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Genotype-guided coumarin dosing: where are we now and where do we need to go next?

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EXPERT OPINION

Genotype-guided coumarin dosing: where are we now and where do we need to go next?

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Introduction: A large proportion of the coumarin dose variability is explained by environmental factors and by common genetic variants in the *VKORC1* and *CYP2C9* genes. Genotype-guided coumarin dosing has been proposed for a more accurate prediction of the coumarin dose in order to reduce the incidence of coumarin-related complications.

Areas covered: This review discusses the current state of coumarin pharmacogenetics, the evidence from recent randomized controlled trials and economic evaluations regarding the possible clinical implementation of genotype-guided coumarin dosing.

Expert opinion: When the *VKORC1* and *CYP2C9* genotypes are available before the start of coumarin therapy in individuals of European ancestry, a genetic-guided algorithm should be used for dose determination. Ethnicity-specific pharmacogenetic algorithms should be tested in other populations. At this moment the evidence is not sufficient to support genotyping before coumarin therapy initiation. Based on results from recent randomized controlled trials, a clinical dosing algorithm could be considered in the initial phase of coumarin treatment. Current economic studies indicate that genotype-guided dosing could be cost-effective, but the clinical implementation of genetic-guided coumarin therapy will depend on the cost of pharmacogenetic tests and the availability of novel oral anticoagulants.

Keywords: coumarins, CYP2C9, dose, genetic-guided, pharmacogenetics, polymorphism, VKORC1, warfarin

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1. Introduction

Interindividual differences in drug response caused by multiple environmental, disease-related and genetic factors can lead to a reduction of efficacy or an increase in adverse reactions to a drug [1]. Identifying risk factors for the stratification of patients who are likely to have poor therapeutic responses may amend therapeutic choices and has the potential to minimize the number of adverse drug reactions [2]. Pharmacogenetics uses individual genetic information for the prediction of pharmacologic effect of a given drug. Among the most widely studied drugs in the field of pharmacogenetics are coumarin derivates, including warfarin, acenocoumarol and phenprocoumon.

Worldwide warfarin is the most prescribed oral anticoagulant for the treatment of patients with venous thrombosis and prophylaxis of thromboembolic complications related to chronic atrial fibrillation and cardiac valves replacement surgery [3]. Acenocoumarol and phenprocoumon are more frequently used only in some European countries [4]. Due to the narrow therapeutic window of coumarins and the large inter- and intrapatient dose variability, treatment with these drugs is associated

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Article highlights.

- Coumarin effectiveness and adverse drug reactions are in part influenced by common genetic polymorphisms in the *VKORC1* and *CYP2C9* genes.
- Genotype-guided algorithms for coumarin dose calculation have been developed and used in randomized clinical trials to evaluate the clinical utility of pharmacogenetic approach.
- Recent RCTs produced discordant results with respect to TTR during genetic-guided coumarin treatment and were not powered to evaluate clinically relevant endpoints, such as bleedings and thromboembolic events. More clinical utility evidence is being expected.
- Overall, there is limited evidence to support performing a pharmacogenetic test before the start of coumarin therapy. If information on *VKORC1* and *CYP2C9* genotypes is already available, the use of genetic-guided coumarin algorithms should be considered.
- There is not enough evidence to conclude with certainty that pharmacogenetic coumarin dosing could be more cost-effective than novel oral anticoagulants, but recent studies indicate that it could be more cost-effective than the standard anticoagulation care.

This box summarizes key points contained in the article.

with an increased rate of bleedings [5]. Warfarin-related bleedings accounted for as much as one-third of hospitalizations for adverse drug events among older adults in the USA [6]. The individual warfarin dose may vary by a factor of 10 among patients, but in most countries the typical starting warfarin dose is fixed (5 mg) and titrations are performed based on the international normalized ratio (INR), which should remain within the 2.0 – 3.0 range for most indications [1].

Among the important determinants of coumarin dose requirement are clinical factors, such as the intake of vitamin K, age, gender, concurrent medication, renal function and comorbidity [7]. However, it has been well-established that the dose of coumarin derivates is substantially influenced by the genotype [8]. In the past years, a more "personalized" approach to coumarin dosing, guided by an individual's genetic information has been investigated in randomized controlled trials (RCTs) and more trials are still ongoing. Furthermore, the availability of novel oral anticoagulants (NOACs) had an impact on use of coumarins and will probably be an important factor for the clinical implementation of genotype-guided coumarin dosing strategies in the future [1].

In this review, the role of genetic factors influencing the coumarin response and the algorithms for calculating the coumarin dose based on genotype are addressed, along with the clinical evidence from recent RCTs examining the genetic-guided coumarin dosing. The review mostly focuses on warfarin; however the findings on pharmacogenetics of acenocoumarol and phenprocoumon are also evaluated. Moreover, economic considerations related to the realization of genotype-guided coumarin dosing in practice are discussed and suggestions for future research are given.

2. Pharmacogenetics of coumarins

2.1 Genetic variants predicting the coumarin dose

Coumarins act in the liver by inhibiting vitamin K epoxide reductase (VKOR), an enzyme converting inactive oxidized vitamin K back to its active reduced form, which is required as a cofactor for functional coagulation factors II, VII, IX, and X (**Figure 1**) [9]. Coumarins exist as a racemic mixture of S- and R- enantiomers, with the S-enantiomer being several times more potent [9]. The two genes that influence warfarin response the most are vitamin K epoxide reductase subunit 1 (*VKORC1*), which encodes the warfarin target VKOR, and the liver enzyme cytochrome p450 2C9 (*CYP2C9*), metabolizing S-warfarin (**Figure 1**) [7,10,11]. Cytochrome p450 2C9 also metabolizes acenocoumarol, but is less important for phenprocoumon, which is primarily metabolized by CYP3A4 [12,13]. The influence of common *CYP2C9* polymorphisms on the warfarin dose was first described in the late 1990s, whereas the effect of *VKORC1* variants was reported in 2005 [14-16]. Since that time, several investigators studied the contribution of common genetic polymorphisms to the variation in coumarin dose requirement and genome-wide association studies (GWAS) were performed, which confirmed the earlier findings (**Table 1**) [10,11,17-19]. The results of the GWAS in acenocoumarol and phenprocoumon showed genetic associations for dose similar to the studies in warfarin (**Table 1**) [17,20].

Scott *et al*. described rare missense mutations the *VKORC1* gene to be associated with warfarin resistance in Ashkenazi and Sephardi Jewish populations, where extremely high doses (> 20 mg/day) are needed for the therapeutic effect [21]. In the general population, functional single nucleotide polymorphisms (SNPs) in the *VKORC1* promoter (-1639G>A, rs9923231) and intron 1 (1173C>T, rs9934438) are responsible for ~ 25% of the warfarin dose variability [22,23]. These two SNPs are in almost complete linkage disequilibrium and similarly predict warfarin dose across all racial groups [24]. The -1639 G allele results in increased *VKORC1* promoter activity and mRNA levels, which leads to a higher warfarin dose requirement by the G carriers in comparison to individuals with the A allele [23,25]. The homozygotes for the A allele have the highest sensitivity to warfarin and require lowest doses [23]. The GG genotype is most common in African-Americans and a higher frequency of the AA genotype is observed in Asians, whereas ~ 50% of individuals of European ancestry have the AG genotype [24].

Most of the genetic variants in the *CYP2C9* gene lead to a reduced activity of the enzyme and an increased sensitivity to warfarin [26]. The most common variants in Europeans *2 (R144C, rs1799853) and *3 (I359L, rs1057910) polymorphisms are located in the exonic regions of *CYP2C9*, whereas the *6 variant (818delA, rs9332131), primarily present in the African-Americans, is a deletion with a reading frame shift [26]. The *CYP2C9* *2 variant is very rare in Chinese

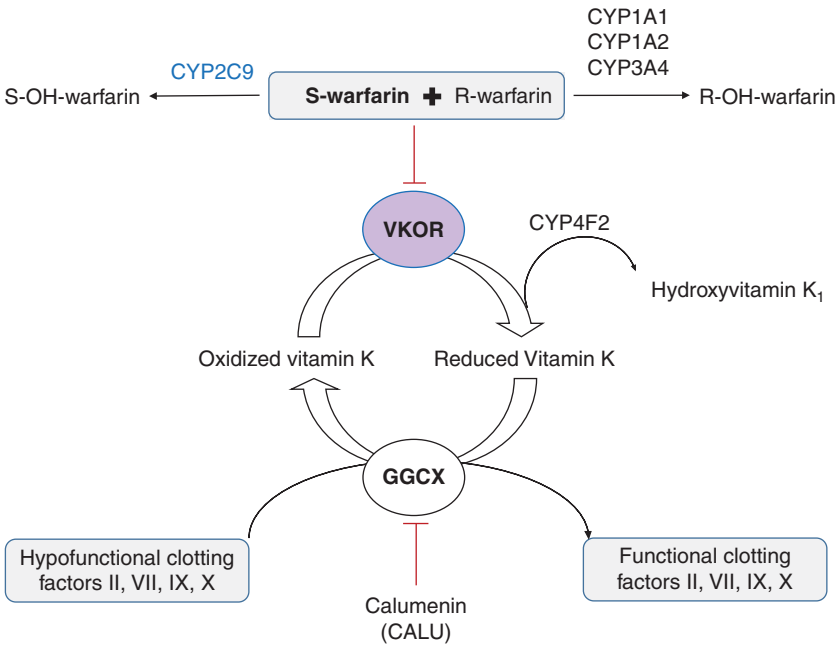

Figure 1. The role of enzymes involved in pharmacodynamics and pharmacokinetics of warfarin. Coumarin dose variation significantly depends on the SNPs in the genes encoding enzymes involved in the vitamin K cycle and the metabolism of warfarin. The most active S-enantiomer of warfarin is primarily metabolized by CYP2C9, whereas R-warfarin is metabolized by several other CYP isoforms [7].

GGCX: Gamma-glutamyl carboxylase; SNPs: Single nucleotide polymorphisms; VKOR: Vitamin K epoxide reductase.

Table 1. Overview of genome-wide association studies of coumarin maintenance dose.

Author, year	Study sample (initial/replication)	Ancestry	Reported genes	Most significant SNP	P-value (most significant SNP)
Warfarin maintenance dose					
Cooper *et al.* (2008) [11]	181/374	European	*VKORC1* *CYP2C9* *CACNA1C*	rs10871454 rs4917639 rs216013	6.2×10^{-13} 9.7×10^{-5} 8.6×10^{-7}
Takeuchi *et al.* (2009) [10]	1053/588	European	*VKORC1* *CYP2C9* *CYP4F2*	rs9923231 rs1057910 rs1799853 rs2108622	3×10^{-181} 3×10^{-79} 1×10^{-31} 3×10^{-10}
Cha *et al.* (2010) [19]	807 low dose, 701 high dose/444	Japanese	*VKORC1* *CYP2C9* *CYP4F2*	rs9923231 rs10509680 rs2108622	9×10^{-31} 3×10^{-8} 4×10^{-7}
Perera *et al.* (2013) [18]	533/432	African-American	*CYP2C18* *CYP2C9* *CYP2C8* *CYP2C19*	rs12777823	5×10^{-12}
Acenocoumarol maintenance dose					
Teichert *et al.* (2009) [17]	1451/287	European	*VKORC1* *CYP2C9* *CYP2C18* *CYP4F2*	rs10871454 rs4086116	2.0×10^{-123} 3.3×10^{-24}

Note: The rs10871454 SNP is in perfect linkage disequilibrium ($r^2 = 1.0$) with the *VKORC1* -1639 G>A rs9923231 SNP [11].

populations [27]. The common *CYP2C9* SNPs account for ~ 10% of the variation in warfarin dose requirement [28]. Altogether the variants in the *CYP2C9* and *VKORC9* explain ~ 35% of the warfarin dose variability, and when they are combined with clinical data, up to 50% of dose variability can be explained [7,9].

A few other genes with smaller effects than *CYP2C9* and *VKORC1* have been associated with warfarin dosing, including *CYP4F2* (V433M, rs2108622), *CYP2C18* (G4A, rs12777823), calumelin (*CALU*) and GGCX (CAA16/17 repeat polymorphism) [18,29,30]. The *CYP4F2* V433M is a non-synonymous polymorphism causing decreased oxidation of vitamin K in the liver and thereby increasing warfarin dose requirement in homozygotes for the variant allele [29]. The association of warfarin dose with *CYP4F2* rs2108622 is present in Europeans and Asians but not in African-Americans, because of a lower allele frequency in this population [19,31].

Cavallari *et al.* reported an association of an SNP in γ- glutamyl carboxylase (GGCX) rs10654848 (CAA) 16 or 17 repeat with a higher warfarin dose in African-Americans [30]. The *GGCX* SNP explained 2% of the warfarin dose variability and is 10 times more frequent in African-Americans than in Caucasians (where the minor allele frequency is 0.27%) [30]. The effect of another SNP, *CYP2C18* rs12777823, on the warfarin dose was also discovered in a population of African-American ancestry [18]. Carriers of the minor A allele had reduced clearance of S-warfarin and lower warfarin doses [18]. It is notable, that despite the same allele frequency across different populations, the effect of rs12777823 was only evident in African-Americans, so it is probably not the causal variant but is inherited together with a rare causal variant in African-Americans [18]. The SNP rs339097 in calumelin (a chaperon protein capable of inhibiting GGCX) has been demonstrated to confer an 11 – 15% higher warfarin dose in African-Americans [32]. This minor allele frequency of rs339097 is ~ 1% in Europeans as opposed to 25% in African-Americans [33].

2.2 Genetic associations of coumarin-related complications

The first 3 – 6 months of warfarin therapy are marked by an increased risk of excessive anticoagulation (INR above therapeutic range) and bleedings [34,35]. Genetic factors influencing warfarin dose contribute to the risk of over-anticoagulation. The *VKORC1* -1639G>A has been associated with higher INR levels during the first month of treatment and with a longer time spent out of the therapeutic INR range, however not all studies found an association of the *VKORC1* SNP with bleeding risk [10,34,36-38]. Several studies showed that *CYP2C9* *2 and *3 polymorphisms were associated with over-anticoagulation and an increased major bleeding risk, particularly in the first week of warfarin therapy [35,36]. A meta-analysis found that the relative bleeding risk for *CYP2C9* *2 was 1.91 (95% CI: 1.16–3.17), for *CYP2C9**3 1.77 (95% CI: 1.07–2.91) and for either variant it was 2.26 (95% CI: 1.36–3.75) [39]. A recent study in Indian population found that carriers of *VKORC1* AA and *CYP2C9* *3 homozygous genotypes were at significantly higher risk of over-anticoagulation (INR > 4) [40]. The study by Tomek *et al.* reported a higher major bleeding risk in carriers of several variant alleles, both during therapy initiation and in a follow-up period of 26 months [41]. A comprehensive meta-analysis including 6272 patients from 22 studies concluded that *CYP2C9**3 was a stronger risk factor for warfarin-related bleeding compared to *CYP2C9**2 and found no significant associations of the *VKORC1* -1639G>A variant with any hemorrhagic complications [42]. The association between the *CYP2C9* (*2 and *3) and *VKORC1* (GA and AA carriers) with over-anticoagulation (INR > 4) was confirmed in this meta-analysis [42]. The effect of *VKORC1* -1639 G>A on over-anticoagulation was shorter than that *CYP2C9* *3, which persisted during the entire treatment period [42].

Increased risk of over-anticoagulation was found in *VKORC1* variant carriers up to 6 months after the start of therapy with acenocoumarol, but no such effect was observed for *CYP2C9* variants [43]. Interestingly, in the same study an increased risk of a subtherapeutic INR was described in *CYP2C9* wild-type individuals during the first month and in *VKORC1* wild-type individuals during 3 months after therapy initiation [43]. This suggests that *VKORC1* and *CYP2C9* wild-type patients might be underdosed when the standard fixed-dose approach is used [43]. In wild-type *VKORC1* and *CYP2C9* phenprocoumon users, the first month of therapy was characterized by an increased risk of underdosing and subtherapeutic INR measurements [44]. Additionally, the risk of overdosing was highest in phenprocoumon users with *VKORC1*or *CYP2C9* variant alleles [44]. However, beyond 1 month of treatment with phenprocoumon, there were no statistically significant differences in the risk of out-of-range INRs between different genotypes [44]. A detailed summary of studies on bleeding risk during coumarin therapy can be found in a recently published review [45].

2.3 Genotype-guided algorithms for the prediction of coumarin dose

Coumarins have become a target for genetic-guided therapy, because only a small number of genetic variants explain such a substantial proportion in coumarin dose variability and the occurrence of hemorrhagic complications. To date, > 40 pharmacogenetic algorithms have been developed for the calculation of warfarin maintenance dose in various populations [45]. The first algorithms only included *CYP2C9* variants and subsequently the information on the *VKORC1* and a few other genes, including *CYP4F2* and *APOE* genotypes, was being used [46,47]. Typically, a pharmacogenetic algorithm also includes demographic characteristics: age, body size, weight, smoking status and the use of amiodarone. Amiodarone intake is an important factor, because this drug inhibits CYP2C9, leading to increased plasma concentrations of warfarin and a higher risk of bleeding. Some pharmacogenetic algorithms include prosthetic valve replacement status, heart failure status, and the amount of vitamin K intake [33]. An algorithm developed by Gage *et al.* explained 57% of warfarin dose variation in Caucasians, but the predictive value of this algorithm was lower (31%) in African-Americans [48].

Another genotype-guided algorithm explained 59% of the dose variability in a Swedish population by the *VKORC1* and *CYP2C9* genotypes, age, race, sex and co-medications capable of increasing the INR [37]. Compared to Caucasians, lower daily doses of warfarin are generally required for Chinese patients [27]. Studies in Chinese populations reported that combining the genetic information on *CYP2C9*3* and *VKORC1* -1639 G>A to the clinical factors could explain 48 – 74% of the warfarin dose variation [27]. At the moment > 10 genetic-guided dosing algorithms have been developed and validated in the Chinese populations [27].

An international group of experts on pharmacogenomics of warfarin (Warfarin Pharmacogenetics Consortium, IWPC) developed a highly reliable warfarin dosing algorithm in a large diverse population from nine countries [49]. The IWPC algorithm predicted 47% of the warfarin dose variation among Caucasians by using the information on *CYP2C9* and *VKORC1* SNPs, age, height, weight, amiodarone use, race and number of CYP enzyme inducers [49]. Earlier studies indicated that pharmacogenetic algorithms in general predict warfarin dose more accurately than do other dosing methods [50]. The warfarin label updated by the FDA in 2010 contains a pharmacogenetic dosing table, which may be used for selection of an initial warfarin dose when the patient's *CYP2C9* and *VKORC1* genotype is available [51]. Finkelman *et al.* reported that a genotype-guided algorithm predicted more doses within 20% of the actual dose than a clinical dosing algorithm, the dosing table on the warfarin label and the 5 mg/day fixed-dose approach [50]. Genetic-guided strategy was particularly more accurate than other dosing approaches in patients requiring low (i.e., ≤ 3 mg/day) or high (i.e., ≥ 7 mg/day) warfarin doses [49]. A guideline for physicians on the interpretation and use of the *CYP2C9* and *VKORC1* genotype was developed by The Clinical Pharmacogenetics Implementation Consortium (CPIC) [52]. The CPIC recommends considering the use of a pharmacogenetic algorithm for warfarin dosing, if genetic information is available [52]. The recommended warfarin dosing algorithm is available online at Warfarindosing.org (http://www.warfarindosing.org).

Compared to warfarin dosing, somewhat less pharmacogenetic-guided algorithms for acenocoumarol and phenprocoumon maintenance dose were created [53-56]. An example is the acenocoumarol and phenprocoumon algorithm by Van Schie *et al.*, developed and validated in a Dutch population [57,58].

3. Genotype-guided coumarin dosing in randomized controlled trials

Despite the promising results of earlier non-randomized studies on pharmacogenetic warfarin dosing, evidence from larger RCTs was required to assess the feasibility of clinical implementation of the genotype-guided approach [3,59]. An overview of recent randomized clinical trials on genotype-guided coumarin dosing is presented in **Table 2**.

At the end of 2013, the results of The Clarification of Optimal Anticoagulation through Genetics (COAG) and The European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) have been simultaneously published in the *New England Journal of Medicine* [60-62]. The COAG trial, conducted in the USA, was a multi-center, double-blinded RCT comparing genotype-guided warfarin dosing with a clinical dosing algorithm [60]. The EU-PACT trial was a single-blinded, multi-center RCT, which had a warfarin and an acenocoumarol-phenprocoumon part and was conducted Sweden, UK, the Netherlands and Greece. Both trials evaluated the effect of genotype-guided dosing strategy on percentage of time therapeutic INR range (TTR) [61,62]. The COAG trial utilized the dose-initiation algorithm by Gage *et al.* and a dose revision algorithm by Lenzini *et al.* after 4 – 5 days [63]. The EU-PACT warfarin trial used the modified IWPC algorithm during therapy initiation in comparison to a standard warfarin loading dose (usual care) and the same dose revision algorithm [61]. The results of these trials turned out to bring slightly more confusion than clarity with respect to the clinical implementation of genetic-guided warfarin dosing. The COAG authors found no between-group differences in the mean TTR after 4 months of therapy [60]. Furthermore, TTR in African-American patients was decreased by 8%-points in the genotype-guided arm [60]. In contrast to COAG, results of the EU-PACT warfarin trial showed a 7%-point increase in the TTR in the genotype guided arm after 3 months of treatment [61]. The EU-PACT acenocoumarol-phenprocoumon trial found a 5% increase in TTR with genetic-guided dosing only during the first 4 weeks of coumarin treatment, but not 12 weeks after the initiation of therapy [62]. Such varying results could be explained by the choice of the control group, the differential influence of genetic variants on dosing in different ethnic groups, the regional variability in clinical practice and, possibly, by the differences in the used algorithms. Choosing the standard of care as a comparator arm in the EU-PACT warfarin trial over a clinical algorithm, including age, co-medications and other factors (as it was done in COAG) has been suggested to contribute to the detection of significant differences in TTR [64]. Furthermore, the ethnical differences between COAG and EU-PACT populations could also in part explain the discordant results [3]. Moreover, the overall number of individuals with variant alleles was greater in EU-PACT than in COAG, which might have had in impact on the findings. Finally, the implemented genotype-guided algorithms were different as well. A more detailed comparison of the EU-PACT and COAG trial design can be found in recently published reviews [3,59].

A few randomized clinical trials have already been performed in Asian populations. Huang *et al.* demonstrated that a pharmacogenetic algorithm allowed more accurate dosing and reduced the time to achieve a therapeutic stable warfarin dose in Chinese patients undergoing heart valve replacement therapy [65]. A randomized controlled trial by

Table 2. Overview of randomized clinical trials on genotype-guided coumarin dosing.

Author, year	N total	Main indication for coumarins (%)	Blinding	Primary endpoint	Genotypes	Dosing strategy		Results
						Pharmacogenetic	Clinical	
Anderson *et al.* (2007) [93]	206	Preoperative orthopedic (60%)	Double-blinded	INR outside 1.8 – 3.2 range	*CYP2C9* *VKORC1*	Regression equation developed by the authors, based on observational data	10-mg warfarin nomogram by Kovacs *et al.* [83]	No differences in primary endpoint; doses were predicted more exact with genotype-guided algorithm
Caraco *et al.* (2008) [94]	191	DVT and PE (66%)	NR	INR 2.0 – 3.0	*CYP2C9*	Based on different algorithms using *CYP2C9* variants	Clinical algorithm by Ageno *et al.* [85]	Genotype-guided arm had higher TTR and less minor bleedings
Burmester *et al.* (2011) [95]	230	Atrial fibrillation (46%)	Single-blinded	INR 2.0 – 3.5	*CYP2C9* *VKORC1* *CYP4F2*	Marshfield Pharmacogenetic model [87]	Dosing according to the Marshfield Anticoagulation Service guidelines	No effect on TTR; more accurate dose prediction by pharmacogenetic model
Borgman *et al.* (2012) [96]	26	DVT (46%)	Single-blinded	INR 1.8 – 3.2	*CYP2C9* *VKORC1*	PerMIT dose calculation software	Standard clinical care by thrombosis service and warfarin nomogram by Kovacs *et al.* [83]	PerMIT led to increase in TTR and a decrease in the frequency of warfarin dose adjustments per INR
Huang *et al.* (2009) [65]	121	Heart valve replacement	Single-blinded	Mean time to reach a stable warfarin maintenance dose	*CYP2C9* *VKORC1*	Pharmacogenetic algorithm developed by the authors	Usual AC: warfarin starting dose 2.5 mg/day with adjustments based on INR	HR for the time to reach stable dose was 1.9 for AC vs genotype-guided dosing
Jonas *et al.* (2013) [97]	109	Atrial fibrillation (34%), DVT (30%)	Double-blinded	INR 2.0 – 3.0 or 2.5 – 3.5	*CYP2C9* *VKORC1*	Washington University School of Medicine pharmacogenetic algorithm	Same algorithm but including only clinical factors	Genotype-guided dosing did not improve TTR or decrease the number of anticoagulation visits
Kimmel *et al.* (2013) [60]	1015	DVT or PE (58%)	Double-blinded	INR 2.0 – 3.0	*CYP2C9* *VKORC1*	Algorithm by Gage *et al.* and a pharmacogenetic dose revision algorithm by Lenzini *et al.* [59]	Clinical dosing algorithm	No difference between arms
Pirmohamed *et al.* (2013) [61]	455	Atrial fibrillation (73%)	Single- blinded	INR 2.0 – 3.0	*CYP2C9* *VKORC1*	Modified IWPC algorithm	Patients aged ≤ 75 year: warfarin 10 mg on day 1 – 3, patients aged > 75 year: warfarin 5 mg on days 1 – 3 with; dosing on days	Genotype-guided dosing superior to clinical care

AC: Anticoagulation care; DVT: Deep venous thrombosis; HR: Hazard ratio; INR: International normalized ratio; NR: Not reported; PE: Pulmonary embolism.

Table 2. Overview of randomized clinical trials on genotype-guided coumarin dosing (continued).

Author, year	N total	Main indication for coumarins (%)	Blinding	Primary endpoint	Genotypes	Dosing strategy		Results
						Pharmacogenetic	Clinical	
Verhoef *et al.* (2013) [62]	548	Atrial fibrillation (83%)	Single-blinded	INR 2.0 – 3.0	*CYP2C9* *VKORC1*	Pharmacogenetic algorithm by van Schie *et al.* [54]	4 – 5 according to local clinical practice Clinical dosing algorithm	No difference between arms
Wang *et al.* (2012) [66]	101	Heart valve replacement	Single-blinded	Mean time to reach a stable warfarin maintenance dose	*CYP2C9* *VKORC1*	Pharmacogenetic algorithm developed by Huang *et al.*	Usual AC: warfarin starting dose 2.5 mg/day with adjustments based on INR	Mean time to reach a stable dose was shorter in the genetic-guided group

AC: Anticoagulation care; DVT: Deep venous thrombosis; HR: Hazard ratio; INR: International normalized ratio; NR: Not reported; PE: Pulmonary embolism.

Wang *et al.* showed similar results favoring the genotype-guided warfarin dosing strategy over a fixed loading dose with adjustments according to INR [66]. At the moment at least three trials for genotype-guided warfarin dosing in the Chinese populations are recruiting participants. One of these studies (ClinicalTrials.gov Identifier NCT01855737) aims to assess the performance of a pharmacogenetic algorithm including *VKORC1*, *CYP2C9* and *CYP4F2* genotypes compared to the actual dose. Another trial will compare a genetic-guided algorithm and using a fixed warfarin dose (standard of practice) with respect to percent time out-of range INRs, TTR, time to reach TTR, warfarin-related bleedings and thromboembolisms (ClinicalTrials.gov Identifier NCT01610141). A trial on genotype-guided warfarin therapy in Chinese elderly people will compare the IWPC dosing algorithm with the standard care using percentage of time in therapeutic INR range as primary outcome (ClinicalTrials.gov Identifier NCT02211326).

Recently, meta-analyses of the largest RCTs have been published to provide more evidence on the effect of the genotype-guided warfarin dosing on thromboembolic and hemorrhagic complications of coumarins [67-71]. The meta-analysis performed by Stergiopoulos *et al.* included data from nine RCTs and a total of 2812 patients receiving warfarin, acenocoumarol or phenprocoumon [67]. The TTR, percentage of time with INR > 4 and the number of bleeding episodes were compared in the genotype-guided arm and the clinical-guided algorithm or the usual care comparator arms [67]. The authors found no statistically significant differences in any of these endpoints, although the TTR definitions and the clinical dosing approaches differed across the included studies [67]. Of note is that the meta-analysis by Franchini *et al.*, which evaluated the same RCTs as the study by Stergiopoulos, concluded that serious bleeding events could be reduced by ~ 50% with the genotype-guided coumarin dosing as compared to the clinical dosing approach [70]. The reasons for such discrepancies might be that the latter study did not include the data from one of the trials into the final analysis, and there were some differences in the study design between the two meta-analyses [67,70].

Another meta-analysis only included RCTs on genotype-guided dosing of warfarin, but not acenocoumarol and phenprocoumon, and pooled the data on TTR, number of bleedings and deaths across 1910 patients in seven trials [68]. In this study the analysis was split for the trials using a fixed coumarin dose or a clinical algorithm as a comparator to the genotype-guided strategy [68]. Compared to fixed-dose strategies (reflecting usual anticoagulation care), the genotype-guided warfarin dosing resulted in an increased TTR, but no significant reduction in the incidences of adverse events and death rates was observed [68]. According to this meta-analysis, the genotype-guided approach was not superior to a non-fixed initial dose that was calculated with clinical algorithms [68]. The meta-analysis by Goulding *et al.* found that genotype-guided warfarin dosing resulted in a statistically

significant reduction of warfarin-related bleedings and thromboembolic events [69]. The differences in the results of these meta-analyses could probably in part be explained by the choice and number of included studies and by different approaches to the analysis of the data [68,69]. The meta-analysis by Tang *et al.* reported an improvement in TTR and a reduction in the number of bleeding events with pharmacogenetic-guided warfarin dosing, showing a significant TTR increase for Asians in a subgroup analysis [71]. Overall, the conflicting (at least to some extent) results of the meta-analyses suggest that even pooled, the data from existing trials might be insufficient to detect statistically significant differences in clinically relevant endpoints.

Ongoing clinical trials powered to detect the effects of genotype-guided dosing on warfarin-related complications are currently underway [72]. In the Genetics Informatics Trial of Warfarin Therapy to Prevent Deep Vein Thrombosis, 1600 elderly patients undergoing elective hip or knee replacement surgery will be genotyped for *VKORC1*-1639G4 A, *CYP2C9**2, and *3 additionally for the *CYP4F2* V433M variant [72]. The IWPC algorithm available on the website WarfarinDosing.org will be used for dosing during a minimum of the first 11 days of treatment for warfarin dose determination [72]. Another RCT in patients older than 65 years (the Warfarin Adverse Event Reduction for Adults Receiving Genetic Testing at Therapy Initiation [WARFARIN] trial) will also compare genetic-guided and clinically guided dosing (Clinicaltrials.gov identifier NCT01305148). The trial anticipates inclusion of 4300 patients and will utilize the incidence of warfarin-related clinical events (major bleedings and thromboembolic events) as the primary endpoint.

4. Cost-effectiveness of genotype-guided coumarin therapy

The evidence of cost-effectiveness is essential for the clinical implementation of genetic-guided coumarin therapy. Since 2003 when the first economic analysis of warfarin pharmacogenetic testing was performed, a number of cost-effectiveness studies aimed to assess the genetic-guided versus clinical coumarin dosing [73-79]. Earlier studies have only evaluated the cost-effectiveness of *CYP2C9* genotyping; however after 2005 the majority of the analyses included *VKORC1* genotyping. Furthermore, before 2010 data on clinical effectiveness of genotyping from RCTs was not available for the analyses and they relied mainly on assumptions [79]. Cost-effectiveness of genetic-guided warfarin therapy ranged from US$171,000 to 347,000 per quality-adjusted life-year (QALY) gained and the willingness to pay was estimated US$50,000 – 100,000 per QALY gained [80]. Meckley *et al.* estimated a 46% chance that genetic-guided dosing would be cost-effective at a threshold of US$50,000 per QALY gained [78]. Patrick *et al.* showed that a 5 % increase in TTR after 3 months of therapy would be required to achieve the incremental cost-effectiveness ratio (ICER) of less than US$100,000 per QALY gained in the USA [75]. To bring the ICER under US$50,000 per QALY gained, a 9% increase in TTR with genetic-guided dosing would be needed [75]. A comprehensive report on the cost-effectiveness analyses performed before 2010 can be found in a previously published review [81]. A cost-effectiveness analysis of pharmacogenetic dosing of phenprocoumon was performed in 2013 by Verhoef *et al.* [82]. This study concluded that the genetic-guided approach slightly increased QALYs in comparison to standard dosing (ICER = €2658 per QALY gained) [82]. A more recent study performed in the EU-PACT acenocoumarol-phenprocoumon data evaluating the cost-effectiveness of genotype-guided versus clinical algorithm in the Netherlands showed that the genetic-guided dosing increased costs by €33 and QALYs by 0.001 [83]. The ICERs for acenocoumarol and phenprocoumon were €28,349 and €24,427 per QALY gained, respectively [83]. The authors concluded that the cost per QALY would be below the willingness to pay threshold of €20,000 if genotyping costs were to decrease to ~ €30 [83]. To make genotyping cost-effective in patients older than 70 years, the costs of the pharmacogenetic test would have to be even lower [83].

The cost-effectiveness of the genetic-guided coumarin dosing can be determined by several factors, including the population where it is tested and the indication, the age of the patients and the cost of the pharmacogenetic test as well as how often it will be used [79]. Currently, one of the most important factors influencing the cost-effectiveness of genotype-guided warfarin therapy is the availability of NOACs, that is, the direct thrombin inhibitors and activated factor X inhibitors (dabigatran, apixaban, and rivaroxaban). Compelling data from RCTs shows that these novel agents can be a good alternative to warfarin for stroke prevention in patients with atrial fibrillation [84,85]. Unlike coumarins, NOACs do not require frequent INR monitoring, but they do have certain limitations, including high costs, the lack of a specific antidote and the anticipated decrease in therapy adherence [1]. The latest cost-effectiveness analyses provide a comparison between the genotype-guided warfarin dosing and treatment with NOACs (**Table 3**). The study by Pink *et al.* used a clinical trial simulation approach to compare genotype-guided dosing with clinical dosing and then performed a discrete-event simulation for comparison of genotype-guided dosing with NOACs [86]. Genotype-guided dosing in this study was more cost-effective than clinical dosing with an ICER of £13,226 (~ €16,792) [86]. However, apixaban would be the most cost-effective option as compared to clinical and genotype-guide dosing algorithms, with an ICER of £20,671 (~€26,245) [86]. Previously it has been shown that the cost-effectiveness of NOACs depends on the INR control in the warfarin comparator group [84]. Supporting this evidence was a study, comparing the genotype-guided and clinical algorithms with dabigatran, which concluded that dabigatran had an ICER of US$13,810 (~€11,173) per QALY gained, but would only be cost effective if TTR is < 64% [87].

An interesting approach to assess the cost-effectiveness of genotype-guided warfarin dosing implied simulation of a

Table 3. Overview of published studies on the cost-effectiveness of genotype-guided warfarin dosing.

Author, year	Comparators	Population	Outcomes	Time horizon	Events included	Perspective	Conclusions
Pink *et al.* (2014) [86]	Clinical algorithm for warfarin Rivaroxaban dabigatran apixaban	Average profile of the AF population in UK	QALYs gained	Lifetime	Stroke, systemic embolism, TIA, major bleed (including intracranial hemorrhage), myocardial infarction	UK National Health Service	Apixaban and genotype-guided warfarin are cost-effective against clinical dosing algorithm. Apixaban had the highest gain in QALYs
You *et al.* (2012) [87]	Usual AC with warfarin Dabigatran	Newly diagnosed AF patients ≥ 65 years old with a high risk for stroke	Total direct medical cost and QALYs gained	Maximum period of 25 years	Dyspepsia, major bleeding, ischemic stroke, myocardial infarction, death	Healthcare payers	Genotype-guided warfarin would be most cost-effective when TTR is > 77% and the utility value of warfarin was the same or higher than that of dabigatran
You (2014) [88]	Usual AC with warfarin Patients with *VKORC1* GA and *CYP2C9* *1*1 were assigned to a NOAC and patients with polymorphisms in *VKORC1* and *CYP2C9* received genetic-guided warfarin	Newly diagnosed AF patients ≥ 65 years old	Total direct medical cost and QALYs gained	Maximum period of 25 years	Major bleeding, ischemic stroke, myocardial infarction, death	Healthcare payers	Compared to usual AC with TTR of 60%, assigning patients by the genotype to either NOACs or pharmacogenetic warfarin was highly cost effective

AC: Anticoagulation care; AF: Atrial fibrillation; NOACs: Novel oral anticoagulants; QALYs: Quality-adjusted life-year.

situation where the decision which anticoagulant to choose would be made based on a warfarin pharmacogenetic test [88]. According to this approach, genotyping would separate *VKORC1* and *CYP2C9* wild-type patients from those with variant alleles and susceptible to over-anticoagulation [88]. The patients with the *VKORC1* GA and *CYP2C9* *1*1 genotype would be prescribed genetic-guided warfarin, whereas the other patients would receive NOAC treatment. In this stratified approach, pharmacogenetic dosing was very cost-effective with an ICER of US$ 2843 per QALY (which is well below than the willingness to pay threshold of US $ 50,000) [88]. The use of genetic-guided dosing was also more cost-effective (ICER = 12,080 US$ per QALY) than the usual anticoagulation care [88].

Prospective, randomized trials are underway to provide clinical utility data for cost-effectiveness analyses. In particular, the Clinical and Economic Implications of Genetic Testing for Warfarin Management study (ClinicalTrials.gov Identifier NCT00964353) aims to assess the clinical effectiveness and cost-effectiveness of genotype-guided warfarin algorithms.

5. Conclusion

Coumarin dose requirements are largely determined by the common genetic variants in the *VKORC1* and *CYP2C9* genes. Over the past decade, in attempt to reduce the number of coumarin-related complications pharmacogenetic dosing algorithms were developed to provide more accurate coumarin doses than clinical algorithms and the usual "one-fits-all" strategy. A few observational and randomized clinical trials suggested the benefit of genetic-guided dosing, whereas some others failed to detect any improvements of the anticoagulation status with this approach. Recent large RCTs of genetic-guided coumarin therapy produced varying results with respect to TTR and were not designed to evaluate clinically relevant endpoints, such as bleedings and thromboembolic events. This will be assessed in ongoing trials. Furthermore, the genetic-guided coumarin dosing must be cost-effective to be able to compete with newly developed anticoagulants. Earlier studies were not sufficient to determine whether or not pharmacogenetic coumarin dosing was cost-effective. Currently, with more clinical effectiveness data available from RCTs, more reliable cost-effectiveness studies can be performed. The data so far indicates that genetic-guided therapy could be more cost-effective than clinical dosing, but this depends on the cost of genetic tests. With suboptimal INR control during coumarin therapy, the cost-effectiveness of NOACs increases.

6. Expert opinion

The environmental and genetic factors defining the coumarin dose required to achieve and maintain therapeutic anticoagulation have been extensively studied. However, the knowledge about these factors is often omitted in clinical practice. The recent RCTs conducted in the USA and Europe, COAG and EU-PACT, aimed to provide more evidence on the clinical utility of pharmacogenetic dosing for coumarin anticoagulants. The differences in trial design and used algorithms and the absence of a third trial arm, which would compare the standard anticoagulation care and the clinical dosing algorithm, complicate the interpretation of the findings. The results of COAG and of some of the recent meta-analyses do not directly support using a pharmacogenetic dosing algorithm before the start of anticoagulation therapy with warfarin [3]. Nevertheless, if the genetic information is already available before the start of treatment, the utilization of the *VKORC1* and *CYP2C9* genotypes for the initial warfarin dose determination should be considered in individuals of European ancestry. When the *CYP2C9* and *VKORC1* genotypes are not known, a clinical algorithm could be considered preferable for the coumarin dose determination [3,8]. The question still remains, whether the implementation of clinical algorithms could take place without the evidence from randomized trials. Furthermore, in the absence of additional data showing that genetic-guided strategy not only improves TTR, but also reduces the number of coumarin-related bleedings and thromboembolic events, there is yet no consensus in current clinical management guidelines to advice for or against *VKORC1* and *CYP2C9* genotyping before the start of coumarin therapy [3]. The recent meta-analyses of RCT on genetic-guided coumarin dosing produced somewhat different results with respect to the coumarin-related complications, which indicate that even pooling the data from several trials might be insufficient to assess the clinically relevant endpoints.

The availability and the cost of a reliable pharmacogenetic test are also important factors that could also influence the implementation of genetic-guided coumarin dosing in clinical practice. It has been suggested that a new point-of-care test for *VKORC1* and *CYP2C9* variants would cost US$50, but the price would be lower if the test is used more often [89]. In the long term, it is possible that pharmacogenetic testing will be a part of standard care and in the meantime several clinics in the USA are using pharmacogenetic data in real-life setting [90]. Data collected through this practice would assist the comparison of outcomes between genotype-guided and clinical dosing [8].

The results of COAG emphasized the importance of developing and utilizing coumarin dosing algorithms specific for certain ethnic groups. In the African-American patients it is especially true because of the different genetic variants that are important for determining the coumarin dose in this ethnical group (e.g., *CYP2C9* *5, *6, *8, and *11). A reliable genetic-guided algorithm for African-Americans, which would include the ethnic-specific *CYP2C9* variants responsible for a lower warfarin dose requirement, is under development [91]. In Asian populations, trials are also currently underway to address the clinical utility of pharmacogenetic coumarin dosing in this ethnic population.

Cost-effectiveness analyses are essential for the decisions surrounding the clinical implementation of coumarin pharmacogenetic testing, especially after the development of direct antithrombin and anti-Xa inhibitors. It is possible that from

the economic perspective pharmacogenetic coumarin dosing for certain indications might be preferable to the use of novel oral anticoagulants [92]. Currently, more cost-effectiveness analyses comparing these two therapeutic options are required.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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