Frequency of CYP2C9 *and* VKORC1 *Gene Polymorphisms and Their Influence on Warfarin Dose in Egyptian Pediatric Patients*

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ORIGINAL RESEARCH ARTICLE

Frequency of *CYP2C9* and *VKORC1* Gene Polymorphisms and Their Influence on Warfarin Dose in Egyptian Pediatric Patients

Mennat-Allah Kamal El-Din · Marwa Salah Farhan · Randa Ibrahim El Shiha · Rania Mohammed Helmy El-Kaffas · Somaia Mohammed Mousa

Abstract

Introduction Warfarin is a widely used anticoagulant that shows a high inter-individual variability in the dose needed to achieve target anticoagulation. In adults, common genetic variants in the cytochrome P450-2C9 (*CYP2C9*) and vitamin K epoxide reductase complex (*VKORC1*) enzymes, in addition to non-genetic factors, explain this dose variability. In children, data about warfarin pharmacogenetics are limited and inconsistent.

Methods *CYP2C9* (**2* and **3*) alleles and the *VKORC1* (*C1173T* and *G-1639A*) polymorphisms were studied by multiplex real time polymerase chain reaction in 41 pediatric patients who received stable warfarin maintenance dose.

Results The allele frequency of the studied genes was *CYP2C9*2* (0.085), *CYP2C9*3* (0.12), *VKORC1 1173T* (0.52), and *VKORC1 −1639A* (0.54). In univariate analysis, patients' age, weight, and height were significantly ($p < 0.0001$) associated with warfarin maintenance dose. However, *CYP2C9* and *VKORC1* gene polymorphisms did not affect warfarin dose. In multivariate analysis, age was found to be the only significant determinant of daily warfarin maintenance dose ($p = 0.045$).

Conclusion Age was the most significant determinant of warfarin dosage in this preliminary study including Egyptian pediatric patients. Further studies involving larger numbers of children are warranted to determine the true impact of genetic factors on warfarin doses in pediatric patients.

Key Points

Age is the most significant determinant of warfarin dosage in children

CYP2C9 or *VKORC1* genotypes may have no significant impact on warfarin dose in this group of pediatric patients

Studies involving larger numbers of pediatric patients are mandatory to determine the true impact of clinical and genetic variables on warfarin dosage in Egyptian children

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1 Introduction

Warfarin is the most frequently used anticoagulant for prevention and treatment of thromboembolic disorders worldwide [1]. Warfarin has a narrow therapeutic range and large inter-individual variability in dose needed to

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reach adequate levels of anticoagulation. This is attributable to clinical, demographic, and environmental factors like age, gender, ethnicity, body mass index, daily vitamin K intake, concomitant diseases, interaction between drugs, and smoking [2]. In adults, genetic factors account for 40–60 % of inter-individual variability in warfarin maintenance dose [3]. Inter-individual warfarin dose variability is influenced by variations in the genes encoding two enzymes: cytochrome P450-2C9 (*CYP2C9*), the enzyme that metabolizes warfarin, and vitamin K epoxide reductase (*VKORC1*), the pharmacologic target of warfarin [4]. In 2007, the Food and Drug Administration (FDA) announced a change in warfarin label, stating that *CYP2C9* and *VKORC1* genotypes may be useful in determining the optimal initial dose of warfarin. The label was further updated in 2010 to include a table describing recommendations for initial dosing ranges for patients with different combinations of *CYP2C9* and *VKORC1* genotypes [1]. It is also recommended to develop population-based warfarin-dosing algorithms that include genetic and non-genetic factors [5]. In children, data about pharmacogenetics of warfarin is limited. To date, few studies have evaluated the effect of genetic and non-genetic factors on warfarin dosage in Egyptians [6–8], none of which includes pediatric patients. The aim of this study is to estimate the effect of the *CYP2C9* (**2* and **3*) alleles and the *VKORC1* (*C1173T* and *G-1639A*) polymorphisms on warfarin dose in Egyptian children.

2 Patients and Methods

The study included 41 Egyptian pediatric (aged $\leq$18 years) patients on warfarin therapy. Patients were invited to participate in the study during their regular follow-up visits to monitor their International Normalized Ratio (INR) at the Hematology Laboratory, New Children Hospital, Cairo University in Cairo, Egypt. Patients were recruited only after informed consents were freely obtained from their guardians after explaining the study. The study was approved by the Ethical Committee of Kasr Al-Ainy School of Medicine, Cairo University and conducted according to the Declaration of Helsinki. At enrolment, all patients were receiving a stable dose of warfarin to achieve the target INR of 2–3. Stable warfarin dose was defined as warfarin requirement that remains constant for 3 consecutive days after achieving the target INR [9]. Patients' age, weight and height, medications used, indications for warfarin therapy, and warfarin maintenance dose were recorded.

Two blood samples were collected from each patient; one on sodium citrate for determination of prothrombin time (PT) and INR and one on EDTA (ethylene diamine tetraacetic acid) for DNA extraction for detection of *CYP2C9* (**2* and **3*) and *VKORC1* (*C1173T* and *G-1639A*) polymorphisms. DNA was extracted using a High Pure polymerase chain reaction (PCR) Template Preparation Kit (Roche Applied Science, Mannheim, Germany). DNA was amplified by PCR on a real-time fluorescence LightCycler instrument in a final volume of 20 μL as described previously [10].

A LightMix kit (Roche Applied Science, Mannheim, Germany, Cat. No. 40-0298-16) was used for simultaneous detection of *CYP2C9*2* and *CYP2C9*3* alleles using fluorogenic hybridization probes (TIB MOLBIOL, Berlin, Germany) according to the manufacturer's instructions. *CYP2C9*2* was detected with a SimpleProbe probe (detected in channel 530) and *CYP2C9*3* was detected with probes labeled with LightCycler Red 640 (detected in channel 640). The genotypes were identified by running a melting curve with specific melting points (T_m) of 58.5 °C for the wild type and 50.5 °C for the mutant allele of *CYP2C9*2* in channel 530 and 48.3 °C for the wild type and 58.3 °C for the mutant allele of *CYP2C9*3* in channel 640.

A LightMix kit (Roche Applied Science, Mannheim, Germany, Cat. No. 40-0302-16) was used for simultaneous detection of *VKORC1 C1173T* and *VKORC1 G-1639A* polymorphisms using fluorogenic hybridization probes (TIB MOLBIOL, Berlin, Germany) according to the manufacturer's instructions. *VKORC1 C1173T* was analyzed with a SimpleProbe probe (detected in channel 530) and *VKORC1 G-1639A* was analyzed with LightCycler Red 640 labeled hybridization probes (detected in channel 640). The genotypes were identified by running a melting curve with T_m of 51.8 °C for the wild type *VKORC1 C1173* and 58.1 °C for the mutant allele *VKORC1 1173T* in channel 530 and 52.6 °C for the wild type *VKORC1 G-1639* and 61.3 °C for the mutant allele *VKORC1 −1639A* in channel 640.

The PCR conditions were initial denaturation at 95 °C for 10 minutes followed by 45 cycles of amplification; denaturation at 95 °C for 5 seconds, annealing at 60 °C for 10 seconds and extension at 72 °C for 15 seconds. Melting curve conditions included two initial hold steps for 20 seconds each at 95 °C then 40 °C followed by temperature rising from 40–85 °C at 0.2 °C/step.

2.1 Statistical Methods

Statistical analysis was performed using Statistical Package for Social Sciences, Version 17.0 (SPSS, Inc., Chicago, IL, USA). Quantitative data were presented as mean ± standard deviation (SD) and were compared using Student's *t* test. Percentages were calculated for categorical data. Pearson's correlation was used to test the relation between

mean daily warfarin maintenance dose and other continuous variables. A linear regression analysis was undertaken to study the relation between warfarin dose as a dependant factor and clinical and genetic variables as independent factors. A p value of <0.05 was considered statistically significant.

3 Results

Forty-one pediatric patients on warfarin therapy were included in the study.

Clinical data, warfarin daily maintenance dose, INR values, and *CYP2C9* (**2* and**3*) and *VKORC1* (*C1173T*) and (*G-1639A*) genotypes of each patient are included (Supplementary table S1, see electronic supplementary material).

Demographic and clinical characteristics of patients are shown in Table 1.

Cardiac valve replacement was the most common indication for warfarin therapy (63.4 %). Most patients (92.7 %) receive drugs that are not known to interact with warfarin.

Genotype and allele frequencies of the *CYP2C9*2, CYP2C9*3, VKORC1 C1173T*, and *VKORC1 G-1639A* polymorphisms are shown in Table 2.

By univariate analysis, the daily warfarin maintenance dose was significantly correlated with age, weight, and height ($p < 0.0001$), meaning that older, heavier, and taller patients required a higher daily warfarin maintenance dose. However, in multivariate analysis including significant variables, only age retained its importance as a determinant of daily warfarin maintenance dose ($p = 0.044$).

Univariate analysis studying the impact of genetic factors on warfarin maintenance dose revealed that there was no significant difference in mean daily warfarin maintenance dosages among patients carrying *CYP2C9*2, CYP2C9*3, VKORC1 C1173T*, or *VKORC1 G-1639A* polymorphisms (Table 3). To evaluate the effect of gene polymorphisms, age, and other clinical variables (sex, weight, and height) on warfarin maintenance dose, a multiple regression analysis was undertaken. *CYP2C9*2, CYP2C9*3, VKORC1 C1173T*, and *VKORC1 G-1639A* polymorphisms were found to have no impact on warfarin maintenance dose in this group of Egyptian children ($p = 0.151$, 0.989, 0.383, 0.576, respectively). Again, age was found to be the only variable that has a significant impact on warfarin dose in a multivariate analysis including genetic and clinical variables ($p = 0.045$).

Table 1 Demographic and clinical characteristics of patients ($n = 41$)

Parameter	Result
Age (mean ± SD), years	6.59 ± 2.97
Sex, *n* (%)	
Males	23 (56.1)
Females	18 (43.9)
Weight (mean ± SD), kg	20.88 ± 6.71
Height (mean ± SD), cm	102.07 ± 17.10
Warfarin maintenance dose (mean ± SD), mg/day	4.88 ± 1.33
PT (mean ± SD), sec	30.51 ± 3.71
INR (mean ± SD)	2.37 ± 0.26

INR international normalized ratio, *PT* prothrombin time

Table 2 Genotype and allele frequencies of the *CYP2C9*2, CYP2C9*3, VKORC1 C1173T*, and *VKORC1 G-1639A* polymorphisms among patients on warfarin ($n = 41$)

Genotype	Frequency *n* (%)
CYP2C9	
**1/*1*	27 (65.9)
**1/*2*	5 (12.2)
**1/*3*	6 (14.6)
**2/*3*	2 (4.9)
**3/*3*	1 (2.4)
**2/*2*	0 (0)
Wild allele (*1)	0.79
Variant allele	
**2*	0.085
**3*	0.12
VKORC1 C1173T	
C/C	8 (19.5)
C/T	23 (56.1)
T/T	10 (24.4)
Wild allele (*C*)	0.48
Variant allele (*T*)	0.52
VKORC1 G-1639A	
G/G	7 (17.1)
G/A	24 (58.5)
A/A	10 (24.4)
Wild allele (*G*)	0.46
Variant allele (*A*)	0.54

4 Discussion

In the current study, we evaluated the effect of clinical and genetic factors on warfarin dose variability in pediatric patients. We found that patients' age, weight, and height were the most significant contributors to differences in warfarin dose, whereas *CYP2C9* and *VKORC1* gene polymorphisms did not affect warfarin dose.

Table 3 Relation between mean daily warfarin dose and genetic variables by univariate analysis

Genetic variables	Warfarin dose, mg (mean ± SD)	*p* Value	95 % CI for the difference between means
*CYP2C9*2*			
Wild ($n = 34$)	4.71 ± 1.27	0.066	−2.09 to 0.71
Variant ($n = 7$)	5.71 ± 1.38		
*CYP2C9*3*			
Wild ($n = 32$)	4.78 ± 1.45	0.385	−1.46 to 0.57
Variant ($n = 9$)	5.22 ± 0.67		
VKORC1 C1173T			
Wild ($n = 8$)	4.88 ± 1.64	0.994	−1.07 to 1.07
Variant ($n = 33$)	4.88 ± 1.27		
VKORC1 G-1639A			
Wild ($n = 7$)	5.00 ± 1.53	0.793	−0.98 to 1.27
Variant ($n = 34$)	4.85 ± 1.31		

However, in multivariate analysis, age was found to be the only significant variable that affects warfarin dosage. In concordance with our study, Nowak-Göttl et al. [9] identified that age is the major determinant of warfarin dose variability, while *CYP2C9* (**2* and **3*) and *VKORC1* (*G-1639GA*) polymorphisms have a minor role on warfarin maintenance dose in children. This finding may be related to age-associated changes in liver size and warfarin pharmacokinetics and pharmacodynamics resulting in increased hepatic clearance of warfarin with subsequent greater dose requirements [11, 12]. In adults, common genetic variants in *CYP2C9* (**2* and **3*) and *VKORC1* (*C1173T* and *G-1639A*), in addition to known non-genetic factors, account for about 50 % of warfarin dose variability [1]. In children, data on warfarin pharmacogenetics are limited and inconsistent. While, Ruud et al. [13] found no association between *CYP2C9* genotypes and warfarin dose in children with cancer, other studies show different results. In a study investigating 120 children from different ethnic origins, the major proportion of the interindividual variability in warfarin dose requirement was attributed to height, *CYP2C9* (**2* and **3*), and *VKORC1* (*G-1639A*) polymorphisms [14]. Similarly, Shaw et al. [15] found that 76.3 % of warfarin dose variability among their cohort of 93 children with a median age of 4.8 years was attributed to weight, indications of therapy, *VKORC1* (*G-1639A*), and *CYP2C9* (**2* and **3*) polymorphisms. Moreau and colleagues [16] investigated 118 children with heart disease and found that 68.3 % of the overall interindividual variability in the warfarin dose was explained by height (48.1 %), *VKORC1* (*G-1639A*) genotypes (18.2 %), and *CYP2C9* genotypes (2.0 %). Kato et al. [17] identified that *VKORC1 C1173T* gene polymorphisms and age were the major factors affecting warfarin dose variability in 48 children of Japanese origin. However, they could not assess the impact of *CYP2C9* genotype on warfarin dose requirement as they found only one child with the *CYP2C9*3* genotype, which has a very low frequency among Asians. In another study, Nguyen et al. [18] investigated 50 children with heart disease and reported that *CYP2C9* polymorphisms did not affect warfarin dose, while *VKORC1 C1173T* polymorphism was an important determinant of warfarin dose. These conflicting results among existing studies may reflect differences in allele frequencies among ethnic groups investigated as allele and genotype frequencies of the *CYP2C9*2, CYP2C9*3*, and *VKORC1* (*C1173T* and *G-1639A*) genes were found to have high inter-ethnic and even intra-ethnic variations [19]. In addition, differences in sample size among the previously mentioned studies may play a role. Our study is limited by the small sample size that makes it difficult to draw a major conclusion from the observed results; however, this is a preliminary study that offers a foundation for further researches aiming at better understanding of warfarin pharmacogenetics in children.

5 Conclusion

In conclusion, our study revealed that age is the most significant determinant of warfarin dosage in children. *CYP2C9* and *VKORC1* genotypes may not have a major impact on warfarin dose in this group of Egyptian pediatric patients. However, the generalization of these observations is limited by the small sample size. These results confer support for the need for further studies involving larger numbers of pediatric patients to determine the true impact of these variants on warfarin dosage in Egyptian children.

Conflict of interest No sources of funding were used to support the writing of this manuscript.

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