Journal of Thrombosis and Haemostasis, **16**: 1732–1742 DOI: 10.1111/jth.14211

ORIGINAL ARTICLE

The pediatric acenocoumarol dosing algorithm: the Children Anticoagulation and Pharmacogenetics Study

H. MAAGDENBERG,* M. B. BIERINGS,† C. H. VAN OMMEN,‡ F. J. M. VAN DER MEER,§ I. M. APPEL,‡ R. Y. J. TAMMINGA,¶ S. LE CESSIE,**†† J. J. SWEN,‡‡ T. VAN DER STRAATEN,‡‡ A. DE BOER* and A. H. MAITLAND-VAN DER ZEE*§§

**Division of Pharmacoepidemiology and Clinical Pharmacology, Faculty of Science, Utrecht Institute for Pharmaceutical Sciences, Utrecht University; †Department of Pediatric Hematology and Stem Cell Transplantation, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht; ‡Department of Pediatric Oncology/Hematology, Sophia Children's Hospital, Erasmus University Medical Center, Rotterdam; §Department of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden; ¶Department of Pediatric Hematology, University Medical Center Groningen, Groningen; **Department of Medical Statistics, Leiden University Medical Center; ††Department of Clinical Epidemiology, Leiden University Medical Center; ‡‡Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden; and §§Department of Respiratory Medicine, Amsterdam Medical Center, University of Amsterdam, Amsterdam, the Netherlands*

To cite this article: Maagdenberg H, Bierings MB, van Ommen CH, van der Meer FJM, Appel IM, Tamminga RYJ, le Cessie S, Swen JJ, van der Straaten T, de Boer A, Maitland-van der Zee AH. The pediatric acenocoumarol dosing algorithm: the Children Anticoagulation and Pharmacogenetics Study. *J Thromb Haemost* 2018; **16**: 1732–42.

Essentials

- A pediatric pharmacogenetic dosing algorithm for acenocoumarol has not yet been developed.
- We conducted a multicenter retrospective follow-up study in children in the Netherlands.
- Body surface area and indication explained 45.0% of the variability in dose requirement.
- Adding the genotypes of *VKORC1*, *CYP2C9* and *CYP2C18* to the algorithm increased this to 61.8%.

Summary. *Background:* The large variability in dose requirement of vitamin K antagonists is well known. For warfarin, pediatric dosing algorithms have been developed to predict the correct dose for a patient; however, this is not the case for acenocoumarol. *Objectives:* To develop dosing algorithms for pediatric patients receiving acenocoumarol with and without genetic information. *Methods:* The Children Anticoagulation and Pharmacogenetics Study was designed as a multicenter retrospective follow-up study in Dutch anticoagulation clinics and children's hospitals. Pediatric patients who used acenocoumarol between 1995 and 2014 were selected for inclusion. Clinical information and saliva samples for genotyping of the genes encoding cytochrome P450 (CYP) 2C9, vitamin K epoxide reductase complex subunit 1 (VKORC1), CYP4F2, CYP2C18 and CYP3A4 were collected. Linear regression was used to analyze their association with the log mean stable dose. A stable period was defined as three or more consecutive International Normalized Ratio measurements within the therapeutic range over a period of ≥ 3 weeks. *Results:* In total, 175 patients were included in the study, of whom 86 had a stable period and no missing clinical information (clinical cohort; median age 8.9 years, and 49% female). For 80 of these 86 patients, genetic information was also available (genetic cohort). The clinical algorithm, containing body surface area and indication, explained 45.0% of the variability in dose requirement of acenocoumarol. After addition of the *VKORC1*, *CYP2C9*, and *CYP2C18* genotypes to the algorithm, this increased to 61.8%. *Conclusions:* These findings show that clinical factors had the largest impact on the required dose of acenocoumarol in pediatric patients. Nevertheless, genetic factors, and especially *VKORC1*, also explained a significant part of the variability.

Keywords: acenocoumarol; adolescent; child; coumarins; infant; pharmacogenetics.

Correspondence: Anthonius de Boer, Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, PO Box 80.082, 3508 TB, Utrecht, the Netherlands
Tel.: +31 6 027 9938
E-mail: a.deboer@uu.nl

Received: 14 March 2018
Manuscript handled by: J. Douketis
Final decision: F. R. Rosendaal, 8 June 2018

Introduction

Vitamin K antagonists (VKAs) can be used to treat or prevent thromboembolic events in pediatric patients.

Dosing of VKAs in pediatric patients is complex, partly because of the developing hemostatic system in these patients. In this population, VKA pharmacokinetics are age-dependent, with younger patients requiring a higher dose per kilogram of body weight [1]. A limited number of studies have investigated the dosing of acenocoumarol in pediatric patients. Therefore, current pediatric guidelines generally use extrapolations of adult dosing recommendations [2–4]. Only one study has investigated the initial acenocoumarol doses needed to achieve the target International Normalized Ratio (INR) in pediatric patients [5]. These initial doses are based on weight and age group, and are incorporated in the guideline of the Dutch Federation of Anticoagulation Clinics [6]. In 2009, Spoor *et al*. studied the initial and maintenance doses in pediatric patients for acenocoumarol and phenprocoumon in the Netherlands. The doses recommended in the guidelines seem to be safe, but are not yet optimal, with percentages of time in which the INR is within the therapeutic range (time in therapeutic range [TTR]) of around 50% during the first year of treatment [7]. Within the cohort of the Children Anticoagulation and Pharmacogenetics Study, we obtained similar results within the first 3 months of acenocoumarol use (54.6%), and higher percentages after the first 3 months of use (> 64%) [8].

There is large interindividual and intraindividual variability in VKA dose requirements. The dose is influenced by many factors, such as height, weight, age, sex, indication for VKA treatment, concurrent drug therapy, and vitamin K intake. Besides these factors, variations in certain genes can influence the dose requirement. The genes that are now known to have the largest influence are those encoding cytochrome P450 (CYP) 2C9 and vitamin K epoxide reductase complex subunit 1 (VKORC1). Single-nucleotide polymorphisms (SNPs) in *CYP2C9* (*CYP2C9*2* and *CYP2C9*3*; rs1799853 and rs1057910, respectively) reduce the metabolism of VKAs, and an SNP in *VKORC1* (rs9934438) increases sensitivity to VKAs. In both cases, the required dose is lower for patients with the variant than for those with the wild-type genotype, to prevent overanticoagulation [9]. The frequency of carrying one or two variant alleles (*CYP2C9*2* or *CYP2C9*3*) in *CYP2C9* is 35.1% in the European population [10]. For *VKORC1*, 61.8% of the European population has one or two variant alleles [10]. Polymorphisms in or flanking *CYP4F2* (rs2108622), *CYP2C18* (rs1998591) and *CYP3A4* (*CYP3A4*1B* and *CYP3A4*22*; rs2740574 and rs35599367, respectively) have also been shown to explain part of the variation in adults [11–14].

For warfarin, a dosing algorithm was constructed for adults by the International Warfarin Pharmacogenetics Consortium [15]. In a study by Biss *et al*., the maintenance dose in pediatric patients was correlated with the calculated doses by the use of this algorithm; however, there was a continuous non-linear overestimation of the dose [16]. This indicated that there is a need for a specific algorithm for pediatric patients. Up to now, several studies have been carried out to create a warfarin pharmacogenetic dosing algorithm for pediatric patients [16–23]. However, to the best of our knowledge, no studies have been carried out to establish an algorithm for acenocoumarol in pediatric patients.

The aim of this study was to develop two dosing algorithms for acenocoumarol in pediatric patients: one algorithm with genetic information, and one without genetic information. The latter can be applied when genotyping is not (yet) available.

Methods

Study design and patient collection

We performed a retrospective follow-up study in four academic pediatric hospitals (Emma Children's Hospital Amsterdam, Wilhelmina Children's Hospital Utrecht, Sophia Children's Hospital Rotterdam, and Beatrix Children's Hospital Groningen) and the Leiden anticoagulation clinic in the Netherlands. Patients who used acenocoumarol for > 1 month between January 1995 and December 2014 and who were aged ≤ 18 years at the time of acenocoumarol use were eligible for participation. The follow-up of a patient ended at the end date of data collection at the anticoagulation clinic (between 11 January 2014 and 10 March 2016), when they reached 19 years of age, when they stopped receiving acenocoumarol therapy, or when they were lost to follow-up. A sample size of 110 patients was required to be able to detect a difference of 1 mg daily between the *CYP2C9*2* and *CYP2C9*3* genotypes ($\alpha = 0.05$ [two-sided]; power = 80%; standard deviation of 2.1 mg in the outcome [16]).

The Medical Ethics Review Committee of the University Medical Center Utrecht decided that the study did not need ethical approval, because non-invasive 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.

Data collection

Participants and/or their parents were asked to fill in a questionnaire. They were asked for their ethnicity, whether breastfeeding had occurred during therapy, and, if applicable, whether the breastfeeding was combined with vitamin K use by the mother.

To obtain complete data for all patients, data were collected at both the hospital where they were treated and at their anticoagulation clinic. Information on the therapeutic INR range (TR), INR values and acenocoumarol

doses was collected at the anticoagulation clinics. Information on the indication for acenocoumarol therapy, date of birth, sex, TR, INR and acenocoumarol doses was collected at the hospital. Furthermore, information on height and weight was collected at the hospital at the start of and during acenocoumarol therapy.

Genotyping

Saliva was used for collection of DNA. Saliva collection packages were sent to the participants after they had signed informed consent forms. The Oragene·DNA (DNA Genotek, Ottawa, Canada) (OG-575) kit for Assisted Collection was used for participants aged between 0 years and 4 years, and the Oragene·DNA (OG-250) kit was used for participants aged > 4 years. Genotyping was performed by the laboratory of the Leiden University Medical Center by use of a LightCycler 480 with a TaqMan SNP genotyping assay (ThermoFisher, Waltham, MA, USA). The following SNPs were genotyped: *VKORC1* rs9934438, *CYP2C9* rs1799853 and rs1057910, *CYP4F2* rs2108622, *CYP3A4* rs35599367 and rs2740574, and *CYP2C18* rs1998591. Only genotypes of the SNPs that were in Hardy–Weinberg equilibrium ($P \geq 0.05$) were included in the analysis.

Outcome and determinants

The outcome of interest was the stable maintenance dose (in mg daily) defined as the mean dose during the first stable period after initiation without missing information on dose and INRs. A stable period was defined as three or more 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.

For the development of the algorithm, the following determinants were used: age at start of stable period, sex, indication for anticoagulation, TR, weight, height, and body surface area (BSA) calculated with the formula of Haycock. For the genotype-guided algorithm, the *VKORC1*, *CYP2C9*, *CYP4F2*, *CYP3A4* and *CYP2C18* genotypes were also used as determinants (number of variant alleles).

Height and weight change continuously in pediatric patients; hence, the last available measurement could be outdated at the start of the stable period. Therefore, we constructed time windows by using the World Health Organization growth tables of height and weight for age [24–27]. For each age, the duration of time in which the mean height increased by ≤ 5 cm and the mean weight by ≤ 2 kg was determined. We considered height and weight to be still valid when measured within the time window. The applied time windows per age at the start of the stable period are shown in Table S1. Only weight and height values that were valid according to the above time windows were used.

Statistical analysis and algorithm development

Two algorithms were generated for acenocoumarol: one with genetic information (genetic model) and one without genetic information (clinical model). Linear regression was used to model the relationships between stable dose as outcome and determinants. Determinants that were univariately associated with outcome ($P < 0.2$) were used as candidate variables for the algorithm. Two transformations of the outcome were considered, i.e. 10-log and square root transformation, to establish whether transformation would, by visual inspection, improve the normal distribution. If this was the case, the transformation by which the outcome was visually most normally distributed was used. A forward stepwise selection procedure was used for the multivariate analysis, in which all determinants with a *P*-value of < 0.05 were entered into the model.

To analyze differences in mean dose between categories of a determinant, a Student's *t*-test or a one-way ANOVA was used.

A sensitivity analysis was conducted in which, instead of a forward stepwise selection procedure, a backward selection procedure was used. A second sensitivity analysis was conducted to check whether defining the stable period more strictly (three or more consecutive INR measurements within the TR over a period of ≥ 4 weeks and no dose changes of > 10%) would lead to different results with regard to R^2, covariates entered in the model, and the β estimates.

The observed mean stable dose was compared with the doses predicted by the genetic model and the doses calculated on the basis of weight and age group as described in the guideline of the Dutch Federation of Anticoagulation Clinics [6]. They were compared by use of a Wilcoxon signed rank test.

Statistical analysis was carried out with SPSS version 23.0.

Results

Patient characteristics

In total, 175 patients were included in the study, of whom 123 had a stable period and dose information available. The patients with valid weight and height information were included in the clinical cohort ($n = 86$). Of these 86 patients, genetic analysis failed in one, and no saliva sample was collected for five. Therefore, for 80 patients, genetic information was available, and these were included in the genetic cohort (Fig. 1). An overview of the characteristics of the cohorts (including indications for anticoagulation) is shown in Table 1. The cohorts consisted of similar numbers of females and males, with median ages of 8.9 years (clinical cohort) and 9.7 years (genetic cohort). Most patients were treated with the standard-intensity (2.5–3.5) or high-intensity (3.0–4.0) TRs that were used at the time

of the study in the Netherlands. No patients were breastfed during the stable period.

Genetic characteristics

The genotype distribution of the SNPs in the five studied genes with the observed mean stable doses are shown in Table 2. Almost all genotypes showed a trend of decreasing dose with an increase in number of variant alleles, except for *CYP3A4*1B*, for which a trend in the opposite direction was shown. All genotypes were in Hardy–Weinberg equilibrium, and all SNPs had call rates of ≥ 99.5%.

Association of clinical and genetic variables with acenocoumarol dose

From the tested transformations, the 10-log transformation of the mean stable dose resulted in the visually most normal distribution, and was therefore used.

High associations existed between log mean stable dose and age (R^2 = 30.5; $P < 0.001$), height (R^2 = 36.9; $P < 0.001$), weight (R^2 = 36.5; $P < 0.001$), and BSA (R^2 = 40.4; $P < 0.001$). Patients with a Fontan circulation required a statistically significantly lower log mean stable dose than the other patients (mean difference of 1.10 mg; 95% confidence interval (CI) [0.60–1.60]; $P < 0.001$), as shown in Fig. 2. This could not be explained by the fact that Fontan patients in general have a lower TR. When Fontan patients were compared with non-Fontan patients stratified per TR group, the mean dose was always lower in Fontan patients than in non-Fontan patients (Table S3). The TR was not statistically significantly associated with the log mean stable dose (R^2 = 4.2%; P = 0.058). The median of the individual mean observed doses per TR seemed to increase when the TR increased, as shown in Fig. 3. However, the observed mean stable doses of patients with a TR of 2.0–3.5 reached from the lowest to the highest observed mean stable dose in all patients in the cohort.

VKORC1, *CYP2C9*2*/*CYP2C9*3* and *CYP3A4*22* were associated with a lower log mean stable dose when the number of variant alleles increased (*VKORC1*, R^2 = 19.2%, $P < 0.001$; *CYP2C9*2*/*CYP2C9*3*, R^2 = 3.9%, P = 0.080; *CYP3A4*22*, R^2 = 4.2%, P = 0.067). The log mean stable dose was statistically significantly higher in patients with the *VKORC1* GG genotype than in patients with the AG genotype (mean difference of 0.93 mg; 95% CI [0.29–1.58]; P = 0.010) or AA genotype (mean difference of 1.35 mg; 95% CI [0.48–2.21]; $P < 0.001$), as shown in Fig. 2. For *CYP2C9*2*/*CYP2C9*3* and *CYP3A4*22*, there were no statistically significant differences in mean stable dose between the genotypes.

No associations with P-values of < 0.2 were found for ethnicity, sex, *CYP4F2*, or *CYP3A4*1B*.

Multivariate linear regression

From the univariate analysis, BSA, Fontan circulation, TR, *VKORC1*, *CYP2C18*, *CYP3A4*22* and *CYP2C9*2*/*CYP2C9*3* were used as candidate variables in the multivariate regression analysis (because $P < 0.2$). For the

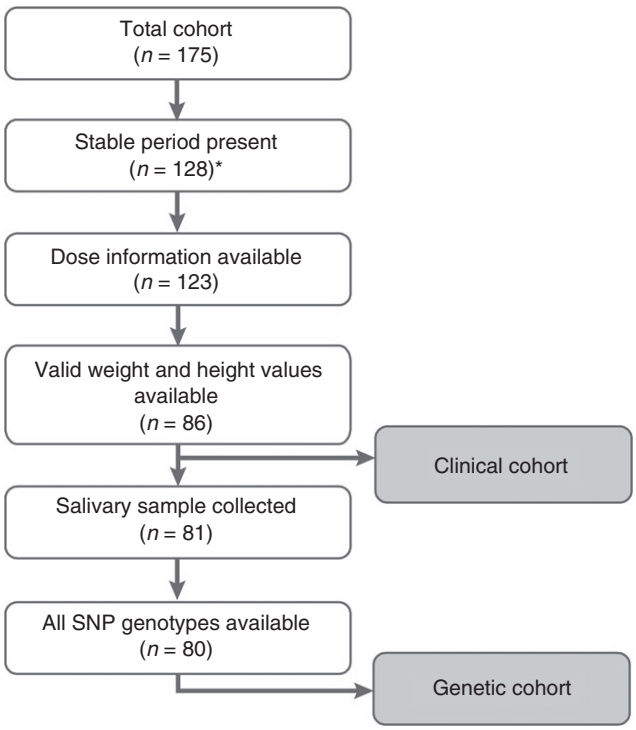

Fig. 1. Flowchart of patients included in the cohort for the clinical algorithm and genetic algorithm. *More information on the 47 patients who were excluded because of missing a stable period can be found in Table S2. SNP, single-nucleotide polymorphism.

Table 1 Patient characteristics at start of stable period

	Clinical cohort* (n = 86)	Genetic cohort* (n = 80)
Female sex, *n* (%)	42 (48.8)	40 (50.0)
Age (years), median (IQR)	8.9 (4.2–13.3)	9.7 (4.2–14.0)
<1, *n* (%)	2 (2.4)	2 (2.5)
1–3, *n* (%)	16 (18.8)	15 (19.0)
4–6, *n* (%)	18 (21.2)	15 (19.0)
7–9, *n* (%)	10 (11.8)	9 (11.4)
10–12, *n* (%)	12 (14.1)	12 (15.2)
13–15, *n* (%)	13 (15.3)	12 (15.2)
16–18, *n* (%)	14 (16.5)	14 (17.7)
Ethnicity, *n* (%)		
European	73 (84.9)	68 (85.0)
Asian	2 (2.3)	2 (2.5)
African	2 (2.3)	2 (2.5)
Others	4 (4.7)	4 (5.0)
Unknown	5 (5.8)	4 (5.0)
Indication for anticoagulation, *n* (%)		
Fontan circulation	26 (30.2)	21 (26.3)
Prosthetic heart valve	24 (27.9)	23 (28.7)
Dilated cardiomyopathy	9 (10.5)	9 (11.3)
Deep vein thrombosis/ pulmonary embolism	18 (20.9)	18 (22.5)
Aneurysm	1 (1.2)	1 (1.3)
Pulmonary hypertension	3 (3.5)	3 (3.8)
Other cardiac indication†	3 (3.5)	3 (3.8)
Cerebral	2 (2.3)	2 (2.5)
TR, *n* (%)		
Extra-low (2.0–2.5)	2 (2.3)	2 (2.5)
Low (2.0–3.0)	18 (20.9)	15 (16.9)
Standard (2.5–3.5)	43 (50.0)	42 (54.5)
High (3.0–4.0)	22 (25.6)	20 (24.7)
Extra high (3.5–4.5)	1 (1.2)	1 (1.3)
BSA‡, median (IQR)	0.98 (0.67–1.38)	1.00 (0.68–1.38)

BSA, body surface area; IQR, interquartile range; TR, therapeutic International Normalized Ratio range. *The genetic cohort is derived from the clinical cohort, with exclusion of six patients without genetic information available. †Consists of supraventricular tachycardia and an unspecified arrhythmia. ‡Calculated with the formula of Haycock.

clinical model, all variables except for the genetic ones were used.

High correlation existed between age, height, weight, and BSA (all $r > 0.872$), which resulted in multicollinearity when more than one was entered into the model. BSA had the highest univariate R^2 of these four variables, and incorporates all variables. Therefore, only BSA was used as a candidate variable in the multivariate linear model.

BSA, Fontan circulation, *VKORC1*, *CYP2C18* and *CYP2C9*2*/*CYP2C9*3* were entered into the genetic model (adjusted R^2 = 61.8%; Table 3). The clinical model containing BSA and Fontan circulation explained 45.0% of the variability in the dose requirement of acenocoumarol.

Sensitivity analysis

Changing the selection procedure to backward selection resulted in exactly the same models.

When the stricter definition of a stable period was used, 47 patients could be included in the analysis. Multivariable analysis resulted in a genetic algorithm consisting of BSA, *VKORC1* genotype, and *CYP2C9* genotype (adjusted R^2 = 62.2%). The coefficients were similar to those generated with the cohort with the less strict definition. The clinical algorithm consisted only of BSA and explained 42.6% of the variability in the dose requirement. Data are shown in Tables S4–S6.

When age was used instead of BSA, 116 patients could be included in the genetic cohort. The same variables with comparable coefficients were found, with an adjusted R^2 of 54.4%. The clinical algorithm (n = 123) with age and Fontan circulation explained only 37.9% of the variability in the dose requirement. Data are shown in Tables S7–S9.

Guideline versus the genetic algorithm

Overall, the current guideline of the Dutch Federation of Anticoagulation Clinics shows a slight trend of underestimating the dose for homozygous wild-type genotypes and overestimating the dose for homozygous variant genotypes (Table 2). For the *VKORC1* AA genotype, homozygous variant type, there was a statistically significant overestimation of the dose according to the guideline as compared with the required observed stable dose (median difference of 0.44 mg; interquartile range [IQR] 0.28–0.98; P = 0.019). Furthermore, for the *CYP2C18* GG genotype, homozygous wild-type, a statistically significant underestimation of the dose was observed (median difference of − 0.20 mg; IQR − 0.88 to 0.25; P = 0.025).

Figure 4 shows that the difference between the predicted and the observed dose was small for most patients (median differences of 0.38 mg [genetic model] and 0.54 mg [clinical model and current guideline]). With the genetic model, 82.5% of the patients had a predicted dose that was ≤ 1 mg higher or lower than the observed dose. For the clinical model and the current guideline, these proportions were 77.5% and 75%, respectively. Two patients (cases 6 and 11) had large overestimations of the dose by the genetic model (differences of > 2 mg). Neither patient had a Fontan circulation or variant *VKORC1*, *CYP2C9* and *CYP2C18* genotypes. However, they had obesity (body mass index [BMI] > 30) and had a distinctively higher BSA than other patients of the same age (Fig. S1). Four patients (cases 16, 55, 62, and 68) had large underestimations of the dose (> 2 mg) by the clinical model and the guideline (and for case 68, also the genetic model). These patients did not have variant *VKORC1*, *CYP2C9* and *CYP2C18* genotypes or a Fontan circulation. However, they had higher observed doses than other patients of similar age (Fig. S2).

Discussion

To the best of our knowledge, this is the first pediatric cohort in which the effects of clinical and genetic

Table 2 Distribution of genotypes and observed, predicted and guideline-based daily dose

	n (%)	Observed dose, median (IQR)*	Predicted dose, median (IQR)*	Guideline-based dose, median (IQR)*†
VKORC1				
GG‡	33 (41.3)	2.52 (1.70–3.64)	2.34 (1.56–3.11)	1.89 (1.47–2.50)
AG	34 (42.5)	1.66 (1.14–2.29)	1.38 (1.22–2.21)	1.44 (1.02–1.95)
AA	13 (16.3)	1.00 (0.71–1.68)	1.35 (0.95–1.48)	1.63 (1.56–2.03)§
*CYP2C9*2/CYP2C9*3*				
CC/AA‡	50 (62.5)	1.88 (1.29–2.83)	1.63 (1.35–2.5)	1.66 (1.32–2.10)
CC/CA	10 (12.5)	1.21 (0.71–2.55)	1.05 (0.91–2.15)	1.71 (1.30–2.35)
CT/AA	17 (21.3)	1.70 (1.14–2.64)	1.64 (1.4–2.05)	1.63 (1.30–2.30)
CT/CA	2 (2.5)	1.51 (0.81–2.22)	1.71 (0.9–2.53)	2.38 (1.55–3.2)
TT/AA	1 (1.3)	1.44 (1.44–1.44)	1.10 (–)	0.90 (–)
CYP2C18				
GG‡	46 (57.5)	1.85 (1.15–3.24)	1.64 (1.35–2.50)	1.56 (1.18–2.04)§
AG	29 (36.3)	1.62 (1.12–2.37)	1.48 (1.07–2.29)	1.76 (1.43–2.50)
AA	5 (6.3)	1.83 (0.71–1.89)	1.56 (0.95–1.87)	2.08 (1.70–2.15)
CYP4F2				
CC‡	40 (50.0)	1.80 (1.29–3.13	–	–
CT	37 (46.3)	1.68 (1.14–2.37)	–	–
TT	3 (3.8)	2.27 (0.81–3.02)	–	–
*CYP3A4*22*				
GG‡	67 (83.8)	1.84 (1.15–2.83)	–	–
GA	13 (16.3)	1.28 (0.81–1.83)	–	–
*CYP3A4*1B*				
TT‡	74 (92.5)	1.70 (1.14–2.63)	–	–
TC	6 (7.5)	1.98 (1.84–2.64)	–	–

CYP, cytochrome P450; IQR, interquartile range; VKORC1, vitamin K epoxide reductase complex subunit 1; –, not applicable. *Doses are shown as mg daily. †The dose is calculated on the basis of the dosing information of the guideline of the Dutch Federation of Anticoagulation Clinics [6]. ‡Wild-type genotype. §Difference between observed and guideline-based dose, $P < 0.05$.

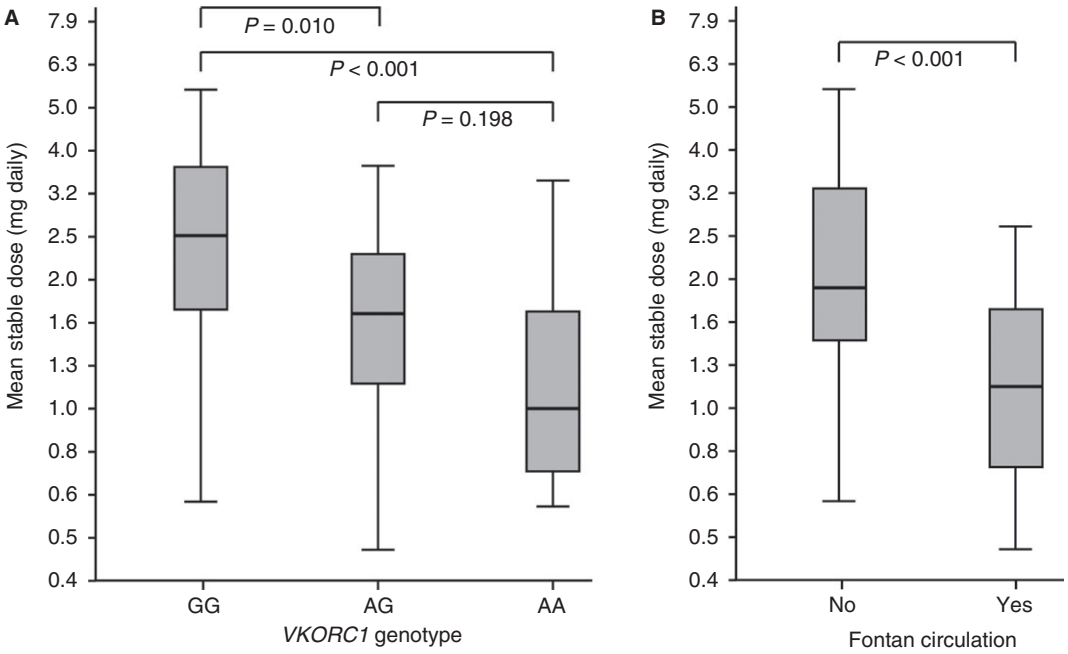

Fig. 2. Differences in mean stable dose between vitamin K epoxide reductase complex subunit 1 (*VKORC1*) genotypes and patients with or without a Fontan circulation. (A) A boxplot of the mean stable dose in mg daily per *VKORC1* genotype in the genetic cohort. (B) A boxplot of patients with and without a Fontan circulation in the clinical cohort (B). The *P*-values shown were calculated by use of the log mean stable dose.

factors on the acenocoumarol dose requirement have been studied. It shows that almost two-thirds of the variability in acenocoumarol dose requirement can be explained by BSA, Fontan circulation, and *VKORC1*, *CYP2C9* and *CYP2C18* genotypes. Almost half of the total variability can be explained by clinical factors.

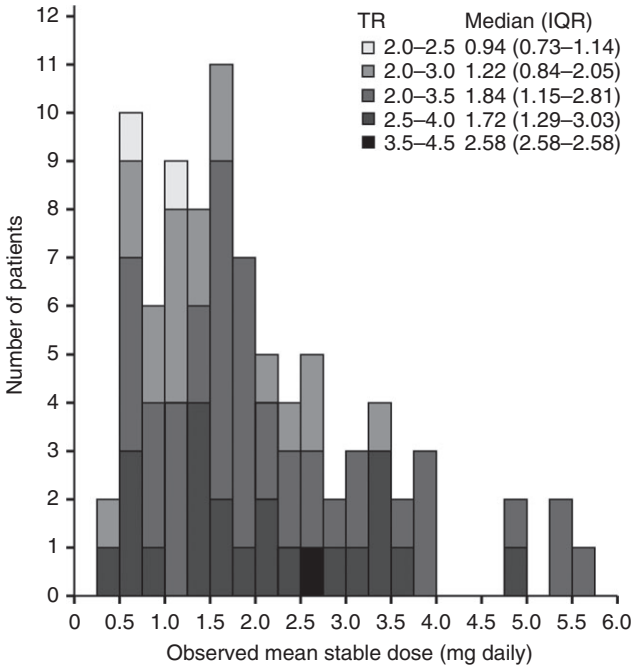

Fig. 3. The observed mean stable dose per therapeutic International Normalized Ratio range (TR) in the clinical cohort. IQR, interquartile range.

Table 3 Genetic and clinical algorithm

	Genetic algorithm* (n = 80)		Clinical algorithm† (n = 86)	
	Coefficients (95% CI)	Univariate unadjusted R^2 (%)	Coefficients (95% CI)	Univariate unadjusted R^2 (%)
Intercept	0.105	–	− 0.061	–
BSA	0.316 (0.228 to 0.404)	38.3	0.319 (0.215 to 0.424)	40.4
Fontan circulation	− 0.102 (− 0.187 to − 0.017)	17.3	− 0.149 (− 0.247 to − 0.051)	22.3
VKORC1	− 0.120 (− 0.171 to − 0.069)	19.2	–	–
CYP2C18	− 0.084 (− 0.146 to − 0.022)	4.4	–	–
*CYP2C9*2* and *CYP2C9*3*	− 0.090 (− 0.155 to − 0.026)	3.9	–	–
Unadjusted R^2 of the algorithm (%)	64.3		46.3	
Adjusted R^2 of the algorithm (%)	61.8		45.0	

BSA, body surface area; CI, confidence interval; CYP, cytochrome P450; VKORC1, vitamin K epoxide reductase complex subunit 1; –, not applicable. *Regression equation: log daily dose (mg) = 0.105 + 0.316 (BSA, m2) − 0.102 (Fontan circulation, yes = 1; no = 0) − 0.120 (number of *VKORC1* variant alleles) − 0.084 (number of *CYP2C18* variant alleles) − 0.090 (number of *CYP2C9*2* and *CYP2C9*3* variant alleles). †Regression equation: log daily dose (mg) = − 0.061 + 0.319 (BSA, m2) − 0.149 (Fontan circulation, yes = 1; no = 0).

With our data, we were able to develop both a clinical and a genetic dosing algorithm for acenocoumarol in pediatric patients.

Age or age-related factors have been shown to be important explanatory factors for the variability in dose requirement in all pediatric pharmacogenetic studies, ranging from 12% to 52.8% [16–21,23]. Mostly, age was used, but sometimes height or weight was also used. We decided to use BSA, which incorporates both height and weight, and had the highest correlation with the required maintenance dose in our cohort. BSA explained 38.3% of the variability in dose requirement. The choice of BSA resulted in a marked decrease in sample size, because a large number of patients had no (valid) BSA information available. The number of patients (80) included in the genetic cohort is lower than the number required (110) on the basis of the sample size calculation. However, we found a standard deviation of 1.26 mg in the outcome for *CYP2C9* (data not shown), which is lower than the 2.1 mg used in the sample size calculation. Consequently, a power of 97.7% was achieved with these 80 patients. The sensitivity analysis in which age was used instead of BSA led to a similar model (same variables and similar coefficients). This shows that the smaller sample size of the cohort when BSA was used did not materially influence the model or decrease the chance of other factors being included in the model.

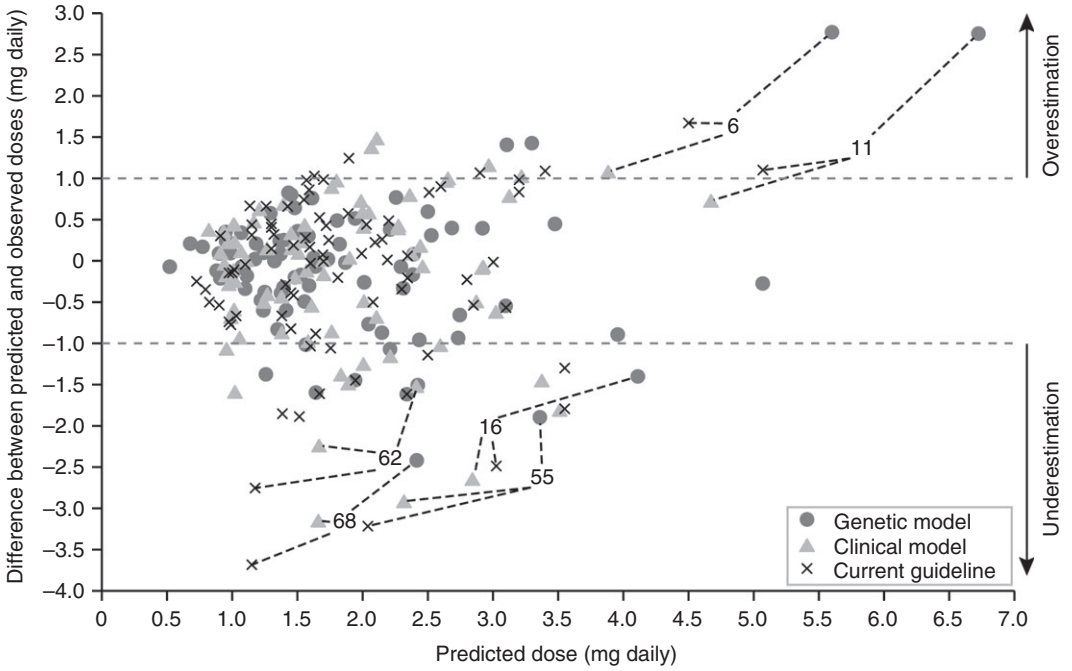

Fig. 4. The differences between predicted and observed doses within the genetic cohort. The differences of the predicted doses with the genetic model (dots), clinical model (triangles) and current guideline (crosses) from the observed mean stable doses are plotted against the predicted dose. There was a large overestimation (> 2 mg) of the dose by the genetic model for cases 6 and 11, and large underestimations (> 2 mg) of the dose by, especially, the clinical model and the guideline for cases 16, 55, 62, and 68.

Patients with a Fontan circulation required a lower acenocoumarol dose than patients without a Fontan circulation. The presence of a Fontan circulation explained 17.3% of the variability. This is substantially higher than the 3.2% and 2.4% found in two other studies [16,21]. Patients with a Fontan circulation were, on average, younger than the other patients and had therefore a lower BSA. Adding Fontan circulation to the model next to BSA resulted in only a minor change in the unadjusted R^2 (6%). Furthermore, the TR could have influenced the dose in patients with a Fontan circulation. In the Netherlands, the range between 2.0 and 3.0 is mostly used for patients with a Fontan circulation (in our genetic cohort, 60% of the patients with this TR had a Fontan circulation). However, we showed that patients with a Fontan circulation required a lower dose even when stratified on the TR. The remaining dose-lowering effect of a Fontan circulation can probably be explained by abnormalities in these patients in liver function and coagulation [28,29].

In line with other studies, *VKORC1* is the most important genetic factor, and explains a larger part of the variability (19.2%) than *CYP2C9*2* and *CYP2C9*3* (3.9%). The percentages of the explained variability fluctuate between the different studies, from 2.8% to 47% [18,20] for *VKORC1*, and from 0.3% to 26.6% for *CYP2C9* [16,18]. The reasons for this fluctuation could be the differences in patient characteristics and the small sample sizes of many studies.

In line with the findings of Teichert *et al.* in adults, we showed an association with the SNP (rs1998591) flanking *CYP2C18* and the acenocoumarol dose [12]. We found that patients with the variant allele needed a lower acenocoumarol dose. The role of this SNP in the metabolism of acenocoumarol is still unclear, which makes interpreting these results difficult.

The ontogeny of the associated CYP450 enzymes and that of the target enzyme of acenocoumarol are similar. They already approach adult levels in early childhood. After birth, the concentration of CYP2C9 quickly increases, and adult values are already approached in the early years of life [30,31]. However, there is variation in expression levels at every age. Little is known about the ontogeny of CYP2C18, but it seems to have a similar ontogeny to that of CYP2C9 [32]. VKORC1 shows low activity in liver tissue in the early prenatal period. After the postnatal period, the activity stabilizes at adult values [33]. Therefore, the influences of ontogeny on the results are most likely minimal.

We did not find a statistically significant association for *CYP4F2*, *CYP3A4*22*, and *CYP3A4*1B*, which is in line with all other pediatric studies [17,21–23]. Furthermore, there was no statistically significant association between the TR and dose requirement. This is in line with the results of three other pediatric studies with warfarin [16,18,21]. Only two studies showed an association between dose and the TR, explaining 4.4% and 18% of the variation in warfarin dose requirement [17,20]. We showed that the observed doses per TR were overlapping substantially

(Fig. 3). Our study was probably underpowered to make a distinction between the overlapping TRs. However, we do not think that the absence of the TR from the model is a problem, because the effect seems to be small. Furthermore, the current guideline also does not use the TR as a factor to determine the dose for a patient [6].

The limitations of our study mainly concern the retrospective data collection. The information on the doses and INRs might be incorrect or incomplete. Furthermore, information on concurrent (interacting) drug therapy was not taken into account. This information was often incomplete or missing from the patient records. No information on the patients' diet (vitamin K intake) was available. Incorporating concurrent drug therapy and diet in the model could have increased the percentage of explained variability in dose requirement. The lack of this information could possibly reduce the external validity. This could also have explained the large underestimation of the predicted doses by the clinical model and the guideline, which was seen in four patients. Furthermore, the number of patients aged < 1 year was very low, which possibly reduces the validity of this model for these patients. The genetic model overestimated the dose for obese patients with a BMI of > 30.

The next step towards clinical use would preferably be the validation of the model in another cohort. However, the low number of pediatric patients using acenocoumarol makes this highly challenging. Therefore, we suggest that the genetic model could also be implemented and evaluated in a clinical setting. We believe that the model can help to get patients more quickly in the TR, allowing the number of INR measurements to be reduced and the TTR to be increased. Using the model without validation does not increase risks. Most pediatric patients start with acenocoumarol in inpatient settings, and dose adjustments can still be made on the basis of INR measurements. Furthermore, we showed a trend for the current guideline to overestimate the dose for patients with a homozygous variant-type genotype of *VKORC1*, *CYP2C9*2*/*CYP2C9*3*, and *CYP2C18*, and to underestimate the dose for patients with a homozygous wild-type genotype for these genes. On the other hand, implementing and evaluating the algorithm in clinical setting costs money for genotyping. Verhoef *et al.* showed that pharmacogenetic dosing of acenocoumarol in adults could slightly increase health, but would only be cost-effective when the costs of genotyping decreased to €30 or less [34]. Furthermore, the costs of genotyping are rapidly decreasing, so prices in this range might be possible in the near future.

For application of the dosing algorithm in clinical practice, tablets of lower strength than the current 1-mg acenocoumarol tablets would be required for young patients needing low doses. In this study, we found that some pharmacies were already manufacturing capsules with lower amounts of acenocoumarol (e.g. 0.5 mg) to meet the dose requirement of the patient. Furthermore, van Schie *et al.* have described how the calculated mean maintenance dose can be achieved with the current available tablets of 1 mg [35]. This method can also be used for tablets of lower strength.

We have provided two dosing algorithms; however, we recommend using the genetic algorithm. When it is not possible to perform genotyping, the clinical model can be used. In Table S9, we also show a model with only age instead of BSA, which could be used when weight and height are unknown. However, the percentage of variability that was explained by the clinical model with age instead of BSA was 7.1% lower (37.9% versus 45.0%). This is a high percentage, which makes it worthwhile to determine height and weight in clinical practice before starting acenocoumarol.

In conclusion, we have shown that clinical factors explain a large proportion of the variability in dose requirement of acenocoumarol in pediatric patients. Furthermore, we have demonstrated that polymorphisms in or flanking *VKORC1*, *CYP2C9* and *CYP2C18* all increase the explained variability. Together, clinical and genetic factors were able to explain 61.8% of the variability. Both the clinical model and the genetic model are expected to improve acenocoumarol therapy in pediatric patients, as compared with the dosing method used today, which is based only on age group and weight.

Addendum

H. Maagdenberg performed the statistical analysis and wrote the manuscript. S. le Cessie supervised the statistical analysis. H. Maagdenberg, M. B. Bierings, A. H. Maitland-van der Zee, and A. de Boer interpreted the results. H. Maagdenberg purified the saliva samples. T. van der Straaten analyzed the saliva samples. All authors critically reviewed the manuscript.

Acknowledgements

The authors thank N. Spoor, J. van der Zee, Z. Sögütoglu, C. de Roon and D. van Bergeijk for all their help with selection of patients eligible for participation and collection of the data. We would also like to thank S. Belitser for her help with the sample size calculation. The authors also thank all of the patients and their parents/legal guardians who participated in this study.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article

Table S1. Applied time windows for height and weight per age at start of stable period.
Table S2. Information on the patients without a stable – distribution of genotypes.
Table S3. Mean stable dose for patients with and without a Fontan circulation stratified per TR within the genetic cohort.
Table S4. Patient characteristics of patients included using a stricter definition of a stable period.
Table S5. Distribution of genotypes within patients included using a stricter definition of a stable period – distribution of genotypes.
Table S6. Genotype-guided algorithm and clinical algorithm using a stricter definition of a stable period.
Table S7. Patient characteristics of patients included using age instead of BSA.
Table S8. Distribution of genotypes within patients included using age instead of BSA.
Table S9. Genotype-guided algorithm and clinical algorithm using age instead of BSA.
Fig. S1. BSA per age.
Fig. S2. Observed mean stable dose per age.

References

1 Bonduel MM. Oral anticoagulation therapy in children. *Thromb Res* 2006; **118**: 85–94.
2 Monagle P, Chan AKC, Goldenberg NA, Ichord RN, Journeycake JM, Nowak-Göttl U, Vesely SK; American College of Chest Physicians. . Antithrombotic therapy in neonates and children: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012; **141**: e737S–801S.
3 Chalmers E, Ganesen V, Liesner R, Maroo S, Nokes T, Saunders D, Williams M. Guideline on the investigation, management and prevention of venous thrombosis in children. *Br J Haematol* 2011; **154**: 196–207.
4 Giglia TM, Massicotte MP, Tweddell JS, Barst RJ, Bauman M, Erickson CC, Feltes TF, Foster E, Hinoki K, Ichord RN, Kreutzer J, McCrindle BW, Newburger JW, Tabbutt S, Todd JL, Webb CL; American Heart Association Congenital Heart Defects Committee of the Council on Cardiovascular Disease in the Young, Council on Cardiovascular and Stroke Nursing, Council on Epidemiology and Prevention, and Stroke Council. Prevention and treatment of thrombosis in pediatric and congenital heart disease: a scientific statement from the American Heart Association. *Circulation* 2013; **128**: 2622–703.
5 Bonduel M, Sciuccati G, Hepner M, Torres AF, Pieroni G, Frontroth JP, Serviddio RM. Acenocoumarol therapy in pediatric patients. *J Thromb Haemost* 2003; **1**: 1740–3.
6 Federatie van Nederlandse Trombosediensten. *De kunst van het doseren – Richtlijn, leidraad en informatie voor het doseren van vitamine K-antagonisten*, 8th edn. Leiden: Federatie van Nederlandse Trombosediensten, 2017.
7 Spoor N, Smiers FJ, van der Meer FJM, Hutten BA, van Ommen CH. Phenprocoumon and acenocoumarol treatment in paediatric patients. *Thromb Haemost* 2012; **108**: 1238–41.
8 Maagdenberg H, Bierings MB, van Ommen CH, van der Meer FJM, Appel IM, Tamminga RYJ, de Boer A, Maitland-van der Zee AH. Characteristics and quality of oral anticoagulation treatment in pediatric patients in the Netherlands based on the CAPS cohort. *J Thromb Haemost* 2018; **16**: 116–24.
9 Schalekamp T, de Boer A. Pharmacogenetics of oral anticoagulant therapy. *Curr Pharm Des* 2010; **16**: 187–203.
10 EMBL-EBI. Ensembl human genome browser. Population genetics. 1000 Genomes Project Phase 3. Available at: http://www.ensembl.org/Homo_sapiens (accessed 5 July 2017).
11 Wang D, Guo Y, Wrighton SA, Cooke GE, Sadee W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. *Pharmacogenomics J* 2011; **11**: 274–86.
12 Teichert M, Eijgelsheim M, Rivadeneira F, Uitterlinden AG, van Schaik RHN, Hofman A, De Smet PAGM, van Gelder T, Visser LE, Stricker BHC. A genome-wide association study of acenocoumarol maintenance dosage. *Hum Mol Genet* 2009; **18**: 3758–68.
13 van Schie RMF, Aoussar A, van der Meer FJM, de Boer A, Maitland-van der Zee AH. Evaluation of the effects of single-nucleotide polymorphisms in CYP3A4 and CYP4F2 on stable phenprocoumon and acenocoumarol maintenance doses. *J Thromb Haemost* 2013; **11**: 1200–3.
14 Daly AK. Optimal dosing of warfarin and other coumarin anticoagulants: the role of genetic polymorphisms. *Arch Toxicol* 2013; **87**: 407–20.
15 International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee M-TM, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD, Johnson JA. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med* 2009; **360**: 753–64.
16 Biss TT, Avery PJ, Brandão LR, Chalmers EA, Williams MD, Grainger JD, Leathart JBS, Hanley JP, Daly AK, Kamali F. VKORC1 and CYP2C9 genotype and patient characteristics explain a large proportion of the variability in warfarin dose requirement among children. *Blood* 2012; **119**: 868–73.
17 Moreau C, Bajolle F, Siguret V, Lasne D, Golmard J-L, Elie C, Beaune P, Cheurfi R, Bonnet D, Loriot M-A. Vitamin K antagonists in children with heart disease: height and VKORC1 genotype are the main determinants of the warfarin dose requirement. *Blood* 2012; **119**: 861–7.
18 Nowak-Göttl U, Dietrich K, Schaffranek D, Eldin NS, Yasui Y, Geisen C, Mitchell LG. In pediatric patients, age has more impact on dosing of vitamin K antagonists than VKORC1 or CYP2C9 genotypes. *Blood* 2010; **116**: 6101–5.
19 Kato Y, Ichida F, Saito K, Watanabe K, Hirono K, Miyawaki T, Yoshimura N, Horiuchi I, Taguchi M, Hashimoto Y. Effect of the VKORC1 genotype on warfarin dose requirements in Japanese pediatric patients. *Drug Metab Pharmacokinet* 2011; **26**: 295–9.
20 Nguyen N, Anley P, Yu MY, Zhang G, Thompson AA, Jennings LJ. Genetic and clinical determinants influencing warfarin dosing in children with heart disease. *Pediatr Cardiol* 2013; **34**: 984–90.
21 Shaw K, Amstutz U, Hildebrand C, Rassekh SR, Hosking M, Neville K, Leeder JS, Hayden MR, Ross CJ, Carleton BC. VKORC1 and CYP2C9 genotypes are predictors of warfarin-related outcomes in children. *Pediatr Blood Cancer* 2014; **61**: 1055–62.
22 Wakamiya T, Hokosaki T, Tsujimoto S-I, Kadota K, Nakano Y, Watanabe S, Iwamoto M, Yanagimachi M, Ito S. Effect of VKORC1, CYP2C9, CFP4F2, and GGCX gene polymorphisms on warfarin dose in Japanese pediatric patients. *Mol Diagn Ther* 2016; **20**: 393–400.
23 Vear SI, Ayers GD, Van Driest SL, Sidonio RF, Stein CM, Ho RH. The impact of age and CYP2C9 and VKORC1 variants on stable warfarin dose in the paediatric population. *Br J Haematol* 2014; **165**: 832–5.

24 World Health Organization. Weight-for-age. Available at: http://www.who.int/childgrowth/standards/weight_for_age/en/ (accessed 8 August 2016).
25 World Health Organization. Length/height-for-age. Available at: http://www.who.int/childgrowth/standards/height_for_age/en/ (accessed 8 August 2016).
26 World Health Organization. Weight-for-age (5–10 years). Available at: http://www.who.int/growthref/who2007_weight_for_age/en/ (accessed 8 August 2016).
27 World Health Organization. Height-for-age (5–19 years). Available at: http://www.who.int/growthref/who2007_height_for_age/en/ (accessed 8 August 2016).
28 Kaulitz R, Luhmer I, Bergmann F, Rodeck B, Hausdorf G. Sequelae after modified Fontan operation: postoperative haemodynamic data and organ function. *Heart* 1997; **78**: 154–9.
29 van Nieuwenhuizen RC, Peters M, Lubbers LJ, Trip MD, Tijssen JG, Mulder BJ. Abnormalities in liver function and coagulation profile following the Fontan procedure. *Heart* 1999; **82**: 40–6.
30 Hines RN. Ontogeny of human hepatic cytochromes P450. *J Biochem Mol Toxicol* 2007; **21**: 169–75.
31 Koukouritaki SB, Manro JR, Marsh SA, Stevens JC, Rettie AE, McCarver DG, Hines RN. Developmental expression of human hepatic CYP2C9 and CYP2C19. *J Pharmacol Exp Ther* 2004; **308**: 965–74.
32 Treluyer JM, Gueret G, Cheron G, Sonnier M, Cresteil T. Developmental expression of CYP2C and CYP2C-dependent activities in the human liver: in-vivo/in-vitro correlation and inducibility. *Pharmacogenetics* 1997; **7**: 441–52.
33 Itoh S, Onishi S. Developmental changes of vitamin K epoxidase and reductase activities involved in the vitamin K cycle in human liver. *Early Hum Dev* 2000; **57**: 15–23.
34 Verhoef TI, Redekop WK, de Boer A, Maitland-van der Zee AH; EU-PACT group. Economic evaluation of a pharmacogenetic dosing algorithm for coumarin anticoagulants in The Netherlands. *Pharmacogenomics* 2015; **16**: 101–14.
35 van Schie RMF, Wessels JAM, le Cessie S, de Boer A, Schalekamp T, van der Meer FJM, Verhoef TI, van Meegen E, Rosendaal FR, Maitland-van der Zee A-H. Loading and maintenance dose algorithms for phenprocoumon and acenocoumarol using patient characteristics and pharmacogenetic data. *Eur Heart J* 2011; **32**: 1909–17.