

Vitamin K antagonists in children with heart disease: height and *VKORC1* genotype are the main determinants of the warfarin dose requirement

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Managing vitamin K antagonist (VKA) therapy is challenging in children because of a narrow therapeutic range and wide inter- and intra-individual variability in dose response. Only a few small studies have investigated the effect of nongenetic and genetic factors on the dose response to VKAs in children. In a cohort study including 118 children (median age 9 years; range, 3 months–18 years) mostly with cardiac disease, we evaluated by

multivariate analysis the relative contribution of nongenetic factors and *VKORC1/CYP2C9/CYP4F2* genotypes on warfarin (n = 83) or fluindione (n = 35) maintenance dose and the influence of these factors on the time spent within/above/below the range. The results showed that height, target international normalized ratio and *VKORC1* and *CYP2C9* genotypes were the main determinants of warfarin dose requirement, accounting for 48.1%,

4.4%, 18.2%, and 2.0% of variability, respectively, and explaining 69.7% of the variability. Our model predicted the warfarin dose within 7 mg/wk in 86.7% of patients. None of the covariates was associated with the time spent above or below the international normalized ratio range. Whether this model predicts accurately the effective maintenance dose is currently being investigated. (*Blood*. 2012; 119(3):861-867)

Introduction

In pediatric patients, vitamin K antagonists (VKAs) are mainly used to prevent thromboembolism after cardiac valve replacement, total cavopulmonary connection, dilated cardiomyopathy, coronary aneurysms after Kawasaki disease, or, less frequently, extra-cardiac diseases.¹⁻³ VKA therapy is challenging in children, because VKAs have a narrow therapeutic range and considerable inter- and intra-individual dose-response variability.² This variability is partly explained by age and other demographic, clinical, and environmental factors such as comedications. In the last decade, an increasing number of genetic variations affecting VKA pharmacodynamics and/or pharmacokinetics were found to have a major impact on the VKA dose in adults.⁴⁻¹⁵ These genetic variations are found in single nucleotide polymorphisms (SNPs) in *VKORC1*, *CYP2C9*, and *CYP4F2*.⁴⁻¹⁵ VKAs exert their anticoagulant effect by inhibiting the enzyme vitamin K epoxide reductase (*VKORC1*), thereby preventing vitamin K recycling and vitamin K–dependent carboxylation of the coagulation factors II, VII, IX, and X.¹⁶ SNPs located in the *VKORC1* gene (*VKORC1*) have the largest effect on the response to VKAs. More specifically, patients carrying the –1639 G > A SNP (rs9923231) located in the functional promoter of *VKORC1* require substantially lower doses than do wild-type patients, and a gene-dose effect has been reported for this genetic variant.^{5,17} The pharmacokinetics of warfarin and other coumarin derivatives

depend mainly on the activity of cytochrome P450 2C9 (*CYP2C9*), a microsomal hepatic enzyme responsible for oxidation of these drugs to inactive metabolites. The effect of *CYP2C9* on non-coumarin VKAs such as fluindione is unclear.¹⁸ Two common SNPs in the *CYP2C9* gene, *CYP2C9**2 (rs1799853) and *CYP2C9**3 (rs1057910), are associated with decreased *CYP2C9* catalytic activity compared with wild-type *CYP2C9**1, and therefore, patients carrying at least one variant allele require lower doses of coumarin derivatives than do wild-type patients.¹⁹ More recently, an SNP (rs2108622) in the *CYP4F2* gene encoding cytochrome 4F2 involved in vitamin K metabolism was shown to be associated with higher warfarin dose requirements.^{10,12,20} Overall, genetic factors accounted for 30%–40% of the dose variability in white adults.^{6,7,9,10,12,13,21-23}

Many studies have assessed genetic variants influencing the VKA response in adults.⁴⁻¹⁵ In contrast, only a few small studies have investigated the effect of the *VKORC1* and/or *CYP2C9* genotype on VKA dose requirements in children.²⁴⁻²⁸ Moreover, no study evaluated the potential influence of pharmacogenetic variables on anticoagulation control.

Herein we report the results of a cohort study of 118 children (age 3 months to 18 years) who were followed in pediatric cardiology departments while receiving long-term VKA treatment.

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Table 1. Weekly VKA maintenance doses (mean ± SD) according to age group and VKA type (n = 118)

	Age > 3 mo and ≤ 3 y	Age > 3 y and ≤ 6 y	Age > 6 y and ≤ 10 y	Age > 10 y and ≤ 14 y	Age > 14 y and ≤ 18 y
Warfarin dose (n = 83)	n = 19	n = 17	n = 15	n = 16	n = 16
mg/wk (mean ± SD)	13.0 ± 5.9	15.0 ± 8.8	21.5 ± 8.5	32.5 ± 15.1	36.5 ± 17.7
mg/kg/wk (mean ± SD)	1.29 ± 0.79	0.82 ± 0.30	0.95 ± 0.34	0.81 ± 0.41	0.91 ± 0.52
Fluidione dose (n = 35)	n = 1	n = 2	n = 12	n = 12	n = 8
mg/wk (mean ± SD)	155	85.0 ± 28.2	120.8 ± 60.6	125.0 ± 57.2	129.4 ± 40.1
mg/kg/wk (mean ± SD)	9.94	3.65 ± 0.87	5.39 ± 2.89	3.65 ± 2.42	2.24 ± 0.86

Our primary objective was to determine the relative contributions of nongenetic and genetic factors (*VKORC1*, *CYP2C9*, and *CYP4F2*) on warfarin or fluidione dose requirements in this cohort. Our secondary objective was to evaluate the potential influence of these factors on the time spent within, above, and below the international normalized ratio (INR) range.

Methods

Study design

From September 2009 to December 2010, consecutive children on VKA therapy were recruited at the National Referral Center for Complex Congenital Heart Diseases (Necker- Enfants-Malades University Hospital, Paris, France). Inclusion criteria were as follows: age, birth to 18 years; VKA therapy for at least the past 2 months; and stable anticoagulation defined as results within the therapeutic range for 3 consecutive INR determinations at 2-week intervals.

Pediatric indications of VKA corresponded to 3 different target INRs: (1) 3.3 for patients with mitral valve replacement; (2) 2.5 for patients with aortic valve replacement, dilated cardiomyopathy, coronary aneurysms after Kawasaki disease, or extracardiac diseases; and (3) 2.2 for patients with total cavopulmonary connection.

Management of anticoagulant therapy in pediatric patients has been a concern at Necker Hospital for many years, with specific training given to all children receiving VKA and their parents. The training consists of information sessions on anticoagulant therapy. In most cases, the children and family members receive an education session on an INR self-measurement system. VKA initiation is performed according to the American College of Chest Physicians Antithrombotic and Thrombolytic Therapy recommendations.¹ When stable INR is achieved, INR is measured at least every 15 days using an INR self-measurement system or at a licensed clinical laboratory. The INR value is given by phone to one of the referent physicians who adjusts the VKA dosage if needed. If a dosage adjustment is required, the INR is further determined at shorter intervals until stable anticoagulation is achieved again (defined in the previous paragraphs). In patients with INR values slightly above or under the therapeutic range, INRs are closely monitored before a dosage adjustment is performed. Supratherapeutic INRs (> 5) are managed according to expert recommendations. In the present study, INR values and the corresponding VKA dosages were prospectively recorded by physicians.

We also recorded demographic data (age, sex, height, and body weight) at the time of the maintenance dose, the indication for VKA therapy, and medications associated with the VKA once stable anticoagulation was achieved. The weekly maintenance dose was defined as the mean VKA dose (in milligrams) required to achieve stable anticoagulation (see previous paragraph). Body surface area (BSA) in square meters was computed as follows: $BSA = (4 \times \text{weight in kilograms} + 7) / (\text{weight in kilograms} + 90)$.

The times spent within, above, and below 3 prespecified INR ranges were determined using the Rosendaal method (ie, assuming a linear variation between 2 consecutive INR determinations).²⁹ These ranges corresponded to INR values for which the physicians did not systematically perform a dosage adjustment. These 3 prespecified INR ranges were: 1.5-3.3 for the 2.2 INR target; 1.8-3.2 for the 2.5 INR target; and 2.5-4 for the 3.3 INR target.

The study was approved by the appropriate ethics committee (Comité de Protection des Personnes-Paris Ile de France IV). Written informed

consent was obtained from each child's parents or legal guardians in accordance with the Declaration of Helsinki.

Sample collection and genotyping

Blood or saliva samples were collected and analyzed at the biochemistry laboratory of the Georges Pompidou European University Hospital (Paris, France). Genomic DNA was extracted from peripheral blood leukocytes using the Blood DNA kit (QIAGEN) or from saliva using the Oragene Kit (Genotek) according to the manufacturers' instructions. Genotyping was performed at the end of the study. Genotyping of the *VKORC1*-1639G > A promoter SNP (rs9923231) capturing most of the *VKORC1* haplotypes was achieved using a real-time PCR allelic discrimination assay with a 7900HT Applied Biosystems thermal cycler.³⁰ *CYP2C9**2 (rs1799853, p.Arg144Cys), and *CYP2C9**3 (rs1057910, p.Ile359Leu) alleles were identified using TaqMan Pre-Developed Assay Reagents (Applied Biosystems) for allelic discrimination. *CYP4F2* rs2108622 genotyping (p.Val433Met) was also performed using an allelic discrimination assay with TaqMan technology (Applied Biosystems).

Statistical analysis

We coded *CYP2C9* SNPs as follows: 0 in wild-type patients, 1 in patients heterozygous for *CYP2C9**2 or *CYP2C9**3, and 2 in patients homozygous for *CYP2C9**2 or *CYP2C9**3 or double heterozygous for both *CYP2C9**2 and *CYP2C9**3. This coding system allowed us to model additive allelic effects. Similarly, all other genotypes were coded 0 (wild-type), 1 (heterozygous), or 2 (mutant homozygous). Indications for VKA therapy were coded based on the 3 target INR groups. Finally, age, body weight, height, and BSA were handled as continuous variables.

Data were described as numbers and percentages for qualitative variables and mean (or median) and SD for quantitative variables. To assess relationships between the weekly maintenance VKA dose and covariables, we first performed a univariate analysis using the 2-sample Wilcoxon test or nonparametric ANOVA (Kruskal-Wallis test) for qualitative variables and the Spearman rank correlation coefficient for quantitative variables. Covariables with *P* values < .20 by univariate analysis were entered into a backward stepwise multiple linear regression model. Covariables with *P* values < .05 in this model were kept in the final model. The same statistical approach was used to evaluate times spent within, above, and below the INR range. Model accuracy was evaluated based on the proportion of individuals whose observed weekly warfarin dose differed by more than 7 mg from the weekly predicted dose. All tests were 2-sided, and *P* < .05 was considered significant. Computations were performed using the SAS Version 9 statistical package.

Results

Patient characteristics and maintenance dose

Between September 2009 and December 2010, we enrolled 120 unrelated patients. Two patients receiving acenocoumarol were not analyzed. The study population comprised 55 girls and 63 boys including more than 90% white and the median age was 9.0 years (range, 3 months-18 years). Of the 118 patients, 83 received warfarin and 35 received fluidione. Table 1 displays the mean VKA dose by age group and VKA type. In the 83 patients on

Table 2. Main characteristics of the 118 pediatric patients according to type of VKA and relationship between these characteristics and the VKA maintenance dose by univariate analysis

	Warfarin (n = 83)		Fluindione (n = 35)	
	N (%) or mean \pm SD (range)	<i>P</i> value*	N (%) or mean \pm SD (range)	<i>P</i> value*
Mean age, y, \pm SD (range)	8.4 \pm 5.6 (3 mo-18 y)	< .0001	11.0 \pm 3.8 (3 y-18 y)	.4957
Sex, M/F, n	46/37	.0082	18/17	.3901
Weight, kg, mean \pm SD (range)	29.5 \pm 19.8 (3.5–81.5)	< .0001	37.8 \pm 17.9 (15.6–76)	.8899
Height, cm, mean \pm SD (range)	120.7 \pm 33.4 (50–183)	< .0001	139.5 \pm 23.6 (92–190)	.5988
Body surface area, m ² , mean \pm SD (range)	0.97 \pm 0.44 (0.22–1.94)	.0134	1.19 \pm 0.36 (0.66–1.87)	.8981
Associated medications, mean \pm SD (range)	0.71 \pm 1.54 (0–5)	NA	0.43 \pm 1.17 (0–5)	NA
Target INR, n (%)†		.1410		.8574
2.2	31 (37)		7 (20)	
2.5	38 (46)		11 (31)	
3.3	14 (17)		17 (49)	
VKORC1 genotype, n (%)		< .0001		.0363
GG	25 (30)		16 (46)	
GA	43 (52)		14 (40)	
AA	15 (18)		5 (14)	
CYP2C9 genotype, n (%)‡		.1582		.0613
*1/*1	53 (64)		22 (63)	
*1/*x	25 (30)		10 (29)	
*x/*x	5 (6)		3 (8)	
CYP4F2 genotype, n (%)		.5182		NA
CC	46 (57)		18 (51)	
CT	26 (32)		8 (23)	
TT	9 (11)		9 (26)	

*By univariate analysis: association with the maintenance dose. Parameters with *P* values in bold were entered in the multivariate model.

†Target INR was 2.2 in patients with total cavopulmonary connection; 2.5 in those with aortic valve replacement, dilated cardiomyopathy, coronary aneurysms after Kawasaki disease, or extracardiac diseases; and 3.3 in those with mitral valve replacement.

‡CYP2C9 genotype: *1/*1, wild-type homozygotes; *1/*x: CYP2C9*2 or CYP2C9*3 heterozygotes; and *x/*x: CYP2C9*2 or CYP2C9*3 homozygotes or compound heterozygotes.

NA indicates not applicable.

warfarin (median age, 9.0 years), the mean weekly maintenance dose was 23.2 \pm 15.0 mg (range, 3.5–84 mg), which corresponded to 0.93 \pm 0.55 mg/kg (range, 0.19–3.47 mg/kg). In the 35 patients on fluindione (median age, 11.0 years), the mean weekly maintenance dose was 123.1 \pm 52.2 mg (range, 45–250 mg), corresponding to 4.07 \pm 2.68 mg/kg (range, 1.15–12.19 mg/kg). When the VKA dose was expressed relative to body weight (mg/kg), we found that very young patients, especially those younger than 3 years, required higher weekly doses than older patients (Table 1). Two patients were younger than 1 year of age (12 and 15 weeks).

The main characteristics of the 118 patients receiving warfarin or fluindione are shown in Table 2. Overall, 38 patients had total cavopulmonary connection with a target INR of 2.2. Forty-nine patients had a target INR of 2.5; of these, 15 had aortic valve replacement, 16 had dilated cardiomyopathy, 6 had coronary aneurysms after Kawasaki disease, and 12 had extracardiac diseases (2 strokes with cyanotic congenital heart disease, 2 pulmonary arterial hypertension, 4 cardiac arrhythmias, 1 lupus, 1 antiphospholipid syndrome, 1 thrombosis with drepanocytosis, and 1 thrombosis with histiocytosis); 31 patients had mitral valve replacement with a target INR of 3.3.

The distribution of the *VKORC1*, *CYP2C9*, and *CYP4F2* genotypes is shown on Table 2. The *VKORC1* and *CYP2C9* allele frequencies did not differ from those found in the general white population (Table 2).^{9,10} No significant deviation from the Hardy-Weinberg equilibrium was observed for any of the SNPs (*P* > .05; Table 2). The allele frequency of the *CYP4F2* gene was as expected in the warfarin-treated group, with no significant deviation from Hardy-Weinberg equilibrium. In the fluindione-treated group, allele frequency differed from that in the general population and

there was a significant deviation from Hardy-Weinberg equilibrium, probably because of the small sample size (n = 35).

All patients receiving VKA for dilated cardiomyopathy were treated with β -blockers, conversion enzyme inhibitor, and spironolactone; in addition, some of them received furosemide or/and digoxin if necessary. All patients treated for coronary aneurysms after Kawasaki disease received low doses of acetylsalicylic acid. No patient had amiodarone or antibiotics at the time of stable anticoagulation as defined above. In the overall cohort (n = 118), the mean number of medications per patient was 0.65 \pm 1.46 (range, 0–5).

Influence of nongenetic and genetic variables on maintenance dose in warfarin-treated patients

Univariate analysis. By univariate analysis, the weekly warfarin maintenance dose (in milligrams) was strongly associated with age, weight, height, and BSA (*P* < .0001; Table 2). The relationship between dose in milligrams and height is shown in the supplemental Figure 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Sex was significantly associated with the weekly warfarin maintenance dose (*P* = .0082; Table 2). Girls required significantly lower doses (mean, 19.3 \pm 1.9 mg) than boys (mean, 27.1 \pm 2.5 mg; *P* = .0246). Among warfarin-treated patients, those with a target INR of 2.2 (n = 31) had significantly lower mean weekly doses (18.5 \pm 9.6 mg) than those in the other 2 INR targets (target INR of 2.5, n = 38, 26.1 \pm 17.2 mg; and target INR of 3.3, n = 14, 27.5 \pm 17.1 mg; Figure 1). Children carrying at least 1 *VKORC1* variant allele required significantly lower doses than wild-type patients. A gene-dose

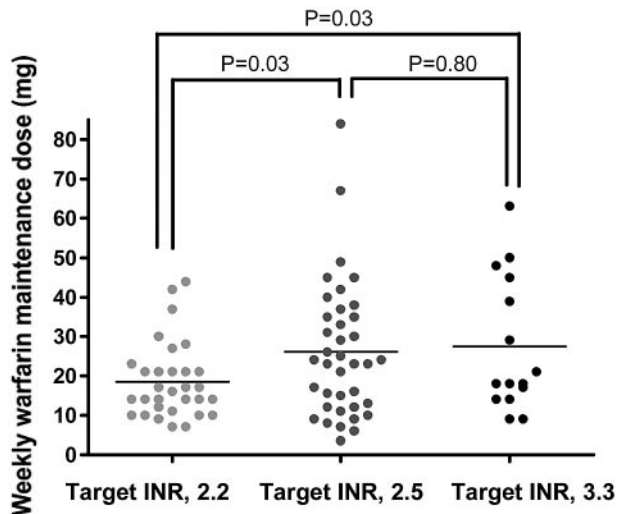


Figure 1. Influence of target INR on weekly warfarin maintenance dose (mg) by univariate analysis. Target INR was of 2.2 in patients with total cavopulmonary connection; 2.5 in those with aortic valve replacement, dilated cardiomyopathy, coronary aneurysms after Kawasaki disease, or extracardiac diseases; and of 3.3 in those with mitral valve replacement.

effect was observed: the weekly maintenance dose was 32.0 ± 3.6 in wild-type patients (GG), 23.0 ± 1.9 mg (-28%) in heterozygotes (GA), and 10.6 ± 0.9 mg (-67%) in mutant homozygotes (AA; $P < .0001$; Figure 2A). There was a trend for an association ($P = .16$) between the presence of 1 or 2 *CYP2C9* variant alleles and a decrease in warfarin dose requirements (Figure 2B).

Multivariate analysis. By multivariate analysis, after statistical adjustment for potential confounding variables, P values were $< .05$ for height, *VKORC1* genotype, target INR, and *CYP2C9* genotype (Table 3). The final model explained 69.7% of the overall interindividual variability in the warfarin dose (Table 3). Height accounted for 48.1% of the variability, *VKORC1* genotype for 18.2%, target INR for 4.4%, and *CYP2C9* genotype for 2.0%.

Stepwise linear multiple regression resulted in the following final model:

Dose in milligrams per week = $(-10.77 + 0.28) \times (\text{height in centimeters} - 5.44) \times (\text{number of } VKORC1 \text{ variant allele}) + (7.83 \text{ if target INR is } 2.5 \text{ or } 11.52 \text{ if target INR is } 3.3-3.29) \times (\text{number of } CYP2C9 \text{ variant alleles})$.

Accuracy of the model. Figure 3 shows the patient distribution according to the difference between the observed weekly maintenance dose and the maintenance dose predicted individually by the model. The difference was ≤ 7 mg/wk in 86.7% of patients; 8% of patients had observed doses at least 7 mg/wk higher than predicted and 5.3% had observed doses at least 7 mg/wk lower than predicted. Given the small number of patients younger than 1 year in our study, this model is probably not applicable in this age group.

Influence of genetic and nongenetic variables on maintenance dose in fluidione-treated patients

The *VKORC1* genotype was the only variable significantly associated with the weekly fluidione maintenance dose ($P = .0363$; Table 2). Because the *CYP4F2* allele frequency differed from that in the general population, this variable was not entered into the statistical analysis. None of the nongenetic factors was significantly associated with the weekly fluidione maintenance dose (Table 2).

Times spent within, above, and below the prespecified INR ranges depending on INR target

Of the 118 patients, 92 had INR values collected routinely during the study period and the mean follow-up was 388 ± 229 days (29-776). Among them, the warfarin-treated patients ($n = 61$) spent $83.0\% \pm 14.6\%$ (range, 45.9%-100%) of the time within the INR range for which no dosage adjustment was systematically performed, $9.6\% \pm 10.5\%$ (range, 0%-34.3%) above this INR range, and $7.1\% \pm 8.9\%$ (range, 0%-36.0%) below this INR range (Table 4). By univariate analysis, the only variable significantly associated with the time spent within the range was the target INR ($P < .0001$). Patients for whom the target INR was of 2.2 spent significantly more time within the INR range than those with the other 2 INR targets ($P < .0001$). Neither the univariate nor the multivariate analysis identified genetic or nongenetic factors associated with the time spent above or below the INR range.

The fluidione-treated patients ($n = 31$) spent $81.4\% \pm 15.7\%$ (range, 36.5%-100%) of the time within the INR range,

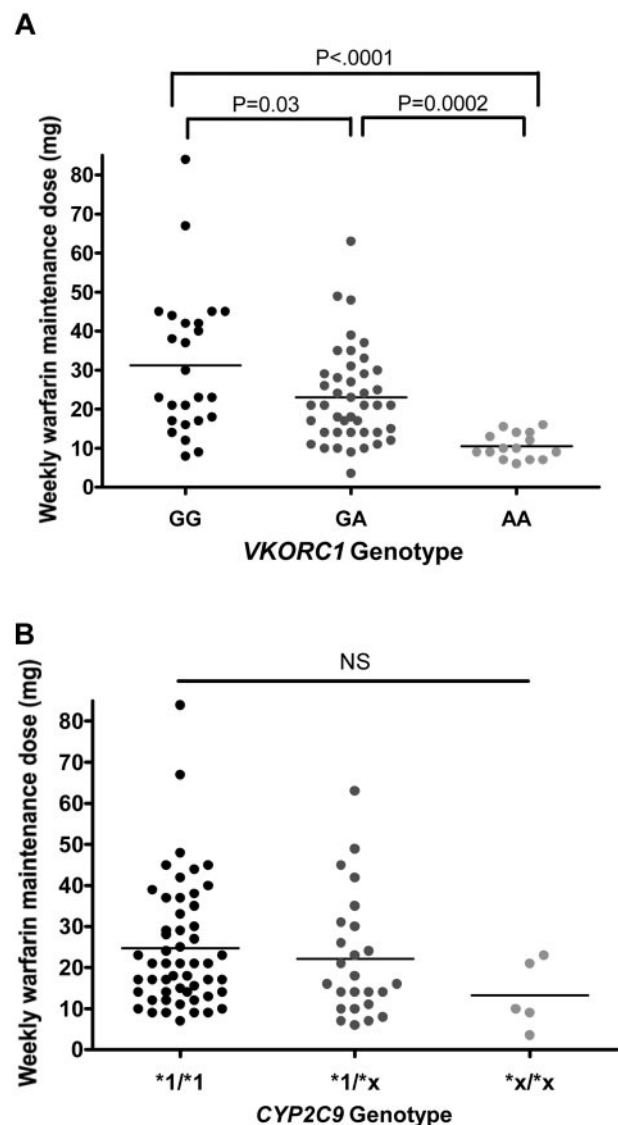


Figure 2. Weekly warfarin dose requirement (mg) by genotype. (A) *VKORC1* genotype: GG wild-type homozygotes, GA heterozygotes, and AA homozygotes. (B) *CYP2C9* genotype: $*1/*1$, wild-type homozygotes; $*1/*x$: *CYP2C9**2 or *CYP2C9**3 heterozygotes; and $*x/*x$: *CYP2C9**2 or *CYP2C9**3 homozygotes or compound heterozygotes. NS indicates nonsignificant.

Table 3. Final multivariate regression model for predicting the weekly warfarin maintenance dose

Predictor	Coefficient	SE	P	Univariate R ²
Intercept	-10.77	4.44		
Height, cm	0.28	0.028	< .0001	0.481
Number of <i>VKORC1</i> variant allele(s)	-5.44	1.41	.0003	0.182
Target INR				
2.2	0	-	-	-
2.5	7.83	2.08	.0003	0.019
3.3	11.52	2.74	< .0001	0.025
Number of <i>CYP2C9</i> variant allele(s)	-3.29	1.55	.0370	0.020

Target INR was 2.2 in patients with total cavopulmonary connection; 2.5 in those with aortic valve replacement, dilated cardiomyopathy, coronary aneurysms after Kawasaki disease, or extracardiac diseases; and 3.3 in those with mitral valve replacement.

Dose (mg/wk) = -10.77 + 0.28 × height (cm) - 5.44 × number of *VKORC1* variant allele(s) + 7.83 (if target INR of 2.5) or 11.52 (if target INR of 3.3) - 3.29 × number of *CYP2C9* variant alleles.

11.4% ± 11.6% (range, 0%-36.7%) above the INR range, and 7.7% ± 11.6% (range, 0%-61.7%) below the INR range. Neither the univariate nor the multivariate analysis identified genetic or nongenetic factors associated with the time spent within, above, or below the INR range.

Discussion

This study is one of the largest cohort studies assessing the impact of genetic and nongenetic factors on VKA dose requirements in pediatric patients with cardiac disease. Our study comprised 118 patients of median age 9 years who were receiving long-term VKA therapy mostly for cardiac disease. In the warfarin-treated patients, we showed that height, target INR, and the *VKORC1* and *CYP2C9* genotypes were significantly associated with dose requirements and explained 69.7% of the overall dose variability in the final model. In our model, nongenetic factors explained most of the variability, with height being the major contributor (48.1%).

To date, only the cohort study of Nowak-Göttl et al in white children assessed the relative impact of the *VKORC1* and *CYP2C9* genotypes in addition to age on VKA dose requirements.²⁷ In this study, in which the main indication for warfarin therapy was venous thrombosis (INR range, 2-3), the multiple linear regression model of daily warfarin dose requirements (in milligrams/kilogram) explained 34% of the dose variability in the warfarin-treated group (n = 34; median age, 15 years).²⁷ Age was by far the main factor (31.2%), with the *VKORC1* and *CYP2C9* genotypes accounting for only approximately 4% of the variability.²⁷ In our study, age was strongly associated with the warfarin dose by univariate analysis, but was not significant in the final model, in which height explained most of the variability (48.1%). This apparent discrepancy may be related to the hypothesis made by Nowak-Göttl et al that age would be the single most important factor in VKA. Because age correlates strongly with weight and

height in pediatric patients, Nowak-Göttl et al entered neither weight nor height into their model. However, weight and height can vary widely across children of the same age, depending on the combination of genetics, sex, cardiovascular disease, nutrition, physical activity, environmental factors, hormones, and lifestyle in each individual.^{1,2,31} Further, these variations in morphology are particularly frequent in the setting of congenital heart diseases with various physiologies. Therefore, we investigated not only age, but also weight and height. When we used the dose in milligrams/kilogram to build the final prediction model, height remained the main determinant of dose variability (data not shown). Finally, both the study by Nowak-Göttl et al and our study showed that the most important contributors to variations in warfarin dose requirements in pediatric patients are age or developmental changes related to age. Conversely, in middle-aged adults, demographic data such as age and body mass index explained less than 20% of the dose variability.^{6,7,9,13,32}

In our cohort, the *VKORC1* and *CYP2C9* genotypes accounted for 18.2% and 2.0% of the warfarin dose variability, respectively. Interestingly, 2 small studies in Japanese patients also found a significant influence of *VKORC1* on warfarin dose requirements.^{25,33} In 31 Japanese patients older than 12 years (median age, 22 years; body weight > 40 kg), Kosaki et al found that *VKORC1* heterozygotes required higher warfarin doses (in milligrams) than *VKORC1* mutant homozygotes after adjustment for the INR value (P = .003).²⁵ In 48 Japanese children (mean age, 6.6 years), Kato et al reported that age and the *VKORC1* genotype were the main factors affecting the relationship between the weight-normalized warfarin dose and the INR value.³³ Nevertheless, the influence of the *CYP2C9* genotype could not be evaluated in these Japanese studies because of the very low allelic frequency of *CYP2C9* variants in this ethnic group.³⁴ In the white pediatric sample studied by Nowak-Göttl et al, the *VKORC1* and *CYP2C9* genotypes explained only a very small part of the warfarin dose variability

Table 4. Follow-up and percentage of time spent within, below, and above the INR range depending on INR target in warfarin-treated patients

	Follow-up, d, mean ± SD (min-max)	Time spent within the INR range, %, mean ± SD (min-max)	Time spent below the INR range, %, mean ± SD (min-max)	Time spent above the INR range, %, mean ± SD (min-max)
INR range 1.5-3.3 (target 2.2; n = 18)	285 ± 203 (29-571)	93.0 ± 9.5 (75.4-1)	4.2 ± 9.5 (0-34.5)	2.8 ± 3.9 (0-12.5)
INR range 1.8-3.2 (target 2.5; n = 14)	323 ± 220 (32-768)	83.5 ± 11.6 (56.4-1)	8.0 ± 8.1 (0-24.1)	8.5 ± 9.1 (0-28.7)
INR range 2.5-4 (target 3.3; n = 29)	511 ± 207 (80-776)	69.8 ± 13.6 (45.9-97.7)	9.3 ± 9.2 (0-35.9)	21.0 ± 10.1 (23.3-34.3)
Total (n = 61)	354 ± 226	83.3 ± 14.6	7.1 ± 8.9	9.6 ± 10.5

Target INR was 2.2 in patients with total cavopulmonary connection; of 2.5 in those with aortic valve replacement, dilated cardiomyopathy, coronary aneurysms after Kawasaki disease, or extracardiac diseases; and 3.3 in those with mitral valve replacement.

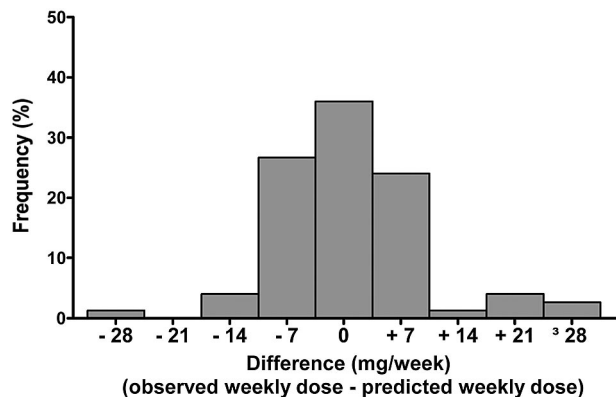


Figure 3. Difference (mg/wk) between the observed warfarin dose and the warfarin dose predicted by the model.

(2.8% and 0.5%, respectively),²⁷ perhaps because of the small sample size (n = 34).²⁸

In our study, the overall contribution of genetic factors (20.2%) to the interindividual variability in the warfarin maintenance dose was smaller than that reported in middle-aged adults (30%-40%)^{6,7,9,10,12,13,21-23} and similar to that found in the elderly (17.5%).³⁵ We did not find an association between warfarin maintenance dose and *CYP4F2* genotype, probably because of the small size of the cohort. Interestingly, in our white pediatric cohort, the *VKORC1* genotype had a far greater impact than the *CYP2C9* genotype, in keeping with our results in very elderly patients.³⁵ These findings might reflect age-related changes in pharmacokinetics and/or liver function, affecting for example the expression or maturation of the liver enzymes VKOR and CYP, which affect the VKA pharmacodynamic target and VKA metabolism.^{24,36} Takahashi et al studied Japanese children and adults to elucidate the developmental changes in the pharmacokinetics and pharmacodynamics of warfarin enantiomers with the goal of establishing pediatric dosage guidelines.²⁴ *CYP2C9* was the only genotype studied, because *VKORC1* had not yet been identified at the time of the study. Liver weight estimated using 3 distinct linear regression curves depending on age was the best parameter for estimating the required warfarin dose in prepubertal patients based on corresponding adult values.²⁴

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A limitation of our study is that most of the patients were whites and therefore our results may not apply to non-white children, because the allele frequencies of SNPs associated with warfarin dose requirements vary across ethnic groups. Another limitation is the small number of fluindione-treated patients, which did not allow us to build a dose prediction model for fluindione. Finally, the patients spent more than 80% of the time within the INR range. This may have impaired our ability to identify factors that significantly influenced the times spent within, above, or below the INR range.

In conclusion, our study provides new information on the contribution of the *VKORC1* and *CYP2C9* genotypes to variations in warfarin response among children with cardiac disease. Our dosing model based on height, target INR, and *VKORC1* and *CYP2C9* genotypes predicted the warfarin dose within 7 mg/wk (1 mg/d) in 86.7% of patients. We are currently validating this model in a prospective cohort of children receiving warfarin therapy. Further work is needed to determine whether this model could minimize the risk of over- and under-anticoagulation, especially at the start of treatment and thus could improve the management of these patients.

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Authorship

Contribution: C.M., F.B., D.L., D.B., and M.-A.L. designed and performed the research, analyzed the data, and wrote the manuscript; and V.S., J.-L.G., C.E., P.B., and R.C. analyzed the data and wrote the manuscript.

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