Physiologically-based pharmacokinetic modeling for the prediction of CYP2D6-mediated gene-drug-drug interactions

STORELLI, Flavia, DESMEULES, Jules Alexandre, DAALI, Youssef

Abstract
The aim of this work was to predict the extent of Cytochrome P450 2D6 (CYP2D6)-mediated drug-drug interactions (DDIs) in different CYP2D6 genotypes using physiologically-based pharmacokinetic (PBPK) modeling. Following the development of a new duloxetine model and optimization of a paroxetine model, the effect of genetic polymorphisms on CYP2D6-mediated intrinsic clearances of dextromethorphan, duloxetine, and paroxetine was estimated from rich pharmacokinetic profiles in activity score (AS)1 and AS2 subjects. We obtained good predictions for the dextromethorphan-duloxetine interaction (Ratio of predicted over observed area under the curve (AUC) ratio (Rpred/obs ) 1.38-1.43). Similarly, the effect of genotype was well predicted, with an increase of area under the curve ratio of 28% in AS2 subjects when compared with AS1 (observed, 33%). Despite an approximately twofold underprediction of the dextromethorphan-paroxetine interaction, an Rpred/obs of 0.71 was obtained for the effect of genotype on the area under the curve ratio. Therefore, PBPK modeling can be successfully used to predict gene-drug-drug interactions (GDDIs). […]

Reference

STORELLI, Flavia, DESMEULES, Jules Alexandre, DAALI, Youssef. Physiologically-based pharmacokinetic modeling for the prediction of CYP2D6-mediated gene-drug-drug interactions. CPT: pharmacometrics & systems pharmacology, 2019, p. 1-10

DOI : 10.1002/psp4.12411
PMID : 31268632
Physiologically-Based Pharmacokinetic Modeling for the Prediction of CYP2D6-Mediated Gene–Drug–Drug Interactions

Flavia Storelli1,2, Jules Desmeules1,2,3,4 and Youssef Daali1,2,3,4,*

The aim of this work was to predict the extent of Cytochrome P450 2D6 (CYP2D6)-mediated drug–drug interactions (DDIs) in different CYP2D6 genotypes using physiologically-based pharmacokinetic (PBPK) modeling. Following the development of a new duloxetine model and optimization of a paroxetine model, the effect of genetic polymorphisms on CYP2D6-mediated intrinsic clearances of dextromethorphan, duloxetine, and paroxetine was estimated from rich pharmacokinetic profiles in activity score (AS)1 and AS2 subjects. We obtained good predictions for the dextromethorphan–duloxetine interaction (Ratio of predicted over observed area under the curve (AUC) ratio ($R_{\text{pred/obs}}$) 1.38–1.43). Similarly, the effect of genotype was well predicted, with an increase of area under the curve ratio of 28% in AS2 subjects when compared with AS1 (observed, 33%). Despite an approximately twofold underprediction of the dextromethorphan–paroxetine interaction, an $R_{\text{pred/obs}}$ of 0.71 was obtained for the effect of genotype on the area under the curve ratio. Therefore, PBPK modeling can be successfully used to predict gene–drug–drug interactions (GDDIs). Based on these promising results, a workflow is suggested for the generic evaluation of GDDIs and DDIs that can be applied in other situations.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ A huge interindividual variability exists in the extent of drug–drug interactions (DDIs), which might be attributed to intrinsic factors affecting drug pharmacokinetics, among them genetic polymorphisms.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ Can physiologically-based pharmacokinetic (PBPK) modeling be used to predict the effect of cytochrome P450 (CYP) CYP2D6 genetic polymorphisms on the extent of DDIs?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
✔ The study demonstrated the good performance of PBPK modeling to predict the effect of CYP2D6 genetic polymorphisms on DDIs using verified initial models and rich pharmacokinetic profiles from dedicated genetic trials to predict the effect of genotype on drug and substrate exposures.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?
✔ Using PBPK modeling to predict the combination of intrinsic (age, organ impairment, genetic polymorphism) and extrinsic factors might help to detect individuals at higher risk of clinically significant DDIs. Future integration of PBPK models in advanced computerized physician order entry systems may therefore help to increase the predictive values of DDI alerts, thereby increasing confidence in model-informed precision medicine.

Drug–drug interactions (DDIs) are of particular clinical interest because of their high prevalence among polymorbid patients and their potential deleterious clinical implications, frequently leading to hospitalizations.1 In particular, cytochromes P450 (CYPs) explain a great amount of pharmacokinetic (PK) DDIs, many drugs being substrates and/or inhibitors or inducers of CYPs. Among them, CYP2D6 is of major clinical relevance as it metabolizes around 20% of marketed drugs and is characterized by a huge interindividual variability, which is mostly explained by genetic polymorphisms (>100 variant alleles have been described so far).2 Despite the development of DDI prediction tools integrated in computerized physician order entry systems, it still remains difficult to predict the clinical relevance of potential DDIs, as shown by the significant discrepancy between the high prevalence of potential DDIs and the low prevalence of actual DDIs leading to clinical harm.3 Indeed, a large interindividual variability exists in the extent of CYP-mediated DDIs, which might be explained by intrinsic factors, such as age, sex, comorbidities, and genetic

1Division of Clinical Pharmacology and Toxicology, Geneva University Hospitals, Geneva, Switzerland; 2Geneva-Lausanne School of Pharmacy, Geneva University, Geneva, Switzerland; 3Faculty of Medicine, Geneva University, Geneva, Switzerland; 4Swiss Center of Applied Human Toxicology, Basel, Switzerland. *Correspondence: Youssef Daali (Youssef.daali@hcuge.ch)
Received: October 16, 2018; accepted: March 22, 2019. doi:10.1002/psp4.12411
polymorphisms. As an example, the extent in the increase of area under the curve (AUC) for venlafaxine when coadministered with quinidine can vary by a 20-fold factor in normal metabolizers of CYP2D6. Therefore, it seems crucial to identify other covariables responsible for this huge variability. This work proposes to use physiologically-based PK (PBPK) modeling to identify individuals at a higher risk of DDIs because of genetic polymorphisms in metabolic enzymes. When the models with genetic data are optimized and fully validated, they can be implemented in computerized physician order entry systems to improve therapeutic management at an individual level. Such a model-informed approach will be increasingly used to improve the efficacy and safety of a treatment for an individual patient.

After several decades of extensive investigations, pharmacogenetic testing is currently being evaluated for routine implementation. It was reported that more than 90% of the population carry at least one actionable genetic variant. These genetic variants can affect the safety and/or efficacy of some drugs. As an example, genetic polymorphisms of CYP2D6 have been shown to affect the clinical outcomes of drug treatments by tamoxifen, opioid analgesics (tramadol, codeine, oxycodone), and antidepressants and antipsychotics. However, not all genetic polymorphisms and medications are responsible for therapeutic accidents. Drugs with a narrow therapeutic window merit special attention, and the addition of other factors such as liver or kidney diseases may potentiate the impact and clinical relevance of DDIs. In addition, recent evidence suggests an impact of genetic polymorphism on the extent of DDIs. The CYP2D6 activity score (AS) system developed by Gaedigk et al. is used to predict a CYP2D6 phenotype from genetic data. According to the Clinical Pharmacogenetic Implementation Consortium, ASs from 1 to 2 predict the extensive metabolizer phenotype. We recently highlighted the differential magnitude of DDIs between homozygous carriers of two fully functional alleles (AS2) and heterozygous carriers of one fully functional with one nonfunctional allele (AS1). Indeed, the AUC ratio of dextromethorphan with paroxetine was about twofold higher in the AS2 subjects when compared with the AS1 subjects.

PBPK models divide the body into physiologically meaningful compartments in which drug-dependent (e.g., physico-chemical properties and in vitro data), physiology-dependent (e.g., physiology, ethnicity, gender, and genetics), and trial-dependent (e.g., dose regimen and concomitant medication) components are taken into account to predict the PK and pharmacodynamics of drugs in special populations. Recently, PBPK models have shown their ability to predict DDI scenarios involving intrinsic factors such as ethnic or genetic polymorphisms in drugs undergoing multiple clearance pathways.

Therefore, the aim of the present work was to evaluate the ability of PBPK modeling to predict the extent of CYP2D6-mediated DDIs in the presence of genetic polymorphisms using available rich PK data in different genotypes. This work addresses the issue of the variability in the extent of DDIs explained by genetic polymorphisms that help identify individuals at a higher risk of DDIs. To the best of our knowledge, this is the first study evaluating the usefulness of PBPK in predicting gene–drug–drug interactions (GDDIs) with specific inhibitors of CYP2D6—duloxetine, paroxetine, and fluoxetine—as well as the specific substrates dextromethorphan, tolterodine, and risperidone.

### MATERIALS AND METHODS

#### General

All simulations were performed using the PBPK modeling software Simcyp version 17 (Certara, Princeton, NJ). The models for dextromethorphan, dextorphan, tolterodine, and fluoxetine were used as provided by the software and modified if required using available clinical data (see Data S1). A model for duloxetine was built based on available in vitro and clinical data. The risperidone model was taken from Vieira et al. All simulations used for model verification perform 10 trials of a given number of subjects.

#### Duloxetine model development and verification

An initial PBPK model for duloxetine was built using available physicochemical, in vitro, and clinical data as described in Table 1. First-order absorption and full PBPK models.
models using the Rodgers and Rowland equation to predict distribution volume were used. CYP1A2-mediated and CYP2D6-mediated intrinsic clearances were calculated using the retrograde calculator provided by the software, assuming that the metabolic clearance of duloxetine was mediated by CYP1A2 and CYP2D6 only. A clinical study that evaluated the exposure change of duloxetine following steady-state administration of paroxetine was used to predict the fraction of duloxetine clearance mediated by CYP2D6 ($f_{\text{m,CYP2D6}}$) from the mechanistic static equation, as follows:

$$f_{\text{m,CYP2D6}} = \frac{1 - AUCR_{\text{obs}}}{AUCR_{\text{obs}} \cdot \frac{k_{\text{deg,CYP2D6,h}}}{k_{\text{deg,CYP2D6,h}} + \frac{K_I}{\text{[I]}}}}$$

(1)

where $k_{\text{deg,CYP2D6,h}}$ is the degradation rate of CYP2D6 in the liver, $[\text{I}]$ is the unbound concentration of paroxetine in the portal vein, $k_{\text{inact}}$ is the maximal CYP2D6 inactivation rate of paroxetine, and $K_I$ is the time-dependent inhibition (TDI) constant as the concentration of inhibitor reaching half $k_{\text{inact}}$. Those values were taken from the paroxetine compound file in Simcyp version 17.

The model was verified with a set of PK and DDI studies that are described in Tables S1 and S2. The contribution of CYP1A2 was verified using a DDI study with fluvoxamine in smokers. To that end, the population used was healthy volunteers in which the frequency of CYP1A2 ultrarapid metabolizers (UMs) was set to 100% (CYP1A2 abundance 94 pmol/mg of microsomal proteins). Because experimental CYP2D6 competitive inhibition constant ($K_I$) values (2–4 μM) resulted in a null DDI prediction, an optimized competitive inhibition constant ($K_I$) value of 0.005 μM was input using sensitivity analysis with available clinical data with desipramine as a victim drug and verified with three independent DDI trials with desipramine, tolterodine, and metoprolol (Table S2).

Optimization of paroxetine model

The TDI parameters of $K_I$ and $k_{\text{inact}}$ in paroxetine library compound file in version 17 were modified using optimized data taking into account the hepatocyte (pH 7.4)-to-plasma (pH 7.0) pH gradient. Briefly, the $K_I$ value was decreased from 0.315 to 0.067 μM, and $k_{\text{inact}}$ was increased from 10.2 to 11.55 hour$^{-1}$. The model was verified for DDI predictions using a set of published DDI clinical trials (Table S3).

Top-down estimation of CYP2D6-mediated intrinsic clearances of dextromethorphan, duloxetine, paroxetine, and fluoxetine

Dextromethorphan and dextrorphan PK profiles in healthy volunteers with different CYP2D6 genotypes with ASs ranging from 1 to 3 were used to estimate the CYP2D6-mediated intrinsic clearance (CLint$_{\text{CYP2D6}}$) of dextromethorphan O-demethylation. In this study, one subject had an AS of 0.5, 14 subjects were carriers of one fully functional with one nonfunctional allele (AS1), 9 subjects were carriers of two fully functional alleles (AS2), and one subject was a carrier of two functional alleles with one duplication (AS3). The CLint$_{\text{CYP2D6}}$ value of dextromethorphan in Simcyp version 17 is 7.275 μL/minute per mg of microsomal proteins. The parameter was refined in each of the genotypes previously to obtain a best fit with the observed concentrations using 100 iterations around 10-fold the initial value mentioned previously, a maximum likelihood objective function, and expectation maximization as the minimization method. As clinical data were obtained in-house, covariates such as age, sex, weight, height, and CYP2D6 genotype were taken into account for the parameter estimation.

Similarly, the genotype-dependent CYP2D6-mediated intrinsic clearance of duloxetine and paroxetine were estimated from available clinical data in AS1 and AS2 healthy volunteers using the initial models’ CLint$_{\text{CYP2D6}}$ values as starting values for the parameter estimation. A total of 100 iterations around 10-fold the initial value were interrogated, and a maximum likelihood objective function and expectation maximization as minimization method were used. As clinical data were obtained in-house, covariates such as age, sex, weight, height, and CYP2D6 genotype were taken into account for the parameter estimation.

Simulations of GDDIs

DDIs studies were simulated in different genotypes using genotype-dependent CYP2D6-mediated clearances of substrates and inhibitors, which were estimated as described previously. Predicted AUC ratio (AUCR) were compared with observed values. Simulated trial characteristics were the same as the observed trials, as described in Table S4. All simulations were run using the Healthy Volunteer population in Simcyp version 17, except for the tolterodine–fluoxetine trial for which the North European white population was used. For extensive metabolizers, including the AS1 and AS2 genotypes, the CYP2D6 phenotype distribution was set to 100% extensive metabolizers (CYP2D6 abundance in liver 8 pmol/mg of microsomal proteins). For poor metabolizers (PMs) and UMs, the CYP2D6 phenotype distribution was set to 100% PMs (i.e., absence of CYP2D6 expression in liver) and 100% UMs (CYP2D6 abundance in liver 16 pmol/mg of microsomal proteins), respectively.

RESULTS

Duloxetine model development and verification

The duloxetine model was developed and validated for single-dose and steady-state exposure using a range of clinical trials in various populations. Based on the observed and simulated PK profiles (as presented in Figure 1), the prediction was judged satisfactory with all observed concentrations between the 5% and 95% percentiles of predicted concentrations. The contribution of CYP2D6 in the clearance of duloxetine ($f_{\text{m,CYP2D6}}$) was estimated to be 39% using Eq. 1 (see the Methods section). Therefore, the contribution of CYP1A2...
was estimated to be 61%, which was confirmed by a predicted mean change of duloxetine exposure in the presence of fluvoxamine in male smokers of 5.49 when compared with a mean observed AUCR of 5.6.\textsuperscript{20} Duloxetine is a moderate inhibitor of CYP2D6.\textsuperscript{18} Using an optimized $K_i$ value estimated from \textit{in vivo} DDI data,\textsuperscript{18} good DDI predictions were obtained.
with tolterodine, metoprolol, and desipramine, as described in Table 2.

**Paroxetine model optimization**
The performance of the optimized paroxetine model was verified in terms of DDI predictions and steady-state exposure (see Table S5 and Figure S1). The observed steady-state PK profile was between 5% and 95% percentiles of predicted concentrations. The predicted change of exposure of CYP2D6 substrates (metoprolol, desipramine, imipramine) following paroxetine dosing were all within a twofold boundary of the observed AUCR and 62.5% restricted limits 75% to 125%.

**Incorporation of genetic effect in CYP2D6-mediated clearance of substrates and inhibitors**
CYP2D6-mediated clearance of victim and inhibitory compounds was modified as a function of genotype following parameter estimation using available clinical data, as described in Table 3. Using rich data from the in-house clinical studies mentioned in the Methods section, the CYP2D6-mediated clearance of dextromethorphan, duloxetine, and paroxetine was 2.36-fold, 1.2-fold, and 1.93-fold higher, respectively, in the AS2 subjects when compared with the AS1 subjects.

**Simulations of GDDIs**
Good predictions for GDDIs were obtained for the dextromethorphan–duloxetine interaction, with a slight overprediction (~40%) of change of substrate exposure when compared with the observed data (Table 4). Despite paroxetine model optimization with regard to TDI parameters, the DDI with dextromethorphan was still underpredicted. However, the effect of genotype was satisfactorily predicted, with \( R_{\text{pred/obs}} \) values for the ratio of AUC increase in the AS2 subjects over the AUC increase in the AS1 subjects of 0.96 for the duloxetine–dextromethorphan interaction and 0.71 for the paroxetine–dextromethorphan interaction.

A slight underprediction of fluoxetine-mediated DDIs was obtained with the CYP2D6 substrates tolterodine and risperidone in non-PMs, whereas good prediction was obtained in PM subjects. For fluoxetine-mediated DDIs, the predicted impact of genetic polymorphisms was in the twofold range of observed values with systematic underprediction (\( R_{\text{pred/obs}} \) of 0.60 and 0.75 for AS1/AS0 and AS2/AS1, respectively).

### Table 2 Simulated and observed drug–drug interactions with duloxetine

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Substrate dose</th>
<th>Duloxetine dose</th>
<th>Simulated AUCR*</th>
<th>Observed AUCR</th>
<th>( R_{\text{pred/obs}} )</th>
<th>Reference study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolterodine</td>
<td>2 mg/12 hours, 9 doses</td>
<td>40 mg/12 hours, 9 doses</td>
<td>2.85 (2.59–2.94)</td>
<td>1.71 (1.31–2.23)</td>
<td>1.67</td>
<td>Ref. 16</td>
</tr>
<tr>
<td>Desipramine</td>
<td>50 mg SD at day 6</td>
<td>30 mg/12 hours, 20 doses</td>
<td>1.75 (1.62–1.78)</td>
<td>2.22 (1.95–2.51)</td>
<td>0.79</td>
<td>Ref. 15</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>100 mg SD at day 17</td>
<td>30 mg day 1 then 60 mg days 2–18</td>
<td>1.75 (1.66–1.82)</td>
<td>2.80 ± 0.31</td>
<td>0.63</td>
<td>Ref. 19</td>
</tr>
</tbody>
</table>

AUCR, ratio of the substrate area under the concentration-time curve in the presence and absence of the inhibitor; \( R_{\text{pred/obs}} \), ratio of model-predicted mean exposure change of substrate to observed value; SD, single dose.

*Geometric mean with 95% confidence interval.

†Geometric mean with 90% confidence interval.

‡Arithmetic mean ± standard deviation.

### Table 3 Optimized values of CYP2D6-mediated clearance (CLint or \( V_{\text{max}} \)) of model substrates and inhibitors as a function of CYP2D6 genotype

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pathway</th>
<th>Initial value</th>
<th>Genotype effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextromethorphan</td>
<td>O-demethylation</td>
<td>CLint = 250.85 μL/minute/mg prot</td>
<td>AS1: −39% AS2: +44% AS3: +470%†</td>
</tr>
<tr>
<td>Tolterodine</td>
<td>5-hydroxylation</td>
<td>( V_{\text{max}} ) = 317 pmol/minute/mg prot</td>
<td>AS0: −100% AS1: −74% AS2: +325%</td>
</tr>
<tr>
<td>Risperidone</td>
<td>9-hydroxylation</td>
<td>CLint = 7.55 μL/minute/pmol CYP2D6</td>
<td>PM: −100% EM: unchanged</td>
</tr>
<tr>
<td>Duloxetine</td>
<td>All</td>
<td>CLint = 13.33 μL/minute/CYP2D6</td>
<td>AS1: −31% AS2: −13%</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>All</td>
<td>( V_{\text{max}} ) = 7.28 pmol/minute/pmol CYP2D6</td>
<td>AS1: −18% AS2: +58%</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>N-demethylation</td>
<td>CLint = 52.65 μL/minute/mg prot</td>
<td>AS0: −100% AS1: +293% AS2: +624%</td>
</tr>
</tbody>
</table>

AS0, activity score 0; AS1, activity score 1; AS2, activity score 2; AS3, activity score 3; CLint, intrinsic clearance; CYP2D6, cytochrome P450 2D6; EM, extensive metabolizer; PM, poor metabolizer; prot, protein; \( V_{\text{max}} \), maximal reaction velocity.

†The CLint value was increased 2.9-fold, and CYP2D6 liver abundance increased twofold.

‡The CLint value was unchanged, and CYP2D6 liver abundance was defined as zero.
DISCUSSION

In this study, we used available rich PK data with different genotypes to estimate the CYP2D6-mediated clearance of dextromethorphan, tolterodine, duloxetine, and paroxetine from beforehand validated PBPK models and subsequently performed genotype-dependent DDI simulations to compare with clinical DDI data.

First, a PBPK model for the moderate CYP2D6 inhibitor duloxetine was developed and validated. Good predictions for single-dose and steady-state exposures in different populations were obtained. The optimized model predicted well CYP1A2 contribution in hepatic clearance, as verified with a DDI trial with the potent inhibitor fluvoxamine in male smokers. Duloxetine has been shown to increase the exposure of the CYP2D6 substrates desipramine and tolterodine up to 2.9-fold with a 60 mg twice-daily regimen, which could not be predicted by the bottom-up approach in Simcyp using experimental Ki values in the range of 2–4 μM. Instead, this value was optimized from in vivo DDI data with desipramine and verified with a set of independent DDI data with desipramine, metoprolol, and tolterodine. To note, the approach of using in vivo optimized rather than in vitro obtained Ki values for the modeling of DDIs was similarly performed previously with fluvoxamine and ciprofloxacin, two CYP1A2 inhibitors for which experimental Ki values resulted in the underprediction of DDIs. The reasons for the underprediction of the DDI with duloxetine from in vitro data remains unclear, and we present herein some possible explanations.

Duloxetine has been previously described as a competitive inhibitor of CYP2D6 lacking of TDI properties, suggesting that duloxetine metabolites are not CYP2D6 inhibitors. Therefore, the underprediction of DDIs cannot be attributed to any TDI mechanism not taken into account in the model. Possible duloxetine accumulation into hepatocytes through active transport might explain the underprediction of DDI, which was not investigated in the present study. Bupropion represents another case of underprediction of CYP2D6-mediated DDIs. Indeed, bupropion is a strong CYP2D6 inhibitor in vivo, which increases the exposure of desipramine by around fivefold. However, in vitro in vivo extrapolation fails to predict such DDIs from experimental data. Recently, Sager et al. showed that concomitant competitive inhibition and CYP2D6 downregulation by bupropion and its metabolites could explain the strong CYP2D6 inhibition observed in vivo and that taking into account the downregulation of CYP2D6 in the prediction of the DDI could improve the in vitro in vivo extrapolation of bupropion-perpetrator DDIs. CYP2D6 has long been considered a noninducible enzyme. Therefore, CYP2D6 induction studies are not recommended by regulatory agencies and not systematically assessed in current drug development. However, recent studies have shown upregulation of CYP2D6 expression during pregnancy, which seems to be related to an enhanced HNF-4α transactivation of the CYP2D6 promoter induced by the decreased expression of small heterodimer partner, a corepressor of various transcription factors, and increased expression of the Krüppel-like factor 9, both occurring during pregnancy. As transcriptional regulation

| Table 4 | Comparison of predicted and observed gene–drug–drug interaction trials |
| Substrate | Inhibitor | CYP2D6 genotype | AUCR | Predicted | Observed | AUCR, Predicted/Observed | R_{pred/obs} | Reference |
| Dextromethorphan | Duloxetine | AS1 | 17 | 2.6 (2.5–2.7) | 1.43 | 1.28 | 1.28 | 0.96 | Ref. 10 |
| | | AS2 | 16 | 3.3 (3.2–3.4) | 1.38 | 1.21 | 1.22 | 0.71 | Ref. 29 |
| | Paroxetine | AS1 | 17 | 4.7 (4.4–5.0) | 0.96 | 0.91 | 0.96 | 0.96 | Ref. 10 |
| | | AS2 | 16 | 5.8 (5.6–6.3) | 1.46 | 1.26 | 1.26 | 0.71 | Ref. 29 |
| | Fluoxetine | AS1 | 2 | 3.3 (3.1–3.4) | 1.25 | 1.27 | 1.27 | 1.06 |
| | | AS2 | 3 | 6.1 (5.9–6.3) | 1.83 | 1.76 | 1.76 | 0.96 |
| | Tolterodine | Fluoxetine | AS0 | 2 | 1.3 (1.3–1.4) | 1.06 | 1.06 | 1.06 | 1.06 |
| | | | AS1 | 3 | 2.0 (1.9–3.0) | 1.71 | 1.71 | 1.71 | 1.06 |
| | | | AS2 | 4 | 2.6 (2.4–2.8) | 1.36 | 1.36 | 1.36 | 1.06 |
| | | PM | 2 | 1.4 (1.3–1.5) | 1.34 | 1.34 | 1.34 | 1.06 |
| | | EM | 7 | 2.9 (2.7–3.1) | 4.2 | 4.2 | 4.2 | 1.06 |

AUCR, area under the concentration-time curve for the substrate; AUCR, ratio of the substrate AUCR in genotype x versus genotype y; CYP2D6, cytochrome P450 2D6; EM, extensive metabolizer; PM, poor metabolizer; R_{pred/obs}, ratio of model-predicted mean exposure change of substrate to observed value.
of CYP2D6 is not systematically investigated during drug development, we found no data regarding any regulation of CYP2D6 by duloxetine. Further investigation of the CYP2D6 downregulation by duloxetine may represent an interesting clue to explain the underprediction of DDIs between duloxetine and CYP2D6 substrates.

In preliminary simulations, the existing paroxetine PBPK model from Simcyp library version 17 resulted in the underprediction of paroxetine exposure with the two-dose regimen used in the dextromethorphan–paroxetine DDI verification study and therefore in the underprediction of DDIs (data not shown), which corroborates with the observed tendency toward underprediction of DDIs between paroxetine and CYP2D6 substrates that was described previously by Marsousi et al. Paroxetine is both a substrate and a strong time-dependent inhibitor of CYP2D6. Therefore, the TDI parameters $K_I$ and $k_{inact}$ were modified to improve the predictions of both paroxetine exposure and the DDIs. Using previously optimized TDI parameters taking into account the hepatocyte-to-plasma pH gradient ($K_I$ was reduced from 0.315 to 0.067 μM, and $k_{inact}$ was increased from 10.2 to 11.55 hour$^{-1}$), we obtained better DDI predictions than previously reported with the Simcyp library model (Table S5). Moreover, the multiple dosing exposure was well recovered as shown in Figure S1, improving confidence in the paroxetine model for the further evaluation of GDDIs. We decided to optimize the paroxetine model by modifying the TDI parameters $K_I$ and $k_{inact}$ because DDI underestimation was linked with an underprediction of paroxetine exposure as well. In the model optimization part of the study, modifying the TDI parameters permitted better recovery of both the paroxetine exposure and magnitude of DDIs. Unfortunately, when simulating the in-house data in which only two close doses of paroxetine were given, an underprediction of the DDI was observed. It is possible that the optimized paroxetine model was only working at steady state and did not capture well the paroxetine single dose. Another parameter difficult to accurately obtain is the intrahepatocyte concentration of the inhibitor that could accumulate through active transport.

At the beginning of the present study, we considered introducing genetic effect in the clearance of CYP2D6 substrates by either playing on CYP2D6 liver abundance or on CYP2D6 enzyme kinetics. In PMs, the CYP2D6 protein is absent, and therefore the CYP2D6 abundance of PM subjects is set to zero. However, immunologic and proteomic studies failed to demonstrate a difference in the amounts of CYP2D6 between activity scores 0.5 to 2, which seems to be mostly related to the high interindividual variability, even within a same genotype. Therefore, there was no evidence supporting a change of functional CYP2D6 abundance for non-PM genotypes, and we rather decided to incorporate the effect of genetic polymorphisms on CYP2D6 enzyme kinetics data (intrinsic clearance (CLint) or maximal reaction velocity ($V_{max}$)). CLint represents the ratio of $V_{max}$ over the affinity constant $K_m$. If the affinity constant is not affected by genetic polymorphism, the effect of modifying $V_{max}$ or CLint is the same. However, it is true that using $K_m$ and $V_{max}$ provides additional information on saturation of the enzyme. The decision of playing with either $V_{max}$ of CLint was based on which parameter was informed in the initial model from the Simcyp database.

We used the full 0–8-hour PK profiles of dextromethorphan and its metabolite dextrorphan obtained from a previous clinical trial in our group to estimate the CYP2D6-mediated

Figure 2 Sensitivity analysis of CYP2D6-mediated clearance of both victim and inhibitors drugs on the extent of drug–drug interactions. AUC, area under the curve; CLint, intrinsic clearance; CYP, cytochrome P450; $V_{max}$, maximal velocity.
O-demethylation CLint in AS1, AS2, and AS3 subjects using individual fitting. Similarly, we used the full PK profiles of duloxetine and paroxetine, both being CYP2D6 substrates as well, from another clinical trial in AS1 and AS2 subjects\textsuperscript{10} to estimate their CYP2D6-mediated clearance. This approach allowed a good prediction of the genetic effect on the extent of DDI between dextromethorphan and paroxetine or duloxetine, although better prediction was obtained with duloxetine when compared with paroxetine. In contrast to duloxetine, whose $f_{\text{mCYP2D6}}$ is around 40\%, paroxetine is importantly metabolized by CYP2D6 ($f_{\text{mCYP2D6}} > 90\%$). Therefore, CYP2D6 genetic polymorphisms affects the enzyme contribution in the clearance of both the victim and the inhibitor drugs, therefore adding uncertainty in both inhibitor exposure and victim drug clearance, which might explain the lower predictive performance with paroxetine.

Similar to paroxetine, we observed an underprediction of fluoxetine-perpetrated DDIs in non-PM subjects, which might be attributable to the CYP2D6-mediated clearance of the inhibitor. It should be noted that fluoxetine CYP2D6-mediated clearance in AS1 and AS2 genotypes was estimated using single fluoxetine through concentration at steady state in a limited number of individuals (only four AS1 and three AS2). It is also interesting to note that fluoxetine is a weak CYP3A4 inhibitor, which explains why the AUCR is higher than one in CYP2D6 PMs. Indeed, the contribution of CYP3A4 in tolterodine and risperidone clearance is increased in subjects lacking CYP2D6, thus resulting in significant DDIs in the presence of even weak CYP3A4 inhibitors mainly cleared by CYP2D6. The authors refer the reader to Vieira et al.\textsuperscript{13} for an insightful discussion about the impact of both comedication and genetic polymorphism on the exposure of drugs undergoing multiple clearance pathways.

Overall, both predictions and observations converged toward the following rule: the higher the activity of CYP2D6, the higher will be the extent of interaction. Indeed, the extent of DDIs is highly dependent on the contribution of the inhibited enzyme, and the higher the activity of CYP2D6 the higher is the contribution of the enzyme in the clearance of the substrate. However, it must be emphasized that numerous CYP2D6 inhibitors, including paroxetine and fluoxetine, are mainly metabolized by CYP2D6 and therefore are also more rapidly eliminated when CYP2D6 activity increases, which has an impact on the extent of DDIs. As illustrated in Figure 2, the extent of a DDI (AUCR) increases as long as the $f_{\text{mCYP2D6}}$ of the substrate increases and might then diminish when the latter saturates, in which case the concentration of the inhibitor becomes a major determinant.

**PERSPECTIVES**

PBPK modeling represents a promising approach to predict the combined effect of multiple intrinsic and/or extrinsic factors affecting drug PK. The extent and clinical impact of DDIs are of huge interindividual variability and poor predictability. Integrating PBPK modeling in advanced computerized physician order entry systems to predict the extent of DDIs as a function of different intrinsic factors, such as age, concomitant medications, organ impairment, and genetic polymorphisms, might help targeting individuals at high risk of clinically significant DDIs to which treatment optimization might be proposed. Previously, the PBPK approach was proposed by Patel et al.\textsuperscript{14} to predict the effect of both race and genotype on drug exposure. Similarly, Vieira et al.\textsuperscript{13} obtained good predictions with PBPK modeling for GDDIs involving drugs with multiple clearance pathways. In this article, we obtained good predictions for the dextromethorphan–duloxetine and the dextromethorphan–paroxetine DDIs in AS1 and AS2 subjects using available rich data from a dedicated GDDI clinical trial in those subjects. However, further optimization of initial PBPK models for the TDI inhibitors paroxetine and fluoxetine appears necessary based on the underestimation of DDIs observed in our study. Indeed, it is only if initial PBPK models are optimized and verified that they can further be used to predict more complex DDIs. Based on these results, we suggest a workflow as depicted in Figure 3. First, PBPK models are developed using available in vitro (bottom-up approach) and clinical data if available (top-down approach) and validated with a range of clinical data, including DDI studies. Available rich PK data for substrates and inhibitors from genetic studies performed during clinical development serve as a basis for the top-down estimation of CYP2D6-mediated clearance in

![Figure 3](image-url)
different genotypes to incorporate the effect of genotype in their clearance. In vitro kinetic experiments in genotyped human liver microsomes might also represent an alternative approach, provided sufficient sample size to account for the high interindividual variability. Finally, GDDIs can be simulated to detect individuals at risk of clinically significant DDIs. In summary, our work demonstrates that PBPK modeling might represent a promising approach to predict the effect of genetic polymorphisms on the extent of DDIs, provided good predictive performance of the initial models and proper evaluation of the genetic effect in the exposure of substrates and inhibitors.

Supporting Information. Supplementary information accompanies this paper on the CPT: Pharmacometrics & Systems Pharmacology website (www.psp-journal.com).

Figure S1. Observed vs. simulated paroxetine steady-state exposure. A total of 10 trials of nine healthy male CYP2D6 extensive metabolizers (age range 20–30 years) were simulated and compared with published data.

Table S1. Characteristics of simulated trials used for duloxetine PK model verification.

Table S2. Characteristics of simulated trials used for duloxetine DDI model verification.

Table S3. Characteristics of simulated trials used for modified paroxetine DDI model verification.

Table S4. Characteristics of simulated GDDI trials.

Table S5. Characteristics of simulated trials used for modified paroxetine DDI model verification.

Data S1. Data set: input model parameters.

Data S2. Supplementary material references.

Acknowledgments. The authors would like to thank Simcyp Limited, a Certara Company for the academic license for the Simcyp Population-based Simulator and providing user support. In particular, the authors would like to thank Mian Zhang, Amaka Ezuruike, and Kim Crewe for their availability to discuss the project.

Funding. This work was supported by a Swiss National Science Foundation grant (SNF 320030_182361).

Conflicts of Interest. The authors declared no competing interests for this work.

Author Contributions. F.S., J.D., and Y.D. wrote the manuscript and designed the research. F.S. performed the research. F.S., J.D., and Y.D. analyzed the data.


