VKORC1 and *CYP2C9* genotype and patient characteristics explain a large proportion of the variability in warfarin dose requirement among children

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Although genetic and environmental factors explain approximately half of the interindividual variability in warfarin dose requirement in adults, there is limited information available in children. In a cross-sectional study of anticoagulated children from 5 tertiary care centers, 120 children with a stable warfarin dose were genotyped for *VKORC1* (−1639G > A; rs9923231), *CYP2C9* (*2 and *3 alleles; rs1799853 and rs1057910), and *CYP4F2* (V433M; rs2108622) polymorphisms. Clinical and demographic features were recorded. Multiple regression analysis of the data showed that, although *CYP4F2* made no contribution to the dose model, 72.4% of the variability in warfarin dose requirement is attributed to by patient height, genetic polymorphisms in *VKORC1* and *CYP2C9*, and indication for warfarin. The recently published International Warfarin Pharmacogenetics Consortium pharmacogenetic-based warfarin dosing algorithm (based on data derived from anticoagulated adults) consistently overestimated warfarin dose for our cohort of children. A similar proportion of the interindividual variability in warfarin dose is explained by genetic factors in children compared with adult patients, although height is a greater predictor in children. A pharmacogenomic approach to warfarin dosing has the potential to improve the efficacy and safety of warfarin therapy in children. However, algorithms should be derived from data in children if their potential benefit is to be realized. (*Blood*. 2012;119(3):868-873)

Introduction

Anticoagulant therapy is being increasingly used in children for the treatment and prevention of thromboembolic events, and warfarin remains the most frequently used agent for long-term anticoagulation in this patient group. Anticoagulation response to a fixed dose of warfarin is notoriously difficult to predict because of interindividual variability in dose requirement. This, together with the drug's narrow therapeutic window, necessitates maintenance of anticoagulation status within a tight therapeutic range facilitated by frequent international normalized ratio (INR) monitoring to ensure the efficacy and safety of therapy.

Oral anticoagulant therapy in children is complicated by numerous factors, including variable age-related dose-response rates, complex underlying health problems, multiple intercurrent illnesses, polypharmacy, and diet, and anticoagulation control is poor in this patient population.[1-3] Studies in adults have shown that warfarin dose requirement is influenced by demographic and genetic factors. The latter include single nucleotide polymorphisms (SNPs) in the vitamin K epoxide reductase (*VKORC1*)[4,5] and cytochrome P450 *CYP2C9* genes[6,7] and, to a lesser extent, other genes involved in vitamin K metabolism and the vitamin K cycle, such as the cytochrome P450 *CYP4F2* gene.[8,9] A pharmacogenomic approach to warfarin dosing has the potential to improve the efficacy and safety of warfarin therapy. This has resulted in the construct of pharmacogenetic-guided dosing algorithms for adult patients.[10,11] Despite substantial interest from physicians who anticoagulate children with warfarin,[12] a pharmacogenomic approach to warfarin therapy has not yet been developed and evaluated in a large pediatric population. We evaluated the effects of genetic, clinical, and demographic factors on maintenance warfarin dose in a cross-sectional design, multicenter study in children with stable anticoagulation.

Methods

Patient recruitment

Children were recruited from 4 United Kingdom sites (Birmingham Children's Hospital, Royal Manchester Children's Hospital, The Newcastle upon Tyne Hospitals NHS Trust, and Royal Hospital for Sick Children, Glasgow) and The Hospital for Sick Children, Toronto, ON. The study was approved by the Regional Ethics Committee, the Medicines and Healthcare products Regulatory Agency, and the institutional review boards at each of the study sites. The study recruited children, 18 years of age or younger, who were anticoagulated for at least 3 months after warfarin initiation and whose target INR range was either 2.0 to 3.0 or 2.5 to 3.5. The stability criterion for inclusion was that no change in warfarin dose had been made for at least the previous 3 consecutive INR measurements over a minimum period of 4 weeks. An additional cohort of children and young adults who

had received warfarin when 18 years of age or younger, and for whom historical data regarding warfarin dose requirement and INR were available, were recruited from one of the participating United Kingdom centers.

Data collection and blood sampling

Written informed consent to take part in the study was obtained from patients 16 years of age or older and from parents/caregivers of children younger than 16 years, in accordance with the Declaration of Helsinki. Details of diet, indication for anticoagulation with warfarin, target INR range, current warfarin dose, and other medication(s) were obtained by questionnaire and review of patients' medical records. Height, weight, and gender were recorded. Ethnicity was reported by the patient or their parent/caregiver, according to categories defined by the investigators. For the cohort of children and young adults who had previously been treated with warfarin during childhood, the aforementioned details were collected by review of patients' medical records and warfarin-dosing records at a time point when the child had been stable on warfarin according to the aforementioned criterion. A venous blood sample (4-8 mL) was collected from each patient and stored in EDTA tubes at −80°C for later genotyping.

Genotyping

Genomic DNA was extracted from whole blood samples according to an established method.[13] Genotyping for *VKORC1* (−1639G > A; rs9923231), *CYP2C9* (*2 and *3 alleles; rs1799853 and rs1057910), and *CYP4F2* (V433M; rs2108622) was performed using the StepOne Real-Time PCR System with TaqMan SNP Genotyping Assays (Applied Biosystems). The *VKORC1* and *CYP2C9* genotype results were validated using control samples that had been genotyped as part of a previous study.[14] *CYP4F2* genotypes were validated by regenotyping 26 samples in duplicate by PCR-restriction fragment length polymorphism analysis as described by Cen et al.[15]

Statistical analysis

Sample size. Because of the absence of data on the impact of genetic polymorphisms on warfarin dose in the pediatric population, a power calculation was based on comparable adult data. To detect a difference of approximately 1 mg in mean warfarin daily dose between the *CYP2C9* polymorphisms, with significance at the .05 level and a power of 80%, it was estimated that a sample size of 120 patients would be required. As the frequency of each of the *VKORC1* genotypes is greater than that of the *CYP2C9* mutant alleles and the effect of *VKORC1* on warfarin dose requirement in the adult population is larger than that of *CYP2C9*, a sample size of 120 was deemed adequate for detecting the effect of *VKORC1* genotype and other significant variables.

Data analysis. Statistical analyses were performed using MiniTab Version 15.0 (Coventry). Mean warfarin daily dose was transformed by taking the square root of each value to obtain a normal distribution, allowing parametric tests to be performed. Associations between warfarin dose and height, weight, body surface area, body mass index, and age were evaluated using the Pearson correlation test. The effect of genotype, indication for warfarin, target INR range, and ethnicity were evaluated using unpaired *t* test or ANOVA. Stepwise regression analysis was used to identify factors contributing to the transformed warfarin dose followed by linear regression to model the relationships of dose with other variables measured. Results are presented as mean ± SD unless stated otherwise. A *P* value of < .05 was taken as statistically significant.

Results

Patient characteristics

Recruitment occurred between April 2009 and December 2010. A total of 120 children with a median age of 11 years (range, 1-18 years) and a median duration of warfarin therapy of 49 months (range, 3-199 months) were recruited, including 8 subjects who were recruited retrospectively using historical data. The patients' demographics, indication for anticoagulation, and target INR range are shown in Table 1. Median warfarin daily dose was 3.4 mg

Table 1. Patient characteristics

	No. (%) of children
Sex	
Male	82 (68.3)
Female	38 (31.7)
Age group	
0-3 y	7 (5.8)
4-6 y	20 (16.7)
7-9 y	20 (16.7)
10-12 y	21 (17.5)
13-15 y	29 (24.2)
16-18 y	23 (19.2)
Ethnic origin	
White	91 (75.8)
Indian/Pakistani	10 (8.3)
Chinese	4 (3.3)
Black Caribbean	3 (2.5)
Black African	3 (2.5)
South-East Asian/Filipino	2 (1.7)
Other*	7 (5.8)
Indication for anticoagulation with warfarin	
Fontan procedure	64 (53.3)
Prosthetic heart valve	18 (15.0)
Coronary aneurysm	11 (9.2)
Dilated cardiomyopathy	6 (5.0)
Deep vein thrombosis/pulmonary embolism	6 (5.0)
Pulmonary hypertension	5 (4.2)
Stroke	2 (1.7)
Other†	8 (6.7)
Target INR range	
2.0-3.0	101 (84.2)
2.5-3.5	19 (15.8)
Total no. of children	120 (100)

*Mixed race, n = 5; Canadian Aboriginal, n = 1; and Middle Eastern, n = 1.

†One patient each with arrhythmia, bidirectional Glenn procedure, cerebral sinovenous thrombosis, left coronary artery to right coronary artery to right ventricular fistula, recurrent transient ischemic attack, transposition of the great arteries, truncal valve replacement, and ventricular assist device.

(range, 0.5-12.5 mg). Figure 1 shows the relationship between warfarin daily dose and age.

Genotyping results

Genotype frequencies for *VKORC1* (−1639G > A; rs9923231), *CYP2C9* (*2 and *3; rs1799853 and rs1057910), and *CYP4F2* (V433M; rs2108622) for the study population are shown in Table 2. All genotypes were in Hardy-Weinberg equilibrium.

Association of demographic and genetic variables with warfarin dose

The square root of warfarin daily dose was highly significantly correlated with body surface area (r = 0.56, *P* < .001, Pearson correlation coefficient), height (r = 0.55, *P* < .001), weight (r = 0.53, *P* < .001), and age (r = 0.53, *P* < .001), with body surface area and height being the most accurate predictors of warfarin dose requirement. Body mass index correlated less closely with warfarin daily dose (r = 0.32, *P* < .001).

The mean warfarin daily dose requirement in children with the *VKORC1* (−1639) GG genotype (5.0 ± 2.2 mg) was significantly higher than in those with GA (3.7 ± 1.9 mg) or AA (2.2 ± 1.1 mg) genotype (*P* < .001, ANOVA; Figure 2). The mean warfarin daily dose requirement in children with homozygous wild-type *CYP2C9* genotype (4.3 ± 2.1 mg) was significantly higher than in those with *1/*3 (2.2 ± 1.1 mg, *P* < .001), *1/*2 (3.7 ± 2.1 mg, *P* = 0.28), *2/*2 (1.3 mg, n = 1), or *2/*3 (1.6 mg, n = 1) genotype (Figure 2). Children who were anticoagulated after a Fontan
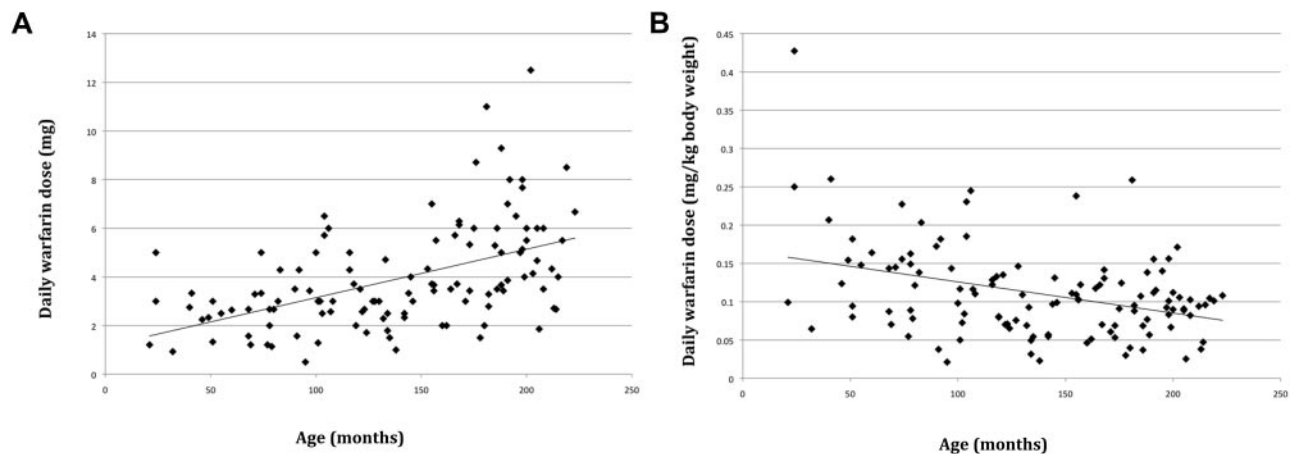
Figure 1. Relationship between age and daily warfarin dose. (A) In milligrams. (B) In milligrams per kilogram body weight.

procedure had a significantly lower mean warfarin daily dose (3.4 ± 1.6 mg) than those who were anticoagulated for other indications (4.4 ± 2.5 mg; $P = .02$, unpaired t test; Figure 2). Although children with a higher target INR range required a higher mean warfarin daily dose than those with a lower target INR range, the effect of target INR range on warfarin dose requirement was not significant (2.5-3.5, 4.5 ± 2.6 mg vs 2.0-3.0, 3.8 ± 2.0 mg, $P = .23$). The mean warfarin daily dose requirement was 5.1 ± 2.8 mg in children with *CYP4F2* (rs2108633) TT genotype and was higher than in those with CT (4.0 ± 2.3 mg) or CC (3.6 ± 1.8 mg) genotype, but the differences were not statistically significant ($P = .12$). Children of Indian/Pakistani origin (n = 10) had a higher mean warfarin daily dose requirement (5.1 ± 2.6 mg) than children of different ethnic origin (3.8 ± 2.1 mg); this was accounted for by the higher frequency of *VKORC1* (−1639) GG genotype in this ethnic group, 8 of 10 (80%) versus 35 of 110 (31.8%) for the rest of the cohort. However, the difference was not statistically significant ($P = .153$, t test). There were too few children for other ethnic groups to permit further subgroup analysis.

According to the regression model, height, indication, and *VKORC1* and *CYP2C9* genotypes made a significant contribution to the variability in warfarin dose requirement ($R^2 = 72.4\%$; Table 3). Both height and body surface area were found to be good predictors of the square root of dose, but in a model where the genetic parameters were included height was found to be the superior predictor and, once height was inclu ded, body surface

area did not significantly improve the fit of the model. Regression coefficient, SE, and P value for target range was 0.065, 0.077 and $P = .395$, and for *CYP4F2* genotype was 0.064, 0.040, and $P = .114$, respectively.

Subgroup analysis using 2 age groups (children < 10 years of age and children ≥ 10 years of age) showed that neither the contribution of each individual factor nor the dosing algorithm differed significantly between children of differing age.

Other variables

Concomitant drug therapy. A total of 83 of 120 (69.2%) children were taking additional prescribed drug(s). Of these children, 16 (13.3%) were taking 1 or more drugs known or suspected to have an effect on warfarin metabolism. These included: trimethoprim/co-trimoxazole, 6 patients; omeprazole, 4 patients; erythromycin/azithromycin, 2 patients; amiodarone, 1 patient; imatinib, 1 patient; prednisolone, 1 patient; iloprost, 1 patient; amitryptiline, 1 patient; sodium valproate, 1 patient; and carbamazepine, 1 patient. Children receiving trimethoprim or co-trimoxazole required a slightly lower warfarin dose than those who were not, but this difference was not statistically significant.

Diet. A total of 117 of 120 (97.5%) of the children reported a normal diet, meaning that they were not vegetarian or vegan or receiving enteral, parenteral, or infant formula feeds. Of the remainder, 1 child (4 years old) was receiving enteral feeding, 1 (14 years old) was vegetarian, and 1 (2 years old) was receiving

**Table 2. Genetic characteristics of study population**

	No. (%) of children
***VKORC1* genotype**	
GG	43 (35.8)
GA	55 (45.8)
AA	22 (18.3)
***CYP2C9* genotype**	
*1/*1	84 (70.0)
*1/*2	17 (14.2)
*1/*3	17 (14.2)
*2/*2	1 (0.8)
*2/*3	1 (0.8)
*3/*3	0 (0.0)
***CYP4F2* genotype**	
CC	61 (50.8)
CT	49 (40.8)
TT	10 (8.3)

Table 3. Contribution of height, *VKORC1*, *CYP2C9**2 and *3 genotypes, and indication for warfarin to regression equation for modeling warfarin daily dose requirements in children

x variable	P	Contribution to model, %
Height	< .001	29.8
VKORC1	< .001	26.6
CYP2C9*3	< .001	12.8
CYP2C9*2	< .001	
Indication	< .001	3.2
Height, *VKORC1*, *CYP2C9**2/*3, indication	< .001	72.4

Regression equation: $\sqrt{\text{dose}} = -0.009 + 0.011$ (height) + 0.357 (*VKORC1*) − 0.478 (*CYP2C9**3) − 0.277 (*CYP2C9**2) + 0.186 (indication). Height: input height in centimeters; *VKORC1* genotype: input 0 for AA, 1 for AG, and 2 for GG; *CYP2C9**3 genotype: input 0, 1, or 2 for the number of *3 alleles; *CYP2C9**2 genotype: input 0, 1, or 2 for the number of *2 alleles; indication: input 0 for Fontan procedure, 1 for other indication. The 95% confidence intervals for the coefficients are: intercept (−0.313, 0.294), height (0.009, 0.013), *VKORC1* (0.284, 0.425), *CYP2C9**3 (−0.335, −0.621), *CYP2C9**2 (−0.148, −0.407), and indication (0.085, 0.29).

Figure 2. Box plots showing the influence of *VKORC1* and *CYP2C9* genotypes, and indication for warfarin, on warfarin dose. Boxes represent the median and interquartile ranges. Vertical lines above and below boxes indicate the minimum and maximum values. Indicated values are mean warfarin doses. FP indicates Fontan procedure; and O, other indication.
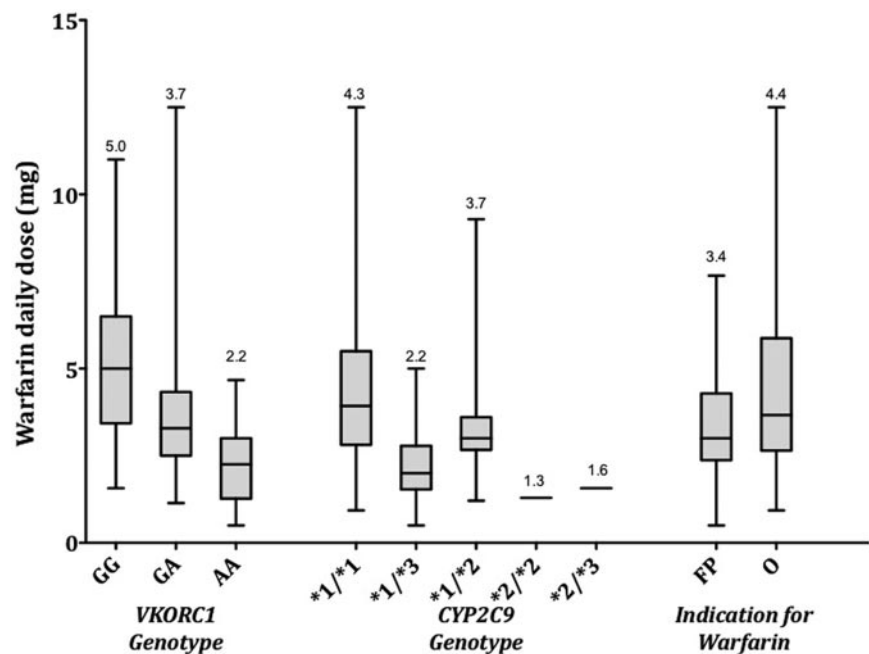
infant formula feed. When the factors in the regression equation were accounted for, none of these children had a warfarin daily dose that was outside of the expected range.

Predictive value of IWPC algorithm

There was a close and highly significant correlation between the actual warfarin maintenance dose and the predicted maintenance dose according to the International Warfarin Pharmacogenetics Consortium (IWPC) algorithm ($r = 0.76$, $P < .001$). However, the algorithm consistently overestimated warfarin dose in our cohort of children by, on average, 1.5 ± 1.4 mg/day (Figure 3).

Discussion

This study is the largest cross-sectional study of the effect of genetic, clinical, and demographic factors on warfarin dose requirement in children reported to date. The results show that a major proportion (72.4%) of the interindividual variability in warfarin dose requirement in children is attributed to by *VKORC1*, *CYP2C9*, height, and indication for warfarin therapy. A recent study published by Nowak-Gottl et al identified only a minor effect of *VKORC1* ($-1639G > A$) and *CYP2C9* (*2, *3) polymorphisms on maintenance warfarin dose in children, 3.7% and 0.4%, respectively.[16] However, only 59 children were included in the analysis, not all were anticoagulated with the same oral coumarin antagonist, there were few children identified as having *CYP2C9* *2 and *3 alleles, and a less robust criterion was used to establish stable anticoagulation status (requiring only 3 consecutive days of stable warfarin dose before subject study participation)[16] than that adopted for our study. A further study by Kato et al in 48 children of Japanese origin identified a 28% lower warfarin dose requirement in children with *VKORC1* -1173TT genotype compared with those with -1173CT or -1173CC genotype.[17] The investigators were unable to assess the impact of *CYP2C9* genotype on warfarin dose requirement as they found only 1 child to be of *CYP2C9**3 genotype. This study was also limited by the small sample size. Neither Nowak-Gottel et al[16] nor Kato et al[17] evaluated the effect of *CYP4F2* polymorphism on pediatric warfarin dose requirement.

In keeping with the studies by Nowak-Gottl et al[16] and Kato et al,[17] body size, in this case measured by height, remains the primary determinant of warfarin dose requirement in a pediatric cohort, contributing 29.8% to interindividual variability. Age and weight were highly correlated with height, but there was a closer correlation between height and warfarin dose. This may be explained by the presence of a positive correlation between liver size and height, resulting in greater warfarin dose requirement because of increased warfarin hepatic clearance[18,19] and possibly greater hepatic availability of coagulation proteins and stored vitamin K with increasing height. The positive correlation of dose with height overrides the smaller effect of younger children requiring a higher warfarin dose per kilogram body weight than older children and adolescents, shown by this (Figure 1) and previous studies.[1,3]

Studies in adults anticoagulated with warfarin have shown that carriers of the common allelic variants (*2 or *3) of the *CYP2C9* are associated with a lower warfarin dose requirement accompanied by a greater tendency to experience hemorrhagic complications during warfarin initiation.[6,7] Studies have shown that the *CYP2C9* polymorphism accounts for 5.7% to 17.5% of interindividual variability in warfarin dose requirement in adults.[14,20,21] The influence of *CYP2C9* on maintenance warfarin dose requirement was similar in our pediatric cohort (in which it accounted for 12.8% of variability) to adults. Our findings are more in keeping with the adult literature than either those of Nowak-Gottl et al[16] or of an earlier study by Ruud et al[22] who found no association between *CYP2C9* genotype and warfarin dose in 29 anticoagulated children, although the latter noted that children with a *CYP2C9* variant allele reached target INR sooner and were more likely to be overanticoagulated than those without. In the Ruud et al study, warfarin doses were not corrected for either body size or age.[22]

Vitamin K epoxide reductase (target enzyme for warfarin) is responsible for the recycling of vitamin K and is encoded by the vitamin K epoxide reductase subunit complex (*VKORC1*). Adults with *VKORC1* (-1639) GG genotype require higher warfarin doses than those with GA or AA genotype.[4,5] *VKORC1* genotype accounts for 15% to 30% of interindividual variability in warfarin
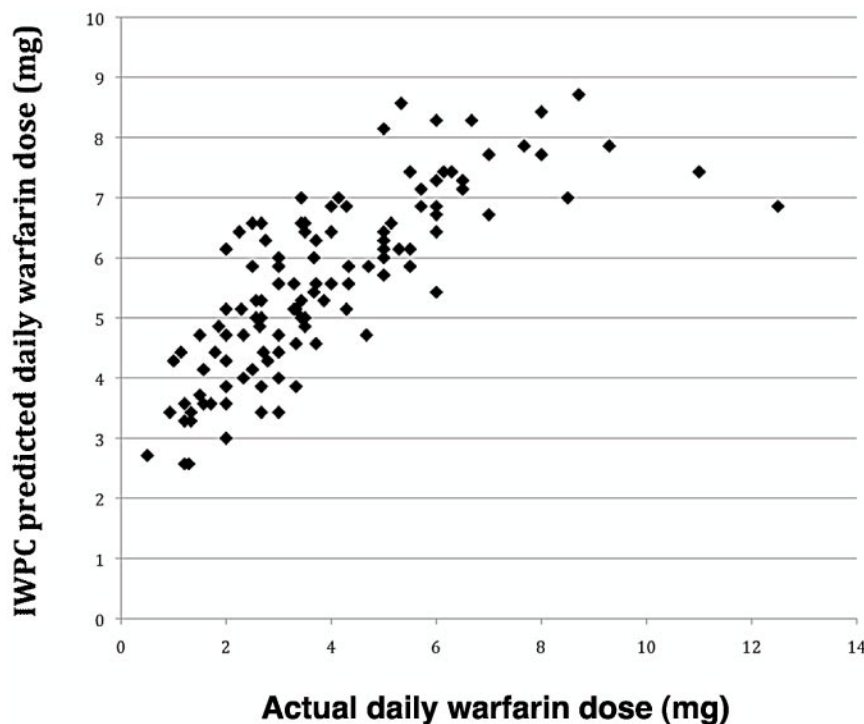
Figure 3. Relationship between actual daily warfarin dose (milligrams) and IWPC predicted daily warfarin dose (milligrams).

dose requirement in adults,[4,14,20] consistent with that seen in our pediatric population in which it accounted for 26.6%.

Indication for warfarin (Fontan procedure) contributed to 3.2% of interindividual variability. A lower warfarin dose requirement in children after a Fontan procedure was previously reported by Streif et al,[1] showing a 25% reduction in dose compared with patients with the same target INR range. The mechanism for the lower dose requirement may be related to abnormal liver function, resulting in reduced warfarin metabolism.[23] Although all of the children in our cohort who had a Fontan procedure were anticoagulated with the lower target INR range of 2.0 to 3.0, target INR range was not an independent variable in terms of its effect on warfarin dose requirement.

Recent studies have identified that the rs2108622 SNP in *CYP4F2* accounts for 2% to 7% of interindividual variability in warfarin dose requirements in adults.[8,9] Although there appears to be no direct role for *CYP4F2* in warfarin metabolism, it has been suggested that it influences vitamin K oxidase activity and therefore hepatic levels of vitamin K.[24] The lack of effect of *CYP4F2* on warfarin dose in our cohort of children may relate to differences in dietary vitamin K intake compared with their adult counterparts. This requires further study of the impact of vitamin K status on warfarin dose requirement in children.

In this study, we found that children of Indian/Pakistan origin tended to require a higher daily warfarin dose than other ethnic groups, which was accounted for by the higher frequency of *VKORC1* (−1639) GG genotype in this ethnic subgroup. This is consistent with data previously reported in adults.[25] Our study was not designed to explore the effect of ethnicity on warfarin dose requirement, which should be evaluated in a larger population.

Based on the study data, we have developed a regression equation for predicting warfarin maintenance dose (Table 3). However, because of the lack of additional eligible patients across the participating clinics, we were unable to test the equation for its accuracy to predict warfarin maintenance dose in an unrelated patient population. Most recently, the IWPC published a pharmaco-

genetic-based warfarin dosing algorithm based on genetic and clinical data derived from a large cohort (n = 4043) of geographically and ethnically diverse adult patients.[10] We found that, despite a good correlation between the predicted maintenance dose according to the IWPC algorithm and the actual dose, the algorithm consistently overestimated warfarin dose in our cohort of children (Figure 3). The overestimation of warfarin dose by the IWPC algorithm can be explained by a greater influence of age on the variability in warfarin dose requirement in children than in adults. This indicates that pharmacogenetic-based dosing algorithms for use in children requiring coumarin therapy should be based on data derived from such patients if the potential benefit of a pharmacogenetic-guided warfarin dosing in children is to be realized. Our dosing equation should now be validated and prospectively evaluated to determine whether it will aid the efficacy and safety of anticoagulant therapy with warfarin in children.

The remaining currently unexplained 27.4% of the interindividual variability in warfarin dose requirement in children may relate to differences in dietary vitamin K status. This is known to influence warfarin dose requirement in adult populations, those with a lower vitamin K status requiring a lower warfarin maintenance dose,[26] an effect that has not yet been studied in a pediatric population.[27] The impact of frequent intercurrent illness was also unaccounted for in the present study. Genome-wide association studies have been applied to adult populations in an attempt to explain some of the remaining interindividual variability in warfarin dose requirement through the contribution of other genes but have failed to identify a significant genetic influence on warfarin dose outside of the *CYP2C9* and *VKORC1* genes.[28] A genome-wide association study is probably even less applicable to a pediatric population in whom a greater proportion of interindividual variability is already explained by the factors included in our algorithm. It is possible that dietary vitamin K status is responsible for a significant proportion of the unexplained variance in warfarin dose requirement in children.

Acknowledgments

Authorship

Contribution: T.T.B., P.J.A., and F.K. designed the study; T.T.B., L.R.B., E.A.C., M.D.W., J.D.G., J.P.H., and F.K. conducted the trial; T.T.B., J.B.S.L., and A.K.D. performed genotyping experiments; T.T.B. and F.K. analyzed and interpreted data; T.T.B. and P.J.A. performed statistical analysis; and all authors wrote the manuscript.

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VKORC1 and *CYP2C9* genotype and patient characteristics explain a large proportion of the variability in warfarin dose requirement among children

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