5	(8)

#### Supplementary method references

- Parkinson A. Regulatory Recommendations for Calculating the Unbound Maximum Hepatic Inlet Concentration: A Complicated Story with a Surprising and Happy Ending. Drug Metab Dispos. 2019;47(7):779–84.
- Britz H, Hanke N, Volz AK, Spigset O, Schwab M, Eissing T, et al. Physiologically-Based Pharmacokinetic Models for CYP1A2 Drug-Drug Interaction Prediction: A Modeling Network of Fluvoxamine, Theophylline, Caffeine, Rifampicin, and Midazolam. CPT pharmacometrics Syst Pharmacol. 2019;8(5):296–307.
- Braeckman RA, Stirtan WG, Soni PN.
   Effect of Icosapent Ethyl (Eicosapentaenoic
   Acid Ethyl Ester) on Omeprazole Plasma
   Pharmacokinetics in Healthy Adults. Drugs R
   D. 2014;14(3):159.
- 4. Hassan-Alin M, Andersson T, Niazi M, Röhss K. A pharmacokinetic study comparing single and repeated oral doses of 20 mg and 40 mg omeprazole and its two optical isomers, S-omeprazole (esomeprazole) and R-omeprazole, in healthy subjects. Eur J Clin Pharmacol. 2005;60(11):779–84.

- Roh HK, Kim PS, Lee DH, Tybring G, Sagar M, Park CS, et al. Omeprazole Treatment of Korean Patients: Effects on Gastric pH and Gastrin Release in Relation to CYP2C19 Geno- and Phenotypes. Basic Clin Pharmacol Toxicol. 2004;95(3):112–9.
- 6. Prichard PJ, Yeomans ND, Mihaly GW, Jones DB, Buckle PJ, Smallwood RA, et al. Omeprazole: a study of its inhibition of gastric pH and oral pharmacokinetics after morning or evening dosage.

  Gastroenterology. 1985;88(1 Pt 1):64–9.
- Li X, Frechen S, Moj D, Lehr T, Taubert M, Hsin C hsuan, et al. A Physiologically Based Pharmacokinetic Model of Voriconazole Integrating Time-Dependent Inhibition of CYP3A4, Genetic Polymorphisms of CYP2C19 and Predictions of Drug-Drug Interactions. Clin Pharmacokinet. 2020:59(6):781–808.
- 8. Shao JG, Jiang W, Li KQ, Lu JR, Sun YY. Blood concentration of pantoprazole sodium is significantly high in hepatogenic peptic ulcer patients, especially those with a poor CYP2C19 metabolism. J Dig Dis. 2009;10(1):55–60.



#### Section II

# (Pre)clinical evaluation of inflammation-induced alterations in drug metabolism



# Chapter 3

Distinct effects of inflammation on cytochrome P450 regulation and drug metabolism: Lessons from experimental models and a potential role for pharmacogenetics

Laura M. de Jong, Wim Jiskoot, Jesse J. Swen, Martijn L. Manson



#### **Abstract**

Personalized medicine strives to optimize drug treatment for the individual patient by taking into account both genetic and non-genetic factors for drug response. Inflammation is one of the non-genetic factors that has been shown to greatly affect the metabolism of drugs – primarily through inhibition of cytochrome P450 (CYP450) drug-metabolizing enzymes - and hence contribute to the mismatch between the genotype predicted drug response and the actual phenotype, a phenomenon called phenoconversion. This review focuses on inflammationinduced drug metabolism alterations. In particular, we discuss the evidence assembled through human in vitro models on the effect of inflammatory mediators on clinically relevant CYP450 isoform levels and their metabolizing capacity. We also present an overview of the current understanding of the mechanistic pathways via which inflammation in hepatocytes may modulate hepatic functions that are critical for drug metabolism. Furthermore, since large inter-individual variability in response to inflammation is observed in human in vitro models and clinical studies, we evaluate the potential role of pharmacogenetic variability in the inflammatory signaling cascade and how this can modulate the outcome of inflammation on drug metabolism and response.

**KEYWORDS:** cytochrome P450 enzyme system, drug metabolism, hepatocytes, inflammation, inter-individual variability, pharmacogenomics, phenoconversion, phenotype, pregnane X receptor

#### Introduction

The clinical outcome of drug treatments can vary greatly between individuals and even within the same individual. Consequently, certain patients may (suddenly) experience reduced efficacy or exhibit an increased risk for developing adverse events (1). While part of this variability can be explained by genetic variations in drug-metabolizing enzymes (DMEs) – mainly stemming from the cytochrome P450 (CYP) enzyme family – other non-genetic factors may also greatly contribute to the observed variability in drug response (2).

Inflammation or disease state is shown to have major effects on the metabolism of drugs through downregulation of CYP enzymes and hence contribute to the mismatch between the genotype predicted drug response and the actual phenotype - a phenomenon better known as phenoconversion (3). However, the impact of inflammation-induced phenoconversion may differ greatly between individual patients and can be dependent on multiple factors. First, the degree of inflammation can significantly influence the extent of CYP suppression (4). Indeed, signature markers of inflammation are often inversely correlated with drug metabolism (5,6). Secondly, the type of inflammation or cytokine profile is an important determinant in the effect of inflammation on drug metabolism. Evidently, interleukin-targeting biologics have shown cytokine-specific successes in reversing the repressing effects of inflammatory cytokines towards CYP proteins (7,8). Thirdly, the extent of inflammation-induced phenoconversion might be dependent on the metabolic pathway of a drug since inflammation is shown to downregulate CYP activities in an isoform-specific manner (9). Lastly, since drug metabolism is also greatly dependent on genetic variability this might be a fourth factor that alters the extent of inflammation-induced phenoconversion in patients (2,10).

To personalize and optimize drug treatments, a better understanding is needed of how inflammation affects pharmacokinetic behavior and clinical effectiveness of drugs. One major hurdle is that the specific effects of inflammation on pharmacokinetics cannot be easily assessed with in vivo studies, due to the presence of many interfering covariables (e.g., age, genetic backgrounds, kidney function, co-medication). Therefore, in vitro liver models may be valuable tools to elucidate the specific effects of inflammation on drug metabolism. Earlier studies with in vitro models have already demonstrated that various inflammatory mediators associated with inflammation and infection can modulate drug

metabolism by reducing the expression of CYPs (2,3,11,12). However, since the effect of inflammation-induced phenoconversion depends on the degree and type of inflammation as well as the metabolic pathway of the drug, it is necessary to better understand the different effects of various pro-inflammatory mediators and focus on the differential sensitivity between CYP isoforms in response to them.

The aim of this literature review is therefore to (1) summarize and update the available evidence on the effects of inflammatory stimuli on CYP expression levels and activity in human in vitro liver models, with a specific focus on type of inflammation and metabolic pathway of the drug. (2) Provide an overview of our current understanding of the mechanistic pathways via which inflammation in hepatocytes modulates hepatic functions (e.g., transcription factors, enzymes, nuclear receptors) that are critical for drug metabolism. (3) Define how genetic variation in these defined mechanistic pathways may modulate the effect of inflammation on drug metabolism and drug response.

## Effects of inflammatory stimuli on CYP expression levels and activity in human in vitro liver models

Experimental laboratory studies have been instrumental for our understanding of how inflammation may modulate drug metabolism in the clinic. Through the use of in vitro hepatocyte models, researchers have investigated which inflammatory mediators can be held responsible for the observed changes in drug metabolism. These studies primarily emphasize the effects of inflammatory stimuli on either the mRNA expression of the major CYPs responsible for drug metabolism or the actual metabolism of probe substrates for these CYPs. Since DMEs show substantial interspecies differences in terms of metabolizing activity and isoform composition, rodent data may not be useful in extrapolation to the clinic (13). Therefore, we describe the effects of inflammatory stimuli on CYPs in relevant human in vitro models, summarized in Table 1.

3

 Table 1
 Regulation of clinically important drug metabolizing cytochrome P450 enzymes by pro-inflammatory stimuli in relevant human in vitro models

	Ref	(14)	(15)					(23)	(16)
	Maximal effect (%)		NS NS	NS	NS				
	Potency (EC <sub>50</sub> ng/mL)								
Effect on drug metabolism	Metabolite		o-OH-atorvastatin acetaminophen	OH-tolbutamide	4'-OH-mephentoin				
Effe	Drug		atorvastatin phenacetin	tolbutamide	S-mephenytoin				
	Dura- tion		72 h						
n	Potency (EC <sub>50</sub> ng/mL)								0.454
Effect on CYP mRNA expression	Maximal effect (%)	CYP3A4 (↓95%) CYP2C9 (↓35%) CYP2C19 (↓40%)	CYP3A4 (↓85%) CYP1A2 (↓76%)	CYP2C9 (↓65%)	CYP2C19 (↓41%)	CYP2D6 (41%)	CYP2E1 (†402%)	CYP3A4 (↓97%) CYP1A2 (↓96%)	CYP3A4 (↓90%) CYP1A2 (↓80%)
Effect on C	Studied concen- tration	10 ng/mL	10 ng/mL					10 ng/mL	0.0006–50 ng/mL
	Dura- tion	24 h	24 h					24 h	48 h
	Model	РНН	РНН					РНН	РНН
	Dura-Stimulus Model tion	IL-6	I					I	ı I

Table 1 continues on next page.

 Table 1
 Continued

			Effect on C	Effect on CYP mRNA expression	п		Effe	Effect on drug metabolism			
snp	Model	Dura- tion	Studied concentration	Maximal effect (%)	Potency (EC <sub>50</sub> ng/mL)	Dura- tion	Drug	Metabolite	Potency (EC <sub>50</sub> ng/mL)	Maximal effect (%)	Ref
	PHH *	48 h	10 ng/mL	CYP3A4 (\$98%) CYP1A2 (\$27%)		72 h	testosterone phenacetin	6β-hydroxytestosterone acetaminophen		176% 122%	(17)
				CYP2C19 (472%)  CYP2C9 (463%)  CYP2D6 (7240%)			5-mepnenytoın tolbutamide dextromethorphan	4 - O.H. mephentoun O.H. tolbutamide dextrorphan		\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	
1	РНН	72 h	0.005–50 ng/mL	CYP3A4 (↓95%) CYP3A5 (↓95%)	0.0032	72 h	testosterone	6β-hydroxytestosterone	0.073	%021	(18)
				CYP1A2 (485%) CYP2C19 (480%)	0.271		phenacetin	acetaminophen	1.25	%06 <b>↑</b>	
				CYP2C9 (490%) CYP2D6 (470%)	0.121						
	PHH/ PHH:KC (10:4)		2-200 ng/ mL	CYP3A4 (?)		72 h	testosterone	6β-hydroxytestosterone		%06↑	(19)
_	PHH:KC*		0.001–10 ng/mL	CYP3A4 (?)		96 h	luminogenic P450- Glo" substrate	proluciferin substrate	0.463	%08↑	(20)
	PHH:KC (10:4)	96 h	0.00625- 5 ng/mL	CYP3A4 (↓95%)		96 h	luminogenic P450- Glo‴ substrate	proluciferin substrate	0.252	%56<↑	(21)

 Table 1
 Continued

			Effect on C	Effect on CYP mRNA expression	u		Effe	Effect on drug metabolism			
alus	Model	Dura- tion	Studied concen- tration	Maximal effect (%)	Potency (EC <sub>50</sub> ng/mL)	Dura- tion	Drug	Metabolite	Potency (EC <sub>50</sub> ng/mL)	Maximal effect (%)	Ref
	HepaRG	24 h	10 ng/mL	CYP3A4 (†95%) CYP3A5 (†90%)		24 h	midazolam	1'-hydroxymidazolam		decreased	(22)
				CYP1A2 (↓80%)			phenacetin	acetaminophen		decreased	
				CYP2C9 (485%)			tolbutamide	OH-tolbutamide		NS	
				CYP2C19 (485%)			S-mephenytoin	4'-OH-mephentoin		decreased	
				CYP2D6 (NS)			propafenone	5-hydroxypropafenone		SN	
				CYP2E1 (NS)							
	HepaRG	24 h	10 ng/mL	CYP3A4 (\$93%) CYP3A5 (\$89%)		72 h	atorvastatin	o-OH-atorvastatin		%08 < ↑	(15)
				CYP1A2 (↓90%)			phenacetin	acetaminophen		%09 < ↑	
				CYP2C9 (↓83%)			tolbutamide	OH-tolbutamide		%09 < ↑	
				CYP2C19 (↓83%) CYP2E1 (NS)			S-mephenytoin	4'-OH-mephentoin		%09 < ↑	
	HepaRG	48 h	0.123–30 ng/mL	CYP3A4 (†99%) CYP1A2 (†90%)	<0.123	72 h	midazolam phenacetin	1'-hydroxymidazolam acetaminophen	2.89	%59 <u>†</u>	(16)
1 1	HepaRG	48 h	10 ng/mL	CYP3A4 (\$\angle > 95\%) CYP1A2 (\$\angle > 95\%)		4 h	midazolam phenacetin	1'-hydroxymidazolam acetaminophen		↓61% ↓68%	(23)

Table 1 continues on next page.

 Table 1
 Continued

			Effect on C	Effect on CYP mRNA expression	u		Eff	Effect on drug metabolism			
Stimulus	Model	Dura- tion	Studied concentration	Maximal effect (%)	Potency (EC <sub>50</sub> ng/mL)	Dura- tion	Drug	Metabolite	Potency (EC <sub>50</sub> ng/mL)	Maximal effect (%)	Ref
	HepaRG	336 h (14 days)	10 ng/mL	CYP3A4 (NS) CYP3A5 (↓80%) CYP1A2 (↓95%)		336 h (14 days)	midazolam phenacetin	1'-hydroxymidazolam acetaminophen		decreased decreased	(22)
				CYP2C9 (NS) CYP2C19 (↓90%)			tolbutamide S-mephenytoin	OH-tolbutamide 4'-OH-mephentoin		NS NS	
				CYP2D6 (NS) CYP2E1 (NS)							
$IL-1\beta$	РНН	24 h	5 ng/mL	CYP3A4 (↓95%) CYP2C9 (NS) CYP2C19 (NS)							(14)
	ЬНН	72 h	0.0001- 10 ng/	CYP3A4 (495%) CYP3A5 (462%)	0.294	72 h	testosterone	$6\beta$ -hydroxytestosterone	0.416	%06 <b>↑</b>	(24)
			m	CYP1A2 (473%) CYP2C9 (479%)	0.531 **		phenacetin	acetaminophen	0.45	<b>165%</b>	
,				CYP2C19 (↓58%) CYP2D6 (↓75%)	0.153 **						

Table 1 Continued

			Effect on (	Effect on CYP mRNA expression	n		Effe	Effect on drug metabolism			
Stimulus	Model	Dura- tion	Studied concen- tration	Maximal effect (%)	Potency (EC <sub>50</sub> 1 ng/mL)	Dura- tion	Drug	Metabolite	Potency (EC <sub>50</sub> ng/mL)	Maximal effect (%)	Ref
	РНН/ РНН:КС (10:4)		0.2–200 ng/mL	CYP3A4 (?)		72 h	Testosterone	6β-hydroxytestosterone		^82%	(19)
	PHH:KC (10:4)	96 h	0.00625– 5 ng/mL	CYP3A4 (↓ > 95%)		96 h	luminogenic P450- Glo" substrate	proluciferin substrate	0.098	%56<↑	(21)
	HepaRG	24 h	5 ng/mL	CYP3A4 (\$97%) CYP3A5 (\$91%)		72 h	atorvastatin	o-OH-atorvastatin		%08<↑	(15)
				CYP1A2 (↓93%)			phenacetin	acetaminophen		%08<↑	
				CYP2C9 (↓90%)			tolbutamide	OH-tolbutamide		%08<↑	
				CYP2C19 (493%)			S-mephenytoin	4'-OH-mephentoin		%08<↑	
				CYP2E1 (↓75%)							
	HepaRG	24 h	1 ng/mL	CYP3A4 (\$98%) CYP1A2 (\$99%)							(23)
IL-18	ЬНН	48 h	1.95–500 ng/mL	CYP3A4 (NS) CYP1A2 (NS)		72 h	midazolam phenacetin	1'-hydroxymidazolam acetaminophen		NS NS	(16)
	HepaRG	48 h	2.06–600 ng/mL	CYP3A4 (NS) CYP1A2 (NS)		72 h	midazolam phenacetin	1'-hydroxymidazolam acetaminophen		NS NS	(16)

Table 1 continues on next page.

 Table 1
 Continued

			Effect on C	Effect on CYP mRNA expression	u		Effe	Effect on drug metabolism			
Stimulus	Model	Dura- tion	Studied concentration	Maximal effect (%)	Potency (EC <sub>50</sub> ng/mL)	Dura- tion	Drug	Metabolite	Potency (EC <sub>50</sub> ng/mL)	Maximal effect (%)	Ref
TNF-α	РНН	24 h	10 ng/mL	CYP3A4 (↓80%) CYP2C9 (NS) CYP2C19 (NS)							(14)
I	* ННА	48 h	10 ng/mL	CYP3A4 (487%) CYP1A2 (45%) CYP2C19 (NS) CYP2C9 (NS) CYP2D6 (440%)		72 h	testosterone phenacetin S-mephenytoin tolbutamide dextromethorphan	6β-hydroxytestosterone acetaminophen 4′-OH-mephentoin OH-tolbutamide		\$\tau_70\%\$\$\tau_72\%\$\$\$\tau_82\%\$\$\$\tau_17\%\$\$\$\tau_42\%\$	(17)
I	HepaRG	24 h	10 ng/mL	CYP3A4 (490%) CYP3A5 (479%) CYP1A2 (487%) CYP2C19 (464%) CYP2C9 (462%) CYP2E1 (454%)		72 h	atorvastatin phenacetin S-mephenytoin tolbutamide	o-OH-atorvastatin acetaminophen 4'-OH-mephentoin OH-tolbutamide		%08 < \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	(15)
TGF-β	ЬНН	24 h	10 ng/mL	CYP3A4 (↓75%) CYP2C9 (↓50%) CYP2C19 (↓50%)							(14)

74

 Table 1
 Continued

			Effect on C	Effect on CYP mRNA expression	u		Effe	Effect on drug metabolism			
Stimulus	Model	Dura- tion	Studied concen- tration	Maximal effect (%)	Potency (EC <sub>50</sub> )	Dura- tion	Drug	Metabolite	Potency (EC <sub>50</sub> ng/mL)	Maximal effect (%)	Ref
IFN-y	РНН	24 h	10 ng/mL	CYP3A4 (↓75%) CYP2C9 (NS) CYP2C19 (NS)							(14)
IL-22	РНН	48 h	10 ng/mL	CYP3A4 (↓70%) CYP1A2 (↓45%) CYP2C9 (↓50%)							(25)
	HepaRG	24 h	10 ng/mL	CYP3A4 (↓75%) CYP1A2 (↓60%) CYP2C9 (↓50%)	1.7	48 h	midazolam phenacetin	1'-hydroxymidazolam acetaminophen		^20%	(25)
IL-2	PHH *		2-200 ng/ mL	CYP3A4 (?)		72 h	testosterone	6β-hydroxytestosterone		NS	(19)
	PHH *	48 h	10 ng/mL	CYP3A4 (NS) CYP1A2 (NS)		72 h	testosterone phenacetin	6β-hydroxytestosterone acetaminophen		NS NS	(17)
				CYP2C19 (NS)			S-mephenytoin	4'-OH-mephentoin		<b>\121%</b>	
				CYP2C9 (NS)			tolbutamide	OH-tolbutamide		NS	
				CYP2D6 (†150%)			dextromethorphan	dextrorphan		<b>122%</b>	
	PHH:KC (10:4) *		200 ng/ mL	CYP3A4 (?)		72 h	testosterone	$6\beta\text{-hydroxytestosterone}$		%021	(19)

Table 1 continues on next page.

Table 1 Continued

			Effect on C	Effect on CYP mRNA expression	ш		Effe	Effect on drug metabolism			
Stimulus	Model	Dura- tion	Studied concen- tration	Maximal effect (%)	Potency (EC <sub>50</sub> ng/mL)	Dura- tion	Drug	Metabolite	Potency (EC <sub>50</sub> ng/mL)	Maximal effect (%)	Ref
	PHH:KC (10:4)		200 ng/ mL	CYP3A4 (?)		96 h	luminogenic P450- Glo" substrate	proluciferin substrate		NS	(21)
IL-12	ЬНН	48 h	10 ng/mL	CYP3A4 (NS) CYP2C19 (NS) CYP2C9 (NS)		48 h	testosterone S-mephenytoin tolbutamide	6β-hydroxytestosterone 4′-OH-mephentoin OH-tolbutamide		SN	(26)
IL-23	ЬНН	48 h	10 ng/mL	CYP3A4 (NS) CYP2C19 (NS) CYP2C9 (NS)		48 h	testosterone S-mephenytoin tolbutamide	6β-hydroxytestosterone 4′-OH-mephentoin OH-tolbutamide		NS	(26)
	PHH:KC (10:4)		200 ng/ mL	CYP3A4 (?)		96 h	luminogenic P450- Glo" substrate	proluciferin substrate		NS	(21)
LPS	РНН	24 h	10 µg/ml	CYP3A4 (↓95%) CYP2C9 (NS) CYP2C19 (NS)							(14)
	HepaRG	48 h	1.37–333 ng/mL	CYP3A4 (\$95%)	<1.37	72 h	midazolam	1'-hydroxymidazolam	7.85	%09↑	(16)

NS = not significant. \* = effect of inflammatory mediator on CYP expression and activity was investigated after treatment with a standard CYP-inducer. \* = excluding non-responders.  $\uparrow$  = increased CYP expression.  $\downarrow$  = decreased CYP expression. ? = effect of inflammatory mediator on CYP mRNA expression was not determined.

76

#### Interleukin-6 (IL-6)

IL-6 is the chief stimulator cytokine in activation of innate immunity in the liver to contribute to host defense (27). Owing to its role as the main cytokine in the acute phase response (APR), multiple studies have focused on investigating the effect of IL-6 on CYP levels in vitro.

#### Maximal Effect (E<sub>max</sub>) (mRNA)

Numerous investigators have confirmed that IL-6 is a potent downregulator of CYP enzymes in both primary human hepatocytes (PHHs) and in the HepaRG cell line, an immortalized human hepatic cell system that retains PHH characteristics but lacks donor variability. Aitken et al. investigated the effect of inflammatory mediators including IL-6 on mRNA expression of CYP2C9, CYP2C19, and CYP3A4 in PHHs (14). Treatment with IL-6 downregulated mRNA levels for all isoforms studied, but simultaneously revealed profound differences in the magnitude of downregulation, as the expression of CYP3A4 was markedly more reduced than that of CYP2C9 or CYP2C19. A similar observation was made by Dickmann et al. (19) and Klein et al. (15) in PHHs and by Tanner et al. (23) in the HepaRG cell line, who all reported that IL-6 exerted the strongest downregulation on CYP3A4, whereas the effects of IL-6 on other CYPs, most notably CYP2D6, seemed to be more limited. It should be noted from the work of Klein et al. that IL-6 may also induce expression of CYP2E1 in PHHs, which could be relevant for the metabolism of certain anesthetics (15). Beyond this exception, IL-6 predominantly reduces CYP expression and thus impairs the biotransformation of a wide range of (pro) drugs that are metabolized through CYP enzymes.

#### Sensitivity between CYPs (mRNA)

The strength of the Dickmann study is that it examined the effects of IL-6 at different concentrations, allowing determination of the potency (EC $_{50}$ ) and thus rank ordering the responsiveness of the major CYP enzymes following IL-6 exposure (19). Through this approach this study was able to demonstrate that the exerted effects of IL-6 in PHH occur at physiological relevant concentrations, as similar concentrations of IL-6 have been detected in the circulation of patients with either chronic or acute inflammation (28,29). Importantly, these investigations revealed that CYP3A4 was also by potency most sensitive to downregulation by IL-6, as IL-6 downregulated CYP3A4 mRNA with an EC $_{50}$  of 0.0032 ng/mL, whereas

a 20- to 500-fold higher concentration of IL-6 was needed for downregulation of other CYPs. A similar difference in CYP sensitivity to IL-6 was observed by Rubin et al. in both PHHs and HepaRG cells (17). Such differences in sensitivity are potentially important as these data suggest that drugs metabolized by CYP3A4 may be affected already at an earlier state during inflammation than drugs that rely on other CYP enzymes.

#### Sensitivity between PHH donors

Another point of attention is the observed interindividual variability in response to IL-6 between donors in a single experimental setup, excluding inconsistencies observed between studies due to model variations or treatment differences (30). For example, Dickmann et al. reported  $EC_{50}$  values for CYP1A2 activity suppression between 0.142 and 4.07 ng/mL (ranging 29-fold) over five donors and a range between 0.0042 and 0.176 ng/mL (ranging 42-fold) for CYP3A4 activity suppression (19). Evers et al. also reported that CYP3A4 downregulation upon IL-6 stimulation varied largely between donors in one experimental setup, reporting  $EC_{50}$  suppression values over approximately a 20-fold range between donors (30). The observed different susceptibility to inflammation between donors may be a consequence of both genetic variability and differences in disease state or medical history of the studied donors.

#### Drug-metabolizing activity

Determination of the cytochrome P450 enzymatic activity is important because beyond the described transcriptional effects, posttranscriptional mechanisms may also contribute to the effects of inflammation on drug metabolism (14,31). As can be observed from Table 1, effects of inflammation have commonly been assessed by measuring metabolite formation of probe substrates of CYP3A4 (midazolam/testosterone), CYP1A2 (phenacetin), CYP2C9 (tolbutamide), CYP2C19 (S-mephenytoin), and CYP2D6 (propafenone/dextromethorfan). Klein et al. showed in PHHs trends for reduced metabolite formation upon IL-6 treatment but statistical power was lacking, presumably because of the heterogeneity of the donors and potential pharmacogenetic variation in CYP450 enzymes as confounding factors (15). The HepaRG cell line lacks interindividual variability and showed stable CYP expression in the control group over at least 72 h, increasing the reproducibility of the results. In this model, the highest suppression of activity

was noted for CYP3A4 as compared to other CYPs, in line with the observed transcriptional downregulation. Tanner et al. showed decreased downregulation of activity for CYP3A4, CYP1A2, and CYP2C19 but not CYP2C9 and CYP2D6 after 24 h (23).

#### **Pathways**

IL-6 may exert its effects in hepatocytes via distinct pathways, as the binding of IL-6 to its receptor initiates cellular signaling pathways via three arms, the Janus kinase (JAK)/STAT protein-3 (STAT3) pathway, the mitogen activated protein kinase (MAPK)/extracellular regulated kinase 1 and 2 (ERK1/2) pathway, and the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway (32). Keller et al. found that, using chemical inhibitors in IL-6 treated PHHs, especially the MAPK/ERK and PI3K/AKT signaling pathways – and not the canonical JAK/STAT pathway – were critical for downregulation of CYP enzymes during inflammation (33). However, it should be noted that the effect of individual kinase inhibitors was tested in only one individual donor. In contrast, Febvre-James et al. found that treatment with the JAK inhibitor ruxolitinib completely reversed the IL-6-mediated suppression of CYP1A2 and CYP3A4 mRNA levels in both HepaRG cells and PHHs, suggesting a prominent role of the JAK/STAT pathway in CYP downregulation (16). As such, multiple signaling arms of the IL-6 pathways can be held responsible for the observed downregulation of CYP enzymes.

#### Long-term studies

Implementing long-term investigation on inflammation-induced CYP suppression in vitro could aid in a better understanding of the chronic inflammation frequently observed in a clinical setting. Long-term investigations on the effect of inflammatory mediators on CYP expression are scarce, especially in PHHs since CYP expression rapidly declines over time in this model (34). Long et al. investigated the effect of IL-6 on CYP3A4 activity in a 3D microreactor platform with PHH and Kupffer cells (21). They tested the effect of tocilizumab, an anti-IL-6 receptor antibody, on inflamed hepatocytes and found that coadministration of tocilizumab with IL-6 after initial 4-day IL-6 treatment prevented the CYP3A4 activity decrease across donors. This highlights that the model is capable of capturing physiological adaptation to inflammation, since CYP3A4 desuppression occurred. Tanner et al. collected data on the long-term effects of IL-6 treatment (14 days) in HepaRG

cells, which resulted in more pronounced downregulation of P450 expression as compared to short-term treatment (23). Still, current studies do not address the impact of long-term low concentrations of cytokines as compared to single high-dose treatment, which leaves an open question.

#### Clinic

Interestingly, the effects of inflammation on drug metabolism in the clinic, most commonly assessed through the IL-6 regulated marker C-reactive protein (CRP), seems to be most reported for CYP3A4 substrates including midazolam, tacrolimus, and/or voriconazole, and less for drugs metabolized through other metabolic pathways. This is in line with data from in vitro hepatocyte models where IL-6 exerts most profound effects on CYP3A4 (4). Altogether, these data confirm isoform specific effects of IL-6 and suggest that drugs metabolized via CYP3A4 may be more prone to the effects of inflammation.

#### Interleukin 1 (IL-1)-family: interleukin-1\beta and interleukin-18

The IL-1 family compromises a group of 11 proteins that play a role in the initiation and regulation of inflammatory responses, of which IL-1 $\beta$  is the most studied member (35).

#### Maximal effect (E<sub>max</sub>) (mRNA)

In PHHs, IL-1 $\beta$  treatment reduced CYP3A4 mRNA expression with 95%, but it had no effect on CYP2C9 or CYP2C19 mRNA levels (14). Protein levels of CYP3A4, but also of CYP2C9, were significantly downregulated after 24 h of treatment with IL-1 $\beta$ . Dickmann et al. showed donor-wide suppression (n = 5) for CYP3A4/A5, however, IL-1 $\beta$ -mediated suppression of other CYP isoforms (CYP2C9, C19, and 1A2) was not consistently observed among all donors (24). The observed nonresponse towards IL-1 $\beta$  of certain CYP isoforms cannot simply be explained by a lack of effect, since IL-1 $\beta$  consistently reduced CYP3A4 expression by > 80% in all donors. Alternatively, because the nonresponse to IL-1 $\beta$  can perhaps be explained by pharmacogenetic variation within these CYP isoforms. IL-18 treatment in HepaRG cells and PHHs did not result in significant downregulation of mRNA levels nor CYP activity (17).

#### Sensitivity between models

Interestingly, although Klein et al. showed that the maximal impact of Il-1β and IL-6 on the mRNA expression of CYP isoforms was comparable in HepaRG cells, IL-1\beta showed an approximate 100-fold higher potency than IL-6 in inducing the same downregulation (15). This described difference in potency might be underlined by the fact that the HepaRG cell line displays morphological heterogeneity, including clusters with nonparenchymal cells which could aggravate or sensitize the response to an inflammatory mediator (36). For example, IL-1β release is associated with activation of the inflammasome in Kupffer cells (37), providing a feed-forward stimulus for production of more inflammatory cytokines which could potentially aggravate cytokine-induced downregulation of CYPs. Indeed, coculturing of Kupffer cells increased responsiveness to IL-1β as compared to monocultures of hepatocytes, as evident from an EC<sub>50</sub> shift from > 5 to 0.098 ng/mL for CYP3A4 suppression upon coculturing, an effect not seen with IL-6 treatment (20,22). Since IL-18 is also reported to mediate its effect through Kupffer cells (38), this can explain the lack of effect on CYPs in HepaRG or PHHs cell models described by Rubin et al. Thus, inclusion of nonparenchymal cells in model systems might increase the responsiveness to IL-1β and IL-18 and hence better reflect the potential effect these inflammatory cytokines may have in an intact human liver.

#### Sensitivity between PHH donors

Looking at the suppression of activity of CYP3A4 and CYP1A2 in PHHs upon IL-1 $\beta$  treatment, again large interdonor variation is evident (24). Dickmann et al. found an average EC $_{50}$  value for two donors of 0.450 ng/mL (three donors showed no response) regarding CYP1A2 activity. For CYP3A4, EC $_{50}$  values for activity ranged from 0.005 to 1.06 ng/mL over five donors.

#### **Pathways**

The effects of IL-1 $\beta$  are presumed to be mediated via activation of the nuclear factor kappa B (NF-kB) pathway (39). Importantly, IL-1 $\beta$  may also rapidly (within 2–4 h) induce IL-6 expression, which raises the possibility that part of the observed actions of IL-1 $\beta$  are actually mediated by IL-6 (40). Interestingly, a recent study by Febvre-James et al. found that the IL-1 $\beta$  repression of CYP enzymes could not be reversed by the JAK inhibitor ruxolitinib, confirming that IL-1 $\beta$  and IL-6 induce distinct pathways upon inflammation and may complement one another in altering drug metabolism (16).

#### Tumor necrosis factor $\alpha$ (TNF- $\alpha$ )

TNF- $\alpha$  is another main cytokine involved in inducing the acute phase response in the liver during inflammation. Hepatocytes express the tumor necrosis factor receptor 1 (TNFR1) that upon binding by TNF-α results in the activation of the major NF-κB pathway and the MAPK/ERK pathway (41). Aitken et al. found that TNF-α treatment induced CYP3A4 mRNA downregulation but not protein downregulation after 24 h (14). They saw no effect on CYP2C9 and CYP2C19 mRNA levels upon TNF-α treatment, but interestingly the CYP2C9 protein levels were reduced by > 95% after 24 h treatment, pointing to a mismatch between the effects on mRNA and protein expression levels. This suggests that posttranscriptional mechanisms, i.e., protein degradation or regulation by miRNAs, are involved in downregulation of CYP protein levels by TNF-α. In line, Dallas et al. reported no effects of TNF-α on CYP2C19 and CYP2C9 mRNA levels, but found significantly downregulated CYP2C19 and CYP2C9 activity (18). Klein et al. found that TNF-α treatment resulted in similar downregulation of CYP gene expression in HepaRG cells as observed with IL-6 treatment, presuming that part of the effect of TNF-α is mediated via nonparenchymal cells (15). After 72 h of exposure to TNF-α, all P450 activities were reduced by more than 80%.

#### Pathogen associated molecular patterns (PAMPs)

PAMPs, such as lipopolysaccharide (LPS), are microbial molecules that can signal immune cells to destroy intruding pathogens associated with infection (42). Upon LPS recognition, the toll like receptor 4 (TLR4) signaling pathway ultimately activates NF-kB. The study by Aitken et al. found LPS to be the most efficacious inflammatory stimulus in downregulating mRNA levels of CYP3A4, and CYP3A4 protein levels were decreased by about 75% of control 24 h after treatment (14). Whereas LPS treatment did not influence mRNA levels of CYP2C9 or CYP2C19, CYP2C9 protein levels were reduced by 80% after 24 h of treatment, again indicating a mismatch between mRNA and protein levels. This is in accordance with data presented by Rubin et al. in HepaRG cells and PHHs, where LPS downregulated CYP3A4 and CYP1A2 mRNA levels in both models (17). LPS showed comparable potency in downregulating CYP3A4 compared to IL-6, but was much less potent in downregulating CYP1A2 levels.

## Other cytokines: transforming growth factor $\beta$ (TGF- $\beta$ ), interferon $\gamma$ (IFN- $\gamma$ ), interleukin-22 (IL-22), interleukin-23 (IL-23), and interleukin-2 (IL-2)

The effect of other pro-inflammatory mediators beyond IL-6, IL-1β, TNF-α, and PAMPs has also been studied in in vitro hepatocyte models. TGF-β, an inflammatory mediator linked to fibrosis, caused significant downregulation of CYP3A4, CYP2C9, and CYP2C19 mRNA levels and subsequent protein levels (only shown for CYP3A4 and CYP2C9) (14). Interestingly, only TGF-β and IL-6 downregulated CYP2C9 mRNA, but protein expression levels of CYP2C9 were strongly downregulated by all inflammatory stimuli tested. IFN-y, a mediator that is associated with the immune response to viral infections, only reduced mRNA levels of CYP3A4 in PHHs. Conversely, IL-22, a pro-inflammatory mediator found in different auto-immune disorders, was found to repress mRNA levels of CYP1A2, CYP3A4, and CYP2C9 in PHHs and HepaRG cells (25). Studies investigating the effect of IL-2 on CYP3A4, 1A2, 2C9, 2C19, and 2D6 expression found no suppression of mRNA levels upon treatment in PHHs (18,20). Interestingly, when culturing the hepatocytes together with Kupffer cells, a concentration-dependent decrease (50–70%) of CYP3A4 activity was observed with IL-2 at 72 h, suggesting that Kupffer cells are essential for the suppressive effect of IL-2 (20). IL-12 and IL-23, pro-inflammatory mediators associated with inflammatory autoimmune responses, did not impact CYP3A4 levels (26) and a coculture model did not change this (22). The effect of other cytokines on CYP expression and activity is yet to be determined.

#### **Summary**

The in vitro data summarized here suggests that direct treatment with inflammatory stimuli can suppress DMEs stemming from the CYP1, CYP2, and CYP3 family. This suppressive effect is most convincingly demonstrated for IL-6, IL-1 $\beta$ , TNF- $\alpha$ , and LPS. CYP3A4 seems to be most susceptible to cytokine-induced downregulation in human in vitro hepatocyte models, whereas CYP2D6 seems to be the least sensitive. The enzyme expression of CYP1A2, CYP2C9, and CYP2C19 was also sensitive to the effects of inflammatory mediators, though higher concentrations of cytokines were in general required to downregulate these enzymes and the response was not always conserved among all studied donors. Interestingly, model-dependent responses were observed which could be reliant on the presence of nonparenchymal

cells. The effect of inflammatory mediators should therefore be divided into direct effects on hepatocytes and indirect effects through inflammatory signaling in nonparenchymal cells.

Importantly, interdonor variation in response to inflammation within the same experimental setup was observed. Translating these findings to the clinic, the consequences of inflammation-induced phenoconversion for drug treatments may differ therefore greatly between individuals and between the metabolic CYP pathways via which drugs are metabolized.

# Mechanistic pathways via which inflammation modulates hepatic functions that are critical for drug metabolism

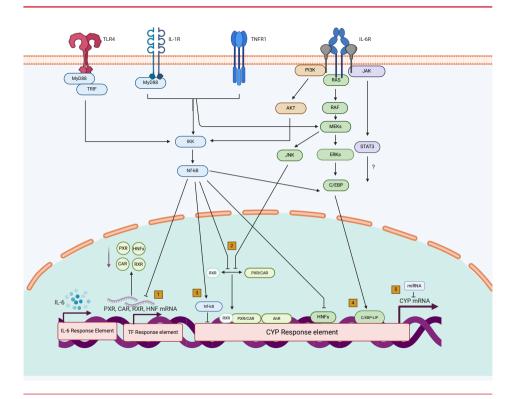
The above described findings from in vitro models show that the sensitivity to inflammation may differ between CYP isoforms and inflammatory stimuli. This implies that distinct mechanisms are involved in the downregulation of CYP enzyme expression and activity.

Mechanistically, regulation of hepatic CYP levels and interactions with CYP gene regulators is complicated and includes a wide variety of ligand-activated transcription factors and mediators. Cytokine-mediated alteration of gene transcription is thought to be the main regulatory mechanism accountable for changing CYP450 activity upon inflammation. It is essential to note that no single common pathway is recognized for all the CYP enzymes and underlying mechanisms are cytokine-specific. Here we describe, summarized in Figure 1, how repression of important CYP enzymes during inflammation may proceed through (1) transcriptional downregulation of transcription factors, (2) interference with dimerization/translocation of (nuclear) transcription factors, (3) altered liver-enriched C/EBP signaling, (4) direct regulation by NF-κB, or (5) post-transcriptional mechanisms.

#### Transcriptional downregulation of transcription factors

Transcription factors involved in the regulation of CYP mRNA levels, including the nuclear receptors pregnane X receptor (PXR), the constitutive androstane receptor (CAR), their dimerization partner retinoid X receptor (RXR), the aryl

hydrocarbon receptor (AhR), as well as human nuclear factors (HNFs) are held responsible for the observed downregulation of DMEs upon inflammation. It is important to distinguish between the role of nuclear transcription factors in the constitutive expression of CYP enzymes versus drug- or inflammation-mediated expression. Here we will focus on the nuclear hormone receptor mechanisms likely to be involved in inflammation-altered CYP expression.



**Figure 1** Mechanistic insights into the effects of inflammation on CYP expression and activity. Transcriptional repression of important CYP enzymes during inflammation may proceed through (1) transcriptional downregulation of nuclear receptors and other transcription factors, (2) interference with dimerization/translocation of nuclear transcription factors, (3) direct regulation by NF-κB, (4) altered liver-enriched C/EBP signaling, or (5) posttranscriptional mechanisms.

#### Downregulation of nuclear receptors

The PXR (gene: *NR1I2*) and the CAR (gene: *NR1I3*) are members of the nuclear receptor superfamily highly expressed in the enterohepatic system of mammals (43). These ligand activated transcription factors have been identified as key

transcriptional regulators of the cytochrome P450 xenobiotic-metabolizing enzymes, mostly for the CYP2C9, CYP2C19, CYP3A4, and CYP3A5 enzyme expression (44). Upon binding with the RXR, the heterodimer nuclear receptor-RXR complex binds to responsive elements present in the 5'-flanking regions of target genes, usually resulting in an upregulation of gene expression aimed at increased metabolism of drugs. Studies have indeed shown that PXR and CAR increase transcription of the human CYP3A4/5, CYP2C9, CYP2C19, and CYP1A2 genes upon drug treatment (45,46,47).

One mechanism by which inflammation changes gene transcription of major DMEs is through repression of the nuclear receptor PXR and CAR. A vast body of evidence shows that inflammation represses PXR levels, leading to downregulation of important CYP enzymes. Pascussi et al. pioneered in showing that IL-6 downregulates PXR mRNA in PHH and inhibits the rifampicine-induced induction of CYP3A4 (48). Upon LPS treatment in HepG2 cells, the mRNA and protein levels of PXR are reduced (49). Mechanistically, a decrease in PXR expression within the nucleus was observed, leading to reduced transactivation of the CYP3A4 promotor and subsequent inhibited transcriptional activity of CYP3A4. Additionally, LPS treatment in mice led to functional repression of PXR's dimerization partner RXR (50). Yang et al. showed that inhibition of a CYP3A4 promotor reporter after IL-6 treatment in human hepatocytes was greater in the presence of PXR than after its knockdown, suggesting a role for PXR in IL-6-facilitated suppression of CYP3A4 (51). Knockdown of PXR in human hepatocytes reversed the IL-6-induced CYP3A downregulation. Furthermore, the authors suggest that downregulation of PXR by inflammatory stimuli is causative for decreased transcription of CYP3A4: a continuous decrease in PXR levels was observed already after 1.5 h of treatment, whereas a significant decrease in CYP3A4 mRNA levels occurred only after 3 h. A likely scenario is that the suppressive effect of inflammation on PXR expression is mediated through NF-kB activation, since Zhou X et al. showed that NF-kB directly interacts with a functional binding site in the PXR promotor to suppress its transcriptional expression (52). Transcriptional downregulation of CAR upon inflammatory stimuli has also been reported. A study by Assenat et al. investigated the negative regulation of CAR via pro-inflammatory cytokines IL-1β and LPS in human hepatocytes (53). IL-1β treatment reduced mRNA levels of CYP2B6, CYP2C9, and CYP3A4 through NF-κB p65 activation. This p65 subunit of the

NF- $\kappa$ B complex interfered with the distal glucocorticoid response element present in the CAR promotor, leading to repressed transcription of CAR. In contrast, the AhR is not substantially affected by IL-6 treatment (15,23). As such, it appears that the response to inflammation is substantial for PXR and CAR and their dimerization partner RXR, but not for AhR.

Still, some debate remains about the role of nuclear receptors in the downregulation of CYP enzymes during inflammation, mostly stemming from conflicting rodent vs. human studies. In the rodent field, a study by Beigneux et al. suggested that downregulation of PXR and CAR was causative for CYP450 downregulation (50), whereas other experiments suggest that downregulation of important P450 enzymes does not necessitate the nuclear receptor PXR. As an example, Richardson et al. found that downregulation of multiple CYP mRNAs was similar in LPS-treated control and PXR-null mice, suggesting a PXR independent mechanism (54). For the human situation, transcription factors responsible for the homeostasis of CYPs are evidently downregulated through inflammation. However, up to what extent downregulation of these transcription factors can actually be held responsible for the inflammation driven changes in expression of DMEs and drug metabolism itself remains to be further investigated.

#### Downregulation of hepatocyte nuclear factors

Hepatocyte nuclear factors (HNFs), including HNF-1 $\alpha$  and HNF-4 $\alpha$ , form another important family of transcription factors. They can modulate CYP expression in the liver through DNA-binding interactions in CYP promotors or via modulation of PXR and CAR expression (55–59). Despite their well-documented role in CYP homeostasis, the contribution of HNFs for the inflammation-induced changes in CYP expression remain, however, scarcely investigated.

The binding activities of HNF-1 $\alpha$  and HNF-4 $\alpha$  to DNA were quickly reduced in rat livers treated with LPS in parallel with downregulated hepatic CYP mRNA levels (60). In HepG2 cells, treatment with IL-6 and IL-1 $\beta$  resulted in a 10% decrease of HNF-4 $\alpha$  activity as a result of an altered phosphorylation status (61). Acute and prolonged treatment with IL-6 reduced mRNA levels of HNF-4 $\alpha$  in HepaRG cells, but this effect was not seen for HNF-1a and the changes shrink into insignificance compared to the observed downregulation of, e.g., PXR (23). In contrast, Klein et al. found that mRNA levels of the HNF-4 $\alpha$  were downregulated ( $\approx$ 40%) by IL-6 only at the early time point of 8 h and seemed to have normalized after 24 h (15).

However, a direct link between the fast transcriptional suppression of P450 genes and the reduced mRNA levels/activity of HNFs is still lacking, questioning a prominent role of transcriptional HNF downregulation as a factor in IL-6-induced DME suppression.

## Interference with dimerization/nuclear translocation of (nuclear) transcription factors

Impairment of the activity of important transcription factors could, in addition to the above described transcriptional repression of transcription factors, also contribute to repression of CYPs during inflammation. Tanner et al. questioned whether transcriptional downregulation of PXR and CAR mRNA levels itself can fully explain the observed downregulation of CYP enzymes (23). They suggested that the transactivation potential of PXR and CAR might be simultaneously influenced by inflammation. They found a clear correlation between downregulated PXR and CYP mRNA levels after short-term treatment with IL-6. However, the reduction in PXR expression following prolonged treatment (14-days) with IL-6 was very modest compared to the downregulation observed for the CYP enzymes. As such, downregulation of nuclear receptor target genes (e.g., CYPs) during inflammation could be a consequence of decreased availability of PXR itself, or an impairment of the translocation/activity of the receptor.

The existence for such interactions between inflammation and hepatic transcription factors (PXR, CAR, and AhR) have been suggested for both the NF-κB pathway and pathways related to IL-6 signaling. A hypothesized mechanism for this is interference of NF-κB with the dimerization of PXR to RXR and subsequent binding to DNA, thereby inhibiting the activity of PXR. The inhibited transcriptional activity of PXR leads to downregulation of DMEs in HepG2 cells (62). As NF-κB interferes with the binding of RXR to PXR, this mechanism of repression by NF-κB may also hold true for more nuclear receptor-controlled systems where RXR is the dimerization partner (e.g., CAR), but no experimental evidence exists that can yet support this. AhR-regulated CYP1A2 is likely not regulated by this mechanism. Studies in mouse hepatoma cells have shown that interactions between the P65 subunit of NF-κB and AhR may result in the formation of an inactive complex, with possible consequences for the translocation to the nucleus (63). In addition, NF-κB has been shown to inhibit transcriptional

activity of AhR by reducing histone acetylation of promotors of CYP enzymes (e.g., CYP1A2), thereby altering the accessibility of the DNA for nuclear transcription factors (64). Thus, activation of the NF-κB pathway may modulate the activity of nuclear transcription factors through changes in dimerization, translocation, or chromatin remodeling.

Kinases involved in the IL-6 signaling pathway can also alter the protein status and translocation of nuclear receptors. Cell signaling protein kinases such as Jun-N-terminal kinase (JNK) and protein kinase C (PKC) can repress the activity of the nuclear receptors PXR and CAR, thereby altering their function and impact on downstream transcriptional CYP activity (65-67). One hypothesized mechanism is that kinases can alter the phosphorylation status of these nuclear receptor proteins. IL-1β treatment induces JNK expression which can phosphorylate RXR, leading to reduced nuclear binding activity and subsequently inhibited RXR-dependent hepatic gene expression (68). Additionally, LPS-induced downregulation of P450 genes was attenuated upon treatment with a specific INK inhibitor in a primary mouse hepatocyte model (69). Thus, JNK can play a role in inflammation-mediated downregulation of nuclear receptors with RXR as partner. This was backed up by findings from Ghose et al., who showed that an increase in JNK signaling is associated with higher export of RXR out of the nucleus upon low-dose LPS treatment, leading to less RXR-mediated hepatic gene expression (70). Additionally, ERK signaling has been proven to impair nuclear translocation of CAR in a mouse primary hepatocyte model (71). Altogether, these findings indicate that kinases play an important role in the regulation of nuclear receptors and their dimerization with RXR, thereby offering a general mechanism for the suppression of genes regulated by nuclear receptors during inflammation. How other important inflammatory cell-signaling components in the IL-6 pathway, such as STAT3, mechanistically regulate CYP repression remains to be investigated.

#### Direct regulation by NF-κB

NF- $\kappa$ B can precisely control the expression of CYP1A1, CYP2B1, CYP2C11, CYP2D5, CYP2E1, and CYP3A7 via interaction with the promotors of these genes, leading to downregulation in most cases (72). For example, Iber et al. reported that the CYP2C11 promotor region contains a low-affinity binding place for NF- $\kappa$ B and mutations in the 3'-end or 5'-end in this NF- $\kappa$ B response element reduced the

binding affinity for NF-κB and subsequently suppressed CYP2C11 transcription by IL-1 or LPS in rat hepatocytes (73). However, experimental evidence for this hypothesis has only been obtained in animal models. Although there is high conservation of CYP enzymes amongst species, the extent and catalytic activity between species differs, highlighting that caution should be taken in extrapolation of results to a human situation (13).

#### Altered liver-enriched C/EBP signaling

The expression and DNA binding activity of the transcription factor C/EBPβ is severely enhanced during the acute phase liver response through activation of the NF-κB pathway (74). One mechanism that is hypothesized to contribute to CYP repression upon IL-6 stimulation is altered balance between two isoforms of the transcription factor C/EBP-β: the liver-enriched transcriptional activating protein (LAP) and the liver-enriched transcriptional inhibitory protein (LIP). The LIP isoform is a shortened variant of C/EBPβ deficient of transactivation activity. Jover et al. found upregulation of the C/EBPβ-LIP protein isoform in HepG2 cells treated with IL-6 (75). They demonstrated that LIP antagonized transactivation of CYP3A4 by the functional LAP isoform. This altered LAP:LIP ratio correlated with a downregulation of CYP3A4 enzyme levels. Martinez et al. showed a novel enhancer site located in the CYP3A4 gene where the LAP isoform can bind and initiate transcription, whereas the antagonizing action of the truncated LIP isoform on LAP resulted in CYP3A4 gene repression, confirming that the LAP:LIP ratio is of importance in regulation of constitutive expression of CYP3A4 (76). A C/ΕΒΡβbased mechanism was also found to be involved in transcriptional repression of CYP2A6 (77). It is yet to be determined whether this mechanism can also explain repression of other CYPs upon IL-6 stimulation in a human model.

#### Posttranscriptional mechanisms (miRNA)

The mechanisms behind downregulation of DMEs upon inflammation, as described above, remain an area of intense study. Increasing attention is being given to the potential post-transcriptional mechanisms that could regulate P450 enzymes in inflammation as well. MicroRNAs (miRNAs) can influence the translation and stability of cellular mRNAs at their 3′-UTR side, offering a broad mechanism for gene expression regulation (78). Previous research has already shown that miRNA

activity regulates phase I and II metabolizing enzymes and transcriptional factors through posttranscriptional modification (31,79,80).

A recent study by Kugler et al. questions whether the previously observed mechanisms are sufficient to explain the huge downregulation of DMEs observed upon inflammation and investigated the possible role of miRNA in this process (81). They performed transfections with five inflammation-associated miRNAs in HepaRG cells and looked at the CYP mRNA levels and activity. They found miRNA-dependent downregulation of several CYP mRNA and expression levels after 96 h, where CYP2C19 and CYP3A4 were amongst the top downregulated genes. Thus, miRNAs might be an extra factor in downregulating drug metabolizing capacity during inflammation. Potentially, this could also explain the sometimes observed mismatch between CYP mRNA levels and CYP protein levels after inflammatory stimuli, as was the case for CYP2C9 in the study from Aitken et al. (14). Since the 3'-UTR region of CYP2C9 can directly be regulated by miR-130b, this could explain the downregulation of CYP2C9 enzyme expression. As such, miRNA regulation could (in part) be responsible for the effects of inflammatory mediators on protein levels in the absence of preceding downregulation of mRNA.

Other post-transcriptional mechanisms, such as the role of nitric oxide in the cytokine-mediated regulation of CYPs were excellently reviewed by Morgan et al. (82).

#### Concluding remark

Concluding from previous sections, we hypothesize that the variation in sensitivity of different CYP enzymes for inflammation stems from the distinct mechanisms that regulate them. It seems like PXR- and CAR-regulated CYP enzymes (3A4/5, 2C9, 2C19) are more sensitive to inflammation, whereas the AhR regulated isoform CYP1A2 is less sensitive. CYP2D6 shows to be least sensitive to inflammation, which might be due to the fact that it is not inducible by nuclear receptors and therefore not sensitive to inflammation-induced alterations of the levels of PXR, CAR, and AhR that regulate the expression of other CYPs (83–85). Most interestingly, deduction of CYP specific inflammatory mechanisms of downregulation can shed light on the distinct sensitivities towards inflammation.

# Pharmacogenetic variation in inflammatory pathways and the effect on drug phamracokinetics

The available data from in vitro experiments with PHHs on drug metabolism have indicated that the response to inflammation or its inflammatory mediators may differ substantially between donors under controlled experimental conditions (19,24,30). This raises the question whether the observed distinct response to inflammation between persons is also observed in the clinic. Clinical studies by van Wanrooy et al. and Vet et al. have shown that the metabolism of voriconazole and midazolam at similar concentrations of CRP and corrected for other known confounding factors may still vary considerably between patients (5,6). These findings from both in vitro models and clinical studies suggests the existence of interindividual variability with regards to the effects of inflammation on drug metabolism. This distinct response towards inflammation between subjects may in part be caused by genetic variability in the described pathways via which inflammation modulates the activity of DMEs.

By presenting examples from the available literature we illustrate how genetic variability within the different elements presented in Figure 1 can modulate the outcomes of the effect of inflammation on drug metabolism and consequently may contribute to the observed interindividual variability in the effect of inflammation.

#### Genetic variation: inflammatory mediators

It is well established that genetic variability within inflammatory mediators (e.g., cytokines) can predispose individuals to an altered susceptibility to immune-related disease (86). For this reason, it is plausible that polymorphisms in cytokine genes could shape the immune response that affects drug metabolism. One prominent example relates to the rs1946518 (-607C/A) variant within the promoter of IL-18 and its effects on the metabolism of the immunosuppressive tacrolimus. Xing et al. and Zhang et al. demonstrated that Han-Chinese patients carrying the AA genotype (19–29% of the patients) exhibited lower concentration/dose (C/D) ratios of tacrolimus within the first month after lung or kidney transplantation than patients with an AC or CC genotype (87,88). Interestingly, this relationship for the rs1946518 variant was exclusively shown for patients expressing CYP3A5\*1 and functionally linked to lower expression of IL-18 mRNA in the liver. These results

imply that the rs1946518 variant reduced the IL-18 driven inflammation in the liver, which prevents the inflammation-induced downregulation of CYP3A5 and consequently reduces the impact of inflammation on drug metabolism in these patients. Importantly, rs1946518 did not modulate C/D ratios in liver transplant patients who were already treated for 1 year with tacrolimus (89). These results suggest that the variant only affects drug metabolism shortly after transplantation when the immune/inflammatory responses are highest. Altogether, this example illustrates that genetic variability within inflammatory mediators has the potential to modulate the effects of inflammation on drug metabolism.

#### Genetic variation: inflammatory receptors

As described above, toll-like receptor (TLR) activation by pathogen-associated molecules may downregulate CYP3A4 expression and modulate drug metabolism. However, TLR activation may also be triggered by endogenous molecules (e.g., DNA) that are released during ischemia-reperfusion injury that develops during organ transplantations (90). Therefore, it has been postulated that genetic variability in TLRs may alter the effect of inflammation and its consequences for drug metabolism. Ou et al. showed that liver transplant patients with the TLR9-rs352139 AA genotype exhibited lower C/D tacrolimus levels than carriers of the AG/GG genotype (91). Subsequent cellular experiments provided functional support for these observations and demonstrated that the TLR9-rs352139 variant impaired TLR9 expression and consequently reduced NF-κB activation. TLR9-rs352139 AA genotype carriers were thus protected from the effects of ischemia-reperfusioninduced inflammation, which resulted in conservation of their metabolic capacity. The opposite effect was observed for carriers of the TLR4-rs1927907-GG phenotype who exhibited higher tacrolimus C/D ratios than AA/AG carriers, indicating that these patients were more susceptible to the effects of inflammation on their drug-metabolizing capacity (91,92). These studies illustrate that genetic variants in receptors can be important modulators of inflammation, which may be particularly relevant for receptors (e.g., IL6R or IL-1R) that are directly involved in the downregulation of CYP enzymes, but this remains to be investigated.

#### Genetic variation: inflammatory transcription factors (NF-κB)

Genetic variability within NF-KB is of great interest given its essential role in inflammatory signaling (93). One common polymorphism in the NFKB1 gene is the promotor -94 ATTG insertion/deletion mutation (rs28362491), with a minor allele frequency of 0.43. Deletion of the ATTG alleles is shown to reduce synthesis of the NF-κB p50 subunit (94). Zhang et al. showed that patients with the NFKB1-94 ATTG ins/ins genotype had higher CYP3A4-metabolized doseadjusted cyclosporine trough concentrations than patients with the -94 ATTG del/del genotype (95). The impact of the same polymorphism in NFKB1 on the pharmacokinetics of lovastatin, a cholesterol-lowering drug mainly metabolized by CYP3A4, was also investigated (96). In accordance, the area under the plasma concentration-time curve (AUC) of the metabolite lovastatin lactone was twofold higher in subjects with two copies of the NFKB1-94 ATTG ins/ins mutation and the plasma clearance was lower as compared to the NFKB1-94del/del genotype. The NFKB1-94del/del mutation may thus impair inflammatory signaling and hence attenuate the inflammation-induced downregulation of CYP3A4. Consequently, patients with the NFKB1-94del/del genotype may perceive milder consequences of inflammation on drug metabolism than people lacking this variant. Since NF-kB is a downstream effector molecule of several inflammatory cytokines, genetic variability has the potential to simultaneously alter the actions of multiple inflammatory mediators on CYP gene expression. The potential impact of genetic variability within NF-κB or within the genes of NF-κB adaptor proteins on the effects of inflammation on drug metabolism is therefore predicted to be greater than genetic variability in the receptors or the mediators themselves.

#### Genetic variation: nuclear receptors (PXR, CAR)

The nuclear receptors PXR and CAR are, as highlighted earlier, important for the transcriptional regulation of CYP450 enzymes. Pharmacogenetic variations within the genes encoding PXR (NR1I2) or CAR (NR1I3) has therefore been thoroughly investigated in relation to their effects on pharmacokinetics and efficacy of drug treatments, as reviewed comprehensively by Mbatchi et al. (97). However, the influence of genetic variants within NR1I2 or NR1I3 has primarily been linked to homeostatic regulation of CYP expression in the absence of inflammation. Until now, it remains therefore largely unclear which genetic polymorphisms

in *NR1I2* or *NR1I3* might be candidates for modulating the effects of inflammation on drug metabolism.

Since PXR is regulated by NF- $\kappa$ B, either through direct transcriptional repression or via interference with RXR-PXR binding, we hypothesize that polymorphisms within NR112 that present themselves in or near NF- $\kappa$ B binding sites might influence the impact of inflammation on drug metabolism (98). For this reason we used the computational databases "gene transcription regulation database" (GTRD) and "Alggen PROMO database" for identification of polymorphisms in NR112 that would be susceptible to the effects of inflammation (99–101). Using information on confirmed NF- $\kappa$ B binding sites by chromatin immunoprecipitation-sequencing (CHIP-seq) or predicted NF- $\kappa$ B binding spots, we were able to identify four common variants (minor-allele frequency > 0.01) in NR112 that are located in or near NF- $\kappa$ B binding spots, as summarized in Table 2. Importantly, the variant NR112-rs3814055 that has initially been linked to a NF- $\kappa$ B binding site was not confirmed by this approach, which is in accordance with observations from Dring et al. who also did not find evidence for a NF- $\kappa$ B binding site positioned at the rs2814055 location (102).

Table 2 SNPs in NR1I2 located in a predicted or confirmed NF-κB binding site#

SNP	Variation	Location	Allele frequency	In binding site (proximity) of:	Distance to binding site (bp)	Binding spot predicted in:
rs10934498	G > A, C, T	intron	G = 0.5024 A = 0.4976	NFκB1-p105 subunit	0	GTRD
rs1403526	A > C, G	Intron	A = 0.64900 G = 0.35100	RelA-p65 subunit	0	Alggen PROMO
rs12721602	G > A	5 -UTR	G = 0.98303 A = 0.01697	RelA-p65 subunit	13	Alggen PROMO
rs1054191	G > A, C	3′-UTR	G = 0.87745 A = 0.12255	NF-κB, NF-κB1 p105	17	Alggen PROMO

 $<sup>^*</sup>$  To cover relevant NF-kB binding sites, we took into consideration the human NF-κB p105 subunit, the NF-κB p100 subunit, and the RelA-p65 subunit binding sites. An arbitrary threshold of 25 base pairs from confirmed or predicted NF-κB binding spot was set to identify relevant NR112 SNPs (Mulero et al. 2019). Matching score to consensus sequence was set at 85% for the Alggen PROMO database. For the GTRD, CHIP-seq derived data was collected from the meta clusters data set. Allele frequencies were obtained from the GnomeAD or 1000Genomes database.

The effects on drug metabolism of these four genetic variants in the NF-kB binding spots in *NR1I2* are sparsely reported in the literature. This may suggest that these variants contribute less than other common SNPs within *NR1I2* (e.g., rs3814055, rs2472677) to the variability of drug metabolism in the absence of inflammation. However, some data is available from studies conducted in cancer patients. Inflammatory reactions are frequently observed in cancer patients and a common cause of phenoconversion (3,103).

Interestingly, in a cohort of 109 patients with colon cancer, the "inflammatory" variant NR1I2 rs10934498 (G > A) was identified, from a panel of NR1I2 variants, as one of the main determinants of Irinotecan pharmacokinetics (104). Irinotecan is a prodrug that is converted into its active metabolite SN-38 and subsequently detoxified into SN-38G. Patient with the rs10934498 AA genotype exhibited reduced SN-38 AUC levels and increased metabolic ratios of SN-38G compared to AG or GG carriers, which indicates that the metabolism of Irinotecan is more conserved in patients with the rs10934498 AA genotype. Based on our observation that rs10934498 is located in an NF- $\kappa$ B binding site, we hypothesize that PXR may no longer be downregulated by inflammation in patients carrying the rs10934498 AA genotype, resulting in a conserved drug-metabolizing activity compared to patients lacking this variant.

Altogether, the computational identification of common "inflammatory" variants within *NR1I2* suggest that genetic variability may modulate PXR-dependent outcomes of inflammatory signaling. However, further (functional) studies are needed to elucidate the impact of these *NR1I2* polymorphisms on drug metabolism in the context of inflammation.

#### Genetic variation: cytochrome P450 enzymes

Ultimately, the output of the inflammatory signaling cascade regulates CYP expression and subsequent drug metabolic capacity. Even though it is well established that genetic polymorphisms in CYP enzymes contribute to the interindividual variability in pharmacokinetics (2), it remains uncertain how and to what extent CYP polymorphisms may modulate the impact of inflammation on drug metabolism. Some studies hint towards a genotype-dependent effect of inflammation-induced phenoconversion, as summarized by Klomp et al. (105). CYP2C19 is highly polymorphic and shown to be affected by inflammation. For

example, in a study of 34 patients with an invasive fungal infection receiving voriconazole, it was shown that the effect of inflammation was modulated by the CYP2C19 genotype: the metabolic ratio of voriconazole and its metabolite was more decreased by inflammation in CYP2C19 ultrarapid metabolizers compared to CYP2C19 intermediate metabolizers (106). Similarly, Ohnishi et al. aimed to investigate the consequences of inflammation for different CYP2C19 genotypes by examining the metabolic ratios of omeprazole and its metabolite in hepatitis C virus (HCV)-positive patients and healthy volunteers (107). The shift in metabolic ratio between healthy patients and HCV-positive patients was largest for genotype-predicted normal metabolizers (21.1-fold change), followed by intermediate metabolizers (12.4-fold change) and least evident for poor metabolizers. Although these examples only illustrate the effects of inflammation on CYP2C19 mediated drug metabolism, and other CYP isoforms remain to be investigated, they clearly indicate that inflammation-induced changes in CYP450-mediated drug metabolism are affected by an individual's CYP metabolizer genotype.

#### **Conclusions**

Concluding, data from in vitro models have been instrumental to elucidate that CYP isoforms show distinct susceptibility to downregulation by inflammatory mediators wherein CYP3A4 seems to be most affected by inflammation, supporting clinical observations on CYP3A4 drug substrates. Additionally, the pattern of downregulation of CYP isoforms was dependent on the inflammatory stimulus. Interestingly, interindividual variability in response to inflammation is observed in both in vitro models and clinical studies. Genetic variability in the described pathways via which inflammation modulates the expression and activity of DMEs might in part explain the distinct response towards inflammation between subjects, but this remains to be further investigated. Ultimately, a better understanding of inflammation-induced phenoconversion may aid in optimizing treatment for the individual patient.

### Acknowledgements

We are very thankful for the assistance of Jan Schoones of the Walaeus library (LUMC) with the literature search. For creation of the figure, BioRender.com. was used.

#### References

- Wilkinson GR. Drug therapy: Drug metabolism and variability among patients in drug response. N Engl J Med. 2005;352:2211–21. doi: 10.1056/ NEJMra032424.
- Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther. 2013;138:103–41. doi: 10.1016/j. pharmthera.2012.12.007.
- Shah RR, Smith RL. Inflammation-induced phenoconversion of polymorphic drug metabolizing enzymes: Hypothesis with implications for personalized medicine. Drug Metab Dispos. 2015;43:400–10. doi: 10.1124/dmd.114.061093.
- 4. Stanke-Labesque F, Gautier-Veyret E, Chhun S, Guilhaumou R. Inflammation is a major regulator of drug metabolizing enzymes and transporters: Consequences for the personalization of drug treatment. Pharmacol Ther. 2020;215:107627. doi: 10.1016/j.pharmthera.2020.107627.
- Vet NJ, Brussee JM, De Hoog M, Mooij MG, Verlaat CWM, Jerchel IS, Schaik RHNV, Koch BCP, Tibboel D, Knibbe CAJ, et al. Inflammation and organ failure severely affect midazolam clearance in critically ill children. Am J Respir Crit Care Med. 2016;194:58–66. doi: 10.1164/rccm.201510-2114OC.
- Van Wanrooy MJP, Span LFR, Rodgers MGG, Van Heuvel ERD, Uges DRA, Van Werf TSD, Kosterink JGW, Alffenaara JWC. Inflammation is associated with voriconazole trough concentrations. Antimicrob Agents Chemother. 2014;58:7098–101. doi: 10.1128/ AAC.03820-14.
- Zhuang Y, de Vries DE, Xu Z, Marciniak SJ, Chen D, Leon F, Davis HM, Zhou H. Evaluation of disease-mediated therapeutic protein-drug interactions between an anti-interleukin-6 monoclonal antibody (sirukumab) and cytochrome P450 activities in a phase 1 study in patients with rheumatoid arthritis using a cocktail approach. J Clin Pharmacol. 2015;55:1386– 94. doi: 10.1002/jcph.561.

- Davis JD, Bansal A, Hassman D, Akinlade B, Li M, Li Z, Swanson B, Hamilton JD, DiCioccio AT. Evaluation of Potential Disease-Mediated Drug-Drug Interaction in Patients with Moderate-to-Severe Atopic Dermatitis Receiving Dupilumab. Clin Pharmacol Ther. 2018;104:1146–54. doi: 10.1002/cpt.1058.
- 9. Gravel S, Chiasson JL, Turgeon J, Grangeon A, Michaud V. Modulation of CYP450 Activities in Patients With Type 2 Diabetes. Clin Pharmacol Ther. 2019;106:1280–9. doi: 10.1002/cpt.1496
- Sim SC, Kacevska M, Ingelman-Sundberg M. Pharmacogenomics of drug-metabolizing enzymes: A recent update on clinical implications and endogenous effects. Pharm J. 2013;13:1–11. doi: 10.1038/tpj.2012.45.
- Aitken AE, Richardson TA, Morgan ET. Regulation of drug-metabolizing enzymes and transporters in inflammation. Annu Rev Pharmacol Toxicol. 2006;46:123–49. doi: 10.1146/annurev. pharmtox.46.120604.141059.
- Zhou J, Li F. Potential pharmacokinetic interactions of therapeutic cytokines or cytokine modulators on small-molecule drugs: Mechanistic understanding via studies using in vitro systems. Drug Metabol Drug Interact. 2014;29:17–28. doi: 10.1515/ dmdi-2013-0028.
- Martignoni M, Groothuis GMM, de Kanter R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol 2006;2:875–94. doi: 10.1517/17425255.2.6.875.
- 14. Aitken AE, Morgan ET. Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. Drug Metab Dispos. 2007;35:1687–93. doi: 10.1124/dmd.107.015511.
- Klein M, Thomas M, Hofmann U, Seehofer D, Damm G, Zanger UM. A systematic comparison of the impact of inflammatory signaling on absorption, distribution, metabolism, and excretion gene expression and activity in primary human hepatocytes and HepaRG Cells. Drug Metab Dispos. 2015;43:273–83. doi: 10.1124/ dmd.114.060962.

- Febvre-James M, Bruyère A, Le Vée M, Fardel O. The jak1/2 inhibitor ruxolitinib reverses interleukin-6-mediated suppression of drug-detoxifying proteins in cultured human hepatocytes. Drug Metab Dispos. 2018;46:131–40. doi: 10.1124/ dmd.117.078048.
- Rubin K, Janefeldt A, Andersson L, Berke Z, Grime K, Andersson TB. HepaRG cells as human-relevant in vitro model to study the effects of inflammatory stimuli on cytochrome P450 isoenzymes. Drug Metab Dispos. 2015;43:119–25. doi: 10.1124/ dmd.114.059246.
- Dallas S, Sensenhauser C, Batheja A, Singer M, Markowska M, Zakszewski C, Mamidi RNVS, McMillia M, Han C, Zhou H, et al. De-Risking Bio-therapeutics for Possible Drug Interactions Using Cryopreserved Human Hepatocytes. Curr Drug Metab. 2012;13:923–9. doi: 10.2174/138920012802138589.
- Dickmann LJ, Patel SK, Rock DA, Wienkers LC, Slatter JG. Effects of interleukin-6 (IL-6) and an anti-IL-6 monoclonal antibody on drug-metabolizing enzymes in human hepatocyte culture. Drug Metab Dispos. 2011;39:1415–22. doi: 10.1124/ dmd.111.038679.
- Sunman JA, Hawke RL, LeCluyse EL, Kashuba ADM. Kupffer cell-mediated IL-2 suppression of CYP3A activity in human hepatocytes. Drug Metab Dispos. 2004;32:359–63. doi: 10.1124/dmd.32.3.359.
- Long TJ, Cosgrove PA, Dunn RT, Stolz DB, Hamadeh H, Afshari C, McBride H, Griffith LG. Modeling therapeutic antibody-small molecule drug-drug interactions using a three-dimensional perfusable human liver coculture platform. Drug Metab Dispos. 2016;44:1940–8. doi: 10.1124/ dmd.116.071456.
- Nguyen TV, Ukairo O, Khetani SR, McVay M, Kanchagar C, Seghezzi W, Ayanoglu G, Irrechukwu O, Evers R. Establishment of a hepatocyte-kupffer cell coculture model for assessment of proinflammatory cytokine effects on metabolizing enzymes and drug transporters. Drug Metab Dispos. 2015;43:774–85. doi: 10.1124/dmd.114.061317.

- Tanner N, Kubik L, Luckert C, Thomas M, Hofmann U, Zanger UM, Böhmert L, Lampen A, Braeuning A. Regulation of drug metabolism by the interplay of inflammatory signaling, steatosis, and xenosensing receptors in HepaRG cells. Drug Metab Dispos. 2018;46:326–35. doi: 10.1124/ dmd 117.078675
- Dickmann LJ, Patel SK, Wienkers LC, Greg Slatter J. Effects of Interleukin 1β (IL-1β) and IL-1β/Interleukin 6 (IL-6) Combinations on Drug Metabolizing Enzymes in Human Hepatocyte Culture. Curr Drug Metab. 2012;13:930–7. doi: 10.2174/138920012802138642.
- Le Vée M, Bruyère A, Jouan E, Fardel O. Janus kinase-dependent regulation of drug detoxifying protein expression by interleukin-22 in human hepatic cells. Int Immunopharmacol. 2020;83:106439. doi: 10.1016/j.intimp.2020.106439.
- Dallas S, Chattopadhyay S, Sensenhauser C, Batheja A, Singer M, Silva J. Interleukins-12 and -23 do not alter expression or activity of multiple cytochrome p450 enzymes in cryopreserved human hepatocytes. Drug Metab Dispos. 2013;41:689–93. doi: 10.1124/ dmd.112.048884.
- 27. Taub R. Liver regeneration: From myth to mechanism. Nat Rev Mol Cell Biol. 2004;5:836–47. doi: 10.1038/nrm1489.
- 28. Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, Zhang P, Liu X, Gao G, Liu F, et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Emerg Microbes Infect. 2020;9:1123–30. doi: 10.1080/22221751.2020.1770129.
- Robak T, Gladalska A, Stepień H, Robak E. Serum levels of interleukin-6 type cytokines and soluble interleukin-6 receptor in patients with rheumatoid arthritis. Mediat Inflamm. 1998;7:347–53. doi: 10.1080/09629359890875.
- Evers R, Dallas S, Dickmann LJ, Fahmi
  OA, Kenny JR, Kraynov E, Nguyen T, Patel
  AH, Slatter JG, Zhang L. Critical review
  of preclinical approaches to investigate
  cytochrome P450-mediated therapeutic
  protein drug-drug interactions and
  recommendations for best practices: A white
  paper. Drug Metab Dispos. 2013;41:1598–
  609. doi: 10.1124/dmd.113.052225.

- Dluzen DF, Lazarus P. MicroRNA regulation of the major drug-metabolizing enzymes and related transcription factors. Drug Metab Rev. 2015;47:320–34. doi: 10.3109/03602532.2015.1076438.
- Reeh H, Rudolph N, Billing U, Christen H, Streif S, Bullinger E, Schliemann-Bullinger M, Findeisen R, Schaper F, Huber HJ, et al. Response to IL-6 trans- A nd IL-6 classic signalling is determined by the ratio of the IL-6 receptor α to gp130 expression: Fusing experimental insights and dynamic modelling. Cell Commun Signal. 2019;17:46. doi: 10.1186/s12964-019-0356-0.
- Keller R, Klein M, Thomas M, Dräger A, Metzger U, Templin MF, Joos TO, Thasler WE, Zell A, Zanger UM. Coordinating Role of RXRα in Downregulating Hepatic Detoxification during Inflammation Revealed by Fuzzy-Logic Modeling. PLoS Comput Biol. 2016;12:e1004431. doi: 10.1371/journal.pcbi.1004431.
- 34. Rodríguez-Antona C, Donato MT, Boobis A, Edwards RJ, Watts PS, Castell JV, Gómez-Lechón MJ. Cytochrome P450 expression in human hepatocytes and hepatoma cell lines: Molecular mechanisms that determine lower expression in cultured cells. Xenobiotica. 2002;32:505–20. doi: 10.1080/00498250210128675.
- Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev. 2018;281:8–27. doi: 10.1111/ imr.12621
- Parent R, Marion MJ, Furio L, Trépo C, Petit MA. Origin and Characterization of a Human Bipotent Liver Progenitor Cell Line. Gastroenterology. 2004;126:1147–56. doi: 10.1053/j.gastro.2004.01.002.
- Szabo G, Petrasek J. Inflammasome activation and function in liver disease. Nat Rev Gastroenterol Hepatol. 2015;12:387– 400. doi: 10.1038/nrgastro.2015.94.
- Bachmann M, Pfeilschifter J, Mühl H.
   A prominent role of interleukin-18 in acetaminophen-induced liver injury advocates its blockage for therapy of hepatic necroinflammation. Front Immunol. 2018;9:161. doi: 10.3389/fimmu.2018.00161.
- Zhou Z, Xu M-J, Gao B. Hepatocytes: A key cell type for innate immunity. Cell Mol Immunol. 2016;13(3):301–15. doi: 10.1038/ cmi.2015.97.

- Cahill CM, Rogers JT. Interleukin (IL)
   1β induction of IL-6 is mediated by a
   novel phosphatidylinositol 3-kinase dependent AKT/IκB kinase α pathway
   targeting activator protein-1. J. Biol Chem.
   2008;283:25900–12. doi: 10.1074/jbc.
   M707692200
- Shi JH, Sun SC. Tumor necrosis factor receptor-associated factor regulation of nuclear factor κB and mitogen-activated protein kinase pathways. Front Immunol. 2018;9:1. doi: 10.3389/fimmu.2018.01849.
- 42. Bianchi ME. DAMPs, PAMPs and alarmins: All we need to know about danger. J Leukoc Biol. 2007;81:1–5. doi: 10.1189/jlb.0306164.
- 43. Xie W, Uppal H, Saini SPS, Mu Y, Little JM, Radominska-Pandya A, Zemaitis MA. Orphan nuclear receptor-mediated xenobiotic regulation in drug metabolism. Drug Discov Today. 2004;9:442–9. doi: 10.1016/S1359-6446(04)03061-2.
- 44. Kojima K, Nagata K, Matsubara T, Yamazoe Y. Broad but distinct role of pregnane x receptor on the expression of individual cytochrome p450s in human hepatocytes. Drug Metab Pharmacokinet. 2007;22:276–86. doi: 10.2133/dmpk.22.276.
- Chen Y, Ferguson SS, Negishi M, Goldstein JA. Identification of constitutive androstane receptor and glucocorticoid receptor binding sites in the CYP2C19 promoter. Mol Pharmacol. 2003;64:316–24. doi: 10.1124/ mol.64.2.316.
- Ferguson SS, Lecluyse EL, Negishi M, Goldstein JA. Regulation of human CYP2C9 by the constitutive androstane receptor: Discovery of a new distal binding site. Mol Pharmacol. 2002;62:737–46. doi: 10.1124/ mol.62.3.737.
- 47. Xie W, Barwick JL, Simon CM, Pierce AM, Safe S, Blumberg B, Guzelian PS, Evans RM. Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. Genes Dev. 2000;14:3014–23. doi: 10.1101/gad.846800.
- 48. Pascussi JM, Gerbal-Chaloin S, Pichard-Garcia L, Daujat M, Fabre JM, Maurel P, Vilarem MJ. Interleukin-6 negatively regulates the expression of pregnane X receptor and constitutively activated receptor in primary human hepatocytes. Biochem Biophys Res Commun. 2000;274:707–13. doi: 10.1006/bbrc.2000.3219.

- Sun HY, Yan YJ, Li YH, Lv L. Reversing effects of ginsenosides on LPS-induced hepatic CYP3A11/3A4 dysfunction through the pregnane X receptor. J Ethnopharmacol. 2019;229:246–55. doi: 10.1016/j. jep.2018.09.041.
- Beigneux AP, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. Reduction in cytochrome P-450 enzyme expression is associated with repression of CAR (constitutive androstane receptor) and PXR (pregnane X receptor) in mouse liver during the acute phase response. Biochem Biophys Res Commun. 2002;293:145–9. doi: 10.1016/ S0006-291X(02)00196-1.
- Yang J, Hao C, Yang D, Shi D, Song X, Luan X, Hu G, Yan B. Pregnane X receptor is required for interleukin-6-mediated down-regulation of cytochrome P450 3A4 in human hepatocytes. Toxicol Lett. 2010;197:219–26. doi: 10.1016/j. toxlet.2010.06.003.
- Zhou X, Li X, Wang X, Jin X, Shi D, Wang J, Bi D. Cecropin B represses CYP3A29 expression through activation of the TLR2/4-NF-κ B/PXR signaling pathway. Sci Rep. 2016;6 doi: 10.1038/srep27876.
- Assenat E, Gerbal-Chaloin S, Larrey D, Saric J, Fabre JM, Maurel P, Vilarem MJ, Pascussi JM. Interleukin 1β inhibits CAR-induced expression of hepatic genes involved in drug and bilirubin clearance. Hepatology. 2004;40:951–60. doi: 10.1002/hep.20387.
- 54. Richardson TA, Morgan ET. Hepatic cytochrome P450 gene regulation during endotoxin-induced inflammation in nuclear receptor knockout mice. J Pharmacol Exp Ther. 2005;314:703–9. doi: 10.1124/jpet.105.085456.
- Chung I, Bresnick E. Regulation of the constitutive expression of the human CYP1A2 gene: Cis elements and their interactions with proteins. Mol Pharmacol. 1995;47:677–85.
- 56. Kamiyama Y, Matsubara T, Yoshinari K, Nagata K, Kamimura H, Yamazoe Y. Role of Human Hepatocyte Nuclear Factor 4α in the Expression of Drug-Metabolizing Enzymes and Transporters in Human Hepatocytes Assessed by Use of Small Interfering RNA. Drug Metab Pharmacokinet. 2007;22:287– 98. doi: 10.2133/dmpk.22.287.

- Tirona RG, Lee W, Leake BF, Lan LB, Cline CB, Lamba V, Parviz F, Duncan SA, Inoue Y, Gonzalez FJ, et al. The orphan nuclear receptor HNF4α determines PXR- and CARmediated xenobiotic induction of CYP3A4. Nat Med. 2003;9:220–4. doi: 10.1038/nm815.
- 58. Martínez-Jiménez CP, Castell JV, Gómez-Lechón MJ, Jover Atienza R. Transcriptional activation of CYP2C9, CYP1A1, and CYP1A2 by hepatocyte nuclear factor 4α requires coactivators peroxisomal proliferator activated receptor-γ coactivator 1α and steroid receptor coactivator 1. Mol Pharmacol. 2006;70:1681–92. doi: 10.1124/ mol.106.025403.
- Ferguson SS, Chen Y, LeCluyse EL, Negishi M, Goldstein JA. Human CYP2C8 is transcriptionally regulated by the nuclear receptors constitutive androstane receptor, pregnane X receptor, glucocorticoid receptor, and hepatic nuclear factor 4α. Mol Pharmacol. 2005;68:747–57. doi: 10.1124/ mol.105.013169.
- Cheng PY, Wang M, Morgan ET. Rapid Transcriptional Suppression of Rat Cytochrome P450 Genes by Endotoxin Treatment and Its Inhibition by Curcumin. J Pharmacol Exp Ther. 2003;307:1205–12. doi: 10.1124/jpet.103.057174.
- 61. Li X, Salisbury-Rowswell J, Murdock AD, Forse RA, Burke PA. Hepatocyte nuclear factor 4 response to injury involves a rapid decrease in DNA binding and transactivation via a JAK2 signal transduction pathway. Biochem J. 2002;368:203–11. doi: 10.1042/bj20020233.
- 62. Gu X, Ke S, Liu D, Sheng T, Thomas PE, Rabson AB, Gallo MA, Xie W, Tian Y. Role of NF-κB in regulation of PXR-mediated gene expression: A mechanism for the suppression of cytochrome P-450 3A4 by proinflammatory agents. J Biol Chem. 2006;281:17882–9. doi: 10.1074/jbc. M601302200.
- 63. Tian Y, Ke S, Denison MS, Rabson AB, Gallo MA. Ah receptor and NF-κB interactions, a potential mechanism for dioxin toxicity. J Biol Chem. 1999;274:510–5. doi: 10.1074/jbc.274.1.510.

- 64. Ke S, Rabson AB, Germino JF, Gallo MA, Tian Y. Mechanism of Suppression of Cytochrome P-450 1A1 Expression by Tumor Necrosis Factor-α and Lipopolysaccharide. J Biol Chem. 2001;276:39638–44. doi: 10.1074/jbc. M106286200.
- Ding X, Staudinger JL. Repression of PXR-mediated induction of hepatic CYP3A gene expression by protein kinase C. Biochem Pharmacol. 2005;69:867–73. doi: 10.1016/j. bcp.2004.11.025.
- Lichti-Kaiser K, Xu C, Staudinger JL.
   Cyclic AMP-dependent protein kinase signaling modulates Pregnane x receptor activity in a species-specific manner. J Biol Chem. 2009;284:6639–49. doi: 10.1074/jbc. M807426200.
- Taneja G, Chu C, Maturu P, Moorthy B, Ghose R. Role of c-Jun-N-terminal kinase in pregnane X receptor-mediated induction of human cytochrome P4503A4 in vitro. Drug Metab Dispos. 2018;46:397–404. doi: 10.1124/dmd.117.079160.
- 68. Li D, Zimmerman TL, Thevananther S, Lee HY, Kurie JM, Karpen SJ. Interleukin-1β-mediated suppression of RXR:RAR transactivation of the Ntcp promoter is JNKdependent. J Biol Chem. 2002;277:31416–22. doi: 10.1074/jbc.M204818200.
- 69. Shah P, Omoluabi O, Moorthy B, Ghose R. Role of adaptor protein toll-like interleukin domain containing adaptor inducing interferon b in toll-like receptor 3- and 4-mediated regulation of hepatic drug metabolizing enzyme and transporter genes. Drug Metab Dispos. 2016;44:61–7. doi: 10.1124/dmd.115.066761.
- Ghose R, Zimmerman TL, Thevananther S, Karpen SJ. Endotoxin leads to rapid subcellular re-localization of hepatic RXRα: A novel mechanism for reduced hepatic gene expression in inflammation. Nucl Recept. 2004;2 doi: 10.1186/1478-1336-2-4.
- Koike C, Moore R, Negishi M. Extracellular signal-regulated kinase is an endogenous signal retaining the nuclear Constitutive Active/androstane Receptor (CAR) in the cytoplasm of mouse primary hepatocytes. Mol Pharmacol. 2007;71:1217–21. doi: 10.1124/mol.107.034538.
- Zordoky B, El-Kadi A. Role of NF-kB in the Regulation of Cytochrome P450 Enzymes. Curr Drug Metab. 2009;10:164–78. doi: 10.2174/138920009787522151.

- Iber H, Chen Q, Cheng PY, Morgan ET. Suppression of CYP2C11 gene transcription by interleukin-1 mediated by NF-κB binding at the transcription start site. Arch Biochem Biophys. 2000;377:187–94. doi: 10.1006/ abbi.2000.1772.
- Ruminy P, Gangneux C, Claeyssens S, Scotte M, Daveau M, Salier JP. Gene transcription in hepatocytes during the acute phase of a systemic inflammation: From transcription factors to target genes. Inflamm Res. 2001;50:383–90. doi: 10.1007/PI.00000260.
- Jover R, Bort R, Gómez-Lechón MJ, Castell JV. Down-regulation of human CYP3A4 by the inflammatory signal interleukin-6: Molecular mechanism and transcription factors involved. FASEB J. 2002;16:1799–801. doi: 10.1096/fi.02-0195fie.
- Martínez-Jiménez CP, Gómez-Lechón MJ, Castell JV, Jover R. Transcriptional regulation of the human hepatic CYP3A4: Identification of a new distal enhancer region responsive to CCAAT/enhancerbinding protein β isoforms (liver activating protein and liver inhibitory protein) Mol Pharmacol. 2005;67:2088–101. doi: 10.1124/ mol.104.008169.
- Pitarque M, Rodríguez-Antona C, Oscarson M, Ingelman-Sundberg M. Transcriptional regulation of the human CYP2A6 gene. J Pharmacol Exp Ther. 2005;313:814–22. doi: 10.1124/jpet.104.081570.
- Jonas S, Izaurralde E. Towards a molecular understanding of microRNA-mediated gene silencing. Nat Rev Genet. 2015;16:421–33. doi: 10.1038/nrg3965.
- Li D, Tolleson WH, Yu D, Chen S, Guo L, Xiao W, Tong W, Ning B. Pharmacoepigenetics. Elsevier; Amsterdam, The Netherlands: 2019. MicroRNA-Dependent Gene Regulation of the Human Cytochrome P450; pp. 129–138.
- 80. Papageorgiou I, Court MH. Identification and validation of microRNAs directly regulating the UDP-glucuronosyltransferase 1A subfamily enzymes by a functional genomics approach. Biochem Pharmacol. 2017;137:93–106. doi: 10.1016/j. bcp.2017.04.017.
- Kugler N, Klein K, Zanger UM. MiR-155 and other microRNAs downregulate drug metabolizing cytochromes P450 in inflammation. Biochem Pharmacol. 2020;171:113725. doi: 10.1016/j. bcp.2019.113725.

- 82. Morgan ET, Skubic C, Lee CM, Cokan KB, Rozman D. Regulation of cytochrome P450 enzyme activity and expression by nitric oxide in the context of inflammatory disease. Drug Metab Rev. 2020;52:455–71. doi: 10.1080/03602532.2020.1817061.
- Rae JM, Johnson MD, Lippman ME, Flockhart DA. Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: Studies with cDNA and oligonucleotide expression arrays. J Pharmacol Exp Ther. 2001;299:849–57.
- 84. Eichelbaum M, Mineshita S, Ohnhaus E, Zekorn C. The influence of enzyme induction on polymorphic sparteine oxidation. Br J Clin Pharmacol. 1986;22:49–53. doi: 10.1111/j.1365-2125.1986.tb02879.x.
- 85. Madan A, Graham RA, Carroll KM, Mudra DR, Alayne Burton L, Krueger LA, Downey AD, Czerwinski M, Forster J, Ribadeneira MD, et al. Effects of prototypical microsomal enzyme inducers on cytochrome P450 expression in cultured human hepatocytes. Drug Metab Dispos. 2003;31:421–31. doi: 10.1124/dmd.31.4.421.
- 86. Hill AVS. Evolution, revolution and heresy in the genetics of infectious disease susceptibility. Philos Trans R Soc B Biol Sci. 2012;367:840–9. doi: 10.1098/rstb.2011.0275.
- 87. Zhang X, Xu J, Fan J, Zhang T, Li Y, Xie B, Zhang W, Lin S, Ye L, Liu Y, et al. Influence of IL-18 and IL-10 Polymorphisms on Tacrolimus Elimination in Chinese Lung Transplant Patients. Dis Markers. 2017;2017:7834035. doi: 10.1155/2017/7834035.
- Xing J, Zhang X, Fan J, Shen B, Men T, Wang J. Association between interleukin-18 promoter variants and tacrolimus pharmacokinetics in Chinese renal transplant patients. Eur J Clin Pharmacol. 2015;71:191–8. doi: 10.1007/s00228-014-1785-8
- 89. Li Y, Zou Y, Cai B, Yang B, Ying B, Shi Y, Wang L. The associations of IL-18 serum levels and promoter polymorphism with tacrolimus pharmacokinetics and hepatic allograft dysfunction in Chinese liver transplantation recipients. Gene. 2012;491:251–5. doi: 10.1016/j. gene.2011.10.008.

- Dar WA, Sullivan E, Bynon JS, Eltzschig H, Ju C. Ischaemia reperfusion injury in liver transplantation: Cellular and molecular mechanisms. Liver Int. 2019;39:788–801. doi: 10.1111/liv.14091
- 91. Ou B, Liu Y, Zhang T, Sun Y, Chen J, Peng Z. TLR9 rs352139 Genetic Variant Promotes Tacrolimus Elimination in Chinese Liver Transplant Patients During the Early Posttransplantation Period. Pharmacotherapy. 2019;39:67–76. doi: 10.1002/phar.2204.
- Wang Z, Wu S, Chen D, Guo F, Zhong L, Fan J, Peng Z. Influence of TLR4 rs1927907 locus polymorphisms on tacrolimus pharmacokinetics in the early stage after liver transplantation. Eur J Clin Pharmacol. 2014;70:925–31. doi: 10.1007/s00228-014-1673-2.
- 93. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduct Target Ther. 2017;2:1–9. doi: 10.1038/sigtrans.2017.23.
- 94. Karban AS, Okazaki T, Panhuysen CIM, Gallegos T, Potter JJ, Bailey-Wilson JE, Silverberg MS, Duerr RH, Cho JH, Gregersen PK, et al. Functional annotation of a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis. Hum Mol Genet. 2004;13:35–45. doi: 10.1093/hmg/ddh008.
- Zhang Y, Li JL, Fu Q, Wang XD, Liu LS, Wang CX, Xie W, Chen ZJ, Shu WY, Huang M. Associations of ABCB1, NFKB1, CYP3A, and NR1I2 polymorphisms with cyclosporine trough concentrations in Chinese renal transplant recipients. Acta Pharmacol Sin. 2013;34:555–60. doi: 10.1038/aps.2012.200.
- Zhao G, Liu M, Wu X, Li G, Qiu F, Gu J, Zhao L. Effect of polymorphisms in CYP3A4, PPARA, NR112, NFKB1, ABCG2 and SLCO1B1 on the pharmacokinetics of lovastatin in healthy Chinese volunteers. Pharmacogenomics. 2017;18:65–75. doi: 10.2217/pgs.16.31.
- 97. Mbatchi LC, Brouillet JP, Evrard A. Genetic variations of the xenoreceptors NR1I2 and NR1I3 and their effect on drug disposition and response variability. Pharmacogenomics. 2018;19:61–77. doi: 10.2217/pgs-2017-0121.
- 98. Mulero MC, Wang VYF, Huxford T, Ghosh G. Genome reading by the NF-κB transcription factors. Nucleic Acids Res. 2019;47:9967–89. doi: 10.1093/nar/gkz739.

- Farré D, Roset R, Huerta M, Adsuara JE, Roselló L, Albà MM, Messeguer X. Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN. Nucleic Acids Res. 2003;31:3651–3. doi: 10.1093/nar/gkg605.
- 100. Messeguer X, Escudero R, Farré D, Núñez O, Martínez J, Albà MM. PROMO: Detection of known transcription regulatory elements using species-tailored searches. Bioinformatics. 2002;18:333–4. doi: 10.1093/ bioinformatics/18.2.333.
- 101. Yevshin I, Sharipov R, Kolmykov S, Kondrakhin Y, Kolpakov F. GTRD: A database on gene transcription regulation—2019 update. Nucleic Acids Res. 2019;47:D100-5. doi: 10.1093/nar/gky1128.
- 102. Dring MM, Goulding CA, Trimble VI, Keegan D, Ryan AW, Brophy KM, Smyth CM, Keeling PWN, O'Donoghue D, O'Sullivan M, et al. The Pregnane X Receptor Locus Is Associated with Susceptibility to Inflammatory Bowel Disease. Gastroenterology. 2006;130:341–8. doi: 10.1053/j.gastro.2005.12.008.
- 103. Slaviero KA, Clarke SJ, Rivory LP. Inflammatory response: An unrecognised source of variability in the pharmacokinetics and pharmacodynamics of cancer chemotherapy. Lancet Oncol. 2003;4:224–32. doi: 10.1016/S1470-2045(03)01034-9.
- 104. Mbatchi LC, Robert J, Ychou M, Boyer JC, Del Rio M, Gassiot M, Thomas F, Tubiana N, Evrard A. Effect of Single Nucleotide Polymorphisms in the Xenobioticsensing Receptors NR1I2 and NR1I3 on the Pharmacokinetics and Toxicity of Irinotecan in Colorectal Cancer Patients. Clin Pharmacokinet. 2016;55:1145–57. doi: 10.1007/s40262-016-0392-5.
- 105. Klomp SD, Manson ML, Guchelaar H-J, Swen JJ. Phenoconversion of Cytochrome P450 Metabolism: A Systematic Review. J Clin Med. 2020;9:2890. doi: 10.3390/ jcm9092890.
- 106. Veringa A, ter Avest M, Span LFR, van den Heuvel ER, Touw DJ, Zijlstra JG, Kosterink JGW, van der Werf TS, Alffenaar JWC. Voriconazole metabolism is influenced by severe inflammation: A prospective study. J Antimicrob Chemother. 2017;72:261–7. doi: 10.1093/jac/dkw349.

107. Ohnishi A, Murakami S, Akizuki S, Mochizuki J, Echizen H, Takagi I. In vivo metabolic activity of CYP2C19 and CYP3A in relation to CYP2C19 genetic polymorphism in chronic liver disease. J Clin Pharmacol. 2005;45:1221–9. doi: 10.1177/0091270005280787



# Chapter 4

CYP and non-CYP drug metabolizing enzyme families exhibit differential sensitivities towards proinflammatory cytokine modulation

Laura M. de Jong, Chandan Harpal, Dirk-Jan van den Berg, Menno Hoekstra, Nienke J. Peter, Robert Rissmann, Jesse J. Swen, Martijn L. Manson



## **Abstract**

Compromised hepatic drug metabolism in response to proinflammatory cytokine release is primarily attributed to downregulation of cytochrome P450 (CYP) enzymes. However, whether inflammation also affects other phase I and phase II drug metabolizing enzymes (DMEs), such as the flavin monooxygenases (FMOs), carboxylesterases (CESs), and UDP glucuronosyltransferases (UGTs), remains unclear. This study aimed to decipher the impact of physiologically relevant concentrations of proinflammatory cytokines on expression and activity of phase I and phase II enzymes, to establish a hierarchy of their sensitivity as compared with the CYPs. Hereto, HepaRG cells were exposed to interleukin-6 and interleukin-1 $\beta$  to measure alterations in DME gene expression (24 h) and activity (72 h). Sensitivity of DMEs toward proinflammatory cytokines was evaluated by determining  $IC_{so}$  (potency) and  $I_{max}$  (maximal inhibition) values from the concentration-response curves. Proinflammatory cytokine treatment led to nearly complete downregulation of CYP3A4 (~98%) but was generally less efficacious at reducing gene expression of the non-CYP DME families. Importantly, FMO, CES, and UGT family members were less sensitive toward interleukin-6 induced inhibition in terms of potency, with IC<sub>50</sub> values that were 4.3- to 7.4-fold higher than CYP3A4. Similarly, 18- to 31-fold more interleukin-1 $\beta$  was required to achieve 50% of the maximal downregulation of FMO3, FMO4, CES1, UGT2B4, and UGT2B7 expression. The differential sensitivity persisted at enzyme activity level, highlighting that alterations in DME gene expression during inflammation are predictive for subsequent alterations in enzyme activity. In conclusion, this study has shown that FMOs, CESs, and UGTs enzymes are less impacted by IL-6 and IL-1 $\beta$  treatment as compared with CYP enzymes.

## Significance statement

While the impact of proinflammatory cytokines on CYP expression is well established, their effects on non-CYP phase I and phase II drug metabolism remains underexplored, particularly regarding alterations in drug metabolizing enzyme (DME) activity. This study provides a quantitative understanding of the sensitivity differences to inflammation between DME family members, suggesting that non-CYP DMEs may become more important for the metabolism of drugs during inflammatory conditions due to their lower sensitivity as compared with the CYPs.

## Introduction

Inflammation is increasingly recognized as a contributor to the regulation and variability of drug clearance in humans, presumably due to alterations in drug metabolism (1,2). More specifically, the widespread elevation of proinflammatory cytokines, such as interleukin (IL)-6 and IL-1 $\beta$  affects gene expression of drug metabolizing enzymes (DMEs) in hepatocytes (3–7), subsequently affecting hepatic drug clearance and efficacy or safety of drug treatments (8). Considering the high prevalence of both acute and chronic inflammatory diseases, it is crucial to take into account how hepatic drug metabolism of both novel and existing drugs can be affected by inflammation.

In vitro studies using human liver models have been instrumental in broadening our understanding of inflammation-induced alterations in drug metabolism and can facilitate in quantifying these effects. A promising approach to predict the subsequent impact of inflammation on drug clearance in vivo involves utilizing in vitro data coupled with physiologically based pharmacokinetic (PBPK) models. This approach has demonstrated its utility in predicting the influence of elevated IL-6 levels on drug clearance, particularly for substrates of the key DMEs cytochrome P450 (CYP) 3A4 and CYP2C19 (9–13). Generating more physiologically relevant quantitative in vitro data will likely aid in utilizing PBPK models to predict the impact of inflammation on drug clearance for substrates of other CYP enzymes and non-CYP mediated pathways (14).

Importantly, it is estimated that clearance of ~25% of the top 200 most prescribed small molecule drugs approved by the FDA is mainly dependent on non-CYP enzymes, with the UDP-glucuronosyltransferase (UGT) family contributing to biotransformation in 45% of the cases (15). However, whereas the impact of proinflammatory cytokines on CYP expression is well established, the potential impact on other DME families, including the UGTs, sulfotransferases, flavin-containing monooxygenases (FMOs), and carboxylesterases (CESs), has received considerably less attention. Yet, it remains unclear to what extent the activity of non-CYP metabolizing enzymes is affected by inflammation, and whether these enzymes exhibit a comparable sensitivity to the effects of inflammatory cytokines as compared with the CYP enzymes.

Another limitation of available in vitro data is that they have mostly focused on the impact of cytokines on the mRNA expression levels of DME enzymes rather

than on their enzymatic activity. While significant changes in the expression of DME mRNA during inflammation have indeed prompted focus on transcription as the primary mechanism underlying changes in metabolic capacity, there is increasing acknowledgment of the influence of post-transcriptional mechanisms on DME activity (1). Consequently, a strong up- or downregulation of mRNA expression observed upon cytokine stimulation may not necessarily translate into similar alterations in enzyme activity. Furthermore, in vitro studies are often conducted using cytokine concentrations that surpass the physiological concentrations observed in patients, compromising clinical translation (16). IL-6 levels typically range from 10 to 1000 pg/ml during inflammatory conditions, and IL-1 $\beta$  can reach up to 50 pg/ml (11,17,18). However, most in vitro studies have exclusively examined the effects of 10 ng/ml IL-6 and 1 ng/ml IL-1 $\beta$ , concentrations that far exceed physiological levels. This underscores the necessity of investigating changes in enzymatic activity upon physiologically relevant concentrations of cytokines to generate reliable quantitative in vitro data.

In this study, we therefore investigated the concentration-dependent effects of IL-6 and IL-1 $\beta$  on both the mRNA expression and activity of CYP and non-CYP DMEs in a relevant human hepatocarcinoma cell line, i.e., in HepaRG cells. Quantifying the impact of inflammatory mediators across various DME families allowed us to establish a hierarchy of their sensitivity. By comparing the effects of IL-6 and IL-1 $\beta$  on transcription versus activity, we shed light on whether alterations in mRNA serve as a reliable predictor of corresponding changes in enzyme activity during inflammation. This information is essential for enhancing our understanding of the impact of inflammation on drug metabolism, and could be implemented in modeling tools aimed at optimizing drug dosing strategies for patients with inflammatory disease.

## Materials and methods

## Reagents and chemicals

William's E Medium with GlutaMAX Supplement and trypsin-EDTA (0.25%) were purchased from ThermoFisher (Waltham, MA, USA). Fetal bovine serum was obtained from Merck (Batch number: 0001663799), penicillin/streptomycin was obtained from Lonza (Basel, Switzerland). Hydrocortisone, DMSO, human

insulin, and primers were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Dulbecco's phosphate-buffered saline (PBS) was obtained from Capricorn Scientific (Ebsdorfergrund, Hessen, Germany). SensiMix SYBR Lo-ROX kit and 10x NH4 Reaction Buffer for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) were purchased from Meridian BioScience (Cincinnati, Ohio, USA). Maxima H minus Reverse transcriptase and 5x RT buffer was purchased from Thermo Scientific (Waltham, MA, USA). Human recombinant IL-6 and human recombinant IL-1 $\beta$  were purchased from Peprotech (London, UK). All cytokines were reconstituted and stored as high concentration stocks according to the manufacturer's instructions. S-mephenytoin, 4'-hydroxymephenytoin, 4'-hydroxymephenytoin-d<sub>2</sub>, diclofenac, 4'-hydroxydiclofac, 4'-hydroxydiclofenac-<sup>13</sup>C<sub>2</sub>, phenacetin, acetaminophen, benzydamine N-oxide, and benzydamine N-oxided<sub>4</sub> were purchased from LGC (Wesel, Germany). Acetaminophen-d<sub>4</sub> was purchased from Alsachim (Illkirch-Graffenstaden, France). Benzydamine was purchased from Sigma-Aldrich (St. Louis, MO, USA). 1'-Hydroxymidazolam was purchased from Cerilliant (Round Rock, Texas, USA) and 1'-hydroxymidazolam-d, from Supelco (St. Louis, Missouri, USA). Midazolam hydrochloride, morphine, morphine-3glucuronide, and morphine-3-gluronide-d, were from Duchefa Farma (Haarlem, the Netherlands). Acetonitrile, methanol, water, and formic acid of LC-MS grade were obtained from Merk (Darmstadt, Germany).

## HepaRG culture and treatment

HepaRG cells at passage 12 (batch HPR101067) were purchased from Biopredict International (Rennes, France) and expanded to set up a working bank according to the provider's instructions. Cells plated in 96-wells plates at a density of 9000 cells/well were first grown in William's E medium GlutaMAX supplemented with 10% fetal bovine serum, 100 U/ml penicillin/streptomycin, 5  $\mu$ g/ml human insulin, and 20  $\mu$ g/ml hydrocortisone for two weeks. Subsequently, cells were cultured for an additional two weeks in the same medium supplemented with 2% DMSO to get fully differentiated cells (19). Cells were maintained at 37°C in 5% CO<sub>2</sub> throughout the experiment.

The fetal bovine serum concentration in the DMSO-containing HepaRG medium was reduced to 1% at 24 hours before treatment with the cytokines IL-6 or IL-1 $\beta$ . Concentrations of IL-6 used for the experiments ranged from 0.0001

ng/ml to 10 ng/ml and from 0.001 pg/ml to 1 ng/ml for IL-1 $\beta$ , respectively. For gene expression analysis, cells were treated with IL-6 or IL-1 $\beta$  for 24 hours prior to lysis. For activity measurements, the cytokine-containing medium was renewed every 24 hours. After 72 hours, the medium was replaced by 2% DMSO-containing serum-free medium with a substrate specific to the DME of interest, as described in detail below. An CyQUANT LDH Cytotoxicity Assay (Thermo Scientific, Wilmington, US) was conducted after 72 hours to evaluate cytotoxicity at the highest concentrations of IL-6 and IL-1 $\beta$ , yielding cytotoxicity levels of 6% and 14%, respectively.

### Human liver biopsies

Human liver biopsies were obtained from the gastroenterology biobank at the Leiden University Medical Center (LUMC, Leiden, the Netherlands), as described elsewhere (20).

### Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated from HepaRG cells or human liver biopsies following the acid guanidinium thiocyanate-phenol-chloroform extraction, as described elsewhere (21). Concentration and purity of RNA was subsequently measured using a NanoDrop 3300 (Thermo Scientific, Wilmington, US). Synthesis of cDNA was performed with 0.5  $\mu$ g RNA input using Maxima H Minus Reverse Transcriptase (Thermo scientific) according to the manufacturer's instructions. RT-qPCR analysis was performed with a QuantStudio 6 Flex System using SYBR Green technology. RT-qPCR samples were run in duplicate. All PCR primers were designed in-house and subsequently checked for amplification efficiency (Supplemental Table 1). Relative mRNA levels were calculated using the comparative  $\Delta\Delta$ Ct method (22). The expression in each HepaRG sample was normalized by subtracting the geometric mean Ct value of the endogenous control genes ribosomal protein lateral stalk subunit P0 (RPLP0), glyceraldehyde-3-phosphate dehydrogenase and  $\beta$ -actin (ACTB) from the target Ct value to obtain the  $\Delta$ Ct (eq. 1).

$$\Delta Ct = Ct(target) - Ct(RPLP0, GAPDH, ACTB)$$
 (1)

Subsequent relative gene expression levels were calculated as  $2^{-\Delta Ct}$ . Fold changes of treated cells as compared to PBS-control cells were calculated using eq. 2 and 3.

$$\Delta\Delta Ct = \Delta Ct(treated) - \Delta Ct(PBS\ control) \tag{2}$$

Fold change = 
$$2^{-\Delta\Delta Ct}$$
 (3)

Data are expressed as mean fold changes  $\pm$  S.E.M. Basal gene expression in HepaRG cells and human liver biopsies presented in Figure 1 and Supplemental Figure 2 are exclusively normalized for *RPLPO*. This is due to the fact that *RPLPO* was identified as a stable endogenous control in liver biopsies, unlike other housekeeping genes (20). Statistical analyses were carried out on  $\Delta$ CT values due to the considerably skewed symmetry of up- and downregulation in the linear fold change.

### DME activities in HepaRG cells

Determination of DME activity was based on the metabolic conversion of probe substrates, i.e., midazolam for CYP3A4, phenacetin for CYP1A2, diclofenac for CYP2C9, S-mephenytoin for CYP2C19, benzydamine for FMO3, and morphine for UGT2B7 using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). CYP2D6 activity could not be determined since HepaRG cells are derived from a CYP2D6 poor metabolizer patient and was thus excluded from our analysis (23). Cells were exposed to 5  $\mu$ M midazolam for 30 minutes, 50  $\mu$ M phenacetin for 2 hours, 10 µM diclofenac for 2 hours, 100 µM S-mephenytoin for 2 hours, 10 µM benzydamine for 4 hours, or 100 µM morphine for 4 hours in serumfree William's E medium supplemented with 2% DMSO. Substrate concentrations were selected below the Michaelis-Menten constant to achieve selective metabolic conversion by the specific DME isoform (24-26). Afterward, cell medium samples containing the probe substrates and their metabolites were collected and mixed with 250 mM formic acid, and immediately frozen at -20 degrees. Notably, UGT2B7 activity samples were mixed with 1 M sodium carbonate and then frozen. For quantification of the metabolites 1'hydroxymidazolam (CYP3A4), acetaminophen (CYP1A2), 4'hydroxydiclofenac (CYP2C9), 4'hydroxymephentoin (CYP2C19), benzydamine-N-oxide (FMO3), or morphine-3-glucuronide (UGT2B7) samples were subjected to LC-MS/MS based analysis. A detailed description of the LC-MS/ MS analysis can be found in the Supplemental Methods 'LC-MS/MS method to quantify CYP activity' or 'LC-MS/MS method to quantify FMO3 and UGT2B7 activity, where MS-specific parameters are listed in Supplemental Tables 2 and 3. CES1 activity was not determined due to the absence of a probe-based analytical

detection method. Enzyme activity data were normalized to the amount of cells per well and presented as the rate of metabolite formation in picomole/min/million cells as compared with untreated cells.

## Statistical analysis

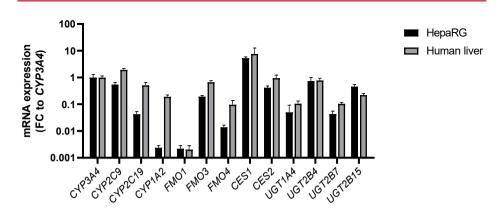
Results were generated from at least four independent experiments. Relative IC<sub>50</sub> of IL-6 and IL-1 $\beta$  for DME expression and activity were determined using GraphPad Prism 9.2.0 software (GraphPad Software, La Jolla, CA, USA) through nonlinear regression on the basis of the four-parameter logistic function (27). In case the concentration-response curve did not reach the lower asymptote upon the highest cytokine stimulation, IC<sub>50</sub> values were determined by directly interpolating from the studied concentration-response curve, without extrapolation for higher cytokine concentrations beyond the range of observed data points. Percentual maximal inhibition (I<sub>max</sub>) values were calculated based upon the upper and lower asymptotes of the concentration-response curves. Statistical significance in IC<sub>50</sub> and I<sub>max</sub> values between DME isoforms was determined by the parametric one-way ANOVA test assuming normal distribution of data and applying the Dunnet's post hoc test for comparison with CYP3A4 in GraphPad Prism 9. Statistical significance between  $IC_{50}$  and  $I_{max}$  values on mRNA and activity was done using an unpaired t test. The criterion was based on the *p*-values and indicated with \*  $p \le 0.05$ , \*\*  $p \le 0.01$ , \*\*\*  $p \le 0.001$  and NS, not significant.

## Results

## Basal mRNA expression of DMEs in HepaRG is comparable to human livers

The mRNA expression levels of four CYP enzymes (*CYP3A4*, *CYP2C9*, *CYP2C19*, *CYP1A2*), five other phase I enzymes (*FMO1*, *FMO3*, *FMO4*, *CES1*, *CES2*), and four phase II enzymes (*UGT1A4*, *UGT2B4*, *UGT2B7* and *UGT2B15*) were analyzed by RT-qPCR in HepaRG cells and biopsies of human livers (Figure 1). Rank order of P450 expression was *CYP3A4* > *CYP2C9* > *CYP2C19* > *CYP1A2* in HepaRG cells and *CYP2C9* > *CYP3A4* > *CYP2C19* > *CYP1A2* in human livers. CYP1A2 expression was relatively low in HepaRG as compared with human livers, consistent with previous characterization studies (23). The rank order of other phase I enzymes expression was *FMO3* > *FMO4* > *FMO1* 

and *CES1* > *CES2*. For the included phase II enzymes, the expression order was UGT2B4 > UGT2B15 > UGT1A4 > UGT2B7. This pattern was consistent in both HepaRG cells and human livers, aligning with previous research (28,29). Thus, the rank order within DME families exhibited strong similarity between human livers and the HepaRG cell model, suggesting that the HepaRG cell model is not only suitable for providing translation input regarding CYP enzymes but also for other DME families



**Figure 1** Basal mRNA expression levels of phase I and phase II drug metabolizing enzymes in HepaRG cells and in human livers. mRNA expression of the gene of interest was normalized to the housekeeping gene RPLP0 and presented as a fold change compared to basal CYP3A4 expression of either HepaRG cells or human livers. All values are means + SEM from 8 independent experiments (HepaRG) or from biopsies of 40 human livers.

# Impact of proinflammatory cytokine treatment on CYP expression and activity

The effect of inflammation on the gene expression and enzyme activity of selected phase I and phase II DMEs was evaluated by determining the IC<sub>50</sub> (potency) and  $I_{max}$  (efficacy) values of IL-6 and IL-1 $\beta$  on individual isoforms.

A concentration-dependent decrease in the relative mRNA expression of all CYP isoforms was observed following treatment with both IL-6 and IL-1 $\beta$ . Among the CYP family members, no substantial differences were noted in the isoform-specific response to cytokine treatment, as evident from the comparable potency and efficacy values (Figure 2A, Table 1). Comparison of IC<sub>50</sub> values and maximum suppression values for IL-1 $\beta$  and IL-6 indicated that in general, IL-1 $\beta$  is much more

potent than IL-6 in suppressing DME gene expression and enzyme activity. This finding corroborates previous research in HepaRG cells (7). We next examined whether the alterations at the DME gene expression level were retained at the enzyme activity level. Indeed, a concentration-dependent decrease was observed for CYP activity of all isoforms (Figure 2B, Table 2). In contrast to the similar potencies of IL-6 and IL-1 $\beta$  in modulating expression levels of different CYP isoforms, there was a distinct potency difference (~10-fold) between the impact of inflammation on CYP2C19 and CYP2C9 enzyme activities as compared with CYP3A4 activity, which was reflected by a higher sensitivity of CYP3A4 activity toward IL-6 and IL-1 $\beta$ .

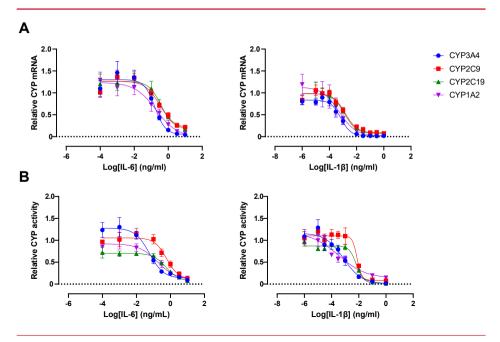


Figure 2 Cytokine concentration-response curves for regulation of CYP isoforms CYP3A4, CYP2C9, CYP2C19, CYP1A2 on expression ( $\bf A$ ) and activity level ( $\bf B$ ). Cells were treated with concentrations of 0.0001 ng/mL to 10 ng/mL (IL-6) or 0.001 pg/mL to 1 ng/mL (IL-1 $\bf \beta$ ) for 24 hours to analyze gene expression alterations via RT-qPCR or for 72 hours to analyze activity alterations via probe substrate metabolism with LC-MS/MS. mRNA and activity data are expressed as fold change of levels found in untreated control cells, arbitrarily set to 1.0. Each data point represents the average  $\pm$  SEM of at least 4 independent experiments. Data were fit with a non-linear regression model.

Table 1 Quantified IC<sub>50</sub> and I<sub>max</sub> values for DME mRNA expression levels obtained from fitting a non-linear regression model on the concentration-effect curves after treatment with IL-6 or IL-1 $\beta$  for 24 hours. The IC<sub>50</sub> values are reported in ng/mL for IL-6 treatment and in pg/mL for IL-1 $\beta$  treatment. One-way ANOVA and Dunnett's post hoc test with comparison to CYP3A4 was done to investigate differences in potency and maximal effect between DME families, for both IL-6 and IL-1 $\beta$  treatment. \* p < 0.05, \*\*\* p < 0.01, \*\*\*\* p < 0.001.

	II	L-6	IL-1β			
	Potency (IC <sub>50</sub> , ng/mL) ± SD	Maximal effect (I <sub>max</sub> ) ± SD (%)	Potency (IC <sub>50</sub> , pg/mL) ± SD	Maximal effect (I <sub>max</sub> ) ± SD (%)		
CYP3A4	$0.14 \pm 0.10$	97 ± 1	$0.35 \pm 0.94$	$99 \pm 2$		
CYP1A2	$0.04 \pm 0.22$	$94 \pm 3$	$0.24 \pm 1.02$	99 ± 1		
CYP2C9	$0.41 \pm 0.29$	$82 \pm 6$	$0.90 \pm 2.05$	$94 \pm 4$		
CYP2C19	$0.27 \pm 0.47$	$86 \pm 5$	$0.98 \pm 1.23$	$94 \pm 6$		
FMO1	$0.57 \pm 0.22$	$84 \pm 11$	$1.80 \pm 4.47$	$97 \pm 3$		
FMO3	$1.00 \pm 1.86^{**}$	55 ± 9***	$6.15 \pm 12.10^{**}$	$84 \pm 6**$		
FMO4	$1.07 \pm 0.95^{**}$	$57 \pm 4***$	9.95 ± 13.56***	80 ± 3***		
CES1	$1.23 \pm 0.30**$	39 ± 15***	no effect	no effect		
CES2	$0.70 \pm 0.30^*$	48 ± 13***	$10.67 \pm 9.32**$	$84 \pm 1^*$		
UGT1A4	$0.61 \pm 0.88^*$	68 ± 17***	$1.93 \pm 7.63$	84 ± 17**		
UGT2B4	$0.76 \pm 0.58^*$	73 ± 7***	$3.28 \pm 14.19^*$	$94 \pm 4$		
UGT2B7	$1.05 \pm 0.42 **$	60 ± 12***	5.01 ± 17.97**	83 ± 10***		
UGT2B15	$0.59 \pm 0.30$	72 ± 13***	$1.53 \pm 6.55$	$97 \pm 2$		

Table 2 Quantified  $IC_{50}$  and  $I_{max}$  values for DME activity obtained from fitting a non-linear regression model on the concentration-effect curves after treatment with IL-6 or IL-1β for 72 hours. The  $IC_{50}$  values are reported in ng/mL for IL-6 treatment and in pg/mL for IL-1β treatment. One-way ANOVA and Dunnett's post hoc test with comparison to CYP3A4 was done to investigate differences in potency and efficacy between DME families, for both IL-6 and IL-1β. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001.

	II	L-6	IL-1β			
	Potency (IC <sub>50</sub> , ng/mL) ± SD	Maximal decrease (I <sub>max</sub> ) ± SD (%)	Potency (IC <sub>50</sub> , pg/mL) ± SD	Maximal decrease (I <sub>max</sub> ) ± SD (%)		
CYP3A4	$0.05 \pm 0.17$	93 ± 2	$0.60 \pm 2.31$	98 ± 1		
CYP1A2	$0.12 \pm 0.11$	$85 \pm 4^*$	$0.43 \pm 3.55$	89 ± 1***		
CYP2C9	$0.55 \pm 0.36***$	$89 \pm 3$	$4.82 \pm 4.59^*$	93 ± 3**		
CYP2C19	$0.52 \pm 0.17***$	$89 \pm 2$	$6.58 \pm 6.27^*$	$99 \pm 0$		
FMO3	$1.28 \pm 1.82^{***}$	29 ± 5***	$1.49 \pm 0.43$	$54 \pm 5***$		
UGT2B7	$1.77 \pm 0.71^{***}$	69 ± 7***	$18.48 \pm 15.54^{**}$	93 ± 2*		

# Non-CYP isoforms are differentially affected by cytokine treatment as compared with CYP isoforms

We next examined the impact of IL-6 and IL-1 $\beta$  treatment on the different members of the most important non-CYP DME families. Sensitivity differences in response to cytokine treatment among DME families were defined by benchmarking potency and efficacy values against CYP3A4, which is recognized as the most important DME in humans because of its clinical importance and high expression (30). Interestingly, gene expression of *FMO3*, *FMO4*, *CES1*, *CES2*, *UGT1A4*, *UGT2B4*, and *UGT2B7* was in terms of potency less sensitive toward the effects of IL-6 as compared with *CYP3A4*, with IC<sub>50</sub> values that were 4- to 9-fold higher than for CYP3A4 (Figure 3A, Table 1). Additionally, while IL-6 elicited a maximal downregulation of only 55  $\pm$  9% for *FMO3*, 57  $\pm$  4% for *FMO4*, 39  $\pm$  15% for *CES1*, and 48  $\pm$  13% for *CES2*, it led to a nearly complete downregulation of 97  $\pm$  1% for *CYP3A4* expression. This difference in efficacy of IL-6 was similarly observed across all members of the *UGT* family, where maximal downregulation ranged from 60  $\pm$  12% to 73  $\pm$  7%.

Similar patterns were observed for the impact of IL-1 $\beta$  on non-CYP DME isoforms. *FMO3*, *FMO4*, *CES2*, *UGT2B4*, and *UGT2B7* exhibited a significantly lower sensitivity to IL-1 $\beta$  as compared with *CYP3A4*, indicating that a, respectively, 18-, 28-, 30-, 9-, and 14-fold higher concentration of IL-1 $\beta$  was needed to exert 50% of the maximal downregulation by this cytokine. Interestingly, IL-1 $\beta$  did not impact *CES1* expression across all concentrations tested. In addition, the maximal inhibitory effect of IL-1 $\beta$  on gene expression levels of *FMO3*, *FMO4*, *CES2*, *UGT1A4*, and *UGT2B7* ranged from 80 ± 3% to 84 ± 17%, which was less as compared with the observed near-complete downregulation of 99 ± 2% of *CYP3A4*.

Importantly, the differential potency and maximal inhibitory impact of inflammatory mediators on different members of the DME families could be confirmed at the enzyme activity level (Figure 3B, Table 2). Compared with CYP3A4 activity, FMO3 activity was less sensitive toward the effects of IL-6, as evident by a 26-fold difference in potency. UGT2B7 activity was even less sensitive toward IL-6, with a 35-fold difference in IC $_{50}$  value as compared with CYP3A4 activity. In addition, maximal inhibition by IL-6 was only  $29 \pm 5\%$  for FMO3, and  $69 \pm 7\%$  for UGT2B7, significantly less than the maximal inhibition of  $93 \pm 2\%$  that was observed for CYP3A4 activity. The maximal downregulation of FMO3 activity following IL-1 $\beta$  treatment was  $54 \pm 5\%$ , which was also less than observed

for the CYP3A4 activity (98  $\pm$  1%). However, IL-1 $\beta$  showed comparable potency toward FMO3 activity inhibition as compared with CYP3A4 activity inhibition, highlighting that the efficacy of IL-1 $\beta$  rather than the sensitivity to IL-1 $\beta$  differed between FMO3 and CYP3A4 activities. UGT2B7 activity displayed lower sensitivity toward IL-1 $\beta$ , which was reflected by a 31-fold difference in IC<sub>50</sub> value as compared with CYP3A4.

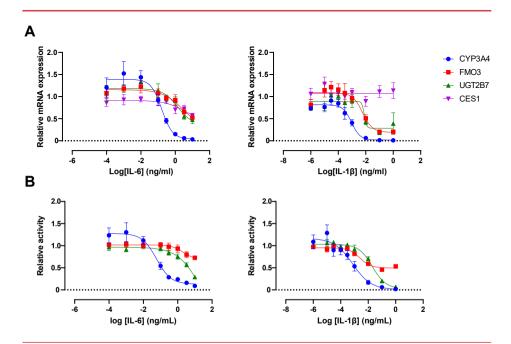


Figure 3 Cytokine concentration-response curves for regulation of CYP3A4, FMO3, UGT2B7 and CES1 on expression (A) and activity level (B). Cells were treated with concentrations of 0.0001 ng/mL to 10 ng/mL (IL-6) or 0.001 pg/mL to 1 ng/mL (IL-1 $\beta$ ) for 24 hours to analyze gene expression alterations via RT-qPCR or for 72 hours to analyze activity alterations via probe substrate metabolism with LC-MS/MS. mRNA and activity data are expressed as fold changes of levels found in untreated control cells, arbitrarily set to 1.0. Each data point represents the average  $\pm$  SEM of at least 4 independent experiments. Data were fit with a non-linear regression model.

# Transcriptional regulation is the main driver of the cytokine-mediated inhibition of DMEs

Several studies have suggested that inflammation-related post-transcriptional mechanisms may modulate CYP activity, which would theoretically result in a mismatch in the overall impact of inflammatory mediators in altering DME gene

expression versus enzyme activity. To investigate whether post-transcriptional modifications induced by inflammation are indeed critical to the effect, acquired IC<sub>50</sub> and I<sub>max</sub> values for DME gene expression and enzyme activity were compared (Figure 4). Overall, there was a strong linear relationship between the potency of IL-6 and IL-1 $\beta$  on DME expression and DME activity (p < 0.0001) (Figure 4A). Importantly, 90% of the variability in DME activity could be explained by changes in transcription ( $R^2 = 0.9$ ), highlighting the strong association between alterations in gene expression and enzyme activity during inflammation. We next compared individual expression versus activity IC<sub>50</sub> values for CYP3A4, CYP2C19, CYP2C9, CYP1A2, FMO3, and UGT2B7, visually presented in Supplemental Figure 1. CYP3A4 activity was more sensitive toward IL-6 induced downregulation compared with CYP3A4 expression, and this was similarly seen for FMO3 activity upon IL-1 $\beta$  treatment. In contrast, CYP2C19 and CYP2C9 expression was more sensitive toward IL-1 $\beta$  treatment as compared with CYP2C19 and CYP2C9 activity. For other isoforms, similar IC<sub>50</sub> values on expression and activity level were found. The maximal impact of IL-6 and IL-1 $\beta$  on expression and activity of the DMEs was highly similar, except for the mismatches observed for FMO3 (Figure 4B).

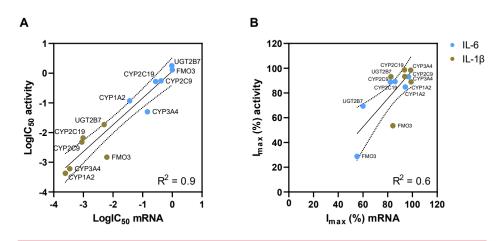


Figure 4 Simple linear regression analysis to investigate the relationship between the impact of IL-6 and IL-1 $\beta$  treatment on DME mRNA expression versus activity for LogIC<sub>50</sub> values (A) and I<sub>max</sub> values (B). The regression line represents the best-fit line calculated from the data, and the dotted lines indicate the 95% confidence interval. Blue dots represent data obtained from IL-6 treated cells, and brown dots represent data obtained from IL-1 $\beta$  treated cells.

# Comparison of $IC_{50}$ values for cytokine-induced CYP changes in HepaRG cells versus two-dimensional (2D) and three-dimensional (3D) primary human hepatocyte (PHH) models

To further highlight the translational value of the HepaRG cell line as in vitro liver model, we compared our quantitative cytokine-induced changes to what has been reported before in 2D and 3D PHH models (4,5,31). Comparing our HepaRG IL-6 IC<sub>50</sub> values with those previously determined for CYP isoforms in 2D/3D PHHs showed good agreement between the results (Table 3). The potency of IL-6 in inducing transcriptional alterations in CYPs in 3D PHH spheroids was almost identical as compared with the potency found in HepaRG cells. The IC<sub>20</sub> data acquired in a 2D PHH model were also comparable. However, it should be noted that basal CYP expression rapidly declines in 2D cultures of PHH, even in the absence of a proinflammatory stimulus (32). The correspondence of our HepaRG IC<sub>50</sub> data does not hold so well for comparing the potency of IL-1 $\beta$  on CYP expression and activity in PHHs. Although we found the most pronounced effects on CYP3A4, similarly to the results in 3D PHHs, IL-1 $\beta$  was much more potent in HepaRG cells as compared with PHHs. This might in part be due to the morphological heterogeneity of HepaRG cells, where biliary-like cells release additional proinflammatory cytokines, amplifying the IL-1 $\beta$  response (33). Indeed, aggravation of the IL-1 $\beta$ , but not the IL-6 response has been demonstrated in hepatocyte coculture models as compared with hepatocytes alone, where a sensitivity increase up to 50-fold was observed for CYP3A4 (34). Taken together, these findings demonstrate that HepaRG cells exhibit comparable sensitivity to IL-6-induced transcriptional changes in CYP enzymes as observed in 2D and 3D PHH models.

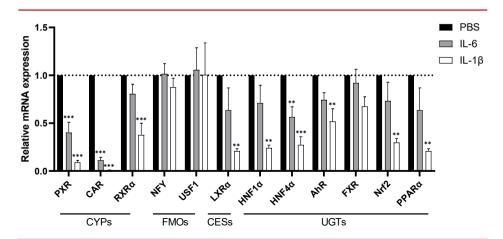
# Cytokine specific effects on nuclear receptors and transcription factors regulating the DMEs

Our data indicates that transcriptional alterations in DME are the primary mechanism underlying inflammation-related changes in CYP enzyme activity in vitro. To gain mechanistic insight into the differential regulation of hepatic gene expression by cytokines, we investigated the effects of IL-6 and IL-1 $\beta$  on a selection of nuclear receptors and transcription factors generally considered to be involved in DME gene expression regulation (Figure 5).

Table 3 Quantified IC<sub>50</sub> values in HepaRG cells from this study as compared to reported values in 2D and 3D PHH models. Data from 3D PHH models was extracted from the publication by Klöditz et al. (2023) and represents the average of 4 independent donors. Data from 2D PHHs was extracted from the studies by Dickmann et al. (2011, 2012) and represents the average of 5 independent donors unless stated otherwise.

			П	II-6					II	$_{ m IL-1}eta$		
	HepaR (IC <sub>50</sub> , ng/	HepaRG C <sub>50</sub> , ng/mL)	3D ] (IC <sub>50</sub> , 1	3D PHH (IC <sub>50</sub> , ng/mL)	2D ] (IC <sub>50</sub> , 1	2D PHH (IC <sub>50</sub> , ng/mL)	Hep (IC <sub>50</sub> , 1	HepaRG (IC <sub>50</sub> , ng/mL)	3D (IC <sub>50</sub> ,	3D PHH (IC <sub>50</sub> , ng/mL)	2D ] (IC <sub>50</sub> , 1	2D PHH (IC <sub>50</sub> , ng/mL)
	mRNA A	Activity	mRNA	mRNA Activity	mRNA*	Activity	mRNA	mRNA Activity	mRNA	Activity	mRNA	Activity
CYP3A4	0.14	0.05	0.46	N.D.	0.003	0.07	0.0004	900000	0.02	N.D.	0.29	0.42
CYP1A2	0.04	0.12	0.03	N.D.	0.27	1.25	0.0002	0.0004	09.0	N.D.	0.53	0.45
CYP2C9	0.41	0.55	0.20	N.D.	0.12	N.D.	0.0009	0.0048	3.95	N.D.	0.23	N.D.
CYP2CI9	0.27	0.52	0.25	N.D.	0.07	N.D.	0.0010	9900.0	0.42	N.D.	0.15###	N.D.

N.D. = not determined. \* data from one donor; \*\* could only be measured in 2 out of 5 donors; \*\*\* could only be measured in 3 out of 5 donors.



**Figure 5** The impact of IL-6 or IL-1 $\beta$  on transcription factors and nuclear receptors that regulate the various DMEs families. Cells were treated with 10 ng/mL IL-6 or 1 ng/mL IL-1 $\beta$  for 24 hours to analyze gene expression alterations via RT-qPCR. Data are expressed as the mean fold change  $\pm$  SEM of mRNA compared to untreated control cells of 6 independent experiments. One way ANOVA with Dunnett post hoc test was performed for every gene separately. \*\* p < 0.01; \*\*\* p < 0.001.

Pregnane X receptor (PXR) and constitutive androstane receptor (CAR) are identified as key transcriptional regulators of the CYP enzymes, with confirmed binding sites in the response elements of human CYP3A4/5, CYP2C9, CYP2C19, and CYP1A2 (35-37). Nuclear factor Y (NFY) and upstream transcription factor 1 (USF1) are essential for constitutive FMO3 transcription via promoter binding (38), while liver X receptor α (LXRα) has recently been identified as regulator of human CES (39). UGT family regulation is isoform-specific, with the aryl hydrocarbon receptor (AhR) and hepatocyte nuclear factor (HNF)  $1\alpha$  implicated in UGT1A4 regulation, farnesoid X receptor (FXR) and peroxisome proliferator activated receptor α (PPARα) in UGT2B4 regulation, and nuclear factor E2-related factor 2 (Nrf2), FXR, HNF4α, HNF1, vitamin D receptor (VDR), and forkhead box protein A1 (FOXA1) in the regulation of UGT2B7 and UGT2B15 (40). Basal gene expression of these regulators in HepaRG cells was confirmed with RT-qPCR (Supplemental Figure 2). PXR and CAR expression was most strongly downregulated, i.e., > 60% by IL-6 treatment and > 90% by IL-1 $\beta$  treatment. IL-1 $\beta$  also downregulated PXR and CAR's binding partner retinoid X receptor  $\alpha$  (RXR $\alpha$ ) (~60%), LXR $\alpha$  (~80%), HNF1 $\alpha$  (~80%), AhR (~50%), Nrf2 (~70%), and PPARα (~80%), which was not seen after IL-6 treatment. Expression of HNF4α was downregulated by ~70% following IL-1 $\beta$  treatment and ~40% by IL-6

treatment. The other regulators FXR, NFY, and USF1 were unaffected by both IL-6 and IL-1 $\beta$ . Sensitivity toward IL-6 and IL-1 $\beta$  was evaluated for PXR and CAR, as these regulators were most affected by cytokine treatment. The IC<sub>50</sub> values for IL-6 treatment were  $0.86 \pm 0.46$  ng/ml for PXR and  $0.38 \pm 0.56$  ng/ml for CAR, while for IL-1 $\beta$  treatment, the IC<sub>50</sub> values were  $4.37 \pm 3.68$  pg/ml for PXR and  $2.50 \pm 7.41$  pg/ml for CAR. Concentration-response curves for PXR and CAR as compared with one of the key genes they regulate, *CYP3A4*, is presented in Supplemental Figure 3.

## Discussion

Proinflammatory cytokine release during inflammatory conditions is associated with compromised metabolism of drugs in the liver. The impact of proinflammatory cytokines on in vitro CYP expression is well-characterized (16). However, less attention has been credited to the effects on non-CYP phase I and phase II drug metabolism, and especially data on the effects of inflammation on DME activity is lacking. Our results demonstrate that members of the non-CYP families FMOs, CESs, and UGTs were less sensitive toward the effects of IL-6 and IL-1 $\beta$  as compared with the CYP family. This differential sensitivity was evident at both the DME gene expression and DME enzyme activity level, highlighting that alterations in transcription during inflammation are highly predictive for subsequent alterations in enzyme activity.

Our concentration-response experiments defined differences in both the potency and efficacy of cytokines in inducing downregulation of expression and activity of individual DME family members. While results from previous in vitro studies at supraphysiological concentrations of IL-6 have hinted toward a more limited impact on UGT isoforms as compared with CYP isoforms (7,41,42), this study is the first to directly compare multiple DME families on both expression and activity. Rank ordering of DME sensitivity highlighted that CYP isoforms exhibited the highest sensitivity to the modulatory effects of IL-6 and IL-1 $\beta$ , whereas members from the FMO, CES, and UGT families consistently showed a lower sensitivity. Importantly, this differential sensitivity was observed for both IL-6 and IL-1 $\beta$  treatment, even though IL-6 and IL-1 $\beta$  induce different inflammatory signaling pathways (43,44) and exert different effects on transcriptional regulators (7).

The mechanisms underlying this differential sensitivity could stem from the differential impact of cytokines on the regulators of the DMEs. IL-6 and IL-1 $\beta$  stimulation of HepaRG cells profoundly and significantly suppress mRNA expression of PXR and CAR by > 60%, whereas presumed transcriptional regulators of UGT and CES enzymes are less impacted, and FMO regulators are not at all impacted by cytokine treatment. Nuclear receptors and transcription factors implicated in DME transcriptional modulation are thus transcriptionally differentially regulated by cytokines, which might underlie the differential sensitivity to inflammation observed for various DME families. In addition to inflammationinduced alterations in gene expression of regulators, a loss of nuclear localization or alterations in the phosphorylation status of regulators has also been proposed, i.e., for the dimerization partner RXRa (41,45). This might explain the observed mismatch between the sensitivity toward proinflammatory cytokines for CYP3A4 expression as compared with expression of the key regulators PXR and CAR. Future studies should aim to investigate whether the transcriptional downregulation concordantly leads to lower transcriptional activation of DME regulators.

Post-transcriptional mechanism related to inflammation may, alongside transcriptional changes, further affect CYP activity (1). For instance, nitric oxide-dependent ubiquitination leading to enhanced proteasomal degradation, or the release of inflammation-related miRNAs, have been implicated in this post-transcriptional regulatory process (46–48). To investigate the importance of post-transcriptional mechanisms in modulating CYP activity under inflammatory conditions, we analyzed the correlation between the impact of IL-6 and IL-1 $\beta$  on DME expression versus DME activity. We found that, in HepaRG cells, alterations in gene expression are highly predictive for alterations in enzyme activity, providing limited evidence for inflammation-associated post-transcriptional modifications of DMEs. Previous studies suggesting the importance of post-transcriptional modifications on CYP activity mainly stem from observed mismatches between mRNA and protein levels in PHHs (49) or from animal studies (1). The time kinetics of alterations in expression versus protein/activity levels could partially account for the observed mismatches, and future studies should therefore evaluate the temporal dynamics of DME expression and activity alterations in response to inflammation. We conducted our activity measurements after 72 hours, in accordance with other studies and considering the reported half-life of CYP3A4,

which is approximately 37 hours (50). However, half-life of CYP2C9 is reported to be 104 hours (50), which could explain why we found a stronger effect of inflammation on *CYP2C9* expression compared with its activity. This finding is thus likely unrelated to post-transcriptional modifications but rather an effect of the protein's half-life. All in all, our results have highlighted that the transcriptional alterations in DME expression are the main driver of the alterations in enzyme activity observed in vitro.

PBPK modeling is increasingly exploited to predict the impact of inflammation or inflammatory diseases on drug clearance. A major advantage of PBPK modeling combined with in vitro to in vivo extrapolation (IVIVE) is the ability to translate in vitro data into biologically relevant parameters for model input to predict clinical inflammation-related alterations in pharmacokinetics. Specifically,  $IC_{50}$  and I walues obtained in vitro can be used to model CYP enzyme dynamics under inflammatory conditions, and this approach has been shown successful for the prediction of disease-drug interactions with CYP substrates in, for example, patients with rheumatoid arthritis, leukemia, or surgical trauma (9,11-13,51). Despite the growing interest in PBPK modeling for non-CYP enzymes, current models predominantly focus on predicting drug-drug interactions rather than the impact of inflammation on non-CYP mediated drug clearance (14). This limitation arises partly due to the scarcity of physiologically relevant quantitative in vitro data on the effects of cytokines on non-CYP enzymes (52,53). To address this gap, we provided  $IC_{50}$  and  $I_{max}$  values for non-CYP enzymes, which can serve as critical inputs for PBPK modeling to better predict inflammation-related changes in non-CYP mediated drug metabolism. Importantly, comparing our HepaRG IL-6 IC<sub>50</sub> values with those previously determined for CYP isoforms in 2D/3D PHHs showed good agreement between the results, enhancing our confidence in the validity of HepaRG data as input for PBPK modeling approaches. Also, our reported IC<sub>50</sub> data are within the physiological range of serum IL-1 $\beta$  and IL-6 in patients experiencing inflammation-related diseases (17). Ultimately, PBPK models, when integrated with robust in vitro data, could serve as a powerful tool for optimizing drug dosing strategies and enhancing therapeutic outcomes in the presence of inflammation.

In the clinic, a differential impact of inflammation on DME family members has been observed, for example in nonalcoholic fatty liver disease (NAFLD) patients,

where hepatic inflammation is an important contributor to disease progression (54). Protein levels of CYPs were lower in diseased patients, but non-CYP enzyme levels remained relatively unchanged, except for select UGTs (55). This was confirmed in other studies which showed CYP2C19 to be most impacted by NAFLD, whereas other DMEs were less affected (56,57). For antifungal agents, a differential impact of inflammation has been demonstrated based on the metabolic route of the drug. Exposure of posaconazole, which is mainly metabolized by UGT1A4, was not influenced by inflammation as assessed by C-reactive protein (CRP) levels (58). Conversely, different studies have demonstrated that trough levels of voriconazole, a substrate for CYP2C19/3A4, are increased during inflammation (59,60). As such, patients with inflammatory conditions may experience variation in pharmacokinetics of concomitant medication depending on the specific DME engaged in the drug's metabolic pathway. Our study suggests that drugs utilizing secondary or alternative routes via non-CYP clearance may be less susceptible to the effects of inflammation as compared with drugs fully metabolized by CYP enzymes.

In conclusion, our study has shown that UGT, FMO, and CES enzymes are less sensitive toward the effects of proinflammatory cytokines IL-6 and IL-1 $\beta$  as compared with the CYP enzymes. Additionally, the findings highlight that transcriptional alterations in the DME expression are highly predictive for the alterations in enzyme activity, arguing against inflammation-related post-transcriptional modifications. Patients suffering from acute or chronic inflammatory diseases may thus be at risk for alterations in their drug metabolism, where the magnitude of the alteration likely depends on the DME family members involved in the clearance route of the drug.

## Acknowledgements

The authors would like to thank and acknowledge Johan van der Reijden and Luuk Hawinkels (Department of Gastroenterology-Hepatology, Leiden University Medical Center) for their excellent support with acquiring and processing the human liver biopsies from the biobank.

## References

- Stanke-Labesque F, Gautier-Veyret E, Chhun S, Guilhaumou R. Inflammation is a major regulator of drug metabolizing enzymes and transporters: Consequences for the personalization of drug treatment. Pharmacol Ther. 2020 Nov 1:215.
- Dunvald ACD, Järvinen E, Mortensen C, Stage TB. Clinical and Molecular Perspectives on Inflammation-Mediated Regulation of Drug Metabolism and Transport. Clin Pharmacol Ther. 2022 Aug 1:112(2):277-90.
- Aitken AE, Richardson TA, Morgan ET. Regulation of drug-metabolizing enzymes and transporters in inflammation. Annu Rev Pharmacol Toxicol. 2006 Feb 10;46(1):123– 49.
- Dickmann LJ, Patel SK, C. Wienkers L, Greg Slatter J. Effects of Interleukin 1β (IL-1β) and IL-1β/Interleukin 6 (IL-6) Combinations on Drug Metabolizing Enzymes in Human Hepatocyte Culture. Curr Drug Metab. 2012 Aug 2;13(7):930–7.
- Dickmann LJ, Patel SK, Rock DA, Wienkers LC, Slatter JG. Effects of interleukin-6 (IL-6) and an anti-IL-6 monoclonal antibody on drug-metabolizing enzymes in human hepatocyte culture. Drug Metab Dispos. 2011 Aug 1;39(8):1415–22.
- Tanner N, Kubik L, Luckert C, Thomas M, Hofmann U, Zanger UM, et al. Regulation of Drug Metabolism by the Interplay of Inflammatory Signaling, Steatosis, and Xeno-Sensing Receptors in HepaRG Cells. Drug Metab Dispos. 2018 Apr 1;46(4):326– 35.
- Klein M, Thomas M, Hofmann U, Seehofer D, Damm G, Zanger UM. A systematic comparison of the impact of inflammatory signaling on absorption, distribution, metabolism, and excretion gene expression and activity in primary human hepatocytes and HepaRG Cells. Drug Metab Dispos. 2015 Jan 1;43(2):273–83.
- Leung JG, Nelson S, Takala CR, Gören JL. Infection and inflammation leading to clozapine toxicity and intensive care: a case series. Ann Pharmacother. 2014;48(6):801–5.

- 9. Xu Y, Hijazi Y, Wolf A, Wu B, Sun YN, Zhu M. Physiologically Based Pharmacokinetic Model to Assess the Influence of Blinatumomab-Mediated Cytokine Elevations on Cytochrome P450 Enzyme Activity. CPT pharmacometrics Syst Pharmacol. 2015 Sep 1:4(9):507–15.
- Stader F, Battegay M, Sendi P, Marzolini C. Physiologically Based Pharmacokinetic Modelling to Investigate the Impact of the Cytokine Storm on CYP3A Drug Pharmacokinetics in COVID-19 Patients. Clin Pharmacol Ther. 2022 Mar 1:111(3):579–84.
- Machavaram KK, Almond LM, Rostami-Hodjegan A, Gardner I, Jamei M, Tay S, et al. A physiologically based pharmacokinetic modeling approach to predict disease-drug interactions: Suppression of CYP3A by IL-6. Clin Pharmacol Ther. 2013 Aug 1;94(2):260–8
- Lenoir C, Niederer A, Rollason V, Desmeules JA, Daali Y, Samer CF. Prediction of cytochromes P450 3A and 2C19 modulation by both inflammation and drug interactions using physiologically based pharmacokinetics. CPT pharmacometrics Syst Pharmacol. 2022 Jan 1;11(1):30–43.
- Jiang X, Zhuang Y, Xu Z, Wang W, Zhou H. Development of a Physiologically Based Pharmacokinetic Model to Predict Disease-Mediated Therapeutic Protein-Drug Interactions: Modulation of Multiple Cytochrome P450 Enzymes by Interleukin-6. AAPS J. 2016 Mar 9;18(3):767-76.
- Ozbey AC, Fowler S, Leys K, Annaert P, Umehara K, Parrott N. PBPK Modelling for Drugs Cleared by Non-CYP Enzymes: Stateof-the-Art and Future Perspectives. Drug Metab Dispos. 2023 Jan 1;52(1):44–55.
- 15. Saravanakumar A, Sadighi A, Ryu R, Akhlaghi F. Physicochemical Properties, Biotransformation, and Transport Pathways of Established and Newly Approved Medications: A Systematic Review of the Top 200 Most Prescribed Drugs vs. the FDA-Approved Drugs Between 2005 and 2016. Clin Pharmacokinet. 2019 Oct 1;58(10):1281–94.

- de Jong LM, Jiskoot W, Swen JJ, Manson ML. Distinct effects of inflammation on cytochrome P450 regulation and drug metabolism: Lessons from experimental models and a potential role for pharmacogenetics. Genes (Basel). 2020 Dec 1:11(12):1–24.
- Coutant DE, Hall SD. Disease-Drug Interactions in Inflammatory States via Effects on CYP-Mediated Drug Clearance. J Clin Pharmacol. 2018 Jul 1;58(7):849–63.
- 18. Jablonska E, Jablonski J, Piotrowski L, Grabowska Z. IL-1beta, IL-1Ra and sIL-1RII in the culture supernatants of PMN and PBMC and the serum levels in patients with inflammation and patients with cancer disease of the same location. Immunobiology, 2001;204(4):508–16.
- Gripon P, Rumin S, Urban S, Le Seyec J, Glaise D, Cannie I, et al. Infection of a human hepatoma cell line by hepatitis B virus. Proc Natl Acad Sci U S A. 2002 Nov 26:99(24):15655–60.
- de Jong LM, Boussallami S, Sánchez-López E, Giera M, Tushuizen ME, Hoekstra M, et al. The impact of CYP2C19 genotype on phenoconversion by concomitant medication. Front Pharmacol. 2023;14.
- 21. Chomczynski P, Sacchi N. Singlestep method of RNA isolation by acid guanidinium thiocyanate-phenolchloroform extraction. Anal Biochem. 1987 Apr;162(1):156–9.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–8.
- Guillouzo A, Corlu A, Aninat C, Glaise D, Morel F, Guguen-Guillouzo C. The human hepatoma HepaRG cells: a highly differentiated model for studies of liver metabolism and toxicity of xenobiotics. Chem Biol Interact. 2007 May 20;168(1):66– 73.
- 24. Court MH, Krishnaswamy S, Hao Q, Duan SX, Patten CJ, Von Moltke LL, et al. Evaluation of 3'-azido-3'-deoxythymidine, morphine, and codeine as probe substrates for UDP-glucuronosyltransferase 2B7 (UGT2B7) in human liver microsomes: specificity and influence of the UGT2B7\*2 polymorphism. Drug Metab Dispos. 2003 Sep 1;31(9):1125–33.

- Spaggiari D, Geiser L, Daali Y, Rudaz S. A cocktail approach for assessing the in vitro activity of human cytochrome P450s: an overview of current methodologies. J Pharm Biomed Anal. 2014 May 1;101:221–37.
- Störmer E, Roots I, Brockmöller J.
   Benzydamine N-oxidation as an index
   reaction reflecting FMO activity in human
   liver microsomes and impact of FMO3
   polymorphisms on enzyme activity. Br J Clin
   Pharmacol. 2000;50(6):553–61.
- Sebaugh JL. Guidelines for accurate EC50/IC50 estimation. Pharm Stat. 2011 Mar;10(2):128–34.
- Hines RN. Developmental and tissuespecific expression of human flavincontaining monooxygenases 1 and 3. Expert Opin Drug Metab Toxicol. 2006;2(1):41–9.
- Izukawa T, Nakajima M, Fujiwara R, Yamanaka H, Fukami T, Takamiya M, et al. Quantitative analysis of UDPglucuronosyltransferase (UGT) 1A and UGT2B expression levels in human livers. Drug Metab Dispos. 2009 Aug;37(8):1759– 68.
- Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Vol. 138, Pharmacology and Therapeutics. Pergamon; 2013. p. 103–41.
- Klöditz K, Tewolde E, Nordling Å, Ingelman-Sundberg M. Mechanistic, Functional, and Clinical Aspects of Proinflammatory Cytokine Mediated Regulation of ADME Gene Expression in 3D Human Liver Spheroids. Clin Pharmacol Ther. 2023 Sep 1;114(3):673–85.
- 32. Kiamehr M, Heiskanen L, Laufer T, Düsterloh A, Kahraman M, Käkelä R, et al. Dedifferentiation of Primary Hepatocytes is Accompanied with Reorganization of Lipid Metabolism Indicated by Altered Molecular Lipid and miRNA Profiles. Int J Mol Sci. 2019 Jun 2;20(12).
- Pinto C, Giordano DM, Maroni L, Marzioni M. Role of inflammation and proinflammatory cytokines in cholangiocyte pathophysiology. Biochim Biophys acta Mol basis Dis. 2018 Apr 1;1864(4 Pt B):1270–8.

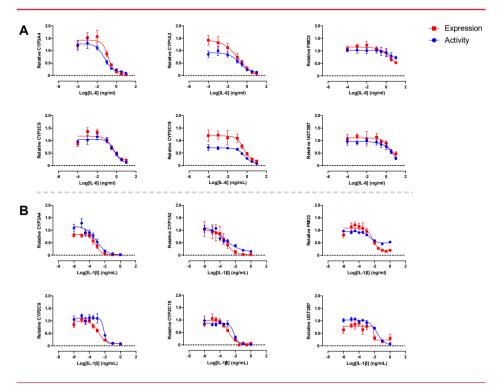
- Nguyen T V., Ukairo O, Khetani SR, McVay M, Kanchagar C, Seghezzi W, et al. Establishment of a hepatocyte-kupffer cell coculture model for assessment of proinflammatory cytokine effects on metabolizing enzymes and drug transporters. Drug Metab Dispos. 2015 May 1;43(5):774–85.
- Xie W, Barwick JL, Simon CM, Pierce AM, Safe S, Blumberg B, et al. Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. Genes Dev. 2000;14(23):3014–23.
- Ferguson SS, Lecluyse EL, Negishi M, Goldstein JA. Regulation of human CYP2C9 by the constitutive androstane receptor: Discovery of a new distal binding site. Mol Pharmacol. 2002 Sep 1;62(3):737–46.
- 37. Chen Y, Ferguson SS, Negishi M, Goldstein JA. Identification of constitutive androstane receptor and glucocorticoid receptor binding sites in the CYP2C19 promoter. Mol Pharmacol. 2003 Aug 1;64(2):316–24.
- Klick DE, Hines RN. Mechanisms regulating human FMO3 transcription. Drug Metab Rev. 2007 Apr;39(2–3):419–42.
- 39. Collins JM, Lu R, Wang X, Zhu HJ,
  Wang D. Transcriptional Regulation of
  Carboxylesterase 1 in Human Liver: Role
  of the Nuclear Receptor Subfamily 1 Group
  H Member 3 and Its Splice Isoforms. Drug
  Metab Dispos. 2022 Jan 1;50(1):43–8.
- Hu DG, Meech R, McKinnon RA, Mackenzie PI. Transcriptional regulation of human UDP-glucuronosyltransferase genes. Drug Metab Rev. 2014 Nov 1;46(4):421–58.
- Keller R, Klein M, Thomas M, Dräger A, Metzger U, Templin MF, et al. Coordinating Role of RXRα in Downregulating Hepatic Detoxification during Inflammation Revealed by Fuzzy-Logic Modeling. Altan-Bonnet G, editor. PLOS Comput Biol. 2016 Jan 4:12(1):e1004431.
- 42. Gramignoli R, Ranade AR, Venkataramanan R, Strom SC. Effects of Pro-Inflammatory Cytokines on Hepatic Metabolism in Primary Human Hepatocytes. Int J Mol Sci. 2022 Dec 1;23(23).
- 43. Weber A, Wasiliew P, Kracht M. Interleukin-1 (IL-1) pathway. Sci Signal. 2010 Jan 19;3(105).
- 44. Schaper F, Rose-John S. Interleukin-6: Biology, signaling and strategies of blockade. Cytokine Growth Factor Rev. 2015 Oct 1;26(5):475–87.

- 45. Ghose R, Zimmerman TL, Thevananther S, Karpen SJ. Endotoxin leads to rapid subcellular re-localization of hepatic RXRalpha: A novel mechanism for reduced hepatic gene expression in inflammation. Nucl Recept. 2004 Aug 16:2(1).
- Ferrari L, Peng N, Halpert JR, Morgan ET. Role of nitric oxide in down-regulation of CYP2B1 protein, but not RNA, in primary cultures of rat hepatocytes. Mol Pharmacol. 2001;60(1):209–16.
- 47. Lee CM, Pohl J, Morgan ET. Dual mechanisms of CYP3A protein regulation by proinflammatory cytokine stimulation in primary hepatocyte cultures. Drug Metab Dispos. 2009 Apr;37(4):865–72.
- 48. Kugler N, Klein K, Zanger UM. MiR-155 and other microRNAs downregulate drug metabolizing cytochromes P450 in inflammation. Biochem Pharmacol. 2020 Jan 1:171
- Aitken AE, Morgan ET. Gene-specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. Drug Metab Dispos. 2007 Sep;35(9):1687–93.
- Willmann S, Lippert J, Sevestre M, Solodenko J, Fois F, Schmitt W. PK-Sim\*: A physiologically based pharmacokinetic "whole-body" model. Drug Discov Today BIOSILICO. 2003;1(4):121–4.
- 51. Machavaram KK, Endo-Tsukude C, Terao K, Gill KL, Hatley OJ, Gardner I, et al. Simulating the Impact of Elevated Levels of Interleukin-6 on the Pharmacokinetics of Various CYP450 Substrates in Patients with Neuromyelitis Optica or Neuromyelitis Optica Spectrum Disorders in Different Ethnic Populations. AAPS J. 2019 May 1;21(3).
- 52. Kenny JR, Liu MM, Chow AT, Earp JC, Evers R, Slatter JG, et al. Therapeutic protein drugdrug interactions: navigating the knowledge gaps-highlights from the 2012 AAPS NBC Roundtable and IQ Consortium/FDA workshop. AAPS J. 2013;15(4):933–40.
- 53. Liu F, Aulin LBS, Manson ML, Krekels EHJ, van Hasselt JGC. Unraveling the Effects of Acute Inflammation on Pharmacokinetics: A Model-Based Analysis Focusing on Renal Glomerular Filtration Rate and Cytochrome P450 3A4-Mediated Metabolism. Eur J Drug Metab Pharmacokinet. 2023 Nov 1;48(6):623–31.

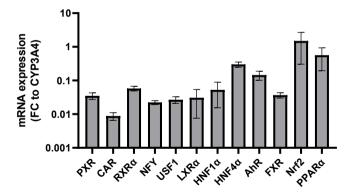
- 54. Song C, Long X, He J, Huang Y. Recent evaluation about inflammatory mechanisms in nonalcoholic fatty liver disease. Front Pharmacol. 2023;14.
- Murphy WA, Adiwidjaja J, Sjöstedt N, Yang K, Beaudoin JJ, Spires J, et al. Considerations for Physiologically Based Modeling in Liver Disease: From Nonalcoholic Fatty Liver (NAFL) to Nonalcoholic Steatohepatitis (NASH). Clin Pharmacol Ther. 2023 Feb 1:113(2):275–97.
- 56. Powell NR, Liang T, Ipe J, Cao S, Skaar TC, Desta Z, et al. Clinically important alterations in pharmacogene expression in histologically severe nonalcoholic fatty liver disease. Nat Commun. 2023 Dec 1;14(1).
- 57. Govaere O, Cockell SJ, Zatorska M, Wonders K, Tiniakos D, Frey AM, et al. Pharmacogene expression during progression of metabolic dysfunction-associated steatotic liver disease: Studies on mRNA and protein levels and their relevance to drug treatment. Biochem Pharmacol. 2024 Oct; 228:116249.
- 58. Märtson AG, Veringa A, Bakker M, van den Heuvel ER, Touw DJ, van der Werf TS, et al. Posaconazole trough concentrations are not influenced by inflammation: A prospective study. Int J Antimicrob Agents. 2019 Mar 1;53(3):325–9.
- 59. Veringa A, ter Avest M, Span LFR, van den Heuvel ER, Touw DJ, Zijlstra JG, et al. Voriconazole metabolism is influenced by severe inflammation: A prospective study. J Antimicrob Chemother. 2017 Jan 1;72(1):261–7.
- Van Wanrooy MJP, Span LFR, Rodgers MGG, Van Heuvel ERD, Uges DRA, Van Werf TSD, et al. Inflammation is associated with voriconazole trough concentrations. Antimicrob Agents Chemother. 2014 Dec 1;58(12):7098–101.

## Supplemental information

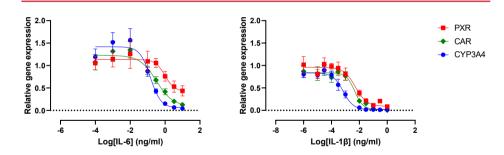
## Supplemental figures



Supplementary Figure S1 Cytokine concentration-response curves for regulation of CYP3A4, CYP2C9, CYP1A2, CYP2C19, FMO3 and UGT2B7 expression and activity by IL-6 (A) and IL-1 $\beta$  (B). Cells were treated with concentrations of 0.0001 ng/mL to 10 ng/mL (IL-6) or 0.001 pg/mL to 1 ng/mL (IL-1 $\beta$ ) for 24 hours to analyze gene expression alterations via RT-qPCR or for 72 hours to analyze activity alterations via probe substrate metabolism with LC-MS/MS. mRNA and activity data are expressed as fold change of levels found in untreated control cells, arbitrarily set to 1.0. Each data point represents the average of at least 4 independent experiments  $\pm$  SEM. Data was fit to a non-linear regression model in Graphpad Prism.



Supplementary Figure S2 Basal mRNA expression levels of DME regulating transcription factors and nuclear receptors in HepaRG cells. mRNA expression of the gene of interest was normalized to the housekeeping gene RPLP0, and presented as a fold change compared to basal CYP3A4 expression in HepaRG cells. All values are means + SEM from 8 independent experiments.



Supplementary Figure S3 Cytokine concentration-response curves for regulation of PXR and CAR as compared to CYP3A4. Cells were treated with concentrations of 0.0001 ng/ml to 10 ng/ml (IL-6) or 0.001 pg/ml to 1 ng/ml (IL-1 $\beta$ ) for 24 hours to analyze gene expression alterations via RT-qPCR. mRNA data is expressed as fold change of levels found in untreated control cells, arbitrary set to 1.0. Each data point presents the average + SEM of at least 4 independent experiments. Data were fit with a non-linear regression model.

## Supplemental tables

## Supplemental Table S1 Primer sequences

	Sequence
CYP3A4	For 5'-TGTGCTGGCTATCACAGATCCTGAC-3' Rev 5'-CAAAAGGCCTCCGGTTTGTGAAGAC-3'
CYP2C19	For 5'-AAAACCAAGGCTTCACCCTGTGATCC-3' Rev 5'-CCGGGAAATAATCAATGATAGTGGGAAA-3'
CYP2C9	For 5'-CTTTCCTCTGGGGCATTATCCATCTTTC-3' Rev 5'-CATAGGAAACTCTCCGTAATGGAGGTCG-3'
CYP1A2	For 5'-GGTTCCTGTGGTTCCTGCAGAAAAC-3' Rev 5'-ATCTTCTCCTGTGGGATGAGGTTGC-3'
FMO1	For 5'-GGGCTCCATGATACCTACAGGAGAAAC-3' Rev 5'-CAGTAGCACAAGCCAAACCAACTGG-3'
FMO3	For 5'-ATTCCCACAGTTGACCTCCAGTCC-3' Rev 5'-GTCTCGCTTTTGCCAAACCATTTGC-3'
FMO4	For 5'-TGGAGGCTACTGAAAAGGAACAGCTC-3' Rev 5'-TCCTTGAGGAACAGAGTGGGATGC-3'
UGT1A4	For 5'-CCTGACAGCCTATGCTGTTCCA-3' Rev 5'-ATGCAGTAGCTCCACACACACCT-3'
UGT2B4	For 5'-CCCTCCTTCCTATGTGCCTGTTGTTATG-3' Rev 5'-TCGAATAAGCCATATGTCAGCTTTTGCC-3'
UGT2B7	For 5'-CATGCAACAGATTAAGAGATGGTCAGACC-3' Rev 5'-CAGCAGCTCACTACAGGGAAAAATAGC-3'
UGT2B15	For 5'-TGGGACTCCTCCTTTATTTCAGCATGG-3' Rev 5'-TGCTGCATCCAGTAACTCGTCATTTAAC-3'
NR1I2	For 5'-GCAGGAGCAATTCGCCATTACTCTG-3' Rev 5'-TAGCAAAGGGGTGTATGTCCTGGATG-3'
NR1I3	For 5'-TGCTTAGATGCTGGCATGAGGAAAG-3' Rev 5'-CTTGCTCCTTACTCAGTTGCACAGG-3'
AHR	For 5'-ATGTATCAGTGCCAGCCAGAACCTC-3' Rev 5'-AGTGGCTGAAGATGTGTGGTAGTCTG-3'
RXRA	For 5'-ATGCAGATGGACAAGACGGAGCTG-3' Rev 5'-AGGACGCATAGACCTTCTCCCTCAG-3'
NR1H4	For 5'-CGGAAATGGCAACCAATCATGTACAGG-3' Rev 5'-CAGACCCTTTCAGCAAAGCAATCTGG-3'
HNF4A	For 5'-AGAGATCCATGGTGTTCAAGGACGTG-3' Rev 5'-CCTTGGCATCTGGGTCAAAGAAGATG-3'
NFYA	For 5'-CGTGGTGAAGGTGGACGATTTTTCTC-3' Rev 5'-TGTCATTGCTTCTTCATCGGCTTGG-3'
USF1	For 5'-ACAAGAAGTACTGCAGGGAGGAAGC-3' Rev 5'-CATTATGCTGAGCCCTGCGTTTCTC-3'

## Supplemental materials and methods

## LC-MS/MS method to quantify CYP activity

Quantification of acetaminophen, 4'hydroxymephentoin, 1'hydroxymidazolam, and 4'hydroxydiclofenac in cell supernatant was done using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system consisting of a Nexera LC-40 high-performance liquid chromatography (HPLC) system equipped with a DGU-403 degassing unit, two LC-40D pumps, a SIL-40C autosampler, and a CTO-40S column oven (Shimadzu, 's-Hertogenbosch, the Netherlands). A Kinetex C18 column (1.7 µM, 50×2.1 mm) (Phenomenex, Utrecht, The Netherlands) with a SecurityGuard Ultra C18, 2.7 µm, 5×2.1 mm cartridge (Phenomenex, Utrecht, The Netherlands) as guard column were used to separate probe metabolites from other analytes present in the sample matrix. Mobile phases consisted of water (A) and methanol (B) both containing 0.1% formic acid. The gradient, with a flow rate of 0.4 ml/min, started at 5% B and increased to 100% B in 4 min, maintaining 100% B for 2 min, and then returned to initial conditions for another 2 min. The column was kept at 50°C and the injection volume was 10 μL or 20 μL depending on the analyte. The HPLC was coupled to a Sciex QTRAP 6500+ mass spectrometer (AB Sciex Netherlands B.V., Nieuwerkerk aan den IJssel, The Netherlands) operating in positive electrospray mode (ESI+).

The MS conditions were as follows: curtain gas 20 psi, collision gas "medium", ion source gas 1 40 psi, ion source gas 2 40 psi, ion spray voltage 5500 V and temperature 550°C. The MS was operated in the multiple reaction monitoring (MRM) mode and was optimized by direct infusion of the standards individually. The optimized MRM transitions, retention time, declustering potential (DP), collision energy (CE) and cell exit potential (CXP) used are summarized in Supplemental Table 2. Analyst software version 1.4 (AB Sciex Netherlands B.V., Nieuwerkerk aan den IJssel, The Netherlands) was used for data analysis.

**Supplemental Table S2** MRM parameters and retention time for the quantified analytes by the LC-MS/MS method

Analyte	Q1 mass (Da)	Q3 mass (Da)	Retention time (min)	DP (V)	CE (V)	CXP (V)
Acetaminophen	152.1	110.0	1.37	46	23	12
Acetaminophen-d <sub>4</sub>	156.1	114.1	1.37	51	23	12
1'hydroxymidazolam	341.9	203.0	3.51	86	35	12
1'hydroxymidazolam-d <sub>4</sub>	345.9	203.0	3.51	81	37	16
4'-hydroxymephenytoin	235.1	150.1	2.70	51	25	10
4'-hydroxymephenytoin-d <sub>3</sub>	238.1	150.1	2.70	41	25	14
4'hydroxydiclofenac	312.0	230.0	4.02	46	43	12
4'hydroxydiclofenac-13C <sub>6</sub>	318.0	236.0	4.02	51	43	12

## LC-MS/MS method to quantify FMO3 and UGT2B7 activity

Quantification of benzydamine N-oxide and morphine-3-glucuronide in cell supernatant was done using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system consisting of a Nexera-X2 ultra high-performance liquid chromatography (UHPLC) system equipped with a DGU-20A degassing unit, three LC-30 pumps, a SIL-30ACMP autosampler, and a CTO-30A column oven (Shimadzu, 's-Hertogenbosch, the Netherlands).

For benzydamine N-oxide, separation was achieved with an Acquity BEH column (1.7  $\mu$ m, 2.1×50 mm) from Waters (Etten-Leur, The Netherlands). Elution of benzydamine-N-oxide was performed using a high pressure gradient, with a flow of 0.4 ml/min, from 5% to 95% acetonitrile with 0.1% formic acid. The column was kept at 40°C and the injection volume was 10  $\mu$ L.

For morphine-3-glucuronide, separation as achieved with a Vision HT Basic column (3  $\mu$ m, 150×3 mm) (Grace, Breda, the Netherlands). An online solid-phase extraction (SPE) method was used to clean the samples, using a Hysphere GP cartridge (Spark Holland, Emmen, the Netherlands). Samples were injected into the SPE column and washed with 1 ml 10 mM ammonium acetate buffer at pH 10 for 1 minute to remove salts and other interferences, after which they were injected into the LC-column. Elution into the LC system was performed with a gradient of 3% to 97% acetonitrile with 0.1% formic acid in 4 minutes, at a flow of 300  $\mu$ L/min and re-equilibrated at 3% acetonitrile. The column was kept at 40°C and the injection volume was 5  $\mu$ L.

The UHPLC was coupled to a TSQ Vantage mass spectrometer (Thermo Fisher, Breda, The Netherlands) operating in positive electrospray mode (ESI+). The MS conditions were as follows: curtain gas 20 psi, collision gas 0.5 atm ion source gas 5 psi, ion spray voltage 3000 V and temperature 350°C. The MS was operated in MRM mode and was optimized by direct infusion of the standards individually. The optimized MRM transitions, retention time, declustering potential (DP) and collision energy (CE) used for both analytes are summarized in Supplemental Table 3. Thermo XCalibur Software LCQuan 2.7 was used for data analysis.

Supplemental Table S3 MRM parameters and retention time for the quantified analytes by the LC-MS/MS method

Analyte	Q1 mass (Da)	Q3 mass (Da)	Retention time (min)	DP (V)	CE (V)
Benzydamine N-oxide	326.2	102.1	4.5	16	9
Benzydamine N-oxide-d <sub>6</sub>	332.2	108.2	4.5	16	8
Morphine-3-glucuronide	462.1	152.9, 201.0, 286.113	4.4	6	62, 48 and 24
Morphine-3-glucuronide-d <sub>3</sub>	465.2	152.9, 201.0, 289.074	4.4	6	62, 48 and 24



# Chapter 5

A systematic review on disease-drug-drug interactions with immunomodulating drugs:

A critical appraisal of risk assessment and drug labelling

Laura M. de Jong, Sylvia D. Klomp, Nicoline Treijtel, Robert Rissmann, Jesse J. Swen, Martijn L. Manson



## **Abstract**

**AIM:** Use of immunomodulating therapeutics for immune-mediated inflammatory dis-eases may cause disease-drug-drug interactions (DDDIs) by reversing inflammation-driven alterations in the metabolic capacity of cytochrome P450 enzymes. European Medicine Agency (EMA) and US Food and Drug Administration (FDA) guidelines from 2007 recommend that the DDDI potential of therapeutic proteins should be assessed. This systematic analysis aimed to characterize the available DDDI trials with immunomodulatory drugs, experimental evidence for a DDDI risk and reported DDDI risk information in FDA/EMA approved drug labelling.

**METHOD:** For this systematic review, the EMA list of European Public Assessment Reports of human medicine was used to select immunomodulating monoclonal anti-bodies (mAbs) and tyrosine kinase inhibitors (TKIs) marketed after 2007 at risk for a DDDI. Selected drugs were included in PubMed and Embase searches to extract reported interaction studies. The Summary of Product Characteristics (SPCs) and the United States Prescribing Information (USPIs) were subsequently used for analysis of DDDI risk descriptions.

**RESULTS:** Clinical interaction studies to evaluate DDDI risks were performed for 12 of the 24 mAbs (50%) and for none of the TKIs. Four studies identified a DDDI risk, of which three were studies with interleukin-6 (IL-6) neutralizing mAbs. Based on (non)clinical data, a DDDI risk was reported in 32% of the SPCs and in 60% of the USPIs. The EMA/FDA documentation aligned with the DDDI risk potential in 35% of the20 cases.

**CONCLUSION:** This systematic review reinforces that the risk for DDDI by immunomodulating drugs is target- and disease-specific. Drug labelling information designates the greatest DDDI risk to mAbs that neutralize the effects of IL-6, Tumor Necrosis Factor alfa (TNF- $\alpha$ ) and interleukin-1 bèta (IL-1 $\beta$ ) in diseases with systemic inflammation.

#### What is already known about this subject

- Inflammation can change the drug metabolizing capacity of individuals and may hence affect drug exposure.
- Immunomodulating therapeutics may, through resolution of inflammation, trigger disease-drug-drug
  interactions (DDDI), for which the EMA and FDA have instructed guidelines for risk assessment.

#### What this study adds

- This is the first study that systematically compared available clinical and non-clinical evidence for the risk assessment of DDDIs to the drug labelling of immunomodulating therapeutics.
- This study reinforces that the risk for DDDI by immunomodulating drugs occurs to be target and disease specific.
- We highlight that the available evidence to determine a DDDI risk is not always reflected in the drug labelling that is approved by the EMA and FDA, and risk assessment differs between regulatory authorities.

## Introduction

Inflammation can contribute to inter-individual variability in drug response, potentially resulting in under- or overexposure of the drug and thereby ineffective treatment or toxicity (1–3). Indeed, in patients with an acute or chronically increased inflammatory status, drug clearance is altered, resulting in phenoconversion (4–7). These changes in drug clearance are attributed to inflammation-associated cytokines that can impair or induce expression of the cytochrome P450 (CYP) enzymes involved in drug metabolism of small molecules (8–10). For example acute COVID-19 infection leads to an isoform specific modulation of CYP activity and studies in rheumatoid arthritis patients have shown increased plasma concentrations of prescribed drugs (11–13).

In the last decades, immunomodulating monoclonal antibodies (mAbs) that target specific cytokines or their receptors have increasingly been deployed in the treatment of immune-mediated inflammatory diseases (IMIDs). These immunomodulating mAbs are not metabolized via CYP enzymes and are therefore also unable to directly induce or inhibit the activities of these metabolic enzymes. For this reason, the risk that mAbs change the pharmacokinetics of concomitant medication and trigger traditional direct drug-drug interactions (DDIs) is generally considered to be low. However, mAbs that resolve inflammation may, through the reversal of cytokine-mediated effects on the expression of drug metabolizing enzymes, restore CYP mediated clearance (14). Immunomodulating mAbs may hence indirectly change the pharmacokinetics of concomitant medication and induce disease-drug-drug interactions (DDDIs).

Immunomodulation may not be restricted to mAbs, but also occur following the administration of small molecules that target downstream signalling pathways of inflammatory mediators. The effects of inflammation on CYPs are presumed to occur via activation of cytokine signalling pathways (10). As such, inhibitors of these pathways might also indirectly reverse the impact of inflammation. In theory, tyrosine kinase inhibitors (TKIs) that interfere with the signalling pathways of cytokines may also be prone to induce DDDIs in patients suffering from an inflammatory disease.

The potential of therapeutic proteins, including mAbs, to trigger DDDIs is acknowledged by both the European Medicine Agency (EMA) and the U.S. Food and Drug Administration (FDA). In 2007, the EMA updated their DDI guidelines

by recommending to assess the potential risk for DDDIs with therapeutic proteins that are either pro-inflammatory cytokines themselves or have the potential to modulate pro-inflammatory cytokines (15). The current FDA guidelines (2020) state that labelling of this type of therapeutic proteins should include a risk analysis in which the potential for DDDIs is defined (16). Input for this risk analysis can be retrieved from in vitro or animal studies, population PK modelling or physiologically based pharmacokinetic (PBPK) modelling, or a dedicated clinical DDDI study (16).

A CYP phenotyping cocktail approach is considered the gold standard for assessing a therapeutic protein's potential for inducing DDDIs. These studies compare the pharmacokinetics of probe substrates for critical CYP enzymes in drug metabolism (e.g. CYP3A4, CYP2D6, CYP2C19, CYP2C9 and CYP1A2) prior and after the start of an immunomodulating mAbs in the intended target population. An advantage of this is that every patient serves as its own control – excluding inter-individual variability in drug metabolism as a confounding factor. Changes in the exposure parameters  $C_{\rm max}$  and  $AUC_{\rm 0-inf}$  of the individual probe substrates that exceed the limits for bioequivalence (80–125%) are an indication that drug metabolism is affected by the investigated drug. Through this approach, the potential of a therapeutic protein to indirectly change drug metabolism of small molecules via immunomodulation can be defined and accordingly inform on the risk of DDDI.

Results from DDDI studies with cytokine-targeting mAbs have been summarized before (2,17,18), but interpretation of these results is limited and not connected to DDDI risk assessment approved by regulatory authorities. To address this gap, this review aimed to provide a systematic overview of all available evidence for DDDIs with immunomodulating drugs and the associated risks stated in the drug labelling information approved by the FDA and EMA between 2007 and 2021. To this end, in this review the results from clinical studies for mAbs and TKIs examining the potential shift in drug exposure following intervention with immunomodulatory therapies are summarized. Secondly, the DDDI risks of therapeutic proteins that are cytokine modulators as described in the EMA's summary of product characteristics (SPC) and the FDA's United States prescribing information (USPI) were analysed and compared to the identified evidence from clinical and non-clinical studies. Finally, the outcome of this analysis was

used to provide recommendations for future assessment of DDDI risks with immunomodulating therapeutics.

## **Methods**

For this systematic review on DDDI studies and labelling information, identification and selection of pharmaceuticals and related studies was performed. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were used to prepare the report (19). The EMA list with European Public Assessment Reports (EPARs) of human medicine was used to identify all authorized pharmaceutical products between January 2007 and November 2021 (20). Only original trade names of drugs were included, thereby excluding biosimilars from the analysis. To identify immunomodulatory drugs, the following pharmaceutical groups were selected: (selective) immunosuppressant, antineoplastic agents, protein kinase inhibitor, interleukin (IL)-inhibitors, monoclonal antibodies, drugs for obstructive airway diseases, and agents for dermatitis. Drugs targeting any cytokine (receptor) were included together with drugs that selectively inhibit the JAK/STAT, MAPK/ MEK/ERK, NF-κB or PI3K/AKT signalling pathways downstream of cytokine receptors, as these pathways have been linked to effects of inflammation on drug metabolism (10). Immunosuppressants without a specific immune-related target were excluded from this analysis. The IUPHAR/BPS Guide to PHARMACOLOGY was used to standardize the nomenclature of all drugs and targets (21).

Pubmed, Cochrane and Embase were with the support of a librarian used to identify all published clinical interaction studies with eligible immunomodulating drugs. Search terms consisted of the drug name together with terms describing interaction studies. Only English language papers with original data were included. Subsequently, ongoing interaction studies wherefore results are available were identified via clinicaltrials.gov (10). Evidence from non-clinical studies on potential DDDI risks was collected and summarized based on the recently reviewed in vitro impact of the targeted cytokines on CYP activity. Only studies utilizing primary human hepatocytes (PHHs) were included for this assessment, since they are considered the golden standard for in vitro studies. Next, the EPAR documents published by the EMA (Annex I, SPC) and the USPI documents published by the FDA of all selected drugs were examined to retrieve information on described

potential risks for DDDIs. The potential risk of each individual drug to induce DDDIs was determined and categorized as 'ves', 'caution', 'no' or 'unknown', based on the provided information. When the SPC or USPI stated: 'perform therapeutic monitoring (TM) of effect or drug concentration' (of victim drug) or 'TM is recommended', the DDDI risk was classified as 'yes'. When the SPC or USPI stated: 'consider performing therapeutic monitoring of effect or drug concentration' the DDDI risk was classified as 'caution'. When SPC or USPI stated: 'clinical significance is unknown' or there was no mention of any DDDI related information, the DDDI risk was classified as 'unknown'. Additionally, the type of studies that were available in literature for assessing DDDI risks - independent from evidence used by regulatory authorities - were determined and classified into the following groups: Class 0: no data, class 1: experimental (in vitro) data; available experimental evidence examining the potential effect of the targeted cytokine to modulate CYP activity in primary human hepatocytes (PHH), class 2: PBPK modelling, class 3: clinical data with a substrate for one CYP enzyme, or class 4: clinical data based on investigations with a probe cocktail for multiple CYP enzymes.

Lastly, the agreement on risk information of mAbs was compared between the SPC and USPI. This analysis was limited to mAbs, since TKI drug labels did not address DDDIs.

# Results

In this systematic review a total of 1573 drugs with an EPAR classified as human medicine between January 2007 and November 2021 were identified. After screening, 37 pharmaceutical products were identified that, based on their mechanism of action, would make them eligible for a DDDI study (Figure 1). Following a review of their EPARs and a literature search in Pubmed and Embase databases in April 2022, conducted clinical CYP interaction study were identified for 12 of the 24 mAbs (50%) and for none of the TKIs (0%) (Table 1). Of these, seven studies exploited a CYP cocktail approach (58%) whereas the other five studies (42%) determined the potential of DDDI using a CYP3A4 substrate (Table 1). There are drugs for which no clinical interaction study was performed, but in the product label a DDDI risk was stated based on non-clinical data (Table 2 & Supplementary Table S1).

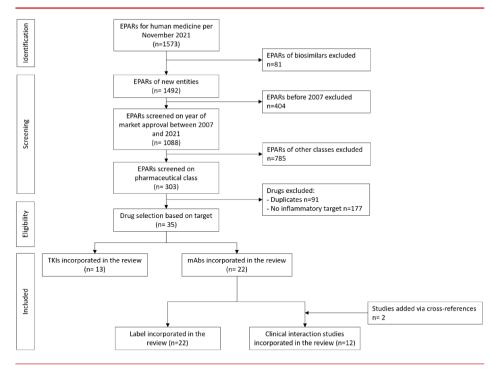


Figure 1 Study flow diagram of the retrieval and review process. Drugs targeting any cytokine (receptor) and drugs that selectively inhibit the JAK/STAT, MAPK/MEK/ERK, NF-κB or PI3K/AKT signalling pathways downstream of cytokine receptors were included in the analysis.

The included immunomodulating mAbs where subdivided based on their target, categorized as acute signalling cytokines, IL-17/IL-23 cytokines, Th2-type cytokines, or Th1-type cytokines (Figure 2). Since TKIs do not target a specific receptor, but rather inhibit the cellular signalling pathways that are initiated after cytokine binding to the receptor, they span multiple categories.

Table 1 Results of performed clinical disease-drug-drug interaction studies. Studies are ordered based on target and study population. Impact of mAb treatment initiation on CYP activity of major CYP enzymes is indicated with colour patterns.

Target	Drug	Study population	ž	Moment of PK evaluation (day 0 = mAb initiation) <sup>d</sup>	CYP3A4€	CYP2C19¢	CYP1A2°	CYP1A2° CYP2D6°	CYP2C9€	Reference
IL-6	Sirukumabª	Rheumatoid arthritis	12	Day 7 & 21 & 42	Midazolam	Omeprazole	Caffeine		Warfarin <sup>g</sup>	(22)
	Tocilizumab	Rheumatoid arthritis	12	Day 7 & 35	Simvastatin					(23)
IL-6Ra	Sarilumab	Rheumatoid arthritis	19	Day 7	Simvastatin					(24)
II-6	Clazakizumab <sup>b</sup> Kidney transpl	Kidney transplant	10	Day 84 & Day 364	Pantoprazole <sup>f</sup> Pantoprazole	Pantoprazole				(25)
IL-17RA	IL-17RA Brodalumab	Psoriasis	20	Day 9	Midazolam					NCT01937260
IL-17A	IL-17A Ixekizumab	Psoriasis	26	Day 8 & 85	Midazolam	Omeprazole	Caffeine	Dextromethorphan Warfarin <sup>g</sup>	Warfarin <sup>g</sup>	NCT02993471
	Secukinumab	Psoriasis	24	Day 8 & 36	Midazolam					(26)
IL-23	Risankizumab	Psoriasis	21	Day 90	Midazolam	Omeprazole	Caffeine	Metoprolol	Warfarin <sup>g</sup>	(27)
	Tildrakizumab Psoriasis	Psoriasis	17	Day 57	Midazolam	Omeprazole	Caffeine	Dextromethorphan	Warfarin <sup>g</sup>	(28)
	Guselkumab	Psoriasis	12	Day 7 & 28	Midazolam	Omeprazole	Caffeine	Dextromethorphan	$S\text{-warfarin}^{g}$	(29)
IL-4Ra	Dupilumab	Atopic dermatitis	13	Day 28	Midazolam	Omeprazole	Caffeine	Metoprolol	S-warfarin $^g$	(30)
IL-2	Daclizumabª	Multiple sclerosis	20	Day 84	Midazolam	Omeprazole Caffeine	Caffeine	Dextromethorphan Warfarin <sup>g</sup>	Warfarin <sup>g</sup>	(31)

application retracted, b applied for market approval, c that completed the study, d baseline is not included, c No effect is when geometric mean ratio (90% CI) of AÙC<sub>pinf</sub> (probe alone vs probe + mAb) is within 80–125% equivalence limits, <sup>f</sup> Involvement of CYP3A4 in pantoprazole metabolism is limited, <sup>g</sup> Warfarin is also metabolized by CYP1A2, CYPC19 and CYP3A4. Colour indicate effect on probe drug exposure: vertical lines green = no change, horizontal lines red = decreased exposure, gridlines orange = increased exposure, clear white = not determined.

5

Table 2 Summary of experimental and clinical evidence for DDDI risks and according labelling information of mAbs documented by the EMA and FDA

							E	Tyna(c) of evidence eveileble for DDDI viel	oiloble for 1	John Inigh
							1ype(s) o	assess	assessment <sup>c</sup>	JUDI Hak
Target	Drug	Indication	Non-clinical evidence from PHHs (9,56,64-67)	Clinical evidence <sup>b</sup>	DDDI risk in SPC	DDDI risk in USPI	Experi- mental data	PBPK modelling	Clinical data with one substrate	Clinical data with multiple probe substrates
Acute IL-6 signalling cytokines	Tocilizumab	RA / COVID-19 / (systemic) JIA / CRS	Yes - CYP1A2 - CYP2C9 - CYP2C19	Yes - CYP3A4 (probe)	Yes	Yes				
IL-6R	Sarilumab	RA	- CYP3A4 -CYP3A4	Yes - CYP3A4 (probe)	Yes	Yes				
IL-6	Siltuximab	GLNH/ MCD	. '	Not conducted	Yes	Yes				
IL-6/ IL-6R	Satralizumab	NMO		Not conducted	Caution	Unknown				
TNF-α	Golimumab	JIA	Yes - CYP1A2	Not conducted	Unknown	Yes				
	Certolizumab pegol	RA / axSpA / PsA / plaque psoriasis	- CYP2C9 - CYP2C19 - CYP2D6 -CYP3A4	Not conducted	Unknown	Unknown				

Table 2 continues on next page.

 Table 2
 Continued

								Type(s) o	Type(s) of evidence available for DDDI risk assessment	nce available for I assessment°	ODDI risk
	Target	Drug	Indication	Non-clinical evidence from PHHs (9,56,64–67)	Clinical evidence <sup>b</sup>	DDDI risk in SPC	DDDI risk in USPI	Experi- mental data	PBPK modelling	Clinical data with one substrate	Clinical data with multiple probe substrates
	$1L-1\beta$	IL-1β Canakinumab	Gouty arthritis /CAPS/ TRAPS/HIDS /MKD/FMF/ Stills disease	Yes - CYP3A4	Not conducted	Yes	Yes				
		Rilonaceptª	CAPS		Not conducted	Yes	Yes				
IL-17/IL- 23 axis	IL- 17RA	Brodalumab	Plaque psoriasis	Not determined	Yes - CYP3A4 (probe)	No	Caution				
	IL-17A	Bimekizumab	IL-17A Bimekizumab Plaque psoriasis		Not conducted	Caution	No drug label				
		Ixekizumab	Plaque psoriasis / PsA / axSpA		No effect (cocktail)	No	Caution				
		Secukinumab	Secukinumab Plaque psoriasis		No effect (cocktail)	No	Caution				

Table 2 Continued

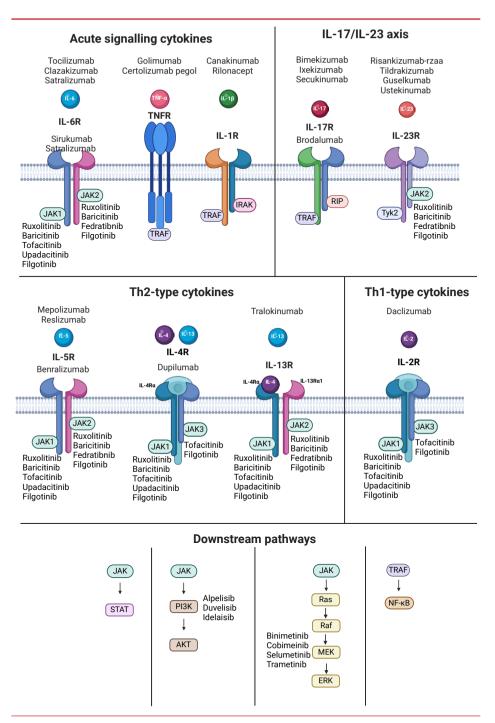
								Type(s) o	Type(s) of evidence available for DDDI risk	ailable for ]	DDDI risk
	Target	Drug	Indication	Non-clinical evidence from PHHs (9,56,64-67)	Clinical evidence <sup>b</sup>	DDDI risk in SPC	DDDI risk in USPI	Experi- mental data	Clini dat PBPK with modelling subst	Clinical data with one substrate	Clinical data with multiple probe substrates
	IL-23	IL-23 Risankizumab Plaque psoriasis	Plaque psoriasis	No	No effect (cocktail)	No	No				
	•	Tildrakizumab	Tildrakizumab Plaque psoriasis		No effect (cocktail)	No	No				
	•	Guselkumab	Plaque psoriasis / PsA	-	No effect (cocktail)	No	Caution				
	IL-23/ IL-12	Ustekinumab	Plaque psoriasis / Crohn's Disease / UC		Ongoing	No	Caution				
Th2-type IL-4Rα cytokines	IL-4Ra	Dupilumab	AD / asthma / CRSwNP	Not determined	No effect (cocktail)	No	Caution				
	IL-13	Tralokinumab	AD	Not determined	Ongoing	Unknown	No drug label				

Table 2 continues on next page.

 Table 2
 Continued

								Type(s) o	Type(s) of evidence available for DDDI risk assessment	nce available for l assessment	DDDI risk
	Target	Drug	Indication	Non-clinical evidence from PHHs (9,56,64–67)	Clinical evidence <sup>b</sup>	DDDI risk in SPC	DDDI risk in USPI	Experi- mental data	PBPK modelling		Clinical data with data multiple with one probe substrate substrates
	IL-5	IL-5 Mepolizumab	Severe EA / CRSwNP / EGPA / HES	Not determined	Not conducted	No	Unknown				
		Reslizumab	EA		Not conducted	No	Unknown				
	IL-5Rα	IL-5Rα Benralizumab	EA		Not conducted	No	Unknown				
Th1-type cytokine	IL-2	Th1-type IL-2 Daclizumab <sup>a</sup> cytokine	MS / allogenic renal transplantation	Yes - CYP2C19 - CYP2D6	No effect (cocktail)	Unknown	No				

on available data in literature on potential modulating effect of cytokine/mAbs on CYP metabolic capacity. DDDI risk categories are classified as: Yes (TM should be ndependent of risks stated in regulation labelling: 0 = no data available. RA = Rheumatoid arthritis, COVID-19 = coronavirus disease 2019, JIA = juvenile idiopathic NMO = Neuromyelitis Optica, axSpA = axial spondylarthritis, PsA = psoriatic arthritis, CAPS = cryopyrin-associated periodic syndromes, TRAPS = tumour necrosis JC = ulcerative colitis, AD = Atopic dermatitis, CRSwNP = chronic rhinosinusitis with nasal polyposis, EA = eosinophilic asthma, CRSwNP = chronic rhinosinusitis application retracted, baltered CYP activity is defined as: when GMR (90% CI) of AUC<sub>0-ini</sub> is beyond equivalence limits 80–125%, c Type of DDDI evidence is based verformed), Caution (consider monitoring for drug/effect), No, or Unknown (clinical significance is unknown or not mentioned). Type of DDDI evidence available, arthritis, CRS = T cell-induced severe or life-threatening cytokine release syndrome, GLNH = Giantlymph node hyperplasia, MCD = multicentric Castleman's disease, actor receptor associated periodic syndrome, HIDS = hyperimmunoglobulin D syndrome, MKD = mevalonate kinase deficiency, FMF = familial Mediterranean fever, with nasal polyps, EGPA = eosinophilic granulomatosis with polyangiitis, HES = hyper eosinophilic syndrome, MS = multiple sclerosis.



**Figure 2** Schematic interpretation of the cytokine pathways targeting monoclonal antibodies and tyrosine kinase inhibitors (68–70).

#### Acute signalling cytokines: IL-6, TNF-α and IL-1β

IL-6, TNF- $\alpha$  and IL-1 $\beta$  are the main cytokines involved in inducing the acute phase response during inflammation (40). Of these, IL-6 is the most studied member, and a vast body of evidence exists showing that IL-6 can impact multiple CYP isoforms (4,8,9,41–43). As such, for mAbs targeting IL-6, it seems important to study the disease-mediated effects of the mAb on the pharmacokinetics of CYP substrates. In patients suffering from active rheumatoid arthritis (RA), IL-6 levels are often elevated in both the systemic circulation and the synovial fluid (44), making this a relevant population to study potential DDDIs elicited by IL-6 targeting mAbs.

#### Interaction studies

Four separate clinical trials investigated the effect of IL-6 neutralization on CYP-mediated drug metabolism of probe substrates (Table 1). In RA patients, sirukumab treatment led to a decrease in exposure (based on AUC $_{\rm inf}$ ) for midazolam (CYP3A4), omeprazole (CYP2C19) and warfarin (CYP2C9) with geometric mean ratios ranging from 65–70%, 55–63% and 81–82% respectively over a period of 1 to 6 weeks (22). In contrast, sirukumab treatment led to an increase in exposure (based on AUC $_{\rm inf}$ ) for caffeine (CYP1A2) with geometric mean ratios ranging from 120–134% over a period of 1 to 6 weeks. In the case of sarilumab and tocilizumab, single dose mAb treatment in RA patients resulted in a decrease in exposure (based on AUC $_{\rm inf}$ ) for simvastatin (CYP3A4 substrate) with a geometric mean ratio of 55% (based on AUC $_{\rm inf}$ ) after 1 week (sarilumab) (24) and geometric mean ratios of 43% to 61% (based on AUC $_{\rm inf}$ ) after 1 and 6 weeks respectively (tocilizumab) (23).

The use of anti-IL-6 mAbs is not restricted to RA. Clazakizumab is an anti-IL-6 mAb currently under investigation for potential benefit in counteracting late antibody-mediated rejection (ABMR), a main reason for renal transplant failure. A sub-study of the phase 2 trial investigated the impact of clazakizumab treatment on the PK of pantoprazole, a CYP2C19 substrate with minor involvement of CYP3A4 in kidney transplant recipients, but found no effect on pantoprazole PK throughout the study period (52 weeks) (Table 1) (25). However, it is important to note that both C-reactive protein (CRP) and IL-6 levels were not elevated in this patient population, and CYP iso-enzyme expression may therefore not have been impacted by elevated IL-6 levels at the start.

Altogether these results imply that IL-6 targeting antibodies have the potential to restore CYP metabolic capacity of CYP3A4, and potentially CYP2C19 and

CYP2C9 in RA patients. Because of this DDDI risk, the plasma levels of concomitant medication might be lower in this treated patient population. In disease populations were baseline IL-6 levels are not elevated, such as renal transplant patients, mAb treatment seem not to interfere with CYP activity.

For the other acute signalling cytokines TNF- $\alpha$  and IL-1 $\beta$ , no drug interaction studies have been performed to the best of our knowledge.

#### **DDDI** risks

The labelling information discussing the potential of a DDDI for acute signalling cytokine targeting mAbs is summarized in Table 2. Experimental evidence in PHH models strongly suggest that IL-6 modulates metabolic capacity of multiple CYP isoforms. Three independent clinical trials indicated a DDDI risk with IL-6 neutralizing antibodies. However, clinical evidence for the reversal of IL-6 mediated effects on metabolic capacity of CYP isoforms other than CYP3A4 is limited, given that only one clinical trial exploited a CYP cocktail approach. Still, the USPIs and the SPCs indicate a clear risk for DDDIs with IL-6 mAbs in the labelling, stating therapeutic monitoring of effect or concentration is warranted, up to weeks after discontinuation of the IL-6 mAb therapy. An exception is satralizumab, where the SPC suggests TDM and the USPI states that the DDDI risk is unknown.

No clinical studies have been performed for mAbs targeting TNF- $\alpha$  or IL-1 $\beta$  to evaluate their potential risk for inducing DDDIs. Risk assessments are thus solely based on experimental findings in PHH models where TNF- $\alpha$  and IL-1 $\beta$  strongly downregulate CYP expression and CYP activity. Consequently, both SPC and USPI of IL-1 $\beta$  targeting antibodies contain a general statement that an increase in cytokine levels during inflammation can alter the activity of CYP enzymes (Table 2). As such, monitoring the effect or active substance concentration is highly recommended for concurrent medicated CYP substrates with a narrow therapeutic window. The USPI label of golimumab, a mAb that neutralizes TNF- $\alpha$ , contains an even more general warning, stating that an effect of golimumab initiation on PK of CYP substrates can be expected. In contrast, the SPCs of golimumab and certolizumab do not mention a potential risk for a DDDI.

#### IL-17/IL-23 axis

The pro-inflammatory IL-17/IL-23 axis has been linked to the pathophysiology of many autoimmune diseases, most notably psoriasis (45). Several mAbs that oppose

the actions of IL-17 or IL-23 have shown to be successful in reducing inflammation and relieving symptoms in psoriasis patients. Because of these anti-inflammatory effects it is considered important to assess the potential for DDDIs of these drugs.

#### Interaction studies

Three clinical trials investigated whether IL-17 neutralization by mAb treatment would impact the PK of CYP substrates (Table 1). A cocktail approach showed that twelve-week ixekizumab treatment did not impact the PK of CYP probe substrates midazolam, omeprazole, caffeine, dextromethorphan, and warfarin in patients with psoriasis (NCT02993471). Secukinumab initiation did not impact CYP3A4 metabolic capacity (26). In contrast, a single subcutaneous dose of brodalumab in patients with moderate to severe plaque psoriasis increased the exposure of midazolam (CYP3A4) with a geometric mean ratio of 124%. (NCT01937260).

Regarding IL-23 neutralization, risankizumab, tildrakizumab and guselkumab treatment in patients did not result in altered CYP metabolic capacity, as all changes were within the bioequivalence limits (28,29). A clinical study evaluating the impact of ustekinumab in patient with Crohn's disease or ulcerative colitis is ongoing (NCT03358706). As such, despite the clinically relevant suppression of IL-17/IL-23 in psoriasis patients, this did not result in altered metabolic capacity of CYPs except for the CYP3A4 alteration by brodalumab.

#### **DDDI** risks

DDDI risks for the IL-17/IL-23 axis targeting therapeutics are summarized in Table 2. No experimental studies were conducted to assess the effect of IL-17 on CYP activity in PHHs (10). Based on data of three clinical trials, the potential for interactions between IL-17 targeting mAbs and co-administrated drugs that rely on CYP-biotransformation in psoriasis patients is very low (Table 1). Based upon these results, the SPC product labels of brodalumab, ixekuzumab and secukinumab indicate no risk for a DDDI, considering that the magnitude of change in midazolam exposure after brodalumab treatment does not require dose adjustments. The SPC of bimekizumab states that therapeutic monitoring of concurrent medication should be considered since no clinical interaction study is performed to inform on the DDDI risk. The USPIs of brodalumab, ixekuzumab and secukinumab contain a general suggestion to monitor the effect when concomitant drugs with a narrow therapeutic window are added on top of IL-17 targeting antibodies, based upon the

general assumption that CYP450 enzyme expression is modulated by inflammatory cytokines. Bimekizumab is not approved by the FDA yet.

Both experimental and clinical data indicate no effect of IL-23 on CYP metabolic capacity (Table 2). The SPC risk labelling for IL-23 targeting antibodies indicates no risk for an altered exposure of concomitant medication after initiation or discontinuation of an IL-23 targeting mAb. For ustekinumab, this conclusion was based on in vitro data since the clinical trial is ongoing. For the other mAbs, the absence of a risk was based on the results of clinical trials. The FDA documentation differs in the risk assessment included in the drug labelling. For ustekinumab, a risk is identified based on the general assumption that cytokines downregulate CYPs. For guselkumab, although the results of the cocktail trial indicate no risk for interactions, the reliability of the results is considered low because of the low number of subjects. Therefore, the USPI still indicates that monitoring the effect or concentration of concurrent mediated small molecule drugs with a narrow therapeutic window should be considered. For risankizumab and tildrakizumab, no DDDI risk is identified based on the results of the cocktail study.

#### Th2-type cytokines

The cytokines IL-4, IL-5 and IL-13 are essential in type 2 immunity and play a central role in the pathogenesis of allergic diseases, through their effects on the synthesis of IgE, eosinophils and epithelial or epidermal cells (46). For the treatment of asthma and atopic dermatitis (AD), mAbs have been developed against either IL-5 signalling (mepolizumab, reslizumab, benralizumab) or the IL-4Ra (dupilumab), that is responsible for the actions of IL-4 and IL-13 (tralokinumab).

#### Interaction studies

One clinical DDDI trial explored the potential shift in CYP-mediated metabolism upon dupilumab treatment, but none of the investigated CYPs were impacted, suggesting a low potential for DDDI with dupilumab (30). For mepolizumab, reslizumab and benralizumab, no DDDI trials were executed. For tralokinumab, a CYP interaction trial is ongoing in patients with moderate to severe atopic dermatitis (NCT03556592).

#### DDDI risks

No experimental studies have assessed the effects of IL-4, IL-5 or IL-13 on the activity of CYP enzymes, though most of the receptors for these cytokines are considered low or absent in the liver (46). Hence the results of the clinical trial investigating the potential modulating effect of dupilumab on CYP metabolic capacity are in line with this (Table 1). Accordingly, in the SPC risk documentation, dupilumab does not exhibit a DDDI risk. Despite the negative results from the cocktail study, the USPI of dupilumab contains a potential risk for a DDDI, based on the general idea of downregulation of CYP activity by cytokines.

For IL-5 neutralizing antibodies, the SPCs state no DDDI risk – where the risk assessment is mainly based on in vitro data. In contrast, the USPIs marks an unknown risk for DDDI for the IL-5(R) targeting antibodies, since no formal drug interaction studies have been performed.

Tralokinumab is not yet authorized for marketing by the FDA and therefore lacks an USPI. The tralokinumab SPC states an unknown risk since the results of the DDDI trial with tralokinumab are not yet publicly available.

#### Th1-type cytokines

IL-2 is a cytokine released from activated T lymphocytes, which effects the proliferation and differentiation of T cells, making it an important member of the Th1 type cytokine response.

#### Interaction studies

Daclizumab is a high-affinity IL-2 receptor blocker that was approved in 2016 for the treatment of relapsing forms of multiple sclerosis but was withdrawn in 2018 after several cases of severe inflammatory brain disease (47–49). The clinical trial evaluating the impact of daclizumab on CYP enzyme activity showed that exposure of substrates of CYP3A4, 1A2, 2C9, 2C19 and 2D6 remained unaltered (31).

#### **DDDI** risks

Both experimental and clinical data of the withdrawn product daclizumab show that IL-2 does not impact CYP activities (Table 2). The SPC does not provide any information on daclizumabs DDDI risk, whereas the USPI indicates no risk based on the interaction trial.

#### Tyrosine kinase inhibitors

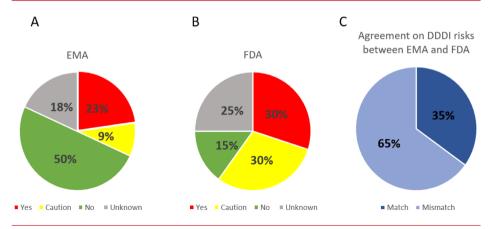
Reversion of the effects of inflammation can also occur by inhibiting the signalling pathways downstream of the receptors that are responsible for the cytokine actions. TKIs that interfere with these cytokine signalling pathways could therefore in theory also induce a DDDI interaction (Figure 2). Through our search, we identified thirteen immunomodulating TKIs that inhibit the JAK/STAT, MAPK/MEK/ERK, Nf-kB or PI3K/Akt pathway(s), whose involvement has been linked to the cytokine-mediated downregulation of CYP enzymes.

#### Interaction studies and DDDI risks

There are no clinical DDDI interaction studies performed for TKIs, and experimental evaluations of a DDDI risk is very limited (Supplementary Table S1). For 7 of the 13 TKIs, a CYP phenotyping cocktail, probe or PBPK study was conducted to determine traditional DDI risks. However, these studies were all conducted in healthy volunteers and not in patients with inflammatory disease, which substantially limits their informative power on the DDDI risk (50–56). Moreover, the SPCs and USPIs only evaluate the traditional DDIs and do not state any inflammation-related interaction risks for these products. The only label that discusses a potential DDDI is the label of tofacitinib, which states that treatment with tofacitinib does not normalize CYP enzyme activity in RA patients and will likely not result in relevant increases in the metabolism of CYP substrates in this population (57). As such, the DDDI risk is expected to be low.

#### EMA vs FDA documented DDDI risks

It is worth noticing that there is discrepancy in DDDI risk assessment for immunomodulatory antibodies between the EMA SPCs and the FDA USPIs (Figure 3). The EMA documentation described a DDDI risk for 32% of the included mAbs, and an absence of a risk in 50% of the cases. The defined risks in the SPC always followed the results of executed cocktail trials. The FDA USPI describes a DDDI risk for 28% of the drugs, and advice to take caution when initiating treatment for 29% of the mAbs – sometimes in contradiction with a negative result from a cocktail trial. No risk for a DDDI is only attributed to 14% of the drugs. Given that the FDA is more conservative in its risk assessment, there is agreement on the DDDI risk in 38% of the cases.



**Figure 3** Summary of the DDDI risks for immunomodulatory mAbs assessed by extracting information from the SPC (A) or USPI (B) and the agreement between them (C).

# Discussion

This systematic review was set out to explore the available evidence for DDDIs with immunomodulating therapeutic antibodies marketed after 2007 and the associated DDDI risk descriptions indicated in the European and American product labels. Additionally, we investigated whether DDDI studies were executed for other type of immunomodulating therapeutics, such as TKIs that inhibit the signalling pathways downstream of inflammatory mediators. This is the first systematic review that links the outcomes of the executed DDDI trials to the risk evaluations stated in the SPCs and USPIs. In short, dedicated DDDI studies were performed for twelve mAbs, where modulating effects on CYP probe substrates were reported for sirukumab (IL-6), tocilizumab (IL-6), sarilumab (IL-6RA) and brodalumab (IL-17RA). The indicated DDDI risk assessment in labels for the mAbs was not always in line with the available experimental and clinical data and showed discrepancies in labelling statements between the SPCs and USPI. Drug labelling indicated the greatest DDDI risk for mAbs that neutralize the effects of IL-6, TNF-α and IL-1β in diseases with systemic inflammation. For TKIs, no DDDI interaction studies were performed, and no DDDI risks were reported in the labelling.

#### Factors that determine DDDI risk

The summarized DDDI studies suggest that the risk for a DDDI is both dictated by the target and the indicated disease population. With respect to drug target, antibodies that impair the actions of IL-6 have consistently shown to alter CYP-dependent metabolism of probe substrates. Both tocilizumab, sirukumab and sarilumab altered CYP metabolic capacity in RA patients, showing that the impaired drug metabolizing capacity during inflammation is (partly) restored after administration of IL-6 targeting mAbs. Importantly, the changes in CYP3A4 metabolic capacity induced by different mAbs were of similar magnitude (~2-fold), indicating a class-effect. The sirukumab trial provided evidence that antagonism of IL-6 in RA patients reversed the IL-6 induced downregulation of not only CYP3A4 but also of CYP2C9 and CYP2C19. In contrast, clinical trials executed with mAbs targeting IL-17, IL-23, IL-4R or IL-2 showed no clinically relevant changes in CYP-mediated metabolism. As such, mAbs that target the acute signalling cytokines appear to have the greatest DDDI risk.

The diseased population is another critical indicator, as the type and degree of systemic inflammation observed in the studied population may determine the potential for DDDIs. No clinically meaningful alterations in CYP metabolizing capacity were observed following the use of immunomodulating antibodies in psoriasis and AD patients. This may be attributed to the type of inflammation in AD and psoriasis patients, as this is characterized by either elevation of type 2 inflammatory cytokines (e.g. IL-4, IL-5 and IL-13) or the IL-17/IL-23 axis cytokines, which are shown not to impact metabolic liver function. Secondly, markers of systemic inflammation, such as C-reactive protein or IL-6, are only elevated in a small proportion of AD or psoriasis patients, and profoundly lower than in patients with RA (58–61). As such, in diseases with only moderate systemic inflammation, the increases in cytokine levels will be insufficient to change CYP expression, simultaneously indicating that the likelihood for a DDDIs within these populations is low.

The importance of conducting a DDDI study in the relevant patient group is emphasized by the discrepancy between the results of mAb treatment in kidney transplant recipients versus RA patients (22–25). In disease populations such as renal transplant recipients, where baseline IL-6 levels are not elevated, the CYP metabolic capacity was unchanged upon IL-6 targeting mAb treatment whereas

significant impact on CYP metabolic capacity was noted for sirukumab, tocilizumab and sarilumab in RA patients. In line with this, the FDA recommends studying the potential DDDI in the population group with the highest inflammatory burden, in order to extrapolate and generalize results to other patient groups (16).

#### **DDDI** risk for TKIs

Immunomodulation is not restricted to therapeutic proteins targeting cytokine (receptors) but may also apply to TKIs that inhibit the signalling pathways of inflammatory mediators. For example, the JAK inhibitors to facitinib and ruxolitinib reduce the plasma levels of IL-6 levels and other pro-inflammatory cytokines, and counteracted the suppressive effects of IL-6 on CYP enzymes in PHHs (47,48,62). Importantly, ruxolinitib was able to fully counteract the downregulatory effects of IL-6 on CYP enzymes, even at supraphysiological concentrations of IL-6 stimulation (47). Considering the increasing use of JAK inhibitors for the treatment of autoimmune disease and other inflammatory diseases, there is a need to determine the risk for DDDI for immunomodulating TKIs, e.g., in COVID-19 patients (49).

The SPCs and USPIs of TKIs did, with exception of tofacitinib, not discuss a potential DDDI risk. TKIs are small molecules, dependent on CYP-mediated biotransformation, and therefore also capable of directly inducing or inhibiting CYP enzymes. In contrast to therapeutic proteins, it is therefore difficult to distinguish traditional DDIs from DDDIs for TKIs. This forms a major hurdle for defining the DDDI risk. Traditional DDIs are evaluated in healthy volunteers, whereas the occurrence of DDDIs may, as earlier discussed, only show in diseased patients. Even though there may be financial constraints, it would be worthwhile to compare the effect of TKIs on a CYP phenotyping cocktail between healthy volunteers and patients with systemic inflammation to reveal the true DDDI potential of immunomodulating TKIs.

#### DDDI risks in drug labels

Since 2007, the SPC and USPI should include labelling language evaluating the risk for a DDDI with therapeutic proteins that are either cytokines themselves or target cytokines (15,16). We classified the reported DDDI risks in drug labels and identified the available data for every mAb and TKI to determine the potential DDDI risk.

Both EMA and FDA documentation identified a DDDI risks for most of the acute signalling cytokine targeting mAbs. In line with experimental data, the various IL-6 mAb trials identified a clear DDDI risk, although clinical evidence for a modulating effect on multiple CYP isoforms is still limited. Interestingly, even though novel mAbs against TNF-α and IL-1β were brought to market after instalment of the renewed DDDI guidelines, no dedicated clinical study has yet investigated the effects of these mAbs on a CYP substrate or CYP cocktail. Importantly, in experimental models, both TNF-α and IL-1β can alter the expression of multiple CYP isoforms (10). Based on this, the SPC and USPIs of canakinumab and rilonacept (both IL-18) contain a general warning message to monitor the effect or drug concentration upon initiation or discontinuation of the mAb in patients treated with medication metabolized by CYP enzymes with a narrow therapeutic window. For mAbs that target beyond the acute signalling cytokines, drug labelling does not report a clear DDDI risk. However, sometimes therapeutic monitoring of drug or effect is advised based on the general assumption that cytokines downregulate drug metabolizing enzymes or the lack of available evidence to base the advice on. Of note, the implementation of the advised therapeutic monitoring of drugs that are at risk for causing a DDDI still needs further investigation, since drug or effect monitoring in clinical practice is currently only available for a select group of drugs.

It is also interesting to note that there is often discrepancy between the stated risks in the EMA and FDA documentation (mismatch in 62% of the labels) and that the authorities do not always base their risk assessment on the same available non-clinical and clinical evidence. The EMA guidelines on DDIs with therapeutic proteins are general in its recommendations and highlight the need for a dedicated in vitro or in vivo interaction studies to assess the potential for a DDDI on a case-by-case basis (15). Subsequently, the EMA documentation always uses the outcomes of clinical DDDI trials as a leading point for their risk analysis. In contrast, the FDA documentation on DDDI risks is more conservative. The USPI often suggests monitoring of therapeutic drug levels or effect, even when the cocktail trial did not identify a risk for a DDDI, thereby often referring to experimental data that showed the impact of cytokines on CYP activity to justify their precaution. This contrasts the statement in the FDA draft guideline for therapeutic proteins where they describe that justification of not including DDDI risk labelling can be based on negative results of a clinical DDDI study (16).

#### Recommendations for assessing future DDDI risks

In vitro studies have been instrumental in dissecting the impact of individual cytokines on CYP enzymes involved in drug metabolism. The utility of in vitro models for predicting clinical DDDI has however been debated during the FDA/IQ consortium workshop in 2012 (63). One particular concern was the limitations of in vitro models for predicting DDDI risk for cytokine targets for which the effect on drug metabolizing capacity may not take place in hepatocytes, but instead develop via immunomodulating effects on other cell types in the liver. Thus, although in vitro PHH models adequately predicted tocilizumab DDDI potential to reverse the IL-6 induced impairment of metabolic CYP capacity (64), the use of such models would not be informative for all cytokine targets. However, liver co-culture platforms have shown to increase our predictive power of in vitro systems. For example, the lack of DDDI risk for IL-23 in experimental co-culture models was confirmed by multiple IL-23 clinical interaction trials (32). One could therefore argue that in vitro system(s), accompanied with physiology-based PK models, could have utility for predicting when clinical DDDI studies with immunomodulatory mAbs are truly needed.

In accordance with the FDAs guidelines which state that justification for a low DDDI risk can be based on results from mAbs with similar targets, considerations on conducted DDDI trials in the same patient population are valuable for assessing the need for a novel DDDI trial (16,64). In the case of IL-23 mAbs, three individual cocktail studies have been performed in psoriasis patients, which all concluded that IL-23 neutralization did not affect CYP metabolic capacity. Considering that DDDI clinical trial patients are scarce (65), novel trials with IL-23 targeting mAbs or biosimilars seem unnecessary.

The potential risks of mAbs for DDDI in clinical trials has been assessed using CYP cocktails or CYP3A4 substrates. The latter approach may have important limitations, as both experimental and clinical studies have indicated that the effects of inflammation on drug metabolism may differ among CYP isoforms (1). CYP3A4 and CYP2C19 mediated metabolism generally declines in the presence of inflammation, whereas CYP2D6 and CYP2C9 mediated metabolism respectively do not change, or even increase during inflammation (10–12). These studies illustrate the distinct sensitivities and opposite effects of inflammation on the different CYP isoforms. Thus, although studies using CYP3A4 probes may

adequately inform on the likelihood of a DDDI, the outcomes of such studies cannot be directly extrapolated to other CYP isoforms and therefore limitedly inform on the DDDI risk for concomitant medication. For future DDDI trials, the cocktail approach would therefore be preferred.

#### Real-world impact in the clinic

Beyond the defined risks for DDDIs documented by the EMA and FDA, it is also important to understand the consequences of DDDIs with immunomodulating therapeutics for clinical practice. The impact of a DDDI is dictated by 1) the magnitude of the inflammation-driven changes in drug exposure and 2) the therapeutic window of the victim drug. Maximum exposure (AUC<sub>0 inf</sub>) alterations due to immunomodulatory antibodies are reported to be 2-fold. Compared to conventional DDIs that rely on CYP induction or inhibition, this magnitude of change is limited. Still, for concurrent drugs with a narrow therapeutic window, the initiation of mAb therapy can still lead to under- or overexposure of the victim drug and potential toxicity or lack of efficacy. To date, only incidental case reports have linked the start of mAb treatment against IL-6 or TNF-α to increased clearance of anti-coagulants and immunosuppressants, and hence reported on the real-world impact of DDDI (66,67). In addition, recent studies have shown that the start of direct-acting antivirals against hepatitis C virus infections or antimalarial agents were associated with reversal of inhibited CYP2C19 activity (68,69). This indicates that these type of DDDIs are not restricted to immunomodulating mAbs, but also involve small molecules. Still, data on the clinical consequences of DDDIs remains scarce and more real-world evidence is needed to better define the true impact of DDDIs for patients in the clinic.

### Study limitations

It should be acknowledged that our systematic literature search has some limitations. First of all, the completeness of the analysis cannot be assured since we were limited to published (clinical trial) studies and some trials are still ongoing. Secondly, the set period of 2007 until now limits our analysis on the DDDI risk information in drug labels to a particular set of immunomodulatory mAbs. Thirdly, we choose to include immunomodulatory drugs that target either a cytokine (receptor) or specific downstream signalling pathway. As such, broader immunosuppressive

drugs were not included in our analysis but might still impact CYP metabolic capacity and thus be at risk for a DDDI.

#### Conclusion

In conclusion, the risk for DDDIs appears to be specific to the targeted cytokine and the intended disease population. SPC and USPI drug information designates the greatest DDDI risk to mAbs that neutralize the effects of IL-6, TNF- $\alpha$  and IL-1 $\beta$  in diseases with systemic inflammation, although for the latter two clinical evidence is lacking. Since in vitro data and already executed DDDI trials with the same target shows predictive value for the outcome of a DDDI risk, these factors should be considered in evaluating the need for a novel DDDI trial for drug labelling. Especially since eligible patient populations for clinical studies are scarce (70). If clinical assessment of a DDDI risk is warranted, this should preferably be conducted through a cocktail approach, since evidence is growing that the impact of inflammation is different for the multiple CYP isoforms. Lastly, efforts are needed to translate the described DDDI risks in drug labelling into guidelines for clinical practice which can ultimately benefit the patient.

# Acknowledgements

We are particularly grateful for the assistance on search terms given by Jan Schoones of the Walaeus library (LUMC). For creation of the graphical abstract and Figure 2, BioRender.com was used.

#### References

- Stanke-Labesque F, Gautier-Veyret E, Chhun S, Guilhaumou R, French Society of Pharmacology and Therapeutics. Inflammation is a major regulator of drug metabolizing enzymes and transporters: Consequences for the personalization of drug treatment. Pharmacol Ther. 2020;215:107627.
- Lenoir C, Rollason V, Desmeules JA, Samer CF. Influence of inflammation on cytochromes P450 activity in adults: A systematic review of the literature. Front Pharmacol. 2021;12(3021):733935.
- Morgan ET. Impact of infectious and inflammatory disease on cytochrome P450-mediated drug metabolism and pharmacokinetics. Clin Pharmacol Ther. 2009;85(4):434–8.
- Lanchote VL, Almeida R, Barral A, et al. Impact of visceral leishmaniasis and curative chemotherapy on cytochrome P450 activity in Brazilian patients. Br J Clin Pharmacol. 2015;80(5):1160–8.
- Shah RR, Smith RL. Inflammation-induced phenoconversion of polymorphic drug metabolizing enzymes: hypothesis with implications for personalized medicine. Drug Metab Dispos. 2015;43(3):400–10.
- Klomp SD, Manson ML, Guchelaar H-J, Swen JJ. Phenoconversion of cytochrome P450 metabolism: A systematic review. J Clin Med. 2020;9(9):2890.
- Darakjian L, Deodhar M, Turgeon J, Michaud V. Chronic inflammatory status observed in patients with type 2 diabetes induces modulation of cytochrome P450 expression and activity. Int J Mol Sci. 2021;22(9):4967.
- Aitken AE, Richardson TA, Morgan ET. Regulation of drug-metabolizing enzymes and transporters in inflammation. Annu Rev Pharmacol Toxicol. 2006;46:123–49.
- Dickmann LJ, Patel SK, Rock DA, Wienkers LC, Slatter JG. Effects of interleukin-6 (IL-6) and an anti-IL-6 monoclonal antibody on drug-metabolizing enzymes in human hepatocyte culture. Drug Metab Dispos. 2011;39(8):1415–22.
- de Jong LM, Jiskoot W, Swen JJ, Manson ML. Distinct effects of inflammation on cytochrome P450 regulation and drug metabolism: Lessons from experimental models and a potential role for pharmacogenetics. Genes. 2020;11(12):1509.

- 11. Lenoir C, Daali Y, Rollason V, et al. Impact of acute inflammation on cytochromes P450 activity assessed by the Geneva cocktail. Clin Pharmacol Ther. 2021;109(6):1668–76.
- 12. Lenoir C, Terrier J, Gloor Y, et al. Impact of SARS-CoV-2 infection (COVID-19) on cytochromes P450 activity assessed by the Geneva cocktail. Clin Pharmacol Ther. 2021;110(5):1358–67.
- 13. Machavaram KK, Endo-Tsukude C, Terao K, et al. Simulating the impact of elevated levels of interleukin-6 on the pharmacokinetics of various CYP450 substrates in patients with neuromyelitis optica or neuromyelitis optica spectrum disorders in different ethnic populations. AAPS J. 2019;21(3):42.
- Ferri N, Bellosta S, Baldessin L, Boccia D, Racagni G, Corsini A. Pharmacokinetics interactions of monoclonal antibodies. Pharmacol Res. 2016;111:592–9.
- 15. Guideline on the clinical investigation of the pharmacokinetics of therapeutic proteins: European Medicines Agency, 2007.
- 16. Drug-Drug Interaction Assessment for Therapeutic Proteins Guidance for Industry: Food and Drug Administration, 2020. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/drug-drug-interaction-assessment-therapeutic-proteins-guidance-industry
- Dunvald AD, Järvinen E, Mortensen C, Stage TB. Clinical and molecular perspectives on inflammation-mediated regulation of drug metabolism and transport. Clin Pharmacol Ther. 2021;112(2):277–90.
- Wang J, Wang YM, Ahn HY. Biological products for the treatment of psoriasis: therapeutic targets, pharmacodynamics and disease-drug-drug interaction implications. AAPS J. 2014;16(5):938–47.
- Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.
- 20. EMA. EPAR of human medicine secondary EPAR of human medicine [Table]. https://www.ema.europa.eu/en/medicines/download-medicine-data#european-public-assessment-reports-(epar)-section

- Alexander SP, Kelly E, Marrion NV, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2017/18: Overview. Br J Pharmacol. 2017;174(Suppl 1):S1–S16.
- 22. Zhuang Y, de Vries DE, Xu Z, et al. Evaluation of disease-mediated therapeutic protein-drug interactions between an anti-interleukin-6 monoclonal antibody (sirukumab) and cytochrome P450 activities in a phase 1 study in patients with rheumatoid arthritis using a cocktail approach. J Clin Pharmacol. 2015;55(12):1386–94.
- 23. Schmitt C, Kuhn B, Zhang X, Kivitz AJ, Grange S. Disease-drug-drug interaction involving tocilizumab and simvastatin in patients with rheumatoid arthritis. Clin Pharmacol Ther. 2011;89(5):735–40.
- 24. Lee EB, Daskalakis N, Xu C, et al. Diseasedrug interaction of sarilumab and simvastatin in patients with rheumatoid arthritis. Clin Pharmacokinet. 2017;56(6):607–15.
- Mühlbacher J, Schörgenhofer C, Doberer K, et al. Anti-interleukin-6 antibody clazakizumab in late antibody-mediated kidney transplant rejection: effect on cytochrome P450 drug metabolism. Transpl Int. 2021;34(8):1542–52.
- 26. Bruin G, Hasselberg A, Koroleva I, et al. Secukinumab treatment does not alter the pharmacokinetics of the cytochrome P450 3A4 substrate midazolam in patients with moderate to severe psoriasis. Clin Pharmacol Ther. 2019;106(6):1380–8.
- 27. Khatri A, Cheng L, Camez A, Ignatenko S, Pang Y, Othman AA. Lack of effect of 12-week treatment with risankizumab on the pharmacokinetics of cytochrome P450 probe substrates in patients with moderate to severe chronic plaque psoriasis. Clin Pharmacokinet. 2019;58(6):805–14.
- Khalilieh S, Hussain A, Montgomery D, et al. Effect of tildrakizumab (MK-3222), a high affinity, selective anti-IL23p19 monoclonal antibody, on cytochrome P450 metabolism in subjects with moderate to severe psoriasis. Br I Clin Pharmacol. 2018;84(10):2292–302.
- 29. Zhu Y, Xu Y, Zhuang Y, et al. Evaluating potential disease-mediated protein-drug interactions in patients with moderate-to-severe plaque psoriasis receiving subcutaneous guselkumab. Clin Transl Sci. 2020;13(6):1217–26.

- Davis JD, Bansal A, Hassman D, et al. Evaluation of potential disease-mediated drug-drug interaction in patients with moderate-to-severe atopic dermatitis receiving dupilumab. Clin Pharmacol Ther. 2018;104(6):1146–54.
- Tran JQ, Othman AA, Wolstencroft P, Elkins J. Therapeutic protein-drug interaction assessment for daclizumab high-yield process in patients with multiple sclerosis using a cocktail approach. Br J Clin Pharmacol. 2016;82(1):160-7.
- 32. Nguyen TV, Ukairo O, Khetani SR, et al. Establishment of a hepatocyte-kupffer cell coculture model for assessment of proinflammatory cytokine effects on metabolizing enzymes and drug transporters. Drug Metab Dispos. 2015;43(5):774–85.
- Dallas S, Sensenhauser C, Batheja A, et al. De-risking bio-therapeutics for possible drug interactions using cryopreserved human hepatocytes. Curr Drug Metab. 2012;13(7):923–9.
- 34. Dickmann LJ, Patel SK, Wienkers LC, Slatter JG. Effects of interleukin 1β (IL-1β) and IL-1β/interleukin 6 (IL-6) combinations on drug metabolizing enzymes in human hepatocyte culture. Curr Drug Metab. 2012;13(7):930–7.
- 35. Sunman JA, Hawke RL, LeCluyse EL, Kashuba ADM. Kupffer cell-mediated IL-2 suppression of CYP3A activity in human hepatocytes. Drug Metab Dispos. 2004;32(3):359–63.
- Long TJ, Cosgrove PA, Dunn RT, et al. Modeling therapeutic antibody-small molecule drug-drug interactions using a three-dimensional perfusable human liver coculture platform. Drug Metab Dispos. 2016;44(12):1940–8.
- Zarrin AA, Bao K, Lupardus P, Vucic D. Kinase inhibition in autoimmunity and inflammation. Nat Rev Drug Discov. 2021;20(1):39–63.
- 38. David M, Ford D, Bertoglio J, Maizel AL, Pierre J. Induction of the IL-13 receptor alpha2-chain by IL-4 and IL-13 in human keratinocytes: involvement of STAT6, ERK and p38 MAPK pathways. Oncogene. 2001;20(46):6660–8.

- Reusswig F, Fazel Modares N, Brechtenkamp M, et al. Efficiently restored thrombopoietin production by Ashwell-Morell receptor and IL-6R induced janus kinase 2/signal transducer and activator of transcription signaling early after partial hepatectomy. Hepatology. 2021;74(1):411–27.
- Zhang JM, An J. Cytokines, inflammation, and pain. Int Anesthesiol Clin. 2007;45(2):27–37.
- 41. Tvedt TH, Lie SA, Reikvam H, et al. Pretransplant levels of CRP and interleukin-6 family cytokines: Effects on outcome after allogeneic stem cell transplantation. Int J Mol Sci. 2016;17(11):1823.
- 42. Klein M, Thomas M, Hofmann U, Seehofer D, Damm G, Zanger UM. A systematic comparison of the impact of inflammatory signaling on absorption, distribution, metabolism, and excretion gene expression and activity in primary human hepatocytes and HepaRG cells. Drug Metab Dispos. 2015;43(2):273–83.
- 43. Tanner N, Kubik L, Luckert C, et al. Regulation of drug metabolism by the interplay of inflammatory signaling, steatosis, and xeno-sensing receptors in HepaRG cells. Drug Metab Dispos. 2018;46(4):326–35.
- 44. Houssiau FA, Devogelaer JP, Van Damme J, de Deuxchaisnes CN, Van Snick J. Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides. Arthritis Rheum. 1988;31(6):784–8.
- 45. Hawkes JE, Yan BY, Chan TC, Krueger JG. Discovery of the IL-23/IL-17 signaling pathway and the treatment of psoriasis. J Immunol. 2018;201(6):1605–13.
- 46. Akdis CA, Arkwright PD, Brüggen MC, et al. Type 2 immunity in the skin and lungs. Allergy. 2020;75(7):1582–605.
- 47. Febvre-James M, Bruyère A, Le Vée M, Fardel O. The JAK1/2 inhibitor ruxolitinib reverses interleukin-6-mediated suppression of drug-detoxifying proteins in cultured human hepatocytes. Drug Metab Dispos. 2018;46(2):131–40.
- Boyle DL, Soma K, Hodge J, et al. The JAK inhibitor tofacitinib suppresses synovial JAK1-STAT signalling in rheumatoid arthritis. Ann Rheum Dis. 2015;74(6):1311–6.
- Kumar D, Trivedi N. Disease-drug and drug-drug interaction in COVID-19: Risk and assessment. Biomed Pharmacother. 2021;139:111642.

- Mohamed MF, Feng T, Enejosa JV, Fisniku
  O, Othman AA. Effects of upadacitinib
  coadministration on the pharmacokinetics of
  sensitive cytochrome P450 probe substrates:
  A study with the modified Cooperstown 5+1
  cocktail. J Clin Pharmacol. 2020;60(1):86–95.
- 51. Ogasawara K, LoRusso PM, Olszanski AJ, et al. Assessment of effects of repeated oral doses of fedratinib on inhibition of cytochrome P450 activities in patients with solid tumors using a cocktail approach. Cancer Chemother Pharmacol. 2020;86(1):87–95.
- 52. Anderson K, Nelson CH, Gong Q, Alani M, Tarnowski T, Othman AA. Assessment of the effect of filgotinib on the pharmacokinetics of atorvastatin, pravastatin, and rosuvastatin in healthy adult participants. Clin Pharmacol Drug Dev. 2021;11(2):235–45.
- 53. Gupta P, Chow V, Wang R, et al. Evaluation of the effect of fluconazole and ketoconazole on the pharmacokinetics of tofacitinib in healthy adult subjects. Clin Pharmacol Drug Dev. 2014;3(1):72–7.
- Bossaer JB, Chakraborty K. Drug interaction between idelalisib and diazepam resulting in altered mental status and respiratory failure. J Oncol Pharm Pract. 2017;23(6):470–2.
- 55. Umehara K, Huth F, Jin Y, et al. Drug-drug interaction (DDI) assessments of ruxolitinib, a dual substrate of CYP3A4 and CYP2C9, using a verified physiologically based pharmacokinetic (PBPK) model to support regulatory submissions. Drug Metab Pers Ther. 2019;34(2):20180042.
- 56. Budha NR, Ji T, Musib L, et al. Evaluation of cytochrome P450 3A4-mediated drugdrug interaction potential for cobimetinib using physiologically based pharmacokinetic modeling and simulation. Clin Pharmacokinet. 2016;55(11):1435–45.
- 57. Tse S, Dowty ME, Menon S, Gupta P, Krishnaswami S. Application of physiologically based pharmacokinetic modeling to predict drug exposure and support dosing recommendations for potential drug-drug interactions or in special populations: An example using tofacitinib. J Clin Pharmacol. 2020;60(12):1617–28.
- Kleiner G, Marcuzzi A, Zanin V, Monasta L, Zauli G. Cytokine levels in the serum of healthy subjects. Mediators Inflamm. 2013:434010.

- 59. Kaminishi K, Soma Y, Kawa Y, Mizoguchi M. Flow cytometric analysis of IL-4, IL-13 and IFN-gamma expression in peripheral blood mononuclear cells and detection of circulating IL-13 in patients with atopic dermatitis provide evidence for the involvement of type 2 cytokines in the disease. J Dermatol Sci. 2002;29(1):19–25.
- 60. Hanifin JM, Thurston M, Omoto M, Cherill R, Tofte SJ, Graeber M. The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. EASI Evaluator Group Exp Dermatol. 2001;10(1):11–8.
- 61. Dowlatshahi EA, van der Voort EA, Arends LR, Nijsten T. Markers of systemic inflammation in psoriasis: a systematic review and meta-analysis. Br J Dermatol. 2013;169(2):266–82.
- Simon F, Garcia J, Guyot L, et al. Impact of interleukin-6 on drug-metabolizing enzymes and transporters in intestinal cells. AAPS J. 2019;22(1):16.
- 63. Kenny JR, Liu MM, Chow AT, et al. Therapeutic protein drug-drug interactions: navigating the knowledge gaps-highlights from the 2012 AAPS NBC Roundtable and IQ Consortium/FDA workshop. AAPS J. 2013;15(4):933–40.
- 64. Zhang X, Schmitt C, Grange S, et al. Diseasedrug interaction studies of tocilizumab with cytochrome P450 substrates in vitro and in vivo. Clin Pharmacol Ther. 2009:735–40.
- 65. Gelinas L, Lynch HF, Bierer BE, Cohen IG. When clinical trials compete: prioritising study recruitment. J Med Ethics. 2017;43(12):803–9.
- 66. Wen H, Chen D, Lu J, et al. Probable drug interaction between etanercept and cyclosporine resulting in clinically unexpected low trough concentrations: First case report. Front Pharmacol. 2020;11:939.
- 67. Clarivet B, Robin P, Pers YM, et al.
  Tocilizumab and mesenteric arterial
  thrombosis: drug-drug interaction with
  anticoagulants metabolized by CYP 450 and/
  or by P-glycoprotein. Eur J Clin Pharmacol.
  2016;72(11):1413–4.
- 68. Pippa LF, Vieira CP, Caris JA, et al. Clinical treatment for hepatitis C reverses CYP2C19 inhibition. Br J Clin Pharmacol. 2021;87(10):4013–9.

- 69. Almeida AC, Elias ABR, Marques MP, et al. Impact of Plasmodium vivax malaria and antimalarial treatment on cytochrome P450 activity in Brazilian patients. Br J Clin Pharmacol. 2021;87(4):1859–68.
- Ueda R, Nishizaki Y, Nojiri S, et al.
   Factors associated with the acceleration of patient enrollment in clinical studies:
   A cross-sectional study. Front Pharmacol. 2021;12:753067.

# Supplemental information

Supplementary Table 1 Summary of experimental and clinical evidence for DDDI risks with TKIs, according to labelling information documented by the EMA and FDA

							Type(s) o	Type(s) of evidence available for DDDI risk assessment <sup>b</sup>	ailable for Di	IDDI
Drug In-	In	ndication	Non-clinical evidence from PHHs (51, 71, 72)	Clinical evidence <sup>a</sup>	DDDI risk in SPC	DDDI risk in USPI	DDDI risk DDDI risk Experimental in SPC in USPI data	PBPK modelling	Clinical data with one substrate	Clinical data with multiple probe substrates
Upadacitinib RA / U	RA / U	RA / PsA / AS / UC / JIA	Not determined	Not conducted	Not Not mentioned mentioned	Not mentioned				
Fedratinib Mye	Mye	Myelofibrosis	Not determined	Not conducted	Not mentioned	Not mentioned				
Ruxolitinib My	My	Myelofibrosis / PV	Yes - CYP1A2	Not conducted	Not mentioned	Not mentioned				
Baricitinib RA / PsA /	RA / PsA /	RA / AD / AS / PsA / COVID-19	- CYP3A4 - CYP3A4	Not conducted	Not Not mentioned mentioned	Not mentioned				
Tofacitinib RA/UC	RA/ UC	RA / PsA / AS/ UC / JIA / AD	Yes - CYP1A2 - CYP2B6 - CYP3A4	Not conducted	No	No				
Filgotinib	R	RA / UC	Not determined	No Not conducted	Not Not mentioned mentioned	Not mentioned				

Supplementary Table 1 continues on next page.

Supplementary Table 1 Continued

							Type(s) of	Type(s) of evidence available for DDDI risk assessment <sup>b</sup>	able for DDI nt <sup>b</sup>	OI risk
Target	Drug	Indication	Non-clinical evidence from PHHs (51, 71, 72)	Clinical evidence <sup>a</sup>	DDDI risk in SPC	DDDI risk DDDI risk in SPC in USPI	Experimental data	PBPK modelling	Clinical data with one substrate	Clinical data with multiple probe substrates
MEK1/ MEK2	Trametinib	Melanoma / NSLC	Not determined	Not conducted	Not mentioned	Not mentioned				
	Cobimetinib	Melanoma		Not conducted	Not mentioned	Not mentioned				
	Binimetinib	Melanoma		Not conducted	Not mentioned	Not mentioned				
	Selumetinib	PN		Not conducted	Not mentioned	Not mentioned				
PI3K	Idelalisib	Leukaemia / FL	Not determined	Not conducted	Not mentioned	Not mentioned				
	Alpelisib	Breast Neoplasms		Not conducted	Not mentioned	Not mentioned				
	Duvelisib	Leukaemia /FL		Not conducted	Not Not mentioned mentioned	Not mentioned				

altered CYP activity is defined as: when GMR (90% CI) of AUC<sub>o-inf</sub> is beyond equivalence limits 80–125%, b Type of DDDI evidence is based on available data in iterature on potential modulating effect of cytokine/mAbs on CYP metabolic capacity. DDDI risk categories are classified as: Yes (TM should be performed), Caution consider monitoring for drug/effect), No, or Unknown (clinical significance is unknown or not mentioned). Type of DDDI evidence available, independent of risks stated in regulation labelling: 0 = no data available. RA = rheumatoid arthritis, PsA = psoriatic arthritis, UC = ulcerative colitis, JIA = Juvenile idiopathic arthritis, AS = ankylosing spondylitis, PV = polycythaemia vera, AD = atopic dermatitis, COVID-19 = coronavirus disease 2019, NSLC = non-small lung cancer, PN = plexiform neurofibromas, FL = follicular lymphoma.

170



# Section III

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



# Chapter 6

Changes in plasma clearance of CYP450 probe drugs may not be specific for altered in vivo enzyme activity under (patho)physiological conditions – how to interpret findings of probe cocktail studies

Laura M. de Jong, A.M. (Marinda) van de Kreeke, Mariam Ahmadi, Jesse J. Swen, Catherijne A.J. Knibbe, J.G. Coen van Hasselt, Martijn L. Manson, Elke H.J. Krekels



# **Abstract**

**BACKGROUND AND OBJECTIVE:** CYP450 (CYP) phenotyping involves quantifying an individual's plasma clearance of CYP-specific probe drugs, as a proxy for in vivo CYP enzyme activity. It is increasingly applied to study alterations in CYP enzyme activity under various (patho)physiological conditions, like inflammation, obesity, or pregnancy. The phenotyping approach assumes that changes in plasma clearance of probe drugs are driven by changes in CYP enzyme activity. However, plasma clearance is also influenced by protein binding, blood-to-plasma ratio, and hepatic blood flow, all of which may change under (patho)physiological conditions.

**METHODS:** Using a physiologically-based pharmacokinetic (PBPK) workflow, we aimed to evaluate whether the plasma clearance of commonly used CYP probe drugs is indeed directly proportional to alterations in CYP enzyme activity (sensitivity), and to what extent alterations in protein binding, blood-to-plasma ratio, and hepatic blood flow observed under (patho)physiological conditions impact plasma clearance (specificity).

**RESULTS:** Plasma clearance of CYP probe drugs is sensitive to alterations in CYP enzyme activity, since alterations in intrinsic clearance between -75 and +150% resulted in near-proportional changes in plasma clearance, except for midazolam in case of > 50% CYP3A4 induction. However, plasma clearance also changed near-proportionally with alterations in the unbound drug fraction, diminishing probe specificity. This was particularly relevant for high protein-bound probe drugs, as alterations in plasma protein binding resulted in larger relative changes in the unbound drug fraction. Alterations in the blood-to-plasma ratio and hepatic blood flow of  $\pm 50\%$  resulted in plasma clearance changes of less than  $\pm 16\%$ , meaning they limitedly impacted plasma clearance of CYP probe drugs, except for midazolam. In order to correct for the impact of non-metabolic determinants on probe drug plasma clearance, an R script was developed to calculate how much the CYP enzyme activity is actually altered under (patho)physiological conditions, when alterations in the unbound drug fraction, blood-to-plasma ratio and/or hepatic blood flow impact probe drug plasma clearance as well.

**CONCLUSIONS:** As plasma protein binding can change under (patho)physiological conditions, alterations in unbound drug fraction should be accounted for when using CYP probe drug plasma clearance as a proxy for CYP enzyme activity in patient populations. The tool developed in this study can support researchers in determining alterations in CYP enzyme activity in patients with (patho)physiological conditions.

## Introduction

CYP450 (CYP) phenotyping is an important tool to characterize an individual's CYP enzyme activity (1). It involves the quantification of an individual's plasma clearance upon administration of a CYP-specific probe drug, as a proxy for individual in vivo CYP enzyme activity. Various probe drugs for evaluating the activity of different CYP isoforms have been utilized, administered either individually or combined in a 'phenotyping cocktail' (2,3). Among the human CYP enzymes, CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 metabolize more than 85% of drugs administered to patients and the assessment of their activity is therefore an integral component of phenotyping assays. In this approach, CYP enzyme activity is derived from the estimated plasma clearance of a probe drug that is primarily metabolized by the respective CYP enzyme, such as midazolam for CYP3A4 or dextromethorphan for CYP2D6. Alternatively, the metabolic ratio, representing the ratio between the CYP-specific metabolite and the unaltered parent compound, is determined, as it is a less resource-intensive surrogate marker for plasma clearance. Findings on changes in plasma clearance or metabolic ratios are subsequently used to quantify how the factors that are studied, i.e. drug-drug interactions or genotype, impact drug metabolism. This methodology is now also increasingly used to explore the impact of (patho) physiological conditions such as inflammation, obesity, or pregnancy on in vivo CYP enzyme activity (4-6).

The implicit assumption made when utilizing the CYP phenotyping approach is that differences in plasma clearance of the CYP probe drugs are sensitive and specific to changes in the enzyme activity of the CYPs they represent. This enzyme activity is generally quantified as intrinsic clearance ( $\mathrm{CL}_{\mathrm{int}}$ ). This means that changes in plasma clearance of the CYP probes are proportional to changes in  $\mathrm{CL}_{\mathrm{int}}$  of the CYP of interest, and that plasma clearance is insensitive to alterations in other physiological parameters. In traditional pharmacokinetic interaction studies investigating drug–drug and/or drug–gene interactions in healthy volunteers, this assumption may be reasonable; however, when studying the impact of (patho) physiological conditions on enzyme activity, this assumption is challenged by the fact that besides the CYP enzyme activity ( $\mathrm{CL}_{\mathrm{int}}$ ), plasma clearance of probe drugs may also be influenced by other non-metabolic determinants, including the fraction of drug that is not bound to plasma protein (fu), the blood-to-plasma ratio

(B/P), and the hepatic blood flow (Oh) (7). Indeed, alterations in the abundance of drug binding plasma proteins that impact protein binding, hematocrit levels that impact B/P, and the cardiac output (CO) that drives Oh have been observed in a range of pathophysiological conditions such as cancer, rheumatoid arthritis (RA) and coronavirus disease 2019 (COVID-19) infection (8-10). As a result, CYP phenotyping studies that aim to assess the consequences of (patho)physiological conditions on CYP enzyme activity could be compromised by simultaneous changes in fu, B/P, and/or Qh, in addition to changes in CL,..... In this context, CL<sub>in</sub>, fu, and B/P are composite parameters that are impacted by both drugspecific and system-specific factors (Figure 1). When studying conditions for which (patho)physiological changes in these non-metabolic determinants may occur, an important question is to what extent clinical CYP phenotyping results from probe drug studies reliably reflect changes in CYP enzyme activity, and to what extent (patho)physiological changes in fu, B/P, and Qh affect the clearance of probe drugs used to phenotype CYP enzyme activity. It is conceivable that (patho)physiological conditions may impact the phenotyping probe drug metrics by mechanisms beyond changes in CL<sub>int</sub> only.

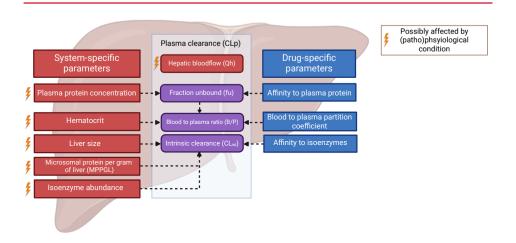
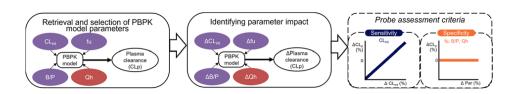


Figure 1 Illustration of how system-specific parameters (red boxes) and drug-specific parameters (blue boxes) drive hepatic plasma clearance (CLp) (white square). Plasma clearance (CLp) of probe drugs is driven by the four parameters presented in the white center square. Purple parameters are influenced by both system-specific and drug-specific parameters. During (patho)physiological conditions, alterations may occur in one or more system-specific parameters, symbolically depicted by a lightning bolt. All these alterations have the potential to affect the plasma clearance of probe drugs.

In this study, we investigate whether the plasma clearance of commonly used CYP phenotyping probe drugs is directly proportional to alterations in CYP enzyme activity, CL., (i.e., probe sensitivity), and to what extent fu, B/P, and Qh impact the plasma clearance (i.e., probe specificity). As delineating and quantifying the impact of alterations in either CL, fu, B/P, or Qh on plasma clearance is not possible in human subjects, physiologically based pharmacokinetic (PBPK) modeling principles are applied, enabling a univariate analysis of how changes in each parameter separately, i.e. CL<sub>int</sub>, fu, B/P, or Qh, affect the plasma clearance of CYP probe drugs. As an illustration, we explore how changes in fu, B/P, or Oh observed in three inflammatory conditions, including chronic inflammation during RA, surgery-associated acute inflammation, or acute COVID-19 infection, impact the plasma clearance of probe drugs. We thereby provide insights and tools that are necessary to interpret the results of CYP phenotyping studies obtained in (patho)physiological conditions correctly, and can help clinicians and clinical pharmacologists to conclude whether a probe drug is suitable to predict altered CYP enzyme activity in conditions that might impact fu, B/P, and Qh.

# Materials and methods

The PBPK-based workflow illustrated in Figure 2 was used to simulate the plasma clearance of CYP phenotyping probe drugs.



**Figure 2** Applied PBPK-based workflow. Parameter values of  $CL_{\rm int}$ , fu, B/P, and Qh of the 13 studied probe drugs were obtained for the PBPK model to predict hepatic  $CL_{\rm int}$ . Each parameter value was subsequently changed univariately to assess the impact of each parameter change on the predicted plasma clearance. Probe sensitivity was defined as a change in plasma clearance that is proportional to a change in  $CL_{\rm int}$ . Probe specificity was defined as an absence of change in plasma clearance with changes in fu, B/P, and Qh. System-specific parameters are shown in red; parameters that are influenced by both drug- and system-specific parameters are shown in purple. PBPK physiologically based pharmacokinetic,  $CL_{\rm int}$  intrinsic clearance, fu fraction unbound, B/P blood-to-plasma ratio, Qh liver bloodflow, CLp plasma clearance.

#### Cytochrome P450 (CYP) probe drugs

Thirteen probe drugs commonly used in phenotyping drug cocktails, representing selective substrates for the six most clinically relevant CYP enzymes, were selected (Online Resource 1 Table 1). This yielded the following CYP enzyme–probe drug combinations for the analysis: CYP3A4: midazolam and quinine; CYP2D6: dextromethorphan and metoprolol; CYP2C19: omeprazole; CYP2C9: diclofenac, flurbiprofen, losartan, s-warfarin and tolbutamide' CYP2B6: bupropion and efavirenz: and CYP1A2: caffeine.

#### Physiologically based pharmacokinetic (PBPK) model for plasma clearance

Hepatic plasma clearance (CLp) was calculated using the dispersion model (Eqs. 1–4), which has been shown to predict plasma clearance well for drugs with both high and low hepatic extraction ratios (11,12). This model was implemented in R version 4.4.1 (The R Foundation for Statistical Computing, Vienna, Austria).

$$CLp = (Qh \cdot E_H) \cdot B/P \tag{1}$$

$$E_H = 1 - \frac{4a}{(1+a)^2 \cdot e^{\frac{a-1}{2D_N}} - (1-a)^2 \cdot e^{-\frac{a+1}{2D_N}}}$$
(2)

$$a = \sqrt{1 + 4R_N \cdot D_N} \tag{3}$$

$$R_N = \frac{fu}{B/P} \cdot \frac{CLint}{Qh} \tag{4}$$

where  $E_H$  is the hepatic extraction ratio;  $D_N$  is the axial dispersion number, which is set to 0.17 (13);  $R_N$  is the efficiency number, which quantifies how effectively a drug is extracted from the blood as it flows through the liver; and  $CL_{int}$ , Qh, and  $CL_{p}$  are expressed in the same units (mL/min).

### Retrieval and selection of PBPK model parameters

The systems-specific parameter Qh and the composite drug- and system-specific parameters  $CL_{int}$ , fu, and B/P were extracted from published sources.  $CL_{int}$  values describing CYP-specific metabolite formation were obtained from in vitro studies in human liver microsomes (HLMs), either by extracting the reported microsomal  $CL_{int}$  ( $CL_{int,mic}$ ) values or calculating  $CL_{int,mic}$  by using the kinetic parameters Vmax (in pmol/min/mg) and Km (in  $\mu$ M), as described in Eq. 5.

180 SECTION III • CHAPTER 6

$$CL_{int,mic} = \frac{Vmax}{Km} \tag{5}$$

For each probe drug, the average  $\mathrm{CL}_{\mathrm{int,mic}}$  (in mL/min/mg microsomal protein) was calculated from multiple studies. Subsequently,  $\mathrm{CL}_{\mathrm{int,mic}}$  values were scaled to whole liver  $\mathrm{CL}_{\mathrm{int}}$  (in mL/min) by using the average weight of a human liver and the milligram protein per gram of liver (MPPGL), as described in Eq. 6.

$$CL_{int} = MPPGL \cdot Weight_{liner} \cdot CL_{int\ mic}$$
 (6)

The fu values were obtained from either the Summary of Product Characteristics (SmPC), available from the European Medicines Agency (EMA), or from the US Food and Drug Administration (FDA) drug label. Due to the considerable variability and experimental challenges associated with accurately determining fu values, an fu range is often reported. The average of this range was initially selected. B/P ratios were obtained from the literature or assumed to be 1 when unavailable.

To ascertain that the clearance predictions by the PBPK model (Figure 2) based on the retrieved parameters for CL<sub>i...</sub>, fu, and B/P were in line with clinically observed plasma clearance values, reported plasma clearance values from studies in healthy volunteers were extracted from the literature for comparison. Since the PBPK model exclusively predicts hepatic metabolic clearance by the primary metabolizing isoenzyme, the comparison involved multiplying the reported clinical plasma clearance by the fraction of the drug eliminated through the main CYPspecific metabolic pathway. In case the initially retrieved parameters yielded a fivefold difference or more in prediction of plasma clearance, changes were made to the parameter values with most uncertainty and with the reported range of values in the literature. This meant that fu was adapted first to a value within the range reported in the SmPC/FDA drug label that yielded a plasma clearance prediction that aligned with the clinically reported plasma clearance that was adjusted for the potential presence of additional clearance routes as described above. If alignment between observed and predicted plasma clearance could not be achieved by changing the fu within the reported range, the  $CL_{int,mic}$  values were adapted within the reported range in HLMs to yield accurate PBPK-predicted plasma clearance.

The final parameter values obtained, including their references, and the evaluation of the predicted plasma clearance can be found in Online Resource 1 Tables 2–5, and Online Resource 2.

### Identifying parameters that impact the plasma clearance of probe drugs

To examine the percentage change in plasma clearance ( $\Delta$ CLp) as a function of univariate changes in each input parameter, CL<sub>int</sub> and fu were varied over a range of -90% to +150%, and B/P and Qh over a range of -50% to +50%. These ranges were chosen to reflect clinically relevant changes in the parameters. Because the fu cannot exceed 100%, the fu values of metoprolol and caffeine were only increased with a maximum of 7% and 56%, respectively (i.e. fucaffeine = 0.64, therefore the maximal percentual increase is  $(\frac{1-0.64}{0.64})*100 = 56\%$ 

For a probe drug to be considered sensitive to changes in  $CL_{int}$ , plasma clearance ideally changes proportionally with changes in  $CL_{int}$ , as illustrated in the 'Sensitivity' panel of Figure 2. On the other hand, to be considered specific, an ideal probe should be insensitive to changes in fu, B/P, or Qh, yielding a desired sensitivity criterion of  $\Delta CLp$  being close to zero across the full range of parameter changes, as illustrated in the 'Specificity' panel of Figure 2.

#### Assessment of probe specificity in the context of inflammatory diseases

We used three inflammatory conditions, i.e. chronic inflammation (RA), acute inflammation (surgery), and acute infection (COVID-19) to assess the suitability of phenotypic probe drugs for the quantification of alterations in enzyme activity under pathophysiological changes in fu, B/P, or Qh. Alterations in parameters fu, B/P, and Qh during RA, surgery, and COVID-19 were extracted from the literature, or, alternatively, when no reported values could be retrieved from the literature, assumptions were made to derive inflammation-induced changes in parameter values. For fu, it was assumed that changes in protein binding of all probe drugs during inflammation were fully dependent on changes in human serum albumin (HSA) concentrations and not on changes in any other drug binding proteins (Eq. 7).

$$fu_{inflammation} = \frac{1}{1 + \frac{\left(1 - fu_{healthy}\right) \cdot \left[HSA_{inflammation}\right]}{\left[HSA_{healthy}\right] \cdot fu_{healthy}}} \tag{7}$$

Inflammation-induced change in B/P were assumed to be only dependent on changes in hematocrit and assessed independently from the change in fu (Eq. 8).

182 SECTION III • CHAPTER 6

$$B/P_{inflammation} = 1 + Hematocrit_{inflammation} \cdot (fu_{healthy} \cdot k_p - 1)$$
 (8)

To calculate Qh alterations during inflammation, it was assumed that the fraction of cardiac output (CO) directed to the liver is similar as for healthy volunteers (Eq. 9).

$$Qh_{inflammation} = 0.25 \cdot CO_{inflammation} \tag{9}$$

When multiple parameter values of HSA, hematocrit, or CO were found, the most extreme reported value was selected to reflect worst-case scenarios. The retrieved and derived pathophysiological changes in fu, B/P, and Qh during chronic inflammation in RA, surgery-related acute inflammation, and acute COVID-19 infection are listed in Online Resource 1 Tables 6 and 7.

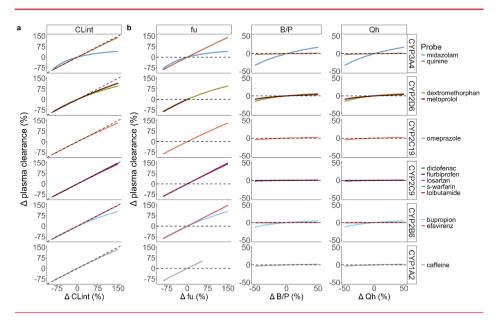
### Results

### Parameters that impact the plasma clearance of probe drugs

The PBPK workflow was used to identify which parameters affect plasma clearance of CYP phenotyping probe drugs. Plasma clearances of quinine, omeprazole, diclofenac, flurbiprofen, losartan, s-warfarin, tolbutamide, efavirenz, and caffeine are highly sensitive to alterations in  $\mathrm{CL}_{\mathrm{int}}$ , showing a proportional change in plasma clearance when decreasing or increasing  $\mathrm{CL}_{\mathrm{int}}$  values over a range of -90% to +150% (Figure 3a). For midazolam, dextromethorphan, metoprolol, and bupropion, decreasing  $\mathrm{CL}_{\mathrm{int}}$  leads to a proportional decrease in plasma clearance, but when the  $\mathrm{CL}_{\mathrm{int}}$  increases more than 50%, these probe drugs exhibit a disproportionally smaller increase in plasma clearance. This disproportional relationship between  $\mathrm{CL}_{\mathrm{int}}$  and plasma clearance is most evident for midazolam, considering an increase of 150% in  $\mathrm{CL}_{\mathrm{int}}$  results in an increase in plasma clearance of only 41%.

Plasma clearance of all probe drugs was also found to be sensitive to both increases and decreases in fu (Figure 3b). On the contrary, alterations in B/P and Qh had minimal impact on plasma clearance of most probe drugs, as indicated by a horizontal line for quinine, omeprazole, diclofenac, flurbiprofen, losartan, s-warfarin, tolbutamide, efavirenz and caffeine (Figure 3b). For midazolam, dextromethorphan, metoprolol, and bupropion, alterations in B/P or Qh led to slight changes in plasma clearance, with a maximum decrease in plasma clearance of -35% at a -50% parameter change for midazolam. Thus, our results highlighted

that plasma clearance of probe drugs is sensitive to alterations in  $\mathrm{CL}_{\mathrm{int}}$ , but might not be specific to alterations in  $\mathrm{CL}_{\mathrm{int}}$  only, considering alterations in the fu also impact plasma clearance.



**Figure 3** PBPK-predicted change in plasma clearance resulting from the univariate changes in each of the indicated parameters. As absolute parameter values differ for each drug, relative changes are depicted, with a value of 0 reflecting no change compared with the value representing a healthy state. Results are presented per parameter (columns) and per cytochrome P450 isoenzyme (rows). The dotted lines indicate the patterns for ideal probe drugs for phenotype assessment [i.e., (a) plasma clearance changes that are proportional to change in CL<sub>int</sub> (sensitivity), and (b) no plasma clearance changes with changes in fu, B/P, and Qh (specificity)]. Please note the different axes between panels for CL<sub>int</sub> and fu compared with B/P and Qh.

### Probe specificity in the context of inflammatory diseases

Alterations in fu that occur during inflammation might impact the specificity of probe drug plasma clearance as a proxy for CYP enzyme activity. Figure 4 shows the probe-specific relative changes in fu for the three selected inflammatory diseases. Evidently, relative changes in fu are dependent on the initial degree of protein binding of the probe drug, as well as the inflammatory condition. The alterations in fu were highest for acute COVID-19 infection, followed by chronic inflammation in RA, and least for surgery-related acute inflammation. The relative changes in fu are smaller for probe drugs with low protein binding, i.e., metoprolol and

184 SECTION III • CHAPTER 6

caffeine, with  $\Delta$ fu between 3 and 4% for metoprolol and between 16 and 21% for caffeine, depending on the inflammatory condition. The fu alterations were larger for the intermediate protein bound drugs dextromethorphan and quinine, with a maximal fu change of 64% for quinine and 46% for dextromethorphan during acute COVID-19 infection. Relative changes in fu during chronic inflammation (RA), acute inflammation (surgery), and acute infection (COVID-19) were largest for the high protein bound drugs midazolam, omeprazole, diclofenac, flurbiprofen, losartan, s-warfarin, tolbutamide, bupropion, and efavirenz.

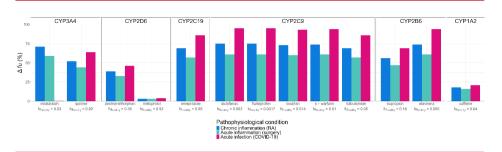
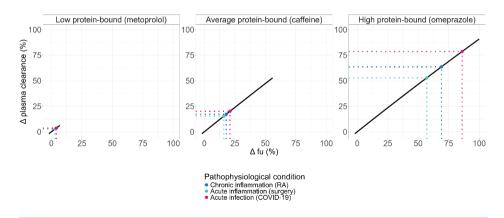


Figure 4 Percentage change in fu during three inflammatory conditions: chronic inflammation (RA), acute inflammation (surgery), and acute infection (COVID-19) for all probe drugs per CYP isoenzyme. Indicated below the probe drug is the fu in a healthy state, showing which probe drugs are low (<0.3), intermediate (0.3–0.7), or high (>0.7) protein-bound. Due to the absence of reported changes in fu for all drugs and all conditions, except for midazolam during acute infection, the changes in fu are derived from reported changes in albumin concentration.

Figure 5 highlights how the univariate changes in fu observed during RA, surgery, and COVID-19 impact the plasma clearance of either a low-protein bound, intermediate protein-bound, or high protein-bound probe drug. The impact of alterations in fu on plasma clearance of a probe drug is dependent on its initial degree of protein binding. The plasma clearance of the low protein-bound drug metoprolol is limitedly affected by the fu changes occurring in these inflammatory conditions, with a maximum plasma clearance change of 4%. For intermediate and high protein-bound probe drugs such as caffeine and dextromethorphan, alterations in fu that occur during acute COVID-19 infection result in plasma clearance changes of 21% and 46%, respectively. As such, plasma clearance of probe drugs with high protein binding are predicted to be most impacted by alterations in fu observed in inflammatory and other (patho)physiological conditions, limiting the specificity of plasma clearance as a proxy for enzyme activity.



**Figure 5** The impact of univariate alterations in fu observed during chronic inflammation (RA), acute inflammation (surgery), and acute infection (COVID-19) on plasma clearance, for the low, intermediate, and high protein-bound drugs metoprolol, caffeine and omeprazole, respectively.

### Tool to interpret phenotyping study results under (patho)physiological conditions

The impact of alterations observed in fu, B/P, and Oh in these three inflammatory conditions on plasma clearance of all probe drugs is outlined in Online Resource 2 Figure 1. Analog to the graphs in Figure 5, these graphs can be used to assess the impact of a univariate change in the three non-metabolic determinants on plasma clearance for each of the probe drugs. When, for a studied (patho)physiological condition, it is evident from Figure 3 that fu impacts the plasma clearance of a probe drug, alterations in this parameter should be accounted for to derive true alterations in CL<sub>int</sub> from measured CLp. There is no analytical solution for this calculation, therefore the R script provided in Online Resource 3 can be used to iteratively derive alterations in CL<sub>int</sub> based on CLp and fu in healthy and (patho) physiological conditions. As an example, if a phenotyping study in diseased patients with caffeine showed a decrease in plasma clearance of 10% and, additionally, an increase in fu of 25% as compared with a healthy population, Figures 3 and 5 can be used to conclude that the change in fu will impact the plasma clearance of caffeine. To derive the change in in vivo CYP1A2 activity from these results, the changes in CLp and fu need to be included in the dispersion model and solved for CL<sub>in</sub>, according to the R script in Online Resource 3. In this example, this would

186 SECTION III • CHAPTER 6

lead us to conclude that the  $\mathrm{CL}_{\mathrm{int}}$  (CYP1A2 activity) decreased 28%. The script also allows for the calculations of  $\mathrm{CL}_{\mathrm{int}}$  in scenarios of changed B/P or Qh, or a combination of changes in the three non-metabolic variables.

### Discussion

In this study, we used a PBPK-based workflow to investigate how sensitive and specific changes in plasma clearance of commonly used CYP probe drugs are in detecting alterations in in vivo CYP enzyme activity, quantified as  $\mathrm{CL}_{\mathrm{int}}$ . This is particularly relevant when the probe drugs are used to assess changes in enzyme activity in the context of (patho)physiological conditions, such as inflammation, obesity, or pregnancy (4–6).

Plasma clearance of all probe drugs was sensitive to alterations in CYP enzyme activity, as changes in plasma clearance demonstrated a close to proportional relationship with alterations in CL,,, except for midazolam. One of the validation criteria for phenotyping metrics states that the metric should not depend on factors beyond enzyme activity, meaning the metric should not be sensitive to, amongst others, the non-metabolic variables fu, B/P, and Qh that were studied in this work (2). Importantly, our results highlight that plasma clearance of all probe drugs was equally sensitive to alterations in protein binding as to alterations in CL,,, highlighting that alterations in fu might impact the phenotyping metric. This will not affect the use of cocktail approaches to assess differences in enzyme activity in scenarios where fu remains unchanged, for example in traditional pharmacokinetic interaction studies investigating drug-drug and/or drug-gene interactions, since these interactions are linked to changes in the levels or activity of CYP enzymes only and studied in healthy volunteers. However, it does indicate that additional methodological approaches are required to account for changes in fu, if the cocktail approach is applied to study scenarios where fu might change, as illustrated in this work for inflammatory conditions. Taking the alterations in fu into consideration as a confounder that can impact the phenotyping metrics will improve the validity of the metric during (patho)physiological conditions. Practically, this would involve the measurement of fu through measuring both total and unbound drug concentrations in patient populations to evaluate how this parameter is impacted as compared with a healthy population. Subsequently, these values can be used as input for the dispersion model to derive changes in enzyme activity ( $\mathrm{CL}_{\mathrm{int}}$ ) from changes in plasma clearance. An R script was provided in Online Resource 3 for the practical implementation of this method.

Midazolam is a widely acknowledged probe for phenotyping in vivo CYP3A4 activity. Interestingly, our results for midazolam show that changes in its plasma clearance are not proportional to changes in CL<sub>int</sub>, when CL<sub>int</sub> increases more than 50%. This might not have implications for, for example, inflammatory conditions, since CYP3A4 activity is known to decrease under these conditions (14). However, midazolam is often utilized to study CYP3A4 induction in clinical DDI studies, when a disproportional relationship between increased CL<sub>int</sub> and midazolam plasma clearance could become relevant, because with high induction the observed increase in midazolam plasma clearance will be less than the increase in in vivo CYP3A4 activity. We found that midazolam clearance is also sensitive to alterations in fu, B/P, and Qh, which is in line with its intermediate extraction ratio (15). The importance of this sensitivity towards non-metabolic determinants was recently highlighted in an obese study population (16). This study showed that midazolam clearance in obese patients was higher as compared with controls, contrary to what would be expected given the decrease in hepatic CYP3A activity with increasing body weight. Given that patients with severe obesity have a higher Qh (17), this study suggested that increased Qh may have a more significant role than CL<sub>int</sub> changes on plasma clearance of midazolam in this patient population. Particularly for midazolam, it may therefore be required to measure and account for alterations in Qh and B/P, as well as for changes in fu, when using it as a phenotyping probe for measuring in vivo CYP3A4 enzyme activity in (patho)physiological conditions.

Our findings on probe sensitivity and specificity are in line with the general notion that clearance of low extraction ratio drugs is limited by both fu and  $\mathrm{CL}_{\mathrm{int}}$ , while drugs with a high extraction ratio are mainly limited by Qh. It is therefore unlikely that probe drugs that are sensitive to changes in  $\mathrm{CL}_{\mathrm{int}}$ , but not sensitive to changes in fu, will ever be identified. One way to circumvent issues with changes in fu, is to select probe drugs with low protein binding, as our results show that low protein binding probe drugs are likely less impacted by alterations in fu as compared with probe drugs that are highly protein bound. Given that information on alterations in fu during inflammatory or other (patho)physiological conditions is scarce, inclusion of fu measurements in study designs will increase our

188 SECTION III • CHAPTER 6

understanding of the magnitude of alterations in fu during disease and whether they impact clearance of probe drugs. In the absence of (literature) data on disease-driven changes in fu, assumptions can be made. We assumed that all probe drugs exclusively bind to HSA and calculated fu changes based on reported alterations in HSA levels in RA, surgery, and COVID-19 patients. This is a limitation considering inflammation is also characterized by upregulation of  $\alpha$ -1-acid glycoprotein (AGP) and other proteins that may impact fu (18). However, the affinity of probe drugs to AGP is unclear, as is the exact value of upregulation under various (patho) physiological conditions. The effects of alterations in drug binding proteins on fu may contradict and counterbalance each other, potentially clarifying why the fu of midazolam remained unaffected in COVID-19 patients, contrary to reported HSA alterations (19). Therefore, measured fu values should be considered to be the golden standard in this context.

Incorporation of the impact of (patho)physiological conditions on drug exposure to advance personalized medicine is a long-standing goal. Several phenotyping cocktail studies have been conducted to characterize how (patho) physiological conditions such as non-alcoholic fatty liver disease, obesity, diabetes or inflammatory conditions can affect drug clearance (4,16,20–24). Incorporation of phenotyping results into clinical guidelines has indeed been demonstrated in special patient groups, e.g. pregnant women, where results from phenotyping studies were utilized to estimate alterations in CYP-mediated drug clearance (25). One important consideration here is that the phenotyping methodology assumes that drug exposure can be predicted by the pharmacokinetics of a relevant probe drug with a shared metabolic pathway. Indeed, the EMA guidelines on DDIs state that results of cocktail studies can be extrapolated to other drugs and can be used to support treatment recommendations in the SmPC (26); however, these results may not always be directly translatable between drugs. Our result highlighted that plasma clearance of probe drugs might also be impacted by alterations in fu, and to a lesser extent B/P and Qh, with the extraction ratio of the drugs determining which parameters are most influential. When findings from phenotyping cocktails in (patho)physiological conditions are used to make inferences about plasma clearance of other drugs, potential differences in fu, B/P, and Qh need to be accounted for, particularly when the extraction ratio of the drug that the finding is extrapolated to is higher. To achieve this, the equations of the dispersion model can be used.

Our results suggest that alterations in fu observed during inflammatory conditions such as COVID-19, RA, or surgery may influence  $\mathrm{CL}_{\mathrm{int}}$  predictions derived from differences in plasma clearance. Of note, our research did not aim to study the impact of inflammation on  $\mathrm{CL}_{\mathrm{int}}$ , but rather to assess the accuracy of the cocktail approach used to quantify such impact. Inflammation is known to affect liver function, induce oxidative stress, and modulate CYP enzyme activity through the release of proinflammatory cytokines (27). While the broader question of how inflammation impacts drug clearance and plasma concentrations has been well-studied by others (28,29), our focus is on providing tools and insights to improve the interpretation of clinical phenotyping studies that assess the overall effects of these inflammation-related mechanisms on CYP enzyme activity.

Our approach focused on the plasma clearance of probe drugs, but the primary metric used in phenotyping studies is the metabolic ratio in plasma or urine at a specific time point. The ratio of metabolite to parent concentrations in plasma indeed increases when plasma clearance increases, however this ratio also changes continuously over time within each individual, causing the method to be sensitive to deviations in sampling time. Moreover, the plasma concentration of both the metabolite and parent drug may be impacted by alterations in distribution volume or equilibration to peripheral tissue, while, in addition, the metabolite concentration may also be impacted by alterations in its elimination rate (26). Especially in diseased study participants, these processes may all be altered. When employing urine sampling, variations in urinary pH and glomerular filtration rate may further impact the measured metabolic ratio (30,31). Considering that all these disease-driven alterations could lead to changes in the metabolic ratio that are independent of changes in enzyme activity, taking multiple plasma samples and calculating plasma clearance may be more appropriate to study in vivo enzyme activity under (patho)physiological conditions. Finally, our approach focused on systemic plasma clearance of probe drugs, while the probe drugs are typically administered orally. Disease-related changes in pre-systemic clearance (i.e., gut and first-pass metabolism) would impact oral bioavailability (F) and thereby the apparent oral clearance (CL/F) that is obtained in cocktail studies. As quantitative information on disease-related changes in presystemic clearance pathways is limited, this could not be included in our PBPK assessment.

190 SECTION III • CHAPTER 6

A limitation that applies to clinical plasma clearance values is that probe drugs are never exclusively eliminated through metabolism by a single isoenzyme and that minor elimination pathways will impact the concentration of the parent compound. The PBPK approach applied in the current analysis allows for studying the major elimination routes in isolation, which would be impossible to do in vivo and which is an advantage for establishing sensitivity and specificity of the drugs in phenotyping cocktails. Due to limited information on intrinsic hepatic transporter activity for most probe drugs, the applied PBPK framework does not incorporate the influence of hepatic influx or efflux transporters, but as far as we know, hepatic transporters are not major contributors to the plasma clearance of the studied probe drugs, which reduces the impact of this limitation on our findings. Finally, we describe the influence of univariate changes in CL, fu, B/P, and Qh on plasma clearance. Multiple parameters could change simultaneously under (patho) physiological conditions, leading to additive, synergistic or antagonistic effects due to the non-linearities in the dispersion model between plasma clearance and the model parameters. Conducting a multivariate analysis investigating all possible combinations of changes in all four variables would exponentially increase the number of scenarios to be evaluated and further challenges the interpretation of the results. Despite the fact that univariate changes in B/P and Qh limitedly impact probe drug plasma clearance, a combination of changes in parameters might have a more significant impact. Specifically, changes in Qh could become more important in scenarios where  $CL_{\mbox{\tiny int}}$  increases, while changes in B/P will be more relevant when the fu is considerably impacted by altered partitioning of drugs into red blood cells. With the provided R script in Online Resource 3, the reader can derive changes in CL<sub>int</sub> for any drug, based on the difference in CLp and any possible combination of changes in fu, B/P, and Qh.

### Conclusion

The PBPK-based simulation workflow utilizing mechanistic equations defining hepatic plasma clearance allowed us to unravel that plasma clearance of 13 commonly used drugs in CYP phenotyping cocktails is highly sensitive to alterations in enzyme activity, except when capturing > 50% CYP3A4 induction with midazolam. However, plasma clearance of all these drugs is also sensitive to

changes in unbound drug fraction, which reduces the specificity of probe drug plasma clearance as a proxy for CYP enzyme. As drug-binding plasma protein levels can change under (patho)physiological conditions, alterations in protein binding should be considered when using probe drug plasma clearance as a proxy for CYP enzyme activity in these patient populations. The provided R script can be used to accurately determine changes in CYP enzyme activity in patients under (patho)physiological conditions by accounting for these alterations.

#### Key points

- CYP450 (CYP) phenotyping entails quantifying an individual's plasma clearance of CYP-specific probe drugs to estimate in vivo CYP enzyme activity. It is increasingly applied to study alterations in CYP enzyme activity under different (patho)physiological conditions.
- Plasma clearance of 13 commonly used CYP probe drugs is sensitive to changes in enzyme activity, but is also affected by variations in the unbound drug fraction, which reduces the specificity of probe drug plasma clearance as a proxy for CYP enzyme activity under (patho) physiological conditions.
- To facilitate the interpretation of results from phenotyping studies, an R script is provided that allows for calculating the alterations in enzyme activity under (patho)physiological conditions by accounting for alterations in the unbound drug fraction, blood-to-plasma ratio and/or hepatic blood flow that might occur.

192 SECTION III • CHAPTER 6

#### References

- Breimer DD, Schellens JH. A "cocktail" strategy to assess in vivo oxidative drug metabolism in humans. Trends Pharmacol Sci. 1990;11:223-5. 10.1016/0165-6147(90)90245-4.
- Fuhr U, Jetter A, Kirchheiner J. Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the "cocktail" approach. Clin Pharmacol Ther. 2007;81:270–83. 10.1038/sj.clpt.6100050.
- de Andrés F, LLerena A. Simultaneous determination of cytochrome P450 oxidation capacity in humans: a review on the phenotyping cocktail approach. Curr Pharm Biotechnol. 2016;17:1159–80. 10.2174/138920 1017666160926150117
- Lenoir C, Daali Y, Rollason V, Curtin F, Gloor Y, Bosilkovska M, et al. Impact of acute inflammation on cytochromes P450 activity assessed by the Geneva Cocktail. Clin Pharmacol Ther. 2021;109:1668–76. 10.1002/ cpt.2146.
- Ghasim H, Rouini M, Safari S, Larti F, Khoshayand M, Gholami K, et al. Impact of obesity and bariatric surgery on metabolic enzymes and P-glycoprotein activity using the Geneva Cocktail approach. J Pers Med. 2023;13:1042. 10.3390/jpm13071042.
- Tracy TS, Venkataramanan R, Glover DD, Caritis SN. Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. Am J Obstet Gynecol. 2005;192:633–9. 10.1016/j. ajog.2004.08.030.
- Espié P, Tytgat D, Sargentini-Maier M-L, Poggesi I, Watelet J-B. Physiologically based pharmacokinetics (PBPK). Drug Metab Rev. 2009;41:391–407. 10.1080/10837450902891360.
- 8. Coutant DE, Hall SD. Disease-drug interactions in inflammatory states via effects on CYP-mediated drug clearance. J Clin Pharmacol. 2018;58:849–63. 10.1002/jcph.1093.
- Djakpo DK, Wang Z, Zhang R, Chen X, Chen P, Antoine MMLK. Blood routine test in mild and common 2019 coronavirus (COVID-19) patients. Biosci Rep. 2020;40:BSR20200817. 10.1042/BSR20200817.

- Santos RC, Figueiredo VN, Martins LC, Moraes CH, Quinaglia T, Boer-Martins L, et al. Infliximab reduces cardiac output in rheumatoid arthritis patients without heart failure. Rev Assoc Médica Bras. 2012;58:698– 702. 10.1590/S0104-4/302012000600015
- Roberts MS, Rowland M. A dispersion model of hepatic elimination: 1. Formulation of the model and bolus considerations. J Pharmacokinet Biopharm. 1986;14:227–60. 10.1007/BF01106706.
- Ridgway D, Tuszynski JA, Tam YK. Reassessing models of hepatic extraction. J Biol Phys. 2003;29:1–21. 10.1023/A:1022531403741.
- 13. Naritomi Y, Terashita S, Kimura S, Suzuki A, Kagayama A, Sugiyama Y. Prediction of human hepatic clearance from in vivo animal experiments and in vitro metabolic studies with liver microsomes from animals and humans. Drug Metab Dispos. 2001;29:1316– 24.
- 14. Vet NJ, Brussee JM, De Hoog M, Mooij MG, Verlaat CWM, Jerchel IS, et al. Inflammation and organ failure severely affect midazolam clearance in critically ill children. Am J Respir Crit Care Med. 2016;194:58–66. 10.1164/ rccm.201510-2114OC.
- Salem F, Abduljalil K, Kamiyama Y, Rostami-Hodjegan A. Considering age variation when coining drugs as high versus low hepatic extraction ratio. Drug Metab Dispos. 2016;44:1099–102. 10.1124/dmd.115.067595.
- 16. Kvitne KE, Robertsen I, Skovlund E, Christensen H, Krogstad V, Wegler C, et al. Short- and long-term effects of body weight loss following calorie restriction and gastric bypass on CYP3A-activity—a nonrandomized three-armed controlled trial. Clin Transl Sci. 2022;15:221–33. 10.1111/ cts.13142.
- 17. Cho S-J, Yoon I-S, Kim D-D. Obesity-related physiological changes and their pharmacokinetic consequences. J Pharm Investig. 2013;43:161–9. 10.1007/s40005-013-0073-4.
- Kremer JM, Wilting J, Janssen LH. Drug binding to human alpha-1-acid glycoprotein in health and disease. Pharmacol Rev. 1988;40:1–47.

- Le Carpentier EC, Canet E, Masson D, Martin M, Deslandes G, Gaultier A, et al. Impact of inflammation on midazolam metabolism in severe COVID-19 patients. Clin Pharmacol Ther. 2022;112:1033–9. 10.1002/cpt.2698.
- Li H, Canet MJ, Clarke JD, Billheimer D, Xanthakos SA, Lavine JE, et al. Pediatric cytochrome P450 activity alterations in nonalcoholic steatohepatitis. Drug Metab Dispos Biol Fate Chem. 2017;45:1317–25. 10.1124/dmd.117.077644.
- Kvitne KE, Krogstad V, Wegler C, Johnson LK, Kringen MK, Hovd MH, et al. Short- and long-term effects of body weight, calorie restriction and gastric bypass on CYP1A2, CYP2C19 and CYP2C9 activity. Br J Clin Pharmacol. 2022;88:4121–33. 10.1111/ bcp.15349.
- 22. Kvitne KE, Åsberg A, Johnson LK, Wegler C, Hertel JK, Artursson P, et al. Impact of type 2 diabetes on in vivo activities and protein expressions of cytochrome P450 in patients with obesity. Clin Transl Sci. 2022;15:2685– 96. 10.1111/cts.13394.
- 23. Lenoir C, Terrier J, Gloor Y, Curtin F, Rollason V, Desmeules JA, et al. Impact of SARS-CoV-2 infection (COVID-19) on cytochromes P450 activity assessed by the Geneva Cocktail. Clin Pharmacol Ther. 2021;110:1358–67. 10.1002/cpt,2412.
- 24. Lenoir C, Rollason V, Desmeules JA, Samer CF. Influence of inflammation on cytochromes P450 activity in adults: a systematic review of the literature. Front Pharmacol. 2021;12:733935. 10.3389/ fphar.2021.733935.
- Zwangerschap/Lactatie n.d. https:// www.farmacotherapeutischkompas.nl/ farmacologie/zwangerschap-lactatie. Accessed 15 Jan 2024.
- European Medicines Agency. Guideline on the investigation of drug interactions. Amsterdam: European Medicines Agency; 2013.
- 27. Stanke-Labesque F, Gautier-Veyret E, Chhun S, Guilhaumou R. Inflammation is a major regulator of drug metabolizing enzymes and transporters: consequences for the personalization of drug treatment. Pharmacol Ther. 2020;215:107627. 10.1016/j. pharmthera.2020.107627.

- Machavaram KK, Almond LM, Rostami-Hodjegan A, Gardner I, Jamei M, Tay S, et al. A physiologically based pharmacokinetic modeling approach to predict disease-drug interactions: suppression of CYP3A by IL-6. Clin Pharmacol Ther. 2013;94:260–8. 10.1038/clpt.2013.79.
- Simon F, Gautier-Veyret E, Truffot A, Chenel M, Payen L, Stanke-Labesque F, et al. Modeling approach to predict the impact of inflammation on the pharmacokinetics of CYP2C19 and CYP3A4 substrates. Pharm Res. 2021;38:415–28. 10.1007/s11095-021-03019-7.
- Jetter A, Kinzig-Schippers M, Skott A, Lazar A, Tomalik-Scharte D, Kirchheiner J, et al. Cytochrome P450 2C9 phenotyping using low-dose tolbutamide. Eur J Clin Pharmacol. 2004;60:165–71. 10.1007/s00228-004-0754-z.
- Fuhr U, Rost KL, Engelhardt R, Sachs M, Liermann D, Belloc C, et al. Evaluation of caffeine as a test drug for CYP1A2, NAT2 and CYP2E1 phenotyping in man by in vivo versus in vitro correlations. Pharmacogenetics. 1996;6:159–76. 10.1097/00008571-199604000-00003.

194 SECTION III • CHAPTER 6

### **Supplemental information**

Supplemental information can be found online at https://doi.org/10.1007/s40262-024-01426-8.



# Chapter 7

General discussion and perspectives

### General discussion

Personalized medicine is anticipated to replace the conventional 'one-size-fitsall' approach to pharmacological treatments, enhancing both the efficacy and safety of therapy. The discovery of genetic variants that impact drug response, and subsequent implementation of guidelines on dose optimization for certain drug-gene pairs has greatly advanced our ability to tailor treatment to individual patients. However, not all variability in drug metabolism can be explained by current PGx. Besides heritable traits, metabolic activity of DMEs is also modulated by non-genetic factors, including concomitant medication and (inflammatory) comorbidities. In order to ultimately incorporate the impact of non-genetic factors into drug metabolizing phenotype predictions, it is imperative to acquire a quantitative understanding of the magnitude and duration of phenoconversion due to non-genetic factors. This thesis explores how non-genetic factors impact hepatic drug metabolism. In section I, we focus on the role of concomitant medication as a contributor to phenoconversion and its impact on drug metabolizer phenotype predictions. Section II delves into (pre)clinical evaluations of inflammationinduced alterations in drug metabolism and the potential of immunomodulating therapeutics to reverse these alterations. Section III moves to in vivo tools for studying alterations in enzyme activity and examines whether the CYP phenotyping cocktail approach accurately reflects alterations in enzyme activity under inflammatory and other (patho)physiological conditions. Together, these sections provide a comprehensive exploration of the non-genetic factors that influence drug metabolism, with the aim of improving drug metabolizing phenotype predictions and ultimately guiding more personalized treatment.

### The prevalence of phenoconversion

It is important to get a grasp of the scale of phenoconversion in order to evaluate its clinical relevance. The scale of phenoconversion is likely dependent on several factors, including the characteristics of the patient population, their underlying comorbidities, and the type and dosage of concomitant medication (1). In the cohort of liver microsomes from 40 different patients included in chapter 2, we observed a 40% concordance between genetically-predicted CYP2C19 phenotypes and measured phenotypes, indicating substantial phenoconversion. This aligns with

198 CHAPTER 7

findings from Kiss et al., who also reported a 40% concordance in a cohort of 114 CYP2C19 genotyped microsomes (2). In both cohorts, a significant increase in the amount of PMs was observed, that was not predicted based on genotype data. This can have significant clinical implications, as alterations in drug PK can be expected when the individual has a limited capacity in the primary metabolic pathway.

Discrepancies between genotype and phenotype are also observed in larger clinical PK studies. Lorenzini et al. found low concordances between genotype-predicted and measured phenotypes across several CYP enzymes including CYP2B6, 2C9, 2C19, 2D6 and 3A4/5, with genetically predicted NMs and UMs generally showing lower concordances compared to IMs and PMs (3). For example, the CYP2C19 PM phenotype was correctly predicted by PGx in 100% of patients, and the IM phenotype in 91% of patient. In contrast, PGx correctly predicted the phenotype in only 33% of NMs and 19% of UMs. These patterns of phenoconversion for key DMEs have been observed across different ethnic groups (4–6). Notably, discrepancies between genotype-predicted and observed CYP2C19 phenotype were also found in a healthy patient population without liver disease or drugs affecting CYP2C19 activity, were only approximately 20% of phenotypes were accurately predicted by PGx (7).

Importantly, the quantification of phenoconversion is highly dependent on the set thresholds between phenotype groups. Currently there is no standardized methodology for defining these thresholds – although efforts are made to address this challenge, at least for CYP2C19 phenotyping (7). As such, the extent of phenoconversion may vary according to the method applied, calling for a more uniform framework for phenotype thresholds to enhance consistency across studies. More real-world data is required to identify the prevalence of phenoconversion across different therapeutic contexts, and more importantly, to understand when a phenotype switch leads to clinically relevant change in efficacy or safety of a drug.

# Section I: Impact of concomitant medication on drug metabolizer phenotype predictions

Phenoconversion due to the use of concomitant medication can reduce the accuracy of PGx-based drug dosing. For example, 32–47% of phenoconversion of CYP2C19 and CYP2D6 could be attributed to concomitant medication use (3). Integrating knowledge of drug-drug and drug-gene interactions remains a complex

challenge. There is however consensus that drug-gene guidelines should consider the influence of CYP inducers and inhibitors on PGx-phenotype predictions. For example, CPIC guidelines on CYP2C19 PGx and proton pump inhibitor dosing warn for a potential interaction when a PPI and a CYP inhibitor/inducer are co-administered chronically in CYP2C19 IMs or PMs, recommending interaction monitoring (8). Similarly, CYP2C19 PGx and clopidogrel guidelines emphasize that the impact of additional drugs in combination with CYP2C19 genotype warrants further investigations (9).

To provide concrete dosing recommendations for these potential DDGIs, it is crucial to gain an understanding of the phenoconversion that occurs following the administration of inhibiting or inducing concomitant medication, and whether this interaction is different for different genotypes. As such, in chapter 2, utilizing human liver microsomes, we quantified the phenoconversion in various CYP2C19 genotype groups following administration of either a strong (fluvoxamine), moderate (omeprazole or voriconazole) or weak (pantoprazole) inhibitor of CYP2C19. The relative CYP inhibition by the inhibitors was consistent across genotypes, but the outcome of phenoconversion varied per genotype, e.g. voriconazole caused IM/PM phenotypes in 50% of genetically-predicted NMs, but in only 14% of genetically-predicted RM patients. We subsequently concluded that the degree of phenoconversion is dependent on 1) the inhibitor strength, since phenoconversion towards a lower metabolic phenotype was more frequent with stronger CYP2C19 inhibitors, and 2) the basal CYP2C19 activity, which is only in part dictated by genotype. These findings were confirmed in a large clinical study in healthy volunteers with a similar objective, which showed that overall more than 80% of volunteers experienced phenoconversion to a lower phenotype upon fluvoxamine and/or voriconazole – with RMs experiencing the greatest shifts in metabolic ratios upon inhibition (7). Consequently, accounting for concomitant medications in phenotyping predictions appears essential for the optimization of PGx-based personalized therapy.

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

Inflammation is shown to have major effects on the metabolism of drugs – primarily through downregulation of CYP enzymes – and hence contribute to

200 CHAPTER 7

phenoconversion (1,10,11). The impact of inflammation on drug metabolism is well recognized but not yet well understood. A more profound understanding of the impact of inflammation on DMEs necessitates better insights into the mechanisms driving these changes. In chapter 3, we summarized how repression of important CYP enzymes during inflammation may proceed through 1) transcriptional downregulation of nuclear factors and other transcription factors which regulate the CYPs, 2) interference with dimerization or translocation of these (nuclear) transcription factors, 3) altered liver-enriched C/EBP signaling, 4) direct regulation of CYP expression by NF-κb, or 5) via post-transcriptional mechanisms. Here, the general consensus is that transcriptional alterations are the main regulatory mechanisms accountable for altered CYP activity during inflammation. This is supported by our analysis on the effects of IL-6 and IL-1β on DME expression and activity in chapter 4, where 90% of variability in DME activity was attributable to transcriptional changes (R2=0.9). These transcriptional changes might, in part, result from the inhibition of transcription factors that regulate DME expression (chapter 4). Consequently, it appears that transcriptional changes are significant drivers of altered enzyme activity in inflammation, at least in an in vitro setting.

Ultimately, we would like to identify for which inflammatory diseases and for which drugs the inflammation-induced changes in metabolism might result in clinically relevant alterations in drug efficacy or safety. Based on evidence assembled in this thesis, we conclude that the impact of inflammation on drug metabolism is multifaceted and contingent upon several critical factors:

1. The type of inflammation or cytokine profile is a key factor determining how DMEs are affected. The evidence assembled through in vitro liver models as summarized in chapter 3 highlights that the pro-inflammatory cytokines IL-6, IL-1 $\beta$ , TNF- $\alpha$  as well as lipopolysaccharides (LPS) exert the strongest suppressive effects on various CYPs, whereas cytokines like IL-22, IL-23 or IL-2 have minimal to no effect. Further supporting this, studies with immunomodulating biologics have shown cytokine-specific successes in reversing the inhibitory effects on CYP-mediated drug clearance. Chapter 5 systematically reviewed 12 clinical studies investigating the potential of immunomodulatory antibodies to counteract inflammation-induced CYP downregulation, with three trials highlighting risks associated with IL-6 targeting mAbs. No changes in PK

of probe drugs was observed following administration of mAbs targeting IL-2, IL-4R or IL-23. Incorporating evidence from in vitro and clinical trials, the EMA and FDA assesses the risk for these DDDIs and advices on this in the drug label. The analyzed labeling information designated the greatest risk for DDDIs to mAbs that neutralize the effects of IL-6, TNF- $\alpha$  and IL-1 $\beta$ , where for the latter two this is mainly based on in vitro work. Collectively, the data from chapter 3 and 5 indicate that patients suffering from inflammatory conditions that are marked by elevated levels of IL-6, IL-1 $\beta$  an TNF- $\alpha$  are likely to experience changes in CYP-mediated drug metabolism – whereas this is less likely in IL-17/ IL-23-axis inflammatory diseases such as psoriasis.

- 2. The degree of inflammation is another determinant influencing the impact of inflammation on DMEs and subsequent clearance, as outlined in chapters 4 and 5. Whereas mAbs targeting IL-6 do result in restored CYP-mediated clearance of probe substrates in RA patients, this is not evident for mAbs targeting IL-6 in patient populations with lower levels of pro-inflammatory cytokines, such as kidney transplant patients. This is supported by the concentration-dependent effects of pro-inflammatory cytokine treatment on DME expression and activity in the HepaRG cell model presented in chapter 4. As such, when pro-inflammatory cytokines are only marginally elevated in the patient population, the risk for an alteration in drug metabolism is low. In line with this, the FDA advises investigating DDDIs in the population with the highest inflammatory burden (12).
- 3. The impact of inflammation and the magnitude of the alteration in drug PK might also be dependent on the **metabolic clearance route of the drug**. Data from in vitro models as summarized in chapter 3 have been instrumental to elucidate that CYP isoforms show distinct susceptibility to downregulation by inflammatory mediators wherein CYP3A4, CYP2C19 and CYP1A2 seems to be most affected by proinflammatory cytokine treatment, supporting clinical observations (13,14). Differences between DME families are also observed. In chapter 4, our concentration-response experiments defined differences in both the potency and efficacy of cytokines in inducing downregulation of

202 CHAPTER 7

individual DME family members. The rank ordering revealed that CYP isoforms were the most responsive to IL-6 and IL-1β modulation, while enzymes from the FMO, CES, and UGT families consistently showed lower sensitivity. This differential sensitivity is confirmed by clinical observations. For example, posaconazole, metabolized by UGT1A4, showed no change in exposure related to CRP levels, whereas voriconazole, metabolized mainly by CYP2C19 and 3A4, exhibited increased trough levels during inflammatory conditions (15–17). This highlights the greater susceptibility of CYP-mediated clearance pathways towards inflammation. These findings suggest that inflammation may differentially affect drug PK depending on the relative contribution of DMEs involved in its clearance pathways. Subsequently, drugs relying on secondary or non-CYP pathways for clearance may be less affected by inflammatory processes than those predominantly metabolized by CYP enzymes.

4. Little is known about how **genetics** might predispose an individual towards the impact of inflammation on drug metabolism, but there are some hints for a genotype-dependent effect of inflammation on drug metabolism (chapter 3). These mainly stem from clinical studies that highlight a greater shift in CYP-specific metabolic ratios upon inflammation in RMs or NMs, as compared to IMs or PMs (18,19). Larger clinical trials that simultaneously investigate inflammatory status and pharmacogenetics are important to decipher whether genotype is a determinant in the impact of inflammation on drug metabolism.

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

Ultimately, it is of interest to understand how the described changes in DME activity during inflammation translate to alterations in drug clearance in patients. Beyond enzyme activity, several factors – including protein binding, the blood-to-plasma ratio, and hepatic blood flow – also drive clearance and may be impacted by inflammation. As discussed in chapter 6, in vivo clearance data obtained using phenotyping cocktail approaches cannot always be directly attributed to changes in metabolism alone. Through PBPK modeling, we demonstrated that plasma

clearance of 13 commonly used phenotyping probes was not only sensitive to alterations in enzyme activity but also to changes in protein binding. Given that drug binding proteins may change under inflammatory conditions, such variations must be taken into account when using probe drugs clearance as a proxy for CYP enzyme activity in patient populations with inflammatory comorbidities. This can be achieved by using the R script provided in chapter 6. In light of phenotyping studies conducted in patient populations with inflammatory disease, the reported absolute percentual changes in CYP activity should be interpreted with care, as they may reflect not only changes in enzyme activity but also shifts in protein binding and/or blood-to-plasma ratio or hepatic blood flow during disease. This was recently confirmed in a cohort of liver cirrhosis patients which showed that the probe drugs used to quantify CYP enzyme activity are impacted by altered protein binding occurring in this disease, limiting the precision of probe drugs (20). As such, interpreting phenotyping results in the context of inflammatory or liver diseases requires a nuanced approach, considering not only enzyme activity but also the broader physiological changes that can influence drug clearance.

### **Perspectives**

Following the great advances in PGx-based drug dosing, this thesis advocates that the CYP genotype should be evaluated within the broader context of the individual patient, considering it a starting point rather than an end point. Incorporating all relevant contributors to CYP metabolic function is critical to refining phenotype predictions that better reflect the real-time metabolizing status of the patient. This approach raises the central question: how can we effectively integrate the impact of non-genetic factors, such as concomitant medication and inflammatory status, into phenotype predictions to advance personalized medicine?

### Evaluating the clinical relevance of inflammation-induced phenoconversion

A critical aspect to answering this question is identifying which drugs in which therapeutic context may be susceptible to clinically relevant alteration in efficacy or safety that requires dose adjustments. Whilst there is lots of evidence for altered drug PK during e.g. inflammatory episodes, there is little evidence for altered outcomes of treatment or more adverse events. Supratherapeutic exposure

204 CHAPTER 7

of clozapine and theophylline during episodes of acute inflammation is linked with concurrent clinical manifestations of drug-related toxicity (21). However, for drugs like midazolam, voriconazole or tacrolimus, alterations in efficacy or increase side effects due to elevated concentrations during inflammation remain scarcely reported. This highlights that future studies should focus on systematically measuring clinical outcomes alongside PK changes during inflammation. This would help to clarify for which drug classes phenoconversion might be clinically relevant.

For inflammatory or metabolic diseases where changes in CYP-mediated clearance are a possible concern, the use of one or more inflammatory markers may inform on the likelihood and risk for clinically meaningful phenoconversion, considering the type and severity of inflammation are important determinants in this effect. Recommended inflammatory markers could include C-reactive protein (CRP), alpha-1-acid glycoprotein (AAG), albumin, IL-1β, IL-6 and TNF-α. For example, mild psoriasis patients have inadequate systemic inflammation to cause a meaningful alterations in CYP-mediated metabolic activity, with CRP levels usually below 10 mg/L (22). For diseases where e.g. CRP levels exceed 20 mg/L, for example in some cancers, the risk might be categorized as 'moderate' (23). Diseases might be put into the highest risk category if a combination of inflammatory markers is strongly altered, e.g. albumin levels dropping below 35 g/L and AAG > 1.2 g/L, such as seen in COVID-19 patients and severe rheumatoid arthritis patients (24–26). While using inflammatory markers to stratify disease-related phenoconversion risk provides a useful framework, it has limitations, including significant interpatient variability in cytokine levels. Nonetheless, it offers a practical starting point for categorizing diseases by their phenoconversion risk

### Time dynamics of phenoconversion

A better understanding of the duration of phenoconversion is necessary in order to estimate how phenotypes of patients might change over time – and when dosing adjustments are necessary or close monitoring may suffice. This proves to be a challenge considering the duration of phenoconversion likely varies based on the underlying cause and the patient's unique physiological response. Duration of concomitant medication-induced phenoconversion is related to dose, duration of use and drug-specific properties like the drug's half-life and affinity towards its

target (1). Importantly, the inhibitory effect of some drugs, such as fluoxetine or paroxetine can persist days to weeks after discontinuation of the drug, complicating a general approach to predicting durations of concomitant medication-induced phenoconversion (27–29).

In contrast, inflammation-induced phenoconversion may have a more variable and potentially shorter duration, particularly in the case of acute inflammation. Here the key question is how long the inflammatory state – and its impact on drug metabolism – will persist. Only a few clinical studies have investigated the link between the resolution of inflammation or infection and the subsequent time dynamics of restoring hepatic metabolic capacity (13,14). Considering the half-life of DMEs likely plays an important role in this process, the field would benefit from a comprehensive analysis of both phase I and phase II DMEs half-lives, as conflicting reports have been published so far (30). Drug-induced resolution of inflammation showed us that the PK parameters of a CYP3A4 substrate given to tocilizumab-treated RA patients resembled that of healthy volunteers after 15 days of anti-inflammatory treatment, which indicates a time frame for the restoration of CYP3A4 activity post-treatment (31). Until the point of more data collection, creating awareness that clinical signs of inflammation, e.g. fever or elevated CRP levels can precede a rise in plasma concentrations of CYP substrates would already be a significant step forward.

#### Accurate measurement techniques to quantify phenoconversion

To effectively address the integration of non-genetic factors into phenotype predictions, it is essential to utilize accurate (measurement) techniques that can quantify phenoconversion. The large-scale implementation of the CYP phenotyping approach in clinical practice would be ideal to study phenoconversion, however this is not realistic considering it is expensive, labor-intensive and very invasive for the patient as it requires additional dosing of probe substrates. One way to circumvent this latter problem is by using endogenous phenotypic biomarkers to assess individual drug metabolism capacity. For example, the potato alkaloid solanidine serves as a sensitive and specific dietary biomarker for CYP2D6 activity (32). Endogenous biomarkers could serve as a valuable technique to quantify phenoconversion in future studies, if validation criteria are met (33).

In vitro hepatic models, such as liver microsomes or hepatocyte cultures allow for the controlled studying of modulation of DME activity under specific

206 CHAPTER 7

conditions. By simulating conditions of phenoconversion, they can yield valuable data to support predictions of alterations in drug metabolism in vivo. However, translating findings from in vitro to in vivo have proven to be complex, as discrepancies often arise. In example, whereas we and others have consistently showed a downregulation of CYP2C9 and CYP2D6 activities upon stimulation with pro-inflammatory cytokines in vitro, in vivo results are conflicting. CYP2C9 activity is shown to increase during acute inflammation (13), diabetes (34) and non-alcoholic fatty liver disease (35), whereas clearance of CYP2D6 probe substrates is mainly unaffected during inflammation in vivo (13). Discrepancies in in vitro versus in vivo studies reporting on comedication-induced phenoconversion are also present, where the magnitude of phenoconversion for voriconazole and fluvoxamine was different (7,36).

PBPK models could aid in a better translation of in vitro to in vivo by integrating patient and system-specific characteristics to study how drug clearance is affected by non-genetic factors. Machavaram et al. pioneered in utilizing in vitro data in PHHs on IL-6-mediated CYP suppression to subsequently predict the impact of IL-6 on CYP3A4 substrates in vivo (37), and more studies followed (38-41). Similar successes have been achieved by utilizing PBPK approaches to predict DDGIs (42-45). These models do heavily rely on accurately determined in vitro kinetic parameters, which can vary significantly between labs, potentially compromising prediction accuracy (46). Additionally, most PBPK models simplify the inflammatory response by focusing on the effect of a single cytokine on CYP activity, overlooking the complex interplay between multiple cytokines and the influence of anti-inflammatory cytokines. They also often exclude the effects of cytokines on drug transporters and extrahepatic metabolism. Despite these limitations, PBPK modeling applications hold great potential to simulate these interactions and lay the groundwork for future research aimed at refining phenotype predictions by incorporating all feasible contributors to CYP metabolic function.

### Integration of phenoconversion into clinical practice: the phenoconversion calculator

So how can we ultimately translate this acquired knowledge into usable clinical guidelines during routine pharmacotherapy? First of all, ensuring uniformity in the implementation of phenoconversion into phenotyping predictions is crucial.

In an attempt to enable consistent classification of CYP2D6 phenoconversion, various web-tools have been created to integrate genotype and drug interactions to ensure the correct clinical phenotype is utilized when making dosing decisions (47,48). This approach is founded on translating different diplotypes into activity scores, which are then adjusted according to the concomitant medication used, and subsequently, a clinical phenotype is inferred. This method has been shown to improve phenotype predictions, e.g. in breast cancer patients taking CYP2D6related comedication (49), but in other patient cohorts there was little added benefit of corrected genotype-predicted activity scores in explaining the overall variability in drug PK (50). This highlights that more data is required to optimize such web-based tools. In example, one of the assumptions in the tools is that a PM phenotype is expected upon strong inhibitor use for all genotype-predicted phenotypes. Our data challenges this assumption and highlights that for some genotypes, an IM phenotype is more likely upon strong inhibition. Additionally, there are some reports that UMs might be less prone towards concomitant medication-induced phenoconversion, at least for CYP2D6 (51,52). More data is essential to capture the nuances in the specificity and strength of concomitant medication on specific CYP enzymes, which can then be use as input for the available tools.

Additionally, the phenotyping scoring system would benefit from an extension with other factors that impact phenotype, e.g. the presence of liver disease and other (inflammation-related) comorbidities. Integrating non-genetic factors into the activity scoring system and subsequent phenotype predications involves systematically evaluating when a phenotypic switch is evident during a certain comorbidity. Clinical trials on the impact of inflammation now mainly focus on how CYP activity is impacted, but future studies should simultaneously evaluate phenotypic switches during various comorbidities, or determine inflammatory marker cut-offs where this will likely happen. One difficulty is that patient medications and comorbidities are dynamic. Thus, the occurrence and extent of phenoconversion may fluctuate over time as interacting drugs are initiated or discontinued, or as underlying diseases emerge or are successfully treated. As such, it would be important for clinicians or pharmacists to evaluate the calculated phenotype prediction in the light of the current situation, and re-evaluate the calculator when necessary.

208 CHAPTER 7

Until there is such a scoring tool available for clinicians, we should spread awareness of the potential impact of phenoconversion on PGx-based phenotype predictions, for example implement a warning into pharmacogenetic guidelines. CPIC guidelines for drug-gene pairs usually do contain a warning that concomitant medication or other patient specific-comorbidities might skew the assigned phenotype. But for example the EMA draft guideline on the implementation of pharmacogenetics could benefit from a warning concerning the impact of nongenetic factors on interpreting phenotypes. Creating awareness could help alert clinicians and other healthcare providers to consider phenoconverting factors when unexpected variations in plasma PK of DME substrates occur.

### Conclusion

This thesis underscores the importance of broadening the scope of CYP phenotype predictions beyond genetic determinants by integrating non-genetic factors such as concomitant medication and inflammatory status. While significant strides have been made in personalized dosing through PGx, refining these predictions to reflect real-time metabolic status remains a critical challenge. This thesis contributes to a deeper quantitative understanding of how inflammation and concomitant medications impact drug metabolism, ultimately supporting the development of more accurate phenotype predictions and advancing personalized dosing strategies.

#### References

- Shah RR, Smith RL. Addressing phenoconversion: The Achilles' heel of personalized medicine. Br J Clin Pharmacol. 2015 Feb 1;79(2):222–40.
- Kiss ÁF, Vaskó D, Déri MT, Tóth K, Monostory K. Combination of CYP2C19 genotype with non-genetic factors evoking phenoconversion improves phenotype prediction. Pharmacol Rep. 2018 Jun 1;70(3):525–32.
- Ing Lorenzini K, Desmeules J, Rollason V, Bertin S, Besson M, Daali Y, et al. CYP450 Genotype—Phenotype Concordance Using the Geneva Micrococktail in a Clinical Setting. Front Pharmacol. 2021 Aug 26;12:730637.
- de Andrés F, Altamirano-Tinoco C, Ramírez-Roa R, Montes-Mondragón CF, Dorado P, Peñas-Lledó EM, et al. Relationships between CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 metabolic phenotypes and genotypes in a Nicaraguan Mestizo population. Pharmacogenomics J. 2021 Apr 1;21(2):140-51.
- De Andrés F, Terán S, Hernández F, Terán E, Llerena A. To Genotype or Phenotype for Personalized Medicine? CYP450 Drug Metabolizing Enzyme Genotype-Phenotype Concordance and Discordance in the Ecuadorian Population. OMICS. 2016 Dec 1;20(12):699–710.
- de Andrés F, Sosa-Macías M, Ramos BPL, Naranjo MEG, LLerena A. CYP450 Genotype/Phenotype Concordance in Mexican Amerindian Indigenous Populations-Where to from Here for Global Precision Medicine? OMICS. 2017 Sep 1;21(9):509–19.
- 7. Abouir K, Exquis N, Gloor Y, Daali Y, Samer CF. Phenoconversion Due to Drug-Drug Interactions in CYP2C19 Genotyped Healthy Volunteers. Clin Pharmacol Ther. 2024 Oct 1;116(4).
- 8. Lima JJ, Thomas CD, Barbarino J, Desta Z, Van Driest SL, Rouby N El, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C19 and Proton Pump Inhibitor Dosing. Clin Pharmacol Ther |.

- Lee CR, Luzum JA, Sangkuhl K, Gammal RS, Sabatine MS, Stein CM, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2C19 Genotype and Clopidogrel Therapy: 2022 Update. Clin Pharmacol Ther. 2022 Nov 1:112(5):959–67.
- Klomp SD, Manson ML, Guchelaar H-J, Swen JJ. Phenoconversion of Cytochrome P450 Metabolism: A Systematic Review. J Clin Med. 2020 Sep 7;9(9):2890.
- 11. Stanke-Labesque F, Gautier-Veyret E, Chhun S, Guilhaumou R. Inflammation is a major regulator of drug metabolizing enzymes and transporters: Consequences for the personalization of drug treatment. Pharmacol Ther. 2020 Nov 1;215.
- U.S. Food and Drug Administration Center for Drugs Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER). Drug-Drug Interaction Assessment for Therapeutic Proteins. Guidance for Industry. FDA Guid. 2020;(August):1-11.
- Lenoir C, Daali Y, Rollason V, Curtin F, Gloor Y, Bosilkovska M, et al. Impact of Acute Inflammation on Cytochromes P450 Activity Assessed by the Geneva Cocktail. Clin Pharmacol Ther. 2021 Jun 1;109(6):1668–76.
- 14. Lenoir C, Terrier J, Gloor Y, Curtin F, Rollason V, Desmeules JA, et al. Impact of SARS-CoV-2 Infection (COVID-19) on Cytochromes P450 Activity Assessed by the Geneva Cocktail. Clin Pharmacol Ther. 2021 Nov 1;110(5):1358–67.
- Märtson AG, Veringa A, Bakker M, van den Heuvel ER, Touw DJ, van der Werf TS, et al. Posaconazole trough concentrations are not influenced by inflammation: A prospective study. Int J Antimicrob Agents. 2019 Mar 1;53(3):325–9.
- 16. Veringa A, ter Avest M, Span LFR, van den Heuvel ER, Touw DJ, Zijlstra JG, et al. Voriconazole metabolism is influenced by severe inflammation: a prospective study. J Antimicrob Chemother. 2017 Jan 1;72(1):261–7.
- Van Wanrooy MJP, Span LFR, Rodgers MGG, Van Heuvel ERD, Uges DRA, Van Werf TSD, et al. Inflammation is associated with voriconazole trough concentrations. Antimicrob Agents Chemother. 2014 Dec 1;58(12):7098–101.

210 CHAPTER 7

- 18. Bolcato L, Khouri C, Veringa A, Alffenaar JWC, Yamada T, Naito T, et al. Combined Impact of Inflammation and Pharmacogenomic Variants on Voriconazole Trough Concentrations: A Meta-Analysis of Individual Data. J Clin Med. 2021 May 13;10(10):2089.
- Klomp SD, Veringa A, Alffenaar JWC, de Boer MGJ, Span LFR, Guchelaar HJ, et al. Inflammation altered correlation between CYP2C19 genotype and CYP2C19 activity in patients receiving voriconazole. Clin Transl Sci. 2024 Jul 1;17(7).
- Duthaler U, Chapuisat F, Hanimann R, Krähenbühl S. Effect of protein binding on the pharmacokinetics of the six substrates in the Basel phenotyping cocktail in healthy subjects and patients with liver cirrhosis. Eur J Pharm Sci. 2024 Nov 1;202.
- Lenoir C, Rollason V, Desmeules JA, Samer CF. Influence of Inflammation on Cytochromes P450 Activity in Adults: A Systematic Review of the Literature. Front Pharmacol. 2021 Nov 16;12.
- Rocha-Pereira P, Santos-Silva A, Rebelo I, Figueiredo A, Quintanilha A, Teixeira F. The inflammatory response in mild and in severe psoriasis. Br J Dermatol. 2004 May;150(5):917–28.
- Coutant DE, Hall SD. Disease-Drug Interactions in Inflammatory States via Effects on CYP-Mediated Drug Clearance. J Clin Pharmacol. 2018 Jul 1;58(7):849–63.
- 24. Chen Y, Chen Y, Zhao L, He H, Wei L, Lai W, et al. Albumin/Globulin Ratio as Yin-Yang in Rheumatoid Arthritis and Its Correlation to Inflamm-Aging Cytokines. J Inflamm Res. 2021;14:5501–11.
- Bartelink IH, Bet PM, Widmer N, Guidi M, Duijvelaar E, Grob B, et al. Elevated acute phase proteins affect pharmacokinetics in COVID-19 trials: Lessons from the CounterCOVID - imatinib study. CPT pharmacometrics Syst Pharmacol. 2021 Dec 1;10(12):1497–511.
- Ben-Hadj-Mohamed M, Khelil S, Ben Dbibis M, Khlifi L, Chahed H, Ferchichi S, et al. Hepatic Proteins and Inflammatory Markers in Rheumatoid Arthritis Patients. Iran J Public Health. 2017 Jul 17;46(8):1071.
- Juřica J, Žourková A. Dynamics and persistence of CYP2D6 inhibition by paroxetine. J Clin Pharm Ther. 2013 Aug;38(4):294–300.

- Liston HL, DeVane CL, Boulton DW, Risch SC, Markowitz JS, Goldman J. Differential time course of cytochrome P450 2D6 enzyme inhibition by fluoxetine, sertraline, and paroxetine in healthy volunteers. J Clin Psychopharmacol. 2002;22(2):169–73.
- Amchin J, Ereshefsky L, Zarycranski W, Taylor K, Albano D, Klockowski PM. Effect of venlafaxine versus fluoxetine on metabolism of dextromethorphan, a CYP2D6 probe. J Clin Pharmacol. 2001 Jan 1;41(4):443–51.
- Yang J, Liao M, Shou M, Jamei M, Yeo K, Tucker G, et al. Cytochrome p450 turnover: regulation of synthesis and degradation, methods for determining rates, and implications for the prediction of drug interactions. Curr Drug Metab. 2008 Jun 24;9(5):384–93.
- Schmitt C, Kuhn B, Zhang X, Kivitz AJ, Grange S. Disease–Drug–Drug Interaction Involving Tocilizumab and Simvastatin in Patients With Rheumatoid Arthritis. Clin Pharmacol Ther. 2011 May 23:89(5):735–40.
- 32. Kiiski JI, Neuvonen M, Kurkela M, Hirvensalo P, Hämäläinen K, Tarkiainen EK, et al. Solanidine is a sensitive and specific dietary biomarker for CYP2D6 activity. Hum Genomics. 2024 Dec 1;18(1).
- Magliocco G, Thomas A, Desmeules J,
   Daali Y. Phenotyping of Human CYP450
   Enzymes by Endobiotics: Current Knowledge
   and Methodological Approaches. Clin
   Pharmacokinet. 2019 Nov 1;58(11):1373–91.
- 34. Gravel S, Chiasson JL, Turgeon J, Grangeon A, Michaud V. Modulation of CYP450 Activities in Patients With Type 2 Diabetes. Clin Pharmacol Ther. 2019 Dec 1;106(6):1280–9.
- 35. Fisher CD, Lickteig AJ, Augustine LM, Ranger-Moore J, Jackson JP, Ferguson SS, et al. Hepatic cytochrome P450 enzyme alterations in humans with progressive stages of nonalcoholic fatty liver disease. Drug Metab Dispos. 2009 Oct;37(10):2087–94.
- 36. de Jong LM, Boussallami S, Sánchez-López E, Giera M, Tushuizen ME, Hoekstra M, et al. The impact of CYP2C19 genotype on phenoconversion by concomitant medication. Front Pharmacol. 2023:14.

- 37. Machavaram KK, Almond LM, Rostami-Hodjegan A, Gardner I, Jamei M, Tay S, et al. A physiologically based pharmacokinetic modeling approach to predict disease-drug interactions: Suppression of CYP3A by IL-6. Clin Pharmacol Ther. 2013 Aug 1;94(2):260– 8
- 38. Xu H, Murray M, McLachlan AJ. Influence of genetic polymorphisms on the pharmacokinetics and pharmaco-dynamics of sulfonylurea drugs. Curr Drug Metab. 2009/10/06. 2009;10(6):643–58.
- 39. Jiang X, Zhuang Y, Xu Z, Wang W, Zhou H. Development of a Physiologically Based Pharmacokinetic Model to Predict Disease-Mediated Therapeutic Protein-Drug Interactions: Modulation of Multiple Cytochrome P450 Enzymes by Interleukin-6. AAPS J. 2016 Mar 9;18(3):767–76.
- Lenoir C, Niederer A, Rollason V, Desmeules JA, Daali Y, Samer CF. Prediction of cytochromes P450 3A and 2C19 modulation by both inflammation and drug interactions using physiologically based pharmacokinetics. CPT pharmacometrics Syst Pharmacol. 2022 Jan 1;11(1):30–43.
- 41. Stader F, Battegay M, Sendi P, Marzolini C. Physiologically Based Pharmacokinetic Modelling to Investigate the Impact of the Cytokine Storm on CYP3A Drug Pharmacokinetics in COVID-19 Patients. Clin Pharmacol Ther. 2022 Mar 1;111(3):579–84.
- 42. Feick D, Rüdesheim S, Marok FZ, Selzer D, Loer HLH, Teutonico D, et al. Physiologically-based pharmacokinetic modeling of quinidine to establish a CYP3A4, P-gp, and CYP2D6 drug-drug-gene interaction network. CPT pharmacometrics Syst Pharmacol. 2023 Aug 1;12(8):1143–56.
- 43. Kanacher T, Lindauer A, Mezzalana E, Michon I, Veau C, Mantilla JDG, et al. A Physiologically-Based Pharmacokinetic (PBPK) Model Network for the Prediction of CYP1A2 and CYP2C19 Drug-Drug-Gene Interactions with Fluvoxamine, Omeprazole, S-mephenytoin, Moclobemide, Tizanidine, Mexiletine, Ethinylestradiol, and Caffeine. Pharmaceutics. 2020 Dec 1;12(12):1–15.
- 44. Wojtyniak JG, Selzer D, Schwab M, Lehr T. Physiologically Based Precision Dosing Approach for Drug-Drug-Gene Interactions: A Simvastatin Network Analysis. Clin Pharmacol Ther. 2021 Jan 1;109(1):201–11.

- 45. Kovar C, Kovar L, Rüdesheim S, Selzer D, Ganchev B, Kröner P, et al. Prediction of Drug-Drug-Gene Interaction Scenarios of (E)-Clomiphene and Its Metabolites Using Physiologically Based Pharmacokinetic Modeling, Pharmaceutics, 2022 Dec 1:14(12).
- 46. Evers R, Dallas S, Dickmann LJ, Fahmi OA, Kenny JR, Kraynov E, et al. Critical review of preclinical approaches to investigate cytochrome P450-mediated therapeutic protein drug-drug interactions and recommendations for best practices: A white paper. Vol. 41, Drug Metabolism and Disposition. American Society for Pharmacology and Experimental Therapy; 2013. p. 1598–609.
- Cicali EJ, Elchynski AL, Cook KJ, Houder JT, Thomas CD, Smith DM, et al. How to Integrate CYP2D6 Phenoconversion Into Clinical Pharmacogenetics: A Tutorial. Clin Pharmacol Ther. 2021 Sep 1;110(3):677–87.
- Bousman CA, Wu P, Aitchison KJ, Cheng T. Sequence2Script: A Web-Based Tool for Translation of Pharmacogenetic Data Into Evidence-Based Prescribing Recommendations. Front Pharmacol. 2021 Mar 18;12.
- Borges S, Desta Z, Jin Y, Faouzi A, Robarge JD, Philips S, et al. Composite functional genetic and comedication CYP2D6 activity score in predicting tamoxifen drug exposure among breast cancer patients. J Clin Pharmacol. 2010;50(4):450–8.
- 50. Lesche D, Mostafa S, Everall I, Pantelis C, Bousman CA. Impact of CYP1A2, CYP2C19, and CYP2D6 genotype- and phenoconversion-predicted enzyme activity on clozapine exposure and symptom severity. Pharmacogenomics J. 2020 Apr 1;20(2):192–201.
- Güzey C, Spigset O. Low serum concentrations of paroxetine in CYP2D6 ultrarapid metabolizers. J Clin Psychopharmacol. 2006 Apr;26(2):211–2.
- Lam YWF, Gaedigk A, Ereshefsky L, Alfaro CL, Simpson J. CYP2D6 inhibition by selective serotonin reuptake inhibitors: analysis of achievable steady-state plasma concentrations and the effect of ultrarapid metabolism at CYP2D6. Pharmacotherapy. 2002;22(8):1001-6.

212 CHAPTER 7



# **Appendices**

Nederlandse samenvatting Curriculum Vitae List of publications

### Nederlandse samenvatting

Niet iedereen reageert zoals gewenst op een geneesmiddelbehandeling. Bij 20-70% van de patiënten die geneesmiddelen gebruiken, wordt niet het beoogde effect bereikt of treden er bijwerkingen op. De traditionele 'one-size-fits-all'-aanpak voor het doseren van geneesmiddelen is daarom achterhaald. Gepersonaliseerde farmacotherapie wordt gezien als de vervanging van deze aanpak, met als doel zowel de werkzaamheid als de veiligheid van geneesmiddelen te verbeteren. Bij gepersonaliseerde farmacotherapie worden de unieke genetische, biologische en klinische kenmerken van een individuele patiënt meegenomen in de selectie van een juist geneesmiddel en de juiste dosering. Zo is bekend dat bijwerkingen kunnen worden verminderd als de dosering wordt afgestemd op het genetische profiel van een patiënt. Dit komt onder andere doordat er variatie bestaat in de activiteit van leverenzymen, zoals de cytochroom P450 (CYP) enzymen, die verantwoordelijk zijn voor de afbraak van geneesmiddelen. Sommige mensen breken een geneesmiddel sneller af dan anderen. Deze verschillen in afbraaksnelheid zijn gedeeltelijk terug te vinden in het DNA. Op basis van de activiteit van CYP-enzymen worden er vier verschillende fenotypen onderscheiden: snelle ((ultra) rapid), normale (normal), verlaagde (intermediate) of langzame (poor) metaboliseerders. Door met deze fenotypes rekening te houden kan een betere dosering worden vastgesteld of kan overgegaan worden op een ander passend medicijn. Voor specifieke combinaties van geneesmiddelen en genetische varianten zijn inmiddels richtlijnen beschikbaar die in de kliniek worden toegepast voor een meer gepersonaliseerde behandeling. Toch kan niet alle variabiliteit in de respons op geneesmiddelen worden verklaard door genetica. Naast erfelijke eigenschappen wordt de capaciteit van de lever om geneesmiddelen af te breken ook beïnvloed door niet-genetische factoren, zoals het gebruik van comedicatie, of de aanwezigheid van een infectie of chronische ontstekingsziekte. Deze niet-genetische factoren kunnen ervoor zorgen dat iemands werkelijke capaciteit om geneesmiddelen af te breken (fenotype) afwijkt van wat op basis van het DNA-profiel zou worden verwacht (genotype). Dit fenomeen wordt fenoconversie genoemd.

Om de invloed van niet-genetische factoren in de voorspellingen van het leverfenotype op te nemen, is het essentieel om een kwantitatief inzicht te krijgen in de omvang en duur van fenoconversie als gevolg van deze factoren. In dit proefschrift wordt de impact van niet-genetische factoren op het metabolisme van geneesmid-

216 APPENDICES

A

delen in de lever onderzocht. In deel I wordt ingegaan op de invloed van comedicatie op de voorspelling van het leverfenotype op basis van genetische informatie. In deel II worden de veranderingen die optreden in het geneesmiddelmetabolisme in de lever tijdens ontsteking behandeld. Dit deel omvat ten eerste een samenvatting van het preklinische bewijs voor de invloed van ontstekingsmediatoren op de levercapaciteit om geneesmiddelen af te breken. Vervolgens onderzoeken we in een in vitro levermodel hoe ontstekingsmediatoren de activiteit van verschillende enzymfamilies beïnvloeden die betrokken zijn bij de afbraak van geneesmiddelen. Ten derde creëren we een systematisch overzicht van de klinische en niet-klinische bewijsvoering voor het omkeren van ontstekingsgerelateerde veranderingen in de metabole capaciteit van de lever na behandeling met immunomodulerende therapieën. Deel III richt zich op de CYP-fenotyperingsmethode, waarmee veranderingen in enzymactiviteit bij mensen worden onderzocht. Hierbij wordt een cocktail van verschillende modelgeneesmiddelen gebruikt om de enzymactiviteit te meten. In dit deel wordt onderzocht hoe nauwkeurig deze methode veranderingen in enzymactiviteit kan detecteren, ook bij patiënten met ontstekingsziekten.

# Sectie I – De invloed van comedicatie op de genetische voorspelling van het leverfenotype

Het gebruik van comedicatie kan de nauwkeurigheid van de dosering van geneesmiddelen op basis van genetica verminderen. Dit komt doordat sommige geneesmiddelen de activiteit van CYP-enzymen beïnvloeden. Remmers zorgen voor een verminderde metabole activiteit van het betreffende enzym, inductoren verhogen juist de metabole activiteit. Hierdoor verandert de metabole levercapaciteit die op basis van genetica was voorspeld (fenoconversie). Een groot klinisch onderzoek toonde aan dat 32–47% van de fenoconversie voor CYP2C19 en CYP2D6 kon worden toegeschreven aan gelijktijdig medicijngebruik met een remmer of inductor. Het integreren van kennis over genetica en comedicatie om zo een goede voorspelling van de levercapaciteit te doen blijft echter een complexe uitdaging. Om concrete doseringsaanbevelingen voor deze potentiële geneesmiddel-geneesmiddel-genotype interacties te kunnen geven, is het cruciaal om inzicht te krijgen in de fenoconversie die optreedt na toediening van remmende of inducerende comedicatie en om te bekijken of deze interactie verschillend is voor verschillende genotypen. Daarom hebben we in **hoofdstuk 2**, met behulp

NEDERLANDSE SAMENVATTING 217

van humane levermicrosomen (kleine blaasjes die enzymen uit het endoplasmatisch reticulum van levercellen bevatten), de fenoconversie gekwantificeerd in verschillende CYP2C19-genotypegroepen na toediening van een sterke remmer (fluvoxamine), matige remmer (omeprazol of voriconazol) of zwakke remmer (pantoprazol) van CYP2C19. De relatieve inhibitie van de CYP2C19-activiteit door de remmers was consistent voor alle genotypen, maar de mate van fenoconversie verschilde per genotype; voriconazol veroorzaakte bijvoorbeeld een verlaagd of langzaam CYP2C19 fenotype bij 50% van de genetisch voorspelde normale metaboliseerders, maar bij slechts 14% van de genetisch voorspelde snelle metaboliseerders. Wij concludeerden vervolgens dat de mate van fenoconversie afhankelijk is van: 1) de sterkte van de remmer, aangezien fenoconversie naar een lager metabool fenotype vaker voorkwam bij sterkere CYP2C19-remmers, en: 2) de basale CYP2C19-activiteit, die deels wordt voorspeld door het genotype, maar ook sterk afhankelijk is van o.a. de aanwezigheid van leverziekten. Het meewegen van de invloed van comedicatie in fenotypevoorspellingen is dus essentieel voor de optimalisatie van op farmacogenetica gebaseerde gepersonaliseerde therapieën.

# Sectie II – (Pre)klinische evaluatie van ontstekingsgerelateerde veranderingen in het metabolisme van geneesmiddelen

Een andere niet-genetische factor die het metabolisme van de lever beïnvloedt, is de aanwezigheid van een ontsteking. Dit kan een ontsteking zijn ten gevolge van een acute infectie, zoals COVID-19, maar ook ten gevolge van een chronische inflammatoire aandoening zoals reuma. De invloed van ontstekingen op het metabolisme van geneesmiddelen wordt algemeen erkend, maar nog niet goed begrepen. Een beter begrip van de invloed van ontsteking op de werking van geneesmiddelafbrekende enzymen begint bij het samenvatten van wat er nu bekend is in de literatuur. Dat is gepresenteerd in **hoofdstuk 3**. In het bijzonder bespreken we het bewijs dat verzameld is met behulp van humane in vitro modellen voor het effect van ontstekingsmediatoren op de hoeveelheid en activiteit van klinisch relevante CYP-enzymen. Hieruit bleek dat CYP-enzymen een uiteenlopende gevoeligheid vertonen voor de effecten van ontstekingsmediatoren, waarbij CYP3A4 het meest wordt beïnvloed door inflammatie. De mate van remming blijkt ook sterk afhankelijk van de ontstekingsmediator. Ook hebben we in dit hoofdstuk samengevat wat de mechanismes zijn die veranderingen in levermetabolisme bewerkstelligen.

218 APPENDICES

A

Onderdrukking van CYP-enzymen tijdens ontsteking kan plaatsvinden door: 1) transcriptionele downregulatie van nucleaire factoren en andere transcriptiefactoren die de CYP's reguleren; 2) interferentie met dimerisatie of translocatie van deze (nucleaire) transcriptiefactoren; 3) verandering in de signalering van leververrijkte CCAAT-enhancer-bindende eiwitten (C/EBP); 4) directe regulatie van CYP-expressie door Nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-кB), of; 5) post-transcriptionele mechanismen. Hierin is de algemeen aanvaarde opvatting dat transcriptionele veranderingen de belangrijkste regulerende mechanismes zijn die verantwoordelijk zijn voor veranderde CYP-activiteit tijdens ontsteking. Uiteindelijk kan een beter begrip van door inflammatie geïnduceerde fenoconversie bijdragen aan het optimaliseren van de behandeling voor de individuele patiënt.

De effecten van ontsteking op de CYP-enzymen zijn het meest onderzocht. Echter, ook andere enzymfamilies, zoals de uridine difosfaat-glucuronosyltransferases (UGT's), flavine-bevattende monooxygenasen (FMO's) en carboxylesterases (CES's), spelen een belangrijke rol bij de afbraak van geneesmiddelen. De mate waarin deze enzymfamilies gevoelig zijn voor de invloed van ontsteking is nog onvoldoende bekend. Daarom gebruiken we in hoofdstuk 4 het HepaRG levermodel om te bestuderen hoe verschillende geneesmiddelmetaboliserende enzymfamilies gevoelig zijn voor de effecten van de ontstekingsmediatoren interleukine (IL)-6 en IL-1β, en stellen een hiërarchie vast van hun sensitiviteit. Concluderend toonde onze studie aan dat UGT-, FMO- en CES-enzymen minder gevoelig zijn voor de effecten van de pro-inflammatoire cytokinen IL-6 en IL-1β dan CYPenzymen. Daarnaast benadrukken de resultaten dat transcriptieveranderingen in de expressie van geneesmiddel-metaboliserende enzymen sterk voorspellend zijn voor veranderingen in enzymactiviteit. Dit pleit tegen het belang van inflammatiegerelateerde post-transcriptionele modificaties. Patiënten met acute of chronische inflammatoire aandoeningen lopen dus mogelijk risico op veranderingen in hun geneesmiddelmetabolisme, waarbij de omvang van deze veranderingen waarschijnlijk afhangt van de betrokken enzymfamilies die verantwoordelijk zijn voor de afbraak van het geneesmiddel.

Immunomodulerende therapieën, zoals monoklonale antilichamen die gericht zijn tegen specifieke cytokines of hun receptoren, worden steeds vaker gebruikt om inflammatoire ziekten te behandelen. Deze therapieën werken door de immuun-

NEDERLANDSE SAMENVATTING 219

respons te beïnvloeden en te reguleren, waardoor ontstekingen in het lichaam worden verminderd. Aangezien inflammatoire mediatoren een sleutelrol spelen in de downregulatie van geneesmiddelmetaboliserende enzymen, kan remming van inflammatie deze effecten op de lever omkeren, wat leidt tot herstel van de metabole activiteit van CYP-enzymen. In hoofdstuk 5 vatten we systematisch samen wat het preklinische en klinische bewijs is voor deze mogelijke interactie, en of het als risico wordt opgenomen in de geneesmiddellabels uitgegeven door de United States Food and Drug administration (FDA) en de European Medicines Agency (EMA). Daartoe worden in deze review de resultaten van klinische studies met monoklonale antilichamen en tyrosinekinaseremmers samengevat, waarbij de potentiële veranderingen in geneesmiddelblootstelling na interventie met immunomodulerende therapieën worden onderzocht. Hieruit bleek dat het interactierisico afhankelijk is van zowel de cytokine die de ontstekingsziekte drijft als de ontstekingsziekte zelf. Het grootste interactierisico werd gedocumenteerd voor de monoklonale antilichamen die de effecten van IL-6, TNF-α en IL-1β neutraliseren bij ziekten met systemische ontsteking, alhoewel voor de laatste twee klinisch bewijs ontbreekt. Hoe tyroskinekinaseremmers die de effecten van ontsteking tegengaan de metabole capaciteit van de lever beïnvloeden, is nog niet onderzocht in patiënten en het interactierisico is dus onbekend. Uit analyse van de geneesmiddellabels bleek dat er in 62% van de gevallen discrepanties bestaan tussen de vermelde risico's door de EMA en FDA en dat de autoriteiten hun risicobeoordeling niet altijd baseren op hetzelfde beschikbare niet-klinische en klinische bewijs. Over het algemeen is de FDA conservatiever in zijn aanpak, en vermeldt de FDA sneller een risico voor een interactie in de geneesmiddellabels.

### Sectie III – In vivo-tools om veranderingen in enzymactiviteit tijdens (ontstekings)ziekten te bestuderen

Om te kwantificeren in welke mate de enzymactiviteit tijdens ziekte verandert in patiënten, wordt vaak de CYP-fenotyperingscocktail toegepast. Met deze aanpak krijgen patiënten een cocktail van modelsubstraten voor bepaalde CYP-enzymen toegediend, waarna de plasmaklaring van dit substraat gebruikt wordt als indicatie voor de activiteit van het desbetreffende CYP-enzym. De klaring van midazolam wordt bijvoorbeeld gebruikt als indicatie voor CYP3A4-activiteit. De fenotyperingsaanpak gaat ervan uit dat veranderingen in de plasmaklaring van modelsub-

220 APPENDICES

4

straten louter worden veroorzaakt door veranderingen in CYP-enzymactiviteit. De plasmaklaring wordt echter eveneens beïnvloed door factoren als eiwitbinding van het modelsubstraat, de bloed-tot-plasma-verhouding en de hepatische bloedtoevoer, die allen beïnvloed kunnen worden door ziekten. Het is daarom van belang om te bestuderen in hoeverre veranderingen in plasmaklaring inderdaad direct te herleiden zijn naar veranderingen in enzymactiviteit in patiënten met bijvoorbeeld een ontstekingsziekte. Hoofdstuk 6 gaat in op deze vraag. Door middel van een hepatisch pysiologically-based pharmacokinetic (PBPK) model konden we aantonen dat de plasmaklaring van CYP-modelsubstraten inderdaad zeer gevoelig is voor veranderingen in CYP-activiteit. Veranderingen in enzymactiviteit van -90% tot +150% leidden vrijwel proportioneel tot dezelfde veranderingen in plasmaklaring. Echter is de plasmaklaring van modelsubstraten eveneens gevoelig voor veranderingen in eiwitbinding. Dit geldt voornamelijk voor modelsubstraten die sterk gebonden zijn aan plasma-eiwitten. Omdat eiwitbinding kan veranderen tijdens inflammatoire omstandigheden, moeten we hiervoor corrigeren wanneer we klaring van modelsubstraten gebruiken als indicatie voor CYP-activiteit in patiënten. Dit kan gedaan worden met het bijgeleverde R-script in hoofdstuk 6.

#### Conclusie

Dit proefschrift onderstreept het belang van het uitbreiden van CYP-fenotypevoorspellingen door, naast genetische informatie, ook de invloed van niet-genetische factoren zoals comedicatie en inflammatoire status mee te wegen. Hoewel de implementatie van farmacogenetica aanzienlijke vooruitgang heeft geboekt in het personaliseren van farmacotherapie, blijft het een uitdaging om deze voorspellingen verder te verfijnen, zodat ze de levercapaciteit om geneesmiddelen af te breken beter weerspiegelen. Dit proefschrift draagt bij aan een dieper kwantitatief inzicht in hoe ontsteking en comedicatie het geneesmiddelmetabolisme beïnvloeden. Hiermee levert het uiteindelijk een bijdrage aan de ontwikkeling van nauwkeurigere leverfenotypevoorspellingen en de optimalisatie van gepersonaliseerde behandelstrategieën.

### Curriculum Vitae

Laura de Jong was born on the 21<sup>st</sup> of September, 1997 in Den Haag. After graduating from Hofstad Lyceum in 2015, she started with her bachelor Bio-Pharmaceutical Sciences at Leiden University, followed by the master Bio-Pharmaceutical Sciences. During her master, she performed an internship at the division of BioTherapeutics under supervision of dr. Menno Hoekstra, where she investigated a potential new target to treat atherosclerosis. She then moved to Belgium for a six-month internship at Janssen Pharmaceutica's Functional Genomics department, focusing on developing an in vitro cell system to study ternary complex formation of PROTACs. Laura completed her Master's degree summa cum laude. In September 2020, she started her PhD at the Leiden Academic Center for Drug Research under the supervision of dr. Martijn Manson, prof.dr. Robert Rissmann and prof.dr. Jesse Swen, aiming to better understand how nongenetic factors affect hepatic drug metabolism.

222 APPENDICES

### List of publications

**de Jong LM**, Jiskoot W, Swen JJ, Manson ML. Distinct Effects of Inflammation on Cytochrome P450 Regulation and Drug Metabolism: Lessons from Experimental Models and a Potential Role for Pharmacogenetics. Genes (Basel). 2020 Dec 16;11(12):1–24. doi: 10.3390/genes11121509.

**de Jong LM**, Klomp SD, Treijtel N, Rissmann R, Swen JJ, Manson ML. A systematic review on disease-drug-drug interactions with immunomodulating drugs: A critical appraisal of risk assessment and drug labelling. Br J Clin Pharmacol. 2022 Oct;88(10):4387–402. doi: 10.1111/bcp.15372.

**de Jong LM**, Boussallami S, Sánchez-López E, Giera M, Tushuizen ME, Hoekstra M, Hawinkels LJAC, Rissmann R, Swen JJ, Manson ML. The impact of CYP2C19 genotype on phenoconversion by concomitant medication. Front Pharmacol. 2023 Jun 8;14:1201906. doi: 10.3389/fphar.2023.1201906

**de Jong LM**, Harpal C, Berg DVD, Hoekstra M, Peter NJ, Rissmann R, Swen JJ, Manson ML. CYP P450 and non-CYP P450 Drug Metabolizing Enzyme Families Exhibit Differential Sensitivities towards Proinflammatory Cytokine Modulation. Drug Metab Dispos. 2024 Nov 15;52(12):1429–37. doi: 10.1124/dmd.124.001867.

**de Jong LM**, van de Kreeke M, Ahmadi M, Swen JJ, Knibbe CAJ, van Hasselt JGC, Manson ML, Krekels EHJ. Changes in Plasma Clearance of CYP450 Probe Drugs May Not be Specific for Altered In Vivo Enzyme Activity Under (Patho) Physiological Conditions: How to Interpret Findings of Probe Cocktail Studies. Clin Pharmacokinet. 2024 Nov;63(11):1585–95. doi: 10.1007/s40262-024-01426-8.

Hoekstra M, Nahon JE, **de Jong LM**, Kröner MJ, de Leeuw LR, Van Eck M. Inhibition of PRMT3 activity reduces hepatic steatosis without altering atherosclerosis susceptibility in apoE knockout mice. Biochim Biophys Acta Mol Basis Dis. 2019 Jun 1;1865(6):1402–9. doi: 10.1016/j.bbadis.2019.02.012.

LIST OF PUBLICATIONS 223

4

**de Jong LM**, Zhang Z, den Hartog Y, Sijsenaar TJP, Martins Cardoso R, Manson ML, Hankemeier T, Lindenburg PW, Salvatori DCF, Van Eck M, Hoekstra M. PRMT3 inhibitor SGC707 reduces triglyceride levels and induces pruritus in Western-type diet-fed LDL receptor knockout mice. Sci Rep. 2022 Jan 10;12(1):483. doi: 10.1038/s41598-021-04524-w.

Hoekstra M, **de Jong LM**, van der Geest R, de Leeuw LR, Krisnamurthi R, Geerling JJ, Van Eck M. LXR Agonist T0901317's Hepatic Impact Overrules Its Atheroprotective Action in Macrophages, Driving Early Atherogenesis in Chow-Diet-Fed Male Apolipoprotein E Knockout Mice. Biomolecules. 2024 Apr 2;14(4):429. doi: 10.3390/biom14040429.

224 APPENDICES