Pharmacogenetics of Coumarin Anticoagulant Therapy

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Abstract Coumarins are effective drugs for treatment and prevention of thromboembolic events. However, their use requires a balancing act between the chance of underdosing which increases the risk of thromboembolic events and the chance of overdosing which increases the risk of haemorrhages. It has been shown that polymorphisms in *VKORC1* and *CYP2C9* explain 35–50 % of the dose variability, although patient characteristics and environmental factors also play a role. In this book chapter we discuss the pharmacogenetics of coumarin derivatives, clinical trials investigating the effectiveness of pre-treatment genotyping and the cost-effectiveness of pharmacogenetic-guided dosing.

Keywords Adverse drug reaction · Pharmacogenetics · Predictive genotyping · Translation · Abacavir · Hypersensitivity · Malignant

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1 Introduction

Coumarin derivatives, such as warfarin, phenprocoumon and acenocoumarol, are very effective in the prevention and treatment of thromboembolic diseases, for example in patients with atrial fibrillation or venous thromboembolism [1–5]. Patients with atrial fibrillation have an annual stroke risk of 4.5 %, which decreases to 1.4 % during treatment with warfarin [1]. Warfarin is the most prescribed coumarin in the world while phenprocoumon and acenocoumarol are the coumarins of first choice in continental Europe [6–8]. Although these drugs have already been on the market for decades, finding the right dose for each patient is still challenging. Coumarins have a narrow therapeutic index, often resulting in an unacceptably low anticoagulant effect with an increased risk of thromboembolism or unacceptably high anticoagulant effect with an increased risk of haemorrhages [9–13]. Furthermore, they are subject to inter- and intra-individual variability in dose requirements [14, 15]. Also, the use of coumarins frequently results in drug-related hospitalisation [16–19]. It has been established that anticoagulation response is affected by environmental, clinical, and genetic factors such as age, height, weight, concurrent drug therapy, morbidities, dietary vitamin K intake, and genetic variation in Cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex subunit 1 (VKORC1) [20–25]. This chapter elaborates on the inter- and intra-patient variability in the response to coumarin derivatives, mainly focusing on the pharmacogenetics of these drugs.

2 Mechanism of Action

Inactive coagulation factors II, VII, IX and X require γ-carboxylation of the glutamic acid (Glu) residues into γ-carboxyglutamic (Gla) residues for their coagulation activity (see Fig. 1) [26–28]. In this process, the γ-carboxylase cofactor vitamin K-hydroquinone is oxidised to vitamin K-epoxide. Vitamin K- epoxide is recycled for the carboxylation of new coagulation factors in a 2-step reduction to vitamin K-hydroquinone [27, 28]. Vitamin K epoxide reductase (VKOR) is the catalyser of the first step in the reduction of vitamin K-epoxide into vitamin K-quinone and also contributes to the second reduction step, in which vitamin K-quinone is further reduced to vitamin K-hydroquinone [27, 28]. Cytochrome P450 4F2 (CYP4F2) is a vitamin K-oxidase and metabolises vitamin K-quinone to hydroxyvitamin K [29]. Coumarins, also called vitamin K antagonists, inhibit the reduction of oxidised vitamin K by binding to a small trans membrane protein in the endoplasmic reticulum called vitamin K epoxide reductase complex 1 (VKORC1), which is part of the VKOR complex [30, 31]. As a result, vitamin K-hydroquinone will not become available for the γ-carboxylation of coagulation factors. Coumarins thus act indirectly on the coagulation factors. The half-lives of the coagulation factors range from approximately 6 h for factor VII to 2.5 days for

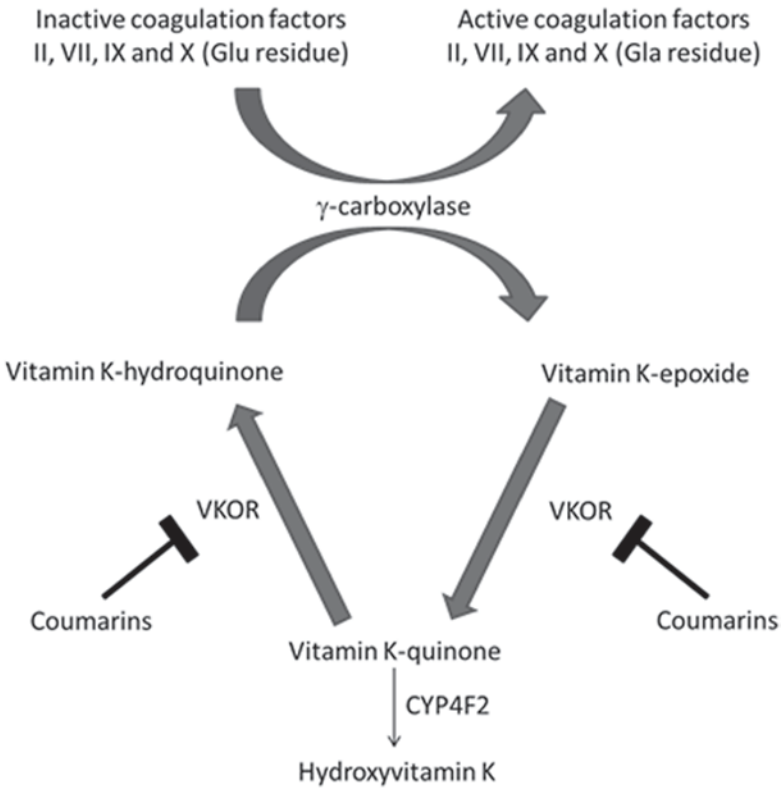

Fig. 1 The mechanism of action

factor II (prothrombin) [32]. This means that the effect of the coumarins in inducing an anticoagulant effect starts 15 h after administration [33] and ends 36–72 h after start of coumarin use [34, 35].

3 Pharmacokinetics

All three coumarin derivatives have a similar chemical structure and belong to the group of 4-hydroxycoumarins. Each coumarin has a single, chiral centre with a R-enantiomeric form or a S enantiomer, which is approximately 2- to 5-fold more potent [36]. Even though the mechanism of action is identical for the three coumarins, there are clear differences in their pharmacokinetic properties and therefore we discuss the pharmacokinetics of the coumarins separately. After administration, all coumarins (except S-acenocoumarol) are absorbed from the gastrointestinal tract with almost complete bioavailability [36].

3.1 Warfarin

Warfarin is metabolised to five different monohydroxylated metabolites (i.e. 4'-, 6-, 7-, 8- and 10-hydroxywarfarin), cis- and trans-dehydro-warfarin, and two diastereomeric alcohols [36, 37]. Metabolism to hydroxylated and dehydro- metabolites is dependent on Cytochrome P450 (CYP) enzymes and occurs in the microsomal fraction of hepatocytes [38], while reduction to alcohols is dependent on NADPH and takes place in the endoplasmic reticulum and cytosol [39, 40]. Different monohydroxylated warfarin metabolites are formed, which suggests involvement of different CYP-isoenzymes. The largest proportion of hydroxylation is catalysed by CYP2C9, resulting in the formation of 7-hydroxywarfarin, the most abundant metabolite. To a much smaller extent, CYP2C8, CYP2C19, CYP1A2 and CYP3A4 are involved [36]. The half-life of warfarin is 24–33 h for S-warfarin and 35–58 h for R-warfarin [36, 41].

3.2 Acenocoumarol

Acenocoumarol is metabolised to 6-, 7-, and 8-hydroxy-acenocoumarol, amino and acetamido acenocoumarol and two diastereometic alcohols [42, 43]. Enzymes involved in the formation of amino and acetamido metabolites and alcohols have not yet been identified. Hydroxylation is dependent on CYP-enzymes [44]. Hydroxylation is catalysed by CYP2C9, the main metabolite being 7-hydroxyacenocoumarol. As for warfarin, CYP2C9 regioselectivity for the 6- and 7- position and stereoselectivity for the S-enantiomer seem to play a role [36]. In contrast, the role of CYP2C19 and CYP1A2 is much smaller [36]. The half-life of acenocoumarol is 1.8 h for S-acenocoumarol—the most potent form—and 6.6 h for R-acenocoumarol [43].

3.3 Phenprocoumon

The metabolites of phenprocoumon are 4'-, 6-, 7- and 8-hydroxy-phenprocoumon and in contrast to warfarin and acenocoumarol all metabolites are hydroxyl-metabolites [36]. The hydroxyl-metabolites are all formed by CYP-enzymes [45, 46]. The 6- and 7-hydroxy phenprocoumon are the most abundant metabolites, 45 and 52%, respectively [36]. The main metabolising enzymes involved are CYP2C9 for approximately 60–65% and CYP3A4 for approximately 35–40% of 6- and 7-hydroxy-phenprocoumon. These CYP-enzymes and CYP2C8 are also involved in the formation of the other metabolites [36]. The half-life of phenprocoumon is much longer compared with the two other coumarins: 110–130 h for S-phenprocoumon (the most potent form) and 110–125 h for R-phenprocoumon [47]. The contribution of CYP2C9 to the metabolism of the different enantiomers of the three coumarins varies [36] and is shown in Fig. 2.

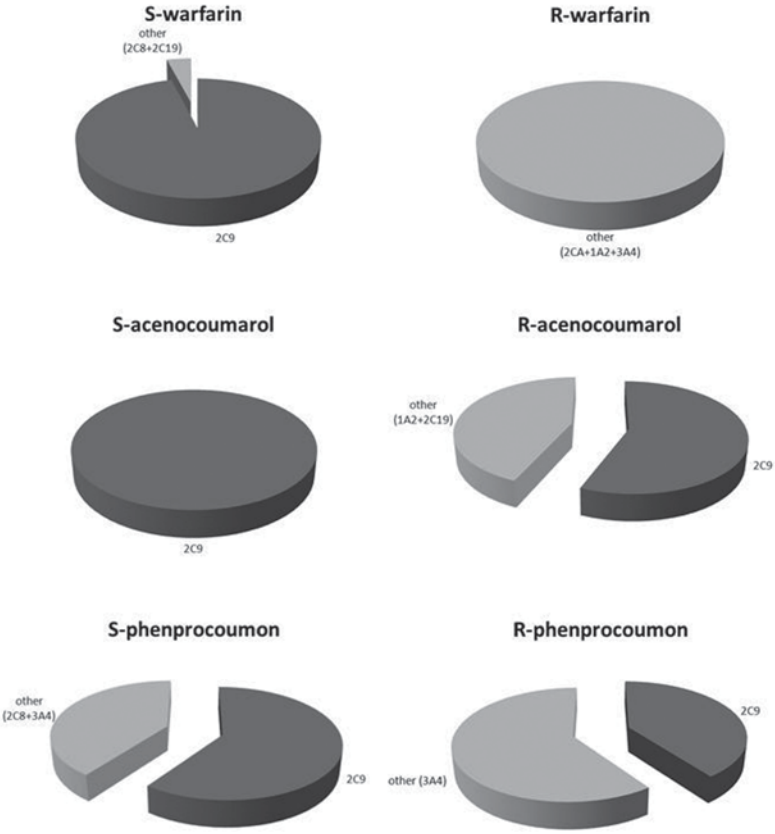

Fig. 2 The contribution of CYP2C9 and other CYP enzymes to the metabolism of the different enantiomers

4 Anticoagulant Therapy

In order to find the most effective and safe balance between underanticoagulation (with a risk of thromboembolic events) and overanticoagulation (with a risk of haemorrhage), a recommendation was made during the first American College of Chest Physicians (ACCP) conference in 1986 that therapy with coumarins should be monitored using the International Normalised Ratio (INR) established by the World Health Organisation [48, 49]. A dose that prolongs the INR to two to three times control (i.e. INR of 2.0–3.0) was recommended for indications such as prophylaxis and treatment of venous thromboembolism, and atrial fibrillation [49]. Higher ranges (i.e. INR of 3.0–4.5) were recommended for other indications including, for example, recurrent venous thrombosis despite adequate anticoagulation [49]. These recommendations are widely accepted and have increased the safety of coumarins [48]. The treatment is often managed by the general practitioner (GP) or a physician

in the hospital. In contrast to most other countries, there are specialised anticoagulation clinics in The Netherlands that follow dosing strategies to maintain the INR between the 2.0 and 3.5 for the low intensity range (e.g. atrial fibrillation, venous thromboembolism) or 2.5 and 4.0 for the high intensity range (e.g. artificial heart valves, recurrent venous thrombosis despite adequate anticoagulation) [28, 50, 51]. Dutch patients regularly visit the anticoagulation clinic for INR measurements and subsequent dose adjustments. Anticoagulation clinics improve the quality of the anticoagulant therapy and are cost saving because haemorrhages and thromboembolic events are prevented more adequately compared to usual clinical care (monitoring by GPs or in the hospital) [52, 53]. In 2010, the Dutch anticoagulation clinics achieved a median percentage time spend in target INR range of 77.9% for patients in the low intensity range and 73.2% for patients in the high intensity range [50]. This is a very high percentage time in range compared with what has been reported in other countries (for example, 63% in the UK, 56% in Germany, and 66% in Austria) and comparable to Sweden (76%) [54], but it still means that over 20% of the time, INRs are above or below the target range. This can be explained by intra-individual dose variability over time, which will be discussed, together with inter-individual variability, in the next paragraph.

5 Inter- and Intra-Individual Dose Variability

The coumarin dose that is optimal for one patient may cause haemorrhages in another patient and thromboembolic events in a third patient. Patients need very different dosages which can differ by up to 10 fold [14]. For example, the maintenance dose of warfarin ranges from 1.5 to 12 mg/day, acenocoumarol from 1 to 9 mg/day and phenprocoumon from 0.75 to 9 mg/day [36]. In addition, the required dose may also change over time in an individual patient. There are several factors that cause inter- and intra-individual variability.

5.1 Patient Characteristics and Environmental Factors

Effects of patient characteristics and environmental factors can roughly be divided into 3 categories: effects on the coumarin dose, effects on the stability of the anticoagulant therapy, and effects on clinical outcomes.

5.1.1 Effects on Coumarin Dose

Coumarin dose requirements decrease with increasing age, but increase with increasing weight and height [25, 55]. Many diseases affect the coumarin dosages as well. Patients with hepatic disorders need lower dosages because the synthesis of

coagulation factors is reduced in these patients because of Vitamin K deficiency, decreased metabolism due to reduction in hepatocyte mass or hypo-albuminaemia [56, 57]. Hyperthyroidism leads to decreased coumarin dosages compared to euthyroidism, while hypothyroidism is associated with a decreased catabolism of vitamin K-dependent coagulation factors, attenuating the response to oral anticoagulant therapy and resulting in increased dose requirements [56]. Heart failure may cause hepatic congestion, resulting in a decreased synthesis of coagulation factors and therefore lower coumarin maintenance dose requirements [56, 58]. Malignancies might affect the coumarin dose by metastatic liver disease, malnutrition, or use of chemotherapy [56]. Fever decreases coumarin dose requirements, probably by increasing degradation of coagulation factors [9]. Dehydration might affect the INR and therefore the coumarin dose by changing the volume of distribution of the coumarins [57]. Hypo-albuminaemia affects the concentration of unbound coumarins and therefore the coumarin dose requirements [57]. Kidney disorders might also affect the albumin concentration and therefore coumarin dose requirements [57]. Comedication use is also of importance and there are many drugs that can increase or decrease the anticoagulation effect and thereby influence the coumarin dose requirements [22, 23, 25, 59–62]. In the Netherlands, clinically relevant drug interactions with coumarins have been described and regulated in the guidelines for anticoagulation clinics [63, 64]. There are two main categories of drug interactions: first, the pharmacokinetic interactions affecting the absorption, distribution or elimination and second, the pharmacodynamic interactions affecting production or metabolism of coagulation factors, or directly affecting coagulation [57]. Besides affecting the coumarin maintenance dose, comedication might also increase the risk of haemorrhages.

5.1.2 Effects on Stability of the Anticoagulant Therapy

Dietary vitamin K intake interferes with the stability of the oral anticoagulant therapy [65]. Daily supplementation of vitamin K intake possibly contributes to a more stable anticoagulant therapy [66–68]. Other nutrition factors can also be of influence [57]. Because vitamin K is a fat-soluble vitamin, the absorption of vitamin K through the intestines is influenced by fat intake and absorption disorders which might result in instability of the anticoagulant therapy. Gavage feeding might cause fluctuating INRs [57, 69]. This could be due to different concentrations of vitamin K in the gavage in comparison to normal diet. Also, vitamin K might bind to proteins in the gavage feeding, or vitamin K might get lost in the preparation of the gavage or due to adsorption to the tube wall. Disorders of the gastrointestinal tract (e.g. vomiting, diarrhea, malabsorption of fat, or antibiotic use which may affect bacteria in the intestines that produce vitamin K) might affect the stability of anticoagulant therapy [57]. Increased levels of stress are thought to be associated with increased INRs and varying amounts of physical exercise may cause a fluctuation in INR as well [57]. Travelling (and any resulting changes in diet or alcohol consumption) and poor compliance might cause instability as well [57, 70].

5.1.3 Effects on Clinical Outcomes

Hematological disorders, such as thrombocytopenia, might affect the anticoagulant therapy by increasing the risk of haemorrhage. In addition, local disorders such as polyps increase the risk of haemorrhage. Malignancies may increase the risk of both venous thromboembolism and haemorrhages [57].

5.2 Pharmacogenetics

In 1992, Rettie et al. reported that CYP2C9 is the main metabolising enzyme of warfarin [71]. CYP1A2 and CYP3A4 were also found to contribute to the metabolism of the drug [71]. Furuya et al. hypothesised that polymorphisms in *CYP2C9* (resulting in proteins with different catalytic activities) might have a major effect on the clearance of the most potent enantiomer (S-warfarin) and therefore might affect the warfarin maintenance dose [72]. They recruited almost 100 patients who attended the anticoagulation clinic for routine INR monitoring. Information on body weight, height, age, sex, drug history, INRs history, indication for coumarin use, and comorbidities was collected. A blood sample was used to determine the *CYP2C9*2* genotype. Of the 94 included patients, 58 (62%) were wild type (*CYP2C9*1/*1*) and 36 (38%) heterozygous for *CYP2C9*2*. There were no patients homozygous for *CYP2C9*2*. Patients carrying the variant allele required significantly lower warfarin dosages than wild type patients (Mann-Whitney U-test, $p = 0.02$). In addition, they found an association between age and warfarin dose requirements. The results suggesting an effect of *CYP2C9* genotypes on the coumarin maintenance dose have since been replicated by many research groups [25, 73–78]. Not only *CYP2C9*2* but also *CYP2C9*3* is a common variant allele in Caucasians that reduces the coumarin maintenance dose significantly [25, 73–78]. The CYP2C9*2 allele frequencies vary from 8 to 19% and the CYP2C9*3 alleles from 3 to 16% in Caucasians [79]. East Asian and African or Afro-American populations show an absence of CYP2C9*2 and a reduced frequency of CYP2C9*3 (79). The *CYP2C9* genotype explains approximately 4.5–17.5% of the coumarin (warfarin, acenocoumarol and phenprocoumon) dose variation [25, 76, 80–85].

Rost et al. and Li et al. identified VKORC1 as a target of the coumarins in 2004 [30, 31]. This introduced a new possibility for explaining the coumarin dose variability. Indeed, many researchers showed decreased coumarin dose requirements if patients carried one or two variant alleles in the *VKORC1* gene [73–75, 82, 86, 87]. Two SNPs in *VKORC1*, the −1639G>A and the 1173 C>T, were found to be associated with decreased warfarin dose requirements [28]. It was demonstrated that promotor SNP −1639G>A causes the variability in VKORC1 activity by suppressing the gene expression, but a role for 1173 C>T could not be excluded because of the complete linkage disequilibrium between the two SNPs [88]. Patients carrying one or two variant alleles have decreased levels of *VKORC1* mRNA in the liver and therefore need lower coumarin dosages compared to wild type patients

[88]. Because the two SNPs are in complete linkage disequilibrium [88, 89], studying either of the two SNPs will give the same results. Allele frequencies for the *VKORC1* variant allele are 37–41 % in Caucasians, 10–12 % in African Americans, and 88–92 % in East-Asians [28].

There are many other genes that could potentially affect the coumarin maintenance dose. The association with the coumarin dose might for example be based on other pharmacokinetic or pharmacodynamic mechanisms, for example by affecting the transport of coumarins or vitamin K or by affecting the vitamin K cycle. In the metabolism of phenprocoumon, other metabolising enzymes, especially CYP3A4, also play an important role [36, 90] and therefore SNPs in the genes encoding for these metabolising enzymes are hypothesised to affect the phenprocoumon dose requirements. However, Teichert et al. did not find an association between *CYP3A4*1B* and the phenprocoumon dose [91]. Another gene that has been associated with coumarin response is *CYP4F2* [91–97], which is a vitamin K oxidase. Patients carrying one or two V433M variant alleles in *CYP4F2* have a reduced capacity to metabolise Vitamin K, resulting in increased vitamin K levels and therefore also resulting in higher coumarin dose requirements when compared to non-carriers [29]. SNPs in *CYP4F2* have a nominal effect on the coumarin maintenance dose; it explains an additional 1–2 % of the coumarin dose requirements [92, 94]. Polymorphisms in the gene encoding γ-glutamylcarboxylase (*GGCX*), which is involved in the carboxylation of coagulation factors, have also been shown to have a minor effect on the coumarin dose [74, 98] however other research groups did not find an association between the coumarin dose and polymorphisms in *GGCX* [99, 100]. Other minor influences on the coumarin maintenance dose might be caused by polymorphisms in the genes encoding for the coagulation factors VII and X [101], epoxide hydrolase (*EPHX1*) [100, 102] which encodes a protein subunit of VKOR, apolipoprotein E (*APOE*) [103–107] which encodes for the protein responsible for the vitamin K uptake, and in protein C (*PROC*) [103] which encodes for protein C, responsible for the inactivation of coagulation factors Va and VIIIa. All these polymorphisms show low or no clinical relevance.

Until now, only *VKORC1*, *CYP2C9* and *CYP4F2* genotypes were found to be associated with the coumarin maintenance dose in genome wide association studies (GWAS) [91, 93, 94, 97]. Ross and co-workers studied the allele frequencies of these genes in different populations and found that there are significant differences between populations worldwide [108]. The allele frequencies of the common and variant alleles of *VKORC1, CYP2C9*2, CYP2C9*3* and *CYP4F2* are shown in Fig. 3. One study also found an association between CYP2C18 and the acenocoumarol dose [97]. Another study of 1496 Swedish patients starting warfarin treatment investigated possible associations between183 polymorphisms in 29 candidate genes and warfarin dose and only found an association for *CYP2C9* and *VKORC1* [83].

CYP2C9 and *VKORC1* genotypes together explain approximately 35–50 % of the coumarin dose requirements [83, 87, 109]. To date, a number of studies have reported the development of pharmacogenetics-guided algorithms for coumarins in order to predict the personalised coumarin dose before start of the anticoagulant

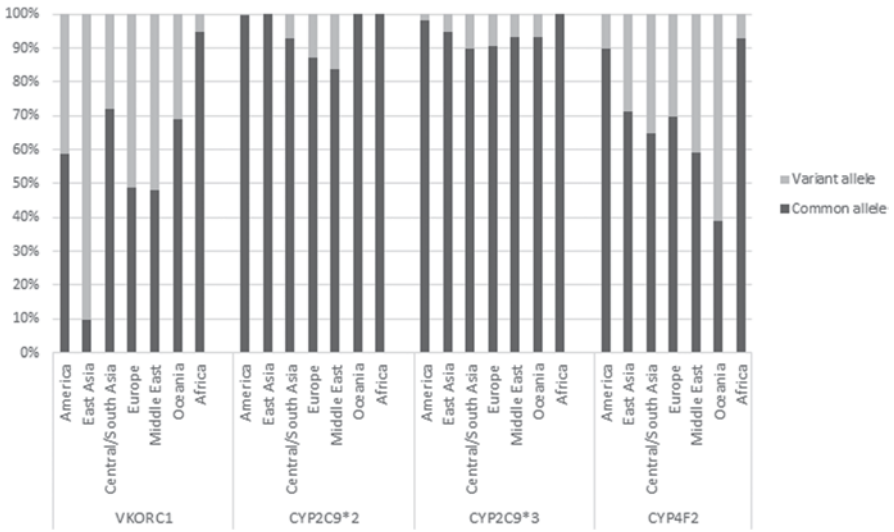

Fig. 3 Allele frequencies of genes associated with coumarin dose requirement among different populations

therapy [25, 76, 80–85]. The predictive value of these algorithms varied from 47 to 60%. Because of ethnic differences in allele frequencies, it can be expected that pharmacogenetic algorithms have a different predictive value in different populations. Several authors have included race as a parameter in their pharmacogenetic-guided algorithm [76, 80, 81, 83]. The International Warfarin Pharmacogenetics Consortium showed that a model that was adjusted for race performed better than specific models for each ethnicity. However, racial differences were not significantly associated with the required dose when genetic information was added to the model [76].

5.2.1 Clinical Trials

In 2005, the first (pilot) randomised trial on pharmacogenetic-guided warfarin dosing in 38 patients was published [110]. These authors reported no differences in percentage time in INR range or the risk of supratherapeutic INR values. In another randomised trial with 191 patients, the time to stable dose was decreased and the time spent in therapeutic range was increased by pharmacogenetic-guided dosing [111]. In both these studies, only *CYP2C9* genotype was assessed and not *VKORC1* genotype. Anderson et al. [112] investigated the impact of genotyping for both *CYP2C9* and *VKORC1* genotypes in 220 patients. No effect on the number of out-of-range INR values could be demonstrated when looking at all patients, but in wild-type

patients and patients carrying multiple variant alleles, genotyping decreased the risk of out-of-range INRs by 10%. In two small randomised trials in Chinese patients, a stable dose was reached faster in patients receiving a pharmacogenetic-guided dose than in patients receiving a standard dose [113, 114]. Burmester et al., compared dosing using a pharmacogenetic algorithm to a clinical algorithm instead of standard dosing and found no differences in percentage time in therapeutic range between the two arms [115]. The Applying Pharmacogenetic Algorithms to Individualise Dosing of Warfarin (Coumagen-II) trial (NCT00927862) showed that pharmacogenetic dosing was superior to standard dosing for percentage time in and out of therapeutic range [116]. During the first month of the treatment, 31% of the INR measurements were below or above the therapeutic range in the intervention group vs. 42% in the control group. The reduction in out-of-range INRs was mainly due to a reduction in INRs below the therapeutic range. The percentage time within the therapeutic range was 69% in the intervention group and 58% in the control group. Also, less serious adverse events (including haemorrhagic and thromboembolic events) occurred in the genotype-guided group (4.5 vs. 9.4%, $p=0.001$). The limitation of this study was the lack of randomised comparison.

The European Pharmacogenetics of Anticoagulant Therapy EU-PACT trial (unique ClinicalTrials.gov Identifiers: NCT01119274, NCT01119261, and NCT01119300) compares a dose algorithm with patient characteristics (or in the case of warfarin standard clinical care) to a dose algorithm with patient characteristics and *VKORC1* and *CYP2C9* genotype [117, 118]. The primary outcome is the time within target INR range. It is the only RCT that investigates all three coumarins (warfarin, phenprocoumon and acenocoumarol). The EU-PACT warfarin arm showed a positive effect of the genotype-guided dosing taking percent time in therapeutic INR range as an outcome. The patients that were genotyped spent 7% more time in range in the first 12 weeks of warfarin therapy compared with the patients in the standard care arm. In the EU-PACT phenprocoumon/acenocoumarol arm there was no statistically significant difference in time in therapeutic range in the first 12 weeks, however there was a statistically significant effect in the first 4 weeks of treatment. Patients in the genotyped arm spend 5% more time within therapeutic range in these first 4 weeks [117]. On the other hand, the Clarification of Optimal Anticoagulation Through Genetics (COAG) (NCT00839657) trial results in no significant difference in the time spent within the therapeutic range in the first 4 weeks of warfarin treatment [119]. These conflicting results are compared in Table 1. One of the reasons for these observed differences might be the comparator, since for warfarin dosage, the genotype guided dose was compared to standard care in the EU-PACT trial, whereas the comparator in the EU-PACT phenprocoumon/acenocoumarol arm and in the COAG trial was a clinical algorithm. Furthermore in the COAG trial it was shown that for African Americans the time in therapeutic range was less in the genotyped arm compared with the clinical algorithm arm. This implies that different algorithms are necessary for different race groups.

Table 1 Overview of randomised clinical trials

	EU-PACT [117]	COAG [119]
Coumarin derivative	Phenprocoumon, acenocoumarol, warfarin	Warfarin
Population	Patients with atrial fibrillation or venous thromboembolism	Patients requiring warfarin therapy with a target INR range of 2–3
Genotypes included	VKORC1, CYP2C9	VKORC1, CYP2C9
Comparator	Clinical algorithm (acenocoumarol, phenprocoumon) Standard care (warfarin)	Clinical algorithm
Number of patients	911	1015
Primary outcome	Percentage time within target INR range	Percentage time within target INR range
Result	Genotype-guided Warfarin Algorithm is superior	No difference

6 Cost-Effectiveness

Clinical trials can provide valuable information about the safety and effectiveness of genotyping before starting coumarin therapy. This information is not only valuable for clinicians but also for policymakers who need to make a decision about whether or not to implement genotype-guided dosing. However, this decision will not only depend on the effectiveness of genotyping, but also on the cost-effectiveness since an important factor for implementation will be reimbursement of the genetic tests. This is the primary reason for performing cost-effectiveness analyses. Some of the cost-effectiveness analyses of genotyping performed in the past have estimated the costs to avoid an adverse event. But for a health insurance company, this way of describing cost-effectiveness makes it difficult to compare with the cost-effectiveness of other drugs for other diseases. Reimbursement authorities therefore often require a so-called *cost-utility analysis* in which the extra costs to gain one quality-adjusted life-year (QALY) are estimated. Since the QALY represents a generic measure of overall health that can be improved by increasing life expectancy and/or quality of life, the cost per QALY gained can therefore be applied for any health technology for any disease area.

One of the first estimates of the cost-effectiveness of genotyping warfarin users was published in 2003. These authors estimated that the cost to avoid one bleeding event were US$5940 [120] if patients were given a dose based on their *CYP2C9* genotype, compared with standard care. Very similar results were obtained by You et al., who calculated a cost-effectiveness ratio of US$5778 per bleeding event avoided [121]. Schalekamp et al. reported that the cost-effectiveness of genotyping acenocoumarol users for their *CYP2C9* genotype was US$5151 per bleeding event avoided [122]. This study focused on the Netherlands, while the other two studies focused on the

US. After the relevance of the *VKORC1* genotype was demonstrated, it was assumed that genotyping the patient for both *CYP2C9* and *VKORC1* genotypes would lead to better dose prediction and therefore a larger effect of pharmacogenetic-guided dosing than genotyping for *CYP2C9* alone. More recent cost-effectiveness analyses therefore also included *VKORC1* genotyping in their assessment. Several authors estimated the cost-utility ratio of genotyping for these two genes compared with standard care in the US [123–127] and reported results that vary from US$60,750 to US$347,000 per QALY gained. Eckman and co-workers performed a meta-analysis of the three trials that were available in 2008 [110–112] and found that pharmacogenetic-guided dosing could reduce the risk of bleeding by 32 % [124]. When they used this data in their economic model, they found that genotyping would cost US$170,000 per QALY gained, a value much higher than the willingness-to-pay thresholds of US$50,000–US$100,000 that are often applied in the US to conclude whether or not an intervention is cost-effective [128]. Sensitivity analyses by Eckman et al. showed that the costs per QALY gained would be less than US$50,000 only if the test would be restricted to patients with a high bleeding risk or if all of the following criteria were met: more bleeding events could be avoided, the test would cost less than US$200 and the results would be available within 1 day. Patrick and co-workers also found that genotyping only patients with a high bleeding risk would increase its chance of being cost-effective [126]. Meckley and co-workers used data from the CoumaGen trial [112] and found a cost-effectiveness ratio of US$60,740 per QALY gained [127]. You et al. reported a much higher cost per QALY gained than previous studies (US$347,000) as well as high costs per life saved (US$1,106,000 per life saved) and high cost per adverse event averted (US$170,000), which combined bleeding events with thromboembolic events [123]. The chance that genotyping would cost less than the US$50,000 threshold was low (38 %) and increased with lower genotyping costs, greater reduction in out-of-range INRs and in specific settings where poor INR control was seen. Using data from the CoumagenII trial [116], in which the time in therapeutic range in the first month was increased by 11 % in the first month, Verhoef et al. reported that pharmacogenetic-guided phenprocoumon dosing would be cost-effective [129] given a cost per QALY gained of 2700 euro.

Recently, novel oral anticoagulant drugs such as dabigatran, rivaroxaban and apixaban have been developed, which appear to be good alternatives to coumarin anticoagulants [130]. You et al. studied the cost-effectiveness of dabigatran and genotype-guided warfarin treatment and showed that dabigatran seems to be a cost-effective treatment [131]. However, they reported that pharmacogenetic-guided warfarin dosing had a higher chance of being cost-effective if it was able to increase the percentage time in target INR range to > 77 %.

The main limitation of the cost-effectiveness studies published up to now has been the lack of robust data from appropriately powered clinical trials [132]. Also, the costs of genotyping *VKORC1* and *CYP2C9* polymorphisms are not clear yet. Previous studies have used costs that vary from US$175 to US$575 when the genotype is determined in the lab and US$50 for a point-of-care test [127, 132, 133]. These costs are expected to decrease over time and with increased usage, which will influence the cost-effectiveness as well. In the analysis by Verhoef and

co-workers, the use of a point-of-care test was assumed, which provides the results within 2 h and costs less than US$50 [133]. In sum, most of the studies found that, pharmacogenetic-guided dosing did not seem to be cost-effective and their results underline the large influence of effectiveness of genotyping and the costs of the test. Genotype-guided dosing will only be cost-effective if the costs of the test can be kept low or if it has a large effect on INR control and related incidences of adverse events. The results also show that genotyping could be cost-effective if it would be used only with specific patients (with a high bleeding risk) or in specific settings (with a low quality of INR control).

A more reliable estimate of the cost-effectiveness or cost-utility of pharmacogenetic-guided coumarin dosing can be calculated after the results of the large RCTs become available. Because of many differences between countries in costs and organisation of anticoagulation services, the cost-effectiveness of genotyping coumarin users probably varies between countries [54]. Therefore it will also be necessary to carry out country-specific analyses in the future.

7 Conclusion

Coumarins are effective drugs for treatment and prevention of thromboembolic events. However, their use requires a delicate balancing act between the chance of underdosing (which increases the risk of thromboembolic events) and the chance of overdosing (which increases the risk of haemorrhages). It has been shown that polymorphisms in *VKORC1* and *CYP2C9* explain a large part (35–50%) of the dose variability but patient characteristics and environmental factors also play a role. Clinical trials have researched the added value and cost effectiveness of pre-treatment genotyping. The results from the trials were not convincing, and at this moment there is not enough evidence to recommend genotyping for CYP2C9 and VKORC1 in routine clinical practice. Recent cost-effectiveness studies have shown that the small improvement of time in therapeutic range does not weigh against the costs of genotyping all patients. However, the cost-effectiveness of the intervention will depend on the costs of genotyping and on the availibility of other anticoagulation therapy such as the Direct Oral Anticoagulants (DOACs) [118].

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