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Clinical Investigation

Genetics-Based Pediatric Warfarin Dosage Regimen Derived Using Pharmacometric Bridging

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BACKGROUND Warfarin dosage regimens using *CYP2C9* and *VKORC1* polymorphisms have been extensively studied in adults and is included in US Food and Drug Administration-approved warfarin labeling. However, no dosage algorithm is available for pediatric patients.

OBJECTIVES To derive a genetics-based pediatric dosge regimen for warfarin, including starting dose and titration scheme.

METHODS A model-based approach was developed based on a previously validated warfarin dosage model in adults, with subsequent comparison to pediatric data from pediatric warfarin dose, genotyping, and international normalized ratio (INR) results. The adult model was based on a previously established model from the CROWN (CReating an Optimal Warfarin dosing Nomogram) trial. Pediatric warfarin data were obtained from a study conducted at the Children's Hospital of Los Angeles with 26 subjects. Variant alleles of *CYP2C9* (rs1799853 or *2, and rs1057910 or *3) and the *VKORC1* single nucleotide polymorphism (SNP) rs9923231 (-1639 G>A) were assessed, where the rs numbers are reference SNP identification tags assigned by the National Center for Biotechnology Information.

RESULTS A pediatric warfarin model was derived using the previously validated model and clinical pharmacology considerations. The model was validated, and clinical trial simulation and stochastic modeling were used to optimize pediatric dosage and titration. The final dosage regimen was optimized based on simulations targeting a high (≥60%) proportion of INRs within the therapeutic range by week 2 of warfarin therapy while minimizing INRs >3.5 or <2.

CONCLUSIONS The proposed pediatric warfarin dosage scheme based on individual *CYP2C9* (alleles *1,*2,*3) and *VKORC1* rs9923231 (-1639 G>A) genotypes may offer improved dosage compared to current treatment strategies, especially in patients with variant *CYP2C9* and *VKORC1* alleles. This pilot study provides the foundation for a larger prospective evaluation of genetics-based warfarin dosage in pediatric patients.

INDEX TERMS *CYP2C9*, pediatrics, pharmacogenetics, *VKORC1*, warfarin

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INTRODUCTION

Management of warfarin therapy is complicated by a narrow therapeutic index and high inter- and intraindividual variability in drug

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disposition and response. Polymorphisms in two genes, cytochrome p450 2C9 (*CYP2C9*) and vitamin K epoxide reductase C1 (*VKORC1*), are involved in the pharmacokinetic (PK) and pharmacodynamics (PD) of warfarin, respectively, and variant alleles have been shown to result in increased international normalized ratio (INRs) and reduced warfarin dose requirements.[1–7] The *CYP2C9* variant alleles *2 and *3 reportedly reduce warfarin clearance to approximately 30% and 15% of normal, respectively.[1,5] Patients with these variant alleles also tend to achieve a stable dosage regimen later and are at a significantly

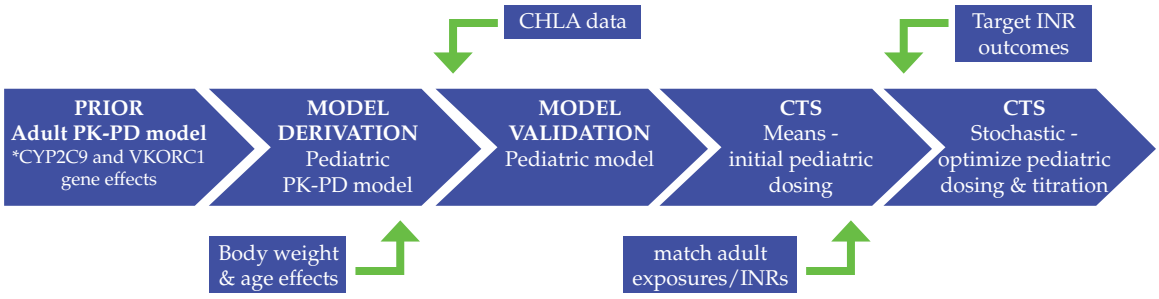

Figure 1. Study Design.
CHLA, Children's Hospital of Los Angeles; CTS, clinical trial simulation; CYP2C9, cytochrome p450 2C9 gene; INR, international normalized ratio; PK-PD, pharmacokinetics-pharmacodynamics; VKORC1, vitamin K epoxide reductase C1 gene

increased risk of bleeding compared with patients with the *CYP2C9*1*1* (homozygous wild-type) genotype.[3] Multiple polymorphisms in the *VKORC1* gene, such as the rs9923231 (-1639 G>A) and the rs9934438 (1173C>T) sites, occur in linkage disequilibrium and have been shown to increase warfarin sensitivity by 30% to 50%.[8–10] Collectively, the *CYP2C9* and *VKORC1* genotypes have been shown to account for approximately 45% of the variability in adult warfarin dose requirements.[2,6,7,11,12]

At the Clinical Pharmacology Advisory Committee meeting of the US Food and Drug Administration (FDA) in 2005, a consensus was expressed on the existence of sufficient mechanistic and clinical evidence to support lower doses of warfarin for patients with variant polymorphisms in the *CYP2C9* and *VKORC1* genes.[13] Subsequently, the warfarin label was updated in 2007 to include recommendations to perform genotype tests in patients for *CYP2C9* and *VKORC1* polymorphisms prior to initiating warfarin therapy.[14] Testing for these polymorphisms is currently performed in adults at some centers,[8] although no dosage algorithm is currently available for pediatric patients.

The objective of this pilot study was to develop a gene-based pediatric warfarin dosage regimen that would include both starting doses and a titration scheme which could then be validated in a larger pediatric patient population. A pharmacometric bridging approach using modeling and simulation was used along with limited available pediatric data.

METHODS

The study design is illustrated in Figure 1. Briefly, data were obtained from a previously reported adult warfarin PK/PD model[15] and from a limited number of pediatric subjects from a pilot study of pediatric warfarin pharmacogenetics conducted at the Children's Hospital of Los Angeles (CHLA), Los Angeles, CA. A pediatric PK/PD model was derived using the prior adult PK/PD model and considering the relationship between drug clearance and body size; the established maturation pattern of drug metabolizing enzymes involved in warfarin disposition; and the mechanism of action of warfarin. The pediatric model was subsequently validated using the CHLA pediatric data, which were not used for model derivation. Initial pediatric warfarin doses were estimated by matching target INRs for typical pediatric subjects with adult data. The pediatric dosage regimen, including starting dose and titration scheme, were then optimized using simulations of several thousand virtual pediatric subjects.

Pediatric Data

Pediatric data were obtained from patients ≤18 years of age who were followed in the warfarin clinic of the Division of Cardiology, CHLA. The protocol was approved by the Institutional Review Board of CHLA and the University of Southern CA, and parental consent and patient assent were obtained before starting any study procedures. Patients were treated according to standard of care and warfarin dosage regimen, and INR logs were recorded during regularly scheduled visits. Patients who received warfarin for less than 7 days were excluded. A blood sample (1 mL) was obtained during routine blood draws and was sent to the University of Southern California Pharmacogenetics Laboratory for genotyping. A vitamin K dietary intake estimate was performed from a food diary. In addition to genotype and diet, patient information collected

included age, weight, height, sex, warfarin dose, INR, other medical illness or medications and adverse events.

Genetic Analysis

DNA samples were extracted from blood samples using a genomic DNA extraction kit (QIAmp DNA blood mini-kit; Qiagen, Mississauga, ON, Canada). Variant alleles of *CYP2C9* (rs1799853 or *2, and rs1057910 or *3) and the *VKORC1* single nucleotide polymorphism (SNP) rs9923231 (-1639 G>A) were assessed, where the rs numbers are reference SNP identification tags assigned by the National Center for Biotechnology Information. SNPs were determined using real-time quantitative polymerase chain reaction assay based on the 5′ nuclease allelic discrimination assay (PRISM 7900 sequence detection system; Applied Biosystems, Foster City, CA). Genomic DNA (10 ng) was mixed with 2.5 µL of gene-specific primers and probes (10× concentrated) and 12.5 µL of polymerase chain reaction universal master mixture (Applied Biosystems) to a final volume of 25 µL. Thermal cycler parameters included 10 minutes at 95°C and 50 cycles involving denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 1 minute. For quality control of genotyping, negative and positive controls were used. Distributions of the *CYP2C9* and the *VKORC1* genotypes were compared to the Hardy-Weinberg theoretical distribution using the chi-square test. A p value of <0.05 was considered statistically significant.

Pediatric Warfarin Model: Derivation and Validation

Prior adult model

Investigators from the FDA developed a warfarin population PK/PD model and dosage scheme in adults in collaboration with Harvard Partners, Boston, MA.[15] The adult model was based on published research of the concentration–effect relationship for warfarin as well as data from the adult warfarin CROWN (CReating an Optimal Warfarin dosing Nomogram) trial.[5,16] The model was developed using data from an initial 271 subjects and subsequently validated in the same trial by using model-derived dosage in 117 subjects.

Pediatric model

Adult PK parameters were scaled to account for body size in pediatrics, using an allometric exponent of 0.75 for systemic and intercompartmental clearances and an exponent of 1 for central and peripheral volumes of distribution. The effect of age on clearance was accounted for based on a relationship previously developed for the maturation of CYP2C9, using warfarin data.[17] The concentration/dose response relationship for warfarin in pediatric patients was assumed to be similar to that of adult patients, as drugs with a related mechanism of action, namely argatroban[18] and low-molecular-weight heparins,[19] show a similar relationship. Therefore, the adult PD model was used in pediatric patients.[15] The derived pediatric population PK/PD model was validated using CHLA pediatric clinical data.

Optimal pediatric dosage, clinical trial simulations

The pediatric PK/PD model was used to investigate optimal warfarin dosage using clinical trial simulations. For purposes of deriving the starting dose, each genotype category was treated independently. All possible combinations of genotypes for *CYP2C9* (the *1 allele in various combinations with the variants *2 and *3) and *VKORC1* rs9923231 (GG, GA or AA where G and A represent the purines guanine and adenine) yielded 18 unique combinations.

A two-step approach was used for determining the optimal dosage. The first step narrowed the starting dose choices based on deterministic simulations in typical subjects within each genotype. The second step consisted of performing stochastic simulations to derive the best starting and titration dosage regimen.

Demographics

The Centers for Disease Control Growth Charts for the United States was used for the simulation of pediatric demographics that included age, sex, and weight.[20] For each unique combination of age and sex, there is a parameter set including a variability component to determine the distribution of body weight. We simulated 100 pediatric patients of different body weights for each combination of age (1 month–17 years old) and sex, resulting in a virtual bank of approximately 48,000 unique pediatric subjects. For preliminary simulations, we considered six typical pediatric subjects, covering the entire pediatric demographic range. The six typical subjects represent the mean body weight and

age, obtained from the virtual bank, for five different body weight/age groups. The typical demographics were 5 kg/1 month old; 8 kg/6 months old; 11 kg/1.5 years old; 16 kg/4 years old; 28 kg/9 years old; and 54 kg/15 years old. For final simulations, subjects were randomly sampled from the virtual bank of pediatric patients. A total of 1000 pediatric subjects for each genotype category were simulated.

Software

Clinical Trial Simulator version 2.2.1 software (Pharsight, Cary, NC) was used for the mean and stochastic simulations to determine pediatric doses. NONMEM version VI (ICON Development Solutions, Ellicott City, MD) with Visual FORTRAN 6 compiler (Compaq Computer Corp., Houston, TX) software was used for simulations during model validation. For NONMEM simulations, random numbers were generated using a six-digit seed. R version 2.9.1 software (R Project for Statistical Computing) was used for data processing, data analysis, and graphics generation.

RESULTS

Pediatric Data

A total of 36 pediatric subjects were included in the CHLA study. Of these, 10 subject records were missing genotype and/or INR log data. Data from the remaining 26 subjects were used for model validation. Cohort demographics are provided in Table 1. The mean age of subjects was 4 years 5 months (range, 4 months–18 years old), and the mean body weight was 23 kg (range, 6.9–84 kg). While there were subjects with the *VKORC1* polymorphisms in the study population, the *CYP2C9* *2 polymorphism was rare and the *3 polymorphism was absent. The target INR range was dependent on indication for warfarin therapy.

A wide range of doses (0.5–6.5 mg/day) were used in the pediatric subjects by the clinicians in the CHLA study. Starting doses ranged from 0.5 to 5 mg/day. The dose and titration choices were independent of patient genotype and were based on clinical judgment. The patient charts revealed that adherence to the dosage prescribed was poor in 4 subjects, and prolonged times (>60 days) were needed to arrive at stable dose in 12 subjects (46%). The median time to achieve a stable INR was 137 days. There were four major bleeding and six minor bleeding events during the study.

Table 1. Characteristics of the Study Population (n=26).

Characteristic	Mean (range) or Number (%)
Age (yr)	4.4 (0.33-18)
Body weight (kg)	23 (6.9-84.1)
Height (cm)	107 (65-189)
BSA	0.81 (0.36-2.1)
Warfarin maintenance dose (mg/kg/day)	0.12 (0.04 - 0.3)
Sex	
Male	16 (61%)
Female	10 (39%)
Ethnicity	
Hispanic	16 (61%)
Caucasian	7 (27%)
African American	2 (8%)
Mixed	1 (4%)
Target INR	
1.5-2.5	13 (50%)
2.0-2.5	5 (19%)
2.5-3.5	8 (31%)
Indication	
Valve replacement	8 (31%)
Fontan procedure	12 (46%)
Kawasaki disease	5 (19%)
Cardiomyopathy	1 (4%)
CYP2C9 genotype	
*1*1	22 (85%)
*1*2	4 (15%)
*1*3 / *2*2 / *2*3 / *3*3	0
VKORC1 rs9923231 genotype	
GG	7 (27%)
GA	8 (31%)
AA	11 (42%)

BSA, body surface area; CYP2C9, cytochrome p450 2C9; INR, international normalized ratio; VKORC1, vitamin K epoxide reductase C1.

Pediatric Warfarin Model

The model validation outcomes for all 26 subjects, including the 5th, 50th, and 95th percentiles of INR predictions by the model and observed INR values, are presented in Figure 2. In 80% of the cases (21 of 26 subjects), the observations lie within the 95% prediction intervals. There was no particular genotype that had a higher probability of failing the validation. Based on these results, the model was determined to be reasonable for use in subsequent simulations to determine an

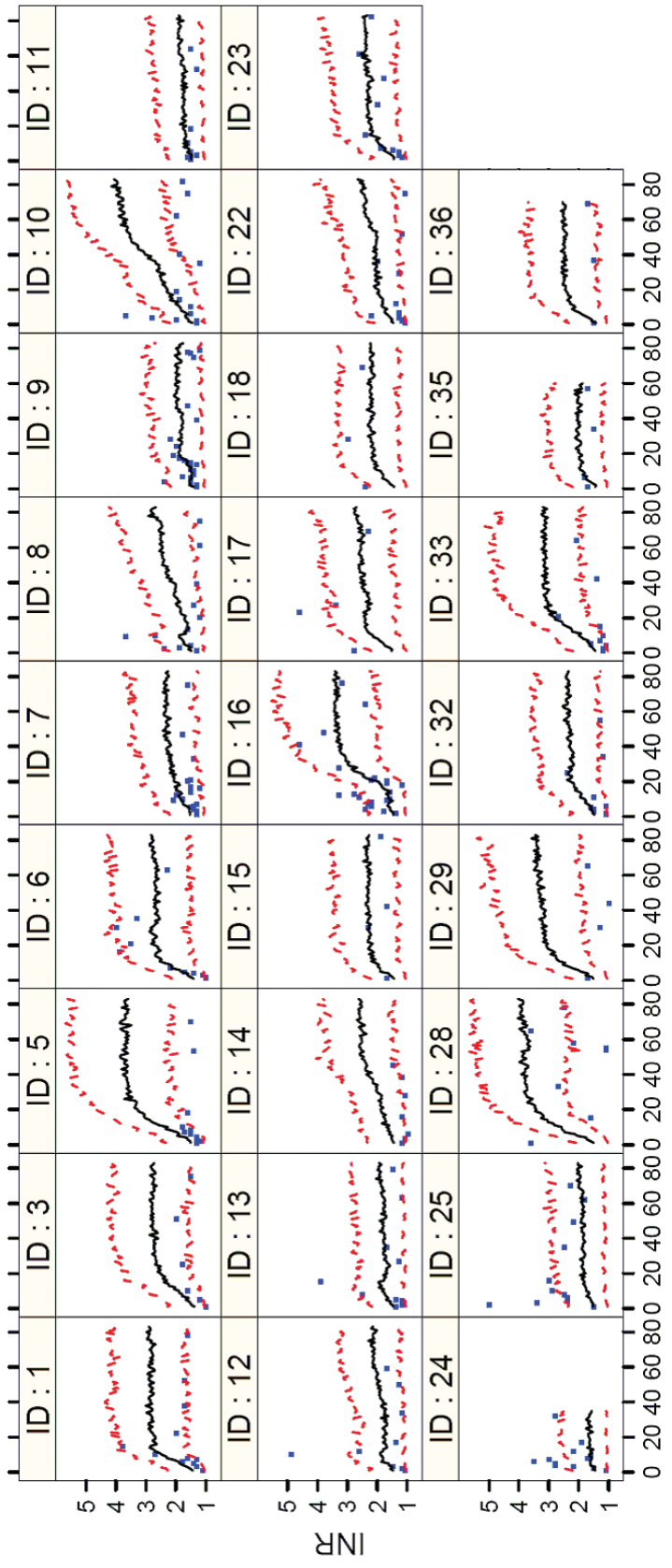

Figure 2. Model validation outcomes shown as predicted and observed INR for the 26 pediatric patients, where the blue square is the observed INR, and the predicted 5th, 50th (median) and 95th percentiles are the lower, middle and top lines respectively.

■ OBS; — P50; ··· P5; ··· P95

Table 2. Final Proposed Pediatric Warfarin Dosage Regimen. To identify the starting warfarin dose in mg/kg/day, choose the correct *CYP2C9* genotype from the top bar, and go down that column until you find the *VKORC1* genotype and patient weight (listed in the far left column).

VKORC1 rs9923231	***CYP2C9*** ***1*1**	***1*2**	***1*3**	***2*2**	***2*3**	***3*3**
GG						
<20 kg	0.13	0.08	0.065	0.035	0.035	0.017
>20 kg	0.09	0.06	0.05	0.027	0.027	0.012
GA						
<20 kg	0.095	0.06	0.05	0.027	0.027	0.015
>20 kg	0.07	0.045	0.035	0.02	0.02	0.01
AA						
<20 kg	0.07	0.05	0.045	0.025	0.025	0.012
>20 kg	0.05	0.04	0.03	0.017	0.017	0.01

CYP2C9, cytochrome p450 2C9; VKORC1, vitamin K epoxide reductase C1

optimal warfarin dosage regimen for pediatrics.

Initial Pediatric Dose

The simulated INR–time profiles and the clearance–body weight relationship for warfarin per our model indicated that two different milligram/kilogram doses for larger (≥20 kg) and smaller (<20 kg) body weight subjects within each genotype category were required. There did not appear to be a need to alter the milligram/kilogram dose based on an age cutoff. The selected initial dosage scheme allowed for targeting an average INR of 2.0 to 2.5 for all genotypes and matched average adult INR profiles.

Final Pediatric Dosage

For the stochastic simulations, the results were evaluated in terms of target INR outcomes across time. The starting dose was refined from the initial doses derived for typical subjects, as suitable for each genotype category. Minor modifications were made to the titration scheme from that suggested for adults.[15] An optimal pediatric warfarin dosage regimen was derived, inclusive of starting dose and titration scheme, to maximize desired INR outcomes. The final proposed dosage regimen is presented in Table 2. The titration scheme used was as follows: for INR <1.8, increase dose by 20%; for INRs ≥1.8 and <3.2, no change in dose; for INRs ≥3.2 to <4.0, decrease dose by 20%; for INRs ≥4.0 to <5.0, decrease dose by 25%; for INRs ≥5.0 to <6.0, decrease dose by 30%; and for INRs ≥6.0, decrease dose by 50%.

The comparison of INR outcomes for genotype-independent and genotype-based dosage is presented in Figure 3 for the four representative genotypes. The genotype-independent dosage regimen results in progressively worse outcomes (dramatic increase in proportions of INR >3.5) as the number of variant *CYP2C9* or *VKORC1* alleles increases. The comparison of INR outcomes between CHLA dosage and our proposed genotype-based dosage are displayed in Figure 4 for all six genotypes present in the CHLA data.

For the patients with both *CYP2C9* *1*1 and *VKORC1* rs9923231 GG homozygous wild type for both genes, the proportions of INR within target therapeutic range were high (60%) at week 2 with the CHLA clinically managed warfarin dosage regimen. However, the percentage of pediatric patients receiving a supratherapeutic INR through 1 month increased to 20%. At the other extreme, a patient with a *CYP2C9* *1*2 and *VKORC1* rs9923231 AA genotype produced percentages of INR that were within target therapeutic range 60% of the time at week 2, but a sharp decline to 30% along with an increase in the percentage of supratherapeutic INR to 50% was observed through 1 month. In contrast, both therapeutic and supratherapeutic INRs were consistently approximately 60% and 10%, respectively, through 1 month with the proposed warfarin dosage regimen.

In the remaining intermediate genotype categories, the percentages of INR within the target therapeutic range were much lower (10%-40%) at week 2 with clinical management and remained lower (below 45%) through 1 month in contrast to that with the proposed genotype-based dosage regimen (60%). Supratherapeutic INRs increased through 1 month up to 10% to 30% in all cases. The proportions of subtherapeutic INRs at 2

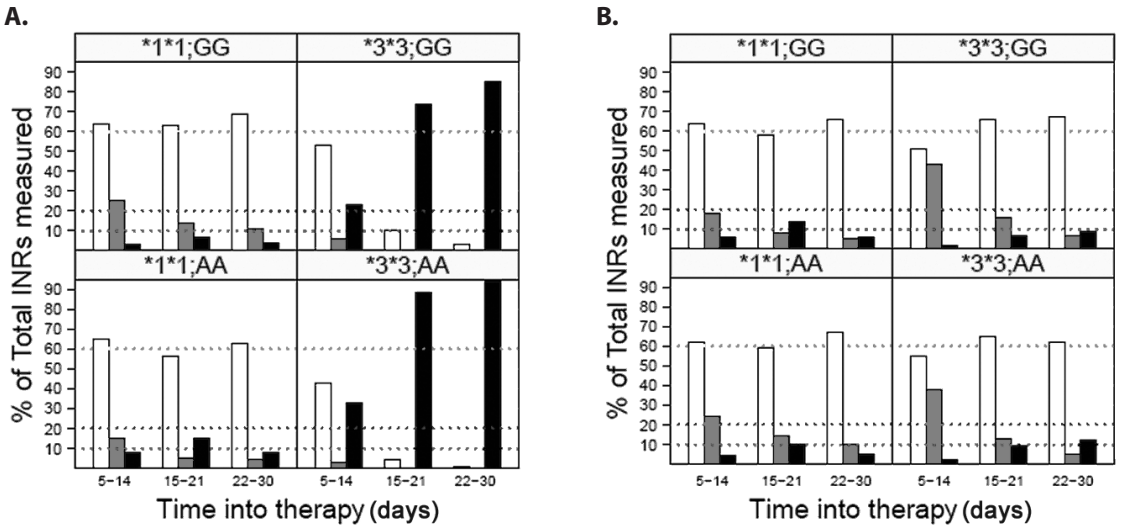

Figure 3. Comparison of the Effect of Genotype-Based and Genotype-Independent Dosage on Patient INR Outcomes Across Time.

The dashed lines represent the arbitrary goals of having 60% of patients within the target INR, with 20% being unacceptable to be outside the target INR, and 10% as acceptable for being outside the target INR.

A: Genotype independent dosing; B: Genotype dependent dosing

□ INR: 2-3; ■ INR<2; ■ INR>3.5

weeks were high (40%–90%) with clinically based dosage and remained high (20%–50%) through 1 month relative to that of the proposed genotype-based dosage regimens (<20%).

Finally, the INR outcomes for all genotype categories were simulated using the proposed pediatric dosage regimens with the imposed restrictions of available warfarin formulation strengths. The lowest dose administered and all dose changes during the titration were limited to a minimum of 0.5 mg. The proportions of INR >3.5 increase sharply as the number of variant *CYP2C9* or *VKORC1* alleles increases.

DISCUSSION

Pediatric warfarin dosage is currently based primarily upon clinical judgment and INR monitoring. While adult warfarin dosage has potentially benefitted from the use of pharmacogenetics based on information from *CYP2C9* and *VKORC1* in some centers, this information has not been applied to pediatric warfarin dosage previously. This study involved leveraging prior information in the form of adult warfarin data, application of knowledge of warfarin PK and PD, developmental pharmacology, and preliminary pediatric warfarin pharmacogenetic information. Pharmacometric methods were used to bridge an adult model and dosage regimen to develop a pediatric warfarin model and propose a dosage regimen. The two primary contributions of the current study are the proposal of a science-based, reproducible pediatric warfarin dosage regimen and a tool that can be used by clinicians and researchers to arrive at a pediatric warfarin dosage regimen based on a target INR.

Based on the developed pediatric warfarin PK/PD model, the *CYP2C9* and *VKORC1* genotypes should have a significant effect on warfarin clearance and INR response and on the required doses to achieve a target INR. In the simulations, using a fixed dose and assuming that each patient belongs to the same genotype category (*CYP2C9**1*1 and *VKORC1* rs9923231 GA) resulted in adverse INR outcomes, particularly for the most variant genotypes (*CYP2C9* *2*3 and *VKORC1* rs9923231 AA). Between the *CYP2C9* (PK) and *VKORC1* (PD) genetic effects, the polymorphisms altering PK had the most significant impact on dosage. There are two explanations for this observation. First, the dual *CYP2C9* *2*2, *2*3, and *3*3 variant genotypes had a large magnitude of effect (–70% to –85% on clearance) relative to that of the *VKORC1* homozygous rs9923231 AA variant genotype (–35% on potency). Second, the warfarin dose was titrated by monitoring the INR and PD response not the drug concentra-

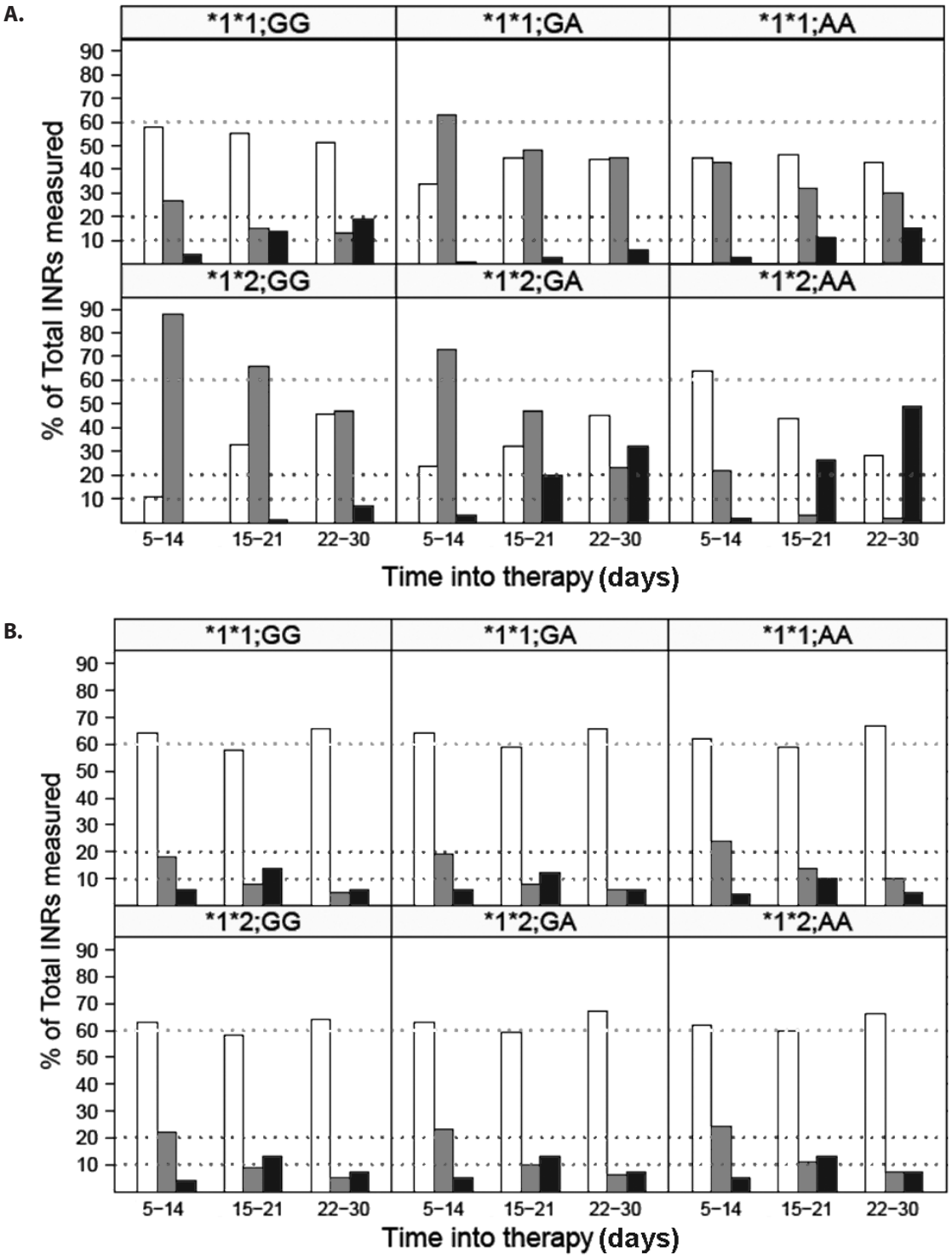

Figure 4. Target INR outcomes across time with CHLA standard of care dosing and with the proposed dosing regimen. Target INR's at CHLA were 1.5 to 2.5 and 2.5 to 3.5, depending on the indication for warfarin therapy. The dashed lines represent the arbitrary goals of having 60% of patients within the target INR, with 20% being unacceptable to be outside the target INR, and 10% as acceptable for being outside the target INR.
A: Children's Hospital of Los Angeles standard dosing; □ Target INR; ▒ INR<target; ■ INR>target
B: Proposed Dosing; □ Target INR: 2.0-3.0; ▒ INR<2.0; ■ INR>3.5

tions. Hence, adjusting starting doses based on genotypes relevant for PK may be more critical. Patients with the *2*2,*2*3, and*3*3 genotypes have a prolonged warfarin half-life (3–6 times longer than that of *1*1 patients), and the starting dose must account for this effect.

While supratherapeutic INRs remained well below 10% initially, they tended to increase over time, especially in patients with variant genetic polymorphisms. Despite generally conservative dosage, the lack of genotyping for *CYP2C9* and *VKORC1* results in a suboptimal starting dose and/or lack of a rational warfarin dose titration scheme. In the CHLA study, a target INR range of 2.5 to 3.5 was used for patients with valve replacements, consistent with previous reports in the literature. However, the choice of a target INR range of 1.5 to 2.5 or 2.0 to 2.5 for patients undergoing the Fontan procedure or for whom Kawasaki disease was diagnosed is institution-specific and different from previous reports.[21–23] For the proposed pediatric warfarin dosage based on pharmacometric modeling, the commonly accepted and used target INR range of 2.0 to 3.0 was selected to maintain consistency with the literature and generalize the outcomes to most settings. However, the modeling and simulation tool developed herein could be used to derive rational pediatric warfarin dosage for optimizing clinical outcomes for any alternative target INR range.

There are limitations to the current research. The most significant limitation may be attributed to the paucity of available clinical data on warfarin use and pharmacogenetics in pediatric patients. A multicenter pediatric warfarin pharmacogenetics trial sponsored by the FDA is now underway. The current pilot study was restricted to a small sample of pediatric subjects (n=26), which was used to validate our model. Additionally, the study population was relatively homogenous and did not represent a wide variety of ethnic groups. Next, Fontan patients were included, and this population may have different warfarin dosage requirements than other populations. The influence of interacting drugs was also not accounted for in the pediatric study population. The model was based on adult data and physiological principles rather than pediatric data, and the proposed dosage was based on simulations. Another limitation of this study is the absence of prospective validation of the dosage regimen. However, despite these limitations, the current study makes efficient use of available information and provides an important first step towards improving pediatric warfarin dosage.

This research is based on certain assumptions. The first assumption is that the concentration–response relationship for warfarin is similar between pediatric and adult patients. Some researchers have suggested intrinsic developmental differences in the coagulation systems,[24,25] precluding extrapolation of dose–response for antithrombotic therapy from adults to the youngest subset of the pediatric population (<2 years old). The second assumption is that *CYP2C9* polymorphisms reduce warfarin clearance to the same extent in pediatric patients as they do in adult patients. However, data regarding how ontogeny affects warfarin PK and PD is not available to formally challenge these assumptions. This is particularly true for *VKORC1* where the patterns of developmental expression are not yet understood.

The goal of this work is to eventually establish an improved standard of care for pediatric patients who require warfarin therapy. A genetics-based warfarin dose nomogram that functions more efficiently than the conventional standard of care would represent a major advance in pediatric pharmacotherapy. Such a nomogram could be made widely available to all clinicians and may enhance the safety and effectiveness of warfarin therapy in pediatric patients.

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ABBREVIATIONS CHLA, Children's Hospital of Los Angeles; CROWN, CReating an Optimal Warfarin dosing Nomogram; *CYP2C9*, cytochrome p450 2C9 gene; FDA, Food and Drug Administration; INR, international normalized ratio; PD, pharmacodynamics; PK, pharmacokinetics;

SNP, single-nucleotide polymorphism; *VKORC1*, vitamin K epoxide reductase C1 gene.

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