

Characterizing variability in warfarin dose requirements in children using modelling and simulation

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Genetic, clinical and demographic factors collectively make a significant contribution to the inter-individual variability in warfarin dose requirement in adults.
- Less is known about the major causes of warfarin dose variability in children, including the relative importance of genetic, clinical and demographic factors.

WHAT THIS STUDY ADDS

- This study shows that *CYP2C9* and *VKORC1* genotype, bodyweight, age, baseline and target INR, and time since initiation of therapy, but not *CYP4F2* genotype, are significant causes of warfarin dose variability in children.
- The model can provide the basis for development of more individualized warfarin dosing in children.

AIMS

Although genetic, clinical and demographic factors have been shown to explain approximately half of the inter-individual variability in warfarin dose requirement in adults, less is known about causes of dose variability in children. This study aimed to identify and quantify major genetic, clinical and demographic sources of warfarin dose variability in children using modelling and simulation.

METHODS

Clinical, demographic and genetic data from 163 children with a median age of 6.3 years (range 0.06–18.9 years), covering over 183 years of warfarin therapy and 6445 INR observations were used to update and optimize a published adult pharmacometric warfarin model for use in children.

RESULTS

Genotype effects in children were found to be comparable with what has been reported for adults, with *CYP2C9* explaining up to a four-fold difference in dose (*CYP2C9* *1/*1 vs. *3/*3) and *VKORC1* explaining up to a two-fold difference in dose (*VKORC1* G/G vs. A/A), respectively. The relationship between bodyweight and warfarin dose was non-linear, with a three-fold difference in dose for a four-fold difference in bodyweight. In addition, age, baseline and target INR, and time since initiation of therapy, but not *CYP4F2* genotype, had a significant impact on typical warfarin dose requirements in children.

CONCLUSIONS

The updated model provides quantitative estimates of major clinical, demographic and genetic factors impacting on warfarin dose variability in children. With this new knowledge more individualized dosing regimens can be developed and prospectively evaluated in the pursuit of improving both efficacy and safety of warfarin therapy in children.

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Introduction

Warfarin is the most commonly prescribed anticoagulant in both adults and children [1]. Although it has been in clinical use for over 60 years, warfarin therapy is still a clinical challenge due to the drug's narrow therapeutic range and large inter- and intra-individual variability in response to a fixed dose. Known factors contributing to the variability in dose requirement in adults include age, body size, co-morbidities, co-medications, vitamin K intake and genetic polymorphisms [2], with over 40% of the variability explained by polymorphisms in the two genes *VKORC1* and *CYP2C9* [3].

Less is known about the relative importance of genetic, clinical and demographic factors on warfarin dose variability in children, but data from small studies have started to emerge. Nowak-Göttl *et al.* [4], studying 34 warfarin-treated children with a median age of 15 years (range 1–19 years), found that age was the most important determinant, explaining 31.2% of the variability in dose requirement, while polymorphisms in *VKORC1* and *CYP2C9* only explained 2.8% and 0.5%, respectively. In contrast, Nguyen *et al.* [5] recently reported that, in 37 children with a mean age of 9.6 years (range 1.8–18.6 years), age explained only 12% and polymorphisms in *VKORC1* and *CYP2C9* accounted for 47% and 5% of the variability, respectively. A larger study by Moreau *et al.* [6] involving 83 children with a mean age of 8.4 years (range 0.2–18 years), reported that height explained 48.1% of the variability in dose requirement and polymorphisms in *VKORC1* and *CYP2C9* accounted for 18.2% and 2%, respectively. In the largest published pharmacogenetic study in children reported by Biss *et al.* [7], including 120 children with a median age of 11.7 years (range 1.7–18 years), height was found to account for 29.8%, *VKORC1* for 26.6% and *CYP2C9* for 12.8% of the variability in warfarin maintenance dose requirement. Hence, published results are inconclusive with regard to the relative importance of individual predictors.

Reported differences in the contribution of *CYP2C9* and *VKORC1* genotype to the variability in warfarin dose requirement in children could partly be explained by differences in minor allele frequencies between studies [8], but also heterogeneity in study design and analysis approaches [2], as well as the small sample sizes, which cause uncertainty in reported point estimates for rare covariate effects. True genotype–phenotype relationships may also be obscured when analyzing data from studies in infants and young children depending on the importance of ontogeny on enzyme activity levels [9]. For some of the polymorphically expressed CYPs, ontogeny has been shown to play a major role in explaining interindividual variability in enzyme activity in newborns and infants, and makes the genotype–phenotype relationship less evident in these age groups [9]. For the *CYP2C9* enzyme published data indicate complete maturation around 6 months of

age [10]. Corresponding information about the ontogeny of the *VKORC1* enzyme is lacking.

Adult patients who possess *CYP2C9* and/or *VKORC1* polymorphisms have been shown to be more sensitive to warfarin and prone to supra-therapeutic INRs during warfarin initiation [3, 11]. A recent study [12] reported that these polymorphisms also play an important role in the early response to warfarin therapy in children. Children with at least one *CYP2C9* variant allele had higher mean peak INR values during the first week of therapy, and a higher proportion of INR values above target range during the first month of therapy, than children with wild-type *CYP2C9*. Children with the *VKORC1* –1639 A/A genotype had higher mean peak INR values and a tendency to more INR values being above target range during the first month of therapy than those with the G/A or G/G genotype [12].

Pharmacometric models developed on adult data are increasingly being used to improve dose predictions in children [13, 14]. Using a pharmacometric approach when analyzing clinical data can alleviate many of the difficulties encountered with data derived from studies in children. A model-based approach can benefit from the possibility to incorporate prior knowledge, and to pool data across studies. The approach also enables use of sparse and unstructured real-life data, rather than relying on data derived from stringent clinical trial settings [15]. It is also possible to bridge from an existing adult model to paediatrics through the use of physiological principles that acknowledge that adults and children are in some aspects similar and not discrete subpopulations [13, 14, 16]. Pharmacometric modelling and simulation are also advocated by regulatory bodies to support the selection of safe and efficacious dosing regimens for children [17, 18].

We have previously developed a PKPD-based model that describes the relationship between warfarin dose and the International Normalized Ratio (INR) response in adults [19, 20], and which takes into account variation in pharmacokinetics (PK) due to age and *CYP2C9* genotype, and variation in pharmacodynamics (PD) due to *VKORC1* genotype. By estimating allele effects rather than genotype effects, the model development is less sensitive to small sample sizes since individuals with two variant alleles are not required for estimation of rare genotype effects. The individual genotype effect is obtained by simply adding the estimated allele effects. We have theoretically adapted the adult model [20] for use in children [21] through allometric weight scaling of clearance and volume of distribution [22], and addition of a published maturation function for *CYP2C9* enzyme activity [10] to handle the influence of enzyme ontogeny in neonates and infants [9]. External validation of the theoretically bridged model on data from 64 children 0–18 years old showed that the model overall performed well, but with a tendency to over predict INR response, or underestimate the dose, in children ≤ 2 years old [21].

The main objective of the present study was to identify and quantify major genetic, clinical and demographic sources of warfarin dose variability in children using modelling and simulation.

Methods

Patients and treatment data

Data from two observational warfarin studies in children with underlying heart disease [7, 21] were used in the current analysis. The two studies are only briefly described here as relevant to this work. One study recruited 93 children at four hospitals in the UK and 27 children at one hospital in Canada; the other included 67 children from four hospitals in Sweden. Warfarin was administered using formulations available in the respective countries: 2.5 mg tablets or extemporaneous 0.1 mg or 0.3 mg capsules in Sweden, 1, 3 or 5 mg tablets in the UK, and 1, 2, 2.5, 3, 4, 5, 6, 7.5 or 10 mg tablets in Canada. Local ethics committees approved the studies and informed consent was obtained from patients 18 years or older or from parents of children below 18 years.

Longitudinal treatment data were collected from hospital records and patient charts, including information on treatment indication, baseline INR, target INR, warfarin doses, INRs, date of birth, height and total bodyweight. All children were genotyped for *VKORC1* -1639 G > A (rs9923231), *CYP2C9* *2 and *3 (rs1799853 and rs1057910) and *CYP4F2* *3 (rs2108622). The patients' ethnic background was self-reported according to pre-specified categories.

One hundred and sixty-three of the 187 children enrolled had sufficiently detailed treatment records to be included in the analysis. Seventy-eight children had data available from treatment start. For the other 85 children a sufficiently long dosing history to ascertain pharmacokinetic steady-state was required before INR observations were included in the analysis. The assumption on steady-state was based on *CYP2C9* genotype and predicted half-lives for S-warfarin, using the following set of rules: at least 7 (*1/*1), 10 (*1/*2), 14 (*1/*3 or *2/*2) or 21 days (*2/*3) of dosing information before inclusion of an INR (no *3/*3 included). The assumption was tested in a sensitivity analysis by doubling the treatment length, with no impact on the results, suggesting that the rules applied were robust.

The measured effect of warfarin therapy is the change in INR from baseline, i.e. if baseline INR is 1.0 and the measured INR is 2.8, the treatment effect is a Δ INR of 1.8. Baseline INR was only available in 35 children and ranged from 1.0–1.7, with a median of 1.2. Attempts were made to estimate baseline INR for those children where this was missing, but the data were not informative enough to separate baseline and EC_{50} when estimated simultaneously. Instead we used the observed baseline when avail-

able, and fixed it to the median value of 1.2 for children with missing baseline values.

Model development

All modelling was performed in NONMEM, version 7 (ICON, ICON Development Solutions, Ellicott City, MD, USA), using the first order conditional estimation method with interaction [23]. Analyses were carried out using an additive residual error model on log-transformed data, and with interindividual variability implemented using exponential models. Model selection was guided by (i) the decrease in the objective function value (the minimization criteria in NONMEM), (ii) the condition number obtained from NONMEM, (iii) graphical goodness-of-fit analysis in Xpose [24], (version 4.3.5 [xpose.sourceforge.net]) and R (version 2.14.2 [www.r-project.org]), (iv) the estimated uncertainty in parameter estimates as reported by the standard error obtained from NONMEM, (v) simulation based diagnostics (prediction corrected visual predictive checks (pc-VPCs)) and (vi) plausibility of parameter estimates. The difference in the objective function value between two hierarchical models is approximately χ^2 -distributed, meaning that a nominal difference of 3.84 corresponds to a *P* value <0.05 for one degree of freedom. Criteria for final model selection included: (i) a statistically significant improvement over a competing simpler model, (ii) a condition number that was reasonably low (<1000), (iii) reasonable sized standard errors, and with 95% confidence intervals not including zero or unity (whatever applicable), (iv) pc-VPCs showing good agreement between observed and model predicted INRs and (v) biologically plausible estimates of model parameters.

Base model

This work is a further development of our published adult PKPD-based warfarin model with S-warfarin as the only exposure predictor for INR response [20], or rather a theoretically scaled version of this model for use in children [21]. Scaling to children was achieved by adding bodyweight to the adult model using allometric scaling [22], and addition of a published maturation function for *CYP2C9* enzyme activity [10]. Both models are adapted according to the KPD-framework, as described by Jacqmin *et al.* [25]. This means that the models operate without PK observations, but make use of available information about variability in PK on a population level, e.g. the known influence of *CYP2C9* genotype on the typical clearance (CL) of S-warfarin. A drawback compared with a full PKPD-model is that it becomes more difficult to separate remaining sources of variability into PK and PD, respectively, and hence less mechanistic interpretation can be made from a KPD-model.

Equation 1 provides the general formula for the prediction of clearance (CL) of S-warfarin as a function of age (*CYP2C9* enzyme ontogeny) and size (bodyweight), and equation 2 provides the formula for the prediction of

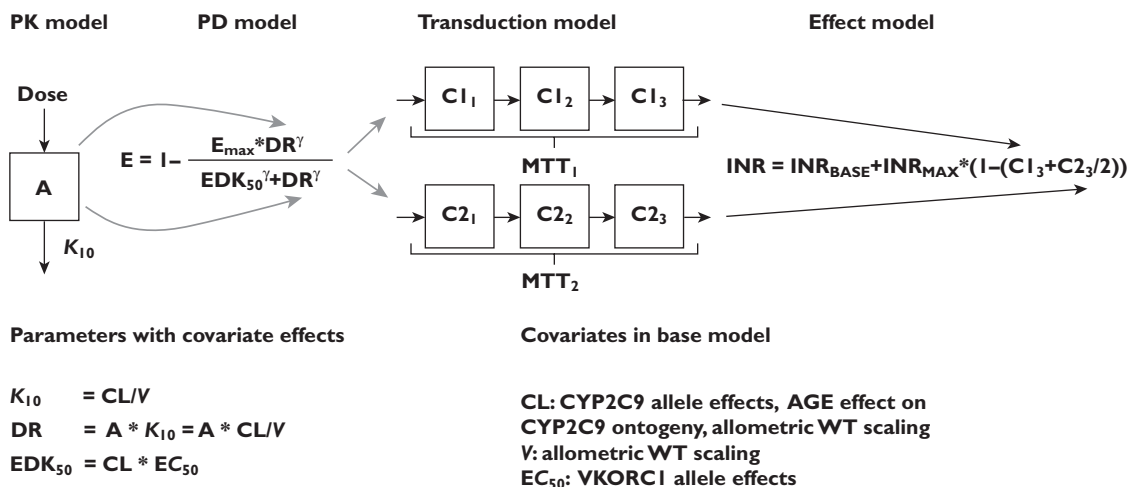


Figure 1

Schematic picture of the bridged warfarin model [21] used as base model in this study and a list of covariate effects included in the base model. The model consists of a one compartment PK model that describes the relationship between warfarin dose and S-warfarin concentrations (PK), and an inhibitory E_{max} -model to describe inhibition of the vitamin K cycle (PD), with S-warfarin as the only predictor of the anticoagulant response. Included also is a transduction model that captures the time delay between the inhibition of the vitamin K cycle, and the decrease in functioning coagulation factors (II, VII, IX and X), and finally an effect model that translates the decrease in functioning coagulation factors to an increase in INR. Compartment A represents amount of warfarin in the body, K_{10} the rate constant governing drug elimination from the body but also distribution to the site of action, here denoted DR for dose rate. EDK_{50} is the dose rate that results in 50% of the maximum effect E_{max} and MTT is the mean transit time through the transduction-model

volume of distribution (V) of S-warfarin as a function of size (bodyweight):

$$CL_i = CL_s \times \left(\frac{BW_i}{BW_s}\right)^{0.75} \times \left(\frac{0.821 \times AGE_i}{0.01 + AGE_i} + 0.21\right) \quad (1)$$

$$V_i = V_s \times \left(\frac{BW_i}{BW_s}\right)^1 \quad (2)$$

CL_i and V_i represent the typical parameter values in an individual with the bodyweight BW_i , and CL_s and V_s represent typical parameter values in an individual with the bodyweight BW_s . AGE_i is the post-natal age expressed in years of an individual with the bodyweight BW_i . Using the published maturation function, CYP2C9 enzyme activity in a typical child is mature and reaches adult values at the age of 6 months.

Figure 1 provides a schematic picture and a brief description of the theoretically scaled warfarin model [21], which served as the base model in this analysis.

Covariate selection

The base model included CYP2C9 genotype, bodyweight and age as predictors of CL, bodyweight as predictor of V and VKORC1 genotype as predictor of EC_{50} . Additional covariates tested when adapting and optimizing the model for children included age, gender, bodyweight,

indication, time since treatment start, dose, and CYP4F2 genotype (tested as allele effects). Covariates were screened for using scatter plots of individual parameter estimates (K_{10} and EC_{50}) against individual covariates, and by generalized additive modelling (GAM) using Xpose [24]. Nested models were compared statistically using a likelihood ratio test on the differences in objective function value (OFV).

Model evaluation

VPCs were used to evaluate the predictive performance of candidate models. The principle of a VPC is to assess graphically whether simulations from a model are able to reproduce both the central trend (the median curve) and the variability (outer percentiles) in the observed data when plotted against an independent variable (e.g. time, age or bodyweight). A prediction corrected VPC (pc-VPC) is a modification of the standard VPC that is appropriate to apply on data collected in studies with an adaptive design [26] as in the case with warfarin. All pc-VPCs were constructed based on 500 simulated replicates of the original dataset design.

Predictions of maintenance dose

The final model was used to predict the warfarin maintenance dose for typical children aged 2, 8 and 14 years old, adopting the approach described by Jönsson & Karlsson [27]. Briefly, this entails (i) defining a target steady-state INR response, in our case 2.5, and (ii) using the model

to estimate the dose required to achieve the target response, conditioned on the population (mean) parameter estimates.

Results

Patient and treatment data

Of 187 children enrolled in the two warfarin studies [7, 21], 163 had sufficiently detailed data records to be included in this analysis. Demographic information, including treatment indication, target INR and genotype frequencies for *VKORC1* -1639 G > A (rs9923231), *CYP2C9* *2 and *3 (rs1799853 and rs1057910) and *CYP4F2* *3 (rs2108622) are shown in Table 1. All genotypes were in Hardy–Weinberg equilibrium. Age and weight for the entire study period ranged from 0.06–18.9 years and 3.6–100.1 kg, respectively. The median dose from all phases of therapy was 2.5 mg (range, 0.125–12.5 mg) or 0.112 mg kg⁻¹ (range, 0.0156–0.585 mg kg⁻¹), respectively. Self-reported ethnic origin included 76.7% White, 7.4% Asian, 4.9% Black

Table 1

Patient and clinical characteristics

Gender, n (% male/female)	105/58 (64/36)
Median age, years (range)	6.3 (0.066–19)
Median weight, kg (range)	20 (3.6–100.1)
<i>CYP2C9</i> genotype, n (%)	
*1/*1	116 (71.2)
*1/*2	20 (12.3)
*1/*3	23 (14.1)
*2/*2	2 (1.2)
*2/*3	2 (1.2)
*3/*3	–
<i>VKORC1</i> genotype, n (%)	
G/G	57 (35.0)
A/G	79 (48.5)
A/A	27 (16.5)
<i>CYP4F2</i> genotype, n (%)	
C/C	87 (53.4)
C/T	66 (40.5)
T/T	10 (6.1)
Treatment indication, n (%)	
Fontan procedure	75 (46.0)
Prosthetic heart valve	34 (20.8)
Dilated cardiomyopathy	15 (9.2)
Coronary aneurysm	11 (6.7)
Other	28 (17.3)
Target INR, n (%)	
2.0–3.0	119 (73.0)
2.5–3.5	32 (19.6)
Other (lower range <2 or upper range >3.5)	8 (4.8)
Missing	4 (2.5)
INR observations, n (%) assayed with	
Owren based method	4298 (66.7)
Quick based method	2147 (33.3)
Total number of children, n (%)	163
Swedish study [21]	64 (39.3)
UK/Canadian study [7]	99 (60.7)

African and 11% other. Treatment data varied in length from 3 weeks up to 10 years per child, comprising a total of 6445 INR observations covering more than 183 treatment years. Figure 2 provides a raw data plot of all INR observations per subject, showing the age span for which treatment data were available. Coherent treatment records that included data after a child had turned 18 years old were also included in the analysis to increase the amount of data per individual. There were in total 55 INR observations from seven subjects that were between 18 and 19 years old.

Final model

The theoretically scaled warfarin model [21] was used as the base model in this analysis. All model parameters in the base model, except the Hill factor in the E_{max}-model, were re-estimated on the paediatric dataset. Data supported addition of two new covariates to the base model: an exponential age-effect on the elimination rate constant *K*₁₀, and an exponential effect of time since treatment start on EC₅₀, the parameter describing sensitivity to warfarin.

The principal impact on *K*₁₀ and EC₅₀, respectively, are shown in Equations 3 and 4:

$$K_{10} = \frac{CL}{V} \times \left(1 + \theta_{\text{Increase at age}=6.3y} \times \exp \left(-(\text{age} - 6.3) \times \frac{\ln 2}{\theta_{\text{Half-life, age-effect on } K_{10}}} \right) \right) \quad (3)$$

$$EC_{50} = EC_{50} \times \left(1 - \theta_{\text{Decrease at time}=0} \times \exp \left(-\text{time} \times \frac{\ln 2}{\theta_{\text{Half-life, time-effect on } EC_{50}}} \right) \right) \quad (4)$$

where $\theta_{\text{Increase at age}=6.3y}$ describes the relative increase in *K*₁₀ in a 6.3 year old child and $\theta_{\text{Half-life, age-effect on } K_{10}}$ the half-life of the age-effect on *K*₁₀, and $\theta_{\text{Decrease at time} = 0}$ describes the decrease in EC₅₀ at treatment start (time = 0) and $\theta_{\text{Half-life, time-effect on } EC_{50}}$ the half-life of the time-effect on EC₅₀. This means that the model predicts that the elimination rate of warfarin (*K*₁₀) is higher than that predicted from bodyweight (allometric weight scaling of CL and V) and age (maturation function on CL). For example, at the age of 2 years the model predicts a *K*₁₀ that would result in a half-life of S-warfarin (*t*_{1/2} = ln2/*K*₁₀) approximately five times shorter than that predicted without this additional age-effect. With the time effect on EC₅₀, the model predicts that children are more sensitive to warfarin at the start of therapy, with an approximate 60% reduction of EC₅₀. This effect showed a half-life of 170 h (7 days), which means that the effect disappears after approximately 4 weeks (four half-lives) of therapy.

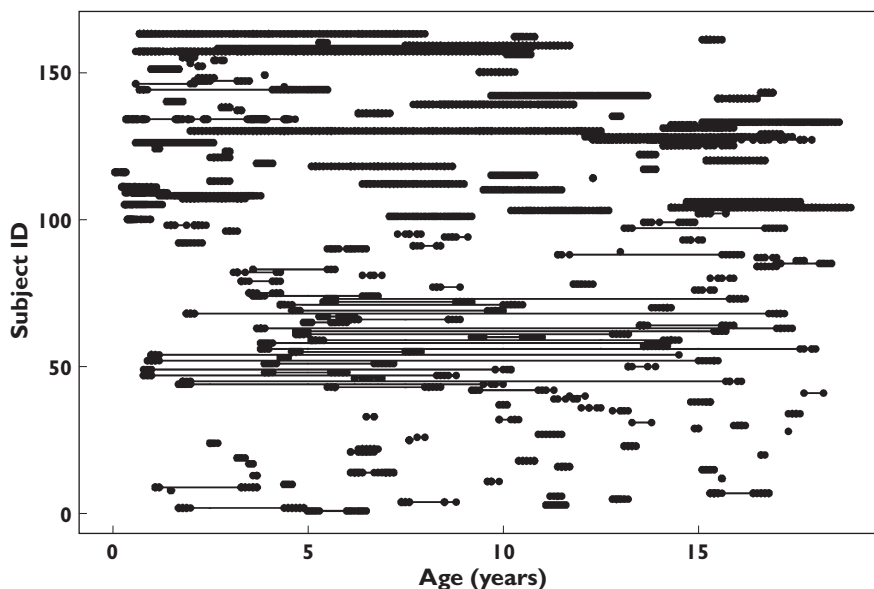


Figure 2

Raw data plot of INR observations included in the analysis, and where the dots along the x-axis show the age span for which observations were available per child. INR observations from several treatment periods in the same child are connected with a line. ID 1–99 represent children from the British/Canadian study and ID 100–163 represent children from the Swedish study

When the two additional covariates were included stepwise in a multivariate model, both remained significant. Following a confirmatory backward stepping approach, both remained significant, with a Δ OFV of 189 and 150 upon removal of the age-effect on K_{10} and the time-effect on EC_{50} , respectively. Also the objective function value dropped from -10586.8 in the base model to -10906.1 in the final model, i.e. a Δ OFV of -319.3 . The final model parameters are reported in Table 2, together with parameter estimates from the base model and adult parameter values [21] as reference. The proportion of unexplained variability in K_{10} dropped from 160% in the base model to 124% in the final model, and the residual error in INR response from 25.4% to 24.9%. ETA shrinkage in K_{10} and EC_{50} was 37.1% and 8.6%, respectively. As shrinkage in K_{10} was relatively high, *post hoc* plots were only used for guidance and all covariate effects were tested formally using the likelihood ratio test. The condition number was 250, which is reasonable and not indicative of over-parameterization.

Attempts were made to re-estimate *CYP2C9* allele effects on CL in children. However, due to the nature of the KPD model, with no input of drug concentrations, primary PK parameters such as CL and V were unidentifiable. The quantitative *CYP2C9* allele effects on CL were therefore assumed to be the same in children as previously estimated for adults [20], an assumption that seems valid at least for children older than 6 months when the enzyme is expected to be fully matured. However, *VKORC1* allele effects on drug sensitivity were successfully re-estimated

in children. The quantitative effect was found to be very similar in children and adults, with a two-fold difference in dose between children with the G/G and A/A genotype. This indicates that the *VKORC1* enzyme is also matured at an early age. The potential influence of genetic variation in the *CYP4F2* gene has not been tested in the adult warfarin KPD-model. The gene codes for the *CYP4F2* enzyme, which is involved in the metabolism of vitamin K, and carriers of a variant allele have been suggested to require a higher warfarin dose [28–30]. When *CYP4F2* genotype was tested as a predictor for dose variability in children, there was a trend suggesting a 9% higher dose per T allele, but with a symmetrical 95% confidence interval ranging from a 3% lower to a 21% higher dose per T allele, hence including zero. In addition, the drop in OFV (-4.2) was only borderline significant, and resulted in a model with a condition number exceeding 2500, which is indicative of over-parameterization. Taken together, this did not support the retention of *CYP4F2* genotype in the final model.

Internal model evaluation

Figure 3 shows pc-VPCs of the final model, both with and without stratification on age, and with stratification on *VKORC1* genotype shown in Figure 4. Additional VPCs with stratification on *CYP2C9* genotype or *CYP4F2* genotype, or without stratification but with bodyweight or age as the independent variable, are included as Supporting Information Figures S1 and S2. Overall the model performed well in predicting the INR response in children, irrespective of

Table 2

Model parameters (%RSE) for (I) base model estimated in adults [21], (II) base model re-estimated in children and (III) final covariate model in children

	(I) Base model with parameters estimated in adults	(II) Base model with parameters estimated in children	(III) Final covariate model in children
Objective function value		-10586.8	-10906.1
Structural model parameters			
EC ₅₀ per VKORC1			
- G allele (mg l ⁻¹)	1.62 (10)	2.05 (4.6)	2.02 (4.3)
- A allele (mg l ⁻¹)	0.770 (9.5)	0.91 (10.4)	0.997 (9.8)
MTT1 (h)	22.6 (3.3)	42.7 (18.7)	37.4 (0.6)
MTT2-MTT1 (h)	87.8 (5.8)	6.2 (295)	156 (6.6)
MTT2 (h)*	110	48.9	193
Hill factor (γ), E _{max} model	1.37 (4.9)	1.37 FIX	1.37 FIX
Age effect on K ₁₀			
- Increase at age 6.3 years (%)			0.879 (35.5)
- Half-life, age-effect on K ₁₀ (years)			0.488 (3.9)
Time effect on EC ₅₀			
- Decrease at time 0 (%)			61.7 (0.7)
- Half-life, time-effect on EC ₅₀ (h)			170 (6.8)
Interindividual variability parameters (%)			
ω _{EC50}	32 (4.3)	36 (6.6)	33.9 (6.4)
ω _{K10}	42 (8.3)	159 (9.9)	124 (11.3)
Residual error parameters (%)			
σ _{INR}	18 (1.7)	25.4 (0.3)	24.9 (0.3)

*MTT2 was not estimated but derived from MTT1 and MTT2-MTT1.

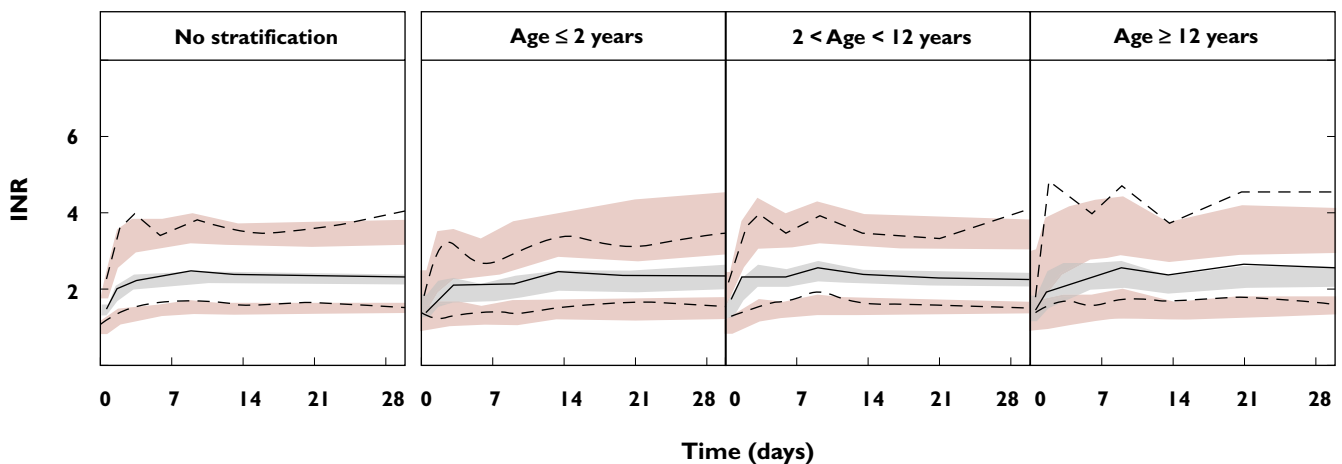


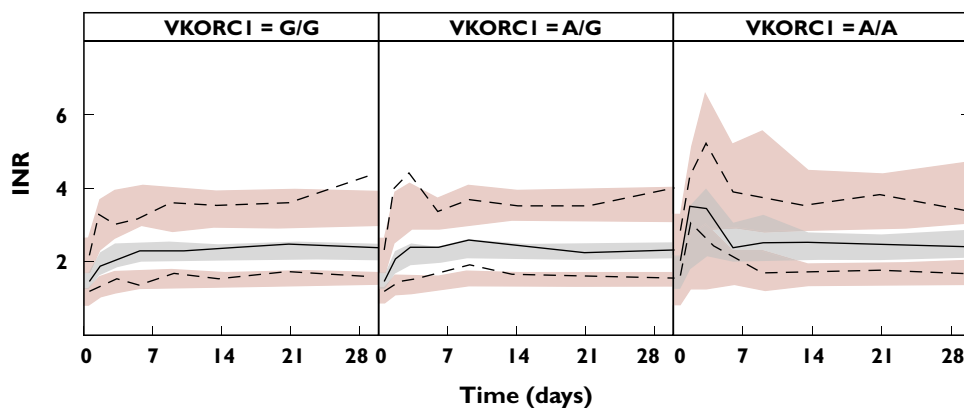
Figure 3

Prediction corrected VPCs for the optimized model applied on data from warfarin treated children. The panel to the left represents data from all children and the three panels to the right after stratification into three age groups; ≤2 years, >2 and <12 years, and ≥12 years. Solid lines denote the medians of observed data and dashed lines denote the 10th and 90th percentiles of observed data. Shaded areas represent 95% confidence intervals of simulated medians (grey) and 80% prediction intervals (pink). A model is considered to have good predictive properties if the lines representing the observed data mainly fall inside the simulated confidence intervals

age, bodyweight, *CYP2C9*, *VKORC1* or *CYP4F2* genotype. Observed INR values (Figure 4 and Supporting Information Figure S1) also confirmed previous findings that children with variant alleles have higher mean peak INR values at the start of therapy than those with wild-type genotypes [12].

Predictions of maintenance dose

Predicted maintenance doses for 54 typical children aged 2, 8 and 14 years old and with all possible combinations of *CYP2C9* and *VKORC1* genotypes, a baseline INR of 1 and a target INR of 2.5 are presented in Table 3. For example, the model predicts a three-fold difference in maintenance


Figure 4

Prediction corrected VPCs but with data stratified on *VKORC1* genotype, with from left to right *VKORC1* G/G (decreased sensitivity to warfarin), *VKORC1* A/G (intermediate sensitivity to warfarin) and *VKORC1* A/A (increased sensitivity to warfarin)

Table 3

Model predicted warfarin maintenance doses in mg (mg kg^{-1}) for typical children 2, 8 or 14 years old, and with different combinations of *CYP2C9* and *VKORC1* genotypes, all with a baseline INR of 1 and a target INR of 2.5. The following bodyweights were assumed for a 2, 8 and 14-year-old: 13 kg, 26 kg and 52 kg, respectively

<i>CYP2C9</i>	<i>VKORC1</i> G/G			<i>VKORC1</i> A/G			<i>VKORC1</i> A/A		
	2 years (13 kg)	8 years (26 kg)	14 years (52 kg)	2 years (13 kg)	8 years (26 kg)	14 years (52 kg)	2 years (13 kg)	8 years (26 kg)	14 years (52 kg)
*1/*1	2.74 (0.211)	5.00 (0.192)	8.43 (0.162)	2.05 (0.158)	3.73 (0.144)	6.29 (0.121)	1.35 (0.104)	2.47 (0.095)	4.16 (0.080)
*1/*2	2.11 (0.162)	3.78 (0.145)	6.35 (0.122)	1.57 (0.121)	2.82 (0.108)	4.74 (0.091)	1.04 (0.080)	1.86 (0.072)	3.13 (0.060)
*1/*3	1.76 (0.135)	3.11 (0.120)	5.24 (0.101)	1.32 (0.101)	2.32 (0.089)	3.91 (0.075)	0.87 (0.067)	1.53 (0.059)	2.58 (0.050)
*2/*2	1.45 (0.112)	2.54 (0.098)	4.27 (0.082)	1.08 (0.083)	1.89 (0.073)	3.19 (0.061)	0.72 (0.055)	1.25 (0.048)	2.11 (0.040)
*2/*3	1.09 (0.084)	1.87 (0.072)	3.15 (0.061)	0.81 (0.062)	1.40 (0.054)	2.35 (0.045)	0.54 (0.041)	0.92 (0.036)	1.56 (0.030)
*3/*3	0.71 (0.055)	1.21 (0.047)	2.04 (0.039)	0.53 (0.041)	0.90 (0.035)	1.52 (0.029)	0.35 (0.027)	0.60 (0.023)	1.01 (0.019)

dose between a typical 2-year-old with a bodyweight of 13 kg and a 14-year-old with a bodyweight of 52 kg, conditioned on the same genotype combinations, despite a four-fold difference in bodyweight. For two children with the same age, bodyweight and *CYP2C9* genotype, the model predicts approximately a two-fold difference in dose between a child carrying the *VKORC1* –1639 G/G genotype and a child homozygous for the variant A allele. For two children who differ only in *CYP2C9* genotype, the model predicts approximately a four-fold difference in dose between subjects having the wild-type genotype (*CYP2C9**1/*1) and those being homozygous for the *3 allele (*CYP2C9**3/*3). Predicted maintenance doses were 10–12% lower if the baseline INR was 1.2 instead of 1, and 25–30% higher if the target INR was set to 3 instead of 2.5.

Discussion

This is the largest study to date, which has evaluated the effect of genetic, clinical and demographic factors on warfarin dose variability in children. Despite warfarin being increasingly used to treat thromboembolic disorders in children, the number of children treated and eligible for pharmacogenetic studies is still limited. Novel approaches, such as modelling and simulation that can incorporate prior knowledge and pool data across studies, have been promoted as one way to improve the quality and robustness of analysis in small patient populations. To our knowledge this is the first time that data from warfarin treated children have been analyzed using modelling and simulation.

We have previously developed a PKPD-based warfarin model from large adult patient populations using population modelling. This model has now been updated and optimized for use in children. A major advantage of analyzing data using population modelling is that all available observations can be used in the analyses, irrespective of when during treatment data were collected (at treatment start, during stable anticoagulation or following dose revisions). To get accurate and reliable results it is imperative that the dose history is correctly reproduced, but this holds true regardless of the method of analysis. In the majority of published warfarin studies in both adults and children, only treatment data from a single time point at a period of stable anticoagulation are included in the analysis. This is likely to introduce a selection bias by excluding the patients who are the most difficult to treat, and also leads to a poor utilization of available treatment data. The definition of stable anticoagulation also varies between studies [2], which further complicates interpretation and comparability of study results. In the current analysis, treatment data from 163 warfarin treated children have been used, including data from all phases of therapy, and representing over 183 treatment years, i.e. on average >1 treatment year/child. A limitation might on the other hand be that our study was restricted to mainly Caucasian children (77%), all with congenital or acquired heart disease. However, the homogeneity of our study population enabled us to detect an increased sensitivity to warfarin at the start of therapy (as a time-effect on EC_{50}). This is in line with results reported by Moffett *et al.* who discovered that cardiac surgery was a risk factor for elevated INR values in children starting warfarin therapy, with an odds ratio of 4.1 (95% CI 1.5, 11.6) [31]. Rahman *et al.* also reported an increased sensitivity to warfarin at the start of therapy in adults undergoing heart valve replacement. The mean maintenance dose (1–3 months after surgery) was 43% higher than the mean initiation dose (5.09 vs. 3.55 mg; $P < 0.001$) [32]. It can therefore be speculated that both children and adults undergoing major surgery are more sensitive to warfarin. Reasons for this could be temporary physiological changes after major surgery that may influence the PK and/or PD of warfarin, combined with a decreased appetite and a lower intake of vitamin K postoperatively. Lack of physical activity postoperatively may also influence dose requirements, since it has been shown that increased physical activity may lead to increased warfarin dose requirements in adults [33].

The most common way to report the contribution of individual factors to warfarin dose requirement is by estimating the percentage in dose variability explained by a given factor [3–7]. However, the clinical utility of this measure, when evaluating predictors important for individualized dosing, may be questioned. The percentage of variability explained by a given factor is a measure of what is most important for the overall variability in the population, and takes into account the effect size of a given factor

and also how common this factor is in the study population. For dose prediction on an individual basis, a more relevant measure would be how much a given factor can influence the dose in an individual patient. *VKORC1* is usually ascribed a more important role on a population level due to the fact that *VKORC1* polymorphisms are more common than *CYP2C9* polymorphisms in most populations [8]. However, we have previously shown that polymorphisms in the *VKORC1* gene typically explain up to a two-fold difference in warfarin dose in adult patients, while the difference in dose between typical individuals with different *CYP2C9* genotypes can be up to 4.2-fold. With the optimized pharmacometric model for children we demonstrate that *CYP2C9* and *VKORC1* genotypes also have comparable quantitative effects on dose variability in children.

Evidence that the *CYP4F2* genotype can influence warfarin dose requirement was first presented in 2008 [28] and later confirmed by others [29, 30]. However, it has been questioned whether the contribution of *CYP4F2* is sufficient for inclusion in pharmacogenetic dosing algorithms [34]. In our study we found a 9% increase in warfarin dose per *CYP4F2* T (*3) allele, but with the 95% CI ranging from a 3% reduction to a 21% increase in dose per T allele. The relatively small effect, together with poor precision in the estimate that also included zero, did not support retention of the *CYP4F2* genotype in the final model. This is also in agreement with results reported by Moreau *et al.* [6] and Biss *et al.* [7], who found no association between warfarin maintenance dose and *CYP4F2* genotype in children.

A general dosing recommendation in children is to adjust the warfarin dose according to bodyweight [1, 4, 5]. The final model also supports that individual dose requirements are dependent on bodyweight, but that the relationship is non-linear. For example, the model only predicts a three-fold difference in dose (in mg) for a four-fold difference in bodyweight, which means that if adult doses are linearly scaled to children this will lead to underdosing. This is in line with findings from other warfarin studies in children, which have concluded that a higher weight-normalized dose is required, especially in the younger age groups [35, 36].

The model-predicted maintenance dose for typical children 2, 8 and 14 years old ranged from 0.019 mg kg⁻¹ to 0.21 mg kg⁻¹ (Table 3). This should be compared with observed maintenance doses of warfarin in children, which are reported to range from 0.03 to 0.39 mg kg⁻¹ by Nowak-Göttl *et al.* [4], from 0.04 to 0.32 mg kg⁻¹ by Nguyen *et al.* [5], from 0.03 to 0.50 mg kg⁻¹ by Moreau *et al.* [6], from 0.02 to 0.43 mg kg⁻¹ by Biss *et al.* [7], and from 0.04 to 0.29 mg kg⁻¹ by Hamberg *et al.* [21]. The upper range of model-predicted warfarin doses was hence lower (0.21 mg kg⁻¹) than reported in these five studies (0.50 mg kg⁻¹). However, this is not unexpected since the model-predicted doses are conditioned on a target INR of 2.5 and limited to children 2–14 years old, whereas

observed doses also included children with a higher target INR and a broader age range (3 months–18.6 years). In addition, the model-predicted doses presented here only represent typical doses and do not take the unexplained between-subject variability into account. Overall the agreement between model-predicted and observed maintenance doses appeared to be good.

In conclusion, we have developed the first PKPD-based pharmacometric model that provides quantitative estimates of genetic, clinical and demographic sources of warfarin dose variability in children. The impact of genotype on dose variability in children was found to be comparable with what has been reported for adults, with *CYP2C9* explaining up to a four-fold difference in dose (*CYP2C9* *1/*1 vs. *3/*3) and *VKORC1* explaining up to a two-fold difference in dose (*VKORC1* G/G vs. A/A), respectively. Bodyweight, age, baseline and target INR, and time since initiation of therapy, but not *CYP4F2* genotype, were also found to influence significantly typical warfarin dose requirements in children. The dependence of warfarin dose requirements on bodyweight is non-linear, and an increased sensitivity to warfarin at the start of therapy can be speculated to only be present after major surgery.

The model provides the basis for development of a computerized tool for *a priori* and *a posteriori* dose-individualization in the pursuit of improving efficacy and safety of warfarin therapy in children. A prototype for a dose individualization tool has already been developed for adults [37]. Our dosing model should now be validated and prospectively evaluated to determine whether it will aid effective and safe warfarin therapy in children. A randomized, controlled clinical trial against standard-of-care would be the ultimate way to investigate if the model could improve INR control in warfarin treated children. However, this is a huge task and is probably best done through an international collaboration that includes children with a diverse clinical and ethnic background.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf and declare: A-K.H. had support from the Ränk family via the Swedish Heart and Lung Foundation and the Swedish Academy of Pharmaceutical Sciences, M.W. had support from the Swedish Research Council (Medicine 523–2008-5568 and 521–2011-2440), the Swedish Heart and Lung Foundation and the Clinical Research Support (ALF) at Uppsala University and T.T.B. had support from Baxter and the Royal College of Pathologists (research training fellowship) for the submitted work. There was no financial relationship with any other organization that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1

Prediction corrected VPCs for the optimized model applied on data from 163 children but stratified on (A) *CYP2C9* genotype or (B) *CYP4F2* genotype. Figure 1A includes from left to right data from children with *CYP2C9*

**1/*1* (high enzyme activity), *CYP2C9* **1/*2* or **1/*3* (intermediate enzyme activity) and *CYP2C9* **2/*2*, **2/*3* or **3/*3* (low enzyme activity). The right panel, representing children with rare *CYP2C9* genotypes, includes data from only four children and results in wide prediction intervals that overlap. Figure 1B includes from left to right data from children with *CYP4F2* **1/*1* (wild-type), *CYP4F2* **1/*3* (one variant allele) and *CYP4F2* **3/*3* (two variant alleles). There is no systematic bias in the VPCs suggesting that a child with the *CYP4F2* variant allele requires a higher warfarin dose

Figure S2

Prediction corrected VPCs for the optimized model applied on data from 163 warfarin treated children but using body weight (A) or age (B) as the independent variable (x-axis) instead of time. Solid lines denote the medians of observed data and dashed lines denote the 10th and 90th percentiles of observed data. Shaded areas represent 95% confidence intervals of simulated medians (grey) and 80% prediction intervals (pink). A model is considered to have good predictive properties if the lines representing the observed data mainly fall inside the simulated prediction intervals