In pediatric patients, age has more impact on dosing of vitamin K antagonists than *VKORC1* or *CYP2C9* genotypes

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Anticoagulation with vitamin K antagonists (VKAs) is problematic because of difficulties in safely managing dosing. Polymorphisms in cytochrome P450 2C9 (*CYP2C9*) and vitamin K epoxide reductase genes (*VKORC1*) have been shown to affect VKA dosing in adults. The association of these polymorphisms on VKA dosing in children has not been investigated. The objective of the study was to assess associations of *CYP2C9* and *VKORC1* polymorphisms and clinical variables on VKA dosing in children. A nonselected cohort of pediatric patients receiving VKA were tested for *CYP2C9* and *VKORC1* polymorphisms, and clinical data were collected. Multiple linear regression modeling was used to assess relationships of VKA dose with genetic and clinical variables. Fifty-nine patients were recruited; 55.9% were receiving warfarin, and 44.1% were on phenprocoumon. There was a negative association of age with VKA dose ($P < .001$). Comparing *VKORC1* genotypes, the AA group required significantly lower daily doses than GG group ($P = .011$). In the full model including age, *VKORC1* and *CYP2C9* genotypes accounted for 38% of dose variation. Age explained 28.3% of VKA dose variations; *VKORC1* and *CYP2C9* explained only 3.7% and 0.4%, respectively. In children, the most critical factor in determining VKA dose is age. *VKORC1*/*CYP2C9* genotypes only marginally explain dose variations. (*Blood*. 2010;116(26):6101-6105)

Introduction

Although vitamin K antagonists (VKAs) have been in use for more than 5 decades, safe management of these drugs remains a major challenge. Bleeding complications are associated with the variation in drug response and account for a significant number of emergency admissions on a yearly basis.[1] Clinical factors such as age, sex, drug interactions, diet, and underlying disorders affect response to VKA.[2] In addition, polymorphisms in the cytochrome P450 2C9 (*CYP2C9*) and vitamin K epoxide reductase gene (*VKORC1*) have a well-described effect on VKA dosing in adults. The CYP2C9 enzyme is responsible for clearance of the S-enantiomer of warfarin and patients with the *CYP2C9*2* or **3* require lower dosages.[3-9] The *VKORC1* gene encodes vitamin K epoxide reductase complex, which recycles reduced vitamin K, an essential component for post translational gamma carboxylation of the vitamin K dependent factors. Therefore, the pharmacodynamics of VKA are affected by *VKORC1*, and it is well described that patients with polymorphisms require lower dosages of VKA.[5-7,10-12] The impact of these 2 genetic markers has resulted in the Food and Drug Administration implementing a label change on warfarin suggesting lower dosages in patients with these polymorphisms.[13] In adults, the variation in dose of VKA is accounted for between 30% and 40% on the genetic factors and between 15%-22% on clinical characteristics.[10,12,14,15]

In children, as in the adult, there are multiple characteristics affecting dose such as age, underlying medical condition, medication and diet.[16,17] However, age is overwhelming the single most important variable affecting dose.[17,18] In the pediatric population, there have been no studies assessing the (1) effect of the VKORC1 and CYP2C9 genetic polymorphisms on variation in VKA dosages or (2) the contribution the genetic polymorphisms relative to the effect of the clinical characteristics on VKA dosages. We hypothesized that, rather than genetic factors, age would be the single most important factor in VKA dosing in children. The current study was designed to address this question.

Methods

Study design

The study was a prospective cohort study of non selected consecutively enrolled children more than 3 months of age receiving VKA (target international normalized ratio [INR] 2.0-3.0) for treatment of objectively confirmed thrombosis treated at the University Children's Hospital, University of Münster, Germany. Written informed consent was obtained from children and/or legal guardians. The present study was performed in accordance with the ethical standards laid down in the updated version of the 1964 Declaration of Helsinki and was approved by the medical ethics committee of the University of Münster, Germany.

Therapeutic VKA doses were defined as the median of 3 consecutive dosages when the patient had achieved stable anticoagulation. Stable anticoagulation was defined by VKA requirement remaining constant for 3 consecutive days after achieving the target INR.[14,15,19] Time to therapeutic range was defined as time in days from first VKA administration until reaching stable values within the target INR range confirmed in a minimum of 3 consecutive daily INR.

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As previously defined, recurrent thrombotic events were defined when site appropriate imaging methods were performed in the acute phase of a new vascular accident that showed fresh thrombotic material within a lumen of the vasculature (ie, a new intraluminal filling defect compared with the previous test).[20] Postthrombotic syndrome (PTS) was defined by objective signs (eg, an increase in calf or ankle circumference by 1-2 cm of normal, dark pigmentation of the skin, venous telangectasia, varicose veins, or open ulcer using a standard scoring system adapted from the adult literature).[21,22] The definition of transient PTS was when PTS was diagnosed but did not persist during the predefined follow-up period. Venography, compression sonography, conventional or spiral computerized axial tomography, magnetic resonance imaging, or perfusion lung scans were the imaging methods used to confirm clinically suspected thrombotic events.

Blood samples

Blood samples were drawn when patients were considered stable on anticoagulation. The blood samples were centrifuged, plasma and buffy coat was separated and after INR testing, the remaining sample was frozen. Data on dose and time to therapeutic range were documented. Demographics were recorded prospectively and included age at time of thrombotic event, age at blood draw, sex, ethnicity, underlying disorder, and location of thrombotic event.

Laboratory methods

Genomic DNA was extracted from patient buffy coats using the QIAamp DNA Blood Kit (QIAGEN) following the manufacturers protocol. The PCR was performed in a 50-μL volume using 0.8μM primer, 0.2mM dNTP, 100 ng of DNA, and 1 U of Taq polymerase. All amplifications were done for 35 cycles followed by a final extension at 72°C for 7 minutes. All enzymes were purchased from New England Biolabs.

VKORC1 genotyping (-1639G>A). The *VKORC1* genotyping was performed as previously described.[14] Amplification was done using an annealing temperature of 59°C and the forward primer 5′-GCCAGCAGGAGAGGGAAATA-3′ and the reverse primer 5′-AGTTTGGACTACAGGTGCCT-3′. Ten microliters of each PCR product was digested overnight at 37°C with the restriction enzyme *Msp1*. Digested PCR products were analyzed on 10% polyacrylamide gels.

CYP2C9 genotyping (CYP2C9*1, *2, *3). The *CYP2C9* genotyping was performed as previously described.[23-25] *CYP2C9*2* genotyping was done using the forward primer 5′-GTATTTTGGCCTGAAACCCATA-3′ and the reverse primer 5′-GGCCTTGGTTTTTCTCAACTC-3′with an annealing temperature of 55°C. The resulting 454-bp product (10 μL) was digested overnight with the restriction enzyme *Ava*II. The *CYP2C9*1* and *CYP2C9*3* genotypes were cut into fragments of 397 bp and 57 bp. The *CYP2C9*2* genotype did not digest with *Ava*II. Genotyping of *CYP2C9*3* was performed by using the forward primer 5′-ATAATAATATGCACGAGGTCCAGAGATGC-3′ and the reverse primer 5′-GATACTATGAATTTGGGACTTC-3′ with an annealing temperature of 55°C. The 141-bp product (10 μL) was digested overnight with the restriction enzyme *Nsi*1. The *CYP2C9*1* and *CYP2C9*2* genotypes were cut into fragments of 110 bp and 31 bp. The *CYP2C9*3* genotype did not digest with *Nsi*1. Digested PCR products were analyzed on 10% polyacrylamide gels.

Statistical analysis

Patient demographics are reported descriptively using median and range (minimum and maximum). The distribution of VKA dose (mg/kg) was transformed by taking the square root of the dose values to produce an approximately symmetric distribution. The study design and the genotype distributions assured more than 80% power to detect a small-to-moderate effect size, a difference by either genotype that is equivalent to a 0.8 standard deviation of the square-root-transformed VKA dose in the study population. Multiple linear regression of the transformed VKA dose was used to assess its relationship with genetic and clinical variables. *VKORC1* genotype was categorized into 3 groups (AA, GA, and GG) and *CYP2C9* genotype into 2 groups for (any mutation 1.2, 1.3 or 2.2, and wild-type 1.1). The multiple linear regression analysis was carried out for the 2 drug types, warfarin and phenprocoumon, separately. In the initial univariate model variables known to influence VKA dosages in adults (*VKORC1*, *CYP2C9*, age, sex, height, weight) as well as INR were assessed. The final multivariate model included only variables with a $P < .2$. Associations between time to therapeutic range with age and across genotypes were assessed using simple linear regression and analysis of variance, respectively. Statistical analyses were performed using Stata 10 statistical software and the MedCalc v11.4 software. Demographic data were analyzed by L.G.M. and U.N.-G., regression modeling and other statistical analysis were performed by Y.Y. and N.S.E. All authors had access to the primary study data.

Results

Study population

A total of 70 children were recruited to the study and complete data were available in 59. The VKA were administered according to recommended guidelines for children.[26] The median INR was 2.3, range 1.4-3.2. The median (minimum-maximum) daily dose in mg/kg for 3 age groups were as follows: (1) 1-5 years 0.26 (0.09-0.39), (2) 5-10 years 0.063 (0.05-0.15), and (3) 10-19 years 0.08 (0.03-0.6). Patient demographics and distributions of the genetic markers are shown on Table 1. The relative distributions of the genotypes are in agreement with the published literature in other patient populations.[7,8,27-31] No child experienced a clinically significant bleed nor recurrence of thrombosis while on VKA. However, after cessation of VKA, 15 (21.4%; 95% confidence interval [CI], 12.5%-32.8%) of children experienced a recurrence and 16 (22.9%; 95% CI, 13.7%-34.5%) of patients had persistent postthrombotic syndrome (eg, edema, superficial collateral circulation, and reduced walking-distance capacity) after a 2-year follow-up.

Associations of VKA dose with genetic and clinical variables

Age was highly correlated with weight and height in this pediatric population, and therefore, weight and height were not entered into the final model with age. Sex and INR were not associated with VKA dose and were removed from the model. Multiple linear regression models of the square root of VKA dose were fit using age and *VKORC1* and *CYP2C9* genotypes as explanatory variables. The resulting regression equations are shown in Table 2. The adjusted analysis showed a negative association of age with VKA dose ($P < .001$). Differences in daily dose were statistically significant for carriers of *VKORC1* AA versus GG genotype; the AA group required significantly lower daily doses than GG group ($P = .011$). Carriers of *VKORC1* GA and GG genotypes were not significantly different ($P = .62$). No association with VKA dose was seen with any mutation in *CYP2C9* genotype ($P = .35$).

The full model including age and *VKORC1* and *CYP2C9* genotypes accounted for 38% of the total variation in VKA dose. According to the partial R^2 of each independent variable, age was by far the greatest contributor to explaining VKA dose variations, with genotypes playing a very minor role (Table 2).

Results of the analysis are consistent irrespective of the 2 VKA drug types, warfarin and phenprocoumon (Table 3). Since the *CYP2C9* has an insignificant effect on VKA dose in our pediatric population, the varied response by drug type seen in adults is not observed here. There was no difference in time to therapeutic range

Table 1. Patient demographics

Median age (minimum-maximum), y	
At time of thrombosis	14.0 (0.1-18.0)
At time of blood sample	15.0 (1.0-19.0)
Median weight (minimum-maximum), kg	61.0 (2.3-101.0)
Sex, n (%)	
Female	32 (54)
Ethnic origin, n (%)	
White	59 (100)
Underlying medical condition, n (%)	
Cardiac	14 (22.0)
Oral contraceptives	9 (15.3)
Infection	6 (8.5)
None	5 (8.5)
Obesity	5 (6.8)
Other	20 (32.2)
Type of thrombosis, n (%)	
Deep vein thrombosis (upper limb, popliteal veins)	20 (33.3)
Pulmonary embolism	19 (31.6)
Renal vein thrombosis	1 (1.7)
Femoral or iliac thrombosis	18 (30)
Hepatic/intracardiac	3 (5.1)
Thromboembolic ischemic stroke/sinovenous thrombosis	9 (15.0)
Recurrence	
n (%)	15 (25.4)
Time to recurrence, median (minimum-maximum), months	126.8 (3.6-58.8)
Postthrombotic syndrome at 2-year follow-up, n (%)	15 (25.4)
VKA type, n (%)	
Warfarin	34 (55.9)
Phenprocoumon	26 (44.1)
Median time to therapeutic range (minimum-maximum), d	7 (2-14)
Median INR at day 7 (minimum-maximum)	2.3 (1.4-3.2)
***VKORC1,* n (%)**	
AA	7 (11.9)
GA	25 (42.4)
GG	27 (45.7)
***CYP2C9,* n (%)**	
1.1	39 (66.1)
1.2	11 (18.6)
1.3	8 (13.6)
2.2	1 (1.7)
2.3/3.3	0 (0)

in relation to the *VKORC1* and *CYP2C9* genotypes ($P = .59$ and .96, respectively) or age ($P = .41$).

Discussion

This study is the first prospective cohort study assessing the relative affect of the *VKORC1* and *CYP2C9* genotypes on VKA dosing in pediatric patients. The results show that in pediatric patients, age is the most important variable in determining VKA dose. In our cohort, as determined by regression modeling, age accounts for 28.3% of the variation in dose, whereas the *VKORC1* and *CYP2C9* account for 3.7% and 0.4%, respectively. Our data provide evidence that genotype effects in children are different from those in adults and assessment of genetic polymorphisms adds little to the explanation of the variation in dose. There was no difference in time to therapeutic range associated with age or phenotypes. These observations were consistent whether the patients received warfarin or phenprocoumon.

In children, age explains 28.3% of variation in VKA dose whereas in adults, age only accounts for 4%-21% of variation.[14,15,19,32] The observation of an age-related association of VKA dose in children is not new, as we and others have shown that of the clinical parameters assessed, age had the most significant effect in dosing of warfarin and acenocoumarin.[17,18] However, neither previous study assessed the relative effect of age in comparison to the *VKORC1* and *CYP2C9* as these genetic polymorphisms have only been identified in the last few years. The novel observation in the current study is the finding that in children, age overwhelms the effect of VKORC1 and CYP2C9 polymorphisms, the 2 variables that have the more profound effect on VKA dosing in adults.

In adults, clinical factors and the genotypes of *VKORC1* and *CYP2C9* have been shown to contribute to the variability of VKA dose requirements. There have been multiple models developed for estimation of VKA dose, in which consistently, all clinical variables, including age account for between 10% and 20% variability on VKA dosing depending on the models.[10,12,14,15,19,32-34] However, the models that include the phenotypes account for between 50% and 60% of the variation, with the phenotypes collectively accounting for between 30% and 50% of variation in dose. Therefore, the *VKORC1* and *CYP2C9* genotypes combined are the most important contributors to differences in VKA dosing in adults. By contrast, in our pediatric study, regression analysis showed that 28.3% of dose variation was associated with age, whereas only 3.7% was associated *VKORC1* and 0.4% with *CYP2C9*. Findings from our study suggest that although inclusion of age would be critical in a pediatric algorithm, inclusion of genotypes, especially *CYP2C9*, would add little to increasing the accuracy of the initial dosing. In fact, use of a regression model derived from an adult population will underestimate the VKA daily dose requirements if applied to children. From our data, an algorithm based on age may be the most useful approach to dosing of VKA in children.

The current study shows a significantly reduced effect on dose (3.7%) of *VKORC1* genotypes in children compared with adults (15%-39%).[10,12,14,15] The explanation for the difference of effect is not exactly clear. However, little is known about vitamin K metabolism over age, although there appears to be differences throughout childhood. For example, it is well described that compared with adult levels, the vitamin K–dependent coagulation factors and inhibitors are markedly decreased at birth and remain decreased over childhood.[35-37] The explanation for this observation has not been elucidated. However, results from the current study indicate that the effect of age on vitamin K metabolism appears to override the effect of the *VKORC1* polymorphisms.

Table 2. Fitted regression equations for modeling daily dose requirements based on age and *VKORC1* and *CYP2C9* genotypes

	Regression equation	*P*	R^2 model, %
Model variable			
Age	$\sqrt{D} = 0.46 - 0.011(\text{age})$	< .001	28.3
VKORC1	$\sqrt{D} = 0.3 - 0.05(VKORC1[AA]) - 0.003\ VKORC1[GA])$	.28	3.7
CYP2C9	$\sqrt{D} = 0.29 - 0.01(CYP2C9)$	.61	0.4
Full model			
Age, *VKORC1*, *CYP2C9*	$\sqrt{D} = 0.49 - 0.013(\text{age}) - 0.08(VKORC1AA]) + 0.01(VKORC1[GA]) - 0.02(Cyp2C9)$	< .001	38.2

Table 3. Regression results for modeling VKA daily dose requirements based on age and genotype according to drug type

	Warfarin		Phenprocoumon	
Model variable	***P***	**R^2 for model, %**	***P***	**R^2 for model, %**
Age	.0006	31.2	.0086	25.5
VKORC1	.63	2.8	.17	10.8
CYP2C9	.69	0.5	.75	0.3
Age, *VKORC1*, *CYP2C9*	.0025	34	.04	35.5

One perceived limitation is the relatively small number of patients tested. However, the study was adequately powered as the range of the CYP2C9 effect supported by our data (-0.06 to 0.02) was highly inconsistent and did not overlap, with the effect seen in adult populations (−0.454 to −0.252).[14] Our observed data are adequately powered to support the following 2 points. First, there is no CYP2C9 effect in children. The lack of association is not a result of lack of power, given the narrow confidence intervals around zero provided by the data. Secondly, the CYP2C9 effect in children is significantly different from that in adults. The clearly disjoint confidence intervals in the adult study compared with the current pediatric study indicates the study was adequately powered.

One limitation of the study is that the study patient population consisted of all white children. The restricted population limits the generalizability of the finding to other populations as the response of VKA dose to the polymorphisms vary in different races.[7,10,12] Future studies need to assess the association of VKA dosing to the polymorphisms in diverse racial pediatric populations.

A study in Japanese pediatric patients showed that the for body weight corrected clearance of the S enantiomer of warfarin was increased over childhood.[38] However, the clearance was similar to that of the adult if the values were normalized to either body surface area or liver weight. The observation was consistent when the patients were tested for *CYP2C9* polymorphisms with the wildtype *CYP2C9*1* pediatric patients clearance being increased in comparison to the adult. In children, the rate of body surface area and liver development is more rapid than body weight.[39] Based on their study findings, the authors speculated that the activity of CYP2C9 as a functional expression per unit weight of the liver is comparable with that of the adult.

One pediatric study from Norway assessed the relationship of *CYP2C9* to VKA dose in 29 children with cancer.[40] The study found no association of the CYP2C9 polymorphisms and VKA dose. However, the time to therapeutic was shorter, and there were more frequent INR about the target level. The limitations of the study were that neither age nor VKORC1 polymorphisms were assessed in relation to the CYP2C9 findings, which as shown in the current study, are the 2 most critical factors in determining VKA dose in children. In addition, most of the children were on steroids that have a well-described influence on VKA.[18,41] Therefore, with these confounding variables it is difficult to interpret the findings of the study.

Conclusion

The results of the current study indicate that, in contrast to the adult population, there is no significant impact of the *CYP2C9* or *VKORC1* genotypes on VKA dosing in children. Furthermore, age is the most significant variable in VKA dosing in children. The lack of effect was seen regardless of whether warfarin or phenprocoumon was administered. These data do not support the screening of children for the *CYP2C9* or *VKORC1* genotypes before initiation of VKA therapy.

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Authorship

Contribution: U.N.-G. performed the research, analyzed and interpreted data, performed statistical analysis, and wrote the manuscript; K.D. performed the research, analyzed and interpreted data, and wrote the manuscript; D.S. performed the research and analyzed and interpreted data; N.S.E. analyzed and interpreted data, performed statistical analysis, and wrote the manuscript; Y.Y. analyzed and interpreted data, performed statistical analysis, and wrote the manuscript; C.G. performed the research and analyzed and interpreted data; and L.G.M. designed and performed the research, analyzed and interpreted data, performed statistical analysis, and wrote the manuscript.

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