

The use of mechanistic DM-PK-PD modelling to assess the power of pharmacogenetic studies – CYP2C9 and warfarin as an example

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What is already known about this subject

- Many studies have shown that genetic polymorphisms of the CYP2C9 gene contribute to some of the variability (around 20%) in warfarin dose requirements and therapeutic response to the drug.
- It is also clear that this effect must be elicited through differences in the plasma (S)-warfarin concentration between individuals of different genotypes, although assessing the effects of any single genotype of CYP2C9 on the kinetics of (S)-warfarin has generally failed.

What this study adds

- The study aims to simulate the impact of genetic polymorphism in CYP2C9 on both the pharmacokinetics (PK) and pharmacodynamics (PD) of (S)-warfarin using a mechanistic, population approach to modelling.
- The outcomes with respect to the design of studies and their statistical power are compared against those of actual reported studies.
- The exercise with warfarin is offered as an example of how prior information on the *in vitro* PK and PD of new drugs might be used in association with knowledge of relevant genetic polymorphisms and their frequencies to carry out virtual clinical studies as an aid to the design, optimization and powering of subsequent real clinical trials assessing the impact of specific genetic differences.

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Aim

To assess the power of *in vivo* studies needed to discern the effect of genotype on pharmacokinetics (PK) and pharmacodynamics (PD) using CYP2C9 and (S)-warfarin as an example.

Methods

Information on the *in vitro* metabolism of (S)-warfarin and genetic variation in CYP2C9 was incorporated into a mechanistic population-based PK–PD model. The influence of study design on the ability to detect significant differences in PK (AUC_{0–12h}) and PD (AUEC_{0–12h} INR) between CYP2C9 genotypes was investigated.

Results

A study size of 90 (based on the natural abundance of genotypes and uniform dosage) was required to achieve 80% power to discriminate the PK of (S)-warfarin between wild type (*1/*1) and the combination of all other genotypes. About 250 subjects were needed to detect a difference in anticoagulant response. The power to detect differences between specific genotypes was much lower. Analysis of experimental comparisons of the PK or PD between wild-type and other individual genotypes indicated that only 21% of cases (20 of 95 comparisons within 11 PD and four PK–PD studies) reported statistically significant differences. This was similar to the percentage expected from our simulations (20%, χ^2 test, $P=0.80$). Simulations of studies enriched with specific genotypes indicated that only three and five subjects were required to detect differences in PK and PD between wild type and the *3/*3 genotype, respectively.

Conclusion

The utilization of prior information (including *in vivo* enzymology) in clinical trial simulations can guide the design of subsequent *in vivo* studies of the impact of genetic polymorphisms, and may help to avoid costly, inconclusive outcomes.

Introduction

(S)-warfarin, which is responsible for most of the anticoagulant activity of racemic warfarin [1], is metabolized by cytochrome P450 2C9 (CYP2C9) [2]. Two allelic variants of this enzyme (*CYP2C9*2* and *CYP2C9*3*) exhibit significantly decreased catalytic activity compared with the wild-type form [3–7]. Accordingly, several studies have assessed the impact of these genetic variations on the pharmacokinetics (PK) of warfarin [8–11] and its pharmacodynamics (PD) with respect to therapeutic response and maintenance dose requirements [8–24]. Using (S)-warfarin as an example, we have developed a mechanistic, population PK–PD model, based on *in vitro* drug metabolism data and information on the frequency and activity of the different allelic forms of CYP2C9, to estimate the statistical power of *in vivo* studies needed to discern the effect of *CYP2C9* genotype on PK and PD. This represents a paradigm for assessing the impact of genetic polymorphisms on the PK and PD of new drugs prior to costly population studies. As such, therefore, it is in keeping with the rationale for clinical trial simulation (CTS) as a ‘smarter’ way of designing actual clinical studies [25, 26]. The present study is a companion to a similar investigation of the impact of genetic polymorphism in CYP2D6 on the PK and PD of dextromethorphan [27].

Methods

Clearance simulation

The *in vitro*–*in vivo* extrapolation (IVIVE) of the clearance of (S)-warfarin in different *CYP2C9* genotypes was achieved using a set of algorithms developed by Simcyp® Ltd (<http://www.simcyp.com>) Utilizing Monte Carlo simulation to establish virtual populations. Input

requirements were the abundances of the different forms of CYP2C9 per mg of microsomal protein [28], milligrams of microsomal protein per gram of liver [29], liver volume [30] and their variances, together with data on the *in vitro* metabolism of (S)-warfarin by wild-type and variant enzymes (see below). Thus, estimates of whole liver intrinsic clearances were made, which were then transformed into whole organ clearances (CL_H) using the ‘well-stirred’ model of hepatic drug clearance [31] (Figure 1). Individual renal clearances were simulated from an average value derived from observed urinary recovery and total clearance [32] considering the level of renal function expected for a given age and weight [33]. Hepatic and renal clearances were combined to derive net clearance. A full description of the methods employed for the IVIVE of individual clearance values is provided by Howgate *et al.* [34]. Specific parameters and modifications to the generic Simcyp® algorithm relevant to this study are indicated below.

Genotype frequencies and activities

A meta-analysis of the enzyme kinetic values in the published literature (Table 1) was carried out to estimate the relative activity of each of the *CYP2C9* alleles with respect to the *in vitro* metabolism of (S)-warfarin. A summary of the studies included in the meta-analysis is given in Table 1 [3–7]. Mean values of the activity of each allelic form were weighted for study size. Percentage decreases in intrinsic clearance ($CL_{U_{int}}$) with respect to wild-type (*1/*1) enzyme were calculated assuming that the *in vitro* activities of heterologously expressed variant enzymes represented those in the respective homozygous genotype. Values of $CL_{U_{int}}$ in heterozygous

Figure 1

Schematic representation of the pharmacokinetic–pharmacodynamic model, indicating propagation of metabolic differences between *CYP2C9* genotypes into (S)-warfarin clearance and its anticoagulant effect. $CL_{U_{int}}$, unbound intrinsic clearance; mppgl, milligrams of microsomal protein per gram of liver; WRF conc, plasma (S)-warfarin concentration; PC, prothrombin complex; R_{syn} , rate of synthesis of PC; k_d , rate constant of degradation of PC; PT, prothrombin time; INR, international normalized ratio

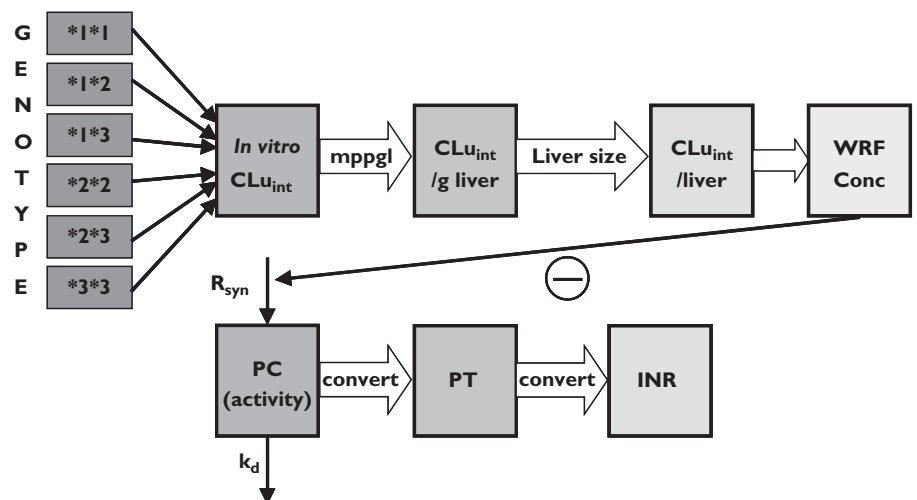


Table 1

Data used to calculate the relative activity of CYP2C9 in each of the genotypes

Allele	No. of observations	V_{max} (pmol min ⁻¹ pmol ⁻¹ P450)	K_m (μ M)	V_{max}^* (pmol min ⁻¹ pmol ⁻¹ P450)	Change in CL _{int} (%)	Reference
*1	2	0.22	18	0.585		Yamazaki <i>et al.</i> [6]
*1	3	0.1333	11.6	0.355		Sullivan-Klose <i>et al.</i> [3]
*1	3	0.282	1.86	0.750		Takahashi <i>et al.</i> [4]
*1	3	0.248	5.8	0.660		Takanashi <i>et al.</i> [7]
*1	3	0.28	2.6	0.745		Takahashi <i>et al.</i> [5]
*2	2	0.11	22	0.293	↓60.6	Yamazaki <i>et al.</i> [6]
*2	3	0.1656	12.5	0.440	↑11.4	Sullivan-Klose <i>et al.</i> [3]
*3	2	0.067	53	0.178	↓90.9	Yamazaki <i>et al.</i> [6]
*3	3	0.181	92.3	0.481	↓83.9	Sullivan-Klose <i>et al.</i> [3]
*3	3	0.111	21.6	0.295	↓87.7	Takanashi <i>et al.</i> [7]
*3	3	0.067	10.4	0.178	↓94.1	Takahashi <i>et al.</i> [5]

All studies were carried out in microsomes from yeast expressing the relevant mutation of the enzyme. * V_{max} corrected with an Inter-System Extrapolation Factor (ISEF) of 2.66 as described by Proctor *et al.* [49].

Table 2

Frequency and relative activity of each of the common CYP2C9 genotypes

Genotype	Frequency (%)	Relative activity (%)
*1/*1	65.3	100
*1/*2	20.4	85
*1/*3	11.6	55
*2/*2	0.9	70
*2/*3	1.4	40
*3/*3	0.4	10

Frequencies are from Lee *et al.* [35]. Relative activities were based on the meta-analysis of the enzyme activities shown in Table 1, and assumptions regarding the heterogeneous genotypes (see Methods).

genotypes were assumed to be the average of those for homozygotes.

The frequencies of each of the six established, common CYP2C9 genotypes in Whites were taken from a review by Lee *et al.* [35] (Table 2). (S)-warfarin elimination was assumed to be mediated exclusively by CYP2C9 with a small contribution from renal clearance (0.0032 l h⁻¹ [32] in the average man). This information

was entered into a classical PK/PD model with physiological elements related to the description of clearance.

PK/PD simulation

Pharmacokinetic model Clearance values for each individual were converted to elimination rate constants (k_i) for input into the PK model using equation 1.

$$k_i = \frac{CL_i}{V_i \cdot BW_i} \quad (1)$$

where CL_i is the clearance of (S)-warfarin in the i^{th} individual, calculated as described above, BW is body weight and V_i is the weight-normalized steady-state volume of distribution assigned to the i^{th} individual. The latter values were determined from the reported mean and standard deviation [36] (Table 3) assuming a log normal distribution. For such a distribution, the geometric mean (GM; μ in log scale) and geometric standard deviation (GSD; σ in log scale) represent the central tendency and variation, respectively. As the reported data refer to the algebraic mean (\bar{x}) and its standard deviation (SD), μ and σ were calculated as follows:

$$\mu = \ln \bar{x} - (0.5 * \sigma^2) \quad (2)$$

$$\sigma = \sqrt{\ln(1 + CV^2)} \quad (3)$$

where \bar{x} is the algebraic mean and

Table 3

Pharmacokinetic and pharmacodynamic (prothrombin complex activity in the blood and prothrombin time) parameters for (S)-warfarin used in the simulations

Parameter	Value*	
Pharmacokinetic	V (l kg ⁻¹)	28.9 (5.5)
	CL_R (renal clearance) (l h ⁻¹)	0.0032
	f_{uB}	0.0113
	t_{lag} (h)	0.8 (0.54)
	ka (h ⁻¹)	3.15 (1.68)
Pharmacodynamic	k_d (h ⁻¹)	0.054 (0.015)
	Cu_{50} (mg l ⁻¹)	0.0026 (0.0015)
	γ	0.90 (0.23)
	a	426 (8.0)
	b	7.75 (0.10)

*Numbers in parentheses indicate standard deviations. For a full description of the use of the parameters see Methods and Appendix. CL_R was taken from Obach [32], all other parameters are those reported by Chan et al. [36].

$$\text{Coefficient of variation (CV)} = \frac{SD}{\bar{x}} \quad (4)$$

These values were entered into Mathcad (Mathsoft, Cambridge, MA, USA) as part of the 'rlnorm' function. This returns a vector of random numbers having a log normal distribution based on the mean and CV value. This procedure was followed for generating all other individual parameter values.

The fraction of the amount of drug reaching the liver escaping hepatic metabolism in each individual ($F_{H,i}$) was determined from:

$$F_{H,i} = \frac{Q_{H,i}}{Q_{H,i} + f_{uB} \cdot CLu_{int,i}} \quad (5)$$

where $Q_{H,i}$ and $CLu_{int,i}$ are hepatic blood flow and the intrinsic metabolic clearance of (S)-warfarin in the i^{th} individual, respectively (estimated using Simcyp® algorithms as described above) and f_{uB} is the unbound fraction of drug in blood calculated from the fraction unbound in plasma (f_u) divided by the blood to plasma concentration drug ratio (B/P). The value of f_u (0.0062) was taken from the literature [36]. Since a value for B/P was not available, it was assumed to be 0.55, representing limited drug uptake into erythrocytes at a haematocrit of 45%.

Plasma concentrations of (S)-warfarin after oral administration of *rac*-warfarin were generated by entering values of the elimination rate constant ($k_{10,i}$) and $F_{H,i}$ into a one-compartment model with first-order absorption and a lag time, adapted from Chan et al. [36] (Equation 1, Appendix) (Figure 1).

Pharmacodynamic model Prothrombin complex activity in the blood (PCA) over time was simulated using an indirect response model in which PCA is the net effect of synthesis and degradation of the complex expressed as a percentage of maximum response [37] (Equation 2, Appendix) (Figure 1 and Table 3). The (S)-isomer of warfarin was assumed to be the active moiety inhibiting synthesis of the complex [1, 38, 39], although some reports have suggested a contribution to activity from the (R)-isomer [36]. PCA was converted to prothrombin time (PT) (Equation 3, Appendix) [36], which, in turn, was converted to International Normalized Ratio (INR) using an equation adapted from Adcock and Duff [40] (Equation 4, Appendix) (Figure 1).

Study design

Individual plasma (S)-warfarin concentration and response vs. time profiles following daily administration of oral warfarin were simulated with hourly sampling times over 24 h. A number of design elements were investigated as described below. As the majority of simulations were undertaken using 'uniform dosage' and 'random recruitment' (see below for definitions), this combination will be referred to as the 'default condition'.

Warfarin dosage Uniform dosage in each individual regardless of *CYP2C9* genotype – Each individual in the virtual population received 3 mg of (S)-warfarin per day. This dose was chosen based on half of the weighted average dose of *rac*-warfarin calculated from Holford et al. [41]. The study design is equivalent to a prospec-

tive clinical trial where each individual receives the same dose, assuming the dose requirement for each *CYP2C9* genotype is not known.

Genotype-related dosage – Several retrospective studies have indicated that the average dose of warfarin given to individuals with varying *CYP2C9* genotypes is different (Table 4). The distribution of warfarin doses in 292 patients treated in two large general hospitals in Merseyside, England, was kindly provided by M. Pirmohammed (University of Liverpool). A random number generator operating on a description of these data by a Weibull function was used to assign a maintenance dose to each virtual individual. The probability of each *CYP2C9* genotype within four different dose bands [low (0–3 mg), medium–low (3.1–4.5 mg), medium–high (4.6–6.4 mg) and high (6.6 + mg)] was adjusted according to a meta-analysis of the data shown in Table 4. Thus, the probabilities shown in Table 5 were used to assign the *CYP2C9* genotype to any given individual at any given dose. This study design will be referred to as ‘genotype-related dosage’.

Subject recruitment strategy Random recruitment – The subjects of most of the published studies of the influence of genetic variation in *CYP2C9* on warfarin PK or PD were not selected for *CYP2C9* genotype. Therefore, the probability of having adequate numbers of a certain genotype in the study population for comparison with other genotypes is dependent on the natural frequency of that genotype in the general population. Virtual populations were simulated based on this ‘random recruitment’ design.

Enriched recruitment – In this case, the virtual populations were enriched with certain genotypes. For example, a population of 10 **3/*3* individuals was compared with a population of 10 wild-type subjects. This design will be referred to as ‘enriched recruitment’. In practice, it would require the prior genotyping of many subjects, particularly with regard to the rarer genotypes, in order to carry out subsequent selective recruitment of small groups of individuals.

Study size Simulated plasma drug concentration- and response-time profiles in the different *CYP2C9* genotypes were compared using a range of population sizes (*n*). For ‘random recruitment’, *n* was varied from 10, 25, 50, 100, 250 to 550, and for ‘enriched recruitment’ it was varied from 2, 3, 5 to 10.

Null hypothesis As a set of control simulations to evaluate the possibility of false-positive outcomes, the null hypothesis that there is no true association of the geno-

Table 4

Data used in a meta-analysis to assign average doses in each *CYP2C9* genotype when simulating ‘genotype-related dosing’

Reference	<i>n</i>	Average dose in each genotype*				Percentage of subjects with each genotype														
		<i>*1/*1</i>	<i>*1/*2</i>	<i>*1/*3</i>	<i>*2/*3</i>	<i>*1/*1</i>	<i>*1/*2</i>	<i>*1/*3</i>	<i>*2/*3</i>	<i>*3/*3</i>										
Siguret <i>et al.</i> [23]	126	3.1 (1.4)	2.7 (1.3)	2.9 (1.1)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Kamali <i>et al.</i> [9]	119	4.06 (1.72)	3.63 (1.78)	2.7 (1.36)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Althall <i>et al.</i> [16]	155	4.68 (2.1)	3.7 (1.9)	2.7 (1.3)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Taube <i>et al.</i> [19]	561	5.01 (2.43)	4.31 (1.94)	3.97 (1.79)	3.04 (1.29)	4.09 (2.09)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Loebstein <i>et al.</i> [11]	156	6.5 (3.2)	5.2 (2.4)	1.6 (0.6)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Higashi <i>et al.</i> [17]	185	5.63 (2.56)	4.88 (2.57)	3.32 (0.94)	4.07 (1.48)	2.34 (0.35)	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Margaglioni <i>et al.</i> [18]	180	6.7 (2.9)	5.2 (2.2)	3.8 (2.0)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

*Mean (standard deviation).

Table 5

The probability of each genotype being found in each of four dose groups, based on a meta-analysis of the data shown in Table 4

Dose group	Genotype	Probability
Low (0–3 mg)	*1/*1	54.005
	*1/*2	20.450
	*1/*3	19.863
	*2/*2	1.229
	*2/*3	3.106
	*3/*3	1.347
Medium (low) (3.1–4.5 mg)	*1/*1	60.028
	*1/*2	22.902
	*1/*3	15.168
	*2/*2	0.922
	*2/*3	0.890
	*3/*3	0.091
Medium (high) (4.6–6.5 mg)	*1/*1	68.430
	*1/*2	23.094
	*1/*3	7.345
	*2/*2	0.425
	*2/*3	0.549
	*3/*3	0.000
High (6.6+ mg)	*1/*1	81.540
	*1/*2	16.443
	*1/*3	1.735
	*2/*2	0.032
	*2/*3	0.252
	*3/*3	0.000

type with drug clearance was tested. These simulations used the same study sizes employed in the ‘random recruitment’ simulations described above ($n = 10, 15, 50, 100, 250$ to 550) and ‘uniform dose’ conditions. Half the population were classed as ‘extensive metabolizers’ (*1*1) and half were ‘poor metabolizers’ (*3*3), but no difference in metabolic capability between the genotypes was introduced.

Influence of VKORC1 genotype Since pharmacodynamic variability in the response to warfarin is known to depend upon the genotype for vitamin K epoxide reductase complex 1 (VKORC1) [42–44] as well as that for CYP2C9, further simulations were carried out to assess its impact on study power. In the default model the variability in k_d was set to 27% [36] (Table 3). To allow for the increase in certainty arising from a knowledge of VKORC1 genotype, this was decreased to 15%. This 44% decrease in k_d was based on the weighted average of the results of studies indicating that the total variability in warfarin dose due to VKORC1 genotype (after subtracting variability due to CYP2C9 genotype) is 18–26%

[42–44]. The simulations were carried out under ‘random recruitment’ and ‘uniform dosing’ conditions.

Data analysis

Twenty simulations were run at each study size involving a total of 79 600 virtual patients. Although PK and PD data were available up to 24 h after dosage in our simulations, values of the areas under the plasma drug concentration–time curves (AUC) and effect (INR)–time curves (AUEC) were calculated up to 12 h (trapezoidal rule) in order to be consistent with the study design of experimental reports on warfarin PK and PD. The probability of detecting statistically significant differences in PK and PD between the wild-type (*1*1) and the combination of the other CYP2C9 genotypes was assessed by comparing values of AUC and AUEC, respectively, using ANOVA (SPSS v. 12, 2003; SPSS Inc., Chicago, IL, USA). The corresponding probabilities of detecting differences in AUC/AUEC between the wild-type and any other single genotype were calculated using Tukey’s *post hoc* test for multiple comparisons. The number of studies out of 20 providing a statistically significant difference in AUC (or AUEC) between CYP2C9 genotypes was recorded as the power of that comparison in that particular trial.

Results

The meta-analysis of the enzyme activities associated with the variant alleles of CYP2C9 indicated mean decreases in $CL_{u_{int}}$ of 15% and 45% compared with wild-type activity, respectively, for every *2 and *3 allele present in an individual. The resultant effects for different CYP2C9 genotypes are shown in Table 2. Propagation of these values through the Simcyp® algorithm resulted in values of unbound oral clearance of (S)-warfarin of 19.9 (*1*1), 17.1 (*1*2), 10.8 (*1*3), 14.8 (*2*2), 7.6 (*2*3) and 1.9 (*3*3) $l h^{-1}$. These predicted values were all within twofold of those reported from *in vivo* studies [8, 9] (Figure 2).

Default conditions (‘uniform dosage’ and ‘random recruitment’)

About 90 subjects were needed to detect a difference in AUC between the wild type (*1*1) and the combination of all other genotypes (Figure 3). The power to differentiate between wild type and any other single genotype was less, to a degree depending on the genotype (Figure 4). For example, about 450 subjects were required to detect a difference in AUC between the *2/*3 genotype and the wild type with a power of 80% (Figure 4), whereas, with the same number of subjects, a

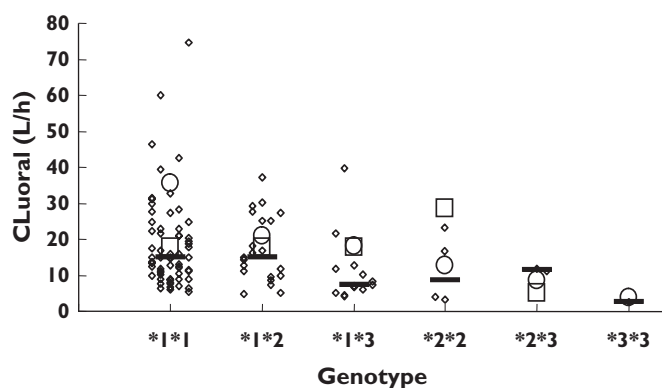


Figure 2

Comparison of observed (Scordo *et al.* [8], \circ ; Takahashi *et al.* [10], \square) and simulated (\diamond ; $n = 100$) values of the unbound oral clearance of (S)-warfarin. Solid lines indicate the medians of the simulated data

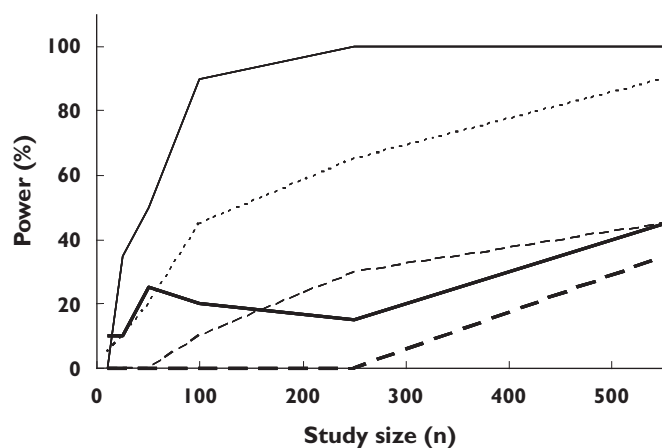


Figure 4

Outcomes of simulations to determine the statistical power of pharmacokinetic studies of (S)-warfarin to detect differences between AUC in wild-type vs. *1/*2 (— 20.4), *1/*3 (--- 11.6), *2/*2 (..... 0.9), *2/*3 (-.-.- 1.4) and *3/*3 (— — 0.4) genotypes under conditions of 'uniform dosage' and 'random recruitment'

power of only about 25% was achieved when comparing the *2/*2 genotype with the wild type (Figure 4). The null hypothesis simulations resulted in 0–5% power to detect differences in the AUC of (S)-warfarin between genotypes.

Corresponding powers to detect differences in AUEC between *CYP2C9* genotypes are shown in Figures 3 and 5. About 250 subjects were required to achieve 80% power to detect a difference between wild-type and any other genotype (Figure 3). The power to detect differences in AUEC between specific genotypes is shown in Figure 5. For example, about 420 subjects are required

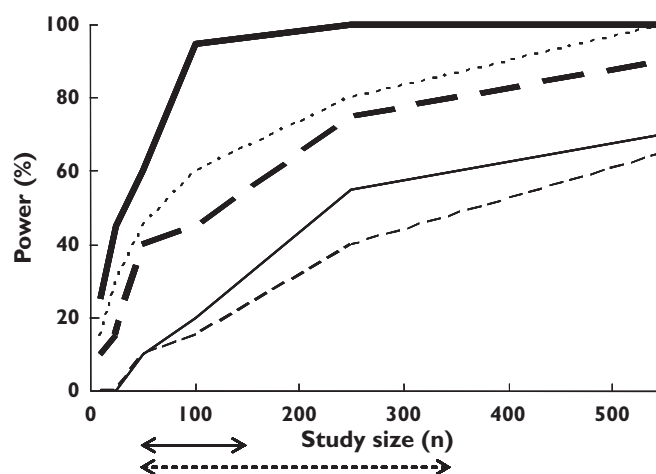


Figure 3

Outcomes of simulations to determine the statistical power of pharmacokinetic (PK) and pharmacodynamic (PD) studies of (S)-warfarin to detect differences between the influence of the *CYP2C9* wild-type genotype and the combination of all other *CYP2C9* genotypes ('random recruitment' conditions). Power with respect to the following conditions is indicated: AUC ('uniform dosage' (—) and 'genotype-adjusted dosage' (---)); AUEC ('uniform dosage' (—) and 'genotype-adjusted dosage' (---)); AUEC ('uniform dosage' and reduced PD variability (.....) due to knowledge of *VKORC1* genotype). The broken arrow indicates the range of sizes of published PD studies and the solid arrow shows the range of sizes of published PK studies

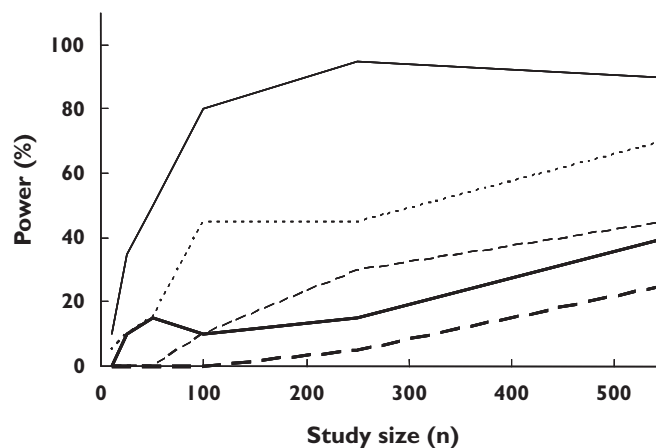


Figure 5

Outcomes of simulations to determine the statistical power of pharmacodynamic studies of (S)-warfarin to detect differences between AUEC in wild-type vs. *1/*2 (— 20.4), *1/*3 (--- 11.6), *2/*2 (..... 0.9), *2/*3 (-.-.- 1.4) and *3/*3 (— — 0.4) genotypes under conditions of 'uniform dosage' and 'random recruitment'

Table 6

The outcomes of reported studies of the pharmacokinetics (PK) and/or pharmacodynamics (PD) of warfarin with respect to *CYP2C9* genotype

Reference	Type of study	Outcome measure	n	Significant difference between *1/*1 and											
				*1/*2	*2/*2	*1/*3	*2/*3	*3/*3	Combo						
Takahashi <i>et al.</i> 2003 [10]	PK	Unbound oral clearance	47	<u>×</u>	25	<u>×</u>	0	<u>×</u>	48	<u>×</u>	20	<u>×</u>	0		
Scordo <i>et al.</i> 2002 [8]	PK	Unbound oral clearance	93	<u>×</u>	20	<u>×</u>	0	×	82	✓	44	✓	10		
Kamali <i>et al.</i> 2004 [9]	PK	Plasma (S)-warfarin concentration	121	✓	20	<u>×</u>	0	×	92	<u>×</u>	48	<u>×</u>	12		
Loebstein <i>et al.</i> 2001 [11]	PK	Plasma clearance	156	<u>×</u>	18	<u>×</u>	0	✓	94	×	52	<u>×</u>	16		
Takahashi <i>et al.</i> 2003 [10]	PD	Weight normalized maintenance dose	47	<u>×</u>	15	<u>×</u>	0	<u>×</u>	48	<u>×</u>	15	<u>×</u>	0		
Khan <i>et al.</i> 2004 [13]	PD	Maintenance dose	53	<u>×</u>	16	<u>×</u>	0	✓	50	<u>×</u>	16	<u>×</u>	0		
Joffe <i>et al.</i> 2004 [15]	PD	Maintenance dose/bleeding rate	73	<u>×</u>	12	<u>×</u>	0	×	64	<u>×</u>	26	<u>×</u>	4	✓	42
Scordo <i>et al.</i> 2002 [8]	PD	Maintenance dose	93	<u>×</u>	10	<u>×</u>	0	✓	76	✓	45	✓	10		
Kamali <i>et al.</i> 2004 [9]	PD	Maintenance dose	121	<u>×</u>	10	<u>×</u>	2	✓	82	<u>×</u>	45	<u>×</u>	12		
Siguret <i>et al.</i> 2004 [23]	PD	Maintenance dose	126	<u>×</u>	11	<u>×</u>	2	×	82	<u>×</u>	45	<u>×</u>	13		
Loebstein <i>et al.</i> 2001 [11]	PD	Maintenance dose	156	✓	12	<u>×</u>	3	✓	86	<u>×</u>	45	<u>×</u>	16		
Tabrizi <i>et al.</i> 2002 [24]	PD	Maintenance dose	153	✓	12	<u>×</u>	3	✓	86	<u>×</u>	45	<u>×</u>	17		
King <i>et al.</i> 2004 [21]	PD	Maintenance dose	159	<u>×</u>	12	<u>×</u>	3	✓	88	<u>×</u>	45	<u>×</u>	18		
Peyvandi <i>et al.</i> 2004 [14]	PD	Mean maintenance dose	175	<u>×</u>	12	<u>×</u>	3	✓	88	<u>×</u>	45	<u>×</u>	20	✓	60
Maragaglione <i>et al.</i> 2002 [18]	PD	Mean maintenance dose	180	✓	12	<u>×</u>	3	✓	88	<u>×</u>	45	<u>×</u>	20		
Higashi <i>et al.</i> 2002 [17]	PD	Maintenance dose/bleeding rate	185	<u>×</u>	12	<u>×</u>	3	×	90	<u>×</u>	45	<u>×</u>	21	✓	62
Lindh <i>et al.</i> 2005 [12]	PD	INR > 3	219	<u>×</u>	14	<u>×</u>	4	×	92	<u>×</u>	45	<u>×</u>	26	✓	68
Sconce <i>et al.</i> 2005 [22]	PD	Maintenance dose	297	✓	20	✓	8	✓	94	<u>×</u>	50	<u>×</u>	32	✓	78
Aquilante <i>et al.</i> 2006 [43]	PD	Maintenance dose	350	×	24	<u>×</u>	12	×	94	<u>×</u>	54	<u>×</u>	35	✓	80

'Combo' signifies comparison between wild type vs. the combination of all other genotypes. Crosses (×) indicate failure of the study to show a statistically significant difference between the corresponding genotype and wild type. Ticks (✓) indicate success of the study in showing a statistically significant difference between the corresponding genotype and wild type. All observations that were consistent with simulated results are underlined (i.e. × for true negative and ✓ for true positive).

for 80% power to detect a difference between the *2/*3 genotype and the wild type. The same number of subjects gave a power of 10% when comparing the *3/*3 genotype with the wild type.

Only 21% (20 of 95) of comparisons within published studies (four PK and 15 PD, with five possible comparisons; see Table 6) reported statistically significant differences in PK or PD between the wild type and any single other genotype. This was similar to the percentage expected from our simulations (20%; 19 of 95 comparisons). There were four false-negative and two false-positive predictions for PK comparisons, and corresponding numbers for PD cases were seven and six (overall a good consistency as indicated by χ^2 test, $P = 0.80$).

'Genotype-related dosage'

Using this model with 'random recruitment', the power to determine differences in drug clearance between wild-type and all other genotypes was the same as that seen under 'default conditions'. This was not the case for AUC (a parameter that is dependent on both dose and clearance) or AUEC, where the powers to determine differences between *CYP2C9* genotypes were decreased under 'genotype-related dosage' conditions compared with 'uniform dosage' (Figure 3). The power to detect a difference in AUEC (comparing wild-type and the combination of other genotypes) with the maximum study size reported in the literature (550 individuals) was 65%.

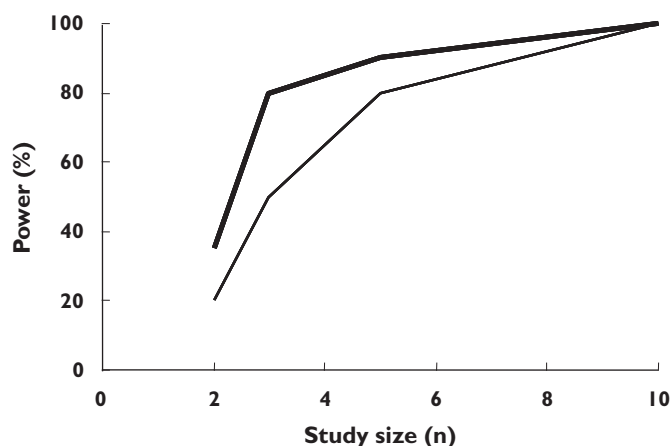


Figure 6

Outcomes of simulations to determine the statistical power of pharmacokinetic and pharmacodynamic studies of (S)-warfarin to detect differences in AUC and AUEC between wild-type and $*3/*3$ genotypes under conditions of 'enriched recruitment'. PK (—), DD (—)

'Enriched recruitment'

To investigate the effect of 'enriched recruitment' under 'uniform dose' conditions, $*3/*3$ individuals were compared directly with wild-type individuals. As expected, this resulted in much higher power compared with that achieved with 'random recruitment'. Thus, to achieve 80% power in detecting a significant difference in AUC between the two genotypes, only three subjects in each group were required (Figure 6). The corresponding number needed to detect differences in AUEC was five per group (Figure 6).

Influence of *VKORC1* genotype

The study powers arising from these simulations were slightly higher than those of the equivalent 'uniform dosage', 'random recruitment' simulations described above (Figure 3).

Discussion

Based on CTS using a mechanistic population PK–PD model incorporating data on *in vitro* drug metabolism, we have examined the power of *in vivo* studies to determine differences in the disposition of (S)-warfarin and its anticoagulant effect in relation to *CYP2C9* genotype. The derivation of metabolic clearance associated with different genotypes from *in vitro* data recovered that reported from *in vivo* studies with good accuracy (Figure 2).

The model projection indicated that, under the assumptions of 'uniform dosage' and 'random recruitment', at least 90 subjects would be required to detect a difference (80% power) in the AUC of (S)-warfarin between wild genotype and the combination of all other genotypes (Figure 3). For 'genotype-related dosage', the power would be much less (over 550 subjects required for a power of 80%). Also, comparisons between the wild-type and specific genotypes would require much higher numbers of subjects (e.g. 420 subjects to achieve 80% power in discriminating PK between wild type and $*2/*3$ under the 'uniform dosage' condition). Study sizes of this order are uncommon in classical PK studies, although they may be achieved in population PK studies using sparse data analysis. Four studies assessing the impact of genetic variation in *CYP2C9* on warfarin PK using randomly selected subjects have been reported. One of these [10] used 47 subjects and, as predicted by our model, failed to detect a difference in PK between wild-type and all other genotypes. Three other studies were successful in discerning a difference between the wild type and some of the genotypes, but not others [8, 9, 11]. For example, each of the three studies had 0% power to differentiate the PK between the wild-type and the $*2/*2$ genotype. Accordingly, none of the studies was successful in observing a significant difference between these genotypes using 93, 121 and 297 subjects (due to insufficient numbers of $*2/*2$ subjects in the study) [8, 9, 11] (Table 6).

With regard to PD outcome, the model projection indicated that under the assumptions of 'uniform dosage' and 'random recruitment', about 250 subjects would be required to achieve 80% power to detect a difference in AUEC between wild type and the combination of all other genotypes (Figure 3). With 'genotype-related dosage' the power decreased to 60% with 550 subjects. All reported studies assessing the impact of genetic variability in *CYP2C9* on warfarin response and dosage requirements were sufficiently powered to detect the contrast between wild-type and the combination of all other genotypes (Table 6). However, as with the PK studies, the power for comparisons between specific genotypes was much lower. For example, with a study size of 121, Kamali *et al.* had a power of around 82% to differentiate the PD of (S)-warfarin between the wild-type and the $*1/*3$ genotype [9], but all other comparisons had powers of <80%. The reported observations were fully consistent with the powers (Table 6). There were four and seven false-negative cases, respectively, in PK and PD studies (indicated by tick marks that are not underlined in

Table 6), where the simulations suggested lack of adequate power to detect differences between wild-type and the respective non-wild-type genotype, but the study found a statistically significant difference. It should be noted that this may happen by chance, as the level of type 1 error is set at 5% and hence every one out of 20 studies may indicate a difference which is not a true difference. However, the 11% rate for false-negative cases *vs.* the 8% rate for false positives (Table 6) may indicate some conservatism in our power calculations. This might reflect some overestimation of the variability in model parameters. The variances in the parameters of the PD model used in our simulations were based on data from only six healthy individuals ($n = 6$). Nevertheless, there was good overall concordance between the predicted and observed percentage of studies (20% *vs.* 21%; P -value of 0.8 from χ^2) successful in differentiating (S)-warfarin PK or PD between the wild type and any single other genotype.

When ‘genotype-adjusted’ conditions were considered, a power of only 60% to detect differences in the AUEC between the wild type and a combination of any other genotype was reached with the maximum number of subjects studied of 550. Accordingly, the simulations indicate that the use of INR as a biomarker to adjust the dose of (S)-warfarin should preclude major differences in therapeutic response between genotypes. This supports the view that genotyping for CYP2C9 is only likely to be of value during the initial phase of warfarin therapy when INR is not yet indicative of final response. Accordingly, Schalekamp *et al.* [45] have recently shown that although other sources of variability such as polymorphism in the vitamin K epoxide reductase (*VKORC1*) gene can explain some of the variability in maintenance doses of acenocoumarol (21.4%), the time to achieve stable acenocoumarol therapy was associated with *CYP2C9* but not *VKORC1* genotype. A similar outcome may apply to warfarin therapy. Our simulations have attempted to assess the increase in study power (with respect to discerning the influence of *CYP2C9* genotype) as a result of having prior knowledge of the *VKORC1* genotype (Figure 3).

The simulations with the ‘enriched-recruitment’ design indicated the need for very small numbers of subjects to separate genotypes with respect to both PK and PD differences (Figure 6), albeit with the requirement for prior screening of many individuals in order to find the rare genotypes. It should be noted that frequent recruitment of individuals with rare genotypes into different trials may pose some practical and ethical problems.

Can the approach that we have used here to simulate the impact of genetic polymorphism on warfarin dosage be applied to new drugs in development? Given adequate prior *in vitro* information on the enzymes, especially cytochromes P450, involved in the metabolism of the compound and estimates of the relative activity of different genotypic forms and their population frequency, it may be possible to predict *in vivo* drug clearance as a function of genotype with reasonable accuracy. Although, in the case of warfarin, we have used *in vivo* information on its volume of distribution, it is possible to estimate this parameter from physico-chemical characteristics and variation in organ size and composition [46, 47]. The degree to which a relevant PD model is available *a priori* may be more limited. However, even crude models based on *in vitro* receptor binding data and, indeed, any information on genetic variability in the target receptors could be informative in a model-building exercise [48]. Thus, in the future, CTS may increasingly utilize prior *in vitro* data in mechanistic PK–PD models. This approach, exemplified by the current study, to calculate statistical power avoids tenuous assumptions about the distributions and variation of primary PK parameters required by many current attempts at CTS.

Appendix

Pharmacokinetic modelling Steady-state plasma concentrations of (S)-warfarin after multiple doses of *rac*-warfarin given every 24 h (τ) were generated using a one-compartment model with first-order absorption and a lag time [36]. Thus, values in the i^{th} individual were given by:

$$C_{\text{warf},i}(t) = \left[\frac{fa_i \cdot F_{H,i} \cdot \left(\frac{D_i}{2}\right) \cdot ka_i}{V_i \cdot BW_i \cdot (ka_i - k_{10,i})} \right] \cdot \left(\frac{e^{-k_{10,i}(t-t_{\text{lag},i})}}{1 - e^{-k_{10,i}\tau}} \right) - \left(\frac{e^{-ka_i(t-t_{\text{lag},i})}}{1 - e^{-ka_i\tau}} \right) \quad (1)$$

where $C_{\text{warf},i}(t)$, t_{lag} , fa_i , $F_{H,i}$, D_i , ka_i , V_i , BW_i and $k_{10,i}$ are, plasma drug concentration at time t , the lag time between administration and appearance of drug in the plasma (Table 3), the fraction of the dose absorbed (set to 1), the fraction escaping first-pass metabolism, the dose, a first-order absorption rate constant (Table 3), the steady-state volume of distribution (Table 3), body weight and a first-order elimination rate constant in the i^{th} individual, respectively. All parameter values were

taken from Chan *et al.* [36] except $F_{H,i}$, BW_i and $k_{10,i}$, which were calculated using the Simcyp[®] algorithm, and D , which was either a uniform daily dose of 3 mg or a dose taken from a population distribution as described in the main text.

Pharmacodynamic modelling The anticoagulant effect of (S)-warfarin was simulated using an indirect response model in which it inhibits prothrombin synthesis, thereby changing prothrombin complex activity (PCA) in the blood as a function of time [36] (Figure 1). The rate of change of PCA is given by:

$$\frac{dPCA_i}{dt} = k_{d,i} \cdot \left[\frac{100}{1 + \left(\frac{Cu_{\text{warf},i}(t)}{Cu_{50,i}} \right)^{\gamma_i}} - PCA_i \right] \quad (2)$$

where $k_{d,i}$ is the degradation rate constant of the prothrombin complex, $Cu_{50,i}$ is the unbound concentration of drug required to produce a 50% inhibition of PCA synthesis and γ_i is a measure of the steepness of the concentration–response curve (Hill coefficient) in the i^{th} individual (Table 3).

PCA was converted to prothrombin time (PT) using the following equation from Chan *et al.* [36]:

$$PCA(\% \text{ normal}) = \frac{a}{PT(\text{seconds}) - b} \quad (3)$$

where a and b are constants with variability (Table 3).

In turn, values of PT were converted to INR values using the following relationship taken from Adcock and Duff [40]:

$$INR = \frac{PT + 0.242}{9.5981}$$

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