BJCP British Journal of Clinical Pharmacology

DOI:10.1111/bcp.12220

Pharmacogenetic-guided dosing of coumarin anticoagulants: algorithms for warfarin, acenocoumarol and phenprocoumon

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Keywords
acenocoumarol, CYP2C9, pharmacogenetics, phenprocoumon, VKORC1, warfarin

Received
13 March 2013

Accepted
17 July 2013

Accepted Article Published Online
7 August 2013

Coumarin derivatives, such as warfarin, acenocoumarol and phenprocoumon are frequently prescribed oral anticoagulants to treat and prevent thromboembolism. Because there is a large inter-individual and intra-individual variability in dose–response and a small therapeutic window, treatment with coumarin derivatives is challenging. Certain polymorphisms in *CYP2C9* and *VKORC1* are associated with lower dose requirements and a higher risk of bleeding. In this review we describe the use of different coumarin derivatives, pharmacokinetic characteristics of these drugs and differences amongst the coumarins. We also describe the current clinical challenges and the role of pharmacogenetic factors. These genetic factors are used to develop dosing algorithms and can be used to predict the right coumarin dose. The effectiveness of this new dosing strategy is currently being investigated in clinical trials.

Introduction

Coumarin derivatives are oral anticoagulants that are prescribed frequently to treat and prevent thromboembolism [1]. This group of drugs was discovered when several cows suffered fatal bleeding after eating stacks of spoiled sweet clover hay in the 1920s [2]. After several years, researchers were able to isolate and synthesize the first coumarin dicumarol. A more potent form of this drug, warfarin, initially used as rat poison, was introduced as an oral anticoagulant in the 1950s and is currently the most widely used oral anticoagulant. Because warfarin and other coumarin derivatives inhibit the vitamin K dependent synthesis of biologically active clotting factors, they are also called vitamin K antagonists.

In this review we will describe the use of different coumarin derivatives, pharmacokinetic characteristics of these drugs and differences amongst the coumarins. We will also describe the current clinical challenges and the role of pharmacogenetic factors. These genetic factors are included in dosing algorithms, which can be used to predict the right coumarin dose for an individual patient. The effectiveness of these new dosing algorithms is currently being investigated in clinical trials.

Coumarin anticoagulants; indications

Coumarins are prescribed for different indications such as treatment and prevention of deep vein thrombosis or pulmonary embolism or prevention of systemic embolism or stroke in patients with prosthetic heart valves or atrial fibrillation [1]. Atrial fibrillation is the most frequent indication and has an estimated prevalence in developed countries of 1.5 to 2% [3]. Patients with this cardiac

arrhythmia have an increased risk of stroke and systemic embolism and warfarin use can reduce this risk by approximately 60% [4]. Anticoagulant therapy is therefore recommended in all patients with atrial fibrillation, except for patients with a very low stroke risk (CHA_2DS_2-VASc score < 1, i.e. patients with no other risk factors such as congestive heart failure, hypertension, age > 65 years, diabetes mellitus, previous stroke or vascular disease) [3].

Patients with a prosthetic heart valve have an increased risk of thromboembolism, caused by an altered blood flow and activation of the coagulation system by exposure of the blood to artificial surfaces [5]. In a systematic review of observational studies major embolism occurred at a rate of 4 per 100 patient-years. This risk was reduced by approximately 75% when patients used a coumarin derivative [6]. Warfarin is more effective in the prevention of thromboembolic events than platelet-inhibitor therapy with aspirin [7]. A combination of a coumarin derivative and an antiplatelet drug has been shown to be even more effective in reducing the risk of death and thromboembolism than a coumarin derivative alone [8].

Venous thromboembolism (deep vein thrombosis or pulmonary embolism) often occurs as a complication after knee or hip replacement surgery [9]. The use of warfarin after discharge from the hospital reduces the risk of this complication [10]. When patients develop a deep vein thrombosis or pulmonary embolism, treatment with an oral anticoagulant is also indicated. Because it takes some time before the normal coagulation factors are cleared from the plasma the effect of coumarins is achieved after a several days. Patients with venous thromboembolism should therefore also start with a low molecular weight heparin for the first few days [11].

Current practice

Because the dose–response can vary between patients (inter-individual variability) and varies over time within one patient (intra-individual variability), frequent monitoring of the anticoagulant effect is required. This can be done by measuring the prothrombin time expressed as the International Normalized Ratio (INR) [12]. For people not using any anticoagulant, this INR should be 1.0. In most countries, the target INR range for patients with atrial fibrillation or venous thromboembolism is 2.0–3.0, which means that the *in vitro* coagulation takes two to three times longer if compared with subjects not using coumarins [13]. For some indications like prosthetic heart valves a higher target range is used (2.5–4.0) [14].

When patients have to initiate coumarin therapy, a standard loading dose is frequently prescribed for the first few days to reach the therapeutic concentration more rapidly. After a few days, the patient's INR is measured to check the response to the coumarin and the dose is adjusted accordingly. When the patient has reached a stable INR within the target range and a stable dose, on average INR measurements will be repeated every 4–6 weeks.

In most countries treatment with coumarin derivatives is managed by the GP or in the hospital (routine practice). In some countries, for example Spain and the Netherlands, the treatment is managed by specialized anticoagulation clinics [15, 16]. The quality of care, assessed as percentage time spent in the therapeutic range, is higher in anticoagulation clinics than in routine practice. In a systematic review and meta-regression in 2006, the percentage time spent in the therapeutic range was 58% in routine practice and 66% in anticoagulation clinics [17]. In the Netherlands all coumarin users, when treated outside the hospital, are treated by an anticoagulation clinic and the target range for patients with atrial fibrillation or venous thromboembolism is 2.0–3.5. The percentage time in this range was as high as 80% for patients using long term anticoagulants in 2011 [18]. Management by anticoagulation clinics has been found to be cost-effective compared with routine practice [19].

Patients using long term anticoagulant therapy can find it bothersome to visit the clinic often for INR measurement. Many of these patients prefer self-monitoring, which is possible using a finger prick point of care test. When a patient only self-tests, the result is forwarded to the physician who will determine the next coumarin dose. With patient self-management, the patients can also adjust the dose themselves, after sufficient training [20].

Challenges

Coumarin derivatives have a small therapeutic window. When the dose is too low, the risk of thromboembolic events remains high and the drug is not effective. When the dose is too high, the risk of bleeding is increased [21]. Bleeding events are the most frequent serious adverse effects of coumarin derivatives. These events can vary from mild haematoma to life-threatening or fatal intracranial haemorrhage. In addition, there is a large inter-individual and intra-individual variability in dose–response. Therefore, giving patients the right dose is challenging. The daily dose can vary up to 10-fold between patients for warfarin (1.5 to 14 mg) as well as for acenocoumarol (1 to 9 mg) or phenprocoumon (0.75–9 mg) [22]. Coumarin use therefore often results in drug-related hospitalization [23, 24].

Which dose is required for a certain patient depends on several factors. The dose can vary between patients because of differences in, for example, age, height, weight, gender, concomitant medication and comorbidities [25–27]. Older patients generally require a lower dose, taller or heavier patients a higher dose. Genetic factors also play an important role here and will be discussed in detail later in this review. The required dose can also vary over time within one patient because of changes in concomitant

medication, diet or health status (fever, vomiting etc.) [28–30]. Many interactions with other drugs exist because of inhibition or induction of the CYP2C9 enzyme [31]. Adherence changes of coumarins, as in many other drugs, also influence the response to the anticoagulants [32].

Pharmacokinetics and differences between coumarins

In Europe, different coumarin derivatives are used of which warfarin, acenocoumarol and phenprocoumon are most frequently prescribed [33]. All coumarin derivatives are 4-hydroxycoumarins. Each coumarin has a single, chiral centre with an S- or an R-enantiomeric form. The drugs are administered as racemic mixtures consisting of 50% of each enantiomer [31]. Although the working mechanism of these drugs is similar, there are some important differences in pharmacokinetics between warfarin, acenocoumarol and phenprocoumon.

All coumarins (except S-acenocoumarol) are absorbed from the gastrointestinal tract with almost complete oral bioavailability. S-acenocoumarol undergoes extensive first pass metabolism. Within a few hours, peak plasma concentrations are reached [31]. Approximately 98–99% of the coumarin is bound to plasma albumin [22]. Metabolism into inactive metabolites takes place in the liver by various hydroxylation reactions, catalyzed by cytochrome P450 (CYP) enzymes.

S-warfarin (the most active form) is mainly metabolized by CYP2C9. R-warfarin is metabolized by several other CYP isoforms [34]. CYP2C9 is also the principal metabolizing enzyme of both acenocoumarol enantiomers, but plays a less important role in phenprocoumon metabolism, where CYP3A4 is also involved [35, 36]. Of these three coumarins, phenprocoumon has the longest elimination half-life of 110–130 h [37]. Warfarin half-life varies from 24–33 h for S-warfarin to 35–58 h for R-warfarin [38]. Acenocoumarol has the shortest half-life. Although the S-enantiomer is more active, the anticoagulation effect of acenocoumarol mainly depends on the R-enantiomer, because of the short half-life of S-acenocoumarol (1.8 h). The elimination half-life of R-acenocoumarol is 6.6 h [39].

Pharmacogenetics

Genetic variants play an important role in the large variation in dose requirements. Certain polymorphisms in two genes (*CYP2C9* and *VKORC1*) can explain approximately one-third of the dose variation [40, 41]. The contribution of *VKORC1* to the variation in dose requirement is larger (approximately 30%) than the contribution of *CYP2C9* (usually less than 10%) [22].

CYP2C9 – pharmacokinetics

Soon after Rettie *et al*. identified CYP2C9 as the main metabolizing enzyme of warfarin in 1992 [42], the effect of the *2 polymorphism on the dose requirement was shown [43]. Aithal *et al*. first described that both *2 and *3 allele carriers required a lower dose and had an increased risk of bleeding [44]. Since publication of this study, many others have investigated the effect of these polymorphisms on warfarin dose requirement and other related outcomes (overanticoagulation, bleeding etc.). Table 1 summarizes some of the evidence on the association between *CYP2C9* genotypes and coumarin dose or bleeding risk. A meta-analysis of pharmacogenetic studies on warfarin revealed that the reduction in warfarin dose requirement varied from 20% for heterozygous carriers of a *2 allele to 78% in homozygous carriers of a *3 allele compared with wild-types [45]. In the studies measuring bleeding risk, carrying

Table 1

Association between *CYP2C9* genotypes and dose or bleeding risk

Reference	Country	*n*	Study type	Association
Warfarin				
Lindh *et al*. 2009 [45]	Various	39 studies	Meta-analysis	Dose reduction:*1*2: 20%, *1*3: 34%, *2*2: 36%, *2*3: 57%, *3*3: 78%
Sanderson *et al*. 2005 [46]	Various	2 or 3 studies	Meta-analysis	Dose reduction *2: 17%, *3: 37% Bleeding risk *2 RR: 1.91, *3 RR: 1.77, *2 or *3: RR 2.26
Acenocoumarol				
Tassies *et al*. 2002 [49]	Spain	325	Observational	Dose reduction *1*2: 16%, *1*3: 36%, *2*2: 1%, *2*3: 27%
Schalekamp *et al*. 2004 [47]	The Netherlands	231	Observational	Dose reduction *2: 1%, *3: 20%
Visser *et al*. 2004 [48]	The Netherlands	1124	Observational	Dose reduction *1*2: 13%, *1*3: 20%, *2*2: 28%, *2*3: 40%
Visser *et al*. 2004 [51]	The Netherlands	996	Observational	Major bleeding risk variant carriers: HR: 1.83
Phenprocoumon				
Hummers *et al*. 2003 [54]	Germany	185	Observational	Bleeding risk *2: OR 0.35, *3: OR 3.10
Schalekamp *et al*. 2004 [53]	The Netherlands	284	Observational	Dose reduction *2: 21%, *3: 25%
Visser *et al*. 2004 [48]	The Netherlands	1124	Observational	Dose reduction *1*2: 10%, *1*3: 17%, *2*2: 33%. In *2*3 patients (n = 3) dose increased by 9%

one or more *CYP2C9* variant alleles was associated with an approximate doubling of bleeding risk compared with the wild-type [46]. Since *CYP2C9* variants influence the pharmacokinetics of coumarins, it is possible that the risk of bleeding in patients carrying a variant allele is not only increased because of the lower dose requirement, but also because of a slower response to changes in dose.

Although less has been published about *CYP2C9* genotypes and acenocoumarol dose than about warfarin, there are several studies confirming the associations found with warfarin, genotypes and bleeding risk for acenocoumarol. The presence of a *CYP2C9* *3 allele reduces the metabolism of the normally clinically inactive S-acenocoumarol and thereby increases the half-life of this enantiomer [39]. Mean acenocoumarol dose requirement is therefore 19–29% lower in carriers of this allele than in wild-types [47], but also 13–15% lower in carriers of a *2 allele [48, 49]. The risk of overanticoagulation is increased in *3 carriers [47, 49, 50]. One study found an increased risk of major bleeding which was seen in *2 and *3 carriers with a hazard ratio of 1.83 [51].

Because CYP2C9 is not the principal metabolizing enzyme of phenprocoumon, one might expect that it would have a less pronounced effect in the pharmacogenetics of phenprocoumon than for warfarin or acenocoumarol [52]. However, Schalekamp *et al*. found a 22–25% decreased dose requirement in *CYP2C9* variant carriers [53]. In one study, both minor and major bleeding risk was increased (OR 3.10) in *3 carriers [54].

VKORC1 – pharmacodynamics

In 2004 the gene coding for the target enzyme of coumarins, Vitamin K epoxide reductase complex subunit 1 (VKORC1), was identified [55, 56]. Since 2005, many authors have studied the effect of *VKORC1* polymorphisms on warfarin and other coumarin doses. A number of polymorphisms in this gene have been studied. Some rare mutations in *VKORC1* are associated with warfarin resistance [57]. More common are mutations that are associated with insensitivity through altered VKORC1 expression. The −1639G>A, in tight linkage disequilibrium with 1173C>T, is associated with the widest range of variation in gene expression and hence enzyme activity within a number of different populations [58]. In a recent meta-analysis, the difference in warfarin dose in relation to genotype for the −1639 polymorphism was compared for a Caucasian and an Asian population [59]. From their results we could calculate that Caucasian patients with one −1639 A allele required a 25% lower dose and patients with two −1639 A alleles a 50% lower dose than patients without this variant allele. This effect was also present in Asian patients, although it was smaller (14 and 38% lower doses, respectively).

Several authors have shown that acenocoumarol dose is also influenced by *VKORC1* genotype. Reitsma *et al*. had already shown in 2005 that Dutch patients carrying one or two variant alleles for the 1173 polymorphism required a 28% and 47% lower dose, respectively, when compared with wild-types [60]. In Greek acenocoumarol users, heterozygous carriers of a variant allele required a 19% lower dose and homozygous carriers a 63% lower dose [61]. Similar percentages were found in a German and Austrian population (25% and 52%) [62], in a Serbian population (27% and 62%) [63] and amongst Lebanese acenocoumarol users (34% and 50%) [64].

Reitsma *et al*. also investigated the influence of *VKORC1* polymorphism on the phenprocoumon dose. Patients with a CT genotype at position 1173 had a 10% lower dose and patients with a TT genotype a 52% lower dose than wild-types (CC) [60]. This effect was also seen in several German and Austrian studies. The dose in phenprocoumon users with one variant *VKORC1* allele was 19–31% lower than in wild type users and 43–51% lower in users with two variant alleles [62, 65–67].

Table 2 summarizes the current evidence on the association between *VKORC1* genotypes and coumarin dose or bleeding risk. Reitsma *et al*. showed an increased bleeding risk in carriers of a *VKORC1* T1173 allele. This effect was larger in phenprocoumon (OR 2.6) than in acenocoumarol (OR 1.2) [60]. Although *VKORC1* genotype was associated with overanticoagulation in a study of warfarin users by Wadelius *et al*., no effect on bleeding risk was found for *VKORC1* polymorphism [40]. In a study by Montes *et al*. the risk of gastrointestinal bleeding was increased in acenocoumarol users carrying a *VKORC1* polymorphism [68]. In a more recent study, the risk of bleeding was also increased in warfarin users with a *VKORC1* variant allele (incidence of 4.9% in AA, 2.3% in AG and 0.47% in GG patients) [69]. However, this increase in risk was limited to the first month of treatment. An increased risk of overanticoagulation (and thereby indirectly an increased bleeding risk) in *VKORC1* variant carriers was also observed in phenprocoumon (limited to the first month also) and acenocoumarol (limited to the first 3–6 months) users [70, 71].

A recent study showed that polymorphisms in *VKORC1* −1639G>A also influence the response to acute vitamin K supplementation in over-anticoagulated patients. The INR decreased faster in patients carrying the G allele [72].

Other genes

The association between coumarin dose and other genes besides *CYP2C9* or *VKORC1* has also been investigated. For example, an effect has been found for *GGCX*, encoding the enzyme catalyzing the carboxylation of vitamin K dependent clotting factors [73], for *APOE*, encoding the vitamin K liver uptake facilitating ligand apolipoprotein E [74], for

Table 2

Association between *VKORC1* genotypes and dose or bleeding risk

Reference	Country	*n*	Study type	Association
Warfarin				
Wadelius *et al*. 2009 [40]	Sweden	1496	Observational	Bleeding risk: no difference between *VKORC1* genotypes
Yang *et al*. 2010 [59]	Various	19 studies	Meta-analysis	Dose reduction: Caucasians: AG: 25%, AA: 50%, Asians: AG: 14%, AA: 38%
Lund *et al*. 2012 [69]	Scotland	557	Observational	Bleeding incidence: GG 0.47%, AG 2.3%, AA 4.9%
Acenocoumarol				
Reitsma *et al*. 2005 [60]	The Netherlands	330	Observational	Dose reduction AG: 28%, AA: 47% Bleeding risk: OR 2.6 in variant carriers
Markatos *et al*. 2008 [61]	Greece	98	Observational	Dose reduction AG: 19%, AA: 63%
Montes *et al*. 2008 [68]	Spain	266	Observational	Gastro intestinal bleeding risk AG: OR 1.18, AA: OR 1.51
Cadamuro *et al*. 2010 [62]	Austria	206	Observational	Dose reduction AG: 25%, AA: 52%
Kovac *et al*. 2010 [63]	Serbia	200	Observational	Dose reduction AG: 27%, AA: 62%
Esmerian *et al*. 2011 [64]	Lebanon	133	Observational	Dose reduction AG: 34%, AA: 50%
Phenprocoumon				
Reitsma *et al*. 2005 [60]	The Netherlands	330	Observational	Dose reduction AG: 10%, AA: 52% Bleeding risk: OR 1.2 in variant carriers
Qazim *et al*. 2009 [66]	Austria	53	Observational	Dose reduction AG: 29%, AA: 49%
Cadamuro *et al*. 2010 [62]	Austria	206	Observational	Dose reduction AG: 21%, AA: 51%
Puehringer *et al*. 2010 [65]	Austria and Germany	185	Observational	Dose reduction AG: 19%, AA: 43%
Geisen *et al*. 2011 [67]	Germany	75	Observational	Dose reduction AG: 31%, AA: 50%

PROC, encoding protein C, which inactivates clotting factor Va and VIIIa [75], for *CYP4F2*, encoding the CYP enzyme that metabolizes vitamin K [76] and for *GATA-4*, encoding the transcription factor involved in the regulation of *CYP2C9* [77]. However, these effects could not always be replicated, or explained only a very small part of the dose variation.

Genotype-guided dosing algorithms for warfarin

The first dosing algorithms incorporating *CYP2C9* genotype were published in 2004 [78–80]. The algorithm by Gage *et al*. was the most extensive and included, in addition to *CYP2C9* genotype, age, body surface area, gender, race, target INR, amiodarone use and simvastatin use. The algorithm explained 39% of the variation in daily warfarin dose. Since that time, more than 30 algorithms have been published based on both *CYP2C9* and *VKORC1* genotype (Table 3). Sconce *et al*. published one of the first algorithms, including *CYP2C9* and *VKORC1* genotypes as well as age and height [81]. This algorithm explained 54% of the warfarin dose variation in a British population. *CYP2C9* genotype alone explained 17.5% of the variation and *VKORC1* genotype 15%. The algorithm by Carlquist *et al*. was developed in an American population and included *CYP2C9* and *VKORC1* polymorphisms, age, weight and gender (r^2 = 0.45) [82]. In 2008, Gage *et al*. published an updated algorithm including *CYP2C9* and *VKORC1* genotype, but also age, body surface area, amiodarone use, target INR, race and smoking status [83]. In a Caucasian population this algorithm explained 57% of the dose variation, but the predictive value was lower (31%) in African-Americans. Wadelius *et al*. were able to explain almost 59% of the variation in a Swedish population, using information on both genotypes, age, race, gender and the number of interacting drugs capable of increasing the INR [40]. The univariate r^2 of *CYP2C9* genotype was approximately 12% and that of *VKORC1* 29%. The International Warfarin Pharmacogenetics Consortium (IWPC) created an algorithm in a more diverse population from nine countries in four continents [84]. Forty-seven percent of the dose variation was explained by *CYP2C9, VKORC1*, age, height, weight, amiodarone use, race and number of CYP enzyme inducers. An alternative measure to the percentage of variation explained by the algorithm (r^2) is the mean absolute error (MAE), although this is not reported for all algorithms. Table 3 also shows this measure for the studies where this measure was reported.

For warfarin, many more algorithms have been published in different populations from several countries, such as the USA [85], UK and Canada [86, 87], Italy [88, 89], Slovenia [90], Singapore [91], Japan [92–94], Korea [95, 96], China [97–100], Indonesia [101], India [102], Oman [103], Brazil [104] and Puerto Rico [105]. Most of these studies have included *VKORC1* and *CYP2C9* genotypes, but some have also included *CYP4F2*, *CCCG* and *APOE* genotypes [89, 99].

The formulae from these studies made it possible to calculate a warfarin maintenance dose. However, only a handful of studies have looked at algorithms for other types of coumarin doses. Avery *et al*. also described how to derive an initiation dose from an adapted version of the

Table 3

Published algorithms to predict the required coumarin dose

Reference	Country	*n*	Type	Genetic parameters	Clinical parameters	R^2	MAE (mg day^{-1})
Warfarin							
Gage *et al.* 2004 [79]	USA	369	M	CYP2C9	Age, gender, BSA, race, target INR, CM	39%	–
Hillman *et al.* 2004 [78]	USA	453	M	CYP2C9	Age, BSA, valve replacement, diabetes	34%	–
Kamali *et al.* 2004 [80]	UK	121	M	CYP2C9	Age	20%	–
Sconce *et al.* 2005 [81]	UK	297	M	CYP2C9, VKORC1	Age, height	54%	–
Carlquist *et al.* 2006 [82]	USA	213	M	CYP2C9, VKORC1	Age, gender, weight	45%	–
Herman *et al.* 2006 [90]	Slovenia	165	M	CYP2C9, VKORC1	Age, BSA	60%	–
Takahashi *et al.* 2006 [92]	Japan	365	M	CYP2C9, VKORC1	Age, weight	57%	–
Tham *et al.* 2006 [91]	Singapore	107	M	CYP2C9, VKORC1	Age, weight	60%	–
Gage *et al.* 2008 [83]	USA	1015	M	CYP2C9, VKORC1	Age, BSA, race, target INR, CM, smoking	57%	1.3
Perini *et al.* 2008 [104]	Brazil	390	M	CYP2C9, VKORC1	Age, weight, heart valve prosthesis, thromboembolic disease, CM	50%	0.99
Wu *et al.* 2008 [85]	USA	92	M	CYP2C9, VKORC1	Age, gender, weight, height, race, CM, smoking	59%	–
IWPC 2009 [84]	Various	4043	M	CYP2C9, VKORC1	Age, height, weight, race, CM	47%	1.19
Huang *et al.* 2009 [97]	China	266	M	CYP2C9, VKORC1	Age, BSA	45%	–
Sasaki *et al.* 2009 [93]	Japan	45	M*	CYP2C9, VKORC1	*	94%*	–
Wadelius *et al.* 2009 [40]	Sweden	1496	M	CYP2C9, VKORC1	Age, gender, race, CM	59%	–
Harada *et al.* 2010 [94]	Japan	97	M	CYP2C9, VKORC1, CYP4F2	Age, white blood cell count, CM	49%	–
Lenzini *et al.* 2010 [107]	Various	969	R	CYP2C9, VKORC1	Age, BSA, race, stroke, target INR, diabetes, CM, dose and INR values	60%	0.79
Wells *et al.* 2010 [87]	Canada	249	M	CYP2C9, VKORC1, CYP4F2	Age, BMI, height, exercise level, CM	58%	–
Avery *et al.* 2011 [106]	UK	671	I	CYP2C9, VKORC1	Age, height, weight, CM	42%	–
Cho *et al.* 2011 [95]	Korea	130	M	CYP2C9, VKORC1	Age, BSA, CM	60%	–
Choi *et al.* 2011 [96]	Korea	564	M	CYP2C9, VKORC1, CYP4F2, GGCX	Age, BSA, gender, INR	35%	–
Gong *et al.* 2011 [86]	UK and Canada	167	I and M	CYP2C9, VKORC1, CYP4F2	Age, weight, gender, CM	42%	1.49
Suriapranata *et al.* 2011 [101]	Indonesia	85	M	CYP2C9, VKORC1	Age, weight, height	21%	–
You *et al.* 2011 [98]	China	100	M	CYP2C9, VKORC1	Age, weight, vitamin K intake	68%	–
Zambon *et al.* 2011 [89]	Italy	274	M	CYP2C9, VKORC1, CYP4F2	Age, BSA	65%	0.97
Cini *et al.* 2012 [88]	Italy	55	M	CYP2C9, VKORC1	Age, height, weight, gender, smoking, vegetable intake, indication, diabetes	44%	1.42
Horne *et al.* 2012 [108]	Various	2022	R	CYP2C9, VKORC1	Age, BSA, CM, stroke, target INR, dose and INR values	72%	0.71
Pathare *et al.* 2012 [103]	Oman	212	M	CYP2C9, VKORC1	Age, weight, gender, indication	62%	0.26
Pavani *et al.* 2012 [102]	India	240	M	CYP2C9, VKORC1	Age, BMI, gender, vitamin K intake	89%	–
Ramos *et al.* 2012 [105]	Puerto Rico	163	M	CYP2C9, VKORC1	Age, indication, CM, dose-adjusted INR	67%	0.79
Wei *et al.* 2012 [99]	China	325	M	CYP2C9, VKORC1, CYP4F2	Age, weight, previous thromboembolism, CM	52%	–
Xu *et al.* 2012 [100]	China	207	R	CYP2C9, VKORC1, CYP4F2	Age, BSA, target INR and INR values	54%	0.59
Acenocoumarol							
Markatos *et al.* 2008 [61]	Greece	98	M	CYP2C9, VKORC1	Age, gender, CM	55%	–
Van Schie *et al.* 2011 [27]	The Netherlands	375	I and M	CYP2C9, VKORC1	Age, height, weight, gender, CM	53%	0.52
Borobia *et al.* 2012 [111]	Spain	147	M	CYP2C9, VKORC1, CYP4F2, APOE	Age, BMI, CM	61%	0.52
Rathore *et al.* 2012 [110]	India	125	M	CYP2C9, VKORC1, CYP4F2, GGCX	Age, weight, height, BSA, gender smoking, indication	41%	0.71
Cerezo-Manchado *et al.* 2013 [112]	Spain	973	M	CYP2C9, VKORC1, CYP4F2	Age, BSA, gender	50%	–
Phenprocoumon							
Van Schie *et al.* 2011 [27]	The Netherlands	559	I and M	CYP2C9, VKORC1	Age, height, weight, gender, CM	56%	0.45
Geisen *et al.* 2011 [67]	Germany	75	M	VKORC1	Age, weight	49%	–

*PKPD model. M, Maintenance dose; R, Refinement; I, Initiation dose; CM, concomitant medication; MAE, mean absolute error.

IWPC algorithm [106]. Gong *et al*. reported both a pharmacogenetic loading and maintenance dose in their publication [86]. When a patient initiates warfarin on a pharmacogenetic-guided dose, it is difficult to know how to adjust this dose after INR measurement. In 2010, a dose refinement algorithm was developed in a combined population from the USA, UK, Sweden and Thailand making use of the first INR measurement [107]. Later, the same group published an algorithm using INR information from days 6 to 11 [108].

Genotype-guided dosing algorithms for acenocoumarol and phenprocoumon

Considerably less has been published on pharmacogenetic-guided algorithms for acenocoumarol and phenprocoumon doses compared with warfarin doses (Table 3). van Schie *et al*. developed a genotype-guided algorithm for both acenocoumarol and phenprocoumon in a Dutch population [27]. The authors also provided loading doses related to the calculated maintenance dose and validated the acenocoumarol algorithm later in a different Dutch population which yielded an r^2 of 52.7% [109]. Other acenocoumarol algorithms were developed in Greek [61], Indian [110] and Spanish [111, 112] populations. [61,110–112] For phenprocoumon, only one other study has developed an algorithm [67]. In a small (n = 75) German population *VKORC1* genotype, age and weight explained 48.6% of the daily phenprocoumon dose variability. *CYP2C9* genotype was not associated with phenprocoumon dose in this study. In the study by van Schie *et al*. the predictive value of this gene was 4.5%, similar to that of acenocoumarol [27].

Evaluation of effectiveness of genotype-guided dosing

Pharmacogenetic-guided dosing was first evaluated by Voora *et al*. [113]. In this study the safety and feasibility of using the dosing algorithm of Gage *et al*. (2004) [79] was investigated in 48 patients. The authors found that this dosing regimen was feasible and improved the time to stable dose in carriers of a *CYP2C9* variant allele. The risk of supratherapeutic INR values was not decreased in this group. A few months later, the first (pilot) randomized trial in 38 patients was published [114]. These authors drew a similar conclusion, reporting that genotyping seemed to be feasible and acceptable to patients and providers. No differences were found in percentage time in INR range or the risk of supratherapeutic INR values. Another randomized trial with 191 patients also investigated the added value of a dose based on *CYP2C9* genotype and found that the time to stable dose was decreased and the time spent in the therapeutic range was increased in the intervention group *vs*. the control group [115].

Anderson *et al*. [116] were the first to investigate the impact of genotyping for both *CYP2C9* and *VKORC1* genotypes, using the algorithm of Carlquist *et al*. [82] and a weighted overview of other observational studies. In this study, 220 patients were included and the patients in the intervention arm required fewer INR measurements and dose adjustments than in the control arm. However, no effect on the number of out of range INR values could be demonstrated when looking at all patients. In wild-type patients and patients carrying multiple variant alleles, genotyping decreased the risk of out of range INRs by 10%. In a randomized controlled study published in 2009, 121 Chinese patients undergoing heart valve replacement surgery were included [97]. Patients who received a dose based on the genotype-guided algorithm spent more time within the target range and required less time to reach a stable dose than patients receiving a standard dose. In China, the standard initiation dose is 2.5 mg day^{-1}. Another Chinese randomized trial using the same algorithm was published in 2012 [117]. The group receiving a loading dose according to this pharmacogenetic algorithm (n = 50) reached stable dosing faster than the group receiving a standard loading dose of 2.5 mg day^{-1} (n = 51).

Several non-randomized prospective studies on pharmacogenetic-guided dosing of warfarin using both *VKORC1* and *CYP2C9* have also been published. Wen *et al*. showed that genotyping for these genes could help to decrease the time to stable dose, although this study did not have a control group [118]. In the study by Lenzini *et al*. the percentage time in the therapeutic range was higher in the genetic group and the risk of adverse events lower compared with the clinical control group [119]. McMillin *et al*. compared [120] two parallel cohorts, one receiving a standard dose and the other receiving a dose based on the algorithm by Sconce *et al*. [81], and found that the outcomes in the two parallel cohorts were not statistically significantly different. In another study, patients in a historical control cohort were more frequently hospitalized for bleeding or thromboembolism than patients whose genotype was reported to the physician [121]. Gong *et al*. found that the differences in time to therapeutic range between genotypes were eliminated when patients were dosed according to a genotype-guided algorithm, but a comparison with a control group was not possible [86].

In a more recent randomized controlled trial by Burmester *et al*., dosing using a pharmacogenetic algorithm was not compared with standard care, but to a clinical algorithm [122]. In both arms, the initial warfarin doses were closer to the stable therapeutic dose than they would have been on a standard dose of 5 mg day^{-1}. No differences between the two arms were found for percentage time in therapeutic range. Also Anderson *et al*. compared two algorithms, both genotype-guided, and could not

Table 4

Current evidence on pharmacogenetic-guided dosing of warfarin

Reference	Country	*n*	Type	Genotypes	Comparator	Effect of genotype-guided dosing
Voora *et al*. 2005 [113]	USA	48	Prospective cohort	CYP2C9	None	Decreased time to stable dose, no effect on overanticoagulation
Hillma *et al*.n 2005 [114]	USA	38	RCT	CYP2C9	Standard care	No effect on % time in therapeutic range or overanticoagulation
Anderson *et al*. 2007 [116]	USA	220	RCT	CYP2C9, VKORC1	Standard care	Fewer INR measurements and dose adjustments were required, no overall effect on out-of-range INRs
Caraco *et al*. 2008 [115]	Israel	191	RCT	CYP2C9	Standard care	Time to stable dose was decreased, time spent in therapeutic range was increased
Wen *et al*. 2008 [118]	Taiwain	108	Prospective cohort	CYP2C9, VKORC1	None	Decreased time to stable dose
Lenzini *et al*. 2008 [107]	USA	412	Prospective cohort	CYP2C9, VKORC1	Clinical algorithm	Increased % time in therapeutic range, decreased risk of adverse events
Huang *et al*. 2009 [97]	China	121	RCT	CYP2C9, VKORC1	Standard care	Decreased time to stable dose, no effect on overanticoagulation, increased % time in therapeutic range
Epstein *et al*. 2010 [121]	USA	896	Prospective cohort	CYP2C9, VKORC1	Historical cohort	Decreased hospitalisation for bleeding or thromboembolism
McMillin *et al*. 2010 [120]	USA	229	Prospective cohort	CYP2C9, VKORC1	Standard care	No statistically significant differences
Burmester *et al*. 2011 [122]	USA	125	RCT	CYP2C9, VKORC1	Clinical algorithm	No differences between the two arms
Gong *et al*. 2011 [86]	UK and Canada	196	Prospective cohort	CYP2C9, VKORC1	None	Eliminated differences in time to therapeutic INR between the genotypes
Anderson *et al*. 2012 [123]	USA	504	RCT + parallel cohort	CYP2C9, VKORC1	Different genotype-guided algorithm	Both algorithms increased % time in therapeutic INR range and decreased the number of out of range INRs
Wang *et al*. 2012 [117]	China	101	RCT	CYP2C9, VKORC1	Standard care	Decreased time to stable dose

find differences between the two groups [123]. However in this study, patients dosed with any of the two pharmacogenetic algorithms (n = 504) spent more time within the target range and had fewer out of range INRs than patients on standard care in a parallel cohort (n = 1911). This is the largest study comparing genotype-guided dosing with standard care to date and probably the only one with sufficient statistical power to detect a significant difference between pharmacogenetic-guided care and standard treatment. However, none of the studies described above (and summarized in Table 4) was able to provide convincing evidence about the clinical significance of genotyping, either because of the small size of the study or a non-randomized comparison. Also, no trials have yet been published describing the impact of genotyping before initiating acenocoumarol or phenprocoumon treatment.

Studies in progress

Some additional clinical trials are currently recruiting patients or have just finished recruiting. In the Clarification of Optimal Anticoagulation through Genetics (COAG) trial, a double-blind randomized clinical trial, the percentage time patients spend in the therapeutic INR range during the first 4 weeks of therapy will be investigated in two groups [124]. The first group will receive a genotype-guided dose based on algorithms using clinical and genetic information. The second group will receive a clinical-guided dose based on algorithms using clinical information only. Patients are currently being recruited from several centres in the USA. The Genetics Informatics trail (GIFT) is a 2 × 2 factorial design trial, comparing a pharmacogenetic algorithm with a clinical algorithm and a high INR target (2.5) with a lower INR target (1.8) [125]. In this study, patients undergoing hip or knee surgery and receiving prophylactic warfarin are being included. The primary outcome is a composite of venous thromboembolism, major bleeding, INR values above 4 or death. Both the COAG trial and the GIFT trial aim to include more than 1000 patients. All aforementioned trials have focused on warfarin. Carcas *et al*. described the study protocol of a trial on acenocoumarol [126]. In this Spanish multicentre, single-blind, randomized trial, 240 patients with venous thromboembolism will be included and followed for 3 months. Patients in the control group will be dosed according to common clinical practice and patients in the intervention group will be dosed according to the algorithm of Borobia *et al*. [111]. The primary endpoint is whether or not the INR at day 7 of acenocoumarol therapy is in the therapeutic range.

The European pharmacogenetics of anticoagulant therapy (EU-PACT) trial is a European trial investigating the added value of genotyping in warfarin, acenocoumarol and phenprocoumon [127]. This trial includes patients with atrial fibrillation or venous thromboembolism initiating warfarin (in the UK and Sweden), acenocoumarol (in Greece and the Netherlands) or phenprocoumon (in the Netherlands, Austria and Germany). Patients are being randomized to either an intervention group or a control group. The acenocoumarol and phenprocoumon control group will receive a dose based on a clinical algorithm; the warfarin control group will receive a dose based on standardized clinical care. The dosing algorithms by van Schie *et al*. and by Avery *et al*. are used to calculate loading and maintenance doses [27, 106]. The primary outcome of this trial is the percentage time in therapeutic INR range during the first 3 months of therapy. Secondary endpoints include percentage time spent with INR of 4 or higher, time to stable dose, time to therapeutic INR, time to and number of adverse events (bleeding or thromboembolism) and cost-effectiveness. A new method will be used to genotype patients for *CYP2C9* and *VKORC1* polymorphisms [128]. This method is a point of care test, providing the results in approximately 1.5 h. This enables physicians to prescribe a pharmacogenetic-guided dose before treatment initiation without delaying the start of the therapy.

Cost-effectiveness

If and when pharmacogenetic-guided coumarin dosing has been shown to be effective and safe, clinical practice guidelines will probably recommend genotyping. However widespread implementation of the dosing strategy will also depend on its cost-effectiveness. The payer, for example a health insurance company, is an important stakeholder in this case. If the genetic test is not reimbursed, patients might not be willing or able to undergo this test and receive a genotype-guided dose. The insurance company may require proper information from cost-effectiveness analyses before considering reimbursement.

A cost-effectiveness analysis (CEA) involves the comparison of the total costs and effectiveness of two or more different treatment strategies. In such an analysis different costs are considered, including not only the costs of genotyping and the cost of monitoring, but also the costs of cardiovascular events that may occur later in time. The effectiveness of genotyping can be defined in different ways. It can be orientated around the reduction in adverse events, in which case the cost-effectiveness of genotype-guided dosing *vs.* clinical-guided (or standard) dosing will be expressed as the extra cost to avoid one adverse event. This is, however, very disease-specific and therefore difficult to compare with treatments in other diseases. For a health insurance company comparability with other treatments may be very valuable when making 'value for money' or budget allocation decisions. For this reason, some payers require a cost-utility analysis (CUA), where the utility of the new treatment is usually expressed in Quality Adjusted Life Years (QALYs). The costs per QALY gained can be compared more easily with treatments in other diseases than the cost per adverse event avoided. Some authors have already investigated the cost per QALY gained by genotype-guided warfarin dosing, but there is still large uncertainty about the effectiveness of a pharmacogenetic-guided algorithm [129, 130]. In the EU-PACT trial, the cost per QALY gained by pharmacogenetic guided dosing will be determined for warfarin, acenocoumarol and phenprocoumon [127]. The costs of genotyping will have an important effect on cost-effectiveness. If the costs are low, genotyping could reduce overall costs if the rate of adverse events is decreased.

Conclusions

Genetic factors play an important role in the response to coumarin derivatives. Dosing algorithms including *CYP2C9* and *VKORC1* genotypes and some clinical factors are able to explain more than half of the variation in coumarin dose requirements. A higher r^2 of the algorithms than what has been found so far, is not expected when more polymorphisms are added, as *CYP2C9* and *VKORC1* (and to a smaller extent *CYP4F2*) are consistently found as the most important determinants of coumarin dose in genome wide association studies [131–134]. The algorithms can be used in clinical practice to predict the right coumarin dose before treatment initiation. The effectiveness of this pharmacogenetic-guided dosing is still uncertain. Currently, novel oral anticoagulants (direct thrombin inhibitors and factor Xa inhibitors) have been developed, which might be good alternatives to coumarin derivatives. A meta-analysis of five large phase III trials revealed that these novel oral anticoagulants compared with coumarins reduced the risk of stroke or systemic embolism by 18% and the risk of haemorrhagic stroke by as much as 49% in patients with atrial fibrillation [135]. These results indicate that these drugs are a promising alternative to coumarin derivatives, especially because patients will not have to be monitored frequently, as is the case with coumarin derivatives. However, these novel oral anticoagulants also have some disadvantages. No biomarker is currently available to monitor the anticoagulant effect of the new drugs. This fact, together with the fact that some of the new drugs have to be taken twice daily, could reduce patient adherence. Secondly, in elderly patients with renal dysfunction, the risk of bleeding is increased because of prolonged half-lives in patients with renal insufficiency [136]. In case of a bleed or if emergency surgery is needed, there is no antidote available yet. However, some studies have been done in healthy volunteers suggesting prothrombin complex

concentrate as a possible antidote [137,138]. Lastly, the costs of novel oral anticoagulants are considerably higher than the costs of coumarins. The costs of the drugs represent only one part of the costs. One also needs to consider the monitoring costs and complication costs etc. It is therefore also necessary to investigate the cost-effectiveness of these drugs. Shah *et al*. showed that a direct thrombin inhibitor was less cost-effective *vs*. warfarin when patients spent more time within the therapeutic range [139]. As pharmacogenetic-guided dosing may increase the time spent within therapeutic range, it would also be very interesting to investigate the cost-effectiveness of the novel oral anticoagulants *vs*. pharmacogenetic-guided dosing of coumarin derivatives. In a cost-utility analysis, You *et al*. concluded that the chance that pharmacogenetic-guided coumarin dosing would be cost-effective would be high if the time spent in therapeutic INR range could be improved from 64% to 77% [140]. The new oral anticoagulants are expected to be used more widely in the coming years. This might influence the role of anticoagulation clinics when these clinics have fewer patients to treat. This can increase the operating costs per patient and also influence the cost-effectiveness of pharmacogenetic-guided dosing.

In conclusion, pharmacogenetics play an important role in the interindividual and intra-individual variation in response to coumarin derivatives. Pharmacogenetic-guided dosing algorithms could be used to predict the required coumarin dose before treatment initiation, but the best evidence of the effectiveness of genotype-guided dosing is still forthcoming. After the clinical effect of genotyping is known, it will be important to consider the cost-effectiveness of genotype-guided coumarin dosing, also when comparing with the new oral anticoagulants.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare TV, WR, AD, RvS, AdB and AMvdZ had support from the European Community's Seventh Framework Programme for the submitted work, no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

This project was funded by the European Community's Seventh Framework Programme under grant agreement HEALTH-F2-2009-223062. The Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute of Pharmaceutical Sciences, employing authors TV, RvS, AdB and AMvdZ, has received unrestricted research funding from the Netherlands Organisation for Health Research and Development (ZonMW), the Dutch Health Care Insurance Board (CVZ), the Royal Dutch Pharmacists Association (KNMP), the private-public funded Top Institute Pharma (http://www.tipharma.nl, includes co-funding from universities, government, and industry), the EU InnovativeMedicines Initiative (IMI), EU 7th Framework Program (FP7), the Dutch Ministry of Health and industry (including GlaxoSmithKline, Pfizer, and others).

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