Physiologically based pharmacokinetic (PBPK) modeling of the role of CYP2D6 polymorphism for metabolic phenotyping with dextromethorphan

Jan Grzegorzewski*, Janosch Brandhorst and Matthias König

Institute for Theoretical Biology, Institute of Biology, Humboldt University, Berlin, Germany

The cytochrome P450 2D6 (CYP2D6) is a key xenobiotic-metabolizing enzyme involved in the clearance of many drugs. Genetic polymorphisms in CYP2D6 contribute to the large inter-individual variability in drug metabolism and could affect metabolic phenotyping of CYP2D6 probe substances such as dextromethorphan (DXM). To study this question, we (i) established an extensive pharmacokinetics dataset for DXM; and (ii) developed and validated a physiologically based pharmacokinetic (PBPK) model of DXM and its metabolites dextrorphan (DXO) and dextrorphan O-glucuronide (DXO-Glu) based on the data. Drug-gene interactions (DGI) were introduced by accounting for changes in CYP2D6 enzyme kinetics depending on activity score (AS), which in combination with AS for individual polymorphisms allowed us to model CYP2D6 gene variants. Variability in CYP3A4 and CYP2D6 activity was modeled based on in vitro data from human liver microsomes. Model predictions are in very good agreement with pharmacokinetics data for CYP2D6 polymorphisms, CYP2D6 activity as described by the AS system, and CYP2D6 metabolic phenotypes (UM, EM, IM, PM). The model was applied to investigate the genotype-phenotype association and the role of CYP2D6 polymorphisms for metabolic phenotyping using the urinary cumulative metabolic ratio (UCMR), DXM/(DXO + DXO-Glu) based on the data. Drug-gene interactions (DGI) were introduced by accounting for changes in CYP2D6 enzyme kinetics depending on activity score (AS), which in combination with AS for individual polymorphisms allowed us to model CYP2D6 gene variants. Variability in CYP3A4 and CYP2D6 activity was modeled based on in vitro data from human liver microsomes. Model predictions are in very good agreement with pharmacokinetics data for CYP2D6 polymorphisms, CYP2D6 activity as described by the AS system, and CYP2D6 metabolic phenotypes (UM, EM, IM, PM). The model was applied to investigate the genotype-phenotype association and the role of CYP2D6 polymorphisms for metabolic phenotyping using the urinary cumulative metabolic ratio (UCMR), DXM/(DXO + DXO-Glu). The effect of parameters on UCMR was studied via sensitivity analysis. Model predictions indicate very good robustness against the intervention protocol (i.e. application form, dosing amount, dissolution rate, and sampling time) and good robustness against physiological variation. The model is capable of estimating the UCMR dispersion within and across populations depending on activity scores. Moreover, the distribution of UCMR and the risk of genotype-phenotype mismatch could be estimated for populations with known CYP2D6 genotype frequencies. The model can be applied for individual prediction of UCMR and metabolic phenotype based on CYP2D6 genotype. Both, model and database are freely available for reuse.
1 Introduction

The cytochrome P450 (CYPs) superfamily of enzymes has a central role in the clearance of many substances and drugs, with the isof orm 2D6 (CYP2D6) being one of the most important xenobiotic-metabolizing enzymes. CYP2D6 is involved in the clearance of around 20% of the most prescribed drugs (Saravanakumar et al., 2019) including antiarrhythmics having a small therapeutic range (e.g., flecainide, procanamide, mexiletine), anticancer agents (e.g., tamoxifen), antidepressants (e.g., citalopram, fluoxetine, duloxetine: venlafaxine), antipsychotics (e.g., aripiprazole, haloperidol, thioridazine), β-blockers (metoprolol), analgesics (tramadol, oxytocin, codeine), and antitussives (dextromethorphan) (Hurtado et al., 2020; Kibaly et al., 2021). CYP2D6-mediated drug response exhibits a particularly large inter-individual variability which poses a challenge for personalized dosage of medication by underdosing on the one hand and toxic side effects on the other. The activity of CYP2D6 is known to be majorly dependent on genetic variants (Berm et al., 2013; Preskorn et al., 2013; Shah and Smith, 2015) with polymorphism of CYP2D6 being related to the risk of adverse effects, non-response during treatment, and death by drug intoxication (Gasche et al., 2004; Kawanishi et al., 2004; Rau et al., 2004; Zackrisson et al., 2010).

In the late 70s, a polymorphism in debrisoquine hydroxylation (Mahgoub et al., 1977) and sparteine oxidation (Eichelbaum et al., 1979) was discovered and subsequently attributed to allelic variants of the CYP2D6 gene. In the following years, CYP2D6 became one of the most studied drug-metabolizing enzymes. Genetic variants were classified into distinct phenotypes and subjects carrying combinations of these variants were categorized as poor metabolizer (gPM), intermediate metabolizer (gIM), rapid metabolizer (gUM), and ultra rapid metabolizer (gUM) (Zanger et al., 2004; Gaedigk et al., 2007; Nofziger and Paulmichl, 2018; Gaedigk et al., 2019). Of note, AS specifics are still under heavy debate and regularly updated (Caudle et al., 2020). A multitude of population studies have been conducted to identify and associate allele variants with metabolic phenotypes within and across populations (Gaedigk et al., 2017). Over 130 CYP2D6 star (*) allele haplotypes have been identified and subsequently cataloged by the Pharmacogene Variation (PharmVar) Consortium into PharmGKB with their respective activity score contribution (Gaedigk et al., 2018b; Whirl-Carrillo et al., 2021).

Various methods exist for the metabolic phenotyping based on test substances. The gold standard is plasma concentration sampling of probe substances and their metabolites at various time points after the administration. (Partial) clearance rates and the relative enzyme activities can be calculated from these plasma time profiles. Simplified methods have been established for many probe substances which do not require repeated sampling of blood, e.g., the (cumulative) metabolic ratios between the probe substance and one or several of its metabolites at a single time point in blood, plasma, or urine are utilized as such proxy measures. Large-scale population studies often tend to employ urinary ratios of metabolites. Alternatively, sampling of saliva and breath are worth considering (De Kesel et al., 2016). Probe substances for metabolic phenotyping of CYP2D6 are debrisoquine, dextromethorphan, metoprolol, or sparteine (Frank et al., 2007; Fuhr et al., 2007). Bufuralol is less popular but well suited for in vitro investigations due to its fluorescent properties (Zanger et al., 2004). Although debrisoquine and sparteine have excellent properties for CYP2D6 phenotyping, they have been withdrawn from clinical use in most countries and are therefore no longer readily available. Frequently in use for the phenotyping of CYP2D6 activity are metoprolol and dextromethorphan.

Dextromethorphan (DMX) is an over-the-counter, antitussive, non-narcotic, synthetic analog of codeine affecting the activity of numerous channels and receptors in the brain that trigger the cough reflex (Silva and Dinis-Oliveira, 2020). It is generally well-tolerated, considered safe in therapeutic dosage, and easily available (Fuhr et al., 2007). Besides therapeutic purposes, DMX is most commonly applied as a probe substance for CYP2D6 phenotyping, alone or with other probe substances in a cocktail. DMX can be administered orally and intravenously, has low bioavailability (≈50%) and a rapid first-pass effect in the intestine and liver. Typically only about half of

Higher activity scores than 2 and additional complexity arise from copy number variation (CNV), chimeras, and hybrids with the pseudo gene CYP2D7. This can result in ambiguities and difficulties in the assignment of the correct diplotype and activity score (Gaedigk et al., 2007; Nozfiger and Paulmichl, 2018; Gaedigk et al., 2019). Of note, AS specifics are still under heavy debate and regularly updated (Caudle et al., 2020). A multitude of population studies have been conducted to identify and associate allele variants with metabolic phenotypes within and across populations (Gaedigk et al., 2017). Over 130 CYP2D6 star (*) allele haplotypes have been identified and subsequently cataloged by the Pharmacogene Variation (PharmVar) Consortium into PharmGKB with their respective activity score contribution (Gaedigk et al., 2018b; Whirl-Carrillo et al., 2021).

Various methods exist for the metabolic phenotyping based on test substances. The gold standard is plasma concentration sampling of probe substances and their metabolites at various time points after the administration. (Partial) clearance rates and the relative enzyme activities can be calculated from these plasma time profiles. Simplified methods have been established for many probe substances which do not require repeated sampling of blood, e.g., the (cumulative) metabolic ratios between the probe substance and one or several of its metabolites at a single time point in blood, plasma, or urine are utilized as such proxy measures. Large-scale population studies often tend to employ urinary ratios of metabolites. Alternatively, sampling of saliva and breath are worth considering (De Kesel et al., 2016). Probe substances for metabolic phenotyping of CYP2D6 are debrisoquine, dextromethorphan, metoprolol, or sparteine (Frank et al., 2007; Fuhr et al., 2007). Bufuralol is less popular but well suited for in vitro investigations due to its fluorescent properties (Zanger et al., 2004). Although debrisoquine and sparteine have excellent properties for CYP2D6 phenotyping, they have been withdrawn from clinical use in most countries and are therefore no longer readily available. Frequently in use for the phenotyping of CYP2D6 activity are metoprolol and dextromethorphan.

Dextromethorphan (DMX) is an over-the-counter, antitussive, non-narcotic, synthetic analog of codeine affecting the activity of numerous channels and receptors in the brain that trigger the cough reflex (Silva and Dinis-Oliveira, 2020). It is generally well-tolerated, considered safe in therapeutic dosage, and easily available (Fuhr et al., 2007). Besides therapeutic purposes, DMX is most commonly applied as a probe substance for CYP2D6 phenotyping, alone or with other probe substances in a cocktail. DMX can be administered orally and intravenously, has low bioavailability (≈50%) and a rapid first-pass effect in the intestine and liver. Typically only about half of

Higher activity scores than 2 and additional complexity arise from copy number variation (CNV), chimeras, and hybrids with the pseudo gene CYP2D7. This can result in ambiguities and difficulties in the assignment of the correct diplotype and activity score (Gaedigk et al., 2007; Nozfiger and Paulmichl, 2018; Gaedigk et al., 2019). Of note, AS specifics are still under heavy debate and regularly updated (Caudle et al., 2020). A multitude of population studies have been conducted to identify and associate allele variants with metabolic phenotypes within and across populations (Gaedigk et al., 2017). Over 130 CYP2D6 star (*) allele haplotypes have been identified and subsequently cataloged by the Pharmacogene Variation (PharmVar) Consortium into PharmGKB with their respective activity score contribution (Gaedigk et al., 2018b; Whirl-Carrillo et al., 2021).

Various methods exist for the metabolic phenotyping based on test substances. The gold standard is plasma concentration sampling of probe substances and their metabolites at various time points after the administration. (Partial) clearance rates and the relative enzyme activities can be calculated from these plasma time profiles. Simplified methods have been established for many probe substances which do not require repeated sampling of blood, e.g., the (cumulative) metabolic ratios between the probe substance and one or several of its metabolites at a single time point in blood, plasma, or urine are utilized as such proxy measures. Large-scale population studies often tend to employ urinary ratios of metabolites. Alternatively, sampling of saliva and breath are worth considering (De Kesel et al., 2016). Probe substances for metabolic phenotyping of CYP2D6 are debrisoquine, dextromethorphan, metoprolol, or sparteine (Frank et al., 2007; Fuhr et al., 2007). Bufuralol is less popular but well suited for in vitro investigations due to its fluorescent properties (Zanger et al., 2004). Although debrisoquine and sparteine have excellent properties for CYP2D6 phenotyping, they have been withdrawn from clinical use in most countries and are therefore no longer readily available. Frequently in use for the phenotyping of CYP2D6 activity are metoprolol and dextromethorphan.

Dextromethorphan (DMX) is an over-the-counter, antitussive, non-narcotic, synthetic analog of codeine affecting the activity of numerous channels and receptors in the brain that trigger the cough reflex (Silva and Dinis-Oliveira, 2020). It is generally well-tolerated, considered safe in therapeutic dosage, and easily available (Fuhr et al., 2007). Besides therapeutic purposes, DMX is most commonly applied as a probe substance for CYP2D6 phenotyping, alone or with other probe substances in a cocktail. DMX can be administered orally and intravenously, has low bioavailability (≈50%) and a rapid first-pass effect in the intestine and liver. Typically only about half of
the dose is recovered in urine over at least 12 h after administration, primarily as glucuronides (Schadel et al., 1995; Capon et al., 1996; Tennené et al., 1999; Strauch et al., 2009). In the systemic circulation, ≈55–65% of DXM is non-specifically bound to plasma proteins (Lutz and Isoherranen, 2012; Taylor et al., 2016).

The biotransformation of DXM is mostly confined to the liver, where DXM is O-demethylated by CYP2D6 to the active metabolite dextrorphan (DXO). Subsequently to O-demethylation, most of the DXO is rapidly transformed via UDP-glucuronosyltransferase (UGT) to dextrorphan O-glucuronide (DXO-Glu) and excreted via the urine. In individuals without any functional variant of CYP2D6, the metabolism of DXM to DXO is extremely slow but still present. Apparently, the O-demethylation is not exclusively mediated by CYP2D6, and it has been demonstrated in vitro that O-demethylation of DXM can be marginally mediated by CYP3A4, CYP3A5 and CYP2C9 (von Moltke et al., 1998; McGinnity et al., 2000; Takashima et al., 2005; Yu and Haining, 2001). In line with this observation, inhibition of CYP2D6, e.g., barely affects poor metabolizer (Pope et al., 2004). The second pathway of DXM metabolization goes via N-demethylation to 3-methoxymorphinan which is mainly catalyzed via CYP3A4. Subsequently, 3-methoxymorphinan and DXO are biotransformed to 3-hydroxymorphinan which is then rapidly transformed via glucuronidation to hydroxymorphinan O-glucuronide and excreted in the urine. The urinary cumulative metabolic ratio (UCMR) of DXM (to its metabolites DXM/(DXO + DXO-Glu)) is a widely applied measure for the in vivo CYP2D6 phenotyping.

An crucial question for metabolic phenotyping and liver function testing is how CYP2D6 polymorphisms affect the pharmacokinetics of DXM and metabolic phenotyping based on DXM, such as the UCMR. The objective of this work was to answer this question by the means of physiologically based pharmacokinetic (PBPK) modeling of DXM.

2 Material and methods

2.1 Pharmacokinetics database of DXM

Pharmacokinetics data of DXM was systematically curated from literature for model development, parameterization, and validation. Curation efforts were mainly focused on concentration-time profiles of DXM, DXO, and DXO-Glu in plasma or serum and their amounts or ratios in urine. The data is accompanied by metadata on the investigated subjects and groups (e.g., CYP2D6 genotype or activity score) and the applied intervention (e.g., dose and application form of DXM). All data was curated using an established curation pipeline (Grzegorzewski et al., 2022) and is available via the pharmacokinetics database PK-DB (https://pk-db.com) (Grzegorzewski et al., 2021). As a first step, a PubMed search for the pharmacokinetics of dextromethorphan in combination with genotyping and/or phenotyping was performed with the search query https://pubmed.ncbi.nlm.nih.gov/?term=dextromethorphan+AND%2Bphenotype+OR+phenotyping%29+AND+genotype. The literature corpus was extended with drug cocktail studies from PK-DB (Grzegorzewski et al., 2022), secondary literature from references, and results from PKPDAI with the search query https://app.pkpdaip.com/?term=dextromethorphan (Gonzalez Hernandez et al., 2021). Data was selected and curated based on eligibility criteria, see below. During the curation process, the initial corpus was updated by additional publications from the references and citations. A subset of the studies only reported pharmacokinetic parameters without timecourses. These studies were curated but not further used in the following analyses.

To be eligible, studies had to report in vivo pharmacokinetics data for adult (age ≥18) humans after administration of DXM or DXM hydrobromide. The application route of DXM was restricted to oral (PO) or intravenous (IV). All application forms (e.g., tablet, capsule, solution) were accepted. No restrictions were imposed on the dosing amount of DXM or coadministrations of other substances. Studies containing coadministrations that inhibit or induce the pharmacokinetics of DXM were identified during the modeling process and excluded. The relevant outcome measures are concentration-time profiles in plasma, serum, and urine amounts of DXM, DXM metabolites, or metabolic ratios of metabolites such as UCMR. Studies containing pharmacokinetic parameters of DXM and its metabolites (e.g., clearance, half-life, AUC) and (urinary cumulative) metabolic ratios of DXM and its metabolites were included. Data containing timecourses and CYP2D6 genotype information were prioritized. Non-healthy subjects were excluded if the disease is known to affect the pharmacokinetics of DXM or DXM metabolites. Study B from the PhD thesis of Frank (2009) highly deviates from the remaining data and was therefore excluded. Further, Wyen et al. (2008) was identified as a duplicate of Study E from the PhD thesis of Frank (2009) and excluded. The final set of curated studies used in the presented analyses is provided in Table 1.

For the selection and evaluation of studies from the literature, the PRISMA-ScR guidelines were adopted where applicable (Tricco et al., 2018). The initial corpus contained 404 studies. After screening, application of eligibility criteria, and prioritization, a total of 47 studies were curated (see Supplementary Figure S1). Of these studies, 36 contained data used in the present work (Table 1).

2.2 PBPK model of DXM

The PBPK model of DXM, DXO, and DXO-Glu (Figure 1) was encoded in the Systems Biology Markup Language (SBML) (Hucka et al., 2019; Keating et al., 2020). For development and visualization, sbmlutils (König, 2021b) and cy3sbml (König et al.,
| Reference            | PK-DB     | PMID            | DXM application | Dosing protocol | Description                                                                 |
|----------------------|-----------|-----------------|-----------------|-----------------|-----------------------------------------------------------------------------|
| Abdelrahman et al.   | PKDB000573| 10340911        | DXM             | Oral (syrup): 0.3 mg/kg | Investigation of terbinafine as a CYP2D6 inhibitor in vivo                  |
| Abdullatif et al.    | PKDB00074 | 20881950        | DXM hydrobromide| Oral (capsule): 30 mg | Assessment of activity levels for CYP2D6*1, CYP2D6*2, and CYP2D6*4 genes by population pharmacoenergetics of dextromethorphan |
| Armani et al.        | PKDB00428 | 10340911        | DXM             | Oral (NR): 30 mg | The antitussive effect of dextromethorphan in relation to CYP2D6 activity   |
| Barnhart. (1980)     | PKDB000575| 7423506         | DXM hydrobromide| Oral (capsule): 30 mg | The urinary excretion of dextromethorphan and three metabolites in dogs and humans |
| Capon et al. (1996)  | PKDB00576 | 8841152         | DXM hydrobromide| Oral (NR): 30 mg | The antitussive effect of dextromethorphan in relation to CYP2D6 activity   |
| Chen et al. (2017)   | PKDB00577 | 28512430        | DXM             | Oral (tablet): 15 mg + water 300 ml | CYP2D6 phenotyping using urine, plasma, and saliva metabolic ratios to assess the impact of CYP2D6*10 on inter-individual variation in a Chinese population |
| Chládek et al. (2000)| PKDB00578 | 11214771        | DXM hydrobromide| Oral (syrup): 30 mg | In vivo indices of CYP2D6 activity: comparison of dextromethorphan metabolic ratios in 4-h urine and 3-h plasma | Bioavailability of dextromethorphan (as dextrorphan) from sustained release formulations in the presence of guaifenesin in human volunteers |
| Demirbas et al. (1998)| PKDB00579| 9840216         | DXM hydrobromide| Oral (sustained release tablet): 60 mg | Lessons from Cuba for global precision medicine: CYP2D6 genotype is not a robust predictor of CYP2D6 ultrarapid metabolism |
| Dorado et al. (2017) | PKDB00580 | 28271978        | DXM             | Oral (NR): 15 mg | Drug metabolism and disposition: the biological fate of chemicals |
| Doroshyenko et al.   | PKDB00138 | 23401474        | DXM             | Oral (capsule): 30 mg | Intravenous dextromethorphan to human volunteers: relationship between pharmacoenergetics and anti-hyperalgesic effect |
| Duedahl et al. (2005)| PKDB00097 | 15661445        | DXM             | Intravenous: 0.5 mg/kg | A phenotype-genotype approach to predicting CYP450 and P-glycoprotein drug interactions with the mixed inhibitor/inducer tipranavir/ritonavir |
| Dumond et al. (2010) | PKDB00499 | 20147896        | DXM             | Oral (solution): 30 mg | Determination of dextromethorphan and dextrophan in human plasma by liquid chromatography/tandem mass spectrometry |
| Edwards et al. (2017)| PKDB00496 | 28808886        | DXM             | Oral (capsule): 30 mg | Determination of competence for CYP2D6 enzyme with dextromethorphan in human plasma by liquid chromatography/tandem mass spectrometry |
| Eichhold et al. (1997)| PKDB00096 | -               | DXM hydrobromide| Oral (syrup): 30 mg | Simultaneous determination of dextromethorphan, dextrophan, and guaifenesin in human plasma using semi-automated liquid/liquid extraction and gradient liquid chromatography tandem mass spectrometry |
| Eichhold et al. (2007)| PKDB00581 | 16939098        | DXM hydrobromide| Oral (solution): 20 mg | Evaluation of pharmacokinetic metrics for phenotyping of the human CYP2D6 enzyme with dextromethorphan metabolic phenotype |
| Frank (2009)         | PKDB00582 | -               | DXM hydrobromide| Oral (capsule): 30 mg | Complexities of CYP2D6 gene analysis and interpretation |
| Gaedigk (2013)       | PKDB00583 | 24151800        | DXM             | Oral (syrup): 0.3 mg/kg | Salivary analysis for determination of dextromethorphan metabolic phenotype |
| Hou et al. (1991)    | PKDB00584 | 2015730         | DXM hydrobromide| Oral (capsule): 50 mg | Floating matrix dosage form for dextromethorphan hydrobromide based on gas forming technique: in vitro and in vivo evaluation in healthy volunteers |
| Hu et al. (2011)     | PKDB00585 | 21050887        | DXM hydrobromide| Oral (sustained release tablet): 30 mg | Determination of cytochrome P450 3A4/5 activity in vivo with dextromethorphan N-demethylation |
| Jones et al. (1996)  | PKDB00586 | 8873685         | DXM hydrobromide| Oral (syrup): 30 mg | Determination of cytochrome P450 3A4/5 activity in vivo with dextromethorphan N-demethylation |
| Kohler et al. (1997) | PKDB00587 | 9429230         | DXM             | Oral (syrup): 20 mg | CYP2D6 genotype and phenotype determination of dextromethorphan and metabolites in serum of healthy controls and of patients under psychotropic medication |
| López et al. (2005)  | PKDB00588 | 16249913        | DXM hydrobromide| Oral (syrup): 30 mg | CYP2D6 genotype and phenotype determination in a Mexican Mestizo population |

(Continued on following page)
The model utilizes ordinary differential equations (ODE) which were numerically solved by sbmlsim (König, 2021a) based on the high-performance SBML simulator libroadrunner (Somogyi et al., 2015; Welsh et al., 2022). It is available in SBML under CC-BY 4.0 license from https://github.com/matthiaskoenig/dextromethorphan-model. Within this work, version 0.9.5 of the model was used (Grzegorzewski and König, 2022).

The model is hierarchically organized with submodels coupled using hierarchical model composition (Smith et al., 2015). The top layer represents the whole body with organs and tissues connected via the blood flow. The lower layer describes substance-related processes within the tissues. Tissues with minor influence on the pharmacokinetics of DXM, DXO, or DXO-Glu are lumped into the ‘rest’ compartment. Intravenous and oral application of DXM appears in the venous and intestinal compartments, respectively. A fraction of DXM is absorbed via the intestinal wall into the systemic circulation. The remainder is excreted via the feces.

The plasma concentration is evaluated at the median cubital vein. The distribution of DXM, DXO, and DXO-Glu between plasma and tissue compartments is based on tissue-to-plasma partition coefficients (Kp) and the corresponding rates of tissue distribution (ftissue).

The metabolism of DXM only includes processes relevant for the simulation of the reported pharmacokinetics data (see Figures 1B,C). Routes of minor contribution such as

NR: not reported, DXM: dextromethorphan.

2012; König and Rodriguez, 2019) were used. The model utilizes ordinary differential equations (ODE) which were numerically solved by sbmlsim (König, 2021a) based on the high-performance SBML simulator libroadrunner (Somogyi et al., 2015; Welsh et al., 2022). It is available in SBML under CC-BY 4.0 license from https://github.com/matthiaskoenig/dextromethorphan-model. Within this work, version 0.9.5 of the model was used (Grzegorzewski and König, 2022).

The model is hierarchically organized with submodels coupled using hierarchical model composition (Smith et al., 2015). The top layer represents the whole body with organs and tissues connected via the blood flow. The lower layer describes substance-related processes within the tissues. Tissues with minor influence on the pharmacokinetics of DXM, DXO, or DXO-Glu are lumped into the ‘rest’ compartment. Intravenous and oral application of DXM appears in the venous and intestinal compartments, respectively. A fraction of DXM is absorbed via the intestinal wall into the systemic circulation. The remainder is excreted via the feces. The plasma concentration is evaluated at the median cubital vein. The distribution of DXM, DXO, and DXO-Glu between plasma and tissue compartments is based on tissue-to-plasma partition coefficients (Kp) and the corresponding rates of tissue distribution (ftissue).

The metabolism of DXM only includes processes relevant for the simulation of the reported pharmacokinetics data (see Figures 1B,C). Routes of minor contribution such as...
FIGURE 1
PBPK model of dextromethorphan (DXM). (A) whole body model consisting of liver, kidney, intestine, forearm, lung and the rest compartment. Organs with minor relevance are not modeled explicitly and lumped into the rest compartment. Organs are coupled via the systemic circulation with arrow width proportional to relative blood flow. DXM can be administered intravenously (IV) or orally (PO) with DXM appearing in the venous blood or intestine, respectively. (B) intestine model consisting of dissolution, absorption and excretion of DXM. Only a fraction of DXM is absorbed with the remainder excreted in the feces. First pass metabolism of DXM via CYP3A4 in the intestine reduces the amount of DXM appearing in the circulation. (C) liver model consisting of DXM $\rightarrow$ DXO conversion via CYP2D6 and CYP3A4 and subsequent glucuronidation to DXO-Glu. (D) kidney model for the urinary excretion of DXM, DXO, and DXO-Glu. Created with BioRender.com.
N-demethylation of DXM in the liver were neglected. Metabolic reactions take place in the intestine and liver and are modeled using irreversible Michaelis-Menten kinetics of the form 

\[ v = \frac{V_{\text{max}}}{K_m + [S]} \]

with \( V_{\text{max}} \) and \( K_m \) for CYP3A4 and CYP2D6 sampled from distributions as described below. The conversion of DXM to DXO can be either catalyzed via CYP2D6 (main route) or CYP3A4 (minor route) in the liver. Reactions with other products than DXM, DXO, and DXO-Glu were modeled as annihilation, i.e., the products of the reaction are not modeled.
explicitly. DXM, DXO, and DXO-Glu are eliminated into the urine via renal excretion.

A subset of model parameters was fitted by minimizing the distance between model predictions and subsets of the data in Figure 4, Figure 5, Figure 6, Figure 7, Figure 8, and Figure 9.

2.3 CYP3A4 and CYP2D6

CYP3A4 and CYP2D6 variability was modeled via correlated bivariate lognormal distributions fitted to in vitro data for CYP2D6 (Yang et al., 2012; Storelli et al., 2019a) and CYP3A4 (Yang et al., 2012), respectively. The data was log10 transformed and a Gaussian, parameterized by the mean (μ) and standard deviation, was fitted by maximum likelihood estimation. The multivariate distribution was realized by a Gaussian copula which in turn was parameterized by Kendall’s tau correlation coefficient from the data (see Figure 2 for data and model).

In order to model the effect of the CYP2D6 AS on the activity, \( V_{\text{max}} \) was assumed to be proportional to the AS, \( V_{\text{max}} \propto \text{AS} \) and \( K_m \) was scaled by the activity score along the first principle component of log10(\( K_m \)) and log10(\( V_{\text{max}} \)) (principal component regression). To model the effect of genetic polymorphisms of CYP2D6, pharmacogenetic variants in the CYP2D6 gene were mapped to their AS and the total activity calculated as the sum of the activity of the two alleles. The genotype-phenotype definitions (i.e. allele variant to AS mapping) were used from PharmGKB (https://www.pharmgkb.org/page/cyp2d6RefMaterials, accessed on 2022-01-10) (Whirl-Carrillo et al., 2021) (Supplementary Table S1).

The stochastic model of CYP2D6 kinetics for a given population consists of a mixture model comprised from the models for each AS weighted by their respective frequency \( P \) (AS), i.e., \( P(V_{\text{max}}, K_m) = \sum_{\text{AS}} P(\text{AS})P(V_{\text{max}}, K_m|\text{AS}), \) To simulate a given AS, the respective \( K_m \) and \( V_{\text{max}} \) values were used (see Figure 2). The variability in pharmacokinetics was simulated by sampling \( K_m \) and \( V_{\text{max}} \) from the CYP3A4 and CYP2D6 distributions. Distributions of CYP3A4 and CYP2D6 parameters were assumed to be statistically independent. To simulate different populations, the AS frequencies for the respective biogeographical population were used from PharmGKB (https://www.pharmgkb.org/page/cyp2d6RefMaterials, accessed on 2022-01-10) (Whirl-Carrillo et al., 2021) (Supplementary Table S2).

2.4 CYP2D6 metabolic phenotype

The metabolic phenotypes ultrarapid metabolizer (UM), extensive metabolizer (EM), intermediate metabolizer (IM), and poor metabolizer (PM) were assigned based on the urinary cumulative metabolic ratio of DXM to total dextromethorphan \( \text{UCMR} = \frac{\text{DXM}}{\text{DXM+DXO}+\text{DXO-Glu}} \) with the following cutoffs: PM: \( \text{UCMR} \geq 0.3 \), IM: \( 0.03 \leq \text{UCMR} < 0.3 \), EM: \( 0.0003 \leq \text{UCMR} < 0.03 \), UM: \( \text{UCMR} < 0.0003 \). Some studies reported the extensive metabolizer as normal metabolizer (NM) with identical cutoffs to the EM. Such data was labeled as EM.

2.5 Sensitivity analysis

A local sensitivity analysis of the effect of model parameters on the UCMR was performed. Individual model parameters \( p \) were varied in both directions by 10% from the base model value \( (p_{-10} \rightarrow p_0 \rightarrow p_{+10}) \) and the change in the state variable describing the UCMR at 8 h (\( q \)) was recorded. The local sensitivity \( S(q, p_i, \text{AS}) \) was calculated for a range of ASs \( (0, 0.25, 0.5, 1, 1.25, 1.5, 2.0, 3.0) \) by the following formula:

\[
S(q, p_i, \text{AS}) = \frac{1}{2} \left( q(p_{-\Delta} - p_{\text{AS}}) - q(p_{+\Delta} - p_{\text{AS}}) \right) \tag{1}
\]

3 Results

Within this work, a physiologically based pharmacokinetic (PBPK) model of DXM was developed and applied to study the role of the CYP2D6 polymorphism on the pharmacokinetics of DXM and metabolic phenotyping using DXM.

3.1 Pharmacokinetics database of DXM

For the development and evaluation of the model, a large pharmacokinetics dataset of DXM and its metabolites, consisting of 36 clinical studies, was established (Table 1). Most of the studies investigated either drug-gene interactions (DGI), drug-drug interactions (DDI), or the interplay of both (i.e., drug-drug-gene interactions). The large majority of studies applied DXM orally \((n = 35)\), whereas only a single publication studied DXM pharmacokinetics after intravenous application \((n = 1)\) (Duedahl et al., 2005). The application form (i.e., solution, syrup, capsule, table), the used DXM dose (2 mg–3 mg/kg), and coadministrations (i.e., phenotyping cocktail, quinidine, cinacalcet hydrochloride, zuojin) vary between studies, as do sampling times and sampled tissues (i.e., urine, plasma, serum). Importantly, plenty of individual UCMR measurements with corresponding CYP2D6 genotype information are contained within this dataset \( (n = 11) \) studies. To our knowledge, this is the first large freely available dataset of pharmacokinetics data for DXM with all data accessible from the pharmacokinetics database (PK-DB) (Grzegorzewski et al., 2021).
TABLE 2 Model parameters in PBPK model of DXM. The complete information is available from the model repository. The prefixes GU__, LI__, and KI__ correspond to the intestine/gut, liver, and kidneys, respectively. Values are either adopted from the references or fitted (F). During the robustness analysis of UCMR, various parameters were scanned (S) and a local sensitivity (SA) was performed, see Section 3.5.

| Parameter | Description | References | Value | Unit | F | S | SA |
|-----------|-------------|------------|-------|------|---|---|----|
| BW        | Body weight | ICRP (2002) (male) | 75    | kg   | ✓ |   |    |
| HEIGHT    | Height      | ICRP (2002) (male) | 170   | cm   | ✓ |   |    |
| HR        | Heart rate  | ICRP (2002) (male) | 70    | 1/min | ✓ |   |    |
| HRrest    | Heart rate (resting) | ICRP (2002) (male) | 70    | 1/min | ✓ |   |    |
| COBW      | Cardiac output per bodyweight | ICRP (2002); de Simone et al. (1997) | 1.548 | ml/s/kg | ✓ | ✓ |    |
| HCT       | Hematocrit  | Vander (2001); Herman (2016) (upper range male) | 0.51  | - |   |   |    |
| Kp_fo_dxm | Tissue/plasma partition coefficient DXM forearm | | 10    | - | ✓ | ✓ | ✓ |
| f_shunting_forearm | Shunting in forearm | | 0.2795 | - | ✓ | ✓ |    |
| FVgu      | Gut fractional tissue volume | Jones and Rowland-Yeo (2013); ICRP (2002) | 0.0171 | l/kg | ✓ |   | ✓ |
| FVki      | Kidney fractional tissue volume | Jones and Rowland-Yeo (2013); ICRP (2002) | 0.0044 | l/kg | ✓ |   | ✓ |
| FVli      | Liver fractional tissue volume | Jones and Rowland-Yeo (2013); ICRP (2002) | 0.021  | l/kg | ✓ | ✓ |    |
| FVla      | Lung fractional tissue volume | Jones and Rowland-Yeo (2013); ICRP (2002) | 0.0076 | l/kg | ✓ |   | ✓ |
| FVsp      | Spleen fractional tissue volume | Jones and Rowland-Yeo (2013); ICRP (2002) | 0.0026 | l/kg | ✓ |   | ✓ |
| FVpa      | Pancreas fractional tissue volume | Jones and Rowland-Yeo (2013); ICRP (2002) | 0.01  | l/kg | ✓ |   | ✓ |
| FVo       | Fore arm fractional tissue volume | | 0.0048 | l/kg | ✓ | ✓ |    |
| FVe       | Venous fractional tissue volume | Jones and Rowland-Yeo (2013); ICRP (2002) | 0.0514 | l/kg | ✓ |   | ✓ |
| FVar      | Arterial fractional tissue volume | Jones and Rowland-Yeo (2013); ICRP (2002) | 0.0257 | l/kg | ✓ |   | ✓ |
| FVpo      | Portal fractional tissue volume | Jones and Rowland-Yeo (2013); ICRP (2002) | 0.001 | l/kg | ✓ |   | ✓ |
| FQgu      | Gut fractional tissue blood flow | Jones and Rowland-Yeo (2013) | 0.146 | - | ✓ |   |    |
| FQki      | Kidney fractional tissue blood flow | Jones and Rowland-Yeo (2013) | 0.19 | - | ✓ |   |    |
| FQh       | Hepatic (venous side) fractional tissue blood flow | Jones and Rowland-Yeo (2013) | 0.215 | - |   |   |    |
| FQlu      | Lung fractional tissue blood flow | Jones and Rowland-Yeo (2013) | 1 | - | ✓ |   | ✓ |
| FQsp      | Spleen fractional tissue blood flow | Jones and Rowland-Yeo (2013) | 0.017 | - | ✓ |   | ✓ |
| FQ6o      | Fore arm fractional tissue blood flow | RNAO (2022) | 0.0146 | - | ✓ | ✓ |    |
| FQpa      | Pancreas fractional tissue blood flow | ICRP (2002) | 0.017 | - | ✓ | ✓ | ✓ |
| tissue_dxm | Vmax tissue distribution DXM | | 1000 | l/min | ✓ | ✓ |    |
| Kp_dxm    | Tissue/plasma partition coefficient DXM | | 8.7346 | - | ✓ | ✓ | ✓ |
| Ka_dis_dxm | DXM rate of dissolution and stomach passage | | 0.0217 | l/hr | ✓ | ✓ | ✓ |
| Mr_dxo    | Molecular weight DXO | CHEBI:29133 | 257.3707 | g/mole |   | ✓ | ✓ |
| tissue_dxo | Vmax tissue distribution DXO | | 100 | l/min | ✓ | ✓ | ✓ |
| Kp_dxo    | Tissue/plasma partition coefficient DXO | | 4 | - | ✓ | ✓ | ✓ |
| Mr_dxo_glu | Molecular weight DXO_glu | CHEBI:32645 | 433.4948 | g/mole |   | ✓ | ✓ |
| tissue_dxo_glu | Vmax tissue distribution DXO_glu | | 3 | l/min | ✓ | ✓ | ✓ |
| Kp_dxo_glu | Tissue/plasma partition coefficient DXO_glu | | 0.08 | - | ✓ | ✓ | ✓ |
| Kl__DXMEX_k | DXM urinary excretion rate | | 0.017 | l/min | ✓ | ✓ | ✓ |
| Kl__DXXOE_k | DXO urinary excretion rate | | 0.3 | l/min | ✓ | ✓ | ✓ |
| Kl__DXXOGUEX_k | DXO glucuronide urinary excretion rate | | 10 | l/min | ✓ | ✓ | ✓ |
| li__DXMCYP2D6_Vmax | DXM CYP2D6 Vmax | | 0.003 | mmol/min/l |   | ✓ | ✓ | ✓ |

(Continued on following page)
3.2 PBPK model of DXM

Within this work, a PBPK model was developed (Figure 1) to study the role of CYP2D6 polymorphism on DXM pharmacokinetics and metabolic phenotyping with DXM. Important model parameters are provided in Table 2. The model is organized hierarchically, with the top layer representing the whole body (Figure 1A) consisting of the liver, kidney, intestine, forearm, lung, and the rest compartment. Organs with minor relevance are not modeled explicitly and lumped into the rest compartment. Organs are coupled via the systemic circulation. DXM can be administered intravenously (IV) or orally (PO) with DXM appearing in the venous blood or intestine, respectively. The intestinal model (Figure 1B) describes dissolution, absorption and excretion of DXM. Only a fraction of DXM is absorbed, with the remainder excreted in the feces. DXM enters the circulatory system by crossing the enterocytes of the intestinal wall. First pass metabolism of DXM via CYP3A4 N-demethylation in the intestine reduces the amount of DXM appearing in the systemic circulation. In the liver model (Figure 1C), DXM gets transformed via O-demethylation to DXO and subsequently transformed to DXO-Glu. The reactions are modeled by Michaelis Menten kinetics and characterized with $K_m$ and $V_{max}$ values. O-demethylation takes place via CYP3A4 and CYP2D6. The $K_m$ and $V_{max}$ of CYP2D6 is modulated via the AS, details can be found in Section 3.3. The kidney model (Figure 1D) describes the urinary excretion of DXM, DXO, and DXO-Glu.

The model allows to predict concentrations and amounts of DXM, DXO, and DXO-Glu depending on CYP2D6 polymorphism, CYP2D6 diplotype, and CYP2D6 AS with amounts and concentrations of DXM, DXO, and DXO-Glu being evaluated in urine or the median cubital vein (plasma).

To our knowledge, this is the first freely accessible, reproducible, and reusable PBPK model of DXM with the model available in SBML from https://github.com/matthiaskoenig/dextromethorphan-model.

### 3.3 CYP3A4 and CYP2D6 variability

Cytochrome P450 enzymes exhibit enormous inter-individual variability in enzyme activity. To account for this variability a stochastic model of CYP2D6 and CYP3A4 activity based on bivariate lognormal distributions of $K_m$ and $V_{max}$ was developed and fitted to experimental data from human liver microsomes (Yang et al., 2012; Storelli et al., 2019a) (see Figure 2).

For the CYP2D6 model, the $V_{max}$ is assumed to be linearly related to the AS with AS = 0 having no CYP2D6 activity. The dispersion of $K_m$ and $V_{max}$ are assumed to be constant for all activity scores. For the mixture model, the frequencies of the individual activity scores $P(AS)$ are adopted from our curated dataset (i.e., relative amount of subjects with reported activity scores and UCMRs). With increasing AS the maximal reaction velocity ($V_{max}$) of DXM conversion via CYP2D6 increases as does the affinity for the substrate DXM ($K_m$ decreases). The

---

**TABLE 2 (Continued) Model parameters in PBPK model of DXM. The complete information is available from the model repository. The prefixes GU__, LI__, KL__ correspond to the intestine/gut, liver, and kidneys, respectively. Values are either adopted from the references or fitted (F). During the robustness analysis of UCMR, various parameters were scanned (S) and a local sensitivity (SA) was performed, see Section 3.5.**

| Parameter | Description | References | Value | Unit | F | S | SA |
|-----------|-------------|------------|-------|------|---|---|----|
| LI__DXMCYP2D6__Km | DXM CYP2D6 Km | Storelli et al. (2019a); Yang et al. (2012) | 0.0079 | mM | ✓ | | |
| LI__cyp2d6__ac | CYP2D6 activity score | | 0.0-3.0 | - | ✓ | ✓ | |
| LI__lambda_1 | Slope of Km by principal component regression of (Km, Vmax) in log space | Storelli et al. (2019a); Yang et al. (2012) | -0.4 | - | ✓ | | |
| LI__DXMCYP3A4__Vmax | Vmax of DXO formation by CYP3A4 | | 0.0004 | mmol/min/l | ✓ | ✓ | |
| LI__DXMCYP3A4__Km | Km of DXO formation by CYP3A4 | Yu and Haining (2001) | 0.157 | mM | ✓ | | |
| LI__DXOU__Vmax | DXO UGT Vmax (glucuronidation) | | 0.8953 | mmol/min/l | ✓ | ✓ | |
| LI__DXOU__Km | DXO UGT Km (glucuronidation) | Lutz and Isoherranen (2012) | 0.69 | mM | ✓ | | |
| GU__F__dxm | Fraction absorbed DXM | Schadel et al. (1995) | 0.55 | - | ✓ | ✓ | |
| GU__K_a__abs__dxm | Ka_abs absorption DXM | | 3.485 | 1/hr | ✓ | ✓ | |
| GU__DXMCYP3A4__Vmax | DXM CYP3A4 Vmax | Yu et al. (1994); Yu and Haining (2001) | 0.0002 | mmol/min/l | ✓ | ✓ | |
| GU__DXMCYP3A4__Km | DXM CYP3A4 Km | | 0.7 | mM | ✓ | | |
| PODOSE | DXM oral dose | | | mg | ✓ | ✓ | |
FIGURE 3

Time-dependency of DXM pharmacokinetics by activity score. (A) DXM plasma concentration, (B) DXO plasma concentration, (C) DXM/DXO plasma ratio, (D) UCMR (DXM/(DXO + DXO-Glu) in urine). Depicted is a subset of data in which 30 mg of DXM was applied orally. The upper rows in the panels depict the data in healthy adults from (Köhler et al., 1997; Abdelrahman et al., 1999; Tamminga et al., 2001; López et al., 2005; Myrand et al., 2008; Frank, 2009; Gaedigk, 2013; Montané Jaime et al., 2013; Chen et al., 2017; Dorado et al., 2017). Cocktail studies are included. Studies containing coadministrations with established drug-drug interactions are excluded. The lower rows depict the respective simulation results. To visualize the large variability in the simulation box plots showing the quartiles alongside the median and outliers for selected time points are used. Variables changed in the simulation are the CYP3A4 and CYP2D6 reaction parameters $K_m$ and $V_{max}$ according to the distributions in Figure 2. For the different activity scores the respective CYP2D6 activity score model was used.
FIGURE 4
Dextromethorphan (DXM) concentration in plasma or serum. Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Schadel et al., 1995; Capon et al., 1996; Eichhold et al., 1997, 2007; Köhler et al., 1997; Tennezé et al., 1999; Duedahl et al., 2005; Nakashima et al., 2007; Nyunt et al., 2008; Frank, 2009; Abduljalil et al., 2010; Dumond et al., 2010; Zawertailo et al., 2010; Oh et al., 2012; Doroshyenko et al., 2013; Lenuzza et al., 2016; Qiu et al., 2016; Armani et al., 2017; Edwards et al., 2017; Yamazaki et al., 2017).
The models of CYP3A4 and CYP2D6 are capable of reproducing the data from the literature, but limited information on CYP2D6 genetics within the data hinders the validation of the AS-specific model.

As motivated in the introduction, even subjects carrying no functional variant of the CYP2D6 gene do metabolize DXM to DXO, however extremely slow. This was implemented in the model via a secondary O-demethylation via CYP3A4 with mean \(K_m\) for DXM adopted from Yu and Haining (2001). The dispersion of \(K_m\) and \(V_{\text{max}}\) is assumed to be identical to the one measured by midazolam in Storelli et al. (2019a) and Yang et al. (2012).

The resulting CYP3A4 and CYP2D6 enzyme model was coupled to the PBPK model and allowed to account (i) for the variability in DXM pharmacokinetics due to the variability in CYPs parameters and (ii) the effect of the AS on CYP2D6 activity and consequently DXM pharmacokinetics.

### 3.4 Effect of CYP2D6 activity score on DXM pharmacokinetics

Model performance was visually assessed for common pharmacokinetic measurements (i.e., DXM, DXO, DXM/DXO in plasma, and DXM/(DXO + DXO-Glu) in urine) and for subjects with reported AS or diplotype (Figure 3). For each AS, a virtual population based on 2,000 \(K_m\) and \(V_{\text{max}}\) samples was created from the stochastic models of CYP3A4 and CYP2D6 model. For every AS, an oral application of 30 mg DXM was simulated and compared to the corresponding data.

**FIGURE 5**
Dextrophan (DXO) concentration in plasma or serum. Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Schadel et al., 1995; Eichhold et al., 1997, 2007; Nakashima et al., 2007; Frank, 2009; Abduljalil et al., 2010; Zawertailo et al., 2010; Oh et al., 2012; Lenuzza et al., 2016).
The model predicts large relative variance within a AS group as well as across different AS groups. With increasing AS, and consequently CYP2D6 activity, plasma DXM decreases (Figure 3A), plasma DXO increases (Figure 3B) and the plasma DXM/DXO decreases (Figure 3C) in very good agreement with the data (Frank, 2009; Chen et al., 2017). The large variability within a AS group is a consequence of the large variability of Km and V_max in CYP2D6 activity of a single AS (see Figure 2). The large overlap between distributions of adjacent AS results in a large overlap in the pharmacokinetics between neighboring AS.

The UCMR (Figure 3D) is very stable over time with a good agreement with the data. With increasing AS, the UCMR decreases and thereby shifts from PM via IM to the EM metabolic phenotype. The UCMR data was pooled independently of the amount of applied DXM (in contrast to A-C only using data from 30 mg oral application) and compared to the simulation as the UCMR endpoint is very robust against the given dose (see Section 3.5).

Overall the model predictions of DXM pharmacokinetics depending on AS are in very good agreement with the available data despite the limited availability of pharmacokinetics timecourses for the low AS 0, 0.25, and 0.5.

To further evaluate the model performance, simulations were compared to pharmacokinetics data for DXM in plasma or serum (Figure 4), DXO in plasma or serum (Figure 5), and DXO + DXO-Glu in plasma or serum (Figure 6), DXM in urine (Figure 7), DXO + DXO-Glu in urine (Figure 8), and the UCMR (Figure 9). With expected variability in mind, the model is capable to reproduce all data from the pharmacokinetics dataset. Minor shortcomings of the model are faster kinetics of DXO + DXO-Glu in plasma (Figure 6).

**FIGURE 6**
Total dextrorphan (DXO + DXO-Glu) concentration in plasma or serum. Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Capon et al., 1996; Köhler et al., 1997; Demirbas et al., 1998; Tennezé et al., 1999; Zawertailo et al., 2010; Hu et al., 2011; Yamazaki et al., 2017).
FIGURE 7
Dextromethorphan (DXM) amount in urine. Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Barnhart, 1980; Schadel et al., 1995; Jones et al., 1996; Nagai et al., 1996; Frank, 2009; Abduljalil et al., 2010).

FIGURE 8
Total dextrorphan (DXO + DXO-Glu) amount in urine. Studies were simulated according to the reported dosing protocol. In case of available activity score information the clinical data is color coded accordingly. Information on metabolizer phenotype (UM, EM, IM, PM) is provided where reported. Data from (Barnhart, 1980; Jones et al., 1996; Frank, 2009; Abduljalil et al., 2010).
3.5 Effect of parameters on metabolic phenotyping via UCMR

Analysis of the effect of parameter changes on UCMR is highly relevant as it can help to identify potential confounding factors and bias in UCMR based phenotyping. Of special importance is the question if there is a dependency on the genetic polymorphism (activity score) of these effects.

To answer this question, model parameters (i.e., liver volume, cardiac output, tissue-to-plasma partition coefficient of DXM, and...
oral dose) were changed in reasonable ranges and the effect on UCMR at 8 h after the application of 30 mg of DXM was investigated (Figure 10A). Independent of the AS, UCMR increased with increasing liver volume and decreased with increasing cardiac output. A change in the tissue-to-plasma partition coefficient of DXM or the amount of oral DXM barely affected the UCMR.

CYP2D6 phenotyping by UCMR is very stable over time as demonstrated in the time course predictions (see 3D and Figure 9) and robust against changes in factors related to the intervention protocol (i.e., dosing amount of DXM, dissolution rate) and to some extent against changes in physiological parameters (see local sensitivity analysis of UCMR in Figure 10B).

Liver volume, heart rate, cardiac output, kidney volume, and kidney elimination rate of DXM altered the UCMR with a similar magnitude as the CYP2D6 reaction parameters. However, the biological variation in these physiological

---

**Figure 10**

Sensitivity analysis of UCMR by activity score. (A) Dependency of UCMR (urinary cumulative ratio of DXM/(DXO-Glu) after 8 h and 30 mg oral DXM) on selected physiological parameters and the DXM dose. Parameter scans were performed for all activity scores. Reference model parameters are depicted as dashed lines. (B) Sensitivity analysis of model parameters. To systematically study the effect of parameter changes the local sensitivity of UCMR was calculated (insensitive parameters with relative change of UCMR smaller than 1% were omitted). Positive sensitivities are depicted in red, negative sensitivities in blue. Parameters were sorted via agglomerative clustering. Representative parameters of the clusters (i.e., liver volume per bodyweight, cardiac output per bodyweight, DXM tissue/plasma partition coefficient, and oral DXM dose) are depicted in A. The local sensitivity for the activity scores in B corresponds to the normalized slope at the dashed lines in A.
parameters is orders of magnitude lower. The sensitivity analysis showed no effect of UGT $V_{\text{max}}$ and $K_m$ on UCMR which is the reason why inter-individual variability of UGT activity was not further investigated in this work. Local sensitivity of UCMR was almost identical at different AS values for almost all parameters, i.e., the effect of physiological parameters is of similar relative magnitude independent of AS. For AS = 0, our model assumptions of minor DXM metabolism by CYP3A4 lead to UCMR not being modulated by CYP2D6 but rather by CYP3A4 activity. Nonetheless, even across studies with non-standardized intervention protocols the UCMR measurements seem to be a good but not perfect endpoint to quantify and compare CYP2D6 enzyme activity. Importantly, our analysis indicates that UCMR measurements can be pooled even across investigations with different intervention protocols (as for instance performed in Figure 3D). This still may lead to biases and errors, e.g., due to differences in the quantification protocol.

**FIGURE 11**

CYP2D6 genotype-, activity score association of the UCMR. Simulation of urinary cumulative ratio of DXM/(DXO-Glu) (UCMR) based on activity score frequencies. UCMR data was measured at least 4 h after the application of DXM (hydrobromide) in healthy adults. Cocktail studies were included in the analysis. Studies containing coadministrations with established drug-drug interactions were excluded. The ranges for metabolic phenotypes (UM, EM, IM, PM) are depicted as gray shaded areas. For timecourse UCMRs, only the latest measurement after administration was included. Data from Köhler et al., 1997; Abdelrahman et al., 1999; Tamminga et al., 2001; López et al., 2005; Myrand et al., 2008; Frank, 2009; Gaedigk, 2013; Montané Jaime et al., 2013; Dorado et al., 2017). (A) Histogram of UCMR data stratified by CYP2D6 activity score. (B) Corresponding simulation results (UCMR at 8 h) from the Monte Carlo simulation with random variables being the enzyme reaction parameter (i.e., $K_m$, $V_{\text{max}}$). See details in Figure 2. (C) Empirical CMFs stratified by the activity scores. (D) Corresponding simulated CMFs stratified by CYP2D6 activity scores. (E) Box plots of observed UCMRs stratified by CYP2D6 activity scores. (F) Box plots of simulated UCMRs stratified by the activity scores. (G) Box plots of observed UCMRs stratified by CYP2D6 diplotypes. (H) Box plots of simulated UCMRs stratified by CYP2D6 diplotype. For D, F, and H, 2,000 samples were simulated for each activity score whereas in B and D a two-fold oversampling with the CYP2D6 activity score frequencies from the UCMR data was performed.
3.6 Effect of CYP2D6 polymorphisms and activity score on UCMR

Next, we tested if the model is able to predict UCMR distributions for given genotypes and AS (Figure 11). Model predictions based on underlying genotype frequencies were compared with the experimental data. UCMR distributions for individual AS groups are well described by the model. The AS impacts the UCMR, with increasing AS resulting in a decrease in UCMR. However, individual AS distributions heavily overlap, as expected, due to the large overlap in CYP2D6 parameter distributions between different AS. The predicted distributions tend to be slightly narrower than the actual data. Possible reasons are many fold (e.g., omitted physiological variation, omitted variation in UGT activity, difficulties in correct genotype assignment, unknown effect modifiers, and biases).

The AS system could be refined to better describe the data. The categorization of CYP2D6 genotypes into discrete activity values (i.e., 0, 0.25, 0.5, 1) is an oversimplification, a continuous activity score would probably perform better. The model and data indicate that gUM (AS ≥ 3) is a very unreliable predictor for ultra rapid metabolism and only gPMs (AS = 0) are almost perfectly distinguishable from other metabolizers, see Figures 11C–F.

Another strength of the presented model is that it enables the prediction of the in vivo phenotype of subjects based on in vitro data.

3.7 Population variability in UCMR

Finally, the model was also capable to predict UCMR distributions for different biogeographical populations (Figure 12) based on the underlying AS frequencies (Supplementary Table S2). Based on the reported frequencies, the UCMR distributions were simulated at 8 h after the application of 30 mg DXM for Oceanian, Near Eastern, American, Latino, Central/South Asian, African American/Afro-Caribbean, Sub-Saharan African, European, and East Asian populations (Figure 12A). Data for Caucasian and East Asian populations (Figure 12B) was used for validation of the predictions (Figure 12C). The data is in good agreement with measurements of Caucasians and East Asians as reported by...
4 Discussion

During the last 20 years various modeling approaches and software solutions were utilized to investigate various aspects of DXM pharmacokinetics, e.g., using GastroPlus (Bolger et al., 2019), P-Pharm (Moghadamnia et al., 2003), SAS (Ito et al., 2010; Chiba et al., 2012), SimCYP (Dickinson et al., 2007; Ke et al., 2013; Sager et al., 2014; Chen et al., 2016; Rougée et al., 2016; Adiwidjaja et al., 2018; Storelli et al., 2019b; Machavaram et al., 2019), MATLAB (Kim et al., 2017), or PK-Sim (Rüdesheim et al., 2022). However, most of the work is difficult/impossible to validate or to build up on due to a lack of accessibility of models and software, and platform-dependency of the models. Here, we provide an openly accessible, reproducible and platform-independent whole-body model of DXM metabolism, which facilitates reusability, extensibility, and comparability.

Apart from that, modeling work which aims for high empirical evidence relies on trustworthy supporting real world data. More and independent sources of data are highly beneficial for the scientific outcomes. For that matter, guidelines like PRISMA for reporting transparency, completeness, and accuracy find very broad endorsement in the field of systematic reviews and meta analysis. The present work faces somewhat similar challenges for the evaluation and selection of data from literature. Therefore, PRISMA-ScR guidelines were adopted where applicable. With this approach, bias within the used dataset could be mitigated or at least identified. Importantly, we supplement our open and accessible model with a large, open, and accessible database of pharmacokinetics data.

The presented PBPK model is able to predict the DXM metabolism of populations and individuals based on their CYP2D6 genotype. It is probably the first model capable to predict individual UCMRs and the expected distributions of UCMR. Moreover, it can reproduce a broad range of reported clinical data on DXM and enables better intuition on how to interpret DXM related pharmacokinetics. E.g., an important message is that CYP2D6 activity is not the only modulator of UCMR, as can be seen by the large variability in activity score and overlap between activity scores. UCMR as a proxy of CYP2D6 metabolic phenotype should therefore be interpreted carefully. The model shows that for extremely low CYP2D6 activity the UCMR is not primarily governed by the CYP2D6 activity. This is consistent with the finding that CYP2D6 inhibition merely affects PMs (Pope et al., 2004).

The current version of the model is already very valuable, still there is plenty of room for improvement. By providing the data and model in open and standardized formats we enable and encourage these improvements by model extensions and updates.

Many of the physiological parameters in the model were fitted or estimated although they could be measured in principle. E.g., relatively low DXM concentrations in plasma suggest substantial extra-vascular binding of DXM. However, tissue-plasma partition coefficients (Kp) are difficult to assess and only limited data is available. Steinberg et al. (1996) reported brain levels to be 68-fold higher and cerebrospinal fluid levels 4-fold lower than serum levels, respectively. Others estimated Kp = 1.65 from n-octanol-water partition coefficients and again others suggested additional trapping mechanisms (i.e. lysosomal trapping) (Bolger et al., 2019). In the model, the DXO-Glu kinetics is a bit to rapid (see Figure 6), probably due to the decision to model tissue distribution uniformly for all organs (i.e., identical Kp and fGlu). We decided for a more parsimonious model. Glucuronides, however, are generally much more polar than their respective non-glucuronides which result in less plasma binding, higher urinary excretion, lower lipid-solubility, and higher water-solubility. Transport into different tissues is affected differently by polarity.

Most important for model improvements would be additional in vitro measurements on the association between CYP2D6 genotype and phenotype which are very limited in literature (Storelli et al., 2019a; Ning et al., 2019; Dalton et al., 2020). Furthermore, simultaneous in vitro and UCMR measurements do not exist the literature. Both would be very important for the validation of the AS system and the development of new models which e.g. take into account structural variation (Dalton et al., 2020). For instance, with the AS system alone it is not possible to explain why CYP2D6 is inhibited differently for different genotypes Qiu et al. (2016).

In conclusion, we developed and validated a PBPK model of DXM and applied it to study the effect of the CYP2D6 polymorphism on metabolic phenotyping.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author. All clinical data of dextromethorphan pharmacokinetics that was used in this work can be found in PK-DB available from https://pk-db.com.

Author contributions

JG and MK designed the study, developed the computational model, implemented and performed the analysis, and wrote the initial draft of the manuscript. JB
provided support with data curation. All authors discussed the results. All authors contributed to and revised the manuscript critically.

Funding

JG, JB, and MK were supported by the Federal Ministry of Education and Research (BMBF, Germany) within the research network Systems Medicine of the Liver (LiSyM, grant number 031L0054). MK and JG were supported by the German Research Foundation (DFG) within the Research Unit Program FOR 5151 “QualiPerF (Quantifying Liver Perfusion-Function Relationship in Complex Resection—A Systems Medicine Approach)” by grant number 436883643 and MK by grant number 465194077 (Priority Programme SPP 2311, Subproject SimLiVa). This work was supported by the BMBF-funded de.NBI Cloud within the German Network for Bioinformatics Infrastructure (de.NBI) (031A537B, 031A533A, 031A538A, 031A533B, 031A535A, 031A537C, 031A534A, 031A532B).

References

Abdelrahman, S., Gotschall, R., Kauffman, R., Leeder, J., and Kearns, G. (1999). Investigation of terbutaline as a CYP2D6 inhibitor in vivo. Clin. Pharmacol. Ther. 65, 465–472. doi:10.1016/S0003-2538(99)00106-5

Abduljalil, K., Frank, D., Gaedigk, A., Klaassen, T., Tomalik-Scharte, D., Jetter, A., et al. (2010). Assessment of activity levels for CYP2D6*1, CYP2D6*2, and CYP2D6*41 genes by population pharmacokinetics of dextromethorphan. Clin. Pharmacol. Ther. 88, 643–651. doi:10.1038/clpt.2010.137

Adiwidjaja, J., Boddy, A. V., and McLachlan, A. J. (2018). A strategy to refine the phenotyping approach and its implementation to predict drug clearance: A physiologically based pharmacokinetic simulation study. CPT. Pharmacometrics Syst. Pharmacol. 7, 798–808. doi: 10.1002/psp4.12355

Armani, S., Ting, L., Sauter, N., Darstein, C., Tripathi, A. P., Wang, L., et al. (2017). Drug interaction potential of osilodrostat (LCI699) based on its effect on the pharmacokinetics of probe drugs of cytochrome P450 enzymes in healthy adults. Clin. Drug Investig. 37, 465–472. doi:10.1007/s40261-017-0497-0

Barnhart, J. W. (1980). The urinary excretion of dextromethorphan and three metabolites in dogs and humans. Toxicol. Appl. Pharmacol. 55, 43–48. doi:10.1016/0041-008X(80)90218-5

Berm, E. J. J., Risselada, A. J., Mulder, H., Hak, E., and Wilffert, B. (2013). Phenocconversion of cytochrome P450 2D6: The need for identifying the intermediate metaboliser genotype. J. Clin. Psychiatry 74, 1025. doi:10.4088/JCP.13B0855

Bolger, M. B., Macwan, J. S., Sarfraz, M., Almukainzi, M., and Lobenberg, R. (2019). The irrelevance of in vitro dissolution in setting product specifications for drugs like dextromethorphan that are subject to lysosomal trapping. J. Pharm. Sci. 108, 268–278. doi:10.1016/j.xphs.2018.09.036

Capon, D. A., Bochner, F., Kerry, N., Mikus, G., Danz, C., and Somogyi, A. A. (1996). The influence of CYP2D6 polymorphism and quinidine on the disposition and antitussive effect of dextromethorphan in humans. Clin. Pharmacol. Ther. 60, 295–307. doi:10.1038/32009-9236/nature00566-9

Caudle, K. E., Sangkuhl, K., Whirt-Carrillo, M., Swen, J. J., Haider, C. E., Klein, T. E., et al. (2020). Standardizing CYP2D6 genotype to phenotype translation: Consensus recommendations from the clinical pharmacogenetics implementation consortium and Dutch pharmacogenomics working group. Clin. Transl. Sci. 13, 116–124. doi:10.1111/cts.12692

Chen, R., Rostami-Hodjegan, A., Wang, H., Berk, D., Shi, J., and Hu, P. (2016). Application of a physiologically based pharmacokinetic model for the evaluation of single-point plasma pharmacotyping method of CYP2D6. Eur. J. Pharm. Sci. 92, 131–136. doi:10.1016/j.ejps.2016.07.001

Chen, R., Zheng, X., and Hu, P. (2017). CYP2D6 phenotyping using urine, plasma, and saliva metabolic ratios to assess the impact of CYP2D6.10 on interindividual variation in a Chinese population. Front. Pharmacol. 8, 239. doi:10.3389/fphar.2017.00239

Chiba, K., Kato, M., Iio, T., Suwa, T., and Sugiyama, Y. (2012). Inter-individual variability of in vivo CYP2D6 activity in different genotypes. Drug Metab. Pharmacokinet. 27, 405–413. doi:10.2133/dmpk.11-rg-078

Chléděk, J., Zamová, G., Beránek, M., and Martináková, J. (2000). In-vivo indices of CYP2D6 activity: Comparison of dextromethorphan metabolic ratios in 4-h urine and 3-h plasma. Eur. J. Clin. Pharmacol. 56, 651–657. doi:10.1007/A1002800000218

Dalton, R., Lee, S.-B., Claw, K. G., Prasad, B., Phillips, B. R., Shen, D. D., et al. (2020). Interrogation of CYP2D6 structural variant alleles improves the correlation between CYP2D6 genotype and CYP2D6-mediated metabolic activity. Clin. Transl. Sci. 13, 147–156. doi:10.1111/ctss.12695

De Keul, P. M. M., Lambert, W. E., and Stone, C. P. (2016). Alternative sampling strategies for cytochrome P450 phenotyping. Clin. Pharmacokinet. 55, 169–184. doi:10.1007/s40262-015-0306-y

de Simone, G., Devereux, R. B., Daniels, S. R., Mureddu, G., Roman, M. J., Kimball, T. R., et al. (1997). Stroke volume and cardiac output in normotensive children and adults. Assessment of relations with body size and impact of overweight. Circulation 95, 1837–1843. doi:10.1161/01.cir.95.7.1837

Demirbas, S., Reyderman, L., and Stavchansky, S. (1998). Bioavailability of dextromethorphan (as dextrophan) from sustained release formulations in the presence of gauafenixin in human volunteers. Biopharm. Drug Dispos. 19, 541–545.

Dickinson, G. L., Rezaee, S., Proctor, N. J., Lennard, M. S., Tucker, G. T., and Rostami-Hodgeman, A. (2007). Incorporating in vitro information on drug metabolisms into clinical trial simulations to assess the effect of CYP2D6 polymorphism on pharmacokinetics and pharmacodynamics: Dextromethorphan as a model application. J. Clin. Pharmacol. 47, 175–186. doi:10.1177/0003235506294279

Dorado, P., González, I., Naranjo, M. E. G., de Andrés, F., Peñas-Lledó, E. M., Calzadilla, L. R., et al. (2017). Lessons from Cuba for global precision medicine: Incorporating in vitro information on drug metabolisms into clinical trial simulations to assess the effect of CYP2D6 polymorphism on pharmacokinetics and pharmacodynamics: Dextromethorphan as a model application. OMICS A J. Integr. Biol. 21, 17–26. doi:10.1089/omi.2016.0146

Doroshyenko, O., Rokitta, D., Zadoyan, G., Klement, S., Schlafke, S., Dierl, A., et al. (2013). Drug cocktail interaction study on the effect of the orally administered lavender oil preparation silexan on cytochrome P450 enzymes in healthy volunteers. Drug Metab. Dispos. 41, 987–993. doi:10.1124/dmd.112.050203

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.1029073/full#supplementary-material
Grzegorzewski, J., Bartsch, F., Köller, A., and König, M. (2022). Pharmacokinetics of the human body. Charm: Springer.

Herman, J. P. (2016). Physics of the human body. Charm: Springer.

Hou, Z.-Y., Pickle, L. W., Meyer, P. S., and Wooley, R. L. (1991). Salivary assay for determination of dextromethorphan metabolic phenotype. Clin. Pharmacol. Ther. 49, 410–419. doi:10.1001/cpt.1991.48

Hu, L., Li, L., Yang, X., Liu, W., Yang, J., Jia, Y., et al. (2011). Floating matrix dosage form for dextromethorphan-hydrobromide based on gas forming technique. In vitro and in vivo evaluation in healthy volunteers. Eur. J. Pharm. Sci. 42, 99–105. doi:10.1016/j.ejps.2010.10.010

Hucka, M., Bergmann, F. T., Chauxiau, C., Dräger, A., Hoops, S., Keating, S. M., et al. (2019). The systems biology markup language (SBML) Language specification for level 3 version 2 core release 2. Integr. Bioinform. 16, 0021. doi:10.1515/ib-2019-0021

Hurtado, I., García-Sempere, A., Péret, S., and Sanfeliú-Cugnos, G. (2020). Increasing trends in opioid use from 2010 to 2018 in the region of Valencia. Spain: A real-world, population-based study. Front. Pharmacol. 11, 612556. doi:10.3389/fphar.2020.612556

ICRP (2002). Basic anatomical and physiological data for use in radiological protection. Reference values. A report of age- and gender-related differences in the anatomical and physiological characteristics of reference individuals. ICRP Publication 89. Ann. ICRP 32, 5–265.

Ito, T., Kato, M., Chiba, K., Okazaki, O., and Sugiyama, Y. (2010). Estimation of the interindividual variability of cytochrome 2D6 activity from urinary metabolic ratios in the literature. Drug Metab. Pharmacokinet. 25, 243–253. doi:10.2133/dm.2013.033

Jones, D. R., Gorski, J. C., Haehner, B. D., O'Mara, E. M., and Hall, S. D. (1996). Determination of cytochrome P450 3A4 activity in vivo with dextromethorphan N-demethylation. Clin. Pharmacol. Ther. 60, 374–384. doi:10.1002/cp.10299

Jou, H., and Rowland-Yeo, K. (2013). Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. CPT Pharmaceutometrics Syst. Pharmac. 2, e63. doi:10.1038/psp.2013.41

Kawashima, C., Lundgren, S., Agren, H., and Bertilsson, L. (2004). Increased incidence of CYF2D gene duplication in patients with persistent mood disorders: Ultrarapid metabolism of antidepressants as a cause of nonresponse. A pilot study. Eur. J. Clin. Pharmacol. 59, 803–807. doi:10.1007/s00028-003-0701-4

Ke, A. B., Nañani, S. C., Zhao, P., Rostami-Hodjegan, A., Isoherranen, N., and Unadkat, J. D. (2013). A physiologically based pharmacokinetic model to predict disposition of CYF2D and CYP1A2 metabolized drugs in pregnant women. Drug Metab. Dispos. 41, 801–813. doi:10.1124/dmd.112.050161

Keating, S. M., Waltemath, D., König, M., Zhang, F., Dräger, A., Chauxiau, C., et al. (2020). SBML Level 3: An extensible format for the exchange and reuse of biomedical models. Mol. Syst. Biol. 16, e9110. doi:10.15252/msb.20199110

Kerry, N., Somogyi, A., Bochner, F., and Mikus, G. (1994). The role of CYF2D in primary and secondary oxidative metabolism of dextromethorphan. In vitro studies using human liver microsomes. Br. J. Clin. Pharmacol. 38, 243–248. doi:10.1111/j.1365-2124.1994.tb04348.x

Kibaly, C., Alderete, J. A., Liu, S. H., Nafse, H. S., Law, P. Y., Evans, C. J., et al. (2021). Oxycodone in the opioid epidemic: High ‘taking,’ warning, and abuse liability. Cell. Mol. Neurobiol. 41, 899–926. doi:10.1007/s10571-020-01013-y

Kim, E.-Y., Shin, S.-G., and Shin, J.-G. (2017). Prediction and visualization of CYF2D genotype-based phenotype using clustering algorithms. Transl. Clin. Pharmacol. 25, 147–152. doi:10.2133/tcpc.2017.25.3.147

Köhler, D., Härter, S., Fuchs, K., Sieghart, W., and Hiesmeyer, H. (1997). CYF2D genotype and phenotype by determination of dextromethorphan and metabolites in serum of healthy controls and of patients under psychotropic medication. Pharmacogenetics 7, 453–461. doi:10.1097/00008571-199712000-00003

König, M., Dräger, A., and Hohlfutter, H.-G. (2012). CySBML: A cytoscape plugin for sbml. Bioinforma. Oxf. Engl. 28, 2402–2403. doi:10.1093/bioinformatics/bts432

König, M. (2021a). Mathematica/brshml: 0.114 - SBML simulation made easy. Available at: https://zenodo.org记录/5531088. [Accessed: 2022-08-23].

König, M., and Rodríguez, N. (2019). Mathematica/ebxml3/Brshml v0.3.0 - SBML for cytoscape. Available at: https://zenodo.org记录/3451313. [Accessed: 2022-08-23].

König, M. (2021b). Shmlutlits: Python utilities for SBML. Available at: https://zenodo.org记录/6099239. [Accessed: 2022-08-23].

Lenuzza, N., Duvall, X., Nicolas, G., Thévenot, E., Joff, S., Videau, O., et al. (2016). Safety and pharmacokinetics of the OME combination of drugs and their metabolites after a single oral dosing in healthy volunteers. Eur. J. Drug Metab. Pharmacokinet. 41, 125–138. doi:10.1007/s10338-014-0259-0
in humans: A cocktail study with dextromethorphan and chloroguanide alone and in combination. Clin. Pharmacol. Ther. 66, 582–588. doi:10.1053/cp.1999.v66.103401001

Tricco, A. C., Lillie, E., Zarin, W., O’Brien, K. K., Colquhoun, H., Levac, D., et al. (2018). PRISMA extension for scoping reviews (PRISMA-ScR): Checklist and explanation. Ann. Intern. Med. 169, 467–473. doi:10.7326/M18-0850

Vander, J. S. (2001). Human physiology: The mechanisms of body function. New York City: McGraw-Hill higher education.

von Moltke, L. L., Greenblatt, D. J., Grassi, J. M., Granda, B. W., Venkatakrishnan, K., Schmader, J., et al. (1998). Multiple human cytochromes contribute to biotransformation of dextromethorphan in vitro: Role of CYP2C9, CYP2C19, CYP2D6, and CYP3A. J. Pharm. Pharmacol. 50, 997–1004. doi:10.1111/j.2042-7158.1998.tb06914.x

Welsh, C., Xu, J., Smith, L., König, M., Choi, K., and Sauro, H. M. (2022). libRoadRunner 2.0: A high-performance sbml simulation and analysis library. arxiv. doi:10.48550/arXiv.2203.01175

Whirl-Carrillo, M., Huddart, R., Gong, L., Sangkuhl, K., Thorn, C. F., Whaley, R., et al. (2021). An evidence-based framework for evaluating pharmacogenomics knowledge for personalized medicine. Clin. Pharmacol. Ther. 110, 563–572. doi:10.1002/cpt.2350

Wyen, C., Fahr, U., Frank, D., Aarnoutse, R. E., Klaassen, T., Lazar, A., et al. (2008). Effect of an antiretroviral regimen containing ritonavir boosted lopinavir on intestinal and hepatic CYP3A, CYP2D6 and P-glycoprotein in HIV-infected patients. Clin. Pharmacol. Ther. 84, 75–82. doi:10.1038/sj.clpt.6100452

Yamazaki, T., Desai, A., Goldwater, R., Han, D., Howieson, C., Akhtar, S., et al. (2017). Pharmacokinetic effects of isavuconazole coadministration with the cytochrome P450 enzyme substrates bupropion, repaglinide, caffeine, dextromethorphan, and methadone in healthy subjects. Clin. Pharmacol. Drug Dev. 6, 54–65. doi:10.1002/cpdd.281

Yang, J., He, M. M., Niu, W., Wrighton, S. A., Li, L., Liu, Y., et al. (2012). Metabolic capabilities of cytochrome P450 enzymes in Chinese liver microsomes compared with those in Caucasian liver microsomes: Hepatic cytochromes P450 of Chinese and Caucasians. Br. J. Clin. Pharmacol. 73, 268–284. doi:10.1111/j.1365-2125.2011.04076.x

Yu, A., and Haining, R. L. (2001). Comparative contribution to dextromethorphan metabolism by cytochrome P450 isoforms in vitro: Can dextromethorphan be used as a dual probe for both CTP2D6 and CYP3A activities? Drug Metab. Dispos. 29, 1514–1520.

Zackrisson, A. L., Lindblom, B., and Ahlner, J. (2010). High frequency of occurrence of CYP2D6 gene duplication/multiduplication indicating ultrarapid metabolism among suicide cases. Clin. Pharmacol. Ther. 88, 354–359. doi:10.1038/ clpt.2009.216

Zanger, U. M., Raimundo, S., and Eichelbaum, M. (2004). Cytochrome P450 2D6: Overview and update on pharmacology, genetics, biochemistry. Naunyn. Schmiedeb. Arch. Pharmacol. 369, 23–37. doi:10.1007/s00210-003-0832-2

Zawertailo, L. A., Tyndale, R. F., Busto, U., and Sellers, E. M. (2010). Effect of metabolic blockade on the psychoactive effects of dextromethorphan. Hum. Psychopharmacol. 25, 71–79. doi:10.1002/hup.1086