Research Article

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Pharmacogenomics

Effects of age and genetic variations in *VKORC1*, *CYP2C9* and *CYP3A4* on the phenprocoumon dose in pediatric patients

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Aim: To study the effects of clinical and genetic factors on the phenprocoumon dose requirement in pediatric patients and to develop a dosing algorithm. **Methods:** Pediatric patients who used phenprocoumon were invited to participate in a retrospective follow-up study. Clinical information and genotypes of genetic variations in *CYP2C9*, *VKORC1*, *CYP4F2*, *CYP2C18* and *CYP3A4* were collected and tested with linear regression for association with phenprocoumon dose requirement. **Results:** Of the 41 patients included in the analysis, age, *VKORC1*, *CYP2C9*2/*3* and *CYP3A4*1B* were statistically significantly associated with dose requirement, and together explained 80.4% of the variability in phenprocoumon dose requirement. **Conclusion:** Our study reveals that age and genetic variations explain a significant part of the variability in phenprocoumon dose requirement in pediatric patients.

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Keywords: adolescent • anticoagulation • child • infant • pharmacogenomics • phenprocoumon • thrombosis

Together with warfarin and acenocoumarol, phenprocoumon is one of the three most commonly used vitamin K antagonists (VKAs). It has a slightly different metabolism than the other two and has the longest half-life of 5 days [1]. Few studies have investigated the use of phenprocoumon in pediatric patients. Currently, the guideline of the federation of Dutch anticoagulation clinics recommends the same starting dose for phenprocoumon as for acenocoumarol without any adjustments for differences between the two VKAs [2]. Spoor *et al.* evaluated the loading and maintenance dosages of acenocoumarol and phenprocoumon in pediatric patients and came to the conclusion that a separate age-related dosing guideline for both VKAs was needed [3]. However, until now, no dosing guideline for phenprocoumon in pediatric patients has been developed.

The VKA dose requirement demonstrates high interindividual variability. An increasing amount of information is becoming available on the factors that are responsible for this variability. In pediatric patients, age and age-related factors (e.g., height and weight) explain a significant part of the variability (12–52.8%) [4–9]. Furthermore, it has been demonstrated that genetic variations in the genes coding for CYP2C9 and VKORC1 explain 0.3–26.6% (*CYP2C9*) and 2.8–47% (*VKORC1*) of the variability in VKA dose requirement [4–9]. Most of these studies were undertaken with patients using warfarin. Only one relatively small study (n = 26) was conducted with patients using

phenprocoumon [6]. This study revealed that age was the most important factor, explaining 25.5% of the variability in dose requirement. Further, there was no statistically significant effect for the genetic variations in *VKORC1* and *CYP2C9* on the phenprocoumon dose [6]. In adults, genetic variations in *VKORC1* and *CYP2C9* have been clearly associated with the phenprocoumon dose [10]. The objective of this study was to undertake a further investigation into the effects of both clinical and genetic factors on the phenprocoumon dose requirement in pediatric patients, and to develop a pharmacogenetic dosing algorithm for phenprocoumon.

Methods

Study design & population

The Children Anticoagulation and Pharmacogenetics Study was designed as a retrospective follow-up study in four academic pediatric hospitals and one anticoagulation clinic in The Netherlands, as previously described in more detail [11,12]. In brief, patients younger than 19 years of age who used phenprocoumon for more than a month between January 1995 and December 2014 were selected and invited to participate in the study. The follow-up of a patient ended at the date of data collection at the anticoagulation clinic (between 11 January 2014 and 10 March 2016), when patients turned 19 years of age, when they stopped phenprocoumon therapy or when they were lost to follow-up.

The Medical Ethics Review Committee of the University Medical Center Utrecht decided that the study did not need ethical approval, because a noninvasive DNA collection was used. The UPPER Institutional Review Board of the Division of Pharmacoepidemiology and Clinical Pharmacology of Utrecht University approved the study protocol. All participants (and/or their parents or legal guardians) provided informed consent before taking part in the study.

Data collection

Information was collected at the hospital and anticoagulation clinics involved in the phenprocoumon treatment of each patient. Data collected at the hospitals included sex, date of birth, weight, height and indication for phenprocoumon treatment. Data collected at the hospitals and anticoagulation clinics included international normalized ratio (INR) values, phenprocoumon doses and the therapeutic INR range (TR). Furthermore, the patients and/or their parents were asked to fill in a questionnaire, on the basis of which information was collected on ethnicity, whether the patient was receiving breastfeeding during therapy and, if applicable, whether this was combined with vitamin K use by the mother.

DNA was collected by means of saliva samples and genotyping was performed by the laboratory of the Department of Clinical Pharmacy and Toxicology of the Leiden University Medical Center using a LightCycler 480, with a TaqMan SNP genotyping assay (ThermoFisher, MA, USA). The following seven SNPs were genotyped: *VKORC1* (rs9934438), *CYP2C9* (rs1799853 and rs1057910), *CYP4F2* (rs2108622), *CYP3A4* (rs35599367 and rs2740574) and *CYP2C18* (rs1998591). Only SNPs with a distribution of the genotypes in Hardy–Weinberg equilibrium ($p \geq 0.05$) were included in the analysis.

Outcome, determinants & data analysis

The outcome of the algorithm was the stable maintenance dose (mg/day) defined as the mean dose during the first stable period after initiation. A stable period was defined as ≥3 consecutive INR measurements within the patient-specific TR over a period of ≥3 weeks. Patients who did not reach a stable period were excluded from the analysis. A 10-log transformation of the outcome was used to obtain a normal distribution.

The following determinants were used for the development of the algorithm: age at start of stable period, sex, indication for anticoagulation and TR. The genotypes of the SNPs in *VKORC1*, *CYP2C9*, *CYP4F2*, *CYP3A4* and *CYP2C18* were also used as determinants (entered as number of variant alleles).

Linear regression was used to model the association between stable dose as outcome and the determinants. Determinants that were univariately associated with the outcome ($p < 0.2$) were used as candidate variables for the algorithm. A one-way analysis of variance (ANOVA) was used to analyze the differences in log mean stable dose between genotypes of candidate SNPs. For the multivariable analysis, a forward stepwise selection procedure was used, in which all determinants with a p-value less than 0.05 were entered in the algorithm. Two sensitivity analyses were conducted. In one a Bonferroni corrected p-value of 0.005 (= 0.05/10) was used for entering a variable into the model. In the other a backward selection procedure was used. The statistical analysis was carried out using IBM SPSS version 24.0.

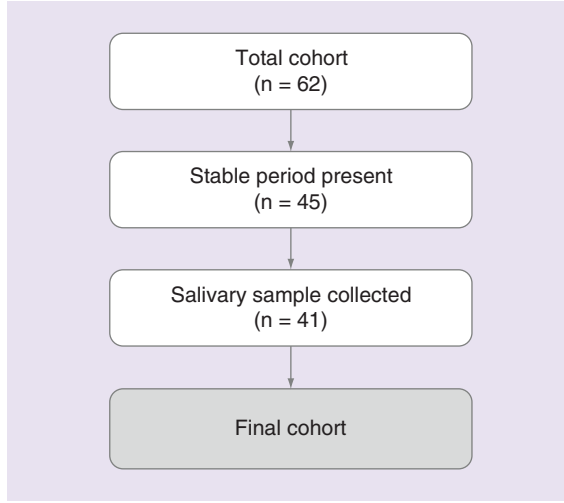

Figure 1. Flowchart of patients included in the analysis. The characteristics of the excluded patients are presented in Supplementary Table 1.

Results

Patient characteristics

A total of 62 patients used phenprocoumon, 41 of whom had a stable period and genetic information available, as presented in Figure 1. There was almost an equal number of females (n = 20, 49%) and males, with a median age of 9.3 years, as presented Table 1. 42% of the patients were 3 years or younger, while 44% were older than 13 years of age. 86% of the patients were of European descent. Phenprocoumon was used for cardiac indications in two-third of the patients. The standard TR of 2.0–3.5 was the most commonly used (53.7%), followed by the high TR of 2.5–4.0 (29.3%). None of the patients received breastfeeding during their stable period. The distributions of the genotypes per SNP (Table 1) were in Hardy–Weinberg equilibrium.

Associations of clinical & genetic variables with the phenprocoumon dose

The log mean stable daily dose was strongly associated with age (unadjusted R^2 = 56.2%; $p < 0.001$). Patients with a Fontan circulation required a lower dose that was not statistically significant (unadjusted R^2 = 6.0; p = 0.12). Sex, ethnicity and TR were not associated with the log mean stable daily dose ($p > 0.2$). From the genotyped SNPs, only *VKORC1* (unadjusted R^2 = 16.9%; p = 0.008), *CYP2C9*2/*3* (unadjusted R^2 = 9.4; p = 0.051) and *CYP3A4*1B* (unadjusted R^2 = 14.9; p = 0.013) were associated with the log mean stable dose. There was a clear decrease in the required dose, with an increase in the number of variant alleles (Figure 2). Only for *CYP2C9*2/*3* there was a larger decrease in dose for patients with one variant allele than for those with two variant alleles, relative to patients without variant alleles.

Phenprocoumon dosing algorithm

Age, Fontan circulation, *VKORC1*, *CYP2C9*2/*3* and *CYP3A4*1B* were candidates in the multivariable regression analysis (because $p < 0.2$). Age, *VKORC1*, *CYP2C9*2/*3* and *CYP3A4*1B* were entered in the genetic algorithm and explained 80.4% (adjusted R^2) of the variability in the dose requirement of phenprocoumon (Table 2). Using the Bonferroni corrected p-value, *CYP3A4*1B* was not entered in the model and the model explained 78.1% (adjusted R^2) of the variability in the dose requirement, as presented in Table 2 of the supplemental material. Using the backward selection procedure instead of the forward stepwise selection procedure resulted in the same algorithm. When clinical factors alone were included, 55.0% (adjusted R^2) of the variability was explained (Table 2).

The influence of including the genotypes becomes clear when comparing the absolute difference of the predicted dose by the clinical and genetic algorithm with the observed dose (clinical algorithm: median difference of 0.35 mg/day, interquartile range [IQR] = 0.16–0.64 mg/day; genetic algorithm: median difference of 0.19 mg/day, IQR = 0.07–0.37 mg/day), as presented in Figure 3. The two patients who were underestimated by both the genetic and clinical algorithms were older (≥16 years of age) and required a distinctive higher dose compared with other patients of the same age – data not shown.

Table 1. Patient characteristics.

Characteristic	Total cohort (n = 41)
Sex (female), n (%)	20 (48.8)
Age at start of SP in years, median (IQR):	9.3 (3.2–17.0)
– <1, n (%)	2 (4.9)
– 1–3, n (%)	15 (36.6)
– 4–6, n (%)	1 (2.4)
– 7–9, n (%)	4 (9.8)
– 10–12, n (%)	1 (2.4)
– 13–15, n (%)	5 (12.2)
–16–18, n (%)	13 (31.7)
BSA‡, median (IQR)	0.97 (0.62–1.23)
European ethnicity, n (%)	35 (85.4)
Indication for anticoagulation, n (%):	
– Fontan procedure	11 (26.8)
– Prosthetic heart valve	8 (19.5)
– Dilated cardiomyopathy	7 (17.1)
– Deep vein thrombosis/pulmonary embolism	10 (24.4)
– Other†	5 (12.2)
TR, n (%):	
– Extra low (2.0–2.5)	1 (2.4)
– Low (2.0–3.0)	6 (14.6)
– Standard (2.0–3.5)	22 (53.7)
– High (2.5–4.0)	12 (29.3)
Genotypes (0/1/2 variant alleles), n:	
– *VKORC1*	14/18/9
– *CYP2C9*2/*3*	26/13/2
– *CYP3A4*1B*	35/6/0
– *CYP3A4*22*	37/4/0
– *CYP4F2*	18/15/8
– *CYP2C18*	25/16/0

†Consists of supraventricular tachycardia (n = 1), prophylactic/postoperative (n = 3) and cerebral event (n = 2).
‡Calculated using the formula of Haycock and only known for n = 18.
BSA: Body surface area; IQR: Interquartile range; SP: Stable period; TR: Therapeutic international normalized ratio range.

Table 2. Clinical and genotype-guided algorithms.

Algorithm component/characteristic	Genetic algorithm†		Clinical algorithm‡ Coefficients (95% CI)
	Coefficients (95% CI)	Univariate unadjusted R^2 (%)	
Intercept	-0.024	–	-0.214
– Age	0.028 (0.023 to 0.034)	56.2	0.030 (0.21 to 0.38)
– *VKORC1*	-0.128 (-0.179 to -0.076)	16.9	–
– *CYP2C9*2 and *3*	-0.113 (-0.178 to -0.049)	9.4	–
– *CYP3A4*1B*	-0.125 (-0.235 to -0.015)	14.9	–
Unadjusted R^2 of the algorithm (%)	82.4		56.2
Adjusted R^2 of the algorithm (%)	80.4		55.0

†Regression equation: Log daily dose (mg) = -0.024 + 0.028 (age, years) – 0.128 (number of *VKORC1* variant alleles) – 0.113 (number of *CYP2C9*2* and **3* variant alleles) – 0.125 (number of *CYP3A4*1B* variant alleles).
‡Regression equation: Log daily dose (mg) = -0.214 + 0.030 (age, years).

Discussion

With our study we were able to develop a dosing algorithm for phenprocoumon in pediatric patients containing both genetic and clinical factors. More than half of the variability in dose requirement was explained by age. The

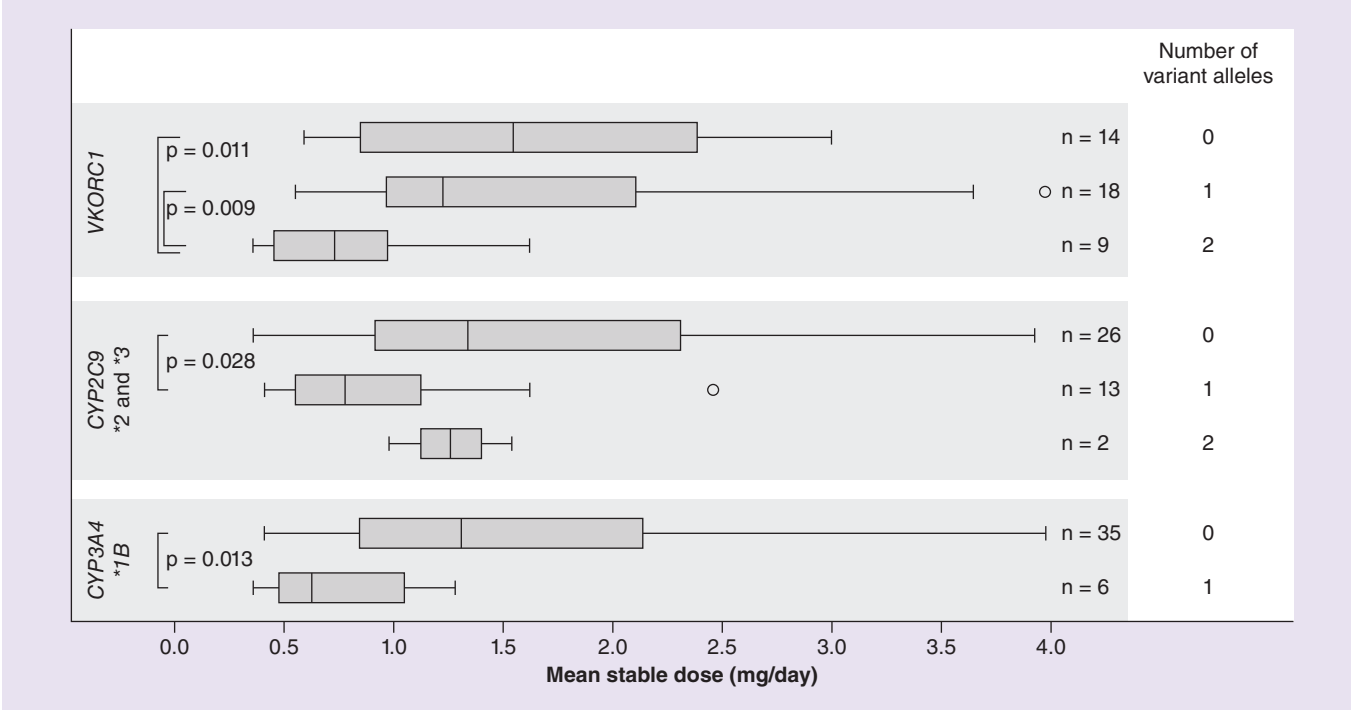

Figure 2. Mean stable dose per number of variant alleles for *VKORC1*, *CYP2C9*2/*3* and *CYP3A4*1B*. The p-values indicated are calculated using the log mean stable dose.

addition of genetic variations in *VKORC1*, *CYP2C9* and *CYP3A4* to the algorithm led to an explanation of 80.4% of the variability in phenprocoumon dose requirement.

Age explained 56.2% of the variability in our cohort. This is slightly higher than what the other studies in pediatric patients found in respect of age or age-related factors (height and weight), which ranges between 12 and 52.8% [4–9]. A possible reason for the higher percentage is our ability to include a relatively large number of young patients (41.5%, <4 years of age). The studies by Shaw *et al.* and Moreau *et al.* – who reported the highest percentages of 52.8% (weight) and 48.1% (height) respectively – also included relatively high percentages of patients under the age of 3 years, in other words, 37.7 and 22.9%, compared with the other published pediatric studies [5,8]. A study in adults revealed that age explained 8.1% of the variability in the phenprocoumon dose requirement [10]. This is substantially lower than the 56.2% we found in our cohort, which can be explained by the fact that age incorporates the difference in body size.

The percentages found for *VKORC1* (16.9%) and *CYP2C9* (9.4%) are in line with the other studies, which found percentages ranging from 2.8 to 47.0%, and 0.3 to 26.6%, respectively [4–9]. To our knowledge, we are the first to demonstrate a statistically significant influence of *CYP3A4*1B* on the required phenprocoumon dose. This SNP was previously studied in adults, demonstrating no statistically significant effect [13–15]. *In vitro*, CYP3A4 has been demonstrated to be an important enzyme for the hydroxylation of phenprocoumon [16]. *CYP3A4*1B* variant alleles have been associated with higher CYP3A4 expression compared with the wild-type [17]. However, multiple *in vivo* studies with drugs that are substrates for CYP3A4, have indicated no altered metabolism linked to *CYP3A4*1B* [18]. We found a dose-lowering effect of *CYP3A4*1B*, which seems counterintuitive and could be a result of multiple testing. When we used a very conservative correction for multiple testing, *CYP3A4*1B* was the only variable that was no longer in the final model. Therefore, replication in a different cohort is required.

Our study has certain limitations. The retrospective study design may have led to misclassification due to misinterpretation of the available data. Moreover, the sample size was relatively small, and the available data were too incomplete to study the effects of concurrent drug therapy, which could explain part of the unexplained variability. This could also be the reason for the underestimation of the required dose by the clinical and genetic algorithm in the case of two patients.

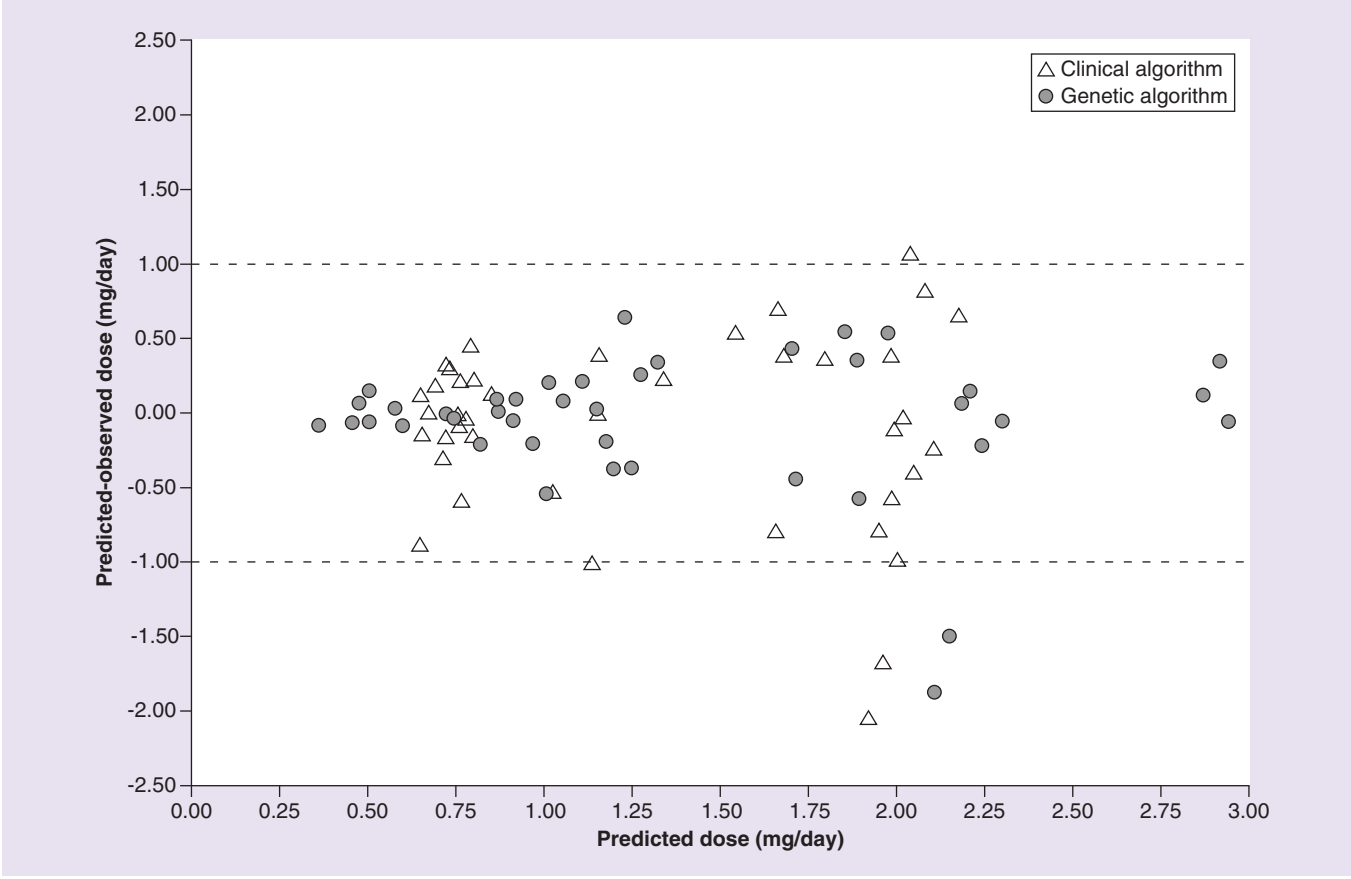

Figure 3. The differences between predicted and observed doses per patient. The differences of the predicted doses with the genetic algorithm (age, *VKORC1*, *CYP2C9*2*/**3*, and *CYP3A4*1B*; circles) and the clinical algorithm (age; triangles) with the observed mean stable doses are plotted against the predicted dose per patient.

In the acenocoumarol cohort of Children Anticoagulation and Pharmacogenetics Study we saw that body surface area explained more of the variability in dose requirement than did age [12]. This could also have been the case for phenprocoumon. We collected height and weight measurements at the start of phenprocoumon use and at random moments during follow-up. The start date of the stable period – at which height and weight measurements should be accurate to be used for developing the model – had not yet been determined at the time that data were collected at the hospitals. Therefore, in less than half of the patients a height and weight measurement was collected that was close enough to the start of the stable period to be accurate at that point in time. Due to the fact that the number of patients in the cohort was already low, we were not able to use height, weight or body surface area in the algorithm.

Our algorithm has been built on a group of pediatric patients treated in different parts of The Netherlands, in which all age groups are represented and includes the most important indications for VKA use. This makes it a representative cohort of pediatric patients using phenprocoumon in The Netherlands. The next step would be to validate the algorithm and to test it prospectively. However, with the low number of pediatric patients starting with phenprocoumon, it is unlikely that the algorithm can be evaluated in a randomized clinical trial. The algorithm can be used as a tool to identify the dose for an individual pediatric patient. At present, it is advised to use the acenocoumarol dosing instructions for starting phenprocoumon therapy. In adults, a higher loading dose is used in phenprocoumon than in acenocoumarol [2]. This suggests that the current way of starting phenprocoumon therapy in pediatric patients would lead to an underestimation of the required dose and a longer period to achieve an INR in TR. This also seems to be the case in our research on the characteristics and quality of acenocoumarol and phenprocoumon therapy in pediatric patients [11]. A lower number of patients using phenprocoumon had an INR in TR within 7 days after start of therapy than patients using acenocoumarol (76.3% for acenocoumarol and 67.7% for phenprocoumon) [11]. This demonstrates the need to optimize the starting dose in the case of phenprocoumon.

We therefore suggest that the genetic algorithm should be used without *CYP3A4*1B* in clinical practice (presented in Table 2 of the supplemental material) and that the algorithm be evaluated and optimized in the future. The effect of *CYP3A4*1B* first needs to be replicated before it can be added to the algorithm and used in clinical practice.

When it comes to implementation, in order for the algorithm to be of benefit, fast genotyping (less than a day) is required. Although in children with congenital heart diseases it is often known well before the start of VKA treatment that such treatment is necessary and therefore more time will be available for genotyping. However, time is not a significant issue. In the EU-PACT trial it was possible to genotype *VKORC1* and *CYP2C9* within 1.5 h using a point-of-care test in a nonlaboratory environment [19]. Furthermore, an increasing number of academic hospitals are offering genetic testing in their laboratories. Genotyping should therefore not be a problem. The costs of genotyping, which are rapidly coming down, should be contrasted to the benefits for the patients. It could be expected that due to a more personalized starting dose the patient would reach TR more quickly and would therefore need to use (low molecular weight) heparins for a shorter period of time. This would lead to a lower burden of injections and fewer INR measurements for the patient.

In conclusion, we have demonstrated that in addition to age, genetic variations in *VKORC1*, *CYP2C9* and *CYP3A4* also explain a significant part of the variability in phenprocoumon dose requirement in pediatric patients (80.4%). The algorithm is likely to improve the current treatment strategy by making it possible for patients to reach TR more quickly.

Summary points

- Genetic and clinical factors influencing the dose of phenprocoumon in pediatric patients have not been widely studied.
- We designed a multicenter retrospective follow-up study in pediatric patients in The Netherlands to study the effects of both clinical and genetic factors on the phenprocoumon dose requirement in pediatric patients and to develop a pharmacogenetic dosing algorithm for phenprocoumon.
- Clinical information was collected from the academic children's hospitals and anticoagulation clinics involved in the treatment of each patient.
- Saliva samples for genotyping were collected to study the relationship between the required phenprocoumon dose and the genotypes of genetic variations in *CYP2C9* (rs1799853 and rs1057910), *VKORC1* (rs9934438), *CYP4F2* (rs2108622), *CYP2C18* (rs1998591) and *CYP3A4* (rs2740574 and rs35599367).
- A total of 41 patients were included in the analysis, with a median age of 9.3 years. 49% were female, and 66% had a cardiac indication for phenprocoumon use.
- Age was positively associated with the required phenprocoumon dose, explaining 56.2% of the variability ($p < 0.001$) in dose requirement.
- Genetic variations in *VKORC1* ($R^2 = 16.9\%$; $p = 0.008$), *CYP2C9*2/*3* ($R^2 = 9.4$; $p = 0.051$) and *CYP3A4*1B* ($R^2 = 14.9$; $p = 0.013$) were all negatively associated with the required phenprocoumon dose.
- The dosing algorithm containing age and the genetic variations in *VKORC1*, *CYP2C9*2/*3* and *CYP3A4*1B* explained 80.4% of the variability in phenprocoumon dose requirement in pediatric patients.

Supplementary data

To view the supplementary data that accompany this paperplease visit the journal website at:

https://www.futuremedicine.com/doi/suppl/10.2217/xxx-xxx-xxx

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Financial & competing interests disclosure

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involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

The manuscript has been checked by an English native speaker.

Ethical conduct of research

The Medical Ethics Review Committee (MERC) of the University Medical Center Utrecht stated that the Medical Research Involving Human Subjects Act (WMO) does not apply to the study and official approval by MERC is not required under the WMO. The UPPER Institutional Review Board of the Division of Pharmacoepidemiology and Clinical Pharmacology of Utrecht University approved the study protocol. All participants (and/or their parents or legal guardians) provided informed consent before taking part.

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