

Quantitative Prediction of the Impact of Drug Interactions and Genetic Polymorphisms on Cytochrome P450 2C9 Substrate Exposure

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Abstract

Background and Objective Cytochrome P450 (CYP) 2C9 is the most common CYP2C enzyme and makes up approximately onethird of total CYP protein content in the liver. It metabolises more than 100 drugs. The exposure of drugs mainly eliminated by CYP2C9 may be dramatically modified by drug–drug interactions (DDIs) and genetic variations. The objective of this study was to develop a modelling approach to predict the impact of genetic polymorphisms and DDIs on drug exposure in drugs metabolised by CYP2C9. We then developed dosing

recommendations based on genotypes and compared them to current Epar/Vidal dosing guidelines.

Methods We created two models. The genetic model was designed to predict the impact of *CYP2C9* polymorphisms on drug exposure. It links the area under the concentration–time curve (AUC) ratio (mutant to wild-type patients) to two parameters: the fractional contribution of CYP2C9 to oral clearance in vivo (i.e. CR or contribution ratio), and the fractional activity of the allele combination with respect to the homozygous wild type (i.e. FA or fraction of activity). Data were available for 77 couples (substrate, genotype). We used a three-step approach: (1) initial estimates of CRs and FAs were calculated using a first bibliographic dataset; (2) external validation of these estimates was then performed through the comparison between the AUC ratios predicted by the model and the observed values, using a second published dataset; and (3) refined estimates of CRs and FAs were obtained using Bayesian orthogonal regression involving the whole dataset and initial estimates of CRs and FAs. Posterior distributions of AUC ratios, CRs and FAs were estimated using Monte-Carlo Markov chain simulation. The drug interaction model was designed to predict the impact of DDIs on drug exposure. It links the AUC ratio (ratio of drug given in combination to drug given alone) to several parameters: the CR, the inhibition ratio (IR) of an inhibitor, and the increase in clearance (IC) due to an inducer. Data were available for 80 DDIs. IRs and ICs were calculated using the interaction model and an external validation was performed. Doses adjustments were calculated in order to obtain equal values for drug exposure in extensive and poor metabolisers and then compared to Epar/Vidal dosing guidelines.

Results CRs were assessed for 26 substrates, FAs for five genotype classes including *CYP2C9**2 and *3 allelic

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variants, IRs for 27 inhibitors and ICs for two inducers. For the genetic model, the mean prediction error of AUC ratios was -0.01 , while the mean prediction absolute error was 0.36 . For the drug interaction model, the mean prediction error of AUC ratios was 0.01 , while the mean prediction absolute error was 0.22 . Of the 26 substrates and *CYP2C9**2 and *3 variants investigated, 30 couples (substrate, genotype) lead to a dose adjustment, as opposed to only ten couples identified in the Epar/Vidal recommendations.

Conclusion These models were already used for *CYP2D6*. They are accurate at predicting the impact of drug interactions and genetic polymorphisms on *CYP2C9* substrate exposure. This approach will contribute to the development of personalized medicine, i.e. individualized drug therapy with specific dosing recommendations based on *CYP* genotype or drug associations.

1 Background

The field of pharmacogenetics was born after analysis of a few rare extreme reactions (phenotypes) in some humans. These phenotypes were inherited diseases, abnormal reactions to drugs or other environmental factors. ‘Polymorphism’ has different meanings in pharmacogenetics. For this study, it means the existence of two or more variants (e.g. alleles and phenotypes) in a population that occurs at a frequency greater than 1%. Several review articles describe genetic variations related to many mechanisms relevant to drug therapy [1–3]. Effects may be on genes that code for metabolising enzymes, transporters, receptors or ion channels. These effects may be described as pharmacokinetic (affecting drug concentrations) or pharmacodynamic (affecting drug action).

Cytochrome P450 (*CYP*) 2C9 is the most common *CYP2C* enzyme [4] and makes up approximately onethird of total *CYP* protein content in the liver [5]. It metabolises more than 100 drugs [6], including coumarin anticoagulants, sulfonylureas and some NSAIDs. The exposure of drugs that are mainly eliminated by *CYP2C9* may be dramatically modified by drug–drug interactions (DDIs) and genetic variations. Gene coding for *CYP2C9* involves numerous inherited polymorphisms. More than 50 single nucleotide polymorphisms have been described in the regulatory and coding regions of the *CYP2C9* gene, but only two coding variants with functional consequences are common (*CYP2C9**2 and *CYP2C9**3). Their allele frequencies are around 11% (*2) and 7% (*3) in Caucasian subjects [6]. They have significantly lower frequencies in African and Asian subjects [7].

Our aim was to undertake a quantitative prediction of the impact of *CYP2C9* polymorphisms and DDIs on drug

exposure. We used the modelling approach developed by Tod et al. [8, 9], combining all available data on 26 *CYP2C9* substrates, five genotypes, 27 inhibitors and two inducers. This approach describes the effect of mutations, inhibitors and inducers using a set of characteristic parameters to predict drug exposure in undocumented situations, after these parameters are estimated. Dosing recommendations based on genotype were written and compared to current Epar/Vidal dosing guidelines [10, 11].

2 Methods

2.1 Impact of *CYP2C9* Genetic Polymorphisms on Drug Exposure: The Genetic Model

A genetic model was designed to perform the quantitative prediction of the impact of *CYP2C9* polymorphisms on drug exposure and is based on a model used to study *CYP2D6* [8]. It relies mostly on in vivo data to avoid extrapolation issues and uses two characteristic parameters: one for the drug (i.e. CR or contribution ratio) and one for the genotype (i.e. FA or fraction of activity). CR_{2C9} is the fraction of oral clearance of the drug metabolised by *CYP2C9*. FA_{2C9} is the fraction of activity of *CYP2C9* resulting from the combination of mutated alleles, relative to the activity of the reference genotype. The metric of interest is the ratio between the area under the drug concentration–time curve (AUC) in patients with mutant genotype and the AUC in patients with wild-type genotype.

Using the equation proposed by Gibbs et al. [12], the ratio of AUC^{XM} (the AUC of the drug in patients with mutated *CYP2C9* alleles: XM may refer to poor metabolisers [PMs], intermediate metabolisers [IMs] or ultra-rapid metabolisers [UMs]) to AUC^{EM} (the AUC of the drug administered alone at the same dose in patients with wild-type genotype [extensive metabolisers; EMs]) was modified as follows (Eq. 1):

$$\frac{AUC^{XM}}{AUC^{EM}} = \frac{1}{1 - CR^{EM} \cdot (1 - FA)} \quad (1)$$

The reference value of FA in EMs who are homozygote wild-type is equal to 1. FA is <1 in IMs and PMs and >1 in UM.

FA is assumed to be the same for all *CYP2C9* substrates and only depends on the allele combination. Each allele is assumed to contribute to the overall activity independently of other alleles. Therefore, if there are m categories of alleles and n_i alleles in each category (Eq. 2):

$$FA = \frac{1}{2} \sum_{i=1}^m n_i \cdot FA_i \quad (2)$$

where FA_i is the fraction of activity of the mutated CYP allele relative to the wild-type CYP allele.

Two reviewers performed a bibliographic search on MEDLINE from January 1966 to December 2011 to identify all available datasets of drug concentrations in humans with *CYP2C9* polymorphisms. Keywords used were “pharmacogenetics”, “genotype”, “cytochrome P450”, “2C9”, “pharmacokinetics”, “pharmacodynamics”, “polymorphism”, “allele” and “metabolism”. No language restrictions were applied. In MEDLINE, we used a species filter to limit the search to “humans”. Major reviews on pharmacogenetics and drug-metabolising enzymes were also systematically searched in MEDLINE. Cross-references and quoted papers were checked in order to update the primary reference list.

A three-step approach was used to estimate CRs and FAs. Initial CRs were estimated with a set of learning data. Then, a second set of data was used for the external validation. The combined set of data was used to refine CR and FA estimates.

The first step was to allocate clinical studies by estimating CRs using pharmacokinetic data from patients with either *CYP2C9**3/*3 or *1/*3 genotypes. If several studies were available for these genotypes, selection criteria of studies included the availability of results for *3/*3 genotype carrier subjects and the number of patients for each genotype group.

The second step was the external validation of initial estimates. This was performed using studies that were not included in the first set of data. We compared the AUC ratios predicted by Eq. 1 to the observed values. Visual examination of the ‘predicted versus observed AUC ratio’ plot was used for external validation. Predicted values that were 50–200 % of observed ratios were considered correct. This limit is frequently used as a criterion for model evaluation in physiologically based pharmacokinetic literature. There is no statistical reason to use this limit, but it is considered relevant from a clinical point of view. A prediction error up to a factor of 2 has no important consequences in most instances. The whole set of initial values (CRs and FAs) was considered valid if 90 % of the predicted AUC ratios were considered correct in the second step.

The third step was to improve estimation of CRs and FAs obtained after orthogonal regression, using all data (initial estimates of CRs and FAs from step 1 and observed AUC ratios used in the first two datasets). The posterior distributions of AUC ratios, CRs and FAs were estimated using Monte-Carlo Markov chain simulation with WinBugs 1.4 software (MRC Biostatistics Unit, Cambridge, UK) (for more information, see Tod et al. [8]). In this regression, we assumed the observed and predicted AUC

ratios followed normal distributions centred at the predicted AUC ratios with a standard deviation of 5. Convergence was assessed by checking the stability of posterior distributions. Goodness of fit was assessed by visual examination of residual scatterplots and posterior distributions. The bias of AUC ratio prediction was evaluated by the mean prediction error. The prediction error is defined as the predicted value minus the observed value. The imprecision of the prediction was assessed by the mean absolute prediction error.

2.2 Impact of CYP2C9-Mediated Drug Interactions on Drug Exposure: The Drug Interaction Model

We carried out a bibliographic search to identify the inhibitors and inducers of CYP2C9. The same search and selection strategy was used as for the genetic model. If genetic status was reported, only data on EMs were considered. If genetic status was not reported, patients were considered as EMs (cf. low prevalence of mutations).

In previous papers [9, 13, 14], AUC* corresponded to data obtained after co-administration of an inhibitor or inducer and AUC to data obtained after administration of the victim drug taken alone at the same dose. The ratio between AUC* and AUC depends on the contribution ratio (CR_{2C9}) defined above and the inhibition ratio (IR_{2C9}) or the apparent increase in clearance (IC_{2C9}) due to the interacting drug.

For an inhibitor (Eq. 3):

$$\frac{AUC^*}{AUC} = \frac{1}{1 - CR \cdot IR} \quad (3)$$

For an inducer (Eq. 4):

$$\frac{AUC^*}{AUC} = \frac{1}{1 + CR \cdot IC} \quad (4)$$

In these equations, IR_{2C9} is a metric of inhibitor potency, ranges from 0 to 1, depends on the dose of the inhibitor and is related to its inhibition constant K_i ; and IC_{2C9} is a metric of inducer potency, depends on the dose of the inducer and ranges from 0 to a maximal value that may be greater than 1. Using the AUC ratio measured in a clinical DDI study, and the CR_{2C9} of the victim drug determined from a pharmacogenetic study, the IR or the IC may be calculated by solving Eqs. 3 and 4. Alternatively, if the AUC ratio and the IR of the inhibitor are known, the CR may be calculated (the ‘interaction method’).

Sometimes, DDIs on CYP2C9 also involve displacement from drug binding to plasma proteins. This approach is valid as long as the AUC ratio of total concentrations (AUC^*/AUC) in Eqs. 3 and 4 is replaced by the AUC ratio of unbound concentrations ($fAUC^*/fAUC$).

For an inhibitor (Eq. 5):

$$\frac{fAUC^*}{fAUC} = \frac{1}{1 - CR \cdot IR} \quad (5)$$

For an inducer (Eq. 6):

$$\frac{fAUC^*}{fAUC} = \frac{1}{1 + CR \cdot IC} \quad (6)$$

IRs or ICs were calculated by solving Eqs. 3, 4, 5 or 6. Then, an external validation was carried out like the one used for the genetic model.

2.3 Impact of Genetic Polymorphisms and Drug Interactions on Dosing Recommendations

We assumed that a substrate with a CR_{2C9} greater than 0.5 may require a dose adjustment. A genetic polymorphism or a DDI leading to an AUC ratio greater than 2 could also require a dose adjustment.

Dose adjustment was calculated in order to obtain equal values for drug exposure in EMs and PMs (Eq. 7):

$$\begin{aligned} \text{Dose adjustment (\%)} &= \left(\frac{AUC^{EM} - AUC^{PM}}{AUC^{PM}} \right) \cdot 100 \\ &= \left(\frac{AUC^{EM}}{AUC^{PM}} - 1 \right) \cdot 100 \quad (7) \end{aligned}$$

Dose adjustments in PMs were then compared to Epar/Vidal dosing guidelines [10, 11] in patients with *CYP2C9* polymorphisms for the 20 substrates with a CR_{2C9} greater than 0.5.

3 Results

3.1 Model Predictions for *CYP2C9* Polymorphisms

The bibliographic search identified 32 relevant articles. Twenty-one of these articles were used for the initial estimates of CRs (first step) and 11 for external validation (second step). For the re-estimations of parameters (third step), all 32 articles were used. The description of each study is given in Appendices 1 and 2 of the Online Resource (drug, dose value, type of study, number of subjects, healthy volunteers or patients, and sampling schedule).

3.2 Initial Estimates for Contribution Ratios (CRs) and Fractions of Activity (FAs)

Initial FA values were estimated from *S*-warfarin which was assumed to be completely metabolised by *CYP2C9* (i.e. $CR = 1$) [15]. The initial FA values for *CYP2C9* genotypes *1/*3, *3/*3, *1/*2, *2/*2 and *2/*3 were 0.55, 0.1, 0.84, 0.68 and 0.39, respectively.

Initial CR values were estimated for 26 substrates (19 drugs and 7 enantiomers). CRs were estimated for 23 of them with pharmacogenetic/pharmacokinetic studies. For indomethacin, naproxen, and tenoxicam, CRs were estimated based on clinical pharmacokinetic data (the fraction of dose recovered as metabolites) and in vitro data. Most pharmacogenetic studies were done in healthy volunteers. The number of subjects included in studies varied between 5 and 108 (for more details, please refer to Appendix 1 of the Online Resource).

3.3 External Validation of Initial Estimates

Eleven pharmacogenetic/pharmacokinetic studies were used for external validation. Six were steady-state studies, one was a multiple-dose pharmacokinetic study, and four were single-dose pharmacokinetic studies. Twenty-four AUC ratios were available for external validation. The number of PMs and IMs varied between 1 and 9 (please see Appendix 2 of the Online Resource). The agreement between predicted and observed AUC ratio is shown in Fig. 1. Two points are outside the acceptable range of prediction. One outlier is underestimated and corresponds to losartan for genotype *CYP2C9**1/*2 [16]. The other one is overestimated and corresponds to *S*-ibuprofen for genotype *2/*3. However, the acceptance criterion for CR and FA initial values was fulfilled ($22/24 = 92\%$ of acceptable predictions). The mean prediction error of the AUC ratio was -0.01 and the mean prediction absolute error was 0.36.

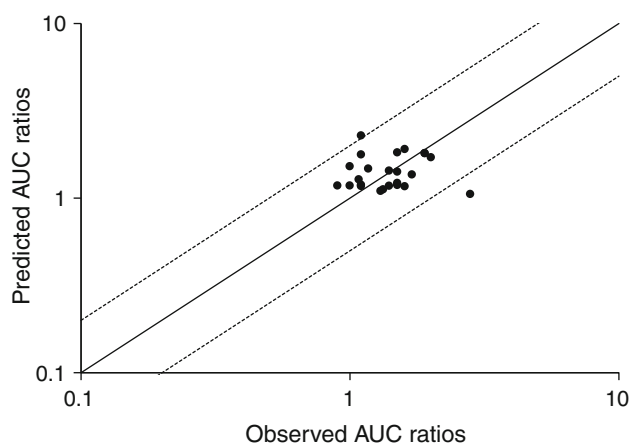


Fig. 1 Predicted versus observed AUC ratios for the external validation set of the pharmacogenetic part. The solid line is the identity line ($y = x$). The upper dotted line represents ($y = 2x$) and the lower dotted line represents ($y = 0.5x$). AUC area under the concentration–time curve

3.4 Final Estimates for CRs and FAs

The final estimates of CRs and FAs are shown in Tables 1 and 2. The agreement between predicted and observed AUC ratio with final estimates of CR_{2C9} and FA_{2C9} is shown in Fig. 2. The mean prediction error of the AUC ratio was -0.05 and the mean prediction absolute error was 0.25.

We found that the relative activity of CYP2C9*2*2 and *3*3 compared with *1*1 are 70 and 13 %, respectively. This is fully consistent with the meta-analysis carried out by Dickinson et al. [17], who reported 70 and 10 %.

3.5 Model Predictions for CYP2C9-Mediated Drug-Drug Interactions

The bibliographic search identified 59 relevant articles. Descriptions of each study are shown in Appendix 3 of the Online Resource (drug, dose value, type of study, number

Table 1 Final estimates of CR_{2C9} and corresponding 90 % confidence intervals

Drug	CR _{2C9}	90 % CI
S-Acenocoumarol	0.99	0.97–1.00
Benzbromarone	0.76	0.64–0.85
Celecoxib	0.97	0.94–0.99
Diclofenac	0.12	0.03–0.26
Fluindione	0.74	0.51–0.91
3S-5R fluvastatin	0.9	0.86–0.95
3R-5S fluvastatin	0.7	0.58–0.8
Flurbiprofen	0.93	0.83–0.98
Glimepiride	0.99	0.97–1.00
Glibenclamide (glyburide)	0.63	0.47–0.76
R-Ibuprofen	0.51	0.31–0.68
S-Ibuprofen	0.7	0.56–0.82
Indomethacin	0.50	0.23–0.77
Irbesartan	0.84	0.65–0.96
Lornoxicam	0.99	0.97–1.00
Losartan	0.4	0.16–0.69
Naproxen	0.22	0.07–0.45
Nateglinide	0.48	0.27–0.66
Phenobarbital	0.99	0.97–1.00
S-Phenprocoumon	0.4	0.16–0.67
Phenytoin	0.72	0.51–0.89
Piroxicam	0.84	0.65–0.95
Tenoxicam	0.24	0.08–0.49
Tolbutamide	0.89	0.83–0.94
Torseamide	0.77	0.67–0.86
S-Warfarin	0.99	0.97–1.00

CR_{2C9} fractional contribution of cytochrome P450 2C9 to oral clearance in vivo

Table 2 Final estimates of FA_{2C9} and corresponding 90 % confidence intervals

CYP2C9 genotype ^a	FA _{2C9}	90 % CI
*1/*2 (105)	0.82	0.72–0.91
*2/*2 (28)	0.70	0.58–0.82
*1/*3 (234 ^b)	0.56	0.49–0.63
*3/*3 (36)	0.13	0.09–0.15
*2/*3 (30)	0.39	0.3–0.44

CYP cytochrome P450, FA_{2C9} fraction of activity of CYP2C9

^a The number given in brackets is the number of subjects that contributed to the estimation of FA_{2C9}

^b The number of subjects was not available for two studies [49, 50]

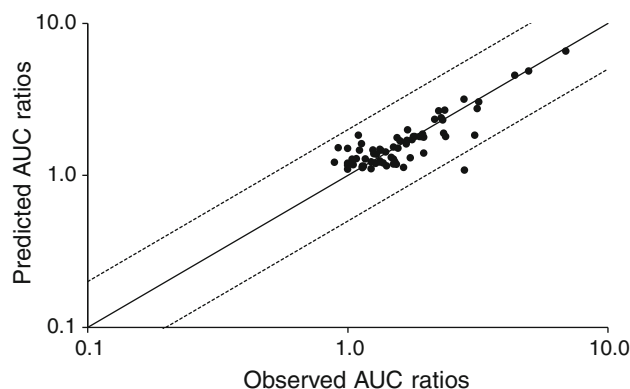


Fig. 2 Predicted versus observed AUC ratios with final estimates of CR_{2C9} and FA_{2C9}. The solid line is the identity line (y = x). The upper dotted line represents (y = 2x) and the lower dotted line represents (y = 0.5x). AUC area under the concentration–time curve, CR_{2C9} fractional contribution of CYP2C9 to oral clearance in vivo, CYP cytochrome P450, FA_{2C9} fraction of activity of CYP2C9

of subjects, healthy volunteers or patients, and sampling schedule). We estimated the CR_{2C9} for four additional drugs (cyclophosphamide: 0.35; meloxicam: 0.59; rosuvastatin: 0.26; and sulfamethoxazole: 0.45) using the interaction method. We calculated the IR_{2C9} for 27 inhibitors and the IC_{2C9} for two inducers. IR_{2C9} values are shown in Table 3. Five inhibitors were actually non-inhibitors (clopidogrel, glibenclamide [glyburide], ibuprofen, losartan and tolbutamide). The IC_{2C9} values of rifampicin (rifampin) 600 mg/day and bosentan 1,000 mg/day were 1.22 and 0.41, respectively.

External validation was based on 46 AUC ratios. The relationship between the predicted and observed AUC ratio is shown in Fig. 3. The mean prediction error of the AUC ratios was -0.01 and the mean absolute prediction error was 0.22. Two AUC ratios (S-warfarin-bucolome and phenytoin-valproate interactions) were predicted using unbound concentrations (Eq. 5). All predicted ratios were 50–200 % of observed values.

Table 3 Final estimates of apparent IR_{2C9} values of CYP2C9 inhibitors

Drug (dosage)	IR_{2C9}
Benzbromarone (50 mg/day)	0.55
Bucolome (300 mg/day)	0.84
Clarithromycin (250 mg)	0.26
Clopidogrel (75 mg)	0.00
Diclofenac (100 mg)	0.09
Fluconazole (100 mg/day)	0.48
Fluconazole (400 mg/day)	0.65
Fluoxetine (20 mg/day)	1.00
Fluvastatin (40 mg/day)	0.20
Gemfibrozil (600 mg)	0.19
Glibenclamide [glyburide] (2.5 mg/day)	0.00
Ibuprofen (400 mg)	0.00
Irbesartan (300 mg/day)	0.05
Lornoxicam (16 mg/day)	0.20
Losartan (50 mg)	0.00
Miconazole (125 mg/day)	0.79
Omeprazole (40 mg/day)	0.28
Phenytoin (300 mg/day)	0.68
Piroxicam (40 mg)	0.13
Sertraline (200 mg/day)	0.17
Simvastatin (20 mg)	0.20
Sulfamethizole (600 mg/day)	0.41
Sulfaphenazole (1,000 mg/day)	0.86
Sulfinpyrazone (400 mg/day)	0.41
Tolbutamide (500 mg)	0.00
Valproate (800 mg/day)	0.61
Voriconazole (800 mg/day)	0.66
Zafirlukast (80 mg)	0.39

CYP cytochrome P450, IR_{2C9} inhibitor ratio of cytochrome P450 2C9

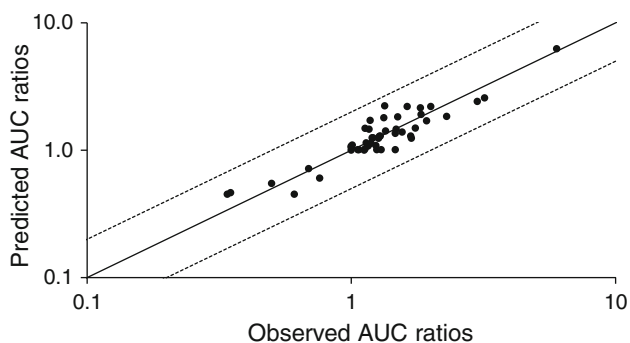


Fig. 3 Predicted versus observed AUC ratios for the external validation set (drug interaction model). The *solid line* is the identity line ($y = x$). The *upper dotted line* represents ($y = 2x$) and the *lower dotted line* represents ($y = 0.5x$). Ratios lower than 1 are inducers. Ratios greater than 1 are inhibitors. AUC area under the concentration–time curve

3.6 Drug Dosage Adjustment

Table 4 summarizes drug dosage adjustments predicted by our models, compared with Epar/Vidal dosing guidelines. Twenty out of 26 substrates included in our models had a CR value greater or equal to 0.5 (Table 1). Only $CYP2C9^{*2/*3}$ and $*3/*3$ genotypes exhibit an AUC higher than twice the AUC of patients with wild-type genotype. With our models, 33 couples (substrate, genotype) may lead to a dose adjustment, as opposed to only ten couples found in Epar/Vidal dosing guidelines [10, 11].

4 Discussion

DDIs and genetic polymorphisms may increase or reduce drug clearance, making it necessary to alter the dose to keep drug exposure in the normal range. The consequences of DDIs and genetic polymorphisms are the same, as long as the predicted AUC ratios (AUC^{XM}/AUC^{EM} and AUC^{*}/AUC) are the same.

The drug interaction model (Eqs. 3 and 4) predicts AUC ratios for about 700 drug combinations, only 10 % of which have been evaluated in clinical studies. Strong interactions were observed for victim drugs with CRs greater than 0.8 (12 drugs), combined with rifampicin or inhibitors with an IR greater than 0.8 (bucolome, fluoxetine and sulfaphenazole).

4.1 Implications on Some CYP2C9 Substrates

This part is focused on the impact of genetic polymorphism. Standard drug doses achieve normal concentrations and effects in homozygous EMs, but may be toxic in PMs (and in heterozygous EMs or IMs) and ineffective in UMs. Genetic polymorphisms may be clinically relevant if the signal of their effect is greater than the noise from all other sources of inter-individual variability and their therapeutic index is narrow.

The pharmacogenetics of blood concentrations, assuming that blood concentrations mirror the concentrations at target sites, is accepted by regulatory authorities. It is possible to find equivalent doses for different genotypes that modify drug kinetics. However, differences in drug effects between genotype groups are almost always smaller than pharmacokinetic differences. Minor dose adjustments are not needed. For some drugs, plasma concentrations correlate poorly with clinical efficacy and empirical dose-finding rather than drug concentrations is used to avoid adverse drug effects.

4.2 Vitamin K Antagonists

The therapeutic use of vitamin K antagonists is difficult because of a narrow therapeutic index: an international

Table 4 Epar/Vidal dosing guidelines and dose adjustments suggested by our model for CYP2C9 poor metabolisers

Drug ^a	CR _{2C9}	Drug dosage adjustments	
		Vidal/Epar	Model prediction: % of dose reduction for CYP2C9 genotypes
S-Acenocoumarol	0.99	Half dose in PM and INR measurement	*3/*3: 85 %; *2/*3: 65 %
S-Warfarin	0.99	INR measurement	*3/*3: 85 %; *2/*3: 65 %
Lornoxicam	0.99	Not available	*3/*3: 85 %; *2/*3: 65 %
Glimepiride	0.99	No recommendation	*3/*3: 85 %; *2/*3: 65 %
Phenobarbital	0.99	Not available	*3/*3: 85 %; *2/*3: 65 %
Celecoxib	0.97	Onethird of dose in PM	*3/*3: 85 %; *2/*3: 60 %
Flurbiprofen	0.93	No recommendation	*3/*3: 80 %; *2/*3: 60 %
Tolbutamide	0.89	No recommendation	*3/*3: 80 %; *2/*3: 55 %
3S-5R fluvastatin	0.89	No recommendation	*3/*3: 80 %; *2/*3: 60 %
Irbesartan	0.84	No recommendation	*3/*3: 75 %; *2/*3: 55 %
Piroxicam	0.84	Caution in PM	*3/*3: 75 %; *2/*3: 55 %
Torseamide	0.77	No recommendation	*3/*3: 70 %; *2/*3: 50 %
Benzbromarone	0.76	Not available	*3/*3: 65 %
Fluindione	0.75	Not available	*3/*3: 70 %
Phenytoin	0.72	No recommendation	*3/*3: 65 %
3R-5S fluvastatin	0.7	No recommendation	*3/*3: 60 %
S-Ibuprofen	0.7	No recommendation	*3/*3: 60 %
Glibenclamide (glyburide)	0.63	Not available	*3/*3: 55 %
R-Ibuprofen	0.51	No recommendation	No adjustment required
Indomethacin	0.50	No recommendation	No adjustment required
Nateglinide	0.48	Caution in PM	No adjustment required

CR_{2C9} fractional contribution of CYP2C9 to oral clearance in vivo, CYP cytochrome P450, INR international normalized ratio, PM poor metabolisers

^a Substrates for which there was no drug dose adjustment recommended either by Epar/Vidal or by our models were not shown

normalized ratio (INR) target between 2.0 and 3.0 is recommended for most indications [18, 19]. The dose-response relationship is not easy to predict, causing frequent bleeding complications or insufficient anticoagulation. CYP2C9 has clearly been established as the predominant catalyst for the metabolism of its more potent S-enantiomer [19]. Vitamin K epoxide reductase complex, subunit 1 (*VKORC1*) genetic polymorphisms are also an important predictor of acenocoumarol pharmacokinetic–pharmacodynamic responses at the initiation of therapy [20].

For S-acenocoumarol and S-warfarin, our model suggests a decrease of the standard dose by 85 % for homozygous CYP2C9*3 patients and 65 % for CYP2C9*2/*3 patients (Table 4). However, Epar/Vidal specifies that the genetic variability related to CYP2C9 represents roughly 14 % of the inter-subject response variability and no drug dosing adjustment is recommended in addition to INR-based dosages and follow-up. In our opinion, a dose adjustment for S-acenocoumarol and S-warfarin could be beneficial for PM patients at the initiation of therapy in relation to pharmacokinetic variability induced by the

CYP2C9 genotype. However, early INR values are routinely determined in clinical practice and dosing is almost continuously adjusted in the early phase of treatment.

Lacut et al. [21] showed that *VKORC1* genotypes strongly affect the anticoagulation effects of fluindione. However, the influence of CYP2C9, CYP4F2 and EPHX1 genotypes on fluindione seems weaker than on S-acenocoumarol and S-warfarin. According to our models, the standard dose should be reduced by 70 % for CYP2C9*3/*3, but there are no specific dosing guidelines for these drugs in Epar/Vidal (Table 4).

4.3 NSAIDs

Dosing guidelines are not well-established for NSAIDs, even though they may cause frequent adverse events, such as hepatotoxicity, gastrointestinal and nephrotoxicity, especially with chronic pain [22]. Our study included six NSAIDs: celecoxib, flurbiprofen, ibuprofen, lornoxicam, naproxen and piroxicam. All but naproxen had a CR_{2C9} value greater than 0.5. We did not find any Epar/Vidal dosing guidelines for four of them (ibuprofen, flurbiprofen,

lornoxicam and naproxen). Piroxicam was recommended to be administered with caution for patients with the *CYP2C9**3 allele. Epar and Vidal recommend a one-third decrease in the standard dose of celecoxib in PMs.

Ibuprofen is one of the most frequently used NSAIDs. Most preparations contain the racemic mixture of *R*- and *S*-ibuprofen [23]. Data from biochemical studies indicate that *CYP2C9* may be the major *S*-ibuprofen hydroxylase, whereas *R*-ibuprofen appears to be metabolised by *CYP2C8* [23]. Our models predict a 2.7-fold increase in AUC for *S*-ibuprofen in *CYP2C9**3/*3 patients, which should lead to a recommended dose reduction of 60 %.

Only one clinical study has assessed the impact of *CYP2C9* polymorphisms on the pharmacokinetics of flurbiprofen but no homozygous PMs (i.e. *2/*3, *3/*3 or *2/*2 genotypes) were included [24]. With our model, the standard dose should be reduced by 80 and 60 % for the *CYP2C9**3/*3 and *CYP2C9**2/*3 genotypes, respectively.

For lornoxicam, our results predicted a sevenfold increase in AUC for *CYP2C9**3/*3 genotype patients compared with *1/*1 genotype patients, suggesting a corresponding recommendation of dose reduction.

Piroxicam is the only NSAID metabolised by *CYP2C9* for which dosing caution is required because of a high risk of dose-related adverse events in PMs. Our results are in line with these guidelines and suggest a dose reduction of 75 and 55 % for the *CYP2C9**3/*3 and *2/*3 genotypes, respectively.

For celecoxib, four clinical studies assessed the impact of *CYP2C9* polymorphisms on pharmacokinetic parameters [25–27]. However, there was high variability between studies. The AUC ratio for *CYP2C9* homozygous *3 allele patients out of wild-type patients varied between 0.91 and 7.78. This may be because of the low number of patients with this genotype included in the studies (a maximum of three patients with the *3/*3 genotype). Our model suggests decreasing the standard dose by 85 % for homozygous *CYP2C9**3 patients and 60 % for *CYP2C9**2/*3 patients and this is in line with Epar/Vidal dosing guidelines.

4.4 Oral Antihyperglycaemic Agents

Oral antihyperglycaemic drugs are prescribed for type 2 diabetes mellitus. Although there is no specific therapeutic concentration range, severe adverse effects can occur if concentrations vary too much [28]. We only found one recommendation for nateglinide when administered to PMs.

According to three clinical studies [29–31], exposure to glibenclamide (AUC) varies between 1.4- and 2.6-fold for heterozygous patients with the *CYP2C9**3 allele, compared with homozygous patients with the wild-type allele.

However, it does not seem to affect the pharmacokinetics of glibenclamide in two studies. Our model suggests being cautious with homozygous *3/*3 patients, in whom the AUC is twofold greater than in wild-type patients.

For nateglinide, data indicate that this substrate is predominantly metabolised by *CYP2C9*, and by *CYP3A4* to a smaller extent. In an interaction trial with sulfapyrazone, a *CYP2C9* inhibitor, a modest increase in nateglinide AUC (~28 %) was observed in healthy volunteers, with no changes in the mean maximum plasma drug concentration and elimination half-life. A more prolonged effect and a potential risk of hypoglycaemia cannot be excluded in patients when nateglinide is co-administered with *CYP2C9* inhibitors. Particular caution is recommended in Epar/Vidal dosing guidelines only when nateglinide is co-administered with more potent *CYP2C9* inhibitors, e.g. fluconazole or gemfibrozil, or in patients who are known to be PMs for *CYP2C9*. With our model, no dose adjustment was required.

Tolbutamide is not often prescribed because of a possible increased risk of cardiovascular mortality and adverse effects. Kirchheiner et al. [32] showed a ratio of AUC^{PM} to AUC^{EM} of more than sixfold in homozygous *CYP2C9**3 patients. Our results suggest a dose reduction of 80 and 55 % for patients with *3/*3 and *2/*3, respectively, but no specific dosing guidelines are proposed in Epar/Vidal.

4.5 Sartans

The most common drug reactions of sartans are headaches, upper respiratory tract infections, diarrhoea, fatigue and cough [33, 34]. For irbesartan, clinical studies show no relationship between the dosage and overall frequency of adverse reactions. The only clinical study we found in the pharmacogenetic literature on irbesartan only included IMs. Our model estimated the CR value at 0.84 for irbesartan, which resulted in a dose reduction of 75 and 55 % for *CYP2C9**3/*3 and *2/*3 genotypes, respectively.

4.6 Anticonvulsant Drugs

Phenytoin exhibits a non-linear relationship between dose and serum concentrations, and the therapeutic index is narrow, with a range usually between 5 and 25 µg/mL. Of the orally administered dose of phenytoin, 70–90 % is oxidized by *CYP2C9*, and a minor amount by *CYP2C19*, leading to *S*-5-(4p-hydroxyphenyl)-5-phenylhydantoin in humans. The relative contribution of *CYP2C19* to phenytoin metabolism increases as phenytoin concentrations increase, due to saturation of *CYP2C9* [35]. We found no Vidal/Epar guidelines on dose adjustment for phenytoin, whereas the AUC ratio of *CYP2C9**3/*3 patients compared to *1/*1 patients is about threefold. Taking into account the

narrow therapeutic index of phenytoin, we think that this increase may be clinically significant. This is in agreement with a retrospective analysis of 269 patients with epilepsy. The maximal dose of phenytoin was stratified by the *CYP2C9* genotype [36]. Carriers of one or two defective *CYP2C9* alleles (i.e. *CYP2C9* heterozygous EMs or PMs) apparently required a 13 and 30 % lower dose, respectively [35]. Other studies also indicated that genetic polymorphisms of *CYP2C9* and *CYP2C19* are closely related to the dose required for the control of seizures in patients [36–40].

Phenobarbital was included in our study, because several pathways (including *CYP2C9*) are responsible for various proportions of total phenobarbital elimination. Although phenobarbital *p*-hydroxylation co-segregates with *CYP2C19* activity [41], our results show that *CYP2C9* is a major pathway for phenobarbital metabolism. This is another reason to monitor the phenobarbital concentration in plasma. However, the use of this drug in developed countries is declining in favour of alternative drugs with improved safety.

4.7 Cholesterol-Lowering Drugs

Kirchheiner et al. [42] studied the impact of *CYP2C9**2 and *3 genotypes on the pharmacokinetics and pharmacodynamics of fluvastatin in healthy volunteers. The pharmacokinetics of both enantiomers showed statistically significant differences according to the number of *3 alleles and no effect on *2 was observed. No correlation with the *CYP2C9* genotype was found with either pharmacokinetics or cholesterol-lowering effects. However, Sabia et al. [43] reported that high fluvastatin concentrations may be related to adverse drug effects such as diarrhoea, headaches and clinically relevant elevations in serum transaminase concentrations. Our results suggest decreasing the dose because we observed a 2.6-fold increase in AUC for the 3*R*-5*S* active enantiomer in patients with the *CYP2C9**3/*3 genotype.

4.8 Limits of the Proposed Modelling Approach

Our models rely on several assumptions. Firstly, the FA of a variant (relative to wild-type) is assumed to be the same with respect to all substrates. This strong assumption has been questioned after results from in vitro experiments [44]. Secondly, the method may only be used for drugs that have linear kinetics. The predictions would be biased if the method is used for drugs exhibiting non-linear kinetics at the usual dosing regimen. The large variability of AUC ratio predictions in XMs and EMs for phenytoin (95 % CI 1.7–4.9) may be explained by its non-linear kinetics. The clinical interpretation of an AUC ratio may also be difficult with respect to drugs that have enantiomers and/or active

metabolites. The quantitative prediction of the impact of an almost complete loss of *CYP2C9* activity on a substrate that is almost exclusively cleared by *CYP2C9* metabolism would probably be imprecise because a small variation in CR and/or FA values may result in a large variation in AUC ratios.

Other limitations are specific to drug polymorphisms. Firstly, the analyses were not conducted for other important variants of *CYP2C9*, such as *5, *6, *8 or *11, because of a paucity of data. These *CYP2C9* alleles were only detected in African populations, such as *CYP2C9**5, *CYP2C9**6, *CYP2C9**8 and *CYP2C9**11 (allele frequencies in African populations of 1.8, <1, 8 and 2.7 %, respectively) [45–47]. A single African subject with epilepsy was identified as carrying a homozygous deletion of the *CYP2C9* gene (*CYP2C9**6), which means it is possible to live without this gene [48]. The allele frequency of this variant was 0.6 % in the African population studied, and this allele was not found in Asian or Caucasian populations. It remains to be seen whether some of the numerous non-coding *CYP2C9* polymorphisms can contribute to the genotypic prediction of the *CYP2C9* metabolic phenotype. Our approach may be extended to these variants once sufficient data become available. Secondly, the impact of genetic polymorphisms may interact with other factors such as race, which is not accounted for in the present models and may result in some bias.

5 Conclusion

As with many complex and new technologies, there are problems and delays in the transfer of scientific pharmacogenetic knowledge from bench to bedside. Specific translational research has to be supported. This modelling approach can only suggest some recommendations in terms of dosing adjustment in patients with specific *CYP2C9* genotypes or co-medications. However, their clinical usefulness in terms of efficacy/toxicity clearly needs to be further quantified and validated. The interest of *CYP2C9* genotyping before drug prescription should be assessed in prospective randomized clinical trials, in which one group of patients is treated with consideration for genotype information (and subsequent dosing adjustment) and compared with another group, in which physicians do not have genotype information and conventional empirical therapy is used. Our models may help design such studies and select study drugs.

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