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Implications of inter-correlation between hepatic CYP3A4-CYP2C8 enzymes for the
evaluation of drug-drug interactions: a case study with repaglinide

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A running head: Impact of enzymes inter-correlation on predicted DDI variability

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Summary

AIMS
Statistically significant positive correlations are reported for the abundance of hepatic drug-metabolising enzymes. We investigate, as an example, the impact of CYP3A4-CYP2C8 inter-correlation on the predicted inter-individual variabilities of clearance and drug-drug interactions (DDIs) for repaglinide using physiologically-based pharmacokinetic (PBPK) modelling.

METHODS
PBPK modelling and simulation was employed using Simcyp Simulator (v15.1). Virtual populations were generated assuming inter-correlations between hepatic CYP3A4-CYP2C8 abundances derived from observed values in 24 human livers. A repaglinide PBPK model was used to predict pharmacokinetic parameters in presence and absence of gemfibrozil in virtual populations, and the results were compared with a clinical DDI study.

RESULTS
Coefficient of variation (CV) of oral clearance was 52.5% in the absence of inter-correlation between CYP3A4-CYP2C8 abundances which increased to 54.2% when incorporating inter-correlation. In contrast, CV for predicted DDI (as measured by AUC ratio before and after inhibition) was reduced from 46.0% in the absence of inter-correlation between enzymes to 43.8% when incorporating inter-correlation: these CVs were associated with 5th/95th percentiles (2.48–11.29 vs. 2.49–9.69). The range of predicted DDI was larger in the absence of inter-correlation (1.55–77.06) than when incorporating inter-correlation (1.79–25.15), which was closer to clinical observations (2.6–12).

CONCLUSIONS
The present study demonstrates via a systematic investigation that population-based PBPK modelling incorporating inter-correlation led to more consistent estimation of extreme values with those observed in inter-individual variabilities of clearance and DDI. As the inter-correlations more realistically reflect enzyme abundances, virtual population studies involving PBPK and DDI should avoid using Monte Carlo assignment of enzyme abundance.
WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

• Current population-based physiologically-based pharmacokinetic (PBPK) models do not consider inter-correlations between the abundances of different enzymes when using Monte Carlo simulations to generate virtual individuals.

• Statistically significant positive correlations are reported for enzyme abundance between certain drug-metabolising enzymes.

WHAT THIS STUDY ADDS

• The study for the first time incorporates inter-correlation of enzymes into population-based PBPK model.

• The impact of inter-correlation was assessed for clearance and DDI variability of the model compound repaglinide.

• Here we present a practical approach to assessing enzyme inter-correlations and their potential impact on drug clearance and DDIs.
Introduction

Pharmacokinetic drug-drug interactions (DDIs) can alter systemic exposure to drugs, and result in reduced treatment efficacy or increased risk of adverse drug reactions (ADRs). Quantitative DDI assessments are important to anticipate the clinical risks associated with DDIs prior to market approval and during post-marketing surveillance. Physiologically-based pharmacokinetic (PBPK) approaches may be used to estimate the risk of DDIs, and validated PBPK models allow the quantitative prediction of the magnitude of DDIs in various clinical situations, including in special populations [1–3]. DDI predictions using validated PBPK models have influenced the labelling recommendations for several drugs, such as ibrutinib and eliglustat [4, 5]. The approaches described above have mainly focused on the prediction of a single ‘average’ outcome for change in systemic exposure. However, in individual subjects, severe ADRs may be induced by the unexpected magnitude of DDIs based on a large inter-individual variability in metabolic clearance [6]. It is therefore important to consider not only the average DDI effect but also the population distribution in DDI outcomes and the theoretically conceivable extremes in outcome in individual subjects [6].

Inter-individual variability in hepatic drug clearance can be anticipated by incorporating sources of variability such as demographic factors (e.g., age and sex), and physiological factors (e.g., hepatic blood flow, enzyme/transporter levels and activity) [7]. Data from clinical pharmacokinetic studies are not amenable to separation of the role of variable intrinsic clearances by various metabolic pathways and their inter-correlations. In contrast, in silico approaches such as PBPK models can provide such information about the contribution of single covariate factors, or about the interaction between them. One of the key physiological factors determining inter-individual variability in hepatic drug clearance is enzyme abundance, which is affected by age, sex, body and liver weight, and dietary habits, as well as genetic polymorphisms. However, without considering the inter-correlation between physiological parameters (e.g. liver volume and hepatic blood flow, and liver volume and kidney volume), physiologically implausible parameter combinations will be generated when sampling from a population distribution that may lead to overestimation in the inter-individual variability of
pharmacokinetic parameters, such as clearance [8]. To account for this, state-of-the-art PBPK models consider the inter-correlation between organ/tissue volumes and blood flows [7, 9].

Up until recently, it has been challenging to consider the inter-correlation between the expression levels of drug-metabolising enzymes and transporters due to the lack of reliable quantitative data. Historically, measuring enzyme and transporter expression levels relied on assessment of gene expression (mRNA) or immuno-quantification assays, which tended to generate semi-quantitative analyses, not amenable to multiplexing, even when robust quality controls were applied, and therefore these methods were generally unable to uncover expression correlations [10]. However, novel methods in quantitative proteomics driven by recent advances in LC-MS technology made it possible to reliably measure multiple enzymes and transporters in individual tissue samples in the same experiment, therefore allowing robust consideration of inter-correlations of these proteins [11]. Although drug-metabolising enzyme inter-correlations have recently been reported [12], the quantitative impact of such relationships on pharmacokinetic outcomes has not been extensively explored. A PBPK model incorporating a CYP3A4-CYP3A5 inter-correlation produced a reasonable oral clearance estimate of tacrolimus, a substrate of both CYP3A4 and CYP3A5, in individuals displaying high or low basal concentration of CYP3A4 [13]. Given a credible range of predicted drug clearance in PBPK models incorporating the reported inter-correlation between two enzymes, the PBPK models could predict the theoretically conceivable extreme risk of DDIs.

This report describes a proof-of-principle study for the prediction of inter-individual variability in drug clearance and DDIs by incorporating inter-correlation between two drug-metabolising enzymes into a PBPK model. To achieve the aim of this study, we investigate, as an example, the impact of CYP3A4-CYP2C8 inter-correlation on the prediction of inter-individual variability in drug clearance and DDIs of repaglinide, a probe substrate for CYP2C8 inhibition studies [14]. Repaglinide, a short acting anti-diabetic drug, is mainly metabolised by CYP2C8 and CYP3A4, and is also a substrate of the hepatic uptake transporter, organic anion transporting polypeptide (OATP) 1B1 [15–17]. Systemic exposure of repaglinide is increased by inhibitors of CYP2C8, CYP3A4, and OATP1B1 [18, 19]. In the present study, we generated virtual populations assuming a
CYP3A4-CYP2C8 inter-correlation, and assessed the effect of the inter-correlation on the prediction of inter-individual variability in clearance and magnitude of DDI of repaglinide using PBPK modelling. Furthermore, theoretically conceivable extreme values of clearance and DDI generated under various assumptions regarding the inter-correlations were investigated and compared with clinical observations.

Methods

Assessing inter-correlation between hepatic CYP3A4 and CYP2C8 abundances

Hepatic CYP3A4 and CYP2C8 abundance data usable for correlation analysis were obtained from the literature (Figure 1A) [20]. The protein expression was measured simultaneously in a set of 24 individual human liver microsomes (HLM) using a multiplexed QconCAT-based proteomic method, designed to allow robust assessment of expression inter-correlation. Individual values for each HLM sample were obtained directly from authors of the original research article. A linear regression model was developed (using IBM SPSS Statistics 24, IBM, NY, USA) to assess the correlation between hepatic CYP3A4 and CYP2C8 protein expression as previously reported [13]. The model was as follows:

\[ \text{CYP2C8 (pmol/mg microsomal protein)} = C0 + C1 \cdot \text{CYP3A4 (pmol/mg)} \]

where \(C0\) is the baseline level of CYP2C8 protein expression, and \(C1\) is the slope. A coefficient of variation (CV) describing residual variability in CYP2C8 protein expression was determined from the sum of squares of the least squares linear regression analysis and the mean relative CYP2C8 abundance of the dataset. It should be noted that assigning CYP2C8 as the dependent variable was arbitrary and does not infer any causality (an alternative of defining CYP3A4 could equally be applied). The correlation was only the manifestation of potential common regulatory pathways (e.g. known regulatory factors such as FXR, PXR, CAR, AhR etc.).
Development of a PBPK model using inter-correlation between CYP3A4 and CYP2C8

PBPK modelling and simulation was employed using the Simcyp® Simulator (v15.1; Certara, Sheffield, UK). Virtual populations, assuming different magnitudes of inter-correlation between CYP3A4 and CYP2C8, were generated by altering physiological parameters in the “healthy volunteers” population template within the Simcyp Simulator population library (Table 1). Hepatic CYP3A4 and CYP2C8 abundances for default healthy volunteers were generated independently using mean population values of CYP3A4 and CYP2C8 of 137 and 24 pmol/mg microsomal protein and associated CVs of 41% and 81% for CYP3A4 and CYP2C8, respectively. Input parameters for CYP3A5 were substituted by the corresponding values of CYP2C8 in population template when assessing inter-correlation between CYP3A4 and CYP2C8. The current version of the Simcyp Simulator allows enzyme inter-correlation where hepatic CYP3A5 abundance is predicted from CYP3A4 abundance based on a linear model. Default parameter values for baseline, slope, and CV of the enzyme inter-correlation module were substituted by parameter values derived from inter-correlation between hepatic CYP3A4 and CYP2C8. Default values of CYP3A5 abundance in gastrointestinal tract and frequency of CYP3A5 extensive metabolisers (EM) were also substituted by values for CYP2C8. This value was set to 1.00 because complete loss-of-function of CYP2C8 variants is very rare [14].

The pre-validated repaglinide compound file in the Simcyp compound library was adapted to use the enzyme inter-correlation module based on an inter-correlation between hepatic CYP3A4 and CYP2C8 (Table 2). Physicochemical parameters (molecular weight, logP<sub>o/w</sub>, acid/base status and pKa), fraction unbound in plasma, and blood/plasma ratio were obtained from data in the literature and public databases [21, 22]. A full-PBPK distribution model for repaglinide was developed using the Rodgers and Rowland method assigning perfusion-limited distribution to all tissues except liver [23]. Permeability-limited distribution of repaglinide into the liver was adopted to account for transporter-mediated intrinsic clearance (CL<sub>int,T</sub>) for the sinusoidal uptake transporter OATP1B1. Default values of V<sub>max</sub> and K<sub>m</sub> for repaglinide metabolism in HLM were 300.8 pmol/min/mg and 2.3 μM for CYP2C8, and 958.2 pmol/min/mg and 13.2 μM for CYP3A4, respectively [15]. The enzyme
kinetic values in HLM were converted into values in microsomes from a recombinant system with transformation of $V_{\text{max}}$ values using default mean abundance data of CYP2C8 and CYP3A4. In the modified repaglinide model for CYP3A4-CYP2C8 correlation, the converted enzyme kinetic values for recombinant CYP2C8 and CYP3A4 were used as parameter values for recombinant CYP3A5 and CYP3A4, respectively. The other parameters were not changed from the pre-validated values.

Gemfibrozil, a clinically relevant CYP2C8 inhibitor, was used for the virtual DDI study. It is mainly metabolised to gemfibrozil 1-0-β glucuronide (Gem-Glu) which is a mechanism-based inhibitor of CYP2C8 [24]. In addition, both parent gemfibrozil and the glucuronide metabolite inhibit OATPB1 in vitro [25] and in vivo [26]. The pre-validated compound files of gemfibrozil and Gem-Glu supplied in the Simcyp compound library were developed with modifications where inhibitory parameter values of CYP2C8 were used as those of CYP3A5 (Table 3).

**Design of virtual studies**

Virtual studies for clinical pharmacokinetics and DDIs of repaglinide were simulated using the default number of trials (10) and modified virtual populations (up to 100 trials). The simulation results were compared to pharmacokinetic data from a published clinical trial in which repaglinide was administered either alone or with pretreatment with gemfibrozil [19]. Observed data for plasma repaglinide concentration profiles were obtained directly from authors of the original research article. The trial design (number of subjects, age range and proportion of females) was replicated as closely as possible to ensure that the characteristics of virtual subjects were matched to those of the clinical trial [19]. An age range of 19-29 years was obtained directly from the authors of the original research article. Replicate virtual trials simulated in ten subjects each (nine males and one female) receiving a single oral dose of repaglinide 0.25 mg were performed in the absence and presence of pretreatment with 600 mg gemfibrozil twice daily. The influence of the number of trials on simulation outcome was assessed by incremental increase of trials from 10 to 100. DDI effects were characterized by ratios between before and after inhibition for maximum concentration ($C_{\text{max}}$) of repaglinide and the area under the concentration-time curve (AUC) from administration to 9 h.
Statistical analyses

Distributions of predicted oral clearance and DDI effects were compared between virtual individuals assuming no correlation and inter-correlation using the Kolmogorov-Smirnov test. CV values for each trial was extracted and the median CV from the sets of virtual trials were used for comparison of the effect of considering or omitting inter-correlations between the enzymes.

Results

Assessing inter-correlation between hepatic CYP3A4 and CYP2C8 abundances

The correlation between CYP3A4 and CYP2C8 protein expression in 24 individual HLMs obtained from Achour et al. [20] is shown in Figure 1B. Linear regression analysis indicated a strong correlation between the two enzymes ($R= 0.85; P<0.0001$). The linear model was described as follows:

$$\text{CYP2C8 (pmol/mg)} = 0.771 + 0.266 \cdot \text{CYP3A4 (pmol/mg)}$$

The residual variability in CYP2C8 abundance was estimated to be 47% based on the residual sum of squares (1715) and mean CYP2C8 abundance of 18.9 pmol/mg derived from the experimental dataset.

Hepatic CYP2C8 and CYP3A4 abundances in virtual individuals

Hepatic CYP2C8 and CYP3A4 protein contents in a population of 100 virtual individuals were generated assuming either no correlation or correlation between the two enzymes with CV of 0%, 47%, and 100% (Figure 2). Default virtual individuals assuming no correlation between two enzymes showed higher CYP2C8/CYP3A4 ratio in virtual individuals with low CYP3A4 protein content (Figure 2A). Incorporation of correlation between CYP2C8 and CYP3A4 resulted in a consistent pattern in CYP2C8/CYP3A4 ratio according to the assigned residual variability (Figure
Hepatic CYP2C8 and CYP3A4 abundances in virtual individuals assuming correlation between the two enzymes with residual variability of 47% (Figure 2C) showed a similar pattern to an actual correlation between HLM CYP3A4 and CYP2C8 protein expression (Figure 1B).

Prediction of pharmacokinetic parameters of repaglinide

Predicted pharmacokinetic parameters, systemic clearance, oral clearance of repaglinide, and the fraction of repaglinide escaping metabolism by the gut (F_G) and by the liver (F_H), were predicted in 1,000 virtual individuals generated assuming no correlation and correlation between the CYP3A4 and CYP2C8 with residual variability of 47% (Figure 3). Systemic and oral clearance, and F_H were affected by the incorporation of correlation between the CYP3A4 and CYP2C8 within the virtual population, but F_G was not. The influence of CYP3A4-CYP2C8 inter-correlation on oral clearance was different among three groups classified by hepatic CYP3A4 abundance in microsomal protein: 5% lower estimate in low expression (<100 pmol/mg), 36% higher estimate in medium expression (100-199 pmol/mg), 107% higher estimate in high expression (≥200 pmol/mg). CV of the predicted oral clearance for individuals with low CYP3A4 expression was greater in the absence of inter-correlation than that assuming inter-correlation (74% vs. 48%), but that for individuals with medium and high expression showed little or no difference between the two populations (medium expression, 62% vs. 56%; high expression, 54% vs. 54%).

Prediction of repaglinide DDI

Simulated plasma repaglinide concentration profiles after a single oral dose of 0.25 mg repaglinide without or with pretreatment with 600 mg gemfibrozil twice daily in 1,000 virtual individuals generated assuming no correlation and correlation between CYP3A4 and CYP2C8 were comparable to observed data from the published clinical trial [19] (Figure 4). Mean values of the predicted AUC and C_max without and with gemfibrozil were within 1.5-fold of the observed values.
(AUC: no correlation, 7.1 vs. 35.0 ng·h/mL; inter-correlation, 6.0 vs. 27.7 ng·h/mL; observed, 4.8 vs. 29.3 ng·h/mL, and C\textsubscript{max}: no correlation, 3.4 vs. 7.3 ng/mL; inter-correlation, 3.0 vs. 6.5 ng/mL; observed, 3.7 vs. 8.1 ng/mL) [19]. Median CVs of the predicted AUC and C\textsubscript{max} without and with gemfibrozil were similar between no correlation and inter-correlation scenarios (without gemfibrozil: AUC, 53.3% and 50.7%; C\textsubscript{max}, 35.7% and 36.9%, and with gemfibrozil: AUC, 31.1% and 35.8%; C\textsubscript{max}, 21.0% and 22.7%), but were lower than the observed values (without gemfibrozil: AUC, 89.8%; C\textsubscript{max}, 71.3%, and with gemfibrozil: AUC, 26.3%; C\textsubscript{max}, 39.7%) [19]. Figure 5 shows the predicted AUC and C\textsubscript{max} ratios (DDI/control) of repaglinide for 100 trials in 10 virtual individuals generated assuming no correlation and in the presence of correlation between hepatic CYP3A4 and CYP2C8, compared with reported clinical data (observed AUC ratio: mean, 6.1; range, 2.6 to 12, and C\textsubscript{max} ratio: mean, 2.2; range, 1.4 to 2.9) [19]. Median values of predicted AUC ratio were similar between virtual populations assuming no correlation and in the presence of inter-correlation (4.72 vs. 4.42), but the 5\textsuperscript{th} and 95\textsuperscript{th} percentiles were different between the two virtual populations (2.48 to 11.29 vs. 2.49 to 9.69). The range of predicted AUC ratio from minimum to maximum was larger for virtual populations assuming no correlation than that for those assuming inter-correlation (1.55 to 77.06 vs. 1.79 to 25.15). Predicted AUC ratios at higher than the maximum observed in clinical data (12-fold) [19] were observed in 39 and 21 virtual individuals from simulations without and with the inter-correlation, respectively. Median values and 5\textsuperscript{th} and 95\textsuperscript{th} percentiles of predicted C\textsubscript{max} ratio were almost the same between virtual populations assuming no correlation and those with the inter-correlation (median, 2.05 vs. 2.02; 5\textsuperscript{th} and 95\textsuperscript{th} percentiles, 1.46 to 3.72 vs. 1.45 to 3.69). However, the range of predicted C\textsubscript{max} ratio from minimum to maximum was larger for virtual populations assuming no correlation than for populations with the inter-correlation (1.18 to 15.15 vs. 1.26 to 6.98). Predicted C\textsubscript{max} ratios of more than the maximum observed in clinical data (2.9-fold) [19] were generated in 145 and 137 virtual individuals without and with the inter-correlation, respectively.

Cumulative distribution plots for predicted oral clearance, AUC ratio, and C\textsubscript{max} ratio are shown for 1,000 virtual individuals generated assuming no correlation and correlation between hepatic CYP3A4 and CYP2C8 (Figure 6). Individual values for CYP3A4-CYP2C8 correlation were
normalised by the corresponding mean values for no correlation. Distributions for predicted AUC ratio were unequal between virtual individuals generated assuming no correlation and inter-correlation ($P = 0.026$), but those for oral clearance and $C_{\text{max}}$ were not significantly different ($P = 0.40$ and $P = 0.18$, respectively). The frequency distributions were wider for predicted oral clearance and tighter for AUC ratio and $C_{\text{max}}$ ratio in the virtual population assuming CYP3A4-CYP2C8 correlation than in the absence of inter-correlation. Extreme values were seen in both ends (1-5% and 95-99%) of the cumulative distribution for the predicted AUC ratio in the absence of inter-correlation (Figures 6E and 6H).

**Inter-individual variability in clearance and DDI of repaglinide**

CVs of oral clearance and predicted DDI (AUC ratio and $C_{\text{max}}$ ratio) in 10 to 100 trials were compared between virtual populations generated assuming no correlation and correlation between CYP3A4 and CYP2C8 (Figure 7). Median CV of oral clearance in the 10 trials was 43.2% in the absence of inter-correlation between enzymes and 49.3% when CYP3A4-CYP2C8 abundances were considered to be inter-correlated. This difference in the median CV of oral clearance became smaller in the 100 trials (52.5% vs. 54.2%). In contrast, median CV of AUC ratio in the 100 trials was reduced from 46.0% in the absence of inter-correlation between the two enzymes to 43.8% when the inter-correlation was incorporated into the virtual population, with the exception of opposite results seen in the minimum number of trials (the 10 trials). Median CV of predicted $C_{\text{max}}$ ratio was almost the same between virtual populations assuming no correlation and inter-correlation regardless of numbers of trials.

**Discussion**

This report constitutes a proof-of-principle study to highlight the importance of inter-correlation between the hepatic amounts of two drug-metabolising enzymes in the prediction of inter-individual variabilities in drug clearance and DDIs. The present study demonstrates, for the first time,
that population-based PBPK modelling incorporating such inter-correlation led to more consistent estimation of extreme values with those observed in inter-individual variability of drug clearance and DDI. It should be the norm, rather than the exception, to consider this information when using population-based PBPK models. Otherwise, a PBPK model not accurately considering the inter-correlation runs the risk of generating implausible combinations of physiological parameters.

Furthermore, a more realistic prediction of inter-individual variability of drug clearance may help to more accurately estimate the theoretically conceivable extreme risks of DDIs. This has clear implications for drug development and clinical drug use. A large inter-individual variability in DDIs usually warrants additional caution in recommendation of dose adjustment even if the average effect of DDI is tolerable based on the safety margin of the substrate drug. Therefore, incorporation of such correlation into a PBPK model should be considered in investigating the DDI risk and is likely to be particularly important for prediction of the clinical consequences of the DDI in individual patients.

A virtual population assuming inter-correlation between CYP3A4 and CYP2C8 was developed based on actual data on HLM enzyme abundance. The abundances of hepatic CYP2C8 and CYP3A4 were compared between virtual individuals generated assuming no correlation and correlation between two enzymes (Figure 2). Simcyp generates individual values of CYP2C8 abundance independently based on population mean and CV by default. Therefore, default virtual individuals with high and low CYP3A4 protein content can be accompanied by underestimated and overestimated values of CYP2C8 abundance, respectively. Although the inter-individual variability of CYP2C8 and CYP3A4 protein expression in liver is high, the protein expression is correlated between the two enzymes [12, 20]. Virtual individuals assuming correlation between the two enzymes with residual variability of 47%, which was estimated from linear regression analysis using hepatic CYP3A4 and CYP2C8 abundance data, were more reflective of an actual correlation between HLM CYP3A4 and CYP2C8 protein expression than those with residual variability of 0% and 100% (Figure 2B-C). These findings indicate that incorporation of an appropriate CV describing residual variability in CYP2C8 protein expression is important to generate virtual individuals assuming inter-correlation between two enzymes. The HLM sample of the individual with the highest CYP2C8 and CYP3A4 abundance (Figure 1A) may be derived.
from a CYP2C8 extensive metaboliser, such as individuals with CYP2C8*1/*3 genotype, who show a high metabolic clearance of repaglinide [27]. However, CYP2C8 genotypes were unknown for the HLM samples used in this analysis.

The PBPK model assuming CYP3A4-CYP2C8 inter-correlation predicted the pharmacokinetic parameters of repaglinide, which is metabolised by CYP2C8 and CYP3A. As expected, the individuals at the extreme ends of CYP3A4 abundance showed similar trends in the assigned CYP2C8 abundances reported for the population in the simulation outcome (Figure 2C). The CYP2C8-mediated pathway displays high clearance under the assumption of inter-correlation when the CYP3A4-mediated pathway shows high clearance, and consequently the overall clearance becomes a higher value. The incorporation of the inter-correlation into a PBPK model produced a greater median CV of oral clearance in the 10 trials of 10 virtual individuals than that in the default virtual individuals (Figure 7A). However, the difference in the median CV of oral clearance became small when the number of trials was increased. This does not mean that the increased number of trials in the default virtual population provided inter-individual variability of oral clearance similar to the virtual population assuming inter-correlation. These findings suggested that the increased number of trials led to increase in the risk of physiologically implausible assignment of higher CYP2C8 abundance in the default virtual individuals with low hepatic CYP3A4 expression, resulting in implausible contribution of CYP2C8-mediated metabolism to oral clearance. Oral clearance was slightly higher in virtual individuals assuming inter-correlation than in the default individuals (Figure 3B). This may be associated with the improvement in simulated plasma concentration profiles of repaglinide when considering the inter-correlation (Figure 4B and 4D). These findings imply that a PBPK model ignoring a significant positive correlation between two enzymes underestimates the inter-individual variability of oral clearance. These results are consistent with a previous report showing that a PBPK incorporating CYP3A4-CYP3A5 inter-correlation predicted more physiologically realistic estimates of population drug clearance and aided in the identification of extreme individuals [13].

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Previous studies have demonstrated that a repaglinide PBPK model was useful to quantitatively predict several repaglinide DDIs, including the complex interaction with gemfibrozil [28–30]. However, these PBPK approaches focused on the prediction of average DDI effects. Here we examined the difference in the outcome of DDI prediction using a repaglinide PBPK model between virtual populations assuming and ignoring CYP3A4-CYP2C8 inter-correlation. Even though the average DDI effect was comparable between the two virtual populations, the inter-individual variability of DDI effect could be more reflective of realistic CYP2C8 abundance in virtual population assuming the inter-correlation (Figure 5). The DDI simulation in a virtual population assuming inter-correlation also provided a smaller maximum DDI effect than that in a virtual population ignoring the correlation. Ignoring a CYP3A4-CYP2C8 inter-correlation produced higher CYP2C8/CYP3A4 ratios in virtual individuals with low CYP3A4 protein (Figure 2A). High CYP2C8/CYP3A4 ratios correspond to a larger contribution of CYP2C8-mediated pathway to repaglinide metabolism, resulting in high sensitivity to inhibition of CYP2C8. These findings suggest that the default model ignoring inter-correlation may overestimate the maximum DDI effect. PBPK simulation assuming inter-correlation between drug-metabolising enzymes may be an important approach to predict the theoretically conceivable extreme effects of DDI. These simulations based on DDI with gemfibrozil do not account for the impact of CYP3A4 inhibition on the inter-correlation because gemfibrozil is only an inhibitor of CYP2C8 and OATP1B1. This effect can be explored using other dual CYP3A4/CYP2C8 substrates, such as montelukast, pioglitazone, and paclitaxel.

Theoretically conceivable inter-individual variability in DDI effect under various assumptions regarding the inter-correlations was investigated and compared with clinical observations [19]. CV of predicted DDI (as measured by AUC ratio) was reduced from 46.0% in the absence of inter-correlation between enzymes to 43.8% when the inter-correlation was incorporated into the virtual population (Figure 7). These CVs were associated with 5th and 95th percentiles of predicted DDI (2.48 to 11.29 vs. 2.49 to 9.69) (Figure 5). Distributions of the predicted AUC ratio were statistically different in populations of 1,000 virtual individuals generated assuming no correlation and inter-correlation. The range of predicted DDI was larger in the absence of inter-correlation (1.55–77.06).
than when incorporating inter-correlation (1.79–25.15), which was closer to clinical observations (2.6–12). Predicted DDI at more than 12-fold in AUC ratio, which is the maximum observed in the clinical study [19], was more frequent in the absence of inter-correlation. Some of the extreme values might be an artifact of the model leading to virtual individuals who do not exist in real populations, though this finding cannot be confirmed unless a larger study is performed. This may be supported by other clinical observations that the maximum of AUC\(_{\text{b-\infty}}\) ratio after pretreatment with 600 mg of gemfibrozil twice daily is 15-fold even when including other clinical trial designs [18, 19, 31].

Prediction of DDIs using PBPK models is usually simulated in 10 (or 20) virtual trials which are used as an indication of consistency between the model and observed data rather than what the model can offer. The lower CVs for predicted DDI considering the inter-correlation were not changed by the increased number of trials except for the opposite results in the minimum number of trials (the 10 trials) (Figure 7B). This result suggests that accurate prediction of inter-individual variability in DDIs is achieved by the incorporation of the inter-correlation into a PBPK model rather than the increase in the number of virtual trials.

PBPK modelling started out along the line of naïve pooled average predictions, mainly because of computational limitations [32]. Precise prediction aiming to individualise dosing is becoming more important [33], and crucially requires the ability to accurately describe inter-individual variability within PBPK modelling. Considering inter-individual variability, a standard deviation is more informative than just an average, and a probability distribution is even more informative [34]. Accurate prediction of inter-individual variability is an important challenge that PBPK modelling needs to address to expand into new areas of application, such as precision dosing and virtual bioequivalence [33, 34], but it requires proteomic data of sufficient quality [11]. Data used in the present analysis of inter-correlation and variability of hepatic CYP3A4 and CYP2C8 abundances were comparable to published meta-analysis data (n = 134) [12] (Spearman correlation, 0.62 vs. 0.68; CV for CYP3A4, 77% vs. 81%; CV for CYP2C8, 87% vs. 68%) [12, 20]. The influence of the regression parameter estimates for inter-correlation on the predicted clearance and DDI of repaglinide was confirmed using a regression model produced by excluding the data point with the
highest value for CYP3A4. Although the regression parameter under exclusion of the single extreme values for high abundance of enzymes resulted in slightly different values of predicted oral clearance, they had a modest effect on predicted inter-individual variabilities in oral clearance, AUC ratio and $C_{\text{max}}$ ratio. The present study does not rule out other unidentified factors for accurate prediction of inter-individual variability in drug clearance and DDIs though it demonstrates the importance of hepatic enzymes inter-correlations.

Many drugs are metabolised by more than one drug-metabolising enzyme, such as CYP and uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes. Uptake and efflux transporters also mediate drug disposition through controlling membrane transport, such as absorption in the small intestine, uptake into hepatocytes, and renal secretion. Therefore, knowledge of the protein expression of drug-metabolising enzymes and drug transporters is important to predict drug clearance and DDIs in more realistic virtual populations. Statistically significant positive correlations can be found in enzyme abundance between not only CYP enzymes, but also between UGT enzymes and across CYP and UGT families [20]. However, there is insufficient data in the literature about inter-correlation between drug-metabolising enzymes and drug transporters and their effects. Therefore, it is unknown whether drug-metabolising enzymes correlate with OATP1B1, which is known to contribute to the disposition of repaglinide. Construction of comprehensive network information for inter-correlation between pharmacokinetics-related proteins is expected to generate more realistic virtual populations.

The current version of the Simcyp Simulator provides an enzyme inter-correlation module based on a simple regression analysis. Regression analysis using centering may be adopted in future studies because it would be useful to infer biological interpretations for the intercept of the regression equation in relation to mean abundance.

In conclusion, incorporation of inter-correlation between the hepatic amounts of two drug-metabolising enzymes into a PBPK model could more accurately predict inter-individual variabilities of drug clearance and DDI, corresponding to protein abundance generated assuming a significant positive correlation between two enzymes in the liver. It is suggested that DDI studies using virtual populations assuming inter-correlation can help to estimate theoretically conceivable extreme risk of
DDIs. PBPK modelling and simulation should be focused on improvement in prediction of not only average values but also inter-individual variability of DDI effect. A middle-out approach to PBPK modelling is used extensively with parameter estimation in order to provide a reasonable characterization of clinical pharmacokinetic data [35]. Without accurately considering inter-correlation, the estimated parameter variability would not be reflective of ‘true’ variability. This produces an increased risk of inaccurate prediction in other scenarios (e.g. DDIs) when perturbing the model except when simply fitting PBPK models to observed clinical data. As the inter-correlations more realistically reflect enzyme abundances, virtual population studies involving PBPK and DDI should avoid using Monte Carlo assignment of enzyme abundance independently, when robust information on correlation exists. In the absence of information on inter-correlation of the enzyme abundance, implications of potential correlation for predicted variability should be borne in mind, where the potential impact can be evaluated as per the approach described herein.

**Competing Interests**

The authors have no competing interests.

**Acknowledgements**

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**Contributors**

manuscript. All authors provided critical revisions to the manuscript and provided their approval for submission.
References


35. Tsamandouras N, Rostami-Hodjegan A, Aarons L. Combining the 'bottom up' and 'top down'
approaches in pharmacokinetic modelling: fitting PBPK models to observed clinical data. Br J
### Table 1
Summary of input population parameters

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CV, coefficient of variation.
Table 2
Summary of input parameters used for repaglinide simulations

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$CL_{int,T}$, transporter-mediated intrinsic clearance; $CL_{PD}$, passive diffusion clearance; $CL_R$, renal clearance; Full PBPK model, full physiologically-based pharmacokinetic model; HLM, human liver microsomes; $K_m$, Michaelis-Menten constant; $K_p$, tissue to plasma partition coefficient; $P_{eff,max}$, human jejunum permeability; $V_{max}$, maximum rate of metabolism; $V_{ss}$, volume of distribution at steady state.
Table 3
Summary of input parameters used for model of gemfibrozil and its metabolite, gemfibrozil 1-O-β glucuronide

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Interactions:
- CYP1A2 $K_i$ (μM): 79.5
- CYP2C8 $K_i$ (μM): 9.0
- CYP2C8 MBI $K_{\text{app}}$ (μM): 19.0
- CYP2C8 MBI $K_{\text{inact}}$ (μM): 13.0
- CYP2C9 $K_i$ (μM): 5.8
- CYP2C19 $K_i$ (μM): 23.3
- CYP3A4 $K_i$ (μM): 210.2
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CL<sub>int</sub>, intrinsic clearance; CL<sub>iv</sub>, in vivo intravenous clearance; CL<sub>R</sub>, renal clearance; PBPK, physiologically-based pharmacokinetic model; HLM, human liver microsomes; K<sub>app</sub>, concentration of mechanism-based inhibitor associated with half maximal inactivation rate; K<sub>i</sub>, concentration of inhibitor that supports half maximal inhibition; K<sub>inact</sub>, inactivation rate of the enzyme; K<sub>m</sub>, Michaelis-Menten constant; V<sub>max</sub>, maximum rate of metabolism; V<sub>ss</sub>, volume of distribution at steady state.
Figure 1. Reported enzyme abundance (A) and correlation plot (B) for CYP2C8 and CYP3A4 protein contents in 24 individual liver samples [20]. Solid and dashed lines represent the regression line and the 95% prediction intervals, respectively. Standard errors for slope and baseline were 0.035 and 2.997, respectively. CV, coefficient of variation.
Figure 2. Hepatic CYP2C8 and CYP3A4 protein contents in a population of 100 virtual individuals generated assuming no correlation (A) and a correlation between the two enzymes with coefficient of variation (CV) of 0% (B), 47% (C), and 100% (D). Correlation plots for CYP3A4 abundance and CYP2C8/CYP3A4 ratio are shown in the insets.
variation (CV) of 0% (B), 47% (C), and 100% (D). Correlation plots for CYP3A4 abundance and CYP2C8/CYP3A4 ratio are shown in the insets.
**Figure 3.** Influence of correlation between hepatic CYP3A4 and CYP2C8 on the prediction of systemic clearance (A), oral clearance (B) of repaglinide, and the fraction of repaglinide escaping metabolism by the gut ($F_G$, C) and by the liver ($F_H$, D). Low, medium and high hepatic CYP3A4 expression relate to abundances of <100, 100-199, and ≥200 pmol/mg microsomal protein, respectively. Blue and red boxes represent simulation of individuals assuming no correlation and a correlation between CYP3A4 and CYP2C8, respectively. Closed circles indicate outliers that are beyond the quartiles by one and a half interquartile range.
Figure 4. Simulated and observed plasma repaglinide concentration profiles after a single oral dose of 0.25 mg repaglinide without (A and B) and with pretreatment (C and D) of 600 mg gemfibrozil twice daily in virtual individuals generated assuming no correlation and a correlation between CYP3A4 and CYP2C8. Simulations are presented as mean of all 10 trials (black solid lines) and 5th and 95th percentiles (grey areas). Observed data extracted from the literature [19] are presented as mean (closed circles) and standard deviation (error bars).
Figure 5. Predicted AUC and C_{max} ratios (DDI/control) of repaglinide for 100 trials in 10 virtual individuals generated assuming no correlation (A and C) and a correlation between hepatic CYP3A4 and CYP2C8 (B and D). Open and closed circles indicate simulated median (range) in each trial, and observed mean (range) extracted from the literature [19], respectively. Dashed and dotted lines represent median and 5th/95th percentiles of total simulated population, respectively.
observed mean (range) extracted from the literature [19], respectively. Dashed and dotted lines represent median and 5\textsuperscript{th}/95\textsuperscript{th} percentiles of total simulated population, respectively.
Figure 6 Cumulative distribution plots for predicted oral clearance (\( CL_{\text{oral}} \)), AUC ratio and \( C_{\text{max}} \) ratio (DDI/control) of repaglinide in 1000 virtual individuals generated assuming no correlation (dotted blue lines) and a correlation (solid red lines) between hepatic CYP3A4 and CYP2C8. Predicted values for CYP3A4-CYP2C8 correlation were normalised by the corresponding mean values for no correlation. Cumulative distributions were shown as central tendency (5-95%; A, B, C), lower end (1-5%; D, E, F), and upper end (95-99%; G, H, I) in frequency distribution. Solid black lines represent cumulative distribution for observed oral clearance, AUC ratio and \( C_{\text{max}} \) ratio which were normalised by the corresponding median values for no correlation [19].

Figure 6. Cumulative distribution plots for predicted oral clearance (\( CL_{\text{oral}} \)), AUC ratio and \( C_{\text{max}} \) ratio (DDI/control) of repaglinide for 1,000 virtual individuals generated assuming no correlation (dotted blue lines) and correlation (solid red lines) between hepatic CYP3A4 and CYP2C8. Predicted values for CYP3A4-CYP2C8 correlation were normalised by the corresponding mean values for no
correlation. Cumulative distributions are shown as central tendency (5-95%; A, B, C), lower end (1-5%; D, E, F), and upper end (95-99%; G, H, I) in frequency distribution. Solid black lines represent cumulative distribution for observed oral clearance, AUC ratio and $C_{\text{max}}$ ratio which were normalised by the corresponding median values for no correlation [19].
Figure 7 Coefficients of variation (CVs) in predicted oral clearance ($\text{CL}_{\text{oral}}$), AUC and $C_{\text{max}}$ ratios (DDI/control) of repaglinide for 10 to 100 trials in 10 virtual individuals generated assuming no correlation (dotted blue line and circles) and a correlation between hepatic CYP3A4 and CYP2C8 (solid red line and circles).
correlation (*dotted blue line and circles*) and correlation between hepatic CYP3A4 and CYP2C8 (*solid red line and circles*).