# Precision anticoagulation with vitamin K antagonists in Children

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# PRECISION ANTICOAGULATION WITH VITAMIN K ANTAGONISTS IN CHILDREN

**Precisie antistolling met vitamine K-antagonisten bij kinderen**
**(met een samenvatting in het Nederlands)**

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# 1 | GENERAL INTRODUCTION

For a long time clinical trials were much rarer in children than in adults. In 2007 the European Medicine Agency implemented the Pediatric Regulation to improve pediatric clinical research.[1] The number of pediatric trials and therewith the number of registrations of new pediatric medicines, dosage forms, and indications increased substantially after the implementation of the regulation.[2] However, this was only marginally affecting the off-label use rate in children in clinical practice. Before the implementation the off-label use rate in children was 18% to 66% for inpatients and 10.5% to 37.5% for outpatients. After the implementation this was decreased to 33.2% to 46.5% (inpatients) and 3.3% to 13.5% (outpatients).[3] This is due to the fact that a large number of the off-label drugs used in pediatric patients are off-patent drugs. The regulation provides incentives for pharmaceutical companies to conduct pediatric clinical trials for new drugs, but also for off-patent drugs. However, the incentives for conducting clinical trials for off-patent drugs seem to be too low. In October 2017, the European Commission reported that only 3 (off-patent drugs) have been granted pediatric use marketing authorization (PUMA) since the implementation of the regulation.[2]

Due to ethical issues and sometimes small numbers of pediatric patients, it is challenging to conduct pediatric clinical trials. Nevertheless, there is a clear need to treat pediatric patients with drugs of which the benefit(s) and risk(s) have been evaluated in children in a valid way. Therefore we should consider more often to expose children to the risks and the burdens of drug research, since without these trials, the off-label use continues and we expose children to potentially unsafe, ineffective or less optimal treatments. Recently, the European Union (EU) and the pharmaceutical industry jointly funded for 140 million euros the Conect4Children project, which aims to generate a trial-network in the member states of the EU to optimize the performance of clinical trials in pediatric patients.[4]

Another option, next to clinical trials, is to use the information of the children who have received off-label treatment. By learning from the response of these patients to treatment in observational studies, optimalization and standardization can at least be sought and the care can be harmonized as much as possible for new patients. In the Netherlands, for the "kinderformularium" scientific literature is systematically reviewed to provide health care professionals with specific information on effective and safe doses in different age groups and also adverse drug events, contraindications and additional information which is important for using drugs in pediatric patients.[5] By providing this information a more uniform prescribing of drugs in children can be reached and and pharmacists are able to perform adequate medication surveillance (verifying dose, interactions, contra-indications etc).

Initiatives like the "kinderformularium" depend on the availability of well-designed randomized and observational studies. However, it is quite challenging to conduct and interpret studies in pediatric patients. Children are continuously changing in body size and composition, and also the physiological systems are developing (e.g. hemostatic and metabolic system).[6,7] The differences between age groups/ developmental stages should be taken into account when designing and analyzing a study with pediatric patients. In addition, as in adults, pediatric patients respond differently to the same medication. Important components are the already mentioned physical differences between pediatric patients, but also genetic variations contribute.[8] In recent years, more and more research has been devoted to study the influence of genetic variations on drug response (pharmacogenetics) in children.[8] By including both genetic and clinical factors in the choice of treatment and dosage, care for children can be further personalized, enabling optimization and safer treatments.

Vitamin K antagonists (VKAs) are an example of an off-patent drug group, which has been on the market already since the 1950s. In the Netherlands, two VKAs are on the market the short-acting acenocoumarol and long-acting phenprocoumon. VKAs are used to treat and prevent thromboembolic events [9]. The total number of pediatric patients using VKAs in the Netherlands has been quite stable over the last six years, approximately 500 users based on reimbursement data of the Dutch National Health Care Institute, as presented in figure 1.[10] In adults who use VKAs, one in five is using phenprocoumon.[11] In pediatric patients this was also the case until 2015. From 2015 onwards this has increased to one in three, as shown in figure 1. Before 2015 young patients of 1-6 years of age were almost exclusively prescribed acenocoumarol. Only 5% to 12% was using phenprocoumon. From 2015 onwards this changed to 44% to 53%. However, acenocoumarol is still the most prescribed VKA in pediatric patients in the Netherlands.

VKA treatment in pediatric patients is challenging. Not only is there a large inter-individual variability caused by genetic and clinical differences, but also a large intra-individual variability over time in dose requirement. The direct oral anticoagulants (DOACs), which are already approved for adults for some years are an attractive alternative. They do not require INR measurements or dose adjustments unless dose adjustments are necessary based on kidney function or interacting drugs. At the moment, the first clinical trials of DOACs in pediatric patients have been completed and many will follow in the coming years. In the Netherlands, the number of users of DOACs (mostly rivaroxaban) in patients of 16-18 years* is rising and in 2017 the number of patients (of 16-18 years of age) was already higher than for phenprocoumon.[10] The use of DOACs in children less than 16 years is very rare. These patients still require VKA treatment.

* Likely to comprise only patients of 18 years of age.

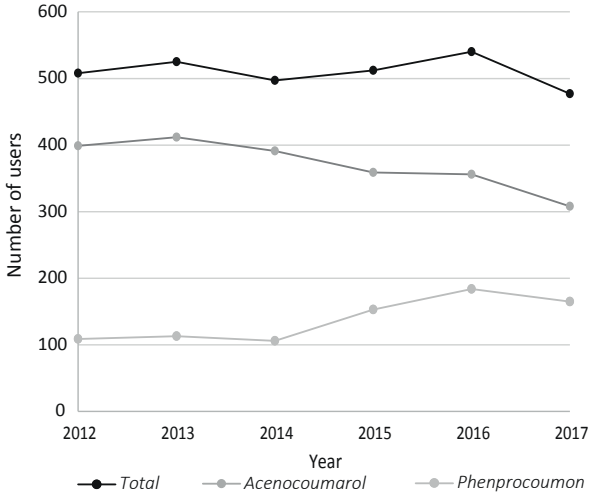


**Figure 1.** Differences in number of acenocoumarol and phenprocoumon users over the last 6 years. [10] Note: the numbers of 2016 and 2017 are not the final number of users in these years, because reimbursement claims could still be made at the time that the data was provided.

The number of studies, which has been conducted with acenocoumarol and phenprocoumon in pediatric patients is low. Only for acenocoumarol one study has been conducted to find the required starting dose in pediatric patients for different age groups.[12] For phenprocoumon this information is lacking and therefore the same starting dose as for acenocoumarol is used.[13] However, in adults a different starting dose is applied for both VKAs.[13] The reported quality of VKA therapy in pediatric patients in the Netherlands has been low with less than 50% of time in which patients were within therapeutic international normalized ratio (INR) range.[14] Factors influencing this quality have not been studied for these two VKAs. Furthermore, in adult and pediatric patients who use warfarin pharmacogenetic dosing models (including both genetic and non-genetic factors) have been developed to correct for inter-individual variability in dose requirement. Pediatric pharmacogenetic dosing models for acenocoumarol and phenprocoumon are lacking.

With 500 pediatric users of VKAs in the Netherlands and with at least years to come before DOACs can possibly become the preferred treatment in pediatric patients it is important to study the use (including quality of anticoagulation) of VKAs in pediatric patients to improve VKA treatment.

## THESIS AIM AND OBJECTIVES

This thesis aimed to study important knowledge gaps in the use of VKA therapy in pediatric patients and to develop dosing algorithms to improve their application.
The objectives were:

- To gain more insight in the quality, safety and effectiveness of VKA treatment in pediatric patients,
- To determine factors influencing anticoagulation stability of pediatric patients using VKA,
- To determine which clinical and genetic factors influence the required dose of acenocoumarol and phenprocoumon in pediatric patients and to develop a dosing algorithm based on these factors.

## OUTLINE OF THIS THESIS

This thesis contains seven manuscripts divided over three chapters. There is a general introduction (**chapter 1**) and a concluding general discussion highlighting the main findings, methodological challenges and future perspectives (**chapter 5**).

**Chapter 2** contains two reviews. The first review (**chapter 2.1**) describes the challenges and potential of personalized medicine in pediatric patients using pharmacogenomics. This is described based on three examples: cisplatin induced ototoxicity in childhood cancer, optimizing VKA dosing, and effective treatment of asthma, which all show a different application of pharmacogenomics (prevention of adverse events, dose requirement, effectiveness). The second review (**chapter 2.2**) describes the use of oral anticoagulants in pediatric patients. Aspects that are discussed are the influence of the development of the hemostatic and metabolic systems, the evidence of DOACs and the current knowledge on dosing algorithms for VKAs in pediatric patients.

**Chapter 3** contains three studies on the quality, effectiveness and safety of VKA therapy in pediatric patients. In the first study (**chapter 3.1**) the incidence of bleeding and thromboembolic events in pediatric patients using warfarin in England is determined. In the second study (**chapter 3.2**) the characteristics of the patients in the Children Anticoagulation and Pharmacogenetics Study (CAPS) cohort are described. Furthermore, the quality, effectiveness and safety of VKA therapy in these patients is studied. In **chapter 3.3** patients within the CAPS cohort who had or had not a stable anticoagulation period are studied to find explanatory factors for not reaching a stable period within the first three months of VKA treatment. Moreover, the quality of anticoagulation control in patients who used both acenocoumarol and phenprocoumon are compared to evaluate if switching improves the quality of anticoagulation.

**Chapter 4** contains two studies. In **chapter 4.1** and **4.2** dosing algorithms are presented for acenocoumarol and phenprocoumon incorporating both genetic and non-genetic factors.

## REFERENCES


1. European Medicines Agency. Paediatric medicines - Paediatric Regulation. 2006. http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000068.jsp&mid=WC0b01ac0580925c45. Accessed 25 Jul 2018.
2. European Commission. State of Paediatric Medicines in the EU - 10 years of the EU Paediatric Regulation. p. 13. Report No.: COM (2017) 626. 2017. https://ec.europa.eu/health/sites/health/files/files/paediatrics/docs/2017_childrensmedicines_report_en.pdf. Accessed 25 Jul 2018.
3. Nordenmalm S, Tomasi P, Pallidis C. More medicines for children: impact of the EU paediatric regulation. Arch Dis Child. 2018 Jun;103(6):557–64.
4. Launch of a new public-private partnership to improve clinical trial infrastructure to facilitate the development of new drugs for children in Europe. conect4children. 2018. https://conect4children.org/news/press-release-launch-of-the-c4c-project/. Accessed 2 Aug 2018.
5. Nederlands Kenniscentrum Farmacotherapie bij Kinderen. Kinderformularium - verantwoording. 2008. https://www.kinderformularium.nl/verantwoording. Accessed 2 Aug 2018.
6. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental Pharmacology — Drug Disposition, Action, and Therapy in Infants and Children. N Engl J Med. 2003 Sep 18;349(12):1157–67.
7. Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L. Maturation of the hemostatic system during childhood. Blood. 1992 Oct 15;80(8):1998–2005.
8. Adam de Beaumais T, Jacqz-Aigrain E. Pharmacogenetics: Applications to Pediatric Patients. Adv Pharmacol San Diego Calif. 2018;83:191–215.
9. Monagle P, Chan AKC, Goldenberg NA, Ichord RN, Journeycake JM, Nowak-Göttl U, et al. 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 Feb;141(2 Suppl):e737S–801S.
10. The Dutch National Health Care Institute. Personal communication - ATC-B01A - number of users (age: 0-18 years; 2012-2017). 2018.
11. The Dutch National Health Care Institute. GIPdatabank . 2004. www.gipdatabank.nl. Accessed 25 Jul 2018.
12. Bonduel M, Sciuccati G, Hepner M, Torres AF, Pieroni G, Frontroth JP, et al. Acenocoumarol therapy in pediatric patients. J Thromb Haemost JTH. 2003 Aug;1(8):1740–3.
13. Federatie van Nederlandse Trombosediensten. De kunst van het doseren - Richtlijn, leidraad en informatie voor het doseren van vitamine K-antagonisten. 8th ed. Leiden: Federatie van Nederlandse Trombosediensten; 2017.
14. Spoor N, Smiers FJ, van der Meer FJM, Hutten BA, van Ommen CH. Phenprocoumon and acenocoumarol treatment in paediatric patients. Thromb Haemost. 2012 Dec;108(6):1238–41.

# 2 | PHARMACOGENOMICS IN PEDIATRIC PATIENTS

# 2.1 Pharmacogenomics in Pediatric Patients: Towards Personalized Medicine

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## ABSTRACT

It is well known that drug responses differ among patients with regard to dose requirements, efficacy, and adverse drug reactions (ADRs). The differences in drug responses are partially explained by genetic variation. This paper highlights some examples of areas in which the different responses (dose, efficacy, and ADRs) are studied in children, including cancer (cisplatin), thrombosis (vitamin K antagonists), and asthma (long-acting β2 agonists). For childhood cancer, the replication of data is challenging due to a high heterogeneity in study populations, which is mostly due to all the different treatment protocols. For example, the replication cohorts of the association of variants in *TPMT* and *COMT* with cisplatin-induced ototoxicity gave conflicting results, possibly as a result of this heterogeneity. For the vitamin K antagonists, the evidence of the association between variants in *VKORC1* and *CYP2C9* and the dose is clear. Genetic dosing models have been developed, but the implementation is held back by the impossibility of conducting a randomized controlled trial with such a small and diverse population. For the long-acting β2 agonists, there is enough evidence for the association between variant *ADRB2* Arg16 and treatment response to start clinical trials to assess clinical value and cost effectiveness of genotyping. However, further research is still needed to define the different asthma phenotypes to study associations in comparable cohorts. These examples show the challenges which are encountered in pediatric pharmacogenomic studies. They also display the importance of collaborations to obtain good quality evidence for the implementation of genetic testing in clinical practice to optimize and personalize treatment.

## INTRODUCTION



Individuals with the same disease will often respond differently to the same drug. Some individuals will have a good response to the drug, while others experience little or no effect. Some patients will experience severe adverse drug reactions (ADRs), whereas others will not. In addition, some patients require a higher or lower dose compared with the standard dose defined in clinical trials to benefit optimally from the drug. In other words, personalizing drug treatment is required. Pharmacogenomics studies the relationship between genetic variation and drug responses. Single nucleotide polymorphisms (SNPs) can lead to changes in the function or the amount of proteins (e.g., enzymes, receptors, ion channels) and therefore in the drug response.[1] Pharmacogenomics covers associations with both germline and somatic mutations. In this review only the influence of germline mutations will be discussed.

The first pharmacogenomic studies were designed as candidate gene studies. The candidate genes are selected based on potential involvement with drug response, such as genes coding for metabolic enzymes and drug target proteins. However, the cause of a different drug response is not always in the potentially involved genes, which makes it difficult to choose candidate genes.

The design of a genome-wide association study (GWAS) is more data driven than the hypothesis-driven candidate gene association studies. In a GWAS, the whole genome of participants is screened for all frequently occurring SNPs. With GWAS, besides SNPs in candidate genes, also previously unknown associations between a specific SNP and a certain response to a drug can be found. The newest innovations in pharmacogenomics, enabled by the rapid improvement of genomics technology, are phenome-wide association studies (PheWAS), whole exome sequencing (WES), and whole genome sequencing (WGS), which bring new opportunities to study the association between response and genetic variants.[2]

Most pharmacogenomic research has been performed in adults. However, it is important to realize that findings in the adult population cannot be applied directly to the pediatric population.[3] Processes and systems (such as the metabolic system and hemostasis and drug biotransformation) are still under development in children.[3,4] Therefore, drugs may act differently in children compared with adults. Although genetic variations remain stable, the contribution to treatment heterogeneity may be different at a younger age. In this article, we highlight examples of pharmacogenomic studies in pediatric patients. Pharmacogenomic research in childhood cancer is, apart from the focus on tumor genetics, focused on predicting which patients will suffer from severe ADRs. In the treatment of thrombosis, the studies have focused on predicting the right anticoagulant dose for each pediatric

**Table 1.** Overview of characteristics of the studies

| | Ross et al.[8] | | Pussegoda et al.[9] | Yang et al.[19] | | Lanvers-Kaminsky et al.[21] | Hagleitner et al.[20] | | Xu et al.[22] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Discovery | Replication | | Radiation | No radiation | | Spanish | Dutch | Discovery | Replication |
| Number of patients | 53 | 109 | 155 | 213 | 41 | 63 | 38 | 110 | 238 | 68 |
| Treatment protocols | NS | NS | NS | SJMB-96; SJMB-03 | SJNB-97; SJNB-05; SJOS-08 | COSS-82/86/91; NB-90; MAHO- 94; MAKEI-89; HIT-91 | NS | NS | SJMB-96; SJMB-03 | SJYC-07 |
| Age, y (median (range)) | | | | | | | | | | |
| Cases | 5 (0-16) | 6 (0-16) | 6 (1-25) | 7.6(3.1-21.6) | 3-12 (0.8-18) | 11.8 (5-22) | 11.5 (4-29) | 15 (5-40) | 8.5 ± 3.8[b] | <5[c] |
| Controls | 9 (0-16) | 9.5 (1-19) | 11(0-18) | 10.1 (3.3-19.8) | 10 (2-13) | 13.9 (5-20) | 14 (7-28) | 15 (7-39.3) | 10.0 ± 4.3[b] | - |
| Sex, male (%) | 67.9 | 57.8 | 49.7 | 66.2 | 70.7 | 60.3 | 55.3 | 50.0 | 62.2 | NS |
| Ethnicity (%) | Caucasian 79.0[a] | | Caucasian 80.0 | White 78.9; Non-white 21.1 | White 61.0; Non-white 39.0 | NS | European ancestry | Dutch ancestry | Mixed population | NS |
| Cancer type | Various cancers | | Various cancers | Medulloblastoma | Neuroblastoma; osteosarcoma | Various cancers (mainly osteosarcoma) | Osteosarcoma | | Brain tumors | |
| Follow up duration, y (median (max) or fixed number of years) Cases/controls | 3(0-18)/2(0-15) [a] | | 5 (0-25)/2(0-16) | 1.7 | NS | 2.5 | NS | 5.2 (0.06-21.3) | 2.1 | 2.1 |
| Craniospinal irradiation (%) | 17.0 | 19.3 | 18.1 | 100 | 0 | NS | 0 | 0 | 100 | NS |
| Concurrent drug therapy | | | | | | | | | | |
| Use of ototoxic antibiotics | Yes | Yes | Yes | NS | NS | NS | Yes | No | NS | NS |
| Vincristine (%) | 39.5[a] | | 49.7 | 100 | 0 | NS | 15.8 | 4.5 | 100 | 100 |
| Otoprotectants (%) | NS | NS | NS | 90.6 | 0 | NS | 0 | 0 | >87 | 0 |

**Table 1.** Continued

| Cumulative cisplatin dose mg/m$^2$ (median (range)) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cases | 360 (180-630) | 400 (120-720) | 400 (92-800) | 300 (77-313) | 390-618 (113-1105) | 412 (120-644) | 504(120-870) | 500 (100-600) | 287 ± 35 [b] | ±300 [c] |
| Controls | 360(180-720) | 410 (100-700) | 400(20-768) | 300 (79-312) | 254 (225-815) | 418 (161-560) | 515(140/720) | 480(200-600) | 289 ± 36 [b] | - |
| Ototoxicity grading scale and comparison groups | CTCAE>1 vs CTCAE=0 | | CTCAE>1 vs CTCAE=0 | CTCAE>0 vs CTCAE=0 + ordinal and Chang 0 vs >0; 2a vs ≥ 2a and ordinal | CTCAE ordinal | NS | CTCAE>1 vs CTCAE=0; SIOP Boston ototoxicity scale | | Chang score>0 vs chang score=0 | |
| Outcome | | | | | | | | | | |
| Association COMT | Yes | Yes | No | No | No | No | Yes | No | No | No |
| Association TPMT | Yes | Yes | Yes | No | No | No | No | No | No | No |

CTCAE, common terminology criteria for adverse events; NS, not specified; SIOP, International Society of Pediatric Oncology; y, years.

[a] Based on combined cohort (discovery + replication) [9]

[b] Mean and standard deviation given instead of median and range

[c] Based on combined cohort (cases + controls)

patient; and in asthma the main issue is to predict the efficacy of a bronchodilator drug. These are representative and extensively studied examples of the earlier mentioned sorts of differences in drug responses (ADRs, dose, and efficacy). These examples will give an insight into the challenges of pharmacogenomic research in children, but will also address the potential of pharmacogenomics to optimize and personalize treatment for children.

## PHARMACOGENOMICS IN CHILDREN

### Childhood Cancer

In 2012, the worldwide estimated number of children under the age of 15 years diagnosed with cancer was 163,300.[5] The mean 5-year survival rates in the US are just above 80 %, but it largely depends on the type of cancer.[6] With the increase in survival rates, the ADRs, which can cause lifelong damage, are becoming increasingly important during and after treatment. Anticancer drugs that are well known for their ADRs are cisplatin (ototoxicity, renal toxicity), anthracyclines (cardiotoxicity), and vincristine (neurotoxicity). These ADRs can have a large impact on quality of life. Many pharmacogenomic studies in the field of childhood cancer have focused on the toxicity of treatment. However, clinical implementation of pharmacogenomic testing is still pending in many centers because of inconclusive study results or uncertainty about whether and for which patients implementation is clinically relevant. We will discuss cisplatin as an example. This drug has been associated with a risk of ototoxicity, which can be very impairing, especially for children who are developing their speech skills.[7] Several candidate gene studies have been conducted to investigate specific SNPs which are associated with an increased or decreased risk of ototoxicity. Variations in the following genes were found to influence the risk of cisplatin-induced ototoxicity: TPMT, COMT, ABCC3, SOD2, GSTT1*1, GSTP1, XPC, LRP2, Otos, SLC22A2, CTR1 and GSTM3*B.[8–18] However, a major issue is the reproducibility of these initial findings. Several groups have conducted relatively small candidate gene studies on the association between ototoxicity and variations in COMT and TPMT in different cohorts.[9,19–21] The cohorts are very heterogeneous (Table 1) and some lack statistical power. For TPMT, the association was replicated in two similar cohorts.[8,9] One small Spanish cohort (n = 38) also showed an association for TPMT; however, because of the lack of power it was not statistically significant (rs12201199, odds ratio (OR) 6.79, 95 % confidence interval (CI) 0.34–13.71).[20] The association with COMT was replicated twice,[8,20] but in one of the studies the association was in the opposite direction.[20] Another problem with COMT and TPMT is the lack of information on the mechanism in which these two enzymes are involved in cisplatin-induced ototoxicity.

Hagleitner et al. conducted a meta-analysis for COMT and TPMT in 2014 and found only a small association for COMT (rs4646316) (OR 1.52, 95 % CI 1.16–1.99, p = 0.003). For the analyzed TPMT mutations there was a trend towards increased risk (rs12201199; OR 2.15, 95 % CI 1.16–1.99, p = 0.003).[20] However, it is debatable if these results give an accurate effect estimation, because of the heterogeneity in populations of the included studies.

Recently, a GWAS failed to find any association for TPMT, COMT or any of the other genes studied in the candidate gene studies.[22] The GWAS study was conducted in 238 pediatric patients with newly diagnosed brain tumors. A strong association was found for a mutation in ACYP2 (rs1872328, hazard ratio 4.5, 95 % CI 2.63–7.69, p = 3.9 × 10−8), which was replicated in a new cohort of 68 patients that was almost similarly treated as the discovery cohort. In the discovery and the replication cohorts, 100 % of the patients that carried at least one mutated allele developed ototoxicity. In the patients with no mutated allele, still more than 70 % developed ototoxicity.[22]

ACYP2 encodes for an acylphosphatase which is, among other places, expressed in the cochlea.[23] The exact mechanism by which this mutation in ACYP2 increases the risk of ototoxicity is still unclear.

The problems with replication of the results found in the different candidate studies has led to an extensive discussion about the underlying reasons.[24–28] The replication issues could be largely due to small sample sizes and differences in the study populations (age, ethnicity, and type of cancer), scoring of ototoxicity, length of follow-up, cumulative dose of cisplatin, and concurrent drug treatment (e.g., use of otoprotectants and craniospinal irradiation) (Table 1). Heterogeneity also existed within the studies, like different treatment regimens and types of cancer. The heterogeneities complicate replicating the results and it is uncertain if the associations found are true or only a result of confounding or bias. At present, only TPMT is mentioned in the label information of cisplatin as a possible contributor to ototoxicity, but no clinical recommendations are provided.[29]

From these studies we can conclude that the mutation in ACYP2 seems to be an important predictor of ototoxicity in children, but that it explains only a small part (12.4 %) of ototoxicity.[22] More research is needed to replicate these findings, and to find practical solutions for the implementation of ACYP2 testing in clinical practice. Studies of the mechanism for TPMT and COMT involvement in cisplatin-induced ototoxicity and independent replication in similar cohorts are required.

For some patients the toxicity is unacceptable (e.g., ototoxicity for a patient who is blind). In such patients, decisions on therapy will be influenced by genetic polymorphisms that enhance the risk of developing toxicity. With the identification of significant risk variants, patients who are at an increased risk can be identified and might be given alternative treatments and/or undergo closer monitoring during

treatment and the follow-up period. Adapting complex treatment regimens in an attempt to reduce side effects is complicated since efficacy must remain intact. Different approaches maybe explored: identifying a protecting agent against ototoxicity is an attractive option. The knowledge gained from the identification of variants that influence the risk of cisplatin-induced ototoxicity can be used to identify new drug targets for protecting agents. This research is promising and will eventually lead to a more personalized anticancer treatment.

**Thrombosis**

In recent years there has been a higher incidence of thrombosis in children,[30] mainly due to intensified medical treatments and increased awareness of the risk of thrombosis. Currently, low molecular weight heparins (LMWHs) and vitamin K antagonists (VKAs) are the two drugs used in clinical practice for the treatment or prevention of venous thrombosis in pediatric patients. The relatively new direct oral anticoagulants (DOACs) are currently being tested in pediatric patients. In 2018, the first phase III studies with DOACs in pediatric patients will be completed. At this time, the VKAs are the only oral anticoagulants which are used for the treatment of thrombosis in pediatric patients.

VKAs inhibit the action of vitamin K epoxide reductase (VKORC1), which leads to lower levels of active vitamin K-dependent clotting factors, and thus to inhibition of the coagulation cascade.[31] In clinical practice, a large variability in dose requirement of VKAs is seen.[32] This is problematic because VKAs also have a narrow therapeutic window. Dosing all patients equally leads to an increased risk of bleeding and thrombotic events. In children, this problem is even more compelling because of the developing hemostatic system and the growing body. In the last decade, many studies have been carried out to explain the large interindividual dose variability in children and adults.[33,34] In addition to clinical factors such as age, weight, and gender, genetic factors play an important role [34]. Mutations in VKORC1 lead to less enzyme production and to a lower dose requirement. Loss-of-function mutations in CYP2C9 (*2 and *3) lead to a decrease in the enzyme activity. The S-isomer of VKAs is almost completely metabolized by CYP2C9; therefore, the mutation leads to a decrease in the required dose.[31,35] To a lesser extent, mutations in CYP4F2 and CYP2C18 have also been found to be (possibly) contributing to the dose variability.[33,35–37]

Seven regression dosing models have been constructed for pediatric patients, almost all for warfarin.[38–44] No pediatric dosing model is available for acenocoumarol. What these pediatric models have in common is that factors related to ontogeny (i.e., age, weight, and height) explain roughly one-third of the dosing variability. The variability explained by the CYP2C9 and VKORC1 genotypes fluctuates between the different models. The CYP2C9 genotype explained 0.4[38] to 12.8 %[39] of the variability

in dose requirement, the VKORC1 genotype 3.7[38] to 47 %.[41] One of the possible explanations is the small sample size of the cohorts ranging from 37 to 120 children. Only two studies included at least 100 patients.[39,44]



Also, two pharmacokinetic/pharmacodynamic (PK/PD) dosing models have been built for pediatric patients.[45,46] Hamberg and Wadelius evaluated the regression and PK/PD models in a retrospective pediatric cohort.[34] Of the evaluated models, the PK/PD model of Hamberg et al.[46] performed best with regards to the proportion of patients for whom the predicted maintenance dose was within ±20 % of the observed dose. Hamberg et al. developed a tool for their model which can run on every computer without licensing for a program and is easy to use.[47] The best performing regression model incorporates the CYP2C9 and VKORC1 genotype, height, and indication and can be used with a simple pocket calculator.[39]

Until now, no randomized controlled trial (RCT) has been conducted with a regression dosing model in children. One trial has just started, in which a PK/PD dosing model is tested against standard dosing.[48] In adults, 12 RCTs have been carried out to evaluate the dosing algorithms.[49] These trials gave conflicting results with regards to improving the time within therapeutic range (TTR) and outcomes such as bleeding and thromboembolic complications. In a recent meta-analysis, a statistically significant increase in TTR and decrease in minor bleeding was found when comparing fixed standard dosing with genotype-guided dosing.[49]

Currently, the American College of Chest Physicians (ACCP) guideline for antithrombotic therapy and prevention of thrombosis does not recommend genotyping before starting VKAs in adults.[50] The FDA follows this recommendation, while still including information on the impact of pharmacogenomics in the drug label.[51] When genetic information is available, the physician can use this to adjust the dose. In pediatric patients, this information should not be used. Studies showed that the adult models overestimate the VKA dose in children.[39,42] Therefore, pediatric models should be used when genetic information is available. An RCT for examining a pediatric regression model does not seem to be a realistic option for determining the usefulness of genotyping before starting a VKA. The numbers of children using these drugs are very low, and therefore such a trial would be very costly and time consuming. We think the pediatric algorithms should be implemented and evaluated in a clinical setting. Using a dosing model can only lead to an increase in the quality of treatment. There are no risks involved, because adjustments of the dose can still be made based on the International Normalized Ratio (INR). The costs of using a model only consist of the price of genetic testing and these costs are already quite reasonable compared with other medical tests, and will probably decrease further over time. It might be possible that genotyping becomes cost effective, because when INR stability increases it is likely that fewer INR measurements will be needed,

and fewer bleeding and thrombotic events will occur. Evaluations should be carried out during implementation in order to determine if the genetic testing is increasing the quality of treatment and/or lowering the costs.

**Asthma**

Asthma is the most common chronic disease in children. Asthma is treated with a stepwise approach.[52] Short-acting β2 agonists (SABA) as needed are prescribed initially to relieve symptoms of bronchoconstriction. Inhaled corticosteroids (ICS) are added to the regimen if asthma symptoms persist to reduce the airway inflammation and are considered to be the cornerstone of asthma treatment.[52] Additionally, long-acting β2 agonists (LABA) or leukotriene receptor agonists (LTRA) can be added if a child's asthma remains insufficiently controlled. Although asthma treatment is effective in many patients, there is a large variability in the level of symptom control or lung function improvement. Already more than 15 years ago, Drazen et al. suggested that up to 80 % of the interindividual variants in drug response in asthmatic patients could be due to genetic variations.[53] Since then, candidate gene approaches and a handful of GWAS studies have described several genetic variants associated with asthma treatment response, yet effect sizes are often small and a successful replication remains rare.[54–56]

Pharmacogenomics of LABA seems closest to clinical implementation. An SNP of interest (ADRB2 Arg16) has been replicated and prospectively tested and the risk genotype is relatively frequent within the population. Variation in the gene that encodes the β2 receptor (ADRB2) is associated with LABA response in children,[57–59] yet not all studies point in the same direction.[60] Nevertheless, a recent meta-analysis of 4226 children of white Northern European and Latino origin showed that this variant (ADRB2 Arg16) was associated with an increased risk of asthma exacerbation when treated with ICS + LABA (OR 1.52, 95 % CI 1.17–1.99; p = 0.0021).[61] In addition, further evidence has been provided by a small prospective study of 62 children with the genetic variation randomized to ICS + LABA or ICS + LTRA. The trial showed that children treated in the ICS + LTRA arm had fewer exacerbations (exacerbation score of −0.39, 95 % CI −0.15 to −0.64; p = 0.049) and school absences (difference in scores of 0.40, 95 % CI −0.22 to −0.58; p = 0.005) compared with the group treated with ICS + LABA.[62] Approximately 16 % of the children with asthma are homozygous for this variant,[57] and may benefit from genotyping before initiation of LABA treatment. Larger trials are necessary to assess the clinical value and cost effectiveness of ADRB2 genotyping.

Defining treatment response in asthma is complicated. Symptoms vary over time and different dimensions of response (lung function, exacerbations, and symptoms) can be associated with different genetic risk profiles.[63] Furthermore,

asthma consists of a heterogeneous population of various distinct phenotypes (e.g., eosinophilic versus neutrophilic asthma), which seems to differ for children and adults. Performing studies in children is therefore of the uttermost importance. Recently, the Pharmacogenomics in Childhood Asthma (PiCA) consortium has been formed to bring asthma researchers in this field together to perform meta-analyses in well-defined joined pediatric asthma cohorts.[61,64]



## CHALLENGES AND FUTURE DIRECTIONS

Although the research field of pediatric pharmacogenomics is rapidly growing, few applications have made it to clinical practice. We have provided examples of three pediatric diseases where pharmacogenomics holds a promise to personalize treatment: childhood cancer, thrombosis, and asthma. These examples illustrate that gathering evidence for a pharmacogenomic association in children is challenging. Replication of genetic associations is complicated by the heterogeneity in both outcome measures and in small study populations in terms of ethnicity, disease phenotype, and age, which leads to underpowered biased studies. To overcome this obstacle, collaborations should be undertaken to enlarge the number of patients studied.

More studies have been performed on pharmacogenomic associations in adults, including a couple of RCTs, but unfortunately these results in adults cannot be simply extrapolated to children. Pharmacogenomic studies in pediatric populations remain essential. The therapeutic goal of a certain treatment is often different for adults and children. In addition, differences in co-medication, diet, and duration of drug use can also lead to dissimilar results. Before data can be extrapolated to children it should be clear if the association is not influenced by ontogeny. Children not only differ from adults in body size, but also in the dynamic expression of metabolic enzymes, drug transporters, and drug targets.[3,65] Furthermore, the organs involved in drug metabolism and elimination (liver and kidney) are under the influence of developmental processes during childhood.[3] Besides these physical differences, the disease can also manifest itself differently in children, as seen, for example, in asthma.[66] These differences make it hard to predict the PK/PD of a drug in children. The drug response can differ between children, but also within one child over time. Therefore, the extrapolation of results between children of different ages should be done with the same caution as the extrapolation of adult data to children. Pediatric patients span a period from birth to adulthood by most definitions. An RCT is still considered the gold standard to collect evidence. However, performing RCTs in children is complicated by the large sample sizes which are required,

especially in rare diseases such as cancer and thrombosis. For example, in the case of VKAs, obtaining the required sample size is a large problem. For the EU-PACT (European Pharmacogenetics of Anticoagulant Therapy) trial in adults, investigating the effectiveness of the pharmacogenomic dosing models for acenocoumarol, phenprocoumon, and warfarin, the calculated sample size was 400 per VKA.[67,68] To put this in perspective, in the Netherlands, currently only 226 children under the age of 15 years use VKAs.[69] To obtain the number of patients needed, international collaborations are essential. Besides the large sample size, the high costs of an RCT need to be considered. This type of research is usually not in the direct interest of pharmaceutical companies, especially if it concerns off-patent drugs. Therefore, it is difficult to find funding for these kinds of trials, and specific financial or other incentives might be required to bridge this obstacle.[70]

As stated in the introduction, the improvement of genomics technology creates opportunities to study pharmacogenomics in new ways. The newest is PheWAS, which is the opposite of GWAS. Instead of studying genetic associations with a predefined phenotype, patients with a certain mutation are the starting point to search for the matching phenotype. Other examples are WES/WGS in which all DNA mutations will be considered, in contrast to GWAS, which is directed to known (frequently occurring) SNPs.

There is no one method better than the others. Which method or combination of methods is the most appropriate depends largely on the research question/situation (e.g., knowledge about drug mechanism, available budget). Findings of a GWAS, for instance, can be subsequently replicated in a candidate gene study, which requires far fewer patients and is less expensive than an additional GWAS.

The progression from gathering evidence to clinical relevance is not easy. Even when an association is strong it does not mean that it is clinically relevant. For example, in the case of ACYP2 and ototoxicity, the association was quite strong, but it still could explain only 12.4 % of the ototoxicity cases. The clinical relevance largely depends on the relative frequency of the risk allele in the population of interest, the disease phenotype, the severity of the outcome, and the risk attribution of the risk-allele to the outcome. Cost effectiveness of a pharmacogenomic test is inevitably necessary to reach clinical implementation. Even when the costs of genetic testing decline, other costs such as the costs of the possible alternative treatment, use of protective agents, and/or extra monitoring should be considered.

To be able to proceed with implementation of pharmacogenomic testing in children, consensus should be reached about what evidence is needed to implement a pharmacogenetic test into clinical practice if RCTs are not feasible. Furthermore, in some cases performing an RCT could be considered unethical. An important example of this is the risk of codeine-induced infant mortality based on a CYP2D6 genotype

of breastfeeding mothers.[71] This has led to a change in the registration of codeine. Codeine is no longer approved for pediatric use in the EU and is contraindicated in women during breastfeeding.[72]

When an RCT is impossible, at least worldwide replication studies are needed to support the generalizability of the association. This is only possible with international collaboration. However, the healthcare systems and availability of treatment options (e.g., differences in authorized VKAs) differ largely between countries and treatment protocols vary between countries, study populations, and over time. This makes finding a comparable replication cohort challenging. Therefore, international treatment harmonization would ease the process of worldwide replication studies.

Strong evidence in adults might support the associations found in pediatric patients. However, because of differences related to ontogeny, adult-derived information should be considered with caution and is not essential. This caution should also be applied when using the dosing guidelines available for adults. As seen in the example for VKAs, using the adult models would lead to an overestimation of the required dose. Pharmacogenomics needs to be considered as valuable information in addition to clinical parameters to guide treatment decisions.

It is important that consensus is reached about the evidence needed for implementation and that healthcare professionals also support these criteria; published, peer-reviewed clinical practice guidelines could be of particular help here. Clinicians need to be appropriately educated on the value of pharmacogenomic testing. Only then will pharmacogenomics be implemented in pediatric clinical practice.

## CONCLUSION

Pharmacogenomics is a promising research field, but has not reached the pediatric clinic yet. International collaborations are needed to gain a more structured approach for pharmacogenomic research in children. When heterogeneity is reduced and research groups work together in order to obtain larger numbers of patients, it is possible to get stronger evidence, both qualitatively and quantitatively. The criteria for implementing a pharmacogenomic test without the presence of a supporting pediatric RCT should be further elaborated by healthcare professionals and researchers. Reaching consensus could lead to easier acceptance by healthcare professionals to the use of these tests in daily clinical practice.

## REFERENCES


1. McLeod HL, Evans WE. Pharmacogenomics: unlocking the human genome for better drug therapy. Annu Rev Pharmacol Toxicol 2001; 41: 101–21.
2. Mooney SD. Progress towards the integration of pharmacogenomics in practice. Hum Genet 2015; 134: 459–65.
3. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental Pharmacology — Drug Disposition, Action, and Therapy in Infants and Children. N Engl J Med 2003; 349: 1157–67.
4. Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L. Maturation of the hemostatic system during childhood. Blood 1992; 80: 1998–2005.
5. American Cancer Society. American Cancer Society. Global Cancer Facts & Figures. 3rd ed. Atlanta, GA: American Cancer Society; 2015.
6. Howlander N, Noone A, Krapcho M, Grashell J, Miller D, Altekruse S, et al. Seer Cancer Statistics Review, 1975-2012 National Cancer Institute, Bethesda, MD, http://seer.cancer.gov/csr/1975_2012/, based on November 2014 SEER data submission, posted to the SEER web site, Apr 2015.
7. Bass JK, Knight KR, Yock TI, Chang KW, Cipkala D, Grewal SS. Evaluation and Management of Hearing Loss in Survivors of Childhood and Adolescent Cancers: A Report From the Children's Oncology Group. Pediatr Blood Cancer 2016; 63: 1152–62.
8. Ross CJD, Katzov-Eckert H, Dubé M-P, Brooks B, Rassekh SR, Barhdadi A, et al. Genetic variants in TPMT and COMT are associated with hearing loss in children receiving cisplatin chemotherapy. Nat Genet 2009; 41: 1345–9.
9. Pussegoda K, Ross CJ, Visscher H, Yazdanpanah M, Brooks B, Rassekh SR, et al. Replication of TPMT and ABCC3 genetic variants highly associated with cisplatin-induced hearing loss in children. Clin Pharmacol Ther 2013; 94: 243–51.
10. Brown AL, Lupo PJ, Okcu MF, Lau CC, Rednam S, Scheurer ME. SOD2 genetic variant associated with treatment-related ototoxicity in cisplatin-treated pediatric medulloblastoma. Cancer Med 2015; 4: 1679–86.
11. Choeyprasert W, Sawangpanich R, Lertsukprasert K, Udomsubpayakul U, Songdej D, Unurathapan U, et al. Cisplatin-induced ototoxicity in pediatric solid tumors: the role of glutathione S-transferases and megalin genetic polymorphisms. J Pediatr Hematol Oncol 2013; 35: e138-143.
12. Oldenburg J, Kraggerud SM, Cvancarova M, Lothe RA, Fossa SD. Cisplatin-induced long-term hearing impairment is associated with specific glutathione s-transferase genotypes in testicular cancer survivors. J Clin Oncol Off J Am Soc Clin Oncol 2007; 25: 708–14.
13. Caronia D, Patiño-García A, Milne RL, Zalacain-Díez M, Pita G, Alonso MR, et al. Common variations in ERCC2 are associated with response to cisplatin chemotherapy and clinical outcome in osteosarcoma patients. Pharmacogenomics J 2009; 9: 347–53.
14. Riedemann L, Lanvers C, Deuster D, Peters U, Boos J, Jürgens H, et al. Megalin genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. Pharmacogenomics J 2008; 8: 23–8.
15. Spracklen TF, Whitehorn H, Vorster AA, Ramma L, Dalvie S, Ramesar RS. Genetic variation in Otos is associated with cisplatin-induced ototoxicity. Pharmacogenomics 2014; 15: 1667–76.
16. Lanvers-Kaminsky C, Sprowl JA, Malath I, Deuster D, Eveslage M, Schlatter E, et al. Human OCT2 variant c.808G>T confers protection effect against cisplatin-induced ototoxicity. Pharmacogenomics 2015; 16: 323–32.
17. Xu X, Ren H, Zhou B, Zhao Y, Yuan R, Ma R, et al. Prediction of copper transport protein 1 (CTR1) genotype on severe cisplatin induced toxicity in non-small cell lung cancer (NSCLC) patients. Lung Cancer Amst Neth 2012; 77: 438–42.

18. Peters U, Preisler-Adams S, Hebeisen A, Hahn M, Seifert E, Lanvers C, et al. Glutathione S-transferase genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. Anticancer Drugs 2000; 11: 639–43.
19. Yang JJ, Lim JYS, Huang J, Bass J, Wu J, Wang C, et al. The role of inherited TPMT and COMT genetic variation in cisplatin-induced ototoxicity in children with cancer. Clin Pharmacol Ther 2013; 94: 252–9.
20. Hagleitner MM, Coenen MJH, Patino-Garcia A, de Bont ESJM, Gonzalez-Neira A, Vos HI, et al. Influence of genetic variants in TPMT and COMT associated with cisplatin induced hearing loss in patients with cancer: two new cohorts and a meta-analysis reveal significant heterogeneity between cohorts. PloS One 2014; 9: e115869.
21. Lanvers-Kaminsky C, Malath I, Deuster D, Ciarimboli G, Boos J, Am Zehnhoff-Dinnesen AG. Evaluation of pharmacogenetic markers to predict the risk of Cisplatin-induced ototoxicity. Clin Pharmacol Ther 2014; 96: 156–7.
22. Xu H, Robinson GW, Huang J, Lim JY-S, Zhang H, Bass JK, et al. Common variants in ACYP2 influence susceptibility to cisplatin-induced hearing loss. Nat Genet 2015; 47: 263–6.
23. Scheffer DI, Shen J, Corey DP, Chen Z-Y. Gene Expression by Mouse Inner Ear Hair Cells during Development. J Neurosci Off J Soc Neurosci 2015; 35: 6366–80.
24. Ratain MJ, Cox NJ, Henderson TO. Challenges in interpreting the evidence for genetic predictors of ototoxicity. Clin Pharmacol Ther 2013; 94: 631–5.
25. Boddy AV. Genetics of cisplatin ototoxicity: confirming the unexplained? Clin Pharmacol Ther 2013; 94: 198–200.
26. Carleton BC, Ross CJ, Bhavsar AP, Amstutz U, Pussegoda K, Visscher H, et al. Role of TPMT and COMT genetic variation in cisplatin-induced ototoxicity. Clin Pharmacol Ther 2014; 95: 253.
27. Carleton BC, Ross CJ, Hayden MR. Genetic markers of cisplatin-induced hearing loss in children. Clin Adv Hematol Oncol 2014; 12: 527–8.
28. Carleton BC, Ross CJ, Bhavsar AP, Lee JW, Visscher H, Rassekh SR, et al. Response to "evaluation of pharmacogenetic markers to predict the risk of Cisplatin-induced ototoxicity." Clin Pharmacol Ther 2014; 96: 158.
29. U.S. Food and Drug Administration (FDA): Centre for Drug Evaluation and Research. Cisplatin label information. 2015. http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/018057s083lbl.pdf. Accessed 2 Feb 2016.
30. Raffini L, Huang YS, Witmer C, Feudtner C. Dramatic increase in venous thromboembolism in children's hospitals in the United States from 2001 to 2007. Pediatrics 2009; 124: 1001–8.
31. Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G, American College of Chest Physicians. Oral anticoagulant therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012; 141: e44S–88S.
32. Streif W, Andrew M, Marzinotto V, Massicotte P, Chan AK, Julian JA, et al. Analysis of warfarin therapy in pediatric patients: A prospective cohort study of 319 patients. Blood 1999; 94: 3007–14.
33. Verhoef TI, Redekop WK, Daly AK, van Schie RMF, de Boer A, Maitland-van der Zee A-H. Pharmacogenetic-guided dosing of coumarin anticoagulants: algorithms for warfarin, acenocoumarol and phenprocoumon. Br J Clin Pharmacol 2014; 77: 626–41.
34. Hamberg A-K, Wadelius M. Pharmacogenetics-based warfarin dosing in children. Pharmacogenomics 2014; 15: 361–74.
35. Shaw K, Amstutz U, Kim RB, Lesko LJ, Turgeon J, Michaud V, et al. Clinical Practice Recommendations on Genetic Testing of CYP2C9 and VKORC1 Variants in Warfarin Therapy. Ther Drug Monit 2015; 37: 428–36.

36. Teichert M, Eijgelsheim M, Rivadeneira F, Uitterlinden AG, van Schaik RHN, Hofman A, et al. A genome-wide association study of acenocoumarol maintenance dosage. Hum Mol Genet 2009; 18: 3758–68.
37. Hirai K, Hayashi H, Ono Y, Izumiya K, Tanaka M, Suzuki T, et al. Influence of CYP4F2 polymorphisms and plasma vitamin K levels on warfarin sensitivity in Japanese pediatric patients. Drug Metab Pharmacokinet 2013; 28: 132–7.
38. Nowak-Göttl U, Dietrich K, Schaffranek D, Eldin NS, Yasui Y, Geisen C, et al. In pediatric patients, age has more impact on dosing of vitamin K antagonists than VKORC1 or CYP2C9 genotypes. Blood 2010; 116: 6101–5.
39 Biss TT, Avery PJ, Brandão LR, Chalmers EA, Williams MD, Grainger JD, et al. 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.
40. Kato Y, Ichida F, Saito K, Watanabe K, Hirono K, Miyawaki T, et al. Effect of the VKORC1 genotype on warfarin dose requirements in Japanese pediatric patients. Drug Metab Pharmacokinet 2011; 26: 295–9.
41. 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.
42. Shaw K, Amstutz U, Hildebrand C, Rassekh SR, Hosking M, Neville K, et al. VKORC1 and CYP2C9 genotypes are predictors of warfarin-related outcomes in children. Pediatr Blood Cancer 2014; 61: 1055–62.
43. Moreau C, Bajolle F, Siguret V, Lasne D, Golmard J-L, Elie C, et al. 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.
44. 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.
45. Lala M, Burckart GJ, Takao CM, Pravica V, Momper JD, Gobburu JVS. Genetics-based pediatric warfarin dosage regimen derived using pharmacometric bridging. J Pediatr Pharmacol Ther 2013; 18: 209–19.
46. Hamberg A-K, Friberg LE, Hanséus K, Ekman-Joelsson B-M, Sunnegårdh J, Jonzon A, et al. Warfarin dose prediction in children using pharmacometric bridging--comparison with published pharmacogenetic dosing algorithms. Eur J Clin Pharmacol 2013; 69: 1275–83.
47. Hamberg A-K, Hellman J, Dahlberg J, Jonsson EN, Wadelius M. A Bayesian decision support tool for efficient dose individualization of warfarin in adults and children. BMC Med Inform Decis Mak 2015; 15: 7.
48. De Montfort University; University hospitals, Leicester. Model-based Versus Traditional Warfarin Dosing in Children. In: ClinicalTrials.gov. Bethesda(MD): National Libary of Medicine (US). 2000. http://clinicaltrials.gov/show/NCT02475863. Accessed 26 Jan 2016.
49. Belley-Cote EP, Hanif H, D'Aragon F, Eikelboom JW, Anderson JL, Borgman M, et al. Genotype-guided versus standard vitamin K antagonist dosing algorithms in patients initiating anticoagulation. A systematic review and meta-analysis. Thromb Haemost 2015; 114: 768–77.
50. Guyatt GH, Akl EA, Crowther M, Gutterman DD, Schuünemann HJ, American College of Chest Physicians Antithrombotic Therapy and Prevention of Thrombosis Panel. Executive summary: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012; 141: 7S–47S.
51. U.S. Food and Drug Administration (FDA): Centre for Drug Evaluation and Research. Label information Coumadin. 2015. http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/009218s115lbl.pdf. Accessed 2 Feb 2016.
52. HGlobal Initiative for Asthma. Global strategy for asthma management and prevention. Updated 2015. http://www.ginasthma.org/local/uploads/files/GINA_Report_2015.pdf. Accessed 2 Feb 2016.
53. Drazen JM, Silverman EK, Lee TH. Heterogeneity of therapeutic responses in asthma. Br Med Bull 2000; 56: 1054–70.

54. Tantisira KG, Lasky-Su J, Harada M, Murphy A, Litonjua AA, Himes BE, et al. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. N Engl J Med 2011; 365: 1173–83.
55. Hosking L, Bleecker E, Ghosh S, Yeo A, Jacques L, Mosteller M, et al. GLCCI1 rs37973 does not influence treatment response to inhaled corticosteroids in white subjects with asthma. J Allergy Clin Immunol 2014; 133: 587–9.
56. Vijverberg SJH, Tavendale R, Leusink M, Koenderman L, Raaijmakers JAM, Postma DS, et al. Pharmacogenetic analysis of GLCCI1 in three north European pediatric asthma populations with a reported use of inhaled corticosteroids. Pharmacogenomics 2014; 15: 799–806.
57. Zuurhout MJL, Vijverberg SJH, Raaijmakers JAM, Koenderman L, Postma DS, Koppelman GH, et al. Arg16 ADRB2 genotype increases the risk of asthma exacerbation in children with a reported use of long-acting β2-agonists: results of the PACMAN cohort. Pharmacogenomics 2013; 14: 1965–71.
58. Basu K, Palmer CNA, Tavendale R, Lipworth BJ, Mukhopadhyay S. Adrenergic beta(2)-receptor genotype predisposes to exacerbations in steroid-treated asthmatic patients taking frequent albuterol or salmeterol. J Allergy Clin Immunol 2009; 124: 1188–1194.e3.
59. Palmer CNA, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 beta2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. Thorax 2006; 61: 940–4.
60. Giubergia V, Gravina L, Castaños C, Chertkoff L. Influence of β(2)-adrenergic receptor polymorphisms on asthma exacerbation in children with severe asthma regularly receiving salmeterol. Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol 2013; 110: 156–60.
61. Turner S, Francis B, Vijverberg S, Pino-Yanes M, Maitland-van der Zee AH, Basu K, et al. Childhood asthma exacerbations and the Arg16 β2-receptor polymorphism: A meta-analysis stratified by treatment. J Allergy Clin Immunol 2016; .
62. Lipworth BJ, Basu K, Donald HP, Tavendale R, Macgregor DF, Ogston SA, et al. Tailored second-line therapy in asthmatic children with the Arg(16) genotype. Clin Sci 2013; 124: 521–8.
63. Wu AC, Tantisira K, Li L, Schuemann B, Weiss ST, Fuhlbrigge AL, Childhood Asthma Management Program Research Group. Predictors of symptoms are different from predictors of severe exacerbations from asthma in children. Chest 2011; 140: 100–7.
64. Vijverberg SJH, Raaijmakers JAM, Maitland-van der Zee AH. ADRB2 Arg16 and the need for collaboration in childhood asthma pharmacogenomics. Pharmacogenomics 2013; 14: 1937–9.
65. Mooij MG, Nies AT, Knibbe CAJ, Schaeffeler E, Tibboel D, Schwab M, et al. Development of Human Membrane Transporters: Drug Disposition and Pharmacogenetics. Clin Pharmacokinet 2016; 55:507-24.
66. Wang F, He XY, Baines KJ, Gunawardhana LP, Simpson JL, Li F, et al. Different inflammatory phenotypes in adults and children with acute asthma. Eur Respir J 2011; 38: 567–74.
67. Verhoef TI, Ragia G, de Boer A, Barallon R, Kolovou G, Kolovou V, et al. A randomized trial of genotype-guided dosing of acenocoumarol and phenprocoumon. N Engl J Med 2013; 369: 2304–12.
68. Pirmohamed M, Burnside G, Eriksson N, Jorgensen AL, Toh CH, Nicholson T, et al. A randomized trial of genotype-guided dosing of warfarin. N Engl J Med 2013; 369: 2294–303.
69. Dutch National Health Care Institute. Drug Information System. 2014. https://www.gipdatabank.nl/databank.asp?tabel=03-lftgesl&geg=gebr&item=B01AA. Accessed 4 Apr 2016.
70. Koch VG. Incentivizing the Utilization of Pharmacogenomics in Drug Development. 2012 15: 263–302.
71. Koren G, Cairns J, Chitayat D, Gaedigk A, Leeder SJ. Pharmacogenetics of morphine poisoning in a breastfed neonate of a codeine-prescribed mother. Lancet Lond Engl 2006; 368: 704.
72. European Medicine Agency. Codeine not to be used in children below 12 years for cough and cold. 2015. http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Codeine_cough_or_cold_in_children/Position_provided_by_CMDh/WC500186159.pdf. Accessed 16 Mar 2016.

# 2.2 Oral anticoagulation treatment in pediatric patients



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## ABSTRACT

In pediatric patients oral anticoagulant agents are used for a variety of rare congenital conditions, after thromboembolic events and prophylactically. Evidence based treatment is challenging, given the small number of cases of highly varying ages and etiology. Treatment is often based on arbitrary extrapolations of recommendations for adults.

This review examines the differences between adults and children with regards to the risk factors, the hemostatic and metabolic system and the recent developments in new and personalized oral anticoagulant treatment in pediatric patients.

## INTRODUCTION

In pediatric patients vitamin K antagonists (VKAs) are used for a variety of generally rare congenital conditions, after thromboembolic events and as prophylaxis, e.g. after cardiac surgery for congenital heart disease.[1,2] The incidence of thrombotic events is quite low between 0.07 and 0.49 events/ 10,000 children[3–6] compared to adults with an incidence of 10 events/ 10,000 adults each year.[7] The recent years a rising trend was seen in the incidence of thrombosis in children,[8,9] mainly due to intensified medical treatments including central venous catheters, intensive chemotherapy (e.g. asparaginase) and increased awareness. VKAs are at this moment the only oral anticoagulants which are recommended in guidelines to prevent and treat thromboembolic events in children. Even though these drugs have been on the market for decades, the use is still off-label and the evidence for treatment of children with VKAs is limited and is largely based on extrapolations of recommendations for adults.



Treatment with VKAs in general has difficulties like high inter and intra-individual variability in dose requirements. In addition, the unavailability of practical dose units especially for neonates further limits their use. Multiple studies have been conducted in pediatric patients to explain the variability in dose requirement and to better predict the starting dose for new patients who start a VKA.[10–19] VKA dosage is monitored with regular international normalized ratio (INR) assays. Optimal INR and duration of treatment are based on limited data in children or extrapolated adult data.

Since 10 years the direct oral anticoagulants (DOACs) are available for adults. These drugs have the advantage of a standard dose and no need for laboratory measurements for dosing. In children the first phase 1 and 2 studies have been completed and phase 3 and 4 studies are currently recruiting patients. The estimated completion date of the first phase 3 study (primary outcomes: all symptomatic recurrent venous thromboembolism and major or clinically relevant non-major bleeding) is January 2019 (NCT02234843).

Evidence in the adult population is not always a reliable source for the treatment of children.[20] Children with thromboembolic events differ from adults with regards to risk factors, coagulation characteristics and drug metabolism. Therefore conclusions from studies in adults are difficult to translate to children. Clinical studies in pediatric cohorts will be important to compare treatments and choose wisely for individual patients.

## MAJOR DIFFERENCES IN RISK FACTORS, HEMOSTASIS AND DRUG METABOLISM

### Risk factors for thromboembolic events in children

Inherited prothrombotic disorders play a diverse role in pediatric thrombosis. Some are in themselves clearly prothrombotic (e.g. protein S, C and antithrombin deficiency), others have an unknown impact, however become apparent in prothrombotic contexts like the use of oral contraceptives (e.g. heterozygous factor V Leiden mutation).

In a large proportion of the cases of pediatric thrombosis an inherited prothrombotic disorder is present.[21,22] However, often the cause is multifactorial.[23] A meta-analysis of case control studies has shown that having any central venous catheter (CVC) or being admitted to the ICU increases the risk of developing a VTE (CVC: odds ratio (OR) 2.12, 95% confidence interval(CI) (2.00-2.25); ICU: 2.14 (1.97-2.32)).[24] Smaller risk effects were shown for mechanical ventilation (1.56 (1.42-1.72)) and length of stay in the hospital (1.03 (1.03-1.03)).[24] In non-case control studies, including retrospective or prospective cohort studies, case series, cross-sectional and registry studies, CVC is the most prevalent acquired risk factors (pooled prevalence 36%, 95%CI (23-4)]) next to oral contraceptive pill use (34% (11-56)), thrombophilia (28% (18-38)), and obesity (26% (8-45)).[24] For neonates and children younger than 6 months of age with a VTE a higher prevalence of central venous catheter was demonstrated, 94% and 77%, respectively.[4,25]

Pediatric patients with a venous thromboembolism differ from adults in that in the majority

A clear difference between children and adults with a venous thrombosis is that for the majority of events in children at least one risk factor is identified and arise proximal to hospitalization. In adults 30-50% of the events are considered idiopathic.[26]

### Development of the hemostatic system

The hemostatic system changes during childhood and adolescence. In fact, the hemostatic system is developing during our entire lifespan.[27] However, most of the maturation of hemostasis takes place in the first 6 months of life. In the neonate the hemostasis is differently balanced than later in life. Neonates have a lower capacity to generate thrombin, a decreased fibrinolytic capacity, and they have a reduced capacity to inhibit coagulation proteins compared to adults.[20]

Among the vitamin K-dependent coagulation factors (factor II, VII, IX and X) there are two distinct patterns of development. The mean level of factor VII is already at 63% of adult level at birth and increases in the first 5 days of life to 85% of adult level.[28,29] However, the levels of factor II, IX and X are all lower than 50% at birth and

gradually increase to 74-81% of adult levels after 6 months of life.[28,29] At the age of 16 years the levels are still statistically significantly lower than in adults.[28]

The activity of thrombin is reduced in children. The cause of this is twofold. Firstly, the capacity to produce thrombin is reduced compared to adults because of the decreased levels of vitamin-K dependent coagulation factors and contact factors [30]. Secondly, the inhibition of thrombin by α2-macroglobulin, which complexes with thrombin, is increased.[28,29] The mean levels of α2-macroglobulin are approximately 2 times higher than in adults throughout childhood and adolescence.[28,29]



The natural anticoagulants protein C and protein S are available at very low levels at birth (36-39%).[29] After 6 months the level of protein S has increased to almost adult level, but protein C remains relatively low at 61% of adult level.[28,29] The level of protein C gradually increases during childhood and adolescence and is almost at adult level at age 16 (86%).[28] Antithrombin is at 60% at birth and reaches adult level at an age of 6 months.[29]

A difference in the development of the hemostatic system between pre-term and full-term infants has been observed.[30] Of the vitamin K-dependent coagulation factors factor II and IX show reduced levels at birth for pre-term compared to full-term infants [30]. At 6 months of age, pre-term and full-term infants have a similar factor II level, but the factor IX level is still lower for pre-term infants.[30] The levels of protein S and C are lower at birth in pre-term infants and reach similar levels as full-term infants at 6 months of age [30]. However, antithrombin is lower at birth and is still at a lower level at 6 months of age for pre-term compared to full-term infants.[30]

All these aberrant levels reflect a hemostatic system, which is balanced in a different way compared to the adult system and it even depends on the gestational age of the infant. This can cause a different response to oral anticoagulants.

**Development of drug metabolism**

Most of the Cytochrome P450(CYP) enzymes are almost undetectable at birth, but rise during the first year of life.[31] For warfarin and acenocoumarol CYP2C9 is the main enzyme involved in the metabolism of these compounds.[32] The onset of expression of CYP2C9 is in the second and third trimester of pregnancy at low levels (10% of adult levels).[33] After birth, the expression gradually increases with a 100-fold inter-individual variability in expression levels during the neonatal period.[33] Between the age of one and ten years CYP2C9 has been shown to be expressed at 40-50% of adult levels.[33] However, a study by Takahashi in Japanese patients showed, that the oral clearance of unbound oral-S-warfarin is the highest during the prepubertal age and decreases at pubertal age to adult levels.[34] Therefore, it has been suggested that CYP2C9 shows a postnatal developmental pattern with low expression before birth, rising within the first 6 months of life to 100% adult

activity and then exceeds adult activity at prepubertal age returning to adult levels by the end of puberty.[35]

For most of the DOACs and for phenprocoumon CYP3A4 is an important metabolizing enzyme.[32,36] CYP3A4 has also been classified as having a postnatal developmental pattern.[37] CYP3A4 is expressed at very low levels during the entire childhood and adolescence and levels increase gradually over time.[38]

## PERSONALIZED DOSING OF VKAs WITH PHARMACOGENETIC MODELS

The inter- and intraindividual variability in dose requirements of children is a major problem when starting treatment with VKAs. Genetic differences can enhance interindividual variability. In adults, genetic variations in especially the genes encoding for Vitamin K epoxide reductase complex subunit 1 (VKORC1) and CYP2C9 have been shown to explain part of the interindividual variability in VKA dose requirement. It involves the single nucleotide polymorphisms *VKORC1* 1173 C>T rs9934438 and -1639 G>A rs9923231, which are in complete linkage disequilibrium, and *CYP2C9*2* rs1799853 and CYP2C9*3 rs1057910. VKORC1 is the target and CYP2C9 the main metabolizing enzyme for VKAs. The genetic variations make patients more sensitive to VKAs and therefore they require lower doses compared to patients without these genetic variations.

Since 2010, multiple studies have been carried out to build regression models to predict the right dosage of VKAs in pediatric patients (Table 1). These models were able to explain 34% to 82% of the inter- and intraindividual variation in dose requirements. Most studies were conducted in children who used warfarin[10–19] and two studies also included patients using phenprocoumon[10] or fluindione.[13] The studies are relatively small in comparison with the studies in adults. There is a large variability in percentage explained of the variation in VKA dose requirement by various genetic and non-genetic factors. Age and age-related factors (e.g. height, weight, body surface area) explained 12% to 72% of the variability in dose requirement. Furthermore, having a Fontan circulation was found to explain 2.4% and 3.2% of the variability in two studies,[12,15] three other studies did not find an association with dose requirement.[14,18,19] Moreover, the target INR (range) was found to be associated with the required dose in halve of the studies (5 studies did and 6 studies did not find an association).[11,13,14,18,19] The reason why the target range (TR) was not always associated with the required dose could have been the result of the relatively small sample sizes. The influence of the TR could be very small because the different TRs are highly overlapping. Larger sample sizes would be required to make a distinction between the TRs in the dose requirement.

**Table 1.** Overview of pharmacogenetic regression models in pediatric patients, with percentages of the variability explained

| | | Genetic | | | Non-genetic | | | Model | |
|---|---|---|---|---|---|---|---|---|---|
| | | CYP2C9 | VKORC1 | CYP4F2 | Age/ weight/ height/ BSA sex | Indication | Target INR (range) | $R^2$ | |
| Nowak-Göttl *et al.*[10] | n=34 w | 0.5%* | 2.8%* | – | Age: 31.2%<br>Sex: NA | – | NA | 34.0% | Not given |
| | n=26 p | 0.3%* | 10.8%* | – | Age: 25.5%<br>Sex: NA | – | NA | 35.5% | Not given |
| | n=60 p + w | 0.4%* | 3.7%* | – | Age: 28.3%<br>Sex: NA | – | NA | 38.2% | Sqrt dose[mg/kg/day] = 0.49 − 0.013(age[year]) − 0.08(VKORC1[AA]) + 0.01(VKORC1[GA]) − 0.02(variant CYP2C9 allele (s); yes=1, no=0). |
| Kato *et al.*[11] | n=48 w | – | A | – | Age: A<br>Weight: A | – | A | – | Model 3: INR = 1.26 + 6.70 × (dose [mg/day]/weight[kg]) x (1+0.105 × [age (year) − 6.6]) × 0.523 (variant VKORC1 allele(s); yes=1, no=0) |
| Biss *et al.*[12] | n=120 w | 26.6% | 12.8% | – | Height: 29.8% | 3.2% | NA | 72.4% | Sqrt dose [mg/day] = -0.009 + 0.011 (height[cm]) + 0.357(number of variant VKORC1 alleles) − 0.478(number of CYP2C9*3 alleles) − 0.277(number of CYP2C9*2 alleles) + 0.186 (Fontan procedure; yes=1, no=0). |
| Moreau *et al.*[13] | n=83 w | 2.0%* | 18.2% | NA | Height: 48.1%;<br>age, weight, sex: A | – | 4.4%* | 69.7% | Dose [mg/week] = (-10.77 + 0.28) × (height [cm] − 5.44) × (number of VKORC1 variant allele) + (7.83 if target INR is 2.5 or 11.52 if target INR is 3.3-3.29) × (number of CYP2C9 variant alleles) |
| | n=35 f | NA | A | NA | NA | – | NA | – | Not given |
| Nguyen *et al.*[14] | n=37 w | 5.0%* | 47% | – | Age:12%; sex: NA;<br>weight, height and BSA: A | NA | 18% | 82.0% | Dose [mg/kg/day] =−0.090 − 0.00060(age[year]) + 0.11(VKORC1CC) + 0.043(VKORC1TC) + 0.045(CYP2C9*1*1) + 0.039(CYP2C9*1*2) + 0.073(Target INR) |
| Shaw *et al.*[15] | n=93 w | 8.9% | 12.2% | NA | Weight: 52.8%; age,<br>height and BSA: A; sex: NA | 2.4% | NA | 76.3% | Sqrt dose [mg/day]=1.711 + 0.014(weight[kg]) × 0.257(number of VKORC1 variant alleles) × 0.127(number of CYP2C9*2 alleles) × 0.463(number of CYP2C9*3 alleles) × 0.161 (Fontan procedure; yes=1, no=0). |
| Vear *et al.*[16] | n=100 w | 6% | 13% | NA | Age: 31%;<br>height: A | – | – | 53% | Log dose [mg/day]=1.098 + 0.027(age[year]) − 1.124(VKORC1A/A) − 0.733(VKORC1G/A) + 0.345(CYP2C9G/G) + 0.031 (age[year] × VKORC1A/A) + 0.037(age[year] × VKORC1G/A) |
| Wang *et al.*[17] | n=47 w | NA | 11.2% | – | Weight: 33.0%;<br>age, BSA, height: A | – | NA | 44.2% | Dose (mg/kg) = 0.133 − 0.002* weight (kg) + 0.026 * VKORC1(1 if CC or CT; 0 if TT) |
| Wakamiya *et al.*[18] | n=45 w | NA; | 27% | NA | Height: 72%; Age: 49%;<br>BSA: 67%; weight: 59% | NA | A | 78.2% | Sqrt dose [mg/day] = 0.235 + 0.011*height − 0.3 VKORC1 TT genotype. |
| Hawcutt *et al.*[19] | n=100 w | A | A | | Age: 29.2%;<br>Sex: NA | NA | A | 41.4% | Multiple regression model is not given, but incorporated age, INR group, CYP2C9*2 (additive), and VKORC1-1639 (additive) |

w, warfarin; f, fluidione; p, phenprocoumon; A, associated; NA, not associated; -, not assessed or reported; sqrt, square root; INR, international normalized ratio; BSA, body surface area; *, not associated univariately (p>0.05).

All studies found an association between VKORC1 genotype and dose requirement of VKAs, although the percentage explained varied from 2.8% to 47%. The CYP2C9 genotype was only found to be associated in 8 of the 10 studies and explained 0.3% to 26.6%. There was a trend towards VKORC1 explaining more of the variability compared with CYP2C9. Four studies also assessed the association between CYP4F2 genotype and the required dose, but none of the studies found an association.[13,15,16,18]

Hamberg et al. and Lala et al. both constructed a pharmacokinetics/pharmacodynamics (PK/PD) model derived from adult data and evaluated the model in a cohort of pediatric patients.[39,40] In the model of Hamberg et al. CYP2C9, VKORC1, age, weight, target INR range (TR) and baseline INR were incorporated in the model.[39] In the model of Lala et al. the same variables were included except TR and baseline INR.[40] In a retrospective evaluation of the different available models in a pediatric patient cohort the PK/PD model of Hamberg et al. performed best with a high proportion (45%) of patients for whom the predicted maintenance dose was within ± 20% of the observed dose. Of the evaluated regression models the model by Biss et al. performed best, 39% of the patients had a predicted maintenance dose within ± 20% of the observed dose.

A few studies did not develop a model, but have merely investigated the influence of genetic factors on the mean maintenance dose. For example in 41 Egyptian pediatric patients no association was found between the mean daily warfarin maintenance dosages and CYP2C9 and VKORC1 genotypes. Age was the only variable that showed a statistically significant association with warfarin dosage.[41] In 37 Japanese pediatric patients a study on the pharmacodynamics and pharmacokinetics of warfarin, was the only pediatric study so far showing that CYP4F2 genotype influenced dose requirement.[42] This study also found a relationship between CYP2C9 genotype and warfarin clearance, but no association between VKORC1 genotype and warfarin sensitivity (INR/warfarin plasma unbound concentration).[42] These two studies were conducted in a small non-European pediatric patients. Both ethnicity and the small number of patients are a probable cause of not finding an association between these CYP2C9 and VKORC1 genotypes and the warfarin dose requirement.

In adults much more research is conducted on constructing models and validating them in prospective clinical trials. In most studies in adults pharmacogenetic models showed a positive effect on the quality of the treatment with VKAs.[43,44]

Two studies calculated how well the adult warfarin model constructed by the International Warfarin Pharmacogenetics Consortium (IWPC)[45] predicted the maintenance dose for a cohort of children.[12,18] The calculated dosages correlated with the real maintenance dosages, but there was a continuous overestimation by

**Table 2.** Results of dabigatran phase 2 studies

| | NCT02223260 | NCT01083732 | NCT00844415 |
|---|---|---|---|
| **Age** | < 1 year of age | 1-<12 years of age | 12-<18 years of age |
| **Indication** | VTE | Primary VTE | Primary VTE |
| **Screened/treated** | 8 | 20/18 | 9 |
| **Completed** | 8 | 18 | 8 |
| **Treatment** | Liquid formulation of dabigatran at an age and weight adjusted dose | Single dose or multidose (twice daily for 3 days) of oral liquid dabigatran (dissolved granules) | Twice daily oral dabigatran for 3 consecutive days at a weight adjusted dose. |
| **Bleeding events** | All type n=0 | n=0 | n=0 |
| **Recurrent TE** | Not assessed | Not assessed | n=1 (7 days after treatment) |
| **SAE** | n=0 | n=0 | n=1 not study drug related |
| **Total other AEs** | n=0 | n=1 in the multidose group (leukopenia and dizziness) | n=2 (1 patient with abdominal discomfort and 1 patient with gastroesophageal reflux and abdominal pain) |
| **Global assessment of acceptability and tolerability – immediately after dosing** | Good n=6;<br>Satisfactory n=1;<br>Bad; n=1 | Good n=6;<br>Satisfactory n=3;<br>Not satisfactory n=6;<br>Bad n= 2;<br>Not assessed n=1 | Not assessed |
| **Taste global assessment of acceptability and tolerability –immediately after dosing** | Not assessed | Satisfactory n=5;<br>Bad taste n=2;<br>Very bad taste n=3;<br>Missing n=8 | Not assessed |

VTE, venous thromboembolism; TE, thromboembolic event; SAE, serious adverse event; AE, adverse event.

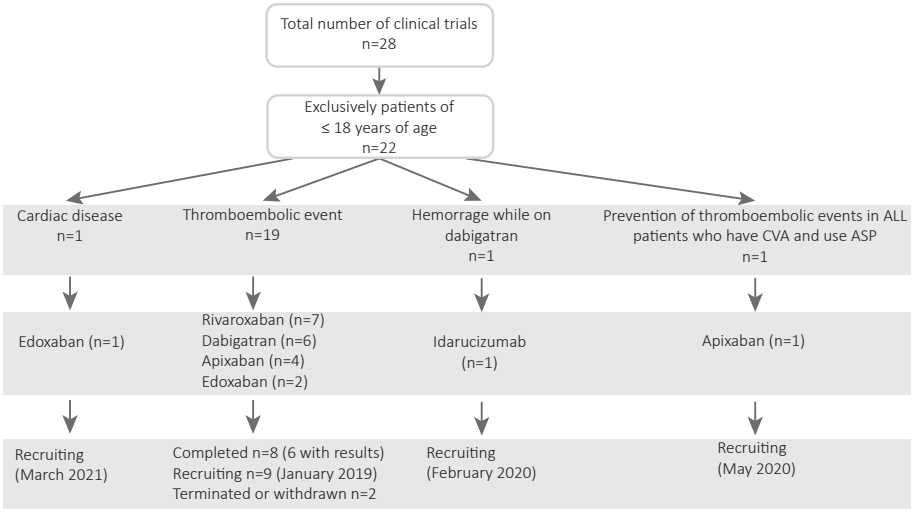


**Figure 1.** Overview of studies carried out on DOACs in children. Source: clinicaltrials.gov.

the model.[12,18] Causes for this overestimation can be the developing hemostatic system, but also metabolic changes as described above.

## DIRECT ORAL ANTICOAGULANTS

In adults four DOACs (dabigatran, rivaroxaban, apixaban and edoxaban) have been approved for the use in preventing and treating thromboembolic events. DOACs act by direct inhibition of factor Xa (rivaroxaban, apixaban, edoxaban) or thrombin (dabigatran). As described, levels of factor II and factor X are changing during childhood and do not reach adult levels until adolescence. Furthermore, the capacity to produce thrombin is reduced and the inhibition of thrombin by α2-macroglobulin is increased in children. Therefore the hemostatic system differs substantially between adults and children on the targets of DOACs, by which the effectiveness and safety can deviate from adult results and also between different pediatric age groups.

Two in vitro studies have been carried out with rivaroxaban in plasma of neonates and children. In plasma of healthy children the prothrombin time, activated partial thromboplastin time (aPTT) and anti-Xa assay did show linearity with adult values and no deviation. In neonates significant deviations were found for prothrombin time and aPTT (only at low concentrations of rivaroxaban) and lag time in thrombin generation. In children and neonates the response to increasing rivaroxaban concentrations was similar to adults.[46,47]

A single in vitro study has been conducted in plasma of children with dabigatran. This study showed a significant longer aPTT, thrombin time and ecarin clotting time in the pediatric groups compared with the adult group. The pediatric groups showed an increased sensitivity to dabigatran using the thrombin time. The response to increasing dabigatran concentrations was similar between the pediatric groups and adults.[48]

On clinicaltrials.gov 28 interventional studies with one of the four DOACs are listed which all include children as participants. Twenty-two studies were exclusively including patients of 18 years or younger. The first phase 1 and 2 studies with DOACs in children have been completed and more clinical trials are currently being conducted (Figure 1). The first results of a phase 3 study will only be available in January 2019 (NCT02234843). In most of the studies patients with thromboembolic events are included. Only 1 study is including patients with a cardiac disease.

Six of the completed phase 2 studies have reported their results. Three studies were conducted with rivaroxaban and three with dabigatran. The three studies with dabigatran included 8 (NCT02223260 and NCT00844415) and 18 (NCT01083732) patients, with respectively patients of <1 year of age, 12 to < 18 years of age

**Table 3.** Results of rivaroxaban phase 2 studies

| | NCT02564718 | NCT02309411 | NCT01684423 |
|---|---|---|---|
| **Age** | < 6 months of age | 6 months to < 6 years of age | 6 to < 18 years of age |
| **Indication** | symptomatic or asymptomatic venous thrombosis | symptomatic or asymptomatic venous thrombosis | symptomatic or asymptomatic venous thrombosis |
| **Screened/treated** | 11/10 | 51/46 | 68/63 |
| **Completed** | 9 | 46 | 62 |
| **Treatment** | Age- and body weight-adjusted 0.5 to 3.2 milligram (mg) twice daily or age- and body weight-adjusted 0.5 to 2.9 mg thrice daily. Both given as a oral suspension of rivaroxaban for 7 days | Age- and body weight-adjusted rivaroxaban twice daily as an oral suspension (n=40) or SOC (n=6) | Doses are age- and body weight adjusted. Group 1 (12-<18 years of age; rivaroxaban tablet once daily for 30 days); Group 2 (12-<18 years; SOC); Group 3 (6-<12 years of age; rivaroxaban tablet once daily for 30 days); Group 4 (6-<12 years of age; rivaroxaban suspension twice daily); Group 5 (6-<12 years of age; SOC). |
| **Bleeding events** | n=0 | n=0 | Group 1 n=3 non-major clinically relevant bleeding; Group 4 n=1 non-major clinical bleeding event; Group 2, 3 and 5 n=0. |
| **Recurrent TE** | n=0 | n=0 | n=0 |
| **SAE** | n=1 in the thrice daily dose group | 2-6 years of age rivaroxaban n=0; 6 months – 2 years of age rivaroxaban n=2 (pyrexia and respiratory disorder); SOC n=1 (headache). | Group 2 n=1 (MS relapse); Group 4 n=2 (hypothalamic-pituitary disorder and Influenza B virus test positive); Group 1,3 and 5 n=0. |
| **Total other AEs** | n=1 in the twice daily dose group (vomiting) | 56.0% 2-6 years rivaroxaban; 73.3% 6 months-2 years rivaroxaban; 50.0% SOC | Group 1: 9/11 (81.8%) Group 2: 8/13 (61.5%) Group 3: 6/13 (46.2%) Group 4: 12/19 (63.2%) Group 5: 2/7 (28.6%) |

VTE, venous thromboembolism; TE, thromboembolic event; SAE, serious adverse event; AE, adverse event.

and 1 to < 12 years of age (Table 2). Dabigatran was relatively well tolerated in all three studies. In one study no adverse events were found (NCT02223260), in another study 2 patients had relative mild adverse events of the gastrointestinal tract (NCT00844415)[49] and in one other study 1 patient developed leukopenia and dizziness (NCT01083732). Only 1 serious adverse event occurred, which was a recurrent venous thromboembolism 7 days after dabigatran was given and was considered not study drug related by the researchers.[49] To be able to dose children with dabigatran, specific pediatric formulations have been developed. In one study the taste was assessed of a formulation in which dabigatran granules were dissolved to form an oral liquid. The taste was graded bad to very bad in 5

of the 10 patients who assessed the taste. The global assessment of acceptability and tolerability was graded satisfactory to good in 7 of the 8 patients in one study (NCT02223260), however was found satisfactory to good in 9/18 patients in another study (NCT01083732).

The three studies with rivaroxaban included 10 (< 6 months of age), 46 (6 to <6 years of age) and 63 (6 to < 18 years of age) patients, respectively NCT02564718, NCT02309411 and NCT01684423 (Table 3). No bleeding events were found in the two studies with the youngest age groups. However in the NCT01684423 trial 4 non-major clinically relevant bleedings were observed (3 in the 12 to < 18 years of age rivaroxaban once daily group and 1 in the 6 to < 12 years of age rivaroxaban suspension twice daily group). None of the studies observed a recurrent thromboembolic event. However, quite a number of patients experienced adverse events in the groups using rivaroxaban. Adverse events were most likely in the trial with the oldest children (NCT01684423). In the group of patients of 12 to <18 years of age 81.8% had an adverse event. In the group of children using rivaroxaban suspensions twice daily 12 out of the 19 patients (63.2%) experienced an adverse event. In the NCT02309411 also 56% and 73.3% of the patients were experiencing at least one adverse event. However, only a relatively small number of patients (n=5) had a serious adverse event in all three studies, including pyrexia, respiratory disorder, hypothalamic-pituitary disorder, and a positive Influenza B virus test.

The in vitro studies show that there are some differences in response to DOACs especially dabigatran which is acting on thrombin. Clinical studies need to establish if children and especially neonates are responding similarly to DOACs as adults on the surrogate and hard endpoints. The small clinical phase 2 studies of dabigatran show that it is relatively well tolerated and no bleeding events occurred. For rivaroxaban larger phase 2 studies have been conducted which show a low number of bleedings, no recurrent thromboembolic events, but a relative large number of adverse events. It seems that the safety and efficacy of both DOACs are promising. The phase 3 studies need to show how the DOACs will perform with longer durations of use and if the number of patients with adverse events are similar to current treatment.

## INDIVIDUALIZED ANTICOAGULANT TREATMENT IN CHILDREN: SUMMARY AND PERSPECTIVE

A child presenting with thrombosis due to an antithrombin deficiency cannot be adequately treated with heparin. Direct oral anticoagulants are an attractive quickly effective treatment, but require a pediatric dose and formulation. This is a rare but clear example of individualized anticoagulant treatment in a child that builds on

experience in adults and children. Studying factors that can be of value to improve pediatric anticoagulant treatment requires networks to acquire sufficient patient numbers. This allows studying the impact of pharmacogenomics in pediatric patients to define dosing algorithms for VKAs. The clear impact of various age categories, especially in neonates further adds to the complexity of the matter. We are currently in an era where many aspects of individualized anticoagulant treatment of children are being explored: using (genetic) dosing algorithms for VKAs, defining dosages for various age cohorts for DOACs, and comparing DOACs to standard treatment with regards to efficacy and safety.



This is an exciting era for anticoagulant treatment, also in children. Only after a phase of joined efforts of many pediatric hematologists worldwide will we be able to pass a judgment on new individualized algorithms for treatment.

## REFERENCES


1. Giglia TM, Massicotte MP, Tweddell JS, Barst RJ, Bauman M, Erickson CC, et al. Prevention and treatment of thrombosis in pediatric and congenital heart disease: a scientific statement from the American Heart Association. Circulation 2013; 128: 2622–703.
2. Monagle P, Chan AKC, Goldenberg NA, Ichord RN, Journeycake JM, Nowak-Göttl U, et al. 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. Andrew M, David M, Adams M, Ali K, Anderson R, Barnard D, et al. Venous thromboembolic complications (VTE) in children: first analyses of the Canadian Registry of VTE. Blood 1994; 83: 1251–7.
4. van Ommen CH, Heijboer H, Büller HR, Hirasing RA, Heijmans HSA, Peters M. Venous thromboembolism in childhood: A prospective two-year registry in The Netherlands. J Pediatr 2001; 139: 676–81.
5. Gibson BES, Chalmers EA, Bolton-Maggs P, Henderson DJ, Lynn R. Thromboembolism in childhood: a prospective two-year BPSU study in the United Kingdom. February 2001-February 2003. Br J Haematol 2004; 125: 1–1.
6. Stein PD, Kayali F, Olson RE. Incidence of venous thromboembolism in infants and children: Data from the National Hospital Discharge Survey. J Pediatr 2004; 145: 563–5.
7. Cushman M. Epidemiology and Risk Factors for Venous Thrombosis. Semin Hematol 2007; 44: 62–9.
8. Raffini L, Huang YS, Witmer C, Feudtner C. Dramatic increase in venous thromboembolism in children's hospitals in the United States from 2001 to 2007. Pediatrics 2009; 124: 1001–8.
9. Boulet SL, Grosse SD, Thornburg CD, Yusuf H, Tsai J, Hooper WC. Trends in venous thromboembolism-related hospitalizations, 1994-2009. Pediatrics 2012; 130: e812-820.
10. Nowak-Göttl U, Dietrich K, Schaffranek D, Eldin NS, Yasui Y, Geisen C, et al. In pediatric patients, age has more impact on dosing of vitamin K antagonists than VKORC1 or CYP2C9 genotypes. Blood 2010; 116: 6101–5.
11. Kato Y, Ichida F, Saito K, Watanabe K, Hirono K, Miyawaki T, et al. Effect of the VKORC1 genotype on warfarin dose requirements in Japanese pediatric patients. Drug Metab Pharmacokinet 2011; 26: 295–9.
12. Biss TT, Avery PJ, Brandão LR, Chalmers EA, Williams MD, Grainger JD, et al. 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.
13. Moreau C, Bajolle F, Siguret V, Lasne D, Golmard J-L, Elie C, et al. 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.
14. 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.
15. Shaw K, Amstutz U, Hildebrand C, Rassekh SR, Hosking M, Neville K, et al. VKORC1 and CYP2C9 genotypes are predictors of warfarin-related outcomes in children. Pediatr Blood Cancer 2014; 61: 1055–62.
16. 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.
17. Wang Z, Zhang L, Huang P, Gu X, Xie X, Wang Y, et al. Weight and the vitamin K expoxide reductase 1 genotype primarily contribute to the warfarin dosing in pediatric patients with Kawasaki disease. Thromb Res 2018; 167: 32–6.

18. Wakamiya T, Hokosaki T, Tsujimoto S-I, Kadota K, Nakano Y, Watanabe S, et al. Effect of VKORC1, CYP2C9, CFP4F2, and GGCX Gene Polymorphisms on Warfarin Dose in Japanese Pediatric Patients. Mol Diagn Ther 2016; 20: 393–400.
19. Hawcutt DB, Ghani AA, Sutton L, Jorgensen A, Zhang E, Murray M, et al. Pharmacogenetics of warfarin in a paediatric population: time in therapeutic range, initial and stable dosing and adverse effects. Pharmacogenomics J 2014; 14: 542–8.
20. Lippi G, Franchini M, Montagnana M, Guidi GC. Coagulation testing in pediatric patients: the young are not just miniature adults. Semin Thromb Hemost 2007; 33: 816–20.
21. Sandoval JA, Sheehan MP, Stonerock CE, Shafique S, Rescorla FJ, Dalsing MC. Incidence, risk factors, and treatment patterns for deep venous thrombosis in hospitalized children: an increasing population at risk. J Vasc Surg 2008; 47: 837–43.
22. Tuckuviene R, Christensen AL, Helgestad J, Johnsen SP, Kristensen SR. Pediatric venous and arterial noncerebral thromboembolism in Denmark: a nationwide population-based study. J Pediatr 2011; 159: 663–9.
23. Klaassen ILM, van Ommen CH, Middeldorp S. Manifestations and clinical impact of pediatric inherited thrombophilia. Blood 2015; 125: 1073–7.
24. Mahajerin A, Branchford BR, Amankwah EK, Raffini L, Chalmers E, van Ommen CH, et al. Hospital-associated venous thromboembolism in pediatrics: a systematic review and meta-analysis of risk factors and risk-assessment models. Haematologica 2015; 100: 1045–50.
25. Newall F, Wallace T, Crock C, Campbell J, Savoia H, Barnes C, et al. Venous thromboembolic disease: A single-centre case series study. J Paediatr Child Health 2006; 42: 803–7.
26. Mahajerin A, Croteau SE. Epidemiology and Risk Assessment of Pediatric Venous Thromboembolism. Front Pediatr 2017; 5: 68.
27. Nowak-Göttl U, Limperger V, Kenet G, Degenhardt F, Arlt R, Domschikowski J, et al. Developmental hemostasis: A lifespan from neonates and pregnancy to the young and elderly adult in a European white population. Blood Cells Mol Dis 2017; 67: 2–13.
28. Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L. Maturation of the hemostatic system during childhood. Blood 1992; 80: 1998–2005.
29. Andrew M, Paes B, Milner R, Johnston M, Mitchell L, Tollefsen DM, et al. Development of the human coagulation system in the full-term infant. Blood 1987; 70: 165–72.
30. Andrew M, Paes B, Johnston M. Development of the hemostatic system in the neonate and young infant. Am J Pediatr Hematol Oncol 1990; 12: 95–104.
31. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental Pharmacology — Drug Disposition, Action, and Therapy in Infants and Children. N Engl J Med 2003; 349: 1157–67.
32. Ufer M. Comparative Pharmacokinetics of Vitamin K Antagonists. Clin Pharmacokinet 2005; 44: 1227–46.
33. Hines RN. Ontogeny of human hepatic cytochromes P450. J Biochem Mol Toxicol 2007; 21: 169–75.
34. Takahashi H, Ishikawa S, Nomoto S, Nishigaki Y, Ando F, Kashima T, et al. Developmental changes in pharmacokinetics and pharmacodynamics of warfarin enantiomers in Japanese children. Clin Pharmacol Ther 2000; 68: 541–55.
35. Blake MJ, Castro L, Leeder JS, Kearns GL. Ontogeny of drug metabolizing enzymes in the neonate. Semin Fetal Neonatal Med 2005; 10: 123–38.
36. Tseng AS, Patel RD, Quist HE, Kekic A, Maddux JT, Grilli CB, et al. Clinical Review of the Pharmacogenomics of Direct Oral Anticoagulants. Cardiovasc Drugs Ther 2018; 32: 121–6.
37. de Wildt SN, Tibboel D, Leeder JS. Drug metabolism for the paediatrician. Arch Dis Child 2014; 99: 1137–42.
38. Stevens JC, Hines RN, Gu C, Koukouritaki SB, Manro JR, Tandler PJ, et al. Developmental expression of the major human hepatic CYP3A enzymes. J Pharmacol Exp Ther 2003; 307: 573–82.

39. Hamberg A-K, Friberg LE, Hanséus K, Ekman-Joelsson B-M, Sunnegårdh J, Jonzon A, et al. Warfarin dose prediction in children using pharmacometric bridging--comparison with published pharmacogenetic dosing algorithms. Eur J Clin Pharmacol 2013; 69: 1275–83.
40. Lala M, Burckart GJ, Takao CM, Pravica V, Momper JD, Gobburu JVS. Genetics-based pediatric warfarin dosage regimen derived using pharmacometric bridging. J Pediatr Pharmacol Ther 2013; 18: 209–19.
41. Kamal El-Din M-A, Farhan MS, El Shiha RI, El-Kaffas RMH, Mousa SM. Frequency of CYP2C9 and VKORC1 gene polymorphisms and their influence on warfarin dose in Egyptian pediatric patients. Paediatr Drugs 2014; 16: 337–41.
42. Hirai K, Hayashi H, Ono Y, Izumiya K, Tanaka M, Suzuki T, et al. Influence of CYP4F2 polymorphisms and plasma vitamin K levels on warfarin sensitivity in Japanese pediatric patients. Drug Metab Pharmacokinet 2013; 28: 132–7.
43. Verhoef TI, Redekop WK, Daly AK, van Schie RMF, de Boer A, Maitland-van der Zee A-H. Pharmacogenetic-guided dosing of coumarin anticoagulants: algorithms for warfarin, acenocoumarol and phenprocoumon. Br J Clin Pharmacol 2014; 77: 626–41.
44. Baranova EV, Verhoef TI, Asselbergs FW, de Boer A, Maitland-van der Zee A-H. Genotype-guided coumarin dosing: where are we now and where do we need to go next? Expert Opin Drug Metab Toxicol 2015; 11: 509–22.
45. International Warfarin Pharmacogenetics Consortium, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, et al. Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med 2009; 360: 753–64.
46. Attard C, Monagle P, Kubitza D, Ignjatovic V. The in-vitro anticoagulant effect of rivaroxaban in neonates: Blood Coagul Fibrinolysis 2014; 25: 237–40.
47. Attard C, Monagle P, Kubitza D, Ignjatovic V. The in vitro anticoagulant effect of rivaroxaban in children. Thromb Res 2012; 130: 804–7.
48. Dietrich K, Stang L, van Ryn J, Mitchell LG. Assessing the anticoagulant effect of dabigatran in children: an in vitro study. Thromb Res 2015; 135: 630–5.
49. Halton JML, Lehr T, Cronin L, Lobmeyer MT, Haertter S, Belletrutti M, et al. Safety, tolerability and clinical pharmacology of dabigatran etexilate in adolescents. An open-label phase IIa study. Thromb Haemost 2016; 116: 461–71.

# 3 | QUALITY AND SAFETY OF VITAMIN K ANTAGONIST THEARAPY

# 3.1 Incidence of bleeding and thromboembolic events in pediatric patients using warfarin in England



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*Manuscript prepared for submission*

## ABSTRACT

**Aims**

This study assesses the incidence of bleeding and thromboembolic events in pediatric patients and characterises the patients with and without such events in England.

**Methods**

Data were obtained from the UK Clinical Practice Research Datalink (CPRD) in the period between April 1997 and February 2016. Using a cohort design, we identified all patients who had ≥1 prescription for warfarin, who were ≤18 years of age and could be linked to both Hospital Episode Statistics and Office for National Statistics mortality data. Patients were followed from the date of the first prescription of warfarin until end of warfarin use, 19 years of age, transfer out of practice, end of data collection or death, whichever came first.

**Results**

In total, 307 patients were included in the analysis (median age 15 years, 46.6% female, 40.7% with a cardiac indication). Bleeding events occurred in 3.33 patients/100 patient years (PY; 95%CI:2.01-5.23) with a total of 5.79 events/100 PY (4.03-8.08). Patients with a bleeding event were on average younger (p=0.025) compared to patients without a bleeding event. Within patients who used warfarin for primary prophylaxis of thromboembolic events, 1.50 patients/100 PY (0.61-3.13) experienced a thromboembolic event with only one event per person (1.47 events/100 PY [0.60-3.06]).

**Conclusions**

The incidence of bleeding and thrombotic events in pediatric patients using warfarin was low. However, younger patients appeared to be more prone to develop a bleeding event. To reduce the incidence of bleeding and thromboembolic events even more, further research is required to elucidate more risk factors.

## INTRODUCTION

Vitamin K antagonists (VKA) are used in pediatric patients to treat or prevent thromboembolic events in patients with congenital or acquired heart diseases. Dosing of VKA is complex with large inter- and intra-individual variability in VKA dose requirement. It is even more challenging in pediatric patients, because of the developing hemostatic system in these patients. Under- and overdosing of VKA can result in thromboembolic events and bleeding events, respectively.

Until now, only one large Canadian study and several smaller studies (<100 patients) have reported on the occurrence of thromboembolic and bleeding events in pediatric patients using warfarin. The incidences of thromboembolic events ranged from 0 to 2.6/100 patients years.[1–6] The incidences of bleeding events ranged from 1.5 to 39/100 patient years (major events 0.5 - 8/100 patient years; minor events 2.3 - 31/100 patient years).[2–7] Some studies did not report incidence rates, but merely reported the number of patients with a bleeding event. The number of patients with minor bleeding events varied from zero patients with event(s) to almost every patient having at least one event.[8–12]



However, the range in incidences is wide. This is probably caused by the small sample sizes of most studies. To our knowledge the incidence of bleeding and thromboembolic events during warfarin use in a large European pediatric population has never been reported. Studying the incidences in large cohorts will give us more insight what the actual incidence of bleeding and thromboembolic events are. Information on the frequency of such events and characteristics of pediatric patients experiencing them can enable the identification of high-risk patients and provide targets for improving management of warfarin therapy in this special patient population.

In this light, we aimed to assess the incidences of bleeding and thromboembolic events during the use of warfarin therapy in pediatric patients and to characterise patients with and without such events in England.

## METHODS

### Data source and study population

Data were obtained from the Clinical Practice Research Datalink (CPRD), an anonymized database containing computerized medical records of primary care practices in the UK, representing around 6.9% of the population.[13] Data recorded in CPRD include demographic information, prescription details, clinical events, preventive care provided, specialist referrals, hospital admissions and

major outcomes since 1987. Primary care diagnoses are recorded in CPRD using a hierarchical clinical coding system (Read codes). For this study, only patients that were eligible for linkage with the national Hospital Episodes Statistics (HES) and mortality data from the Office for National Statistics (ONS) were included. The HES data contain details of all admissions to National Health Service (NHS) hospitals in England from April 1997.[14] The ONS provided data for the causes of death and the exact date as recorded on death certificates by a medical doctor from January 1998.[15] HES and ONS mortality data are deterministically linked to primary care data using a combination of identifiers including the patient's unique NHS number, gender, date of birth and postal code. About 75% of all practices in England (57% of all UK CPRD practices) are linked to HES and ONS.

The study was designed as a retrospective cohort study in the period from April 1997 to February 2016. Patients who had at least one prescription of warfarin and were less than 19 years of age were identified. Only patients with linkage to the HES and ONS were included in the analysis. Patients were followed from the index date (first prescription) until they discontinued warfarin therapy, transferred out of practice, reached the age of 19 years, end of data collection or death, whichever came first.

**Exposure**

For each patient, we identified all prescriptions for warfarin prescribed by a general practitioner. Based on the information available in CPRD, it was not possible to exactly determine the duration of each warfarin prescription. Instead, the median time between prescriptions of warfarin was calculated for each patient. This median duration was subsequently trimmed to a minimum of 28 days and a maximum of 84 days. For patients with only one prescription of warfarin the duration was set to 28 days. Also, INR measurements were used as a proxy for continuing warfarin treatment.

Because patients are expected to use warfarin continuously until there is no longer an indication for its use, we assumed a continuous exposure to warfarin from the first prescription until the end date of the last prescription as primary exposure definition. A patient was considered to have discontinued therapy when no new prescription or INR measurement was available within 1 year from the end date of the previous warfarin prescription or INR measurement. The end date of this treatment period was based on the date of last prescription or INR measurement plus the individual median duration of a warfarin prescription.

In a sensitivity analysis, the exposure definition was adjusted to see the impact of assuming that patients were unexposed to warfarin after a gap of 14 days. A patient could have multiple exposure periods. As with the primary exposure definition, patients were considered to have discontinued therapy when the gap was longer

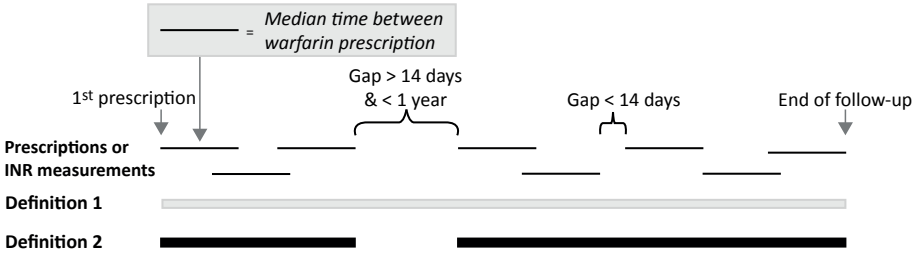



**Figure 1.** Exposure definitions. Definition in which everyone is assumed exposed from first prescription of warfarin until end of follow-up (definition 1) and sensitivity analysis without the assumption of continuously exposure during follow-up (definition 2). Definition 1 has one exposure period. Definition 2 has 2 exposure periods to warfarin.



### Outcomes

Bleeding and thromboembolic events were defined by hospital diagnoses at discharge in HES (ICD-10 codes) or by a relevant Read code for bleeding or thromboembolic events. Fatal events, cause of death being a bleeding or thromboembolic event, were identified using the linked mortality data from the ONS and deaths recorded in CPRD.

### Other variables

Descriptive covariates measured at baseline included age, sex, indication for warfarin use, body surface area (BSA), body mass index (BMI), and the history of bleeding and thromboembolic events. During warfarin use concurrent anticoagulant/antiplatelet drug therapy, use of vitamin K, and total duration of warfarin use was determined.

For calculating BSA and BMI, the most recent record of weight, height and BMI to initiation of warfarin was determined. The time window, which was allowed between the measurement and the initiation of warfarin treatment, depended on the age of the patient at the time of cohort entry. Time windows were constructed using the World Health Organisation growth tables of height and weight for age.[16–19] For each age the duration of time in which the mean height increased no more than 5 cm and the mean weight no more than 2 kg was determined. The time window for BMI was kept on the smallest time window (height or weight) at each age. The applied time windows can be found in the supporting information Table S1. Only when height and weight were assessed within this time window, they were used for calculating the BSA (formula of Haycock) and BMI. If a BMI-value was entered in the age-specific relevant time window for assessing BMI, this measurement was used.

Indications for use of warfarin were assessed by reviewing both the CPRD and HES records before and up to 14 days after the first prescription of warfarin. The

most likely indication, which was the closest to the first prescription of warfarin use, was used as the indication for warfarin therapy.

Concurrent antiplatelet drug therapies evaluated were aspirin, clopidogrel, and dipyridamole. For each of these drugs it was determined whether there was at least one prescription during the use of warfarin.

The history of the study outcomes was determined by reviewing if there were any recorded outcome-related Read codes or ICD-10 codes before initiation of warfarin treatment. Patients with a thromboembolic event as indication for warfarin use automatically had a history of thromboembolic events.

**Data analysis**

All events with the same Read code were clustered when recorded within 14 days (bleeding events) or 40 days (thromboembolic events) of each other to reduce the possibility of counting the same event multiple times. Also, similar ICD codes and Read codes were clustered manually when they occurred within 14 days. Incidence rates were calculated as the number of events divided by the number of years of warfarin use. Two numerators were used: one including only the first event of each patient and one including all events during follow-up. For calculating the incidence using only the first event as the numerator, the patient was censored at this event. The incidences for bleeding and thromboembolic events were analyzed separately. With the available information, it was not possible to distinguish the indication from recurrent events. Therefore, only the incidence of thromboembolic events in patients using warfarin for primary prophylaxis was determined. Primary prophylaxis was defined as patients having no history of thromboembolic events or a thromboembolic event at start of warfarin therapy. Patients were also excluded from this analysis when the indication for warfarin use was unknown, since a thromboembolic event could have been the indication.

The characteristics of patients with and without a bleeding event were compared using t-tests/ Mann-Whitney U-test for continuous variables and Chi-squared test and/or the Fisher's exact test for categorical variables, as appropriate. A p-value of $<0.05$ was considered statistically significant.

Two sensitivity analyses were conducted. The first sensitivity analysis was to determine the effect of another exposure definition, as described above. A second sensitivity analysis was performed to determine the effect of including only patients with one year of prior history in CPRD available.

CPRD has a privacy policy in which no cell should be reported which contains less than 5 patients/events. If this was the case, this will be depicted as <5. IBM SPSS 24.0 was used for data analysis.

**Scientific approval**

The study protocol was approved by the Independent Scientific Advisory Committee for Medicines & Healthcare products Regulatory Agency Database Research on the 8th of June 2015, protocol number 15_043R.



## RESULTS

We identified 693 patients with a first prescription of warfarin of whom 307 patients were included in the analysis (Figure 2). The cohort consisted of a nearly equal number of females (46.6%) and males, with a median age of 15 years (Table 1).

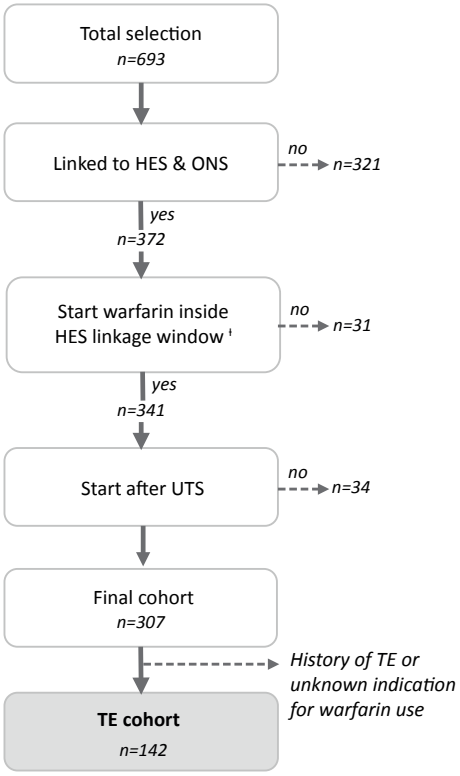



**Figure 2.** Flowchart of included patients. UTS, up to standard; ONS, Office of National Statistics; HES, Hospital Episode Statistics. ǂ HES linkage window 01-04-1997 until 29-02-2016.

**Table 1.** Patient characteristics at start of warfarin therapy

| | **Total cohort** | |
|---|---|---|
| | **n=307** | |
| **Sex (female),** n (%) | 143 | (46.6) |
| **Age at start in years,** median (IQR) | 15 | (5-16) |
| <1, n (%) | 18 | (5.9) |
| 1-3, n (%) | 34 | (11.1) |
| 4-6, n (%) | 43 | (14.0) |
| 7-9, n(%) | 12 | (3.9) |
| 10-12, n (%) | 18 | (5.9) |
| 13-15, n (%) | 46 | (15.0) |
| 16-18, n(%) | 136 | (44.3) |
| **BMI (kg/m²),** median (IQR)† | 20.73 | (17.70-24.30) |
| **BSA (m²),** median (IQR)† | 0.27 | (0.21-0.29) |
| **Indication for warfarin use,** n (%) | | |
| Cardiac | 125 | (40.7) |
| Thromboembolic event | 142 | (46.3) |
| Other | 25 | (8.1) |
| Unknown | 15 | (4.9) |
| **Duration of current use (years),** median (IQR) | 0.56 | (0.24-1.78) |
| <3 months, n (%) | 77 | (25.1) |
| 3-6 months, n (%) | 61 | (19.9) |
| 6-12 months, n (%) | 63 | (20.5) |
| > 1 year, n (%) | 106 | (34.5) |
| **History of bleeding,** n (%) | 41 | (13.4) |
| **History of thromboembolic events,** n (%) | 150 | (48.9) |
| **Concurrent antiplatelet use‡,** n (%) | 16 | (5.2) |

BSA, body surface area; BMI, body mass index.
† Available for n=69.
‡ Includes aspirin and dipyridamole.

Furthermore, 16.9% of the patients was below the age of 4 years and 59.3% was older than 13 years of age. The most common indications for warfarin use were of cardiac nature (40.7%) or a thromboembolic event (46.3%). A more detailed description of the indications can be found in the supporting information Table S2. A substantial proportion of patients used warfarin for less than 3 months (25.1%) or for more than 1 year (34.5%). A history of bleeding was present in 13.4% of the

patients. A history of thromboembolic events, this could also be the indication for the warfarin use, was present in 48.9% of the patients. Sixteen patients concurrently used antiplatelet drugs, predominantly aspirin. No vitamin K was prescribed by a general practitioner during warfarin therapy.

**Bleeding events**

In total 17 patients experienced one or more bleeding events with an incidence of 3.33/100 patient years (Table 2). When taking all events (n=32) into account the incidence rate increased to 5.79/100 patient years. The most common bleeding events were epistaxis (7 patients with a total of 7 events) and gastrointestinal bleedings (<5 patients with a total of 7 events). Other types of bleeding (<5 patients) were hematuria, abnormal vaginal bleeding, cerebral bleeding, hemothorax, and spontaneous ecchymosis. Patients who experienced a bleeding event often had multiple events (41% had ≥2 events; maximum of 7 events). A large proportion of the patients had their first bleeding within the first few weeks of warfarin treatment (Figure 3). In 24% of the patients with a bleeding event this occurred within 15 days after the start (median time to first event 8.1 months). Patients with a bleeding event were younger (median of 6 versus 15 years, p=0.025) and were using warfarin for a longer period (median 3.30 versus 0.55 years, p=0.004) compared to patients without a bleeding event. The proportion of patients with a history of bleeding events before the start of warfarin therapy was similar in patients with a bleeding (11.8%) and patients without a bleeding event (13.4%). A similar number of patients with a bleeding event had an indication for warfarin use of cardiac nature (n=7) or were using warfarin after a thromboembolic event (n=6). None of the patients with a bleeding event were concurrently using an antiplatelet agents during warfarin treatment.



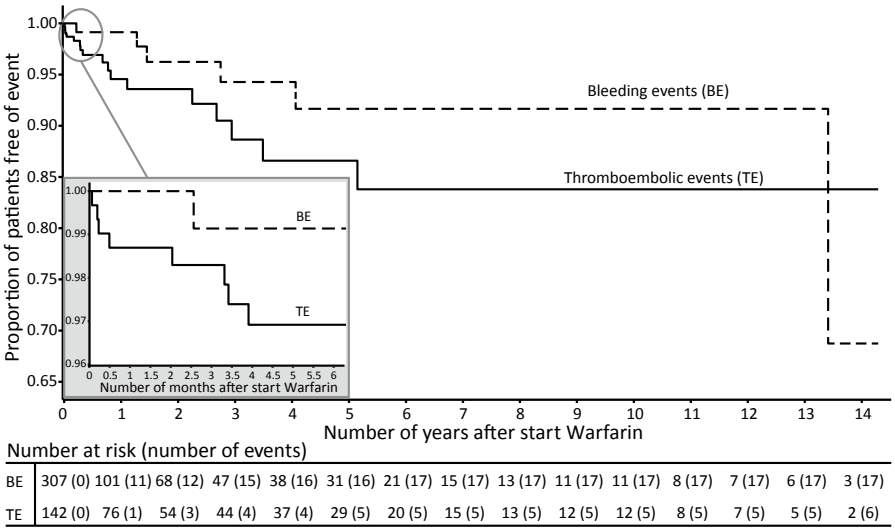
**Table 2.** Incidence of thromboembolic and bleeding events

| | Total number of patients | Number events | PY | IR/100 PY (95% CI) |
|---|---|---|---|---|
| **First event** | | | | |
| Bleeding | 307 | 17 | 510 | 3.33 (2.01 - 5.23) |
| Thromboembolic | 142 | 6 | 399 | 1.50 (0.61 - 3.13) |
| **All events** | | | | |
| Bleeding | 307 | 32 | 552 | 5.79 (4.03 - 8.08) |
| Thromboembolic | 142 | 6 | 408 | 1.47 (0.60 - 3.06) |

IR, incidence rate; PY, patient years; CI, confidence interval.

**Thromboembolic events**

There were 6 patients using warfarin for primary prophylaxis who experienced a thromboembolic event during warfarin therapy (incidence rate 1.50/100 patient years), see Table 2. These patients had only one event per person, yielding an incidence rate of 1.47 events/100 patient years. The six thromboembolic events which occurred, included among others acute myocardial infarction and pulmonary embolism. These thromboembolic events occurred between 0.21 to 13.4 years after the start (median 2.1 years), see Figure 3. Most patients with a thromboembolic event had a cardiac disease leading to warfarin use.




Number at risk (number of events)

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BE | 307 (0) | 101 (11) | 68 (12) | 47 (15) | 38 (16) | 31 (16) | 21 (17) | 15 (17) | 13 (17) | 11 (17) | 11 (17) | 8 (17) | 7 (17) | 6 (17) | 3 (17) |
| TE | 142 (0) | 76 (1) | 54 (3) | 44 (4) | 37 (4) | 29 (5) | 20 (5) | 15 (5) | 13 (5) | 12 (5) | 12 (5) | 8 (5) | 7 (5) | 5 (5) | 2 (6) |

**Figure 3.** First occurrence of bleeding and thromboembolic events after start of warfarin therapy.

**Sensitivity analysis**

Similar incidence rates for bleeding and thromboembolic events were found when the exposure definition was changed to exclude the gaps between the exposure periods of warfarin (see supporting information Table S3). When calculating the incidences only for patients with a prior history in CPRD of one year before the start of warfarin treatment (n=212) less patients experienced one or more bleeding events (n=9; 2.52/100 patient years) and thromboembolic events (n<5; 1.10/100 patient years), results are shown in the supporting information Table S4.

**Fatal events**

Less than 5 patients died during the use of warfarin. Causes of death were a thromboembolic event, an underlying heart disease, or unknown.

## DISCUSSION

Results from this retrospective cohort study showed that the incidence of bleeding (3.33/100 patient years) and thromboembolic events (1.50/100 patient years) in pediatric patients using warfarin is quite low. However, some patients experienced many bleeding events. When taking multiple events per patient into account the incidence increased to 5.79 bleeding events per 100 patient years.



The method for calculating the incidence of bleeding events differs between previously conducted studies. In some, the total number of events was used, while in others only the number of patients having one or more events counted in the numerator. This can have a substantial impact on the incidence rates, because patients with one event tend to have subsequent events, as was shown in our study. We therefore calculated both.

The incidence of patients with a bleeding event of 3.33/100 patient years is in line with the findings of Streif et al. They found an incidence of 2.8 patients with a bleeding event per 100 patient years in a similar Canadian cohort of 319 pediatric patients with diverse indications.[3] The incidence of 5.79 bleeding events/100 patient years is quite low compared to the incidences found by two other studies. One study in 38 pediatric patients with Kawasaki disease found an incidence of 8 major bleeding events/100 patient years and 31 minor bleeding events/100 patient years.[7] Another study in 25 patients with prosthetic heart valves found an incidence of 4 major bleeding events/100 patient years.[2] Unfortunately, with the available information we were not able to classify bleeding events in minor and major events.

We found that especially in the first few weeks the incidence of bleeding events was high. We showed that 24% of the patients with this outcome had their first bleeding event within 15 days after the start of warfarin treatment. This finding is in line with the study by Shaw et al. who reported that of the 7 observed major bleeding events 4 occurred within the first 6 weeks of use.[20]

The reported incidence of thromboembolic events varies in literature from no events to 2.6/100 patients years.[1,2,4–6] The incidence rate of 1.50/100 patient years found in our study is in the middle of this range. The study by Streif et al. found no events in patients with primary prophylaxis.[3] Five smaller pediatric studies (one study in patients with cardiomyopathy and 4 studies in patients with prosthetic heart valves) found incidences of 0 (3 times), 2.3, and 2.6/100 patient years.[1,2,4–6] Although

the incidence of thromboembolic events we found was low, the optimum would be that no events occur during treatment. Other studies showed that this is possible.[1,2,4–6] Low quality of anticoagulation control could be a reason for the occurrence of the events. Unfortunately, the number of available INR measurements within CPRD was too low to study anticoagulation control, as only 20% of the patients in our study had more than 10 INR measurements available. The lack of INR data does not mean that patients are not monitored, but is explained by the fact measurements outside the general practice (e.g. at anticoagulation clinics or hospitals) are not collected in CPRD. Strengths of this study are that we could define a relatively large cohort of pediatric patients of all ages and with a broad range of indications for warfarin therapy using a large, well renowned data source for observation epidemiological studies. Furthermore, we included data from both general practice and hospital, which gives a total view on the incidence of clinical and non-clinical bleeding and thromboembolic events.

Using an administrative database such as CPRD has the advantages of identifying many pediatric patients spread over a large geographical area at relatively modest costs. However, it also has the disadvantage of not necessarily having all information of clinical interest available.

We were not able to calculate the incidence of thromboembolic events for patients with secondary prophylaxis. Some general practitioners appeared to repeat the indication multiple times in the database. Therefore, with the available information we were not able to distinguish the indication from new recurrent thromboembolic events.

Potentially, we could be missing the first period of warfarin therapy. The first prescription of warfarin by the general practitioner was in at least a quarter of the patients more than one month after the date that the indication was diagnosed. There could be multiple reasons for this observation. The first and most common cause was that patients were hospitalized and got warfarin prescribed by the specialist at the start of treatment (this could take up to a few months). A second cause could be, that some patients were not treated in a general practice connected to CPRD at the start of warfarin therapy and moved to a CPRD connected general practice (this could be years after the start of warfarin therapy). Missing the information of the first period of warfarin therapy could have resulted in an underestimation of the incidence of bleeding events, since the risk of bleeding events is especially high in the beginning of treatment.

Due to unavailability of information on medication given in the hospital, we were unable to determine if a patient was using (low molecular weight) heparin at the time of a bleeding event. For three bleeding events, it might be possible that (low molecular weight) heparin was given at the time of the event, based on the

diagnoses at discharge. However, this is unlikely for all the other bleeding events. We therefore think that not having this information available only has minimal impact on the incidence rate of bleeding events found in our study.

For confidentiality reasons, we are not able to report on the specific number and type of thromboembolic and bleeding events occurring in our cohort, nor on specific causes of death. From a clinical point of view, we think it is good to emphasize we observed a rather heterogeneous picture of outcome events without evidence of any clustering within certain types of events.

The integrated HES database we used for this study contained only the first decimal digit of the ICD-10 code. Some codes distinguish between bleeding, thromboembolic or other events in the second or third decimal digit. Therefore, we were not able to include some of the ICD codes that describe these events. This could have resulted in an underestimation of the number of events and the incidences of both bleeding and thromboembolic events. However, as these events should also be captured by the data in CPRD, we think that the effect on the incidences is low.



Due to missing the daily dose information of warfarin, the exact warfarin exposure period was unknown. Therefore, we assumed that everyone was exposed continuously from first to last prescription of warfarin. However, our sensitivity analysis showed that similar incidences were obtained when warfarin exposure was more strictly defined. This shows that it was valid to make the initial assumption of continuous exposure from first to last prescription of warfarin. The incidences found in patients with at least one year of prior history in CPRD were lower compared to the incidences found in the total cohort. However, the number of events was low and the confidence intervals overlap with the incidence rates found in the full cohort. In addition, all patients with an age of less than 1 year were excluded, due to the inability to have at least one year of prior history in CPRD, which could have reduced the incidences of bleeding events.

In conclusion, the incidence of bleeding and thromboembolic events in pediatric patients using warfarin is low. However, a large proportion of the bleeding events occurred during the first few weeks of warfarin use and younger patients appeared to be more prone to developing a bleeding event. Further research is needed to elucidate more risk factors of bleeding and thromboembolic events in pediatric patients and to be able to optimize the care around warfarin treatment in this vulnerable group of patients.

## REFERENCES


1. Monagle K, Jones S, King I, Weintraub R, Monagle P, Newall F. Anticoagulation of cardiomyopathy in children. Thromb Res. 2014;134:255–8.
2. Wong CS, Batchelor K, Bua J, Newall F. Safety and efficacy of warfarin in paediatric patients with prosthetic cardiac valves: a retrospective audit. Thromb Res. 2011;128:331–4.
3. Streif W, Andrew M, Marzinotto V, Massicotte P, Chan AK, Julian JA, et al. Analysis of warfarin therapy in pediatric patients: A prospective cohort study of 319 patients. Blood. 1999;94:3007–14.
4. Bradley SM, Sade RM, Crawford FA, Stroud MR. Anticoagulation in children with mechanical valve prostheses. Ann Thorac Surg. 1997;64:30-34; discussion 35-36.
5. Stewart S, Cianciotta D, Alexson C, Manning J. The long-term risk of warfarin sodium therapy and the incidence of thromboembolism in children after prosthetic cardiac valve replacement. J Thorac Cardiovasc Surg. 1987;93:551–4.
6. Bradley LM, Midgley FM, Watson DC, Getson PR, Scott LP. Anticoagulation therapy in children with mechanical prosthetic cardiac valves. Am J Cardiol. 1985;56:533–5.
7. Manlhiot C, Brandão LR, Somji Z, Chesney AL, MacDonald C, Gurofsky RC, et al. Long-term anticoagulation in Kawasaki disease: Initial use of low molecular weight heparin is a viable option for patients with severe coronary artery abnormalities. Pediatr Cardiol. 2010;31:834–42.
8. Hawcutt DB, Ghani AA, Sutton L, Jorgensen A, Zhang E, Murray M, et al. Pharmacogenetics of warfarin in a paediatric population: time in therapeutic range, initial and stable dosing and adverse effects. Pharmacogenomics J. 2014;14:542–8.
9. Monagle P, Cochrane A, Roberts R, Manlhiot C, Weintraub R, Szechtman B, et al. A multicenter, randomized trial comparing heparin/warfarin and acetylsalicylic acid as primary thromboprophylaxis for 2 years after the Fontan procedure in children. J Am Coll Cardiol. 2011;58:645–51.
10. Massicotte P, Julian JA, Gent M, Shields K, Marzinotto V, Szechtman B, et al. An open-label randomized controlled trial of low molecular weight heparin compared to heparin and coumadin for the treatment of venous thromboembolic events in children: the REVIVE trial. Thromb Res. 2003;109:85–92.
11. Biss T, Avery PJ, Walsh PM, Kamali F. Warfarin treatment outcomes in children monitored at home with a point-of-care device. Thromb Haemost. 2011;105:1103–5.
12. Levy DM, Silverman ED, Massicotte MP, McCrindle BW, Yeung RSM. Longterm outcomes in patients with giant aneurysms secondary to Kawasaki disease. J Rheumatol. 2005;32:928–34.
13. Herrett E, Gallagher AM, Bhaskaran K, Forbes H, Mathur R, van Staa T, et al. Data Resource Profile: Clinical Practice Research Datalink (CPRD). Int J Epidemiol. 2015;44:827–36.
14. NHS Digital. Hospital Episode Statistics (HES).2012.http://www.hesonline.nhs.uk. Accessed 22 Dec 2017.
15. NHS Digital. Mortality data from the Office for National Statistics (ONS).2014. http://www.hscic.gov.uk/onsmortality. Accessed 22 Dec 2017.
16. World Health Organization. Weight-for-age. http://www.who.int/childgrowth/standards/weight_for_age/en/. Accessed 8 Aug 2016.
17. World Health Organization. Length/height-for-age. http://www.who.int/childgrowth/standards/height_for_age/en/. Accessed 8 Aug 2016.
18. World Health Organization. Weight-for-age (5-10 years). 2007. http://www.who.int/growthref/who2007_weight_for_age/en/. Accessed 8 Aug 2016.
19. World Health Organization. Height-for-age (5-19 years). 2007. http://www.who.int/growthref/who2007_height_for_age/en/. Accessed 8 Aug 2016.
20. Shaw K, Amstutz U, Hildebrand C, Rassekh SR, Hosking M, Neville K, et al. VKORC1 and CYP2C9 genotypes are predictors of warfarin-related outcomes in children. Pediatr Blood Cancer. 2014;61:1055–62.

## SUPPORTING MATERIAL

**Table S1.** Time windows height, weight and BMI

| | Valid time window (months) | |
|---|---|---|
| **Age** | **Height, weight and BMI** | |
| <1 | 1 | |
| 1 | 2 | |
| 2 | 4 | |
| 3 | 6 | |
| 4 | 7 | |
| 5 | 8 | |
| 6 | 9 | |
| 7 | 10 | |
| | **Height** | **Weight and BMI** |
| 8 | 10 | 9 |
| 9 | 10 | 7 |
| 10 | 10 | 6 |
| 11 | 9 | 6 † |
| 12 | 10 | 6 † |
| 13 | 8 | 6 † |
| 14 | 8 | 6 † |
| 15 | 10 | 6 † |
| 16 | 12 | 6 † |
| 17 | 18 | 6 † |
| 18 | 24 | 6 † |

BMI, body mass index.
† Kept on 6 months.

**Table S2.** Detailed indications for the cohort of warfarin users (n=307)

| Indication for anticoagulation | Number of patients, n (%) | |
|---|---|---|
| **Cardiac** | 125 | (40.7) |
| Valve | 34 | (11.1) |
| Fontan circulation | 15 | (4.9) |
| Cardiomyopathy | 10 | (3.3) |
| Arrhythmia | 5 | (1.6) |
| Kawasaki disease | 6 | (2.0) |
| Other | 10 | (3.3) |
| Unclear, cardiac problems in past | 45 | (14.7) |
| **Thromboembolic event** | 142 | (46.3) |
| Cerebral | 21 | (6.8) |
| Other TE | 109 | (35.5) |
| Thrombophlebitis around start | 12 | (3.9) |
| **Other**[‡] | 25 | (8.1) |
| **Unknown** | 15 | (4.9) |

[‡] Consists of prophylactic, nephrotic syndrome, cancer, hemolytic uremic syndrome and a clotting factor disorder.

**Table S3.** Results sensitivity analysis 1 – change in exposure definition

| | Total number of patients | Number events | PY | IR/100 PY (95% CI) |
|---|---|---|---|---|
| **First event** | | | | |
| Bleeding | 307 | 17 | 439 | 3.87 (2.33, 6.08) |
| Thromboembolic event | 142 | 5 | 340 | 1.47 (0.54, 3.26) |
| **All events** | | | | |
| Bleeding | 307 | 29 | 474 | 6.12 (4.17, 8.67) |
| Thromboembolic event | 142 | 5 | 348 | 1.43 (0.53, 3.18) |

IR, incidence rate; PY, patient years; CI confidence interval.

**Table S4.** Results sensitivity analysis 2 – adding inclusion criterion ≥1 year of prior history in CPRD

| | Total number of patients | Number events | PY | IR/100 PY (95% CI) |
|---|---|---|---|---|
| **First event** | | | | |
| Bleeding | 212 | 9 | 358 | 2.52 (1.23, 4.62) |
| Thromboembolic event | 97 | <5 | 273 | 1.10 (0.28, 3.00) |
| **All events** | | | | |
| Bleeding | 212 | 12 | 378 | 3.18 (1.72, 5.40) |
| Thromboembolic event | 97 | <5 | 273 | 1.10 (0.28, 3.00) |

IR, incidence rate; PY, patient years; CI confidence interval.

# 3.2 Characteristics and quality of oral anticoagulant treatment in pediatric patients in the Netherlands based on the CAPS cohort



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## ABSTRACT

**Background**

The use of vitamin-K antagonists (VKAs) in pediatric patients is rare and information on the quality and safety of treatment with acenocoumarol and phenprocoumon is limited.

**Objectives**

To assess the quality, safety and effectiveness during the first year of acenocoumarol and phenprocoumon treatment in pediatric patients in the Netherlands.

**Methods**

The Children Anticoagulation and Pharmacogenetics Study (CAPS) was designed as a multicenter retrospective follow-up study. Patients who used acenocoumarol or phenprocoumon at an age of ≤ 18 years, were selected from four pediatric hospitals and one anticoagulation clinic in the Netherlands. The quality of treatment was assessed by calculating the percentage of time in therapeutic INR range (TTR) for the first month and for every 3 months of use during the first year of treatment. Effectiveness and safety were assessed by the number of thromboembolic and bleeding events.

**Results**

In total, 213 patients participated, of whom 187 (155 acenocoumarol; 32 phenprocoumon) were included in this analysis. The mean TTR was 47.0% and 51.4% in the first month of use for acenocoumarol and phenprocoumon, respectively. After the first 3 months the mean TTR for both VKAs was above 64%. In 14.6% (acenocoumarol) and 31.3% (phenprocoumon) of the patients a bleeding event occurred during the first year of treatment; no thromboembolic events were reported.

**Conclusions**

The quality of anticoagulation treatment was low during the first month of use and leaves room for improvement. After the first month it increased to an acceptable level. However, bleeding events occurred frequently during the first year.

## INTRODUCTION

Vitamin-K antagonists (VKAs) are used in children to treat or prevent thromboembolic events.[1] Other than those seen in adults, pediatric indications are dominated by congenital heart disease and its complications. Worldwide, warfarin is the most prescribed VKA. Therefore, most VKA-related research was focused on the use of warfarin in adults. However, in several countries, including the Netherlands, Germany and Spain, acenocoumarol and/or phenprocoumon are used.

In relation to the pharmacokinetics and pharmacodynamics of drugs, it is commonly known that children cannot be considered as small-sized adults.[2] The metabolic and hemostatic systems are still in development, which influences the response to VKAs. Most knowledge on the kinetics and effectiveness of VKAs is obtained from adult patients. As a result, guidelines on the use of VKAs in children are based on relatively low-grade-quality evidence.[1,3,4] This makes it challenging to assess the required dose of VKAs in children, which can impair the quality, effectiveness and safety of the treatment.



The percentage of time in the therapeutic international normalized ratio (INR) range (TTR) is a frequently used parameter for the quality of VKA treatment. Anticoagulation is stated to be poor when the TTR is below 60–65%, because below this range VKAs and antiplatelet therapy have been shown to result in similar antithrombotic effectiveness in a population of adult patients with atrial fibrillation.[5] When the TTR is above 70% the anticoagulation control is defined as high by the European Society of Cardiology.[6] These definitions of anti-coagulation control are all based on studies in adults. Considering there is no information available for pediatric patients, this is currently the only definition that can be used to classify the quality in pediatric patients.

The reported TTR in children using a VKA, mostly warfarin, varies between 39 and 92.9%.[7-14] The level of the TTR is largely dependent on differences in patient populations (i.e. age and genetic composition) and the management of VKA therapy (patient self-testing, patient self-management [self-testing and self-dosing], management by an anticoagulation clinic or general care strategies).[7,15] Moreover, other factors play a role, such as the method of calculating the TTR, the type of VKA, if the TTR includes the initiation period of the VKA or not and the predefined therapeutic INR range (TR).[16-18]

This article presents the characteristics from the Children Anticoagulation and Pharmacogenetics Study (CAPS). Up to now acenocoumarol and phenprocoumon have not been widely studied in children. We assessed the quality, safety and effectiveness during the first year of acenocoumarol and phenprocoumon treatment in the Netherlands.

## METHODS

### Study design

The study protocol from CAPS was approved by the UPPER Institutional Review Board of the Division of Pharmacoepidemiology and Clinical Pharmacology of Utrecht University. CAPS is a multicenter retrospective follow-up study in four pediatric hospitals in Amsterdam, Utrecht, Rotterdam and Groningen, and the Leiden anticoagulation clinic, in the Netherlands. CAPS was designed to study the pharmacogenetics of acenocoumarol and phenprocoumon in children. Children aged 18 years or younger, who used one of the two VKAs after 1 January 1995, were invited to participate. Patients (and/or their parents or legal guardians if appropriate for the age of the patient) who provided written informed consent were eligible for participation. 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 they became 19 years of age, when they stopped VKA therapy or when they were lost to follow-up. For these first analyses only the first year of use was taken into account. Patients were excluded from these analyses when the start date of VKA use was unknown or when no (valid) INR information was available within the first year. All data were collected using a digital form in the study database. No standardized method for information collection was available, because every hospital had a somewhat different system for storing the data (on paper/electronically). INR values, dosing information, indication, TR and weight and height were all retrospectively collected from the patient records of the hospital and the anticoagulation clinic(s) managing the VKA therapy of the patient. Furthermore, information was collected at the time that the informed consent was given by a short patient questionnaire, including questions about weight and height at the start of VKA use.

**Table 1.** Criteria for calculating the quality parameters

| | Time to INR in TR | TTR + number of INR measurements | | | Number of dose changes of >10% |
|---|---|---|---|---|---|
| | | *First month* | *months 1-3* | *Months 4-6/ 7-9/ 10-12* | |
| INR within 5 days | x | x | x | - | - |
| No missing dose and INR information for ≥ 7 days during transition from hospital to anticoagulation clinic | x | x | x | - | - |
| Number of INRs/month required | - | ≥ 3 | ≥ 2 | ≥ 1 | - |
| No hospital readmission for surgery | | x | x | - | - |
| ≥90% of the daily dosages should be available for the specific period | - | - | - | - | x |

INR, international normalized ratio; TR, therapeutic INR range; TTR, percentage time in therapeutic INR range; x, required; -, not required.

**Quality assessment**

For primary assessment of the quality of treatment, only patients using acenocoumarol or phenprocoumon for the first time during follow-up were included. To assess the quality of treatment, four parameters were calculated: the time to an INR in TR, TTR and the percentage of time below and above TR. Additionally, the number of INR measurements and the number of dose changes of more than 10% in mean daily dose between two INR measurements were calculated. The parameters were calculated for the first month and every 3 months during the first year of treatment. Patients had to fulfil the criteria specified in Table 1 to be included in the calculation of the specified parameter. The TTR was calculated using the Rosendaal method.[19] This method assumes linearity between two INR measurements. When there were 28 days or more between two INR measurements, linearity was no longer assumed and the time between these two INR measurements was not included in the TTR.



**Effectiveness and safety assessment**

Effectiveness and safety were assessed during the first year of treatment by the number of INRs below 2 (no therapeutic effect expected) or above 6 (increased risk of bleeding events[20]), the use of vitamin K, and by reviewing the free text of the patient's records at the anticoagulation clinics for mention of both clinical or non-clinical bleeding or thrombotic events. Furthermore, the hospital records regarding correspondence on outpatient consultations, discharge letters and clinical notes during a hospital stay were checked for one of these events. Thromboembolic events were defined as new (recurrent/incident) thromboembolic events after the start of the VKA. Bleeding events were defined as all events describing an abnormal bleed somewhere in the body. All events were manually coded as types of event based on the location in the body.

**Statistical analysis**

To compare the characteristics of the acenocoumarol and phenprocoumon cohorts, a chi-squared test, an independent sample *t*-test or a Mann–Whitney *U*-test was used. Spearman correlation, independent sample *t*-test or one-way anova were used to assess the associations between number of INR measurements, number of dose changes of more than 10%, age, TR, and patient self-testing with the TTR. Because of the low sample size these analyses were performed on all patients, without distinguishing between the two VKAs. Also, the difference in time below, within and above TR between patients with and without a bleeding event was tested using an independent sample *t*-test. The data were analyzed using the statistical software SPSS version 23.

## RESULTS

In total, 573 pediatric patients who used a VKA, were identified and invited in writing to participate in the study. We were able to get in contact with 485 patients, and of these patients, 213 gave informed consent. Of these patients, 172 started with acenocoumarol, 34 with phenprocoumon and seven with warfarin (see Fig. 1). Seventeen acenocoumarol and two phenprocoumon patients were excluded as a result of an unknown start date and/or no (valid) available INR measurements during the first year of VKA use. Furthermore, the seven patients who started on warfarin were excluded. Table 2 provides an overview of the characteristics of the acenocoumarol and phenprocoumon cohorts. The characteristics of the two cohorts differed statistically significantly in the distribution of age ($P$ = 0.001), indication for VKA use ($P$ < 0.001) and duration of VKA use ($P$ = 0.005). An important difference between the two cohorts was the percentage of patients using the VKA for a cardiac indication. For acenocoumarol this was 63%; for phenprocoumon it was 38%. This difference in indication for VKA use also had an effect on the duration of use, which was more than 1 year for 58.1% of patients on acenocoumarol and 28.1% of patients on phenprocoumon (Table 2).

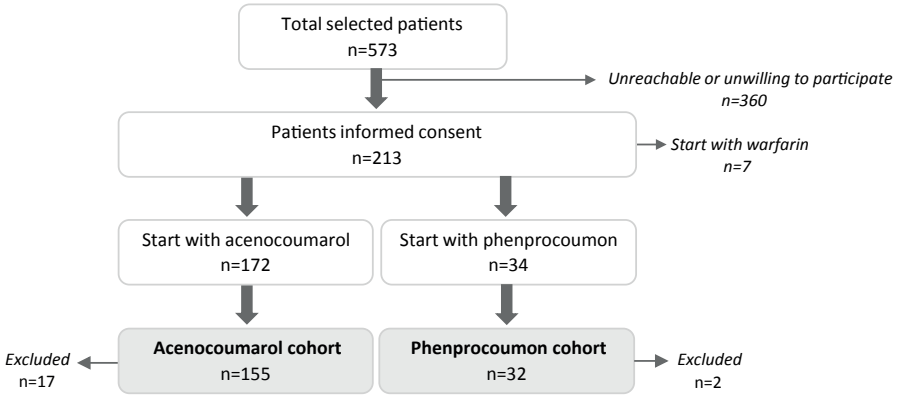


**Figure 1.** Flowchart of the patients included in the acenocoumarol and phenprocoumon cohort.

**Table 2.** Characteristics of the patients starting with acenocoumarol or phenprocoumon therapy

| | Acenocoumarol (n=155) | | Phenprocoumon (n=32) | | P-value |
|---|---|---|---|---|---|
| **Gender (female),** n (%) | 75 | (48.4) | 17 | (53.1) | 0.625 |
| **Age at start of VKA use in years,** n (%) | | | | | 0.001 |
| <1 | 24 | (15.5) | 8 | (25.0) | |
| 1-3 | 37 | (23.9) | 7 | (21.9) | |
| 4-6 | 22 | (14.2) | 1 | (3.1) | |
| 7-9 | 17 | (11.0) | 0 | (0) | |
| 10-12 | 18 | (11.6) | 0 | (0) | |
| 13-15 | 22 | (14.2) | 5 | (15.6) | |
| 16-18 | 15 | (9.7) | 11 | (34.4) | |
| **European ethnicity,** n (%) | 131 | (84.5) | 29 | (90.6) | 0.580 |
| **Indication for anticoagulation,** n (%) | | | | | <0.001 |
| Fontan procedure | 40 | (25.8) | 5 | (15.6) | |
| Prosthetic heart valve | 32 | (20.6) | 0 | (0) | |
| Dilated cardiomyopathy | 18 | (11.6) | 7 | (21.9) | |
| Deep vein thrombosis/ pulmonary embolism | 48 | (31.0) | 13 | (40.6) | |
| Aneurysm | 4 | (2.6) | 0 | (0) | |
| Pulmonary hypertension | 2 | (1.3) | 0 | (0) | |
| Cerebral* | 4 | (2.6) | 2 | (6.3) | |
| Prophylactic after surgical procedure† | 0 | (0) | 5 | (15.6) | |
| Other cardiac indication‡ | 6 | (3.9) | 0 | (0) | |
| Antiphospholipid syndrome | 1 | (0.6) | 0 | (0) | |
| **BMI at the start of VKA use§,** median (IQR) | 15.7 | (14.2-17.6) | 16.4 | (15.1-21.1) | 0.064 |
| **BSA at the start of VKA use§¶ ,** median (IQR) | 0.80 | (0.57-1.31) | 1.25 | (0.61-1.83) | 0.109 |
| **TR,** n (%) | | | | | 0.770 |
| Extra low (2.0-2.5) | 9 | (5.8) | 2 | (6.3) | |
| Low (2.0-3.0) | 27 | (17.4) | 4 | (12.5) | |
| Standard (2.0-3.5) | 82 | (52.9) | 21 | (65.6) | |
| High (2.5-4.0) | 33 | (21.3) | 5 | (15.6) | |
| Extra High (3.5-4.5 (5)) | 4 | (2.6) | 0 | (0.0) | |
| **Duration of use,** n (%) | | | | | 0.005 |
| <3 months | 20 | (12.9) | 10 | (31.3) | |
| 3-6 months | 33 | (21.3) | 8 | (25.0) | |
| 6-12 months | 10 | (6.5) | 3 | (9.4) | |
| >1 year | 90 | (58.1) | 9 | (28.1) | |
| Unknown | 2 | (1.3) | 2 | (6.3) | |
| **Patient self-testing,** n (%) | 67 | (43.3) | 11 | (34.4) | 0.433 |
| **Patient self-monitoring,** n (%) | 12 | (7.7) | 0 | (0) | 0.225 |
| **Switching between VKA,** n (%) | 17 | (11.0) | 2 | (6.3) | 0.167 |

VKA, vitamin K antagonist; BMI, body mass index; IQR, interquartile range; BSA, body surface area; TR, therapeutic international normalized ratio range. * consists of sinus thrombosis, cerebrovascular accident, cerebrovascular insufficiency with brainstem infarction. † consist of prophylactic use after a stent placement (n=2) or orthopedic surgery (n=3). ‡ consist of supraventricular tachycardia, arrhythmia, Blalock-Taussig shunt, and impaired left ventricular function. § known for n=151 (acenocoumarol) and n=29 (phenprocoumon). ¶ calculated using the formula of Haycock.

In the first year of the VKA treatment, patient self-testing occurred in 43.3% of patients for acenocoumarol and 34.4% for phenprocoumon. Only a small proportion of the patients using acenocoumarol (7.7%) had complete self-management (self-testing and self-dosing).

**Quality of treatment**

Within 7 days more than two-thirds of the patients had an INR within TR; within 14 days this percentage increased to more than 80%. The overall quality of treatment, as expressed by the TTR, was 47.0% and 51.4% for users of acenocoumarol and phenprocoumon, respectively, during the first month of treatment (Fig. 2). When considering the first 3 months of treatment the TTR was 54.6% for acencocoumarol and 63.0% for phenprocoumon. After the first 3 months of treatment the TTR ranged between 64.7% and 69.1% for acenocoumarol and 65.8% and 75.4% for phenprocoumon in the 3-month periods thereafter. At the beginning of treatment, when out of TR, time was most often spent below TR. Later in treatment, this was shifting to a more equal division of the time out of TR between above and below TR (Fig. 2).

During the first month a large number of INR measurements was carried out (more than twice each week). In the months thereafter, this decreased to less than once a week. The frequency of dose changes of more than 10% decreased over time, from a median of 2.3 (acenocoumarol) and 3.2 (phenprocoumon) dose changes per month in the first 3 months to less than one dose change per month in the last 3 months of the first year (Table 3).

The TTR was negatively correlated ($P < 0.05$) with the number of INR measurements in the first 9 months of VKA treatment and with the number of dose changes during the first year (Table S1). For age, a positive correlation existed with the TTR in the first 3 months ($r = 0.398$, $P < 0.001$). In the rest of the first year of VKA treatment this effect was less clear, but there was still a trend towards a higher TTR with increasing age (Table S1). There was a trend for higher mean TTRs in patients who used patient self-testing compared with patients who were tested by an anticoagulation clinic. Only for months 7 to 9 was the difference (12%) statistically significant (Table S2). There was a statistically significant difference ($P < 0.001$) between the TTR and the different TRs (Table S3). The patients with an extra low (2.0–2.5) or high (3.5–4.5/5) TR had a lower TTR compared with patients with a standard TR (2.0–3.5), which persisted throughout the year (Fig. 3).

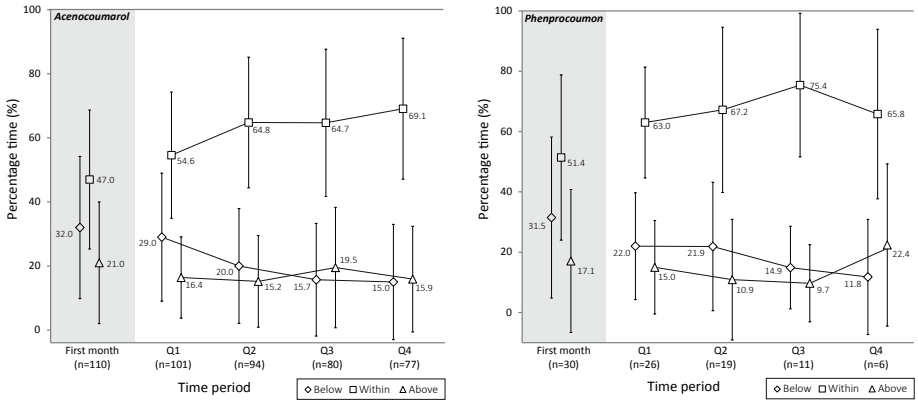


**Figure 2**. The mean percentage of time below, within and above therapeutic INR range within the first month and month 1-3 (Q1), month 4-6 (Q2), month 7-9 (Q3) and month 10-12 (Q4) of the first year of acenocoumarol (left) and phenprocoumon (right) treatment.
INR, International Normalized Ratio; Q, Quarter.



### Effectiveness and safety of treatment

During the first year of follow-up no (recurrent) thromboembolic events occurred. Bleeding events were quite common for both VKAs. They occurred at least once in 14.8% of the patients using acenocoumarol and in 31.3% of the patients using phenprocoumon (Table 4). The most commonly reported bleeding events were nosebleeds and (unexplained) bruising. None of the events could be explained by an INR value above 6 at the last measurement before the event. However, there seems to be a trend that patients with a bleeding event over the first year of treatment had a higher or equal percentage within, a lower or equal percentage below and a higher or equal percentage of time above therapeutic range compared with patients without a bleeding event (Table S4). Only in the third quarter of the first year was the percentage of time below therapeutic range statistically significantly lower in the patients with a bleeding event compared with the patients without a bleeding event ($P = 0.001$).

Although there were no (recurrent) thromboembolic events during the first year, more than 90% of the patients experienced at least once an INR of less than 2 (Table 4). For both VKAs around one-third of the total number of INRs per patient was below 2. INRs higher than 6 occurred less frequently. These were present at least once in 58.1% and 34.4% of the acenocoumarol and phenprocoumon users, respectively. Furthermore, these INRs made up a small fraction of the total INRs per patient (7.9% for acenocoumarol and 4.0% for phenprocoumon). Not all INRs above 6 resulted in vitamin K administration. Only 8.4% of the patients were treated

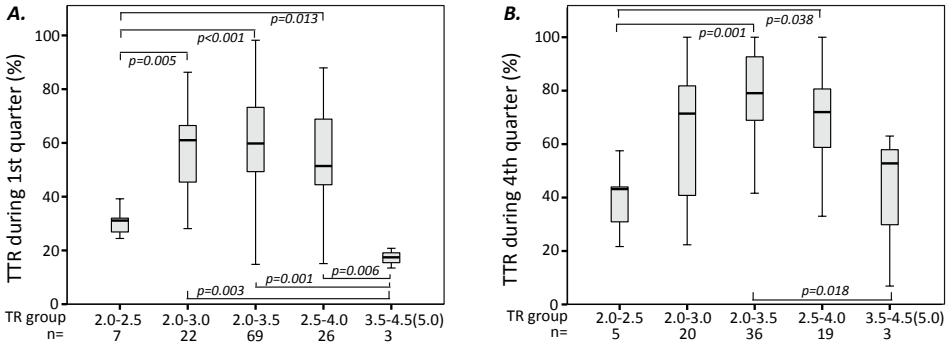


**Figure 3.** Percentage of time in therapeutic INR range (TTR) by therapeutic range (TR) among users of acenocoumarol and phenprocoumon combined. **A.** During the first three month of vitamin K antagonist (VKA) use. **B.** During the last three months of the first year of VKA use.

with vitamin K in the acenocoumarol cohort, which was statistically significantly ($P < 0.001$) lower than the 34.4% of the patients in the phenprocoumon cohort.

## DISCUSSION

The study shows that the quality (defined as the TTR) of VKA treatment in pediatric patients in the Netherlands is acceptable after the first 3 months of use. The TTR ranged between 64.7% and 69.1% for acenocoumarol and 65.8% and 75.4% for phenprocoumon. However, the TTR was lower during the first month (± 50%). Furthermore, the frequency of INR measurements and dose changes of more than 10% was high during the first 3 months of VKA treatment. Although the INR was frequently out of range and also in the extreme areas (< 2 and > 6), this was not associated with bleeding or thromboembolic events. However, a large proportion of the patients (14.8% for acenocoumarol and 31.3% for phenprocoumon) still had at least one bleeding event during the first year. No thromboembolic events were reported.

In adults, high (above 4–5) or low (below 2) INRs are correlated with major bleedings and thromboembolic events, respectively.[20] We were not able to find such associations. However, we did see that patients with bleeding events tended to have more time above range and less time below range, mostly not statistically significant, compared with patients without a bleeding event. However, because of the low number of events we didn't have enough power to study this. The number of complications could have been higher. Because of the retrospective nature of the study such complications may not have been retrieved. Another possibility

**Table 3.** Treatment quality during the first year of treatment with acenocoumarol and phenprocoumon

| | Acenocoumarol | | n = 155 | Phenprocoumon | | n = 32 |
|---|---|---|---|---|---|---|
| **Achieving TR** | | | 139 | | | 31 |
| ≤ 7 days, n (%)* | 106 | (76.3) | | 21 | (67.7) | |
| ≤ 14 days, n (%)* | 131 | (94.2) | | 27 | (87.1) | |
| Number of days, median (IQR) | 4.0 | (2.0-7.0) | | 4.0 | (3.0-12.0) | |
| **Number of INRs per month,** median (IQR) | | | | | | |
| <1 month | 10.0 | (7.0-13.0) | 110 | 9.0 | (7.0-12.0) | 30 |
| Month 1-3 | 6.0 | (4.7-7.7) | 101 | 6.2 | (4.3-10) | 26 |
| Month 4-6 | 3.7 | (2.7-4.3) | 94 | 4.3 | (2.3-5.0) | 19 |
| Month 7-9 | 3.3 | (2.5-4.3) | 80 | 4.3 | (2.0-4.3) | 11 |
| Month 10-12 | 3.0 | (2.3-4.0) | 77 | 2.7 | (2.0-3.3) | 6 |
| **Number of dose changes >10% per month,** median (IQR) | | | | | | |
| Month 1-3 | 2.3 | (1.3-3.3) | 152 | 3.2 | (1.7-4.0) | 32 |
| Month 4-6 | 0.8 | (0.3-2.0) | 110 | 0.7 | (0.3-3.0) | 21 |
| Month 7-9 | 0.7 | (0.3-1.7) | 91 | 1.0 | (0.0-2.7) | 14 |
| Month 10-12 | 0.7 | (0.0-1.3) | 81 | 0.3 | (0.0-1.0) | 11 |

INR, international normalized ratio; TR, therapeutic INR range; IQR, interquartile range. * The percentages are based on the number of patients fulfilling the criteria for the parameter during that specific period, not on the total cohort.

**Table 4.** Effectiveness and safety parameters during first year of acenocoumarol and phenprocoumon treatment

| | Acenocoumarol (n = 155) | | | | Phenprocoumon (n = 32) | | | |
|---|---|---|---|---|---|---|---|---|
| | n (%) | | Mean rate* | Mean fraction of total INRs‡, % | n (%) | | Mean rate* | Mean fraction of total INRs‡, % |
| **Thrombotic events** | 0 | (0) | 0 | - | 0 | (0) | 0 | - |
| **Bleeding events** | 23 | (14.8) | 1.35 | - | 10 | (31.3) | 1.50 | - |
| **Type of bleeding event** | | | | - | | | | - |
| Nosebleed | 13 | (8.4) | 4 | - | 2 | (6.3) | 2 | - |
| Bruising with unknown cause | 7 | (4.5) | 2 | - | 1 | (3.1) | 1 | - |
| Increased bruising on impact | 5 | (3.2) | 1 | - | 2 | (6.3) | 2 | - |
| Hematuria | 2 | (1.3) | 1 | - | 0 | (0) | 0 | - |
| Blood in stool (melena) | 1 | (0.6) | 1 | - | 1 | (3.1) | 1 | - |
| Hematemesis | 0 | (0) | 0 | - | 1 | (3.1) | 1 | - |
| Eye bleeding | 0 | (0) | 0 | - | | 1 (3.1) | 1 | - |
| Other† | 0 | (0) | 0 | - | 3 | (9.4) | 1 | - |
| **INR<2** | 153 | (98.7) | 8.96 | 33.2 | 30 | (93.8) | 8.93 | 28.3 |
| **INR>6** | 90 | (58.1) | 2.03 | 7.9 | 11 | (34.4) | 1.45 | 4.0 |
| **Use of vitamin K** | 13 | (8.4) | 1.15 | - | 11 | (34.4) | 1.18 | - |

INR, international normalized ratio; *n*, number of patients experiencing at least one event; -, not applicable.* For type of bleeding event the maximum rate is shown instead of mean rate. † consists of: bleeding at implantable cardioverter-defibrillator (n=1) and prolonged bleeding after injury (n=2) ‡ fraction of INR<2 or >6 of the total INR measurements of a patient.

is that events were prevented because of the intensive monitoring policy of the anticoagulation clinics with children on VKAs. In our cohort, INR measurements were carried out more often (three INR measurements/month) than recommended in the adult ACCP guideline of one INR measurement per 4–12 weeks,[21] and the minimum of one INR measurement per month recommended for children.[4] This allows rapid dose adjustments when needed. Furthermore, the hemostatic system is still developing during childhood.[22] This might well be related to differences in risks of bleeding and thromboembolic events in comparison to adults.

Our TTRs were mostly higher (47–75.4%) than the TTRs found by Spoor et al. of just under 50% during the first year of treatment in children using acenocoumarol and phenprocoumon in the Netherlands.[11] A possible explanation for this difference is the larger proportion of patients in their cohort with a duration of follow-up/use of less than 3 months (46% compared with 16% in our cohort). In the first months, INR values show more fluctuation than in later periods, which reduces the overall TTR. This is also supported by our TTRs in the first month/first 3 months, which were around 50%. The TTRs of 65–75% after the first 3 months of treatment in our study are similar to the overall TTR of 63% found in the similar warfarin cohort of Biss et al.[8] Our TTRs during the different time periods after the start of VKA therapy are also similar to the TTRs found in adults, changing from 54% in the first month to 75% after the first 3 months.[23]

As expected, there was a clear association between the TR and the TTR during the first year. The TTR in the lower and narrower TR of 2.0–2.5 and the higher TR of 3.5–4.5 (5.0) were significantly lower compared with the TTR in children with a TR of 2.0–3.5. This is in line with the findings of previous studies in adults.[17, 18] The study by Meier et al. showed that a narrow TR of 2.0–2.5 led to more INRs below 2 compared with a TR of 2.0–3.0.[18] Gadisseur et al. showed that a TR of 2.5–3.5 resulted in a significantly higher TTR than a TR of 3.0–4.0.[17] However, for both extreme TRs the numbers of patients were very low in our cohort. Furthermore, it is possible that patients were dosed with the aim of achieving a different TR than the one stated in the patient record, which reduces the calculated TTR. Interestingly, patients with the same indication often had different TRs stated in the patient records (data not shown). This indicates that possibly individual patient characteristics or the preference of the physician has an influence on the choice of the TR.

Although the TTR was on average indicative of an acceptable anticoagulation control, the standard deviation was large for both VKAs. For patients with low TTRs it is important to identify the causes (patient-specific and/or management-related factors) and to search for a way to improve these TTRs. Although we did not find any thromboembolic events in the first year, we did find that a large proportion of patients experienced bleeding events. Furthermore, the very frequent INR

measurements and dose changes of more than 10% were common in the patients with lower TTRs, which can impair patient satisfaction and increase costs.

Earlier studies have shown that patients who self-test and self-manage can improve quality of life, patient satisfaction and TTR.[12, 14, 24] As a result of the large standard deviations of the TTRs, we only found a statistically significant association between patient self-testing and the TTR during the seventh to ninth months of use. However, there was a clear trend of higher TTRs after the first 3 months for patients who were self-testing their INRs. Involving patients in their anticoagulation control by self-testing or self-management might be a way to improve the TTR, especially during the maintenance phase.

A meta-analysis of all randomized controlled trials in adults has shown that using a pharmacogenetic dosing algorithm increases the TTR, especially in the first months of use.[25] No such pediatric dosing algorithm exists yet for acenocoumarol and phenprocoumon. With the data from CAPS we will develop a pediatric pharmacogenetic-guided dosing algorithm for both VKAs. With this model it will be easier to predict the appropriate starting dose for individual patients, which might reduce the number of INR measurements and dose changes and increase the TTR.



A limitation of this study was the small number of patients, especially for phenprocoumon. Phenprocoumon is far less frequently used as a first VKA in pediatric patients in the Netherlands compared with acenocoumarol. Furthermore, the retrospective data collection might have resulted in incomplete or misinterpretation of data. With the available data we were not able to study the cause of low TTRs, such as diet, fever and/or concurrent drug therapy. Some information about concurrent drug therapy was available, but was too incomplete to use in the analysis. A strength of our study is that our cohort was composed of patients from different sites in the Netherlands, making it a representative sample of the Dutch pediatric population using VKAs. With the provided information on patient and treatment characteristics, physicians from other countries should be able to translate the results to their own situation.

In conclusion, the overall quality of acenocoumarol and phenprocoumon treatment in pediatric patients in the Netherlands is acceptable, but can be improved. Especially during the first month, the quality of VKA treatment is low, and during the first year of treatment a substantial number of bleeding events occurs. Developing a dosing algorithm can improve VKA anticoagulation and increase patient satisfaction, with fewer INR measurements, dose adjustments and possibly bleedings.

## REFERENCES


1. Monagle P, Chan AKC, Goldenberg NA, Ichord RN, Journeycake JM, Nowak-Göttl U, et al. 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.
2. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology — drug disposition, action, and therapy in infants and children. *N Engl J Med* 2003; 349: 1157–67.
3. Chalmers E, Ganesen V, Liesner R, Maroo S, Nokes T, Saunders D, et al. 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, et al. 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. Connolly SJ, Pogue J, Eikelboom J, Flaker G, Commerford P, Franzosi MG, et al. Benefit of oral anticoagulant over antiplatelet therapy in atrial fibrillation depends on the quality of international normalized ratio control achieved by centers and countries as measured by time in therapeutic range. *Circulation* 2008; 118: 2029–37.
6. De Caterina R, Husted S, Wallentin L, Andreotti F, Arnesen H, Bachmann F, et al. Vitamin K antagonists in heart disease: current status and perspectives (Section III). Position paper of the ESC Working Group on Thrombosis-Task Force on Anticoagulants in Heart Disease. *Thromb Haemost* 2013; 110: 1087–107.
7. Jones S, Newall F, Manias E, Monagle P. Assessing outcome measures of oral anticoagulation management in children. *Thromb Res* 2011; 127: 75–80.
8. Biss TT, Avery PJ, Walsh PM, Kamali F. Comparison of "time within therapeutic INR range" with "percentage INR within therapeutic range" for assessing long-term anticoagulation control in children. *J Thromb Haemost* 2011; 9: 1090–2.
9. Hawcutt DB, Ghani AA, Sutton L, Jorgensen A, Zhang E, Murray M, et al. Pharmacogenetics of warfarin in a paediatric population: time in therapeutic range, initial and stable dosing and adverse effects. *Pharmacogenomics J* 2014; 14: 542–8.
10. Murray JM, Hellinger A, Dionne R, Brown L, Galvin R, Griggs S, et al. Utility of a dedicated pediatric cardiac anticoagulation program: the Boston Children's Hospital experience. *Pediatr Cardiol* 2015; 36: 842–50.
11. 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.
12. Bauman ME, Black K, Bauman ML, Bruce AAK, Kuhle S, Bajzar L, et al. EMPoWarMENT: Edmonton pediatric warfarin self-management pilot study in children with primarily cardiac disease. *Thromb Res* 2010; 126: e110–5.
13. Bauman ME, Massicotte MP, Kuhle S, Siddons S, Bruce AA. EMPoWARed: Edmonton pediatric warfarin self-management study. *Thromb Res* 2015; 136: 887–93.
14. Jones S, Monagle P, Manias E, Bruce AAK, Newall F. Quality of life assessment in children commencing home INR self-testing. *Thromb Res* 2013; 132: 37–43.
15. Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G; American College of Chest Physicians. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 2008; 133: 160S–98S.
16. Schmitt L, Speckman J, Ansell J. Quality assessment of anticoagulation dose management: comparative evaluation of measures of time-in-therapeutic range. *J Thromb Thrombolysis* 2003; 15: 213–6.

17. Gadisseur APA, van der Meer FJM, Adriaansen HJ, Fihn SD, Rosendaal FR. Therapeutic quality control of oral anticoagulant therapy comparing the short-acting acenocoumarol and the long-acting phenprocoumon. *Br J Haematol* 2002; 117: 940–6.
18. Meier DJ, Sonnad SS, Merz JC, Fay WP. Comparison of narrow versus standard target INR ranges [Abstract]. *J Am Coll Cardiol* 2002; 39: 272–272.
9. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briët E. A method to determine the optimal intensity of oral anticoagulant therapy. *Thromb Haemost* 1993; 69: 236–9.
20. Cannegieter SC, Rosendaal FR, Wintzen AR, van der Meer FJM, Vandenbroucke JP, Briet E. Optimal oral anticoagulant therapy in patients with mechanical heart valves. *N Engl J Med* 1995; 333: 11–7.
21. Guyatt GH, Akl EA, Crowther M, Gutterman DD, Schuünemann HJ. American College of Chest Physicians Antithrombotic Therapy and Prevention of Thrombosis Panel. Executive summary: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012; 141: 7S–47S.
22. Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L. Maturation of the hemostatic system during childhood. *Blood* 1992; 80: 1998–2005.
23. Erkens PMG, ten Cate H, Büller HR, Prins MH. Benchmark for time in therapeutic range in venous thromboembolism: a systematic review and meta-analysis. *PLoS One* 2012; 7: e42269.
24. Bradbury MJE, Taylor G, Short P, Williams MD. A comparative study of anticoagulant control in patients on long-term warfarin using home and hospital monitoring of the international normalised ratio. *Arch Dis Child* 2008; 93: 303–6.
25. Belley-Cote EP, Hanif H, D'Aragon F, Eikelboom JW, Anderson JL, Borgman M, et al. Genotype-guided versus standard vitamin K antagonist dosing algorithms in patients initiating anticoagulation. A systematic review and meta-analysis. *Thromb Haemost* 2015; 114: 768–77.

## SUPPORTING INFORMATION

**Table S1.** The association between TTR and number of INRs, number of dose changes of more than 10% and age during the first year of VKA use

| | Number of INRs | | | Number of dose changes of >10% | | | Age | | |
|---|---|---|---|---|---|---|---|---|---|
| TTR in Month | n | r | p-value | n | r | p-value | n | r | p-value |
| 1-3 | 127 | -0.275 | 0.002 | 127 | -0.294 | 0.001 | 127 | 0.398 | <0.001 |
| 4-6 | 113 | -0.390 | <0.001 | 110 | -0.412 | <0.001 | 113 | 0.181 | 0.055 |
| 7-9 | 91 | -0.213 | 0.042 | 86 | -0.335 | 0.002 | 91 | 0.364 | <0.001 |
| 10-12 | 83 | -0.213 | 0.053 | 82 | -0.473 | <0.001 | 83 | 0.209 | 0.058 |

TTR, percentage time in therapeutic INR range; VKA, vitamin K antagonist; sd, standard deviation.

**Table S2.** The difference in mean TTR between patients who do and do not use self-testing during the first year of VKA use

| | Patient self-testing | | | |
|---|---|---|---|---|
| TTR in Month | n | no, *mean (sd)* | yes, *mean (sd)* | p-value |
| 1-3 | 127 | 56.8 (20.1) | 54.3 (18.3) | 0.550 |
| 4-6 | 113 | 62.8 (21.5) | 68.7 (21.5) | 0.154 |
| 7-9 | 91 | 59.0 (26.7) | 71.3 (19.0) | 0.018 |
| 10-12 | 83 | 66.4 (21.1) | 70.6 (23.1) | 0.400 |

TTR, percentage time in therapeutic INR range; VKA, vitamin K antagonist; sd, standard deviation.

**Table S3.** The mean TTR of patient with different TRs during the first year of VKA use

| | | TR | | | | | |
|---|---|---|---|---|---|---|---|
| TTR in month | n | 2.0-2.5, *mean (sd)* | 2.0-3.0, *mean (sd)* | 2.0-3.5, *mean (sd)* | 2.5-4.0, *mean (sd)* | 3.5-4.5 (5), *mean (sd)* | p-value |
| 1-3 | 127 | 30.4 (5.1) | 57.3 (15.6) | 60.8 (19.4) | 54.8 (16.9) | 17.2 (3.7) | <0.001 |
| 4-6 | 113 | 37.5 (19.4) | 63.1 (20.4) | 71.3 (20.2) | 64 (18.3) | 30.3 (11.0) | <0.001 |
| 7-9 | 91 | 35.3 (13.0) | 65.2 (17.9) | 69.6 (22.4) | 72.4 (23.1) | 28.5 (14.7) | <0.001 |
| 10-12 | 83 | 39.4 (13.7) | 64.0 (23.3) | 78.3 (17.5) | 68.1 (19.3) | 40.9 (29.9) | <0.001 |

TR, therapeutic INR range; TTR, percentage time in therapeutic INR range; VKA, vitamin K antagonist; sd, standard deviation.

**Table S4.** Differences in percentage time below, within and above therapeutic INR range between patient with and without bleeding events

| Percentage of time (%) | | Bleeding event | | | | Independent sample t-test |
|---|---|---|---|---|---|---|
| | | No | | Yes | | |
| | | *mean (sd)* | n | *mean (sd)* | n | p-value |
| **First month** | *Below* | 33.6 (22.7) | 112 | 25.3 (23.7) | 28 | 0.090 |
| | *Within* | 47.3 (22.4) | 112 | 50.6 (25.5) | 28 | 0.336 |
| | *Above* | 19.2 (18.6) | 112 | 24.1(25.0) | 28 | 0.492 |
| **Month 1-3** | *Below* | 29.3 (18.7) | 100 | 21.5 (22.3) | 27 | 0.068 |
| | *Within* | 54.6 (19.0) | 100 | 62.5 (21.3) | 27 | 0.968 |
| | *Above* | 16.1 (13.0) | 100 | 16.0 (14.5) | 27 | 0.064 |
| **Month 4-6** | *Below* | 21.3 (18.9) | 90 | 16.6 (16.2) | 23 | 0.273 |
| | *Within* | 65.0 (21.4) | 90 | 66.0 (22.8) | 23 | 0.419 |
| | *Above* | 13.7 (13.8) | 90 | 17.4 (20.5) | 23 | 0.839 |
| **Month 7-9** | *Below* | 18.0 (18.4) | 70 | 7.8 (8.1) | 21 | 0.001 |
| | *Within* | 63.4 (22.5) | 70 | 74.6 (24.4) | 21 | 0.825 |
| | *Above* | 18.6 (17.2) | 70 | 17.6 (22.5) | 21 | 0.053 |
| **Month 10-12** | *Below* | 14.8 (16.3) | 68 | 15.1 (24.9) | 15 | 0.949 |
| | *Within* | 68.9 (20.6) | 68 | 68.7 (29.4) | 15 | 0.979 |
| | *Above* | 16.4 (16.7) | 68 | 16.2 (20.4) | 15 | 0.975 |

INR, International Normalized Ratio; IQR, interquartile range; sd, standard deviation.

# 3.3 Factors influencing anticoagulation stability of vitamin K antagonists in pediatric patients

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*Manuscript in preparation*

## ABSTRACT

**Background**

A large proportion of pediatric patients treated with vitamin K antagonists (VKAs) does not obtain stable therapeutic International Normalized Ratio (INR) values within a few months of treatment. Therefore, this study aims to identify explanatory factors for not obtaining a stable anticoagulation period within 3 months after the start of acenocoumarol or phenprocoumon and to study whether switching between these two VKAs affects anticoagulation control, expressed as the percentage time in therapeutic INR range (TTR), and INR stability.

**Methods**

The patient cohort of the Children Anticoagulation and Pharmacogenetics Study was used. It consists of pediatric patients who used acenocoumarol and/or phenprocoumon after January 1995. Clinical and genetic information was collected and tested with Cox regression for association with obtaining a stable anticoagulation period. A stable period was defined as ≥3 consecutive INRs within therapeutic range over a period of ≥3 weeks.

The half-year period before and after switching between acenocoumarol and phenprocoumon was compared at patient level on TTR and INR stability.

**Results**

In total 157 patients (median age 6.0 years; 51.6% female) were included in the analysis of whom 43% had a stable period within 3 months of treatment. Age was positively (per year increase the hazard ratio (HR) was 1.09, 95%CI [1.05-1.13], $p<0.001$) associated with obtaining a stable period. Female sex (0.60 [0.37-0.97], $p=0.037$), hospitalized patients (0.46 [0.24-0.57], $p=0.017$), or patients with an interruption in their VKA therapy for ≥3 days (0.50 [0.25-0.98], $p=0.044$) had a lower chance of obtaining a stable period. Patients who switched between acenocoumarol and phenprocoumon (n=12) had often a higher TTR and lower variability in INRs when using phenprocoumon comparing the 3 months before with the 3 months after switching.

**Conclusion**

Younger and female children have less often a stable anticoagulation period compared to older and male children. Patients often showed improvement in TTR and INR stability when switched from acenocoumarol to phenprocoumon. Further research is required to determine if the use of phenprocoumon in pediatric patients could lead to a clinically relevant improvement of anticoagulation control.

## INTRODUCTION

Over the years more pediatric patients become in need of anticoagulation treatment to prevent and treat thromboembolic events.[1] However, the quality of anticoagulation treatment has not been widely studied in pediatric patients. Vitamin K-antagonists (VKAs) are currently the most commonly used oral anticoagulant drugs in pediatric patients in daily practice. Previously it has been shown that the anticoagulation control is acceptable after the first 3 months of treatment in pediatric patients on VKAs in the Netherlands.[2] During the first three months of anticoagulation the percentage time in therapeutic range (TTR) was suboptimal and age appeared to be positively correlated with the TTR. However, the TTR describes how well a patient is controlled during a certain time period which does not exclude a high variability in International Normalized Ratio's (INRs) and substantial under or over coagulation during that time period. An important measure to study the quality of anticoagulation is the time it takes to reach a stable anticoagulation period. In previous research we have shown that approximately one third of acenocoumarol and phenprocoumon users never reach a stable period when defined as ≥3 consecutive INRs within therapeutic INR range [TR] during at least 3 weeks.[3,4] There may be several reasons for not obtaining a stable period, such as frequent interruptions of VKA therapy, non-adherence, infections, varying vitamin K intake, or a very short duration of use. Also the choice of type of VKA can play a role. In a study by Fihn *et al.* it was shown that adult patients on phenprocoumon, had a higher percentage of INRs within TR, required fewer INR measurements and had more stable INR values compared to patients using acenocoumarol.[5]

Therefore, the current study has two objectives: (1) to identify explanatory factors for not obtaining a stable period within 3 months after the start of acenocoumarol or phenprocoumon and (2) to study whether switching between acenocoumarol and phenprocoumon affects the anticoagulation control (expressed as TTR) and INR stability.

## METHODS

### Data source and study population

The data of the Children Anticoagulation and Pharmacogenetics Study (CAPS) was used. CAPS was designed as a retrospective follow-up study in which pediatric patients aged 18 years or younger, who used acenocoumarol and/or phenprocoumon after January 1st 1995 were included at four academic pediatric hospitals and one anticoagulation clinic. The study is described in more detail in

earlier publications.[2–4] The medical records at the hospitals and anticoagulation clinics of the selected patients were used to retrieve all clinical and anticoagulation treatment related information. Furthermore, patients were asked to collect a saliva sample at home for genotyping of VKORC1 (rs9934438), CYP2C9 (rs1799853 and rs1057910), CYP4F2 (rs2108622), CYP3A4 (rs35599367 and rs2740574) and CYP2C18 (rs1998591). The follow-up of the patients started at the start of VKA treatment and ended at the date of data collection at the anticoagulation clinics (between 11-01-2014 and 10-03-2016), when a child became 19 years of age, when VKA therapy was stopped, or when a child was lost to follow-up, whatever came first. The study was reviewed by the Medical Ethics Review Committee of the University Medical Center Utrecht which decided that the study did not need ethical approval, because a non-invasive DNA collection method was used. The UPPER Institutional Review Board of the Division of Pharmacoepidemiology and Clinical Pharmacology of Utrecht University approved thereafter the study protocol. All participants (and/or their parents or legal guardians) provided informed consent before taking part.

**Outcome and determinants and statistical analysis**

The outcome for the first objective was a stable anticoagulation period, which was defined as ≥3 consecutive INRs within TR during at least 3 weeks. Patients were excluded when they were unable to obtain a stable period based on the available data (e.g. the time period between the first and last measured INR was less than three weeks), when they started with VKA treatment with warfarin, when the start date of VKA treatment was unknown or when the first INR was measured more than 5 days after VKA start. Patients who switched to another VKA or had a gap of more than 28 days without VKA treatment were censored at the last INR before one of these events occurred.

Determinants collected were age, sex, TR, indication for VKA use, type of VKA, and having one or two variant alleles of the studied genetic variations. Furthermore, the number of INR measurements during the first 3 months of treatment, duration of follow-up, occurrence of hospitalizations after the primary hospitalization in which the VKA was started, and if patients had at least one interruption in their VKA therapy for ≥ 3 days were determined. Using Cox regression these variables were tested univariately and adjusted for age and gender to study the association with a stable anticoagulation period. A p-value of $<0.05$ was considered statistically significant.

Age has shown to be strongly associated with TTR in our previous study.[2] Therefore, we were interested if any differences in parameters for anticoagulation control and determinants like hospitalizations were visible between age groups. Children were stratified into three age groups: < 1 year, 1-9 years and 10-18 years of

age. For categorical variables a chi-squared test or Fisher's exact test was used. For continuous variables a one-way ANOVA or Kruskal-Wallis test was used.

For the second objective only patients switching between acenocoumarol and phenprocoumon were included. Patients were excluded when they had less than 2 months of follow-up for one of the two VKAs or when there was a gap of more than a week in the treatment information directly before the switch. In the first month of VKA treatment the anticoagulation control is often suboptimal based on TTR and INR variability and therefore the first 28 days of VKA treatment were excluded from the analysis. We evaluated VKA treatment in the 3 months and 3-6 months before and after the switch. For each period the time in therapeutic range, the proportion of INRs in TR, the time-weighted variance in INRs and the mean interval between INR measurements were determined. These were only calculated if ≥ 3 INRs were available. For calculation of the time-weighted variance in INRs the formula of Fihn *et al.* was used.[5]

The data was analyzed using the statistical software SPSS version 25 and visualized using R version 3.5.0.



## RESULTS

**Stable anticoagulation period within the first 3 months of VKA treatment**

In total 213 patients were included in CAPS of whom 157 were eligible for the analysis for the first objective. Most patients were excluded due to missing INR measurements in the first 5 days of treatment or being unable to obtain a stable period due to insufficient follow-up data, as shown in figure 1. In total 68 (43.3%) patients had a stable period within the first 3 months of VKA treatment. Only 41.2% of the patients with a stable period had a good anticoagulation control (TTR>70%), on the other hand 10.3% of them had poor anticoagulation control (TTR<50%), as shown in table 1.

Older children had an increased chance to reach a stable anticoagulation period (per year increase the adjusted HR was 1.1 (1.0-1.1), p<0.001). Patients of 1-9 years of age (adjusted HR 4.9, 95%CI = 1.5-16.1, p=0.009) and patients of 10-18 years (adjusted HR 9.6, 95%CI = 2.9-31.2, p<0.001) had statistically significantly higher chances of obtaining a stable period compared to patients of less than 1 year of age, as shown in table 1 and figure 2. Patients younger than 1 year were more likely to have a poor TTR of less than 50% and a high percentage (>30%) of time below TR, as shown in table 2. Furthermore, they had more frequent INR measurements compared to the older age groups. Variance in INRs was not shown to be statistically significantly different between the age groups. However, the variance in INRs in the first month of treatment was the highest in the patients < 1 year of age and the lowest in patients of 10 to 18 years of age.

**Table 1 .** Patient characteristics and their association with achieving a stable anticoagulation period

| | **Stable period within 3 months after start** | | | | **Unadjusted** | | **Adjusted for age and sex** | |
|---|---|---|---|---|---|---|---|---|
| | **No (n=89)** | | **Yes (n=68)** | | **HR (95%CI)** | **p-value** | **HR (95%CI)** | **p-value** |
| **TTR > 70%,** n (%) | 1 | (1.1) | 28 | (41.2) | - | - | - | - |
| **TTR < 50%,** n (%) | 63 | (70.8) | 7 | (10.3) | - | - | - | - |
| **Time to SP (days),** median (IQR) | NA | | 34 | (15.5-62) | - | - | - | - |
| **Number of switchers,** n (%) | 12 | (13.5) | 4 | (5.9) | - | - | - | - |
| Acenocoumarol to phenprocoumon | 12 | (13.5) | 2 | (2.9) | | | | |
| Phenprocoumon to acenocoumarol | 0 | (0) | 2 | (2.9) | | | | |
| **Age at start of VKA (years),** median (IQR) | 4.0 | (0.5-10.7) | 10.4 | (3.8-15.2) | 1.08 (1.04-1.13) | **<0.001** | 1.09 (1.05-1.13) | **<0.001** |
| <1, n(%) | 26 | (29.2) | 3 | (4.4) | Ref. | NA | Ref. | N.A. |
| 1-9, n(%) | 40 | (44.9) | 28 | (41.2) | 4.72 (1.44-15.54) | **0.011** | 4.88 (1.48-16.07) | **0.009** |
| 10-18, n(%) | 23 | (25.8) | 37 | (54.4) | 8.86 (2.73-28.75) | **<0.001** | 9.59 (2.95-31.22) | **<0.001** |
| **Sex,** n(%) | | | | | | | | |
| male | 39 | (43.8) | 37 | (54.4) | Ref. | NA | Ref. | NA |
| female | 50 | (56.2) | 31 | (45.6) | 0.72 (0.44-1.16) | 0.171 | 0.60 (0.37-0.97) | **0.037** |
| **TR,** n(%) | | | | | | | | |
| 2.0-2.5 | 10 | (11.5) | 0 | (0.0) | - | - | - | - |
| 2.0-3.0 | 12 | (13.8) | 16 | (23.5) | Ref. | NA | Ref. | NA |
| 2.0-3.5 | 44 | (50.6) | 39 | (57.4) | 0.83 (0.46-1.48) | 0.520 | 0.71 (0.39-1.28) | 0.255 |
| 2.5-4.0 | 17 | (19.5) | 13 | (19.1) | 0.67 (0.32-1.40) | 0.290 | 0.54 (0.26-1.13) | 0.100 |
| 3.5-4.5 | 2 | (2.3) | 0 | (0.0) | - | - | - | - |
| Multiple TRs | 2 | (2.3) | 0 | (0.0) | - | - | - | - |
| **Indication,** n(%) | | | | | | | | |
| Cardiac | 54 | (60.7) | 40 | (58.8) | Ref. | NA | Ref. | NA |
| TE | 30 | (33.7) | 24 | (35.3) | 1.16 (0.70-1.92) | 0.577 | 1.07 (0.64-1.78) | 0.808 |
| Other | 5 | (5.6) | 4 | (5.9) | 1.33 (0.47-3.75) | 0.586 | 1.11 (0.39-3.15) | 0.845 |
| **VKA,** n(%) | | | | | | | | |
| Acenocoumarol | 75 | (84.3) | 51 | (75.0) | Ref. | NA | Ref. | NA |
| Phenprocoumon | 14 | (15.7) | 17 | (25.0) | 1.64 (0.95-2.85) | 0.078 | 1.58 (0.90-2.78) | 0.111 |
| **Hospitalization,** n(%) | | | | | | | | |
| No | 61 | (68.5) | 57 | (83.8) | Ref. | NA | Ref. | NA |
| Yes | 28 | (31.5) | 11 | (16.2) | 0.45 (0.24-0.87) | **0.038** | 0.46 (0.24-0.87) | **0.017** |
| **Interruption of VKA treatment for ≥3 days,** n(%) | | | | | | | | |
| No | 67 | (75.3) | 58 | (85.3) | Ref. | NA | Ref. | NA |
| Yes | 22 | (24.7) | 10 | (14.7) | 0.60 (0.31-1.18) | 0.137 | 0.50 (0.25-0.98) | **0.044** |
| **Number of INRs,** median (IQR) | 18 | (14-27) | 17 | (13-19) | 0.94 (0.91-0.97) | **0.001** | 0.95 (0.92-0.99) | **0.015** |
| **Time of follow-up (days),** median (IQR) | 85 | (63-89) | 86 | (82-90) | 1.02 (1.00-1.04) | 0.116 | 1.02 (1.00-1.04) | 0.117 |

CI, confidence interval; HR, hazard ratio; IQR, interquartile range; INR, International Normalized Ratio; NA, not applicable; SP, stable period; TR, therapeutic INR range; TTR, Time in therapeutic INR range; VKA, vitamin K antagonist; -, not analyzed.

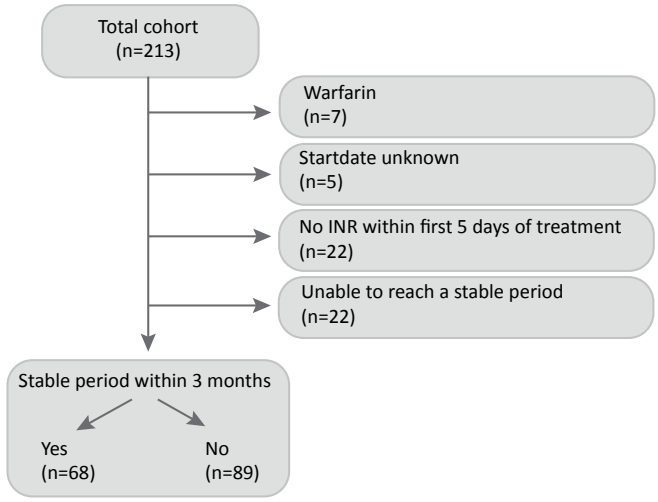


**Figure 1.** Flowchart objective 1.



**Table 2 .** Differences in treatment related variables between the three age groups

| Age | < 1 year (n=29) | | 1-9 years (n=68) | | 10-18 years (n=60) | | p-value |
|---|---|---|---|---|---|---|---|
| TTR < 50%, n(%) | 21 | (72.4) | 29 | (42.6) | 20 | (33.3) | **0.002** |
| Time below TR >15%, n(%) | 26 | (89.7) | 52 | (76.5) | 34 | (56.7) | **0.003** |
| Time below TR >30%, n(%) | 24 | (82.8) | 28 | (41.2) | 24 | (40.0) | **<0.001** |
| Time above TR >15%, n(%) | 13 | (44.8) | 36 | (52.9) | 28 | (46.7) | 0.685 |
| Time above TR >30%, n(%) | 4 | (13.8) | 10 | (14.7) | 9 | (15.0) | 0.989 |
| Variability in INRs during the first month, median (IQR)† | 1.35 | (0.54-1.74) | 1.18 | (0.61-2.38) | 0.74 | (0.48-1.52) | 0.063 |
| Variability in INRs during month 2 and 3, median (IQR)†‡ | 0.40 | (0.24-1.05) | 0.42 | (0.26-0.59) | 0.32 | (0.19-0.62) | 0.439 |
| Time interval between INRs (days), mean (sd) | 3.84 | (1.74) | 4.58 | (1.85) | 5.81 | (2.14) | **<0.001*** |
| Mean time interval < 7 days, n(%) | 26 | (89.7) | 61 | (89.7) | 47 | (78.3) | 0.158 |
| Follow-up time in days, median (IQR) | 85 | (74-89) | 86 | (78-90) | 85 | (77-89) | 0.660 |
| Hospitalizations, n(%) | 10 | (34.5) | 17 | (25.0) | 14 | (23.3) | 0.513 |
| Interruption of VKA treatment for ≥3 days, n(%) | 5 | (17.2) | 12 | (17.6) | 15 | (25.0) | 0.528 |

INR, international normalized ratio; IQR, interquartile range; TR, therapeutic INR range; TTR, percentage time within therapeutic INR range; VKA, vitamin K antagonist.

* One-way ANOVA (<1 vs 1-9 years, p=0.201; <1 vs 10-18 years, p<0.001; 1-9 vs 10-18 years, p=0.001)

† Calculated using the time-weighted variability in INRs formula of Fihn *et al*.[5]

‡ Only available for n=27 <1 year of age, n=66 1-9 years of age, and n=54 10-18 years of age due to < 3 INRs available.

Gender was statistically significantly associated with obtaining a stable period after adjustment for age. Female patients had a lower chance of obtaining a stable period (adjusted HR 0.60, 95%CI = 0.37-0.97, p=0.037) compared to male patients, see table 1. TR and indication for VKA use were not associated with obtaining a stable period. More patients on phenprocoumon obtained a stable period (54.8% for phenprocoumon compared to 40.5% for acenocoumarol), however this was not statistically significant (adjusted HR 1.6, 95%CI = 0.9-2.8, p=0.11). Having one or two variant alleles of either of the genetic variations in VKORC1, CYP2C9, CYP4F2, CYP3A4, or CYP2C18 did not change the chance of obtaining a stable period, see supplemental material Table S1.

Being hospitalized during VKA treatment reduced the chance of obtaining a stable period (adjusted HR: 0.5 (0.2-0.9), p=0.017, as did an interruption of VKA treatment for 3 or more days (adjusted HR: 0.50 (0.25-0.98), p=0.044). The number of INR measurements was negatively associated with obtaining a stable period (adjusted HR: 0.95 95%CI = 0.92-0.98, p=0.015).

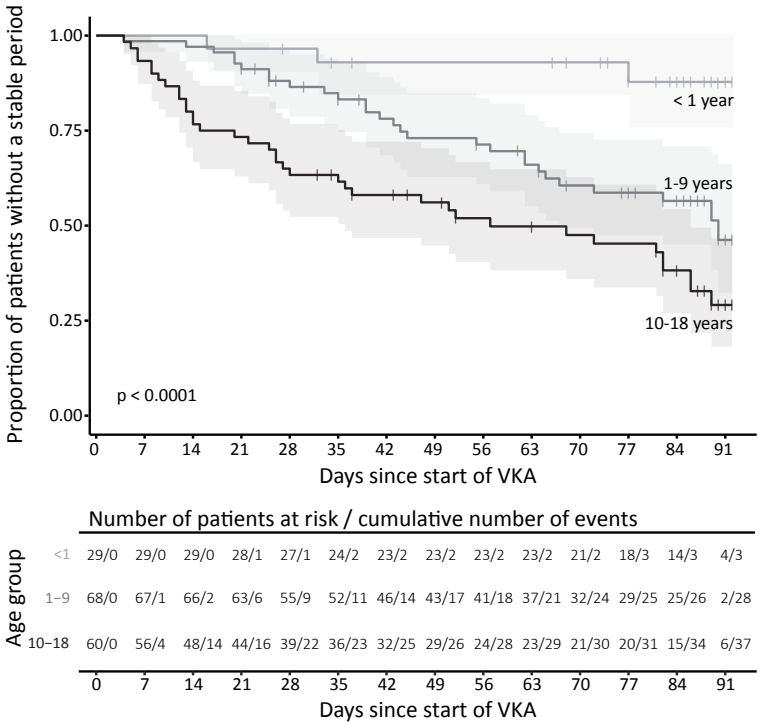


**Figure 2**. Proportion of patients without a stable anticoagulation period over the first 3 months of treatment.

**Effect of switching between VKAs**

As shown in table 1, more patients without a stable period (13.5%) switched to another VKA compared to patients with a stable period (5.9%). All patients without a stable period were switching from acenocoumarol to phenprocoumon. Within the CAPS cohort, a total of 24 patients switched between acenocoumarol and phenprocoumon of whom 12 were eligible for analyzing their treatment in the period before and after the switch. Of these 12 patients, 11 patients switched from acenocoumarol to phenprocoumon and one patient the other way around. It is a diverse group of patients of which the age at switch varies between 0.7 to 18.6 years of age, 4 (33.3%) were female, and the indications for VKA use were mainly of cardiac nature (Fontan circulation [n=3], valve replacement [n=4], and cardiomyopathy [n=4]), as shown in table 3. The most common TRs were 2.5-4.0 (n=6) and 2.0-3.5 (n=4).

All INRs of each of the 12 patient are shown in figure 3. Half of the patients switched in the first year of VKA treatment. In table 4 the quality parameters of the 6 months before and after switch are given per patient. For seven patients who started on acenocoumarol the TTR was less than 70% in the 3 months preceding the switch to phenprocoumon. They all had an increase in TTR in the 3 months after the switch and for 4 patients the increase was even to a TTR of more than 70%. Four patients who started on acenocoumarol had a high TTR of more than 70% in the 3 months before the switch and they all retained a high TTR of more than 70% after switch. The patient switching from phenprocoumon to acenocoumarol had a very high TTR in the 3 months preceding the switch of 92.7% and in the 3 months after switch the TTR dropped to 46.4%. However, in the following 3 months the TTR increased to 76.4%.

The variation in INRs was lower in the 3 months after the switch compared to the 3 months before the switch for 10 patients switching from acenocoumarol to phenprocoumon. The lower variation was still present in the 4-6 months after switching for 6 of the 10 patients. For the patient switching from phenprocoumon to acenocoumarol an opposite effect was seen. The variation in INRs was lower in the months before the switch compared to the 3 months after the switch.

No clear effect was seen in the average time interval between INR measurements, although the total number of INRs was lower in 3 months after the switch compared to the 3 months before the switch in 7 of the 11 patients switching from acenocoumarol to phenprocoumon. The percentage of INRs in TR showed similar changes as the TTR.

**Table 3.** Characteristics of switchers

| Patient ID | Age at switch (years) | Sex | Indication | TR | Duration of follow-up (first to last INR in years) | | Reason for stop |
|---|---|---|---|---|---|---|---|
| | | | | | Acenocoumarol | Phenprocoumon | |
| **Switchers from acenocoumarol to phenprocoumon** | | | | | | | |
| 1 | 0.7 | F | Fontan circulation | 2.0-2.5 | 0.3 | 1.6 | Switch to other anticoagulant/ antiplatelet |
| 2 | 1.2 | M | Valve replacement | 2.5-4.0 | 0.7 | 7.1 | Deceased |
| 3 | 3.1 | M | Fontan circulation | 2.0-3.5 | 0.8 | 8.5 | End of study |
| 4 | 11.2 | M | Cardiomyopathy | 2.0-3.5 | 0.5 | 1.9 | Heart transplantation |
| 5 | 13.5 | F | Valve replacement | 2.5-4.0 | 0.4 | 5.6 | Turning 19 years of age |
| 6 | 10.8 | M | Valve replacement | 2.5-4.0 | 0.5 | 8.3 | Turning 19 years of age |
| 7 | 8.9 | F | Valve replacement | 2.0-3.5 | 5.5 | 6.2 | End of study |
| 8 | 18.4 | M | Cardiomyopathy | 2.5-4.0 | 1.5 | 0.6 | Turning 19 years of age |
| 9 | 16.9 | F | Thromboembolic event | 2.5-4.0 and 2.0-3.5 | 1.2 | 1.1 | Switch to other anticoagulant/ antiplatelet |
| 10 | 18.6 | M | Cardiomyopathy | 2.5-4.0 | 2.2 | 0.4 | Turning 19 years of age |
| 11 | 9.1 | M | Fontan circulation | 2.0-3.0 | 1.2 | 1.4 | End of study |
| **Switcher from phenprocoumon to acenocoumarol** | | | | | | | |
| 12 | 16.5 | M | Cardiomyopathy | 2.0-3.0 | 0.8 | 1.9 | Heart transplantation |

F, female; INR, international normalized ratio range; M, male; TR, therapeutic INR range.

**Table 4.** Quality parameters in the 3 and 3-6 months before and after the switch

| Patient ID | TTR, % | | | | Number of INRs in TR, % (total INRs) | | | | Variability in INRs† | | | | Mean time interval between INRs (days) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | -6 | -3 | +3 | +6 | -6 | -3 | +3 | +6 | -6 | -3 | +3 | +6 | -6 | -3 | +3 | +6 |
| **Switchers from acenocoumarol to phenprocoumon** | | | | | | | | | | | | | | | | |
| 1 | NA | 31.6* | 63.1 | 35.9 | NA (0) | 27.3 (11) | 60.0 (10) | 25.0 (12) | NA | 1.14 | 0.18 | 0.28 | NA | 6.4 | 9.3 | 7.6 |
| 2 | 41.8 | 45.0 | 76.7 | 58.0 | 31.6 (38) | 38.9 (18) | 61.1 (18) | 50.0 (22) | 2.09 | 1.29 | 0.88 | 2.25 | 2.3 | 5.1 | 5.1 | 4.0 |
| 3 | 83.1 | 61.0 | 91.8 | 75.7 | 84.6 (13) | 53.3 (15) | 80.0 (10) | 57.1 (14) | 0.41 | 0.66 | 0.34 | 0.94 | 7.0 | 6.2 | 9.1 | 6.2 |
| 4 | 46.0* | 55.9 | 70.2 | 92.9 | 55.6 (9) | 40.6 (32) | 64.3 (14) | 92.9 (14) | 1.55 | 1.29 | 0.43 | 0.23 | 6.3 | 2.7 | 6.8 | 6.5 |
| 5 | NA | 70.9 | 81.2 | 92.1 | NA (1) | 62.5 (16) | 72.7 (11) | 100 (5) | NA | 1.30 | 0.41 | 0.10 | NA | 5.9 | 8.4 | 17.5 |
| 6 | 68.4* | 84.1 | 75.1 | 87.9 | 71.4 (7) | 66.7 (6) | 66.7 (9) | 91.7 (12) | 0.55 | 0.31 | 0.37 | 0.56 | 9.3 | 15.4 | 11.0 | 7.6 |
| 7 | 86.3 | 80.7 | 75.1 | 85.1 | 91.7 (12) | 63.6 (11) | 66.7 (9) | 87.5 (8) | 0.16 | 0.93 | 0.40 | 0.31 | 8.0 | 7.7 | 8.8 | 11.7 |
| 8 | 63.4 | 68.3 | 69.0 | 45.7 | 46.2 (13) | 55.6 (9) | 88.9 (9) | 40.0 (10) | 0.75 | 0.47 | 0.19 | 0.61 | 7.1 | 9.5 | 8.6 | 9.4 |
| 9 | 89.0 | 64.8 | 100.0 | 85.6 | 85.7 (7) | 66.7 (9) | 100 (7) | 83.3 (6) | 0.12 | 0.44 | 0.09 | 0.15 | 12.8 | 10.4 | 13.8 | 14.6 |
| 10 | 64.1 | 74.5 | 78.3 | NA | 75.0 (4) | 85.7 (7) | 75.0 (4) | NA (0) | 0.13 | 0.32 | 0.23 | NA | 23.3 | 14.0 | 21.0 | NA |
| 11 | 83.5 | 64.3 | 66.0 | 90.5 | 60.0 (15) | 69.2 (13) | 68.4 (19) | 84.6 (13) | 0.52 | 0.33 | 0.29 | 0.15 | 5.7 | 7.0 | 4.8 | 7.0 |
| **Switcher from phenprocoumon to acenocoumarol** | | | | | | | | | | | | | | | | |
| 12 | 86.4 | 92.7 | 46.4 | 76.4 | 87.5 (8) | 83.3 (6) | 44.4 (9) | 85.7 (7) | 0.06 | 0.05 | 0.10 | 0.06 | 11.0 | 17.6 | 9.6 | 12.8 |

INR, international normalized ratio; TR, therapeutic INR range; TTR, percentage time within therapeutic INR range.
* Not the full 3 months of data available. † Calculated using the time-weighted variability in INRs formula of Fihn *et al*.[5]

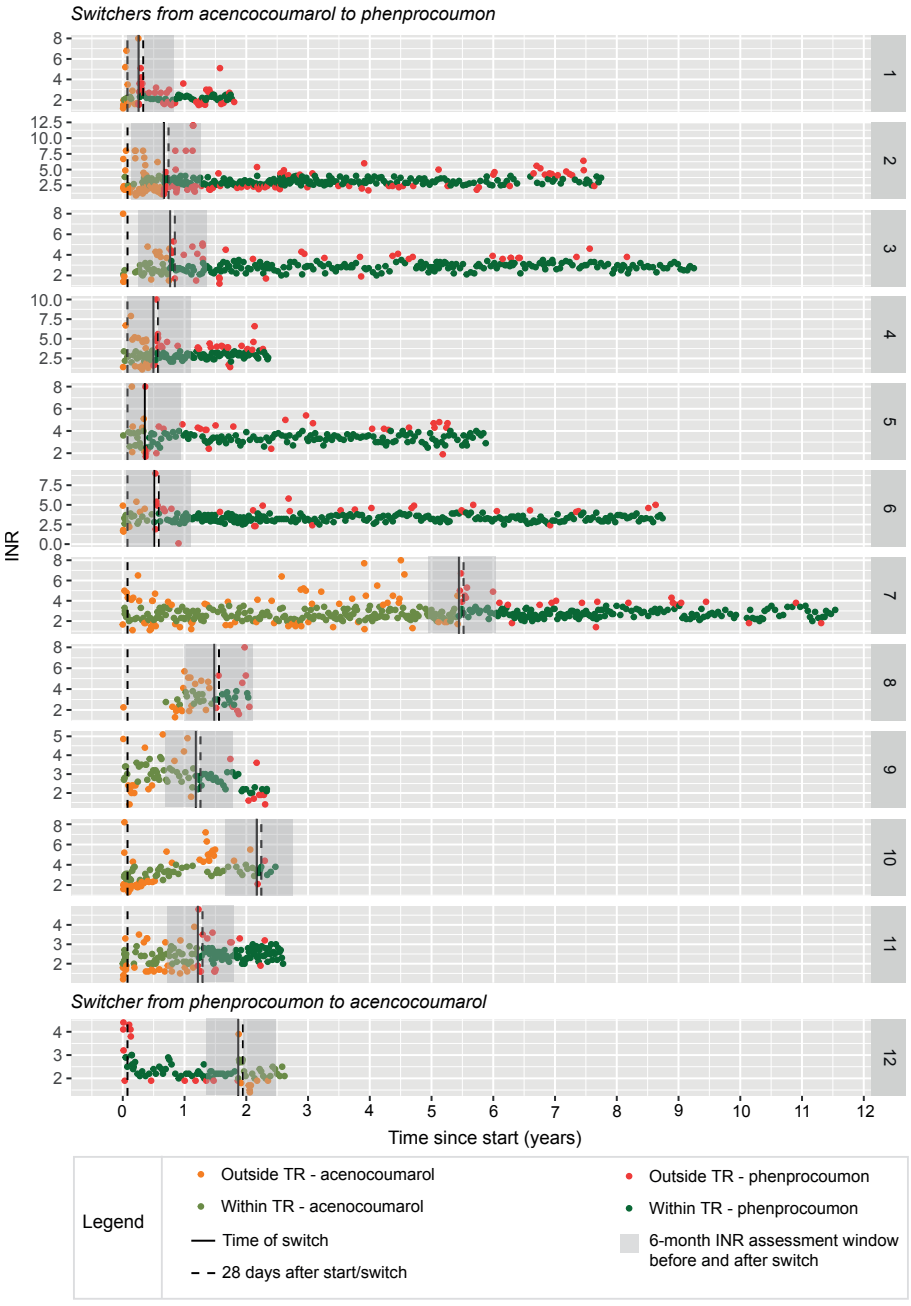


**Figure 3.** International normalized ratio's (INRs) during the whole follow-up of acenocoumarol and phenprocoumon per patient indicating which INRs were within (green) and outside (orange/red) the therapeutic INR window of that patient at the given point in time.

## DISCUSSION

In this study we have shown that pediatric patients under the age of 1 year are less likely to obtain a stable period compared with older patients. Also girls have a lower chance of reaching a stable period within the first 3 months of use. Furthermore, being hospitalized or having an interruption of VKA therapy reduced the chance of having a stable period. 13% of the patients without a stable period switched later in treatment from acenocoumarol to phenprocoumon. Patients who switched between acenocoumarol and phenprocoumon had often a higher TTR and lower variability in INRs on phenprocoumon in the 3 months before compared to the 3 months after the switch.

Our finding that young patients are less likely to have a stable period within the first 3 months of treatment is in line with the positive association of age with the TTR in the first 3 months of treatment as described in a previous paper of CAPS.[2] However, another study by Hawcutt *et al.* in pediatric patients using warfarin did not show an association between age and the TTR in the first 6 months of treatment [6] The median age of the patients in the cohort studied by Hawcutt *et al.* was substantially lower (median of 2.3 years; IQR 4.2) than in our cohort (median of 6.0 years; IQR[2.2-13.4]).[6] This probably explains that no association between age and the TTR was found. There could be multiple explanations for our findings. We have shown that 72.4% of the patients <1 year of age have a poor TTR and 82.8% spend more than 30% of their time below TR. This indicates that they are most likely under dosed. This could be due to inadequate dose increases due to cautiousness to prevent the occurrence of high INRs and also inexperience to dose such young patients could contribute. Furthermore, a slightly higher percentage of patients younger than 1 year of age was hospitalized after their primary hospitalization in which the VKA was started, which also lowers the chance of a stable period. Other factors that were not studied like variations in vitamin K intake, concurrent drug use, and adherence to VKA therapy could also have contributed.

Interruptions of VKA therapy for more than 3 days or a hospitalization led to a decrease of more than 50% of the chance of obtaining a stable period. This could be expected because hospitalization leads to the transfer of the anticoagulation management from anticoagulation clinic to hospital and back which could lead to miscommunication and incorrect VKA dosing. Furthermore, an interruption of VKA therapy could be required and an intercurrent disease (e.g. infection) could also influence stability of the INR. After an interruption of VKA therapy and /or intercurrent disease it will take time to obtain INRs back within TR.

Female patients had a lower chance of obtaining a stable anticoagulation period for which we could not find an explanation in literature. Only one study in pediatric

patients has studied the relationship between gender and TTR and found no association.[6]

The number of INRs was negatively associated with obtaining a stable period within the first 3 months of treatment. This is most likely the result of confounding by indication. Patients which are unstable are more likely to have more frequent measurements.

We did not show an effect of the type of VKA on the chance of obtaining a stable anticoagulation period, which could be due to relative low number of patients using phenprocoumon. The effect, although not statistically significant, was in the direction of a higher chance of a stable period for patients using phenprocoumon. Studies have shown that adult patients using phenprocoumon have a higher percentage of INRs within TR compared to patients using acenocoumarol.[5,7] Patients using phenprocoumon also have a higher TTR.[7] Furthermore, they require fewer INR measurements and have more stable INR values compared to patients using acenocoumarol.[5] The longer half-life of phenprocoumon (144 hours) compared to acenocoumarol (8-10 hours), has been suggested to be the reason for this higher anticoagulation control and stability of INR values.[5]



Our results within the patients switching from acenocoumarol to phenprocoumon show similar changes with regards to the TTR, percentage of INRs within TR and the variability in INRs over time.

Our study has some limitations. Due to the retrospective study design and the information sources used, some variables which could influence INR stability could not be taken into account. No information was available on adherence and the moment of INR measurement relative to the moment of intake of the VKA. Especially for patients using acenocoumarol, adherence and the moment of intake can influence the stability of the INRs. A study by van Geest-Daalderop *et al.* showed that for acenocoumarol the moment of intake relative to the moment of INR measurement has a large impact on the value of the INR.[8] For phenprocoumon this is less crucial because of the longer half-life. Furthermore, no information was available on the amount of vitamin K intake and the data on concurrent drug use and the occurrence of infections was incomplete. All these factors could have influenced the ability of a patient to obtain a stable period.

For the second objective also some other limitations exist. Patients switching from acenocoumarol to phenprocoumon most likely switch because of unstable INR values. Therefore, these patients are likely to benefit most from switching to phenprocoumon. Furthermore, in most patients the TTR improves the longer the VKA is used. Therefore it would be expected that the TTR is already better at the moment that the patients switches to phenprocoumon. However, the switch might reset this improvement. This is supported by the fact that a similar effect was seen

in both patients who switched within the first year of treatment and in patients who switched later on. Furthermore, the one patient who switched from phenprocoumon to acenocoumarol showed the opposite effect. Further research in a larger cohort of pediatric patients is required to determine if the effect on INR stability is clinically relevant.

We did not perform statistical analyses to compare the anticoagulation quality parameters before and after the switch between VKAs. Only a small number of patients was available and the patients were very heterogeneous with respect to indications for anticoagulation and TRs. Therefore, we chose to present the information per patient which gives more insight in what the effect of a switch is than analyzing these patients as a group.

In conclusion, we have shown that young and female pediatric patients have less often a stable anticoagulation period compared to older and male pediatric patients. Furthermore, pediatric patients switching from acenocoumarol to phenprocoumon often showed an improvement in TTR and INR stability.

## REFERENCES


1. Raffini L, Huang YS, Witmer C, Feudtner C. Dramatic increase in venous thromboembolism in children's hospitals in the United States from 2001 to 2007. Pediatrics 2009; 124: 1001–8.
2. Maagdenberg H, Bierings MB, van Ommen CH, van der Meer FJM, Appel IM, Tamminga RYJ, et al. 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.
3. Maagdenberg H, Bierings MB, van Ommen CH, van der Meer FJM, Appel IM, Tamminga RYJ, et al. The pediatric acenocoumarol dosing algorithm: the Children Anticoagulation and Pharmacogenetics Study. J Thromb Haemost 2018; 16: 1732-1742.
4. Maagdenberg H, Bierings MB, van Ommen CH, van der Meer FJM, Appel IM, Tamminga RYJ, et al. Effects of age and genetic variations in VKORC1, CYP2C9, and CYP3A4 on the phenprocoumon dose in pediatric patients. 2018; 19: 1195-1202.
5. Fihn SD, Gadisseur AAP, Pasterkamp E, van der Meer FJM, Breukink-Engbers WGM, Geven-Boere LM, et al. Comparison of control and stability of oral anticoagulant therapy using acenocoumarol versus phenprocoumon. Thromb Haemost 2003; 90: 260–6.
6. Hawcutt DB, Ghani AA, Sutton L, Jorgensen A, Zhang E, Murray M, et al. Pharmacogenetics of warfarin in a paediatric population: time in therapeutic range, initial and stable dosing and adverse effects. Pharmacogenomics J 2014; 14: 542–8.
7. Gadisseur APA, van der Meer FJM, Adriaansen HJ, Fihn SD, Rosendaal FR. Therapeutic quality control of oral anticoagulant therapy comparing the short-acting acenocoumarol and the long-acting phenprocoumon. Br J Haematol 2002; 117: 940–6.
8. van Geest-Daalderop JHH, Hutten BA, Péquériaux NCV, Haas FJLM, Levi M, Sturk A. The influence on INRs and coagulation factors of the time span between blood sample collection and intake of phenprocoumon or acenocoumarol: consequences for the assessment of the dose. Thromb Haemost 2007; 98: 738–46.

## SUPPLEMENTAL MATERIAL

**Table S1.** Results cox regression for each SNP

| | Stable period within 3 months after start | | | | Unadjusted | | Adjusted for age and sex | |
|---|---|---|---|---|---|---|---|---|
| **Stable period** | **No (n=89)** | | **Yes (n=68)** | | **HR (95%CI)** | **p-value** | **HR (95%CI)** | **p-value** |
| **Number of variant alleles,** n(%) | | | | | | | | |
| **VKORC1** | | | | | | | | |
| 0 | 33 | (37.1) | 25 | (36.8) | Ref. | NA | Ref. | NA |
| 1 or 2 | 51 | (57.3) | 38 | (55.9) | 1.07 (0.64-1.77) | 0.802 | 1.10 (0.66-1.83) | 0.715 |
| Unknown | 5 | (5.6) | 5 | (7.4) | - | - | - | - |
| **CYP2C9*2 and *3** | | | | | | | | |
| 0 | 56 | (62.9) | 39 | (57.4) | Ref. | NA | Ref. | NA |
| 1 or 2 | 27 | (30.3) | 25 | (36.8) | 1.12 (0.68-1.86) | 0.652 | 1.07 (0.64-1.77) | 0.801 |
| Unknown | 6 | (6.7) | 4 | (5.9) | - | - | - | - |
| **CYP2C18** | | | | | | | | |
| 0 | 52 | (58.4) | 36 | (52.9) | Ref. | NA | Ref. | NA |
| 1 or 2 | 32 | (36.0) | 28 | (41.2) | 1.08 (0.66-1.76) | 0.773 | 0.94 (0.56-1.55) | 0.795 |
| Unknown | 5 | (5.6) | 4 | (5.9) | - | - | - | - |
| **CYP3A4*1B** | | | | | | | | |
| 0 | 75 | (84.3) | 58 | (85.3) | Ref. | NA | Ref. | NA |
| 1 | 9 | (10.1) | 6 | (8.8) | 0.80 (0.35-1.86) | 0.604 | 0.94 (0.40-2.19) | 0.885 |
| Unknown | 5 | (5.6) | 4 | (5.9) | - | - | - | - |
| **CYP3A4*22** | | | | | | | | |
| 0 | 70 | (78.7) | 58 | (85.3) | Ref. | NA | Ref. | NA |
| 1 | 14 | (15.7) | 6 | (8.8) | 0.55 (0.24-1.27) | 0.160 | 0.56 (0.24-1.30) | 0.174 |
| Unknown | 5 | (5.6) | 4 | (5.9) | - | - | - | - |
| **CYP4F2** | | | | | | | | |
| 0 | 37 | (41.6) | 35 | (51.5) | Ref. | NA | Ref. | NA |
| 1 or 2 | 47 | (52.8) | 29 | (42.6) | 0.75 (0.46-1.23) | 0.258 | 0.78 (0.48-1.28) | 0.322 |
| Unknown | 5 | (5.6) | 4 | (5.9) | - | - | - | - |

HR, hazard ratio; CI, confidence interval; NA, not applicable; -, not analyzed.

# 4 | FACTORS INFLUENCING THE QUALITY AND DOSE OF VITAMIN K ANTAGONIST THERAPY

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

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## ABSTRACT

**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.

## 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.

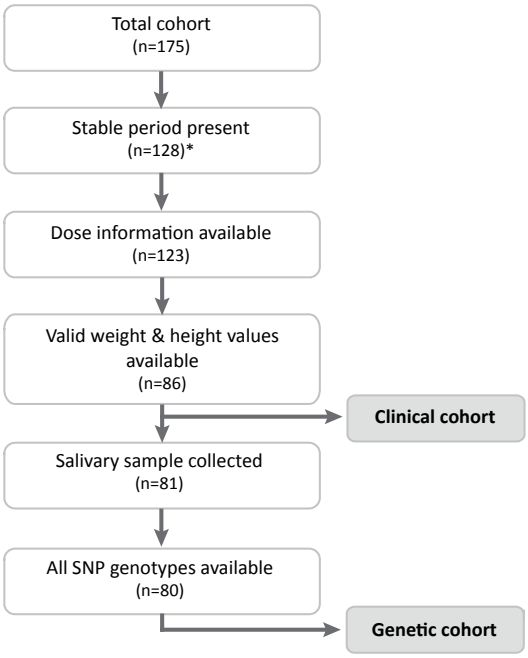


**Figure 1.** Flowchart of patients included in the cohort for the clinical algorithm and genetic algorithm.

* More information on the 47 patients who were excluded based on missing a stable period can be found in the supporting information Table S9.

**Table 1.** Patient characteristics at start of stable period

| | **Clinical cohort*** | | **Genetic cohort*** | |
|---|---|---|---|---|
| | **(n=86)** | | **(n=80)** | |
| **Sex (female),** 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) |

IQR, interquartile range; TR, therapeutic International Normalized Ratio range; BSA, body surface area.

*The genetic cohort is derived from the clinical cohort, excluding 6 patients without genetic information available.

† Consists of supraventricular tachycardia and an unspecified arrhythmia.

‡Calculated using the formula of Haycock.

**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.

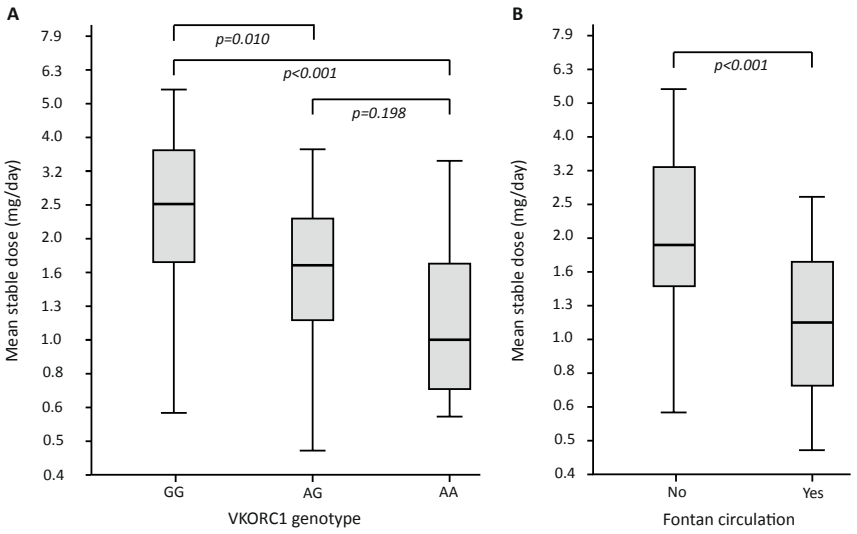


**Figure 2.** Differences in mean stable dose between VKORC1 genotypes and patients with or without a Fontan circulation. A boxplot of the mean stable dose in mg/day per VKORC1 genotype in the genetic cohort (**A**) and a boxplot of patients with and without a Fontan circulation in the clinical cohort(**B**). The p-values shown are calculated using the log mean stable dose.

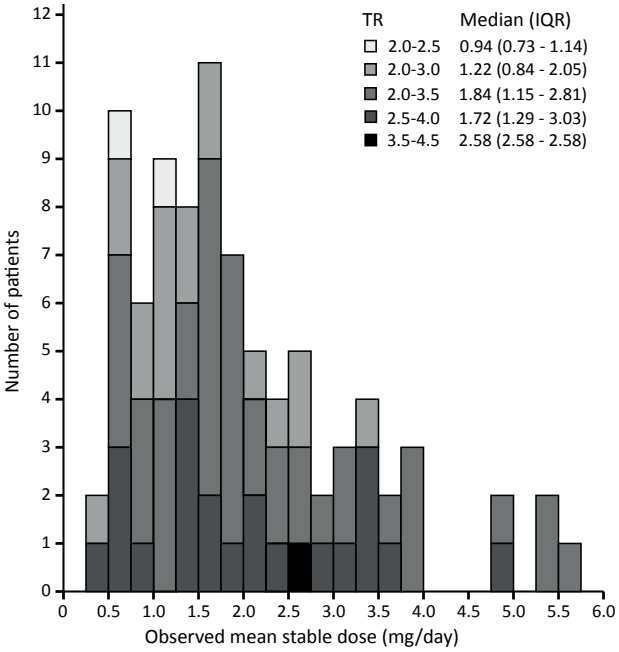


**Figure 3.** The observed mean stable dose per TR in the clinical cohort.

**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/*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) | - | - |

BSA, body surface area; CI, confidence interval; -, not applicable.
*Doses are shown in mg/day.
‡ Difference between observed and guideline based dose, $p<0.05$.
‡The dose is calculated using the dosing information of the guideline of the Dutch Federation of anticoagulation clinics[6].



### 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 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 R2 of these four variables, and incorporates all variables. Therefore, only BSA was used as a candidate variable in the multivariate linear model.

**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, 0.404) | 38.3 | 0.319 ( 0.215, 0.424) | 40.4 |
| Fontan circulation | -0.102 (-0.187, -0.017) | 17.3 | -0.149 (-0.247, -0.051) | 22.3 |
| VKORC1 | -0.120 (-0.171, -0.069) | 19.2 | - | - |
| CYP2C18 | -0.084 (-0.146, -0.022) | 4.4 | - | - |
| CYP2C9*2 and *3 | -0.090 (-0.155, -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; - not applicable.

* Regression equation: log daily dose (mg) = 0.105 + 0.316 (BSA, $m^2$) – 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 *3 variant alleles).

† Regression equation: log daily dose (mg) = -0.061 + 0.319 (BSA, $m^2$) – 0.149 (Fontan circulation, yes=1; no=0).

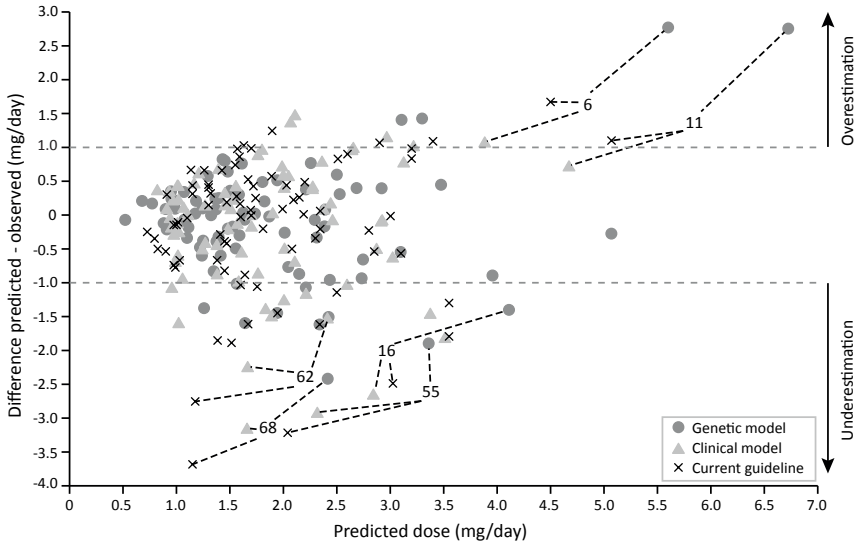


**Figure 4.** The differences between predicted and observed doses within the genetic cohort.
The differences of the predicted doses with the genetic model (dots), clinical model (triangles) and current guideline (crosses) with the observed mean stable doses are plotted against the predicted dose. Large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

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 R2 = 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 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. 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.

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.

## 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, et al. 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, et al. 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, et al. 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, et al. 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, et al. 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, et al. 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, et al. 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, et al. 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, et al. 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, et al. 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, et al. 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, et al. 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, et al. 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. http://www.who.int/childgrowth/standards/weight_for_age/en/. Accessed 8 Aug 2016.
25. World Health Organization. Length/height-for-age. http://www.who.int/childgrowth/standards/height_for_age/en/. Accessed 8 Aug 2016.
26. World Health Organization. Weight-for-age (5–10 years). 2007. http://www.who.int/growthref/who2007_weight_for_age/en/. Accessed 8 Aug 2016.
27. World Health Organization. Height-for-age (5–19 years). 2007. http://www.who.int/growthref/who2007_height_for_age/en/. Accessed 8 Aug 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, et al. 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, et al. Loading and maintenance dose algorithms for phenprocoumon and acenocoumarol using patient characteristics and pharmacogenetic data. *Eur Heart J* 2011; 32: 1909–17.

# SUPPORTING INFORMATION

## Height and weight valid time windows

**Table S1.** Applied time windows for height and weight per age at start of stable period.

| Age | Valid time window (months) | |
|---|---|---|
| | Height & weight | |
| <0.5 | 1 | |
| 0.5 | 2 | |
| 1 | 3 | |
| 1.5 | 4 | |
| 2 | 5 | |
| 2.5 | 6 | |
| 3 | 6 | |
| 3.5 | 7 | |
| 4 | 8 | |
| 4.5 | 8 | |
| 5 | 9 | |
| 6 | 9 | |
| 7 | 10 | |
| | Height | Weight |
| 8 | 10 | 9 |
| 9 | 10 | 7 |
| 10 | 10 | 6 |
| 11 | 9 | 6 * |
| 12 | 10 | 6 * |
| 13 | 8 | 6 * |
| 14 | 8 | 6 * |
| 15 | 10 | 6 * |
| 16 | 12 | 6 * |
| 17 | 18 | 6 * |
| 18 | 24 | 6 * |

*Kept on 6 months.

## Patients without a stable period

**Table S2.** Information on the patients without a stable period

| Possible reasons for no stable period | n=47 | Reasons for end of follow-up |
|---|---|---|
| Impossible to have a SP (<21 days) | 12 | Lost to follow up (n=9) or switch to phenprocoumon (n=3) |
| Short periods of data < 2 months | 12 | Lost to follow up (n=2), stopped (n=4), switch to aspirin (n=3) or phenprocoumon (n=3) |
| Medium periods of data 2-3 months | 13 | Stopped (n=12) or switch to phenprocoumon (n=1) |
| No obvious reason | 10 | Stopped (n=7), switch to aspirin (n=1) or switch to phenprocoumon (n=2)* |

* Medium (min, max) duration of follow-up time 6.3 (3.1,14.8) months

## Fontan and TR

**Table S3.** Mean stable dose for patients with and without a Fontan circulation stratified per TR within the genetic cohort

| TR | Fontan | n | Mean stable dose (mg/day) |
|---|---|---|---|
| 2.0-2.5 | No | 1 | 1.14 |
| | Yes | 1 | 0.73 |
| 2.0-3.0 | No | 6 | 2.08 |
| | Yes | 9 | 1.34 |
| 2.0-3.5 | No | 30 | 2.55 |
| | Yes | 12 | 1.40 |
| 2.5-4.0 | No | 18 | 2.20 |
| | Yes | 2 | 1.24 |
| 3.5-4.5 | No | 1 | 2.58 |
| | Yes | 0 | NA |

TR, Therapeutic International Normalized Ratio range.

## Algorithms using a stricter definition of a stable period

**Table S4.** Patient characteristics of patients included using a stricter definition of a stable period

| | **Clinical & genetic cohort** | |
|---|---|---|
| | **n=47** | |
| **Sex (female),** n (%) | 28 | (59.6) |
| **Age at start of the stable period in years,** median (IQR) | 9.5 | (4.7, 14.2) |
| <1, n (%) | 1 | (2.2) |
| 1-3, n (%) | 6 | (13.0) |
| 4-6, n (%) | 13 | (28.3) |
| 7-9, n (%) | 5 | (10.9) |
| 10-12, n (%) | 4 | (8.7) |
| 13-15, n (%) | 9 | (19.6) |
| 16-18, n (%) | 8 | (17.4) |
| **Ethnicity,** n (%) | | |
| European | 38 | (80.9) |
| Asian | 2 | (4.3) |
| African | 2 | (4.3) |
| Others | 3 | (6.4) |
| Unknown | 2 | (4.3) |
| **Indication for anticoagulation,** n (%) | | |
| Fontan procedure | 11 | (23.4) |
| Prosthetic heart valve | 13 | (27.7) |
| Dilated cardiomyopathy | 5 | (10.6) |
| Deep vein thrombosis/ pulmonary embolism | 12 | (25.5) |
| Aneurysm | 1 | (2.1) |
| Pulmonary hypertension | 2 | (4.3) |
| Other cardiac indication* | 2 | (4.3) |
| Stroke | 1 | (2.1) |
| **TR,** n(%) | | |
| Extra low (2-2.5) | 2 | (4.3) |
| Low (2-3) | 7 | (14.9) |
| Standard (2.5-3.5) | 25 | (53.2) |
| High (3.0-4.0) | 12 | (25.5) |
| Extra high (3.5-4.5) | 1 | (2.1) |
| **Maintenance dose in mg/kg bodyweight,** median (IQR) | 0.07 | (0.04, 0.10) |
| **BSA†,** median (IQR) | 0.96 | (0.69, 1.40) |

IQR, interquartile range; BSA, body surface area; TR, therapeutic International Normalized Ratio range.

* consists of supraventricular tachycardia (n=1) and an unspecified arrhythmia (n=1).

† calculated using the formula of Haycock.

**Table S5.** Distribution of genotypes within patients included using a stricter definition of a stable period

| | n (%) |
|---|---|
| **VKORC1** | |
| *GG* | 21 (44.7) |
| AG | 19 (40.4) |
| AA | 7 (14.9) |
| **CYP2C9 *2/*3** | |
| *CC/AA* | 29 (61.7) |
| CC/CA | 6 (12.8) |
| CT/AA | 9 (19.1) |
| CT/CA | 2 (4.3) |
| TT/AA | 1 (2.1) |
| **CYP2C18** | |
| *GG* | 27 (57.4) |
| AG | 17 (36.2) |
| AA | 3 (6.4) |
| **CYP4F2** | |
| *CC* | 21 (44.7) |
| CT | 24 (51.1) |
| TT | 2 (4.3) |
| **CYP3A4*22** | |
| *GG* | 39 (83.0) |
| GA | 8 (17.0) |
| **CYP3A4*1B** | |
| *TT* | 45 (95.7) |
| TC | 2 (4.3) |

*Italic* is wild type genotype.

**Table S6.** Genotype-guided algorithm and clinical algorithm using a stricter definition of a stable period

| | **Genotype-guided algorithm[*] (n=47)** | | **Clinical algorithm[†] (n=47)** | |
|---|---|---|---|---|
| | **Coefficients (95%CI)** | **Univariate unadjusted $R^2$, %** | **Coefficients (95%CI)** | **Univariate unadjusted $R^2$, %** |
| **Intercept** | 0.069 | | -0.105 | |
| BSA[c] | 0.312 ( 0.208, 0.415) | 43.9 | 0.350 (0.231, 0.469) | 43.9 |
| VKORC1 | -0.118 (-0.186, -0.051) | 22.5 | NA | NA |
| CYP2C9*2 and *3 | -0.113 (-0.189, -0.037) | 6.1 | NA | NA |
| **Unadjusted $R^2$ of the algorithm,** % | 62.2 | | 43.9 | |
| **Adjusted $R^2$ of the algorithm,** % | 59.5 | | 42.6 | |

BSA, body surface area; SE, standard error; CI, confidence interval.

[*] Regression equation: log daily dose (mg) = 0.069 + 0.312 (BSA, $m^2$) – 0.118 (number of VKORC1 variant alleles) – 0.113 (number of CYP2C9 *2 and *3 variant alleles).

[†] Regression equation: log daily dose (mg) = -0.105 + 0.350 (BSA, $m^2$)

## Algorithms using age instead of BSA

**Table S7.** Patient characteristics of patients included using age instead of BSA

| | **Genetic cohort** | | **Clinical cohort** | |
|---|---|---|---|---|
| | **n=116** | | **n=123** | |
| **Sex (female),** n (%) | 58 | (50.0) | 61 | (49.6) |
| **Age at start of the stable period in years,** median (IQR) | 8.0 | (3.8, 13.6) | 7.9 | (3.9, 13.3) |
| <1, n (%) | 3 | (2.6) | 3 | (2.5) |
| 1-3, n (%) | 26 | (22.6) | 27 | (22.1) |
| 4-6, n (%) | 24 | (20.9) | 27 | (22.1) |
| 7-9, n (%) | 11 | (9.6) | 12 | (9.8) |
| 10-12, n (%) | 17 | (14.8) | 17 | (13.9) |
| 13-15, n (%) | 14 | (12.2) | 15 | (12.3) |
| 16-18, n (%) | 20 | (17.4) | 21 | (17.2) |
| **Ethnicity,** n (%) | | | | |
| European | 98 | (84.5) | 103 | (83.7) |
| Asian | 4 | (3.4) | 4 | (3.3) |
| African | 2 | (1.7) | 3 | (2.4) |
| Others | 7 | (6.0) | 7 | (5.7) |
| Unknown | 5 | (4.3) | 6 | (4.9) |
| **Indication for anticoagulation,** n (%) | | | | |
| Fontan procedure | 32 | (27.6) | 37 | (30.1) |
| Prosthetic heart valve | 30 | (25.9) | 31 | (25.2) |
| Dilated cardiomyopathy | 14 | (12.1) | 14 | (11.4) |
| Deep vein thrombosis/ pulmonary embolism | 22 | (19.0) | 23 | (18.7) |
| Aneurysm | 3 | (2.6) | 3 | (2.4) |
| Pulmonary hypertension | 6 | (5.2) | 6 | (4.9) |
| Other cardiac indication* | 6 | (5.2) | 6 | (4.9) |
| Cerebral | 3 | (2.6) | 3 | (2.4) |
| **TR,** n(%) | | | | |
| Extra low (2-2.5) | 4 | (3.4) | 4 | (3.3) |
| Low (2-3) | 23 | (19.8) | 26 | (21.1) |
| Standard (2.5-3.5) | 59 | (50.9) | 61 | (49.6) |
| High (3.0-4.0) | 27 | (23.3) | 29 | (23.6) |
| Extra high (3.5-4.5) | 3 | (2.6) | 3 | (2.4) |
| **Maintenance dose in mg/kg bodyweight,** median (IQR) | 0.07 | (0.04, 0.09) | 0.06 | (0.04, 0.09) |
| **BSA‡,** median (IQR) | 1.00 | (0.68, 1.38) | 0.98 | (0.67, 1.38) |

IQR, interquartile range; BSA, body surface area; TR, therapeutic International Normalized Ratio range.

* consists of BT shunt (n=1), impaired left ventricular function (n=1), supraventricular tachycardia (n=3) and an unspecified arrhythmia (n=1).

‡ calculated only for n= 86 patients (clinical cohort) and n= 80 patients (genetic cohort) using the formula of Haycock.

**Table S8.** Distribution of genotypes within patients included using age instead of BSA

| | n (%) |
|---|---|
| **VKORC1** | |
| *GG* | 42 (36.2) |
| AG | 53 (45.7) |
| AA | 21 (18.1) |
| **CYP2C9 *2/*3** | |
| *CC/AA* | 71 (61.2) |
| CC/CA | 14 (12.1) |
| CT/AA | 27 (23.3) |
| CT/CA | 2 (1.7) |
| TT/AA | 2 (1.7) |
| **CYP2C18** | |
| *GG* | 64 (55.2) |
| AG | 43 (37.1) |
| AA | 9 (7.8) |
| **CYP4F2** | |
| *CC* | 58 (50.0) |
| CT | 53 (45.7) |
| TT | 5 (4.3) |
| **CYP3A4*22** | |
| *GG* | 100 (86.2) |
| GA | 16 (13.8) |
| **CYP3A4*1B** | |
| *TT* | 109 (94.0) |
| TC | 7 (6.0) |

*Italic* is wild type genotype.

**Table S9.** Genotype-guided algorithm and clinical algorithm using age instead of BSA

| | Genotype-guided algorithm* (n=116) | | Clinical algorithm† (n=123) | |
|---|---|---|---|---|
| | **Coefficients (95% CI)** | **Univariate unadjusted $R^2$, %** | **Coefficients (95% CI)** | **Univariate unadjusted $R^2$, %** |
| **Intercept** | 0.254 | | 0.074 | |
| Age | 0.021 (0.014, 0.028) | 29.5 | 0.020 (0.013, 0.028) | 30.5 |
| Fontan circulation | -0.164 (-0.244, -0.084) | 22.2 | -0.186 (-0.277,-0.096) | 25.2 |
| VKORC1 | -0.133 (-0.180, -0.086) | 16.4 | NA | NA |
| CYP2C18 | -0.079 (-0.133, -0.025) | 1.8 | NA | NA |
| CYP2C9*2 and *3 | -0.092 (-0.152, -0.032) | 1.6 | NA | NA |
| **Unadjusted $R^2$ of the algorithm,** % | 56.4 | | 38.9 | |
| **Adjusted $R^2$ of the algorithm,** % | 54.4 | | 37.9 | |

SE, standard error; CI, confidence interval.

* Regression equation: log daily dose (mg) = 0.254 + 0.021(Age, year) – 0.164 (Fontan circulation, yes=1; no=0) – 0.133 (number of VKORC1 variant alleles) – 0.079 (number of CYP2C18 variant alleles) – 0.092 (number of CYP2C9 *2 and *3 variant alleles).

† Regression equation: log daily dose (mg) = 0.074 + 0.020 (Age, year) – 0.186 (Fontan circulation, yes=1; no=0).

**Patients with large over or underestimation of the dose**

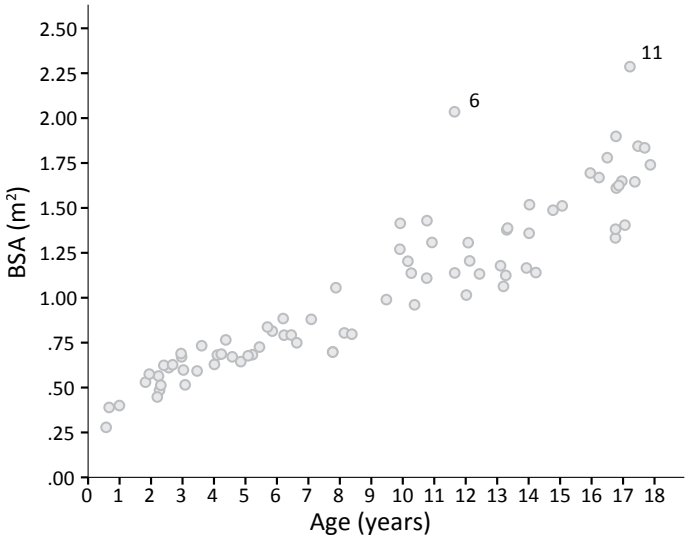


**Figure S1.** Body surface area (BSA) per age. The two patients (number 6 and 11) who had a large overestimation of the dose (>2 mg/day) predicted with the genetic algorithm were both obese. This is also translated in the distinctive higher BSAs compared to the other patient of the same age.

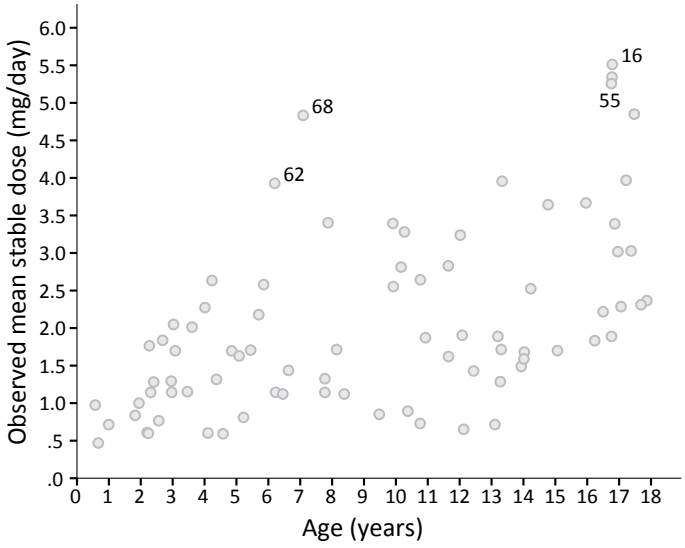


**Figure S2.** Observed mean stable dose per age. The four patients (16, 55, 62, 68) with large underestimations of the dose (>2 mg/day) predicted with the clinical model and the guideline (68 also with the genetic model) all had higher observed doses compared to the other patients within the cohort.

# 4.2 Effect of age and genetic variations in VKORC1, CYP2C9, and CYP3A4 on the phenprocoumon dose in pediatric patients

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## ABSTRACT

**Aims**

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.

## INTRODUCTION

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 five 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% to 26.6% (*CYP2C9*) and 2.8% to 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 and population

The Children Anticoagulation and Pharmacogenetics Study (CAPS) 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 one 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 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 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 single nucleotide polymorphism (SNP) genotyping assay (ThermoFisher). 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 and 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 analysis 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.

## 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. Forty-two percent of the patients were 3 years or younger, while 44% were older than 13 years of age. Eighty-six percent 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.



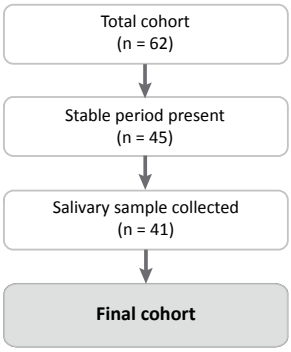


**Figure 1.** Flowchart of patients included in the analysis. The characteristics of the excluded patients are presented Table S1 of the supplemental material.

**Table 1.** Patient characteristics

| | 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**[b]**,** 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[a] | 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 | |

BSA, body surface area; TR, therapeutic International Normalized Ratio range. [a] Consists of supraventricular tachycardia (n=1), prophylactic/postoperative (n=3), and cerebral event (n=2). [b] Calculated using the formula of Haycock and only known for n=18.

### Associations of clinical and 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.

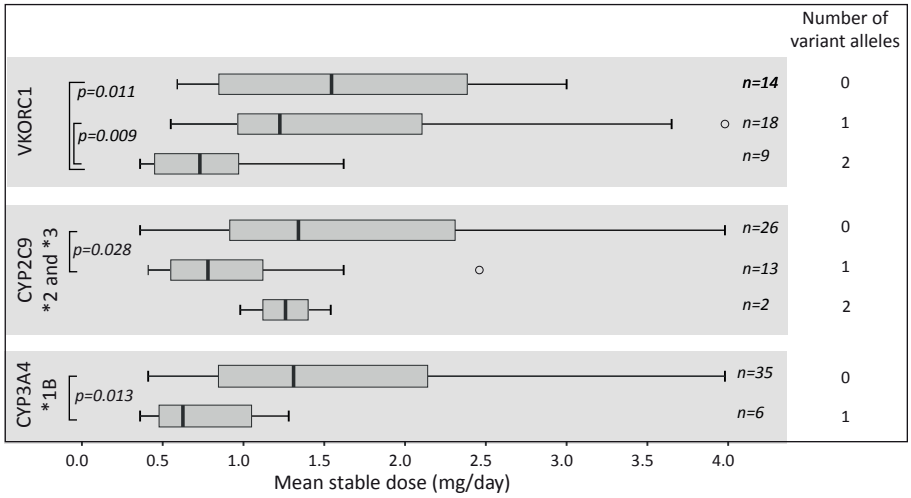


**Figure 2.** Mean stable dose per number of variant alleles for *VKORC1, CYP2C9*2/*3* and *CYP3A4*1B*. The p-values indicated are calculated using the log mean stable dose.

4.2

**Table 2.** Clinical and genetic dosing algorithms

| | **Genetic algorithm[a]** | | **Clinical algorithm[b]** |
|---|---|---|---|
| | **Coefficients (95%CI)** | **Univariate unadjusted $R^2$, %** | **Coefficients (95%CI)** |
| **Intercept** | -0.024 | - | -0.214 |
| Age | 0.028 ( 0.023, 0.034) | 56.2 | 0.030 (0.21, 0.38) |
| VKORC1 | -0.128 (-0.179, -0.076) | 16.9 | - |
| CYP2C9*2 and *3 | -0.113 (-0.178, -0.049) | 9.4 | - |
| CYP3A4*1B | -0.125 (-0.235, -0.015) | 14.9 | - |
| **Unadjusted $R^2$ of the algorithm,** % | 82.4 | | 56.2 |
| **Adjusted $R^2$ of the algorithm,** % | 80.4 | | 55.0 |

CI, confidence interval; -, not applicable.

[a] Regression equation: log daily dose (mg) = -0.024 + 0.028 (age, y) – 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).

[b] Regression equation: log daily dose (mg)= -0.214 + 0.030 (age, y)

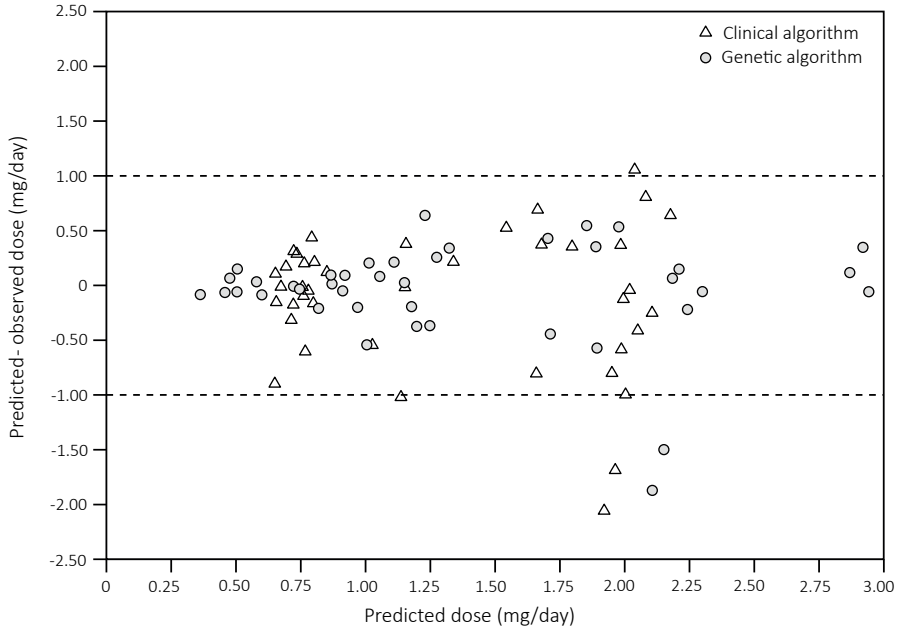


**Figure 3**. The differences between predicted and observed doses per patient. The differences of the predicted doses with the genetic algorithm (age, *VKORC1*, *CYP2C9*2/*3*, and *CYP3A4*1B*; circles) and the clinical algorithm (age; triangles) with the observed mean stable doses are plotted against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

### 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 S2 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 to other patients of the same age – data not shown.

## 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 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 three years, i.e. 37.7% and 22.9%, compared to 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 to 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.

In the acenocoumarol cohort of CAPS we saw that body surface area (BSA) 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 BSA 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 seven 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 hours using a point-of-care test in a non-laboratory environment. [19] Furthermore, an increasing number of academic hospitals are offering genetic testing in their laboratories. Genotyping should not therefore 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.

## REFERENCES


1. Weitz JI. Blood Coagulation and Anticoagulant, Fibrinolytic, and Antiplatelet Drugs. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman & Gilman's: The Pharmacological Basis of Therapeutics. 12th ed. New York, NY: McGraw-Hill Education; 2015.
2. Federatie van Nederlandse Trombosediensten. De kunst van het doseren - Richtlijn, leidraad en informatie voor het doseren van vitamine K-antagonisten. 8th ed. Leiden: Federatie van Nederlandse Trombosediensten; 2017.
3. 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.
4. 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.
5. Shaw K, Amstutz U, Hildebrand C, Rassekh SR, Hosking M, Neville K, et al. VKORC1 and CYP2C9 genotypes are predictors of warfarin-related outcomes in children. Pediatr Blood Cancer 2014; 61: 1055–62.
6. Nowak-Göttl U, Dietrich K, Schaffranek D, Eldin NS, Yasui Y, Geisen C, et al. In pediatric patients, age has more impact on dosing of vitamin K antagonists than VKORC1 or CYP2C9 genotypes. Blood 2010; 116: 6101–5.
7. Biss TT, Avery PJ, Brandão LR, Chalmers EA, Williams MD, Grainger JD, et al. 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.
8. Moreau C, Bajolle F, Siguret V, Lasne D, Golmard J-L, Elie C, et al. 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.
9. 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.
10. van Schie RMF, Wessels JAM, le Cessie S, de Boer A, Schalekamp T, van der Meer FJM, et al. Loading and maintenance dose algorithms for phenprocoumon and acenocoumarol using patient characteristics and pharmacogenetic data. Eur Heart J 2011; 32: 1909–17.
11. Maagdenberg H, Bierings MB, van Ommen CH, van der Meer FJM, Appel IM, Tamminga RYJ, et al. 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.
12. Maagdenberg H, Bierings MB, van Ommen CH, van der Meer FJM, Appel IM, Tamminga RYJ, et al. The pediatric acenocoumarol dosing algorithm: the Children Anticoagulation and Pharmacogenetics Study. J Thromb Haemost 2018; 16: 1732-1742.
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 JTH 2013; 11: 1200–3.
14. Teichert M, Eijgelsheim M, Uitterlinden AG, Buhre PN, Hofman A, De Smet PAGM, et al. Dependency of phenprocoumon dosage on polymorphisms in the VKORC1, CYP2C9, and CYP4F2 genes. Pharmacogenet Genomics 2011; 21: 26–34.
15. Botton MR, Viola PP, Bandinelli E, Leiria TLL, Rohde LEP, Hutz MH. A new algorithm for weekly phenprocoumon dose variation in a southern Brazilian population: role for CYP2C9, CYP3A4/5 and VKORC1 genes polymorphisms. Basic Clin Pharmacol Toxicol 2014; 114: 323–9.
16. Ufer M, Svensson JO, Krausz KW, Gelboin HV, Rane A, Tybring G. Identification of cytochromes P450 2C9 and 3A4 as the major catalysts of phenprocoumon hydroxylation in vitro. Eur J Clin Pharmacol 2004; 60: 173–82.

17. Amirimani B, Ning B, Deitz AC, Weber BL, Kadlubar FF, Rebbeck TR. Increased transcriptional activity of the CYP3A4*1B promoter variant. Environ Mol Mutagen 2003; 42: 299–305.
18. Lee S-J, Goldstein JA. Functionally defective or altered CYP3A4 and CYP3A5 single nucleotide polymorphisms and their detection with genotyping tests. Pharmacogenomics 2005; 6: 357–71.
19. van Schie RMF, Wadelius MIA, Kamali F, Daly AK, Manolopoulos VG, de Boer A, et al. Genotype-guided dosing of coumarin derivatives: the European pharmacogenetics of anticoagulant therapy (EU-PACT) trial design. Pharmacogenomics 2009; 10: 1687–95.

## SUPPLEMENTAL MATERIAL

**Table S1.** Patient characteristics of the excluded patients

| | **Excluded patients (n=21)** | |
|---|---|---|
| **Sex (female),** n (%) | 12 | (57.1) |
| **Age at start of SP in years,** median (IQR) | 8.6 | (0.3, 15.8) |
| <1, n (%) | 8 | (38.1) |
| 1-3, n (%) | 2 | (9.5) |
| 4-6, n (%) | 0 | (0.0) |
| 7-9, n (%) | 1 | (4.8) |
| 10-12, n (%) | 1 | (4.8) |
| 13-15, n (%) | 4 | (19.0) |
| 16-18, n (%) | 5 | (23.8) |
| **European ethnicity,** n (%) | 18 | (85.7) |
| **Indication for anticoagulation,** n (%) | | |
| Fontan procedure | 2 | (9.5) |
| Prosthetic heart valve | 2 | (9.5) |
| Dilated cardiomyopathy | 5 | (23.8) |
| Deep vein thrombosis/ pulmonary embolism | 7 | (33.3) |
| Other | 5 | (23.8) |
| **TR,** n (%) | | |
| Extra low (2.0-2.5) | 2 | (9.5) |
| Low (2.0-3.0) | 0 | (0.0) |
| Standard (2.0-3.5) | 15 | (71.4) |
| High (2.5-4.0) | 4 | (19.0) |

SP, stable period; TR, therapeutic INR range.

**Table S2.** Genetic algorithm excluding *CYP3A4*1B*

| | **Genetic algorithm excluding *CYP3A4*1B*[a]** |
|---|---|
| | Coefficients (95%CI) |
| **Intercept** | -0.046 |
| Age | 0.030 ( 0.024, 0.036) |
| VKORC1 | -0.137 (-0.191, -0.083) |
| CYP2C9*2 and *3 | -0.116 (-0.185, -0.048) |
| **Unadjusted $R^2$ of the algorithm,** % | 79.8 |
| **Adjusted $R^2$ of the algorithm,** % | 78.1 |

[a] Regression equation: log daily dose (mg) = -0.024 + 0.030 (Age, y) – 0.137 (number of *VKORC1* variant alleles) – 0.116 (number of *CYP2C9 *2 and *3* variant alleles).

# 5 | GENERAL DISCUSSION

Pediatric patients are a vulnerable group of patients. Often evidence on efficacy and safety of medication within this group of patients is limited which makes treating them a challenge. Clinical trials with pediatric patients often suffer from low inclusion rates. This results in drugs, which are prescribed off-label and dosages are extrapolated based on results in adults. For vitamin K antagonists (VKAs) this is not different. To date, acenocoumarol and phenprocoumon are still used off-label in children. Information on quality of use and safety of acenocoumarol and phenprocoumon is minimal and for phenprocoumon evidence based information on the required starting dose for pediatric patients of different age groups is lacking.

In adults, a large amount of work has been done to gain insight in the role of variation in genes on dose requirement of VKAs.[1] Several studies with warfarin in pediatric patients were performed during recent years.[2] However, because of small numbers of patients and differences in patient populations it is difficult to find similar results with regards to which extent non-genetic and genetic variables influence VKA dose requirements. Studies on pharmacogenetics of acenocoumarol and phenprocoumon in pediatric patients are lacking.

In this thesis we described the quality of use, effectiveness and safety of the current oral anticoagulation care in children and provide insight in the possibilities for improvement. Furthermore, we developed dosing algorithms for both acenocoumarol and phenprocoumon for pediatric patients incorporating both genetic and non-genetic factors.

## THE CHILDREN ANTICOAGULATION AND PHARMACOGENETICS STUDY (CAPS)



In 2013 we started with CAPS. In this study we selected patients who were using acenocoumarol or phenprocoumon when 18 years of age or younger between 1995 and 2014. We selected them in four university children's hospitals (Amsterdam, Utrecht, Rotterdam, Groningen) and one anticoagulation clinic (Leiden). From all patients we collected anticoagulation clinic and hospital data related to their anticoagulation treatment. All the included 213 patients were asked to collect a saliva sample at home by themselves or with help from their parents. These samples were genotyped in the laboratory of the Leiden University Medical Center. We considered 7 genetic variations in 5 candidate genes (VKORC1, CYP2C9, CYP4F2, CYP2C18 and CYP3A4) relevant. Furthermore, the patients were asked to fill in a short questionnaire to collect information that was not available in the patient files (e.g. if they received breastfeeding during VKA treatment).

## MAIN FINDINGS

**Personalized medicine in pediatric patients**

In the last decade there has been a rising interest in personalized medicine using pharmacogenetics in pediatric patients. In **chapter 2.1** the challenges and potential of pharmacogenomics in children are described. The three examples discussed are: cisplatin induced ototoxicity, optimizing VKA dosing and effective treatment of asthma. They all show that replication is challenging due to heterogeneity in study populations and also small sample sizes are a challenge in childhood cancer and thrombosis. However, all have the potential to improve childhood care. In cisplatin induced ototoxicity, the need of identifying risk factors is clear. Ototoxicity is a very impairing toxicity, especially in children who are developing their speech skills and is unacceptable in children who are already visually impaired. A mutation in ACYP2 has been shown to be an important predictor of ototoxicity, but explains only a small part (12.4%) of the ototoxicity.[3]

In asthma, the ADRB2 Arg16 mutation with regards to long-acting β2 agonist response is closest to clinical implementation. Currently, the PUFFIN trial is carried out in the Netherlands to assess the value of ADRB2 genotyping in children with asthma who are not well controlled on low dose inhaled steroids.[4] It is expected that genotyping will lead to faster improvement of asthma control compared with usual care. For VKAs the evidence to guide warfarin dosing using VKORC1 and CYP2C9*2 and*3 genotype is strong, but has not (yet) been implemented.[5]

Quite a number of small pharmacogenetic studies in children using warfarin have been conducted and have provided dosing models as described in **chapter 2.2**. However, the predictive performances of the developed models was studied in an independent pediatric cohort and showed that the best model among the developed dosing models predicted only for 45% of patients their dose within ± 20% of the observed stable maintenance dose.[2] Both the small sample sizes and heterogeneity of the studied children reduce external validity and make replication and prospective validation highly challenging and urge for international collaborations. Furthermore, consensus needs to be found on how much evidence is required for clinical implementation.

**Quality and safety of VKA treatment**

VKA under and over dosing are associated with the risk of thromboembolic and bleeding events, respectively in adults.[6] The reported incidences of thromboembolic and bleeding events in pediatric patients varies considerably between studies and the latest relatively large study with more than 100 patients dated from 1999.[7] In **chapter 3.1** we have used the UK Clinical Practice Research Datalink

(CPRD) database to assess these incidences and found that bleeding events in pediatric patients (≤ 18 years of age) occurred in 3.33 patients/100 patient years and that epistaxis and GI bleedings were the most common bleeding events. Furthermore, we have shown that bleeding events occurred often during the first weeks of treatment. 24% of the patients with a bleeding event had their first bleeding within 15 days after start. In children using VKAs for primary prophylaxis, thromboembolic events occurred less often: in 1.50 patients/100 patient years. In **chapter 3.2** we have also shown in the CAPS cohort that bleeding events were more common than thromboembolic events. Furthermore, a similar proportion of bleeding events occurred within the first weeks of treatment as was shown in the CPRD study. Of the patients with a bleeding event who were using acenocoumarol 22% had their first bleeding within 15 days after start. For phenprocoumon this was 20% (data was not shown in **chapter 3.2**).

The quality of VKA treatment in children has been reported in multiple studies. The quality varies from poor to high and this was largely dependent on after how much time after start of therapy the quality was measured and how it was measured (percentage time in therapeutic range [TTR] or proportion of INRs in therapeutic range).[8–15] Only one study was conducted in the Netherlands and showed poor quality. Patients were on less than 50% of time within therapeutic INR range (TR).[12] We showed in **chapter 3.2** that the quality, in terms of TTR, is mainly poor in the first 3 months of treatment (55% to 63%) of treatment and thereafter it rises to an acceptable to high quality (65% to 75%). Younger age was associated with lower TTR in the first 3 months of use and younger children had a lower chance of reaching a stable anticoagulation period as was shown in **chapter 3.3**. Young children also spend more of their time below TR compared to older children.

In adults there is evidence that patients on phenprocoumon have more stable INRs and therefore have a higher quality of treatment compared to acenocoumarol.[16,17] This is also in line with our observation in **chapter 3.2** that patients who switched from acenocoumarol to phenprocoumon had often an improved TTR and also a decrease in the variability of INRs.

**Personalized dosing of VKAs**

VKAs are known for their high inter- and intra-individual variability in dose requirements. In adults genetic variations have been shown to explain a large part of the variability in dose requirement next to clinical factors like age.[18] Dosing algorithms were developed and tested in RCTs.[18] In children dosing models were mainly developed for warfarin as described in **chapter 2.2**. We therefore developed for both acenocoumarol and phenprocoumon dosing algorithms (**chapter 4.1 and 4.2**). Age and age-related factors (e.g. weight, height, and body surface area)

have a large impact on the required dose in children. They explained 38% to 56% of the interindividual variability in dose requirement of acenocoumarol and phenprocoumon. Also genetic variations in CYP2C9 and VKORC1 were important for both VKAs. Furthermore, CYP2C18 for acenocoumarol and CYP3A5*1B for phenprocoumon explained an additional part of the variability in dose requirement. For phenprocoumon we were able to make a model, which explained 80.4% of the variability, and 61.8% for acenocoumarol.

## METHODOLOGICAL LIMITATIONS AND CHALLENGES

**Pediatric patient population**

Studying pediatric patients has its challenges. The number of pediatric patients who have an indication for VKA use is low. Furthermore, the developmental changes during ageing make young patients quite different from older patients.[19] Therefore, a sufficient number of patients of different age groups are required to obtain valid results for all pediatric patients. It was challenging to obtain sufficient sample sizes to answer the research questions posed in this thesis.

**Study design**

In this thesis, four of the studies have been conducted within the CAPS cohort (**chapter 3.2, 3.3, 4.1 and 4.2**) and one study in the CPRD database (**chapter 3.1**). Both the CPRD study and the CAPS study were retrospective follow-up studies. This study design has the advantage of acquiring a sufficient sample size easier and is relatively cheap. However, the disadvantages of the study design are that important variables were not measured and that some information was incomplete.

The outcome of the CAPS study was based on the dose information and INRs available in the patient files of the hospitals and anticoagulation clinics. Dose information was provided to us as daily dose calendars or as mean dose and the corresponding dose step. Each dose step corresponds with a daily dose schedule, by which we were able to recover the given doses on each day between two visits. Deviations from planned doses were provided in notes and were if needed interpreted and taken into account. However, misinterpretation of the provided dose information could still have occurred, as could have non-compliance. Both could have led to erroneous daily doses in our database.

The phenprocoumon and acenocoumarol dosing algorithms were able to explain 80.4% and 61.8% of the required dose, respectively (**chapter 4.1** and **4.2**). The unexplained variability might be partly explained by missing information on factors like vitamin K intake (diet and supplements) and concurrent drug use. Also

the TTR found in the first 3 months was quite low (<50%) in some patients, which might also be due to missing information on these factors. However, it could also be caused by the physical condition of the patient (e.g. infections leading to fever and/ or diarrhea in some cases). Information on infections was most likely missing in a considerable proportion of the patients, because they were not routinely reported by all anticoagulation clinics.

With a short questionnaire we have attempted to fill at least part of the missing information on vitamin K intake. Studies have shown that there is a large difference in vitamin K in breast milk and formula milk.[20] Neonates have limited stores of vitamin K at birth and are at risk for vitamin K deficiency bleedings.[21] Therefore, all babies receive vitamin K at birth and babies who are breast fed are administered additional oral vitamin K during the first 3 months after birth.[22] We asked the patients and/ or their parents if they could recall if and when the use of a VKA was combined with breastfeeding and if so, whether the mother was taking vitamin K at the same time.

None of the patients, who reported they had received breastfeeding, were breastfed at the time of the stable period. However, patients could have omitted this question or didn't fill the entire questionnaire, because filling the questionnaire was not required for participation. Furthermore, we do not have information on vitamin K intake in food or as supplement to adjust for fluctuations in vitamin K levels.

Weight and height were by design of the study collected at the hospitals before the analysis of stable period was performed. Per protocol only weight and height were collected at the start of VKA treatment, because it was expected that the stable anticoagulation period would be close to the start. However, when collecting the data we observed that sometimes a substantial time period could be between treatment start and stable period and therefore we started also to collect additional height and weight measurements during VKA treatment, when available, for the remaining patients. In the analyzing phase it appeared for many patients that valid height and weight data were missing (30% acenocoumarol and 56% phenprocoumon patients). For acenocoumarol we still were able to develop a model with body surface area (BSA). However, for phenprocoumon this was not possible. We showed in **chapter 4.1** that BSA explained more of the variability in dose requirement than age. It is therefore likely that this will also be the case for phenprocoumon and that when BSA would have been available, even more of the variability could have been explained.



**Patient selection and generalizability**

Patients were selected from academic hospitals and one anticoagulation clinic. This anticoagulation clinic is closely connected to an academic hospital. Normally, this could lead to only selecting patients with severe indications. However, it is

uncommon that a child would start a VKA treatment outside of an academic children's hospital, because of the complex nature of the indications for VKA. Furthermore, the indications for VKA treatment we observed in the CAPS cohort are similar to other studies in children with VKA treatment.[23] Therefore, the included patients were likely to be representative for the total pediatric population using VKAs.

The quality and safety of current treatment in the Netherlands as described in **chapter 3.2** is largely depending on the anticoagulation management system. In the Netherlands there is a widespread network of anticoagulation clinics. Also the degree of patient involvement influences the results found. A relatively large proportion of patients was self-measuring their INRs (43% for acenocoumarol and 34% for phenprocoumon) and only a relatively small proportion of patients (8% for acenocoumarol and 0% for phenprocoumon) was self-managing (self-measuring and self-dosing). Self-measuring seemed to increase the TTR in the CAPS cohort (**chapter 3.2**). Other studies have shown that patients who use self-measuring and self-management have an improvement in quality of life, patient satisfaction and TTR.[13,15,24] These factors could influence the possibility to translate our findings to the care management system in other countries and should be taken into account when translating the results to other management system situations.

In our study we did not study the differences in the calendar year of VKA start with regards to the TTR. The quality might have differed in the first years of the study period compared to more recent years, because of developments like self-measuring/ self-management.

Furthermore, the TRs that were used at the time of the study in the Netherlands were deviating from the internationally used TRs. Roughly 75% of the patients was dosed on the most common TRs of 2.0-3.5 and 2.5-4.0 which recently have been changed into 2.0-3.0 and 2.5-3.5 to follow international standards. In **chapter 3.2** we did not see a large difference in the quality between patients with TRs of 2.0-3.0, 2.0-3.5 and 2.5-4.0. Also in the algorithms the TR was not associated with the dose, probably because other factors have a larger impact on dose requirement than the TR in itself. Therefore, it is unlikely that the quality of anticoagulation control will be very different when applying the international TR standards.

In the CAPS cohorts used for the development of the dosing algorithms, the number of children of less than 1 year of age was very low (2 patients for acenocoumarol and 2 patients for phenprocoumon). Therefore, the external validity of the dosing models in this young age group will be low. Studies have shown that this age group is different from other age groups. This group requires the highest dose per kilogram bodyweight.[25] Preferably we would have liked to study these infants separately.

## FUTURE PERSPECTIVES

**Implementation of the VKA dosing models**

There is much debate what short of evidence is required before pharmacogenetics could be implemented in clinical practice. Based on the pyramid of evidence a RCT, which shows clear benefit would be preferred. However, small sample sizes and also ethical considerations make this an unfeasible requirement in many pediatric studies and would prevent that children will also benefit from improvements in clinical practice. It might be unethical to conduct a RCT in children when the evidence of improvement is clearly shown in observational studies and/ or in RCTs with adults. Therefore, more feasible evidence should be aimed for. When there is strong evidence in adults and similar results have been shown in observational studies in pediatric patients and when each result of the genetic test has a treatment option, either dose adjustment or an available alternative treatment, genetic testing could be implemented in clinical practice. For warfarin the Clinical Pharmacogenetics Implementation Consortium (CPIC) has stated that in pediatric patients strong evidence exists for the relevance of CYP2C9*2 and *3 and for VKORC1 –1639G>A (rs9923231) or 1173C>T (rs9934438) genotypes to guide warfarin dosing in European children.[5] When considering that in **chapter 4.1** and **4.2** similar results were found for acenocoumarol and phenprocoumon as in the warfarin pediatric studies it might be argued that there is also enough information to use these genotypes to guide acenocoumarol and phenprocoumon dosing. However, these dosing algorithms have never been validated in another cohort of pediatric patients. Therefore, implementation should be piloted by specialists who start on regular basis new children on VKAs. They should collaborate to evaluate the models and adjust the models if necessary. For the evaluation it is important that relevant information for VKA treatment (like age, height, weight, INRs, daily VKA doses, TR, health status (e.g. infections), hospital admissions, vitamin K administrations, interacting co-medication) are documented in at least the first three months of VKA treatment. To collect this information both patients/parents and health care practitioners need to contribute. Digital support with daily or weekly electronical recording of this information is important. It is expected that after one or two years enough children (> 50 per VKA) would have been started with VKAs in the Netherlands to be able to evaluate how well the model for each VKA was able to predict the required dose. If needed the algorithm can be updated. Depending on the outcomes of the evaluation another evaluation period might be necessary.

The pharmacogenetic tests should preferably also be cost-effective to be implemented, and have a limited budget impact. A cost-effectiveness study to the use of pharmacogenetic testing of VKA in pediatric patients has not been performed

yet. Verhoef et al. showed that pharmacogenetic testing for VKAs in adults (50-90 years of age) would be cost-effective when the genetic testing costs would be 30 euros or less.[26] In children, it will most likely be cost-effective even when genetic testing costs would be higher than 30 euros, because they are expected to live many more years to benefit from the prevented complications. The budget impact each year of performing the genetic tests will be low. The costs for genotyping used by Verhoef et al. in 2014 was 40 euros.[26] From data of the Dutch National Health Care Institute we know that around 500 pediatric patients are using a VKA each year.[27] Only a small proportion of them will be new users during a year and require the pharmacogenetic testing. Even when half of them would be new starters this would only cost 10,000 euros per year. This is the situation in which pharmacogenetic testing is specifically done for VKAs. However, in the future probably patients will be genotyped for a broad range of genetic variations or even sequenced for their entire inherited genome which will be stored in a pharmacogenetics passport. When such a pharmacogenetic passport is created the cost-effectiveness of using the genetic information in clinical decision making will be much more favorable than now calculated for individual pharmacogenetic tests.[28]

The risk of using the pharmacogenetic dosing algorithms in clinical practice is that the model over or underestimates the required dose and results in a bleeding or thromboembolic event. However, current recommendations for the starting doses of acenocoumarol and phenprocoumon also over and underestimate the required doses as was shown for acenocoumarol in **chapter 4.1**. The highest risk associated with under or overdosing by the dosing model used, is during the first few days of treatment. After these first days the risks are similar to when no dosing algorithm would have been used, because dosing will be guided by the INR value. In the first few days of VKA treatment, most patients are concurrently using a low molecular weight heparin (LMWH) and are hospitalized. When the INR is still too low the LMWH will prevent the occurrence of thromboembolic events. The LMWH will be stopped when the INR is in TR. When the INR is too high the next dose of VKA can be lowered, not given at all or vitamin K can be given. The chance that this results in more severe bleedings compared to using the current starting dose is minimal.

Facilities for pharmacogenetic testing are increasingly implemented in (academic) laboratories. However, there are also easy options available with point-of-care devices, which can be used in a non-laboratory environment. In the EU-PACT trial it was shown that with a point-of-care device it was possible to genotype patients within 1 day.[29] Rapid genotyping is required at the start of VKA treatment because treatment with VKA cannot be delayed.

Getting children specific dosages is also important, because the tablets available have often a dosage that is too high for administration in young pediatric patients. For

phenprocoumon an oral suspension is available at three compounding pharmacies in the Netherlands. For acenocoumarol only the 1 mg tablets are available. If lower dosages are required, pharmacies with a compounding facility could manufacture capsules by themselves with lower strengths. In a few patients within CAPS, we saw that sometimes powders were used. It is important to note that powders have the disadvantage that the active compound can remain in the mouth, and therefore the full dose will not be available for absorption. In that case the dose calculated will most likely result in an INR which is lower than would be was expected. For acenocoumarol it is preferable that also a formulation for an oral suspension would be developed.

**DOACs**

The last few years clinical trials with DOACs in pediatric patients have started and the first have been completed in children who had a venous thrombosis (**chapter 2.2**). However, VKAs are also used for the prevention of thromboembolic events in children with cardiac diseases. Currently, only one phase 3 trial in which edoxaban is compared with standard of care (low molecular weight heparin (LMWH) and/or VKA) has started (NCT03395639) for the prevention of thromboembolic events in children at risk because of a cardiac disease. This study is expected to be completed in the beginning of 2021.

DOACs were on the Dutch market from 2008 onwards. At first, DOACs were only reimbursed when started by a specialist which resulted in low number of patients starting on DOACs compared to VKAs in the years after the introduction. In September 2016, the Dutch College of General Practitioners (NHG) stated that the benefits and risk of DOACs were similar to VKAs and that general practitioners should be allowed to start DOACs.[30] After this statement, the reimbursement changed accordingly. This has largely increased the number of patients starting with DOACs. Currently, adult patients who start with oral anticoagulants are more often prescribed a DOAC than a VKA.[31]

Presently the first results of six phase 2 studies in pediatric patients of 0 to <18 years of age are available which are described in **chapter 2.2**. In these trials DOACs were only used for a short period of time, varying from one up to 30 days. More evidence is required from larger studies to determine efficacy, safety and tolerability when DOACs are used for a longer period of time. DOACs have the advantage of no INR measurements and no dose adjustments unless necessary based on kidney function or interacting drugs. However**,** a disadvantage is a lack of antidotes studied in children. Antidotes are relevant when a major bleeding occurs or when an acute surgical intervention is necessary. For VKAs coagulation can be relatively quickly restored with vitamin K, 4-factor prothrombin complex concentrate or fresh frozen

plasma. Furthermore, for adult patients with heart valve replacements DOACs have not been approved and these patients have also been excluded in pediatric trials. In the CAPS cohort 21% of the patients was using acenocoumarol for prevention of thromboembolism after a heart valve replacement. For this group of patients VKAs will remain to be the oral anticoagulant of choice. Time will tell if and to which extent DOACs will be implemented in the anticoagulant treatment of pediatric patients to treat and prevent thromboembolic events.

**Future research and recommendations**

Future research should focus on explaining the still unexplained 20 to 40% of the variability in dose requirement of VKAs. We will never be able to explain all variability, but there seems to be room for increasing the percentage of explained variability, especially for acenocoumarol. As already mentioned, this variability could be caused by clinical factors, which were missing from our data. Especially, concurrent drug use could be of influence on the dose requirement. It could even be the case that other drug-drug interactions exist than in adults due to the development of the metabolic system. When the developed dosing models for acenocoumarol and phenprocoumon would be evaluated it would be very important to also record the concurrent drugs used during VKA treatment and to add this, if necessary, to the model. Furthermore, other genetic variations, which were not studied, could play a role. However, quite a number of studies have been conducted and have not led to new common genetic variations which are important in a Caucasian population. Only for patients with other ethnicities other genetic variations might be of interest to study further.[32]

As already mentioned, the number of patients younger than 1 year of age was too low to study their dose requirement separately. It would be highly interesting to study if the drug response is also depending on the gestational age. Andrew et al. showed that the development of the hemostatic system differs between pre-term or full-term babies.[33,34] Also the metabolic system is at a different stage when a baby is born pre-term compared to full-term babies,[19] which may alter the metabolism of VKAs and therefore dose requirement. Furthermore, we showed that a low age is associated with a lower time in therapeutic INR range (**chapter 3.2**). Possibly the differences in pre-term and full-term babies could also contribute in causing more inter and intra-individual variability in VKA dose requirement.

We observed a large variation in TRs for various indications. However, we also showed that the quality was lower for non-standard TR (extra low or high). The incidence of thromboembolic events during VKA treatment is low, as was shown in the CPRD study (**chapter 3.1**). Also within CAPS (**chapter 3.2**) no thromboembolic events occurred during the first year of treatment. Although the patients were

often out of range and at the beginning of treatment especially undertreated. This raises the question if high TRs are necessary in children. The working group Anti-TRoMbotic TheraPy in Children with a CardiOLogical Anomaly (TRAMPOLINE) has made a big step in applying a uniform TR for patients with the same cardiac indication. In principle when a VKA is indicated, the TR of 2-3 is standard and only with a thromboembolic event during VKA treatment or with additional risk factors the higher TR of 2.5-3.5 is recommended.[35,36] Agreement on which TR should be used for each indication and only using these two standard TRs will probably improve anticoagulation quality.

Currently, the quality of anticoagulation in children is not monitored specifically. The Federation of Dutch anticoagulation clinics reports the quality of anticoagulation treatment of all patients, not stratified by age, in their annual report. With this report developments in the quality of anticoagulation can be monitored and actions can be taken to improve it when necessary. It is important to also report the quality of anticoagulation for children separately (stratified on age groups). This would provide the required insight to evaluate if progress is made to optimize their treatment.

## CONCLUSION

In this thesis, we have reported the quality of use, effectiveness and safety of current oral anticoagulation treatment in pediatric patients with acenocoumarol and phenprocoumon. We showed that the quality in the first 3 months of use based on TTR was low and should be improved. Bleeding events were common in the first year of treatment and often occurred in the first few weeks of treatment. Thromboembolic events during treatment were rare. Younger pediatric patients were more likely to have a low TTR in the first 3 months of VKA use and to develop a bleeding event. Furthermore, we have developed dosing algorithms for acenocoumarol and phenprocoumon, which incorporate both genetic and non-genetic factors. These models can be used to predict the required dose and it is hypothesized that this will improve anticoagulation control especially in the first 3 months of treatment by improving the time to reach stable INRs within TR. Piloting the use of this model in clinical practice is required to evaluate the performance of these models in new pediatric patients starting with VKA treatment. After this evaluation the models can be adjusted if needed and be further implemented.

## REFERENCES


1. Baranova EV, Verhoef TI, Asselbergs FW, de Boer A, Maitland-van der Zee A-H. Genotype-guided coumarin dosing: where are we now and where do we need to go next? Expert Opin Drug Metab Toxicol 2015; 11: 509–22.
2. Marek E, Momper JD, Hines RN, Takao CM, Gill JC, Pravica V, et al. Prediction of Warfarin Dose in Pediatric Patients: An Evaluation of the Predictive Performance of Several Models. J Pediatr Pharmacol Ther 2016; 21: 224–32.
3. Xu H, Robinson GW, Huang J, Lim JY-S, Zhang H, Bass JK, et al. Common variants in ACYP2 influence susceptibility to cisplatin-induced hearing loss. Nat Genet 2015; 47: 263–6.
4. Vijverberg SJ, Pijnenburg MW, Hövels AM, Koppelman GH, Maitland-van der Zee A-H. The need for precision medicine clinical trials in childhood asthma: rationale and design of the PUFFIN trial. Pharmacogenomics 2017; 18: 393–401.
5. Johnson JA, Caudle KE, Gong L, Whirl-Carrillo M, Stein CM, Scott SA, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update. Clin Pharmacol Ther 2017; 102: 397–404.
6. Cannegieter SC, Rosendaal FR, Wintzen AR, van der Meer FJM, Vandenbroucke JP, Briet E. Optimal Oral Anticoagulant Therapy in Patients with Mechanical Heart Valves. N Engl J Med 1995; 333: 11–7.
7. Streif W, Andrew M, Marzinotto V, Massicotte P, Chan AK, Julian JA, et al. Analysis of warfarin therapy in pediatric patients: A prospective cohort study of 319 patients. Blood 1999; 94: 3007–14.
8. Jones S, Newall F, Manias E, Monagle P. Assessing outcome measures of oral anticoagulation management in children. Thromb Res 2011; 127: 75–80.
9. Biss TT, Avery PJ, Walsh PM, Kamali F. Comparison of 'time within therapeutic INR range' with 'percentage INR within therapeutic range' for assessing long-term anticoagulation control in children. J Thromb Haemost 2011; 9: 1090–2.
10. Hawcutt DB, Ghani AA, Sutton L, Jorgensen A, Zhang E, Murray M, et al. Pharmacogenetics of warfarin in a paediatric population: time in therapeutic range, initial and stable dosing and adverse effects. Pharmacogenomics J 2014; 14: 542–8.
11. Murray JM, Hellinger A, Dionne R, Brown L, Galvin R, Griggs S, et al. Utility of a dedicated pediatric cardiac anticoagulation program: the Boston Children's Hospital experience. Pediatr Cardiol 2015; 36: 842–50.
12. 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.
13. Bauman ME, Black K, Bauman ML, Bruce A a. K, Kuhle S, Bajzar L, et al. EMPoWarMENT: Edmonton pediatric warfarin self-management pilot study in children with primarily cardiac disease. Thromb Res 2010; 126: e110-115.
14. Bauman ME, Massicotte MP, Kuhle S, Siddons S, Bruce A a. K. EMPoWARed: Edmonton pediatric warfarin self-management study. Thromb Res 2015; 136: 887–93.
15. Jones S, Monagle P, Manias E, Bruce AAK, Newall F. Quality of life assessment in children commencing home INR self-testing. Thromb Res 2013; 132: 37–43.
16. Fihn SD, Gadisseur AAP, Pasterkamp E, van der Meer FJM, Breukink-Engbers WGM, Geven-Boere LM, et al. Comparison of control and stability of oral anticoagulant therapy using acenocoumarol versus phenprocoumon. Thromb Haemost 2003; 90: 260–6.
17. Gadisseur APA, van der Meer FJM, Adriaansen HJ, Fihn SD, Rosendaal FR. Therapeutic quality control of oral anticoagulant therapy comparing the short-acting acenocoumarol and the long-acting phenprocoumon. Br J Haematol 2002; 117: 940–6.
18. Belley-Cote EP, Hanif H, D'Aragon F, Eikelboom JW, Anderson JL, Borgman M, et al. Genotype-guided versus standard vitamin K antagonist dosing algorithms in patients initiating anticoagulation. A systematic review and meta-analysis. Thromb Haemost 2015; 114: 768–77.

19. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental Pharmacology — Drug Disposition, Action, and Therapy in Infants and Children. N Engl J Med 2003; 349: 1157–67.
20. Greer FR, Marshall S, Cherry J, Suttie JW. Vitamin K status of lactating mothers, human milk, and breast-feeding infants. Pediatrics 1991; 88: 751–6.
21. Van Winckel M, De Bruyne R, Van De Velde S, Van Biervliet S. Vitamin K, an update for the paediatrician. Eur J Pediatr 2009; 168: 127–34.
22. Federatie van Nederlandse Trombosediensten. De kunst van het doseren - Richtlijn, leidraad en informatie voor het doseren van vitamine K-antagonisten. 8th ed. Leiden: Federatie van Nederlandse Trombosediensten; 2017.
23. Biss TT, Avery PJ, Brandão LR, Chalmers EA, Williams MD, Grainger JD, et al. 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.
24. Bradbury MJE, Taylor G, Short P, Williams MD. A comparative study of anticoagulant control in patients on long-term warfarin using home and hospital monitoring of the international normalised ratio. Arch Dis Child 2008; 93: 303–6.
25. Bonduel M, Sciuccati G, Hepner M, Torres AF, Pieroni G, Frontroth JP, et al. Acenocoumarol therapy in pediatric patients. J Thromb Haemost JTH 2003; 1: 1740–3.
26. 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.
27. The Dutch National Health Care Institute. Personal communication - ATC-B01A - number of users (age: 0-18 years; 2012-2017). 2018.
28. Verbelen M, Weale ME, Lewis CM. Cost-effectiveness of pharmacogenetic-guided treatment: are we there yet? Pharmacogenomics J 2017; 17: 395–402.
29. van Schie RMF, Wadelius MIA, Kamali F, Daly AK, Manolopoulos VG, de Boer A, et al. Genotype-guided dosing of coumarin derivatives: the European pharmacogenetics of anticoagulant therapy (EU-PACT) trial design. Pharmacogenomics 2009; 10: 1687–95.
30. van den Donk M, de Jong J, Geersing G-J, Wiersma T. NHG-Standpunt Anticoagulantia. Cumarinederivaten en DOAC's voortaan gelijkwaardig. Huisarts Wet 2016; 59: 406–9.
31. van den Heuvel JM, Hövels AM, Büller HR, Mantel-Teeuwisse AK, de Boer A, Maitland-van der Zee AH. NOACs replace VKA as preferred oral anticoagulant among new patients: a drug utilization study in 560 pharmacies in The Netherlands. Thromb J 2018; 16.
32. Cai L-L, Huang W-Q, Su Z-Y, Ye H-M, Wang L-S, Wu Y, et al. Identification of two novel genes SLC15A2 and SLCO1B3 associated with maintenance dose variability of warfarin in a Chinese population. Sci Rep 2017; 7: 17379.
33. Andrew M, Paes B, Milner R, Johnston M, Mitchell L, Tollefsen DM, et al. Development of the human coagulation system in the healthy premature infant. Blood 1988; 72: 1651–7.
34. Andrew M, Paes B, Milner R, Johnston M, Mitchell L, Tollefsen DM, et al. Development of the human coagulation system in the full-term infant. Blood 1987; 70: 165–72.
35. Werkgroep Anti-TRoMbotic TheraPy in Children with a CardiOLogIcal Anomaly ("TRAMPOLINE"). Richtlijn: Antistolling in de kindercardiologie. https://www.eenheidintensievezorg.nl/wiki-picu/antistolling/richtlijn-antistolling-in-de-kindercardiologie/. Accessed 25 Jul 2018.
36. Werkgroep Anti-TRoMbotic TheraPy in Children with a CardiOLogIcal Anomaly ("TRAMPOLINE"). Samenvatting richtlijn antistolling na kindercardiochirurgie | Eenheid Intensieve Zorg. https://www.eenheidintensievezorg.nl/wiki-picu/antistolling/samenvatting-richtlijn-antistolling-na-kindercardiochirurgie/. Accessed 2 Aug 2018.

# 6 | APPENDICES

# 6.1 English summary

Conducting pediatric clinical trials is challenging which results in the necessity of off-label use of drugs in children (**chapter 1**). In 2007, the Pediatric Regulation (Regulation (EC) No 1901/2006) came into force in the European Union to increase the number of clinical trials in children. Next to clinical trials, observational studies can provide physicians with new evidence to improve the treatment of children. The vitamin K antagonists (VKAs) acenocoumarol and phenprocoumon are being used off label to treat and prevent thromboembolic events in children. Minimal information is available on the use and effects in daily pediatric practice. Large variability in the dose requirements of VKAs is seen. Studies in adults have shown that genetic and clinical differences explain a substantial part of the interpatient variability in VKA dose requirement. Studying the influence of genetic variations on drug response (pharmacogenetics) in pediatric patients in general is challenging, but also has the potential to improve childhood care. In **chapter 2.1** we have described these challenges and potentials for three different applications of pharmacogenetics to personalize medicine in pediatric patients. To predict the occurrence of adverse events (cisplatin induced ototoxicity), to predict the optimal dose for a patient (VKA dose requirement) and to predict if a treatment will be effective (asthma treatment). These three examples all show that replication is challenging due to heterogeneity in study populations and also because of small sample sizes in childhood cancer and thrombosis. A mutation in the ACYP2-gene has been shown to be an important predictor of ototoxicity in children using cisplatin, but explains only a small part (12.4%) of ototoxicity. In asthma, the ADRB2 Arg16 mutation with regards to long-acting β2 agonist response is closest to clinical implementation. Its value is currently studied in the PUFFIN trial in the Netherlands in children with asthma who are not well controlled on low dose inhaled corticosteroids. For VKAs the evidence to guide warfarin dosing in children using VKORC1 and CYP2C9*2 and*3 genotype is strong, but has not (yet) been implemented. In **chapter 2.2** we described the differences between adults and children with regards to the risk factors for thrombosis, the hemostatic and metabolic system and recent developments in new and personalized oral anticoagulant treatment in pediatric patients. For warfarin multiple pediatric dosing algorithms have been developed including both genetic and clinical factors. However, for acenocoumarol and phenprocoumon these were lacking. Furthermore, it appeared that more insight in the quality, safety and effectiveness of VKA treatment in pediatric patients and factors influencing anticoagulation stability of pediatric patients using VKA was required.

## THE CHILDREN ANTICOAGULATION AND PHARMACOGENETICS STUDY (CAPS)

Therefore, the Children Anticoagulation and Pharmacogenetics Study (CAPS) was designed. This multicenter retrospective follow-up study included children (≤18 years of age) who were treated with acenocoumarol or phenprocoumon between 1995 and 2014. Patients were selected in four university children's hospitals (Amsterdam, Utrecht, Rotterdam, Groningen) and one anticoagulation clinic (Leiden). From all patients we collected treatment related data at the anticoagulation clinics and hospitals involved in their VKA treatment. Participating patients collected a saliva sample by themselves or by their caregivers to determine their genotype for 7 genetic variations in 5 candidate genes (VKORC1, CYP2C9, CYP4F2, CYP2C18, and CYP3A4).

## QUALITY, EFFECTIVENESS AND SAFETY OF VKA THERAPY IN PEDIATRIC PATIENTS

**Chapter 3** describes three studies on the quality, effectiveness and safety of VKA therapy in pediatric patients. In **chapter 3.1**, the incidence of bleeding and thromboembolic events in pediatric patients using warfarin in England was assessed using the UK Clinical Practice Research Datalink (CPRD) database. We found that 3.33 patients/ 100 patient years (PY) developed a bleeding event and that a large proportion of the patients had their first event within the first few weeks of treatment. Among patients who used warfarin for primary prophylaxis a thromboembolic event occurred in 1.50 patients/100 PY. Furthermore, younger patients appeared to be more prone to develop a bleeding event. **Chapter 3.2** describes the characteristics of the patients and their VKA treatment (quality, effectiveness and safety) in the CAPS cohort. The mean percentage of time in therapeutic INR range (TTR) was 47.0% and 51.4% in the first month of use for acenocoumarol and phenprocoumon, respectively. After the first 3 months the mean TTR for both VKAs was increasing to more acceptable percentages of more than 64%. Especially in the first 3 months of treatment, the TTR was clearly associated with the age of the patient. The younger the patient the lower the TTR. In 14.6% (acenocoumarol) and 31.3% (phenprocoumon) of the patients a bleeding event occurred during the first year of treatment. No thromboembolic events were reported during the first year of treatment. In **chapter 3.3** patients within the CAPS cohort who did or did not have a stable anticoagulation period were studied to find explanatory factors for not reaching a stable period within the first three months of VKA treatment. A stable

period was defined as ≥3 consecutive INRs within therapeutic range over a period of ≥3 weeks. Age was positively associated with obtaining a stable period (the hazard ratio (HR) per increase in year was 1.09, 95%CI [1.05-1.13], p<0.001). Females (HR=0.60 [0.37-0.97], p=0.037), hospitalized patients (HR=0.46 [0.24-0.57], p=0.017), or patients with an interruption in their VKA therapy for ≥3 days (HR=0.50 [0.25-0.98], p=0.044) had a lower chance of obtaining a stable period.

Moreover, the quality of anticoagulation control in patients who used both acenocoumarol and phenprocoumon were compared to evaluate the influence on the type of VKA. We found that patients who switched between acenocoumarol and phenprocoumon had often a higher TTR and lower variability in INRs when using phenprocoumon comparing the 3 months before with the 3 months after switching.

## DOSING ALGORITHMS FOR ACENOCOUMAROL AND PHENPROCOUMON

**Chapter 4** describes the dosing algorithms for acenocoumarol (**chapter 4.1**) and for phenprocoumon (**chapter 4.2**) incorporating both genetic and non-genetic factors. For acenocoumarol we found that 45% of the variability in dose requirement was explained by the body surface area of a pediatric patient and the indication for the use of the acenocoumarol. By adding genetic variations in VKORC1, CYP2C9 and CYP2C18 to the algorithm, the percentage of variability explained increased to 61.8%. For phenprocoumon the age of the patient explained 56.2% of the variability in dose requirement. This increased to 80.4% by adding genetic variations in VKORC1, CYP2C9 and CYP3A4.

In **chapter 5** the results of the studies are summarized, were placed into broader perspective of clinical implications and required further research. The work in this thesis showed that the quality in the first few months of VKA treatment should be improved. The TTR is low and we also showed that the patients with bleeding events often have their first bleeding event within the first few weeks of treatment. Furthermore, young pediatric patients were more prone for a lower TTR and were also at higher risk of developing a bleeding event. The developed dosing algorithms for acenocoumarol and phenprocoumon could assist in improving the quality of anticoagulation control in the first months of treatment.

# 6.2 Nederlandse samenvatting

Het uitvoeren van klinische studies bij kinderen is een uitdaging die leidt tot de noodzaak van off-label gebruik van geneesmiddelen bij kinderen (**hoofdstuk 1**). In 2007 werd de Pediatric Regulation (Verordening (EG) nr. 1901/2006) in de Europese Unie van kracht om het aantal klinische studies bij kinderen te verhogen. Naast klinische studies kunnen observationele studies artsen nieuwe informatie verschaffen om de behandeling van kinderen te verbeteren. De vitamine K-antagonisten (VKA's) acenocoumarol en fenprocoumon worden off-label gebruikt om trombo-embolische events bij kinderen te behandelen en te voorkomen. Er is minimale informatie beschikbaar over het gebruik en de effecten bij kinderen in de dagelijkse praktijk. Er wordt grote variabiliteit in de benodigde doses van VKA's gezien. Studies bij volwassenen hebben aangetoond dat genetische en klinische verschillen een aanzienlijk deel van de variabiliteit tussen patiënten in de dosisbehoefte van VKA verklaren. In het algemeen is het bestuderen van de invloed van genetische variaties op de geneesmiddelrespons (farmacogenetica) bij pediatrische patiënten een uitdaging, maar heeft ook het potentieel om de behandeling van kinderen te verbeteren. In **hoofdstuk 2.1** hebben we deze uitdagingen en mogelijkheden beschreven voor drie verschillende toepassingen van farmacogenetica bij pediatrische patiënten waarbij de behandeling mogelijk gepersonaliseerd kan worden. De eerste is om het optreden van bijwerkingen van een behandeling te voorspellen (cisplatine geïnduceerde ototoxiciteit). De tweede is om de optimale dosis voor een patiënt te voorspellen (dosisbehoefte van VKA's). En de derde is om te voorspellen of een behandeling effectief zal zijn (astmabehandeling). Deze drie voorbeelden tonen allemaal aan dat replicatie van de gevonden resultaten een uitdaging is vanwege de heterogeniteit in de studiepopulaties en ook vanwege de relatief kleine aantallen patiënten in de studies bij kinderen met kanker en trombose. Van een mutatie in het ACYP2-gen is aangetoond dat het een belangrijke voorspeller is van ototoxiciteit bij kinderen die cisplatine gebruiken, maar verklaart slechts een klein deel (12,4%) van patiënten die ototoxiciteit ontwikkelen. Bij astma is de ADRB2 Arg16-mutatie in relatie tot de respons op de behandeling met langwerkende β2-agonisten het dichtst in de buurt van klinische implementatie. De klinische waarde ervan wordt momenteel bestudeerd in de PUFFIN-studie in Nederland bij kinderen waarbij de astma niet voldoende onder controle is bij gebruik van een lage dosis inhalatiecorticosteroïden. Voor VKA's is het bewijs voor het gebruik van het VKORC1- en CYP2C9*2 en *3-genotype voor het bepalen van de benodigde warfarine doses in kinderen sterk, maar dit is (nog) niet geïmplementeerd in de praktijk. In **hoofdstuk 2.2** hebben we de verschillen tussen volwassenen en kinderen beschreven met betrekking tot de risicofactoren voor trombose, het hemostatische en metabole systeem en recente ontwikkelingen in nieuwe en gepersonaliseerde orale antistollingsmiddelen bij kinderen. Voor

warfarine zijn meerdere doseringsalgoritmen voor kinderen ontwikkeld die zowel genetische als klinische factoren bevatten. Voor acenocoumarol en fenprocoumon ontbraken deze echter. Verder bleek dat meer inzicht noodzakelijk is in de kwaliteit, veiligheid en effectiviteit van VKA-behandeling bij kinderen en factoren die met de stabiliteit van de antistolling van VKA in kinderen samenhangen.

## DE CHILDREN ANTICOAGULATION AND PHARMACOGENETICS STUDY (CAPS)

Daarom werd de *Children Anticoagulation and Pharmacogenetics Study* (CAPS) opgezet. Deze multicenter retrospectieve follow-up studie omvatte kinderen (≤18 jaar) die met acenocoumarol of fenprocoumon waren gestart tussen 1995 en 2014. Patiënten werden geselecteerd in vier universitaire kinderziekenhuizen (Amsterdam, Utrecht, Rotterdam, Groningen) en één trombosedienst (Leiden). Van alle patiënten hebben we medische gegevens verzameld in de trombosediensten en ziekenhuizen die betrokken waren bij hun behandeling met VKA's. Deelnemende patiënten stuurden een speekselmonster waarmee wij hun genotype konden bepalen voor 7 verschillende genetische variaties in 5 genen (VKORC1, CYP2C9, CYP4F2, CYP2C18 en CYP3A4).

## KWALITEIT, EFFECTIVITEIT EN VEILIGHEID VAN HET GEBRUIK VAN VKA'S BIJ KINDEREN

**Hoofdstuk 3** beschrijft drie onderzoeken naar de kwaliteit, effectiviteit en veiligheid van de behandeling met VKA's bij kinderen. In **hoofdstuk 3.1** werd de incidentie van bloedingen en trombo-embolische events bij kinderen die warfarine gebruikten in Engeland beoordeeld met behulp van de UK Clinical Practice Research Datalink (CPRD)-database. We vonden dat 3,33 patiënten per 100 patiëntjaren (pj) een bloeding ontwikkelden en dat bij een groot deel van de patiënten hun eerste bloeding tijdens de eerste paar weken van de behandeling optrad. Jongere patiënten bleken een hoger risico te hebben op het ontwikkelen van een bloeding. Onder patiënten die warfarine gebruikten voor primaire profylaxe trad een trombo-embolisch event op bij 1,50 patiënten per 100 pj. **Hoofdstuk 3.2** beschrijft de patiëntkenmerken en de behandeling met VKA's van de patiënten in het CAPS-cohort. Er wordt zowel ingegaan op kwaliteit als ook op de effectiviteit en veiligheid van de behandeling. Het gemiddelde percentage tijd in het therapeutisch INR-bereik (TTR) was respectievelijk 47,0% en 51,4% in de eerste maand van

gebruik voor acenocoumarol en fenprocoumon. Na de eerste 3 maanden steeg de gemiddelde TTR voor beide VKA's tot meer acceptabele percentages van meer dan 64%. Vooral in de eerste 3 maanden van de behandeling was de TTR duidelijk geassocieerd met de leeftijd van de patiënt. Hoe jonger de patiënt hoe lager de TTR. Bij 14,6% (acenocoumarol) en 31,3% (fenprocoumon) van de patiënten trad een bloeding op tijdens het eerste jaar van de behandeling. Er werden geen trombo-embolische events gemeld tijdens het eerste jaar van de behandeling. In **hoofdstuk 3.3** werden de patiënten van het CAPS-cohort die al dan niet een stabiele periode van antistolling hadden, onderzocht om verklarende factoren te vinden voor het niet bereiken van een stabiele periode binnen de eerste drie maanden van VKA-behandeling. Een stabiele periode werd gedefinieerd als ≥3 opeenvolgende INR-waarden binnen het therapeutisch bereik over een periode van ≥3 weken. Leeftijd was positief geassocieerd met het verkrijgen van een stabiele periode (de hazard ratio (HR) per toename in levensjaar was 1.09, 95% CI [1.05-1.13], p <0.001). Meisjes (HR = 0,60 [0,37-0,97], p = 0,037), patiënten die waren opgenomen in het ziekenhuis (HR = 0,46 [0,25-0,57], p = 0,017), of patiënten met een onderbreking in hun VKA-behandeling gedurende ≥3 dagen (HR = 0,50 [0.25-0.98], p = 0.044) hadden een lagere kans op het verkrijgen van een stabiele periode. Bovendien werd de kwaliteit van de VKA behandeling bij patiënten die zowel acenocoumarol als fenprocoumon gebruikten vergeleken om te beoordelen of het type VKA invloed heeft op de kwaliteit. We vonden dat patiënten die switchten tussen acenocoumarol en fenprocoumon vaker een hogere TTR en lagere variabiliteit in INR-waarden hadden wanneer ze fenprocoumon gebruikten waarbij gekeken werd naar de 3 maanden voor en de 3 maanden na het switchen.

## DOSERINGSALGORITMEN VOOR ACENOCOUMAROL EN FENPROCOUMON



**Hoofdstuk 4** beschrijft de doseringsalgoritmen voor acenocoumarol (**hoofdstuk 4.1**) en voor fenprocoumon (**hoofdstuk 4.2**) waarbij zowel genetische als niet-genetische factoren zijn opgenomen in de algoritmen. Voor acenocoumarol vonden we dat 45% van de variabiliteit in dosisbehoefte werd verklaard door de lichaamsoppervlakte van een kind en door de indicatie voor het gebruik van acenocoumarol. Door genetische variaties in VKORC1, CYP2C9 en CYP2C18 aan het algoritme toe te voegen, steeg het percentage verklaarde variabiliteit tot 61,8%. Voor fenprocoumon verklaarde de leeftijd van het kind 56,2% van de variabiliteit in de dosisbehoefte. Dit steeg tot 80,4% na toevoeging van de genetische variaties in VKORC1, CYP2C9 en CYP3A4.

In **hoofdstuk 5** zijn de resultaten van de onderzoeken samengevat, geplaatst in een breder perspectief van klinische implicaties en het benodigde verdere onderzoek. Het werk in dit proefschrift heeft aangetoond dat de kwaliteit in de eerste paar maanden van de behandeling met VKA's verbeterd dient te worden. In de eerste paar weken van de behandeling is de TTR laag en treedt ook vaak de eerste bloeding van een patiënt op. Jonge kinderen hebben een hogere kans op een lagere TTR en hebben ook een hoger risico op het ontwikkelen van een bloeding. De ontwikkelde doseringsalgoritmen voor acenocoumarol en fenprocoumon zouden kunnen helpen bij het verbeteren van de kwaliteit van antistolling in de eerste maanden van de behandeling.

## 6.3 Dankwoord

Het is eindelijk klaar! Wat ooit als extra project tijdens mijn master Farmacie begon, is nu eindelijk afgerond. Er zit een kaft om, het boek kan dicht. Het was een lange weg en het einde leek maar niet te naderen. Wat ben ik ontzettend dankbaar voor alle steun die ik van iedereen gekregen heb.

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# 6.5 List of publications

**Related to this thesis**

Maagdenberg H, Bierings MB, van Ommen CH, van der Meer FJ, Appel IM, Tamminga RY, Cessie SL, Swen JJ, der Straaten TV, Boer A, Maitland-van der Zee AH. Effects of age and genetic variations in VKORC1, CYP2C9 and CYP3A4 on the phenprocoumon dose in pediatric patients. Pharmacogenomics. 2018 Oct;19(15):1195-1202.

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 Sep;16(9):1732-1742.

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 Jan;16(1):116-124.

Maagdenberg H, Vijverberg SJ, Bierings MB, Carleton BC, Arets HG, de Boer A, Maitland-van der Zee AH. Pharmacogenomics in Pediatric Patients: Towards Personalized Medicine. Paediatr Drugs. 2016 Aug;18(4):251-60.

**Unrelated to this thesis**

Co-author of three chapters in the book "Farmacie, van pillen tot patiëntenzorg".

- Hamidpopal J, Maagdenberg H. De reis van een geneesmiddel door het lichaam. In: Farmacie, van pillen tot patiëntenzorg. Culemborg: Twin Media BV; 2015. p 23-35.
- Maagdenberg H, van Lanen H. De patiënt. In: Farmacie, van pillen tot patiëntenzorg. Culemborg: Twin Media BV; 2015. p 49-62.
- Lau C, van Lanen H, Maagdenberg H. Alternatieve geneeswijzen. In: Farmacie, van pillen tot patiëntenzorg. Culemborg: Twin Media BV; 2015. p 139-147.

# 6.5 About the author

Hedy Maagdenberg was born on the 26th of July 1988 in Leidschendam, the Netherlands. In 2007, she obtained her pre-university diploma (*cum laude*) at Stichtse Vrije School in Zeist. In the same year she started studying Pharmacy at Utrecht University.

In her second year of the bachelor she started to participate in het honours program, which she continued during the master. As a result of the honours program she became a co-author of the book "Farmacie, van pillen tot patiëntenzorg".

In 2011, she obtained her BSc degree and started with the master Pharmacy. During her second year of the master she came in contact with prof. Anke-Hilse Maitland-van der Zee and started writing the study protocol for the Children Anticoagulation and Pharmacogenetics Study (CAPS). CAPS was designed to develop a dosing algorithm for vitamin K antagonists in children taking both non-genetic and genetic factors into account. During the following years of her master she made a start with CAPS. After obtaining her MSc degree she continued her work for CAPS in the form of a PhD-trajectory at the division of Pharmacoepidemiology and Clinical Pharmacology at Utrecht University, under the supervision of prof. Anthonius de Boer, prof. Anke-Hilse Maitland-van der Zee and dr. Marc Bierings. Besides doing her PhD-research she was also teaching within the bachelor and master Pharmacy for 1.5 days a week. In 2017, she went to Vancouver to work on a project in the research group of prof. Bruce Carleton for 3.5 months. The focus of this project was to study the genetic risk factors associated with the development of severe mucositis in children with cancer who were treated with methotrexate.

In January 2018, she started working at the Amsterdam University Medical Center on the mucositis project as a postdoctoral researcher funded by the group of prof. Bruce Carleton. Currently, she is working as advisor at the Dutch National Healthcare Institute (ZIN).