



## Experimental and clinical pharmacology

# Pharmacogenetics of warfarin – is testing clinically indicated?

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### Summary

**Pharmacogenetics is genetic testing to optimise prescribing for individual patients. Warfarin is a potential candidate for pharmacogenetic testing as it is commonly used, has a narrow therapeutic window and its mechanism of action and elimination pathways involve receptors and enzymes that are polymorphic. Polymorphism is found in vitamin K epoxide reductase and cytochrome P450 2C9. Pharmacogenetic testing is not yet routine because alone it does not predict all the variability in a patient's response to warfarin so its contribution to improved clinical outcomes is uncertain.**

Key words: anticoagulation, cytochrome P450 system, vitamin K.

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### Introduction

Pharmacogenetics refers to testing based on an individual patient's genetic variation for the purpose of prescribing drug therapy. If it can successfully individualise treatment, pharmacogenetics could have the potential to vastly improve health outcomes. However, there is a long scientific journey from noting a genetic alteration in a drug target or metabolising enzyme to predicting a clinically relevant change in health outcomes.

A patient's response to warfarin is influenced by their genome, so pharmacogenetics could be used to determine warfarin sensitivity. However, there are a myriad of non-genetic factors affecting the relationship between warfarin dose and health outcomes.

### Warfarin

Warfarin is the most commonly prescribed anticoagulant drug for the prophylaxis and treatment of venous and arterial thromboembolic disorders. It is now routinely used by many patients with atrial fibrillation. There is therefore interest in whether

testing for genetic variations in warfarin metabolism could be useful for predicting the optimum dose, reducing bleeding risk and reducing the time to achieve a therapeutic prothrombin time (expressed as the international normalised ratio (INR)).

The efficacy and safety of warfarin is critically dependent on maintaining the INR within the therapeutic range.<sup>1</sup> Treatment may be ineffective if the INR is low, but there is a sharp increase in the risk of bleeding when the INR is above the upper limit of the therapeutic range.<sup>2</sup> However, with current management patients remain on average within their target range for only two-thirds of the time.<sup>3</sup> This is likely to be because current warfarin-dosing algorithms do not incorporate genetic and environmental factors that affect warfarin concentrations and effects.

Different patients can have highly variable responses to the same dose of warfarin. In order to understand the wide inter- and intra-patient variability in response, it is necessary to consider the pharmacokinetics and pharmacodynamics of warfarin and the effect of age, size and diet.

### Cytochrome P450 2C9

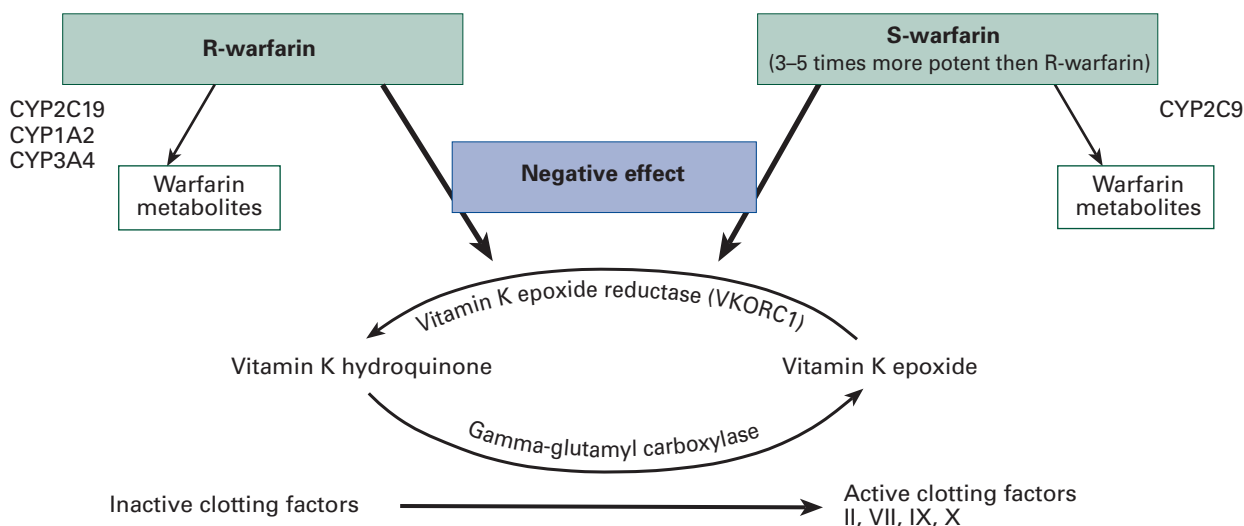
Warfarin is an equal mixture of the enantiomers S-warfarin and R-warfarin, with S-warfarin being approximately 3–5 times more potent than R-warfarin. Metabolism of S-warfarin occurs through the cytochrome P450 2C9 enzyme, while metabolism of the less potent R-warfarin occurs through CYP2C19, CYP1A2 and CYP3A4 (see Fig. 1).<sup>4</sup>

Patients who metabolise warfarin normally are homozygous for the usual (wild-type) allele CYP2C9\*1. Two other clinically relevant single nucleotide polymorphisms have been identified in CYP2C9 (\*2 and \*3). These result in reduced enzymatic activity and therefore reduced warfarin metabolism. The \*2/\*2 homozygous genotype leads to a 12% reduction in CYP2C9 activity and the \*3/\*3 homozygous genotype has less than 5% of wild-type CYP2C9 activity. These single nucleotide polymorphisms are relatively common in Caucasians. Approximately 1% of the population are homozygous for CYP2C9\*2 and 22% are heterozygous carriers of this allele. The corresponding figures for CYP2C9\*3 are 0.4% and 15%. Another 1.4% of people are compound heterozygotes (CYP2C9\*2\*3).

Fig. 1

### Schematic diagram of the action of warfarin

Warfarin is administered as a racemic mixture of S and R enantiomers. Cytochrome P450 2C9 inactivates the more potent S-warfarin enantiomer. Warfarin inhibits vitamin K epoxide reductase, preventing recycling of vitamin K leading to partially carboxylated sub- or non-functional coagulation proteins.



Patients requiring a low dose of warfarin (1.5 mg daily or less) have a high likelihood of having a CYP2C9 variant allele (\*2 or \*3) and an increased risk of major bleeding complications.<sup>5</sup> A number of studies have shown that knowing the patient's genotype helps in both predicting the optimal dose of warfarin and achieving the target INR more quickly.<sup>6,7,8</sup> However, using this knowledge to predict dose may not necessarily reduce bleeding events.<sup>7</sup>

### Vitamin K 2,3 epoxide reductase complex

Even after adjusting the warfarin dose for the variability in CYP2C9 status, there is still an amount of dosing variability in patients who have similar CYP2C9 alleles. This variability appears to be partly attributable to genetic polymorphisms in the C1 sub-unit of the vitamin K 2,3 epoxide reductase complex (VKORC1). This enzyme complex is the rate-limiting step in the vitamin K-dependent gamma carboxylation system which activates clotting factors. Warfarin exerts its anticoagulant effect by inhibiting VKORC1 (Fig. 1).

A number of common polymorphisms in non-coding sequences have been identified in VKORC1. Polymorphisms of this receptor are associated with a need for lower doses of warfarin (see Table 1).<sup>9</sup> The VKORC1 genotype alone may explain nearly 40% of the variability in response to warfarin.<sup>10</sup>

### Other genetic mutations

It is theoretically possible that point mutations in the genes for CYP2C9 or VKORC1 add to the variability in warfarin requirements when patients start therapy. There are at least two models which have demonstrated that the CYP2C9 and VKORC1 genotypes, together with known factors such as age and body size, only explain half to two-thirds of the inter-individual

variability in warfarin requirements.<sup>8,11</sup> Although this is an improvement on current non-pharmacogenetic algorithms, at least one-third of the variability is still unaccounted for. There are at least 30 other genes involved in the pharmacodynamics of warfarin which may explain this variability, including polymorphisms in apolipoprotein E, multidrug resistance 1 (MDR1), genes encoding vitamin K-dependent clotting factors and possibly genes encoding additional components of the vitamin K epoxide reductase complex.

### Environmental factors that affect warfarin dosage requirements

One of the difficulties with focusing solely on the effect of polymorphisms in the metabolising pathways of S-warfarin and vitamin K is that there are a number of non-genetic factors that affect the INR (Tables 1 and 2). Age, racial group and sex are well known, but increasingly recognised yet understudied is the effect of dietary and gut-derived vitamin K.

### Vitamin K

Vitamin K is an essential cofactor for the normal production of clotting factors II, VII, IX and X. By inhibiting VKORC1, warfarin reduces the regeneration of vitamin K and thereby inhibits the activation of vitamin K-dependent clotting factors. It is known that a patient's vitamin K status when starting warfarin affects the time to reach a therapeutic INR. In addition, a daily dietary intake of more than 250 microgram reduces warfarin sensitivity. Interesting from a therapeutic perspective is the finding that giving patients with an unstable INR daily doses of vitamin K 150 microgram decreases the variability of INR and increases the time in the target range.<sup>12</sup>

Table 1

**Factors associated with lower warfarin requirements**

Factor	Effect
Age	Reduced requirements with age may be secondary to smaller liver size with age
Reduced vitamin K intake, e.g. starvation	Inadequate vitamin K to activate clotting factors
<b>Genotypes</b>	
VKORC1 3673	The AA genotype affects warfarin requirement less than GA or GG genotypes
CYP2C9 *2 or *3 CYP2C9 *2 and *3	Both heterozygotes of *2 or *3, or homozygotes of *2 and *3 result in reduced warfarin requirements
<b>Medical conditions</b>	
Advanced malignancy	Reduced requirements may be due to liver metastases, lower body weight and drug interactions
Malabsorption syndromes	Affects vitamin K production and absorption in gut
Liver disease	Affects synthetic functions of liver including production of clotting factors and warfarin metabolism
Heart disease	Causes hepatic congestion, resulting in abnormal liver function and reduced clotting factor synthesis
Pyrexia	Increases warfarin sensitivity by enhancing the rate of degradation of vitamin K-dependent clotting factors
Hyperthyroidism	Thyroxine increases the affinity of warfarin for receptor sites, decreasing production of vitamin K-dependent clotting factors. It also catabolises these factors more quickly.
Some racial groups	May be independent or secondary to known racially divergent CYP2C9 or VKORC1 mutations, different diet or additional factor
Gender	Gender did not make any significant contribution to the regression models, but it is likely that the differences in warfarin requirements noted clinically are attributable to females' smaller body size
Factor VII deletion genotype	Mildly lower reduction
Factor X insertion genotype	Small reduction
VKORC1	vitamin K epoxide reductase
CYP	cytochrome P450

**Is pharmacogenetic testing appropriate when prescribing warfarin?**

Clinicians require easily available information that can help them to predict an individual's warfarin requirements with close to 100% accuracy in both the induction and maintenance phases of therapy. This is especially relevant when starting treatment as this is when the risk of bleeding due to over-anticoagulation is high. The induction regimens in current use (such as modified Fennerty regimens<sup>13</sup>) are only partly successful in achieving the target INR, especially in older people.<sup>14</sup>

Knowing the patient's CYP2C9 and VKORC1 status predicts less than half of the variation in the response to warfarin. Better predictions are achieved by incorporating pharmacogenetics into a dosing algorithm such as that based on the regression model of Sconce.<sup>11</sup> In this model the variables age, height, and the CYP2C9 and VKORC1 genotypes were the best predictors for estimating the starting dose of warfarin. This algorithm also confirmed that the mean warfarin daily dose requirement would be significantly lower with some genotypes.

As an example of the model's utility, the estimated daily

Table 2

**Factors associated with higher warfarin requirements**

Factor	Effect
Increased body weight	Higher total and lean body weight increase warfarin requirements, possibly through their effect on increasing body surface area
Smoking	Increased metabolism, particular of the R-enantiomer
Cytochrome P450 2C9 inducers	Induce metabolism of the S-enantiomer
High dietary vitamin K	Difficulty of carboxylating clotting factors with warfarin
Hypothyroidism	Decreased catabolism of vitamin K-dependent clotting factors

warfarin dosage requirement for a 170 cm tall, 90-year-old man with CYP2C9\*1/\*3 and VKORC1-AA genotypes is more than six times lower than that for a 30-year-old patient of the same height with the CYP2C9 wild type and VKORC1-GG genotypes.

This model is a marked improvement on current algorithms, but it still only explains 55% of the variability in dose requirements. However, a recent paper has shown that, despite the shortcomings, a pharmacogenetics algorithm is clinically helpful to predict appropriate initial doses of warfarin in high-risk patients.<sup>15</sup>

## Cost-effectiveness

As with all new technologies, it is important to evaluate the incremental cost-effectiveness of pharmacogenetics testing versus standard clinical practice. Pharmacogenetic testing for warfarin is relatively cheap compared to other new health technologies. The extra costs of this service include the polymerase chain reaction tests for the three CYP and two VKORC genes and the costs of clinical interpretation, estimated at \$75–80 per person, with a turnaround time of three hours. The efficacy of the tests is measured as the reduction in the number of expensive adverse effects, time in hospital and improvement in quality of life due to less frequent INR monitoring. None of this has been accurately quantified in a prospective study, yet it is clear that even a reduction in hospital stay by one day would provide a sizeable cost offset. However, while testing seems relatively good value for money, there are additional issues to consider, for example the cost of screening all potential warfarin users. Additionally, although the prevalence of heterozygotes is relatively high (approximately 30% for CYP2C9), patients with a null genotype (those likely to get life-threatening and expensive adverse effects) are rare (less than 1%). The detection rate for a genotype associated with serious adverse events is therefore low. Lastly, we know that clinical outcomes such as bleeding are rare in patients followed in anticoagulation clinics because warfarin therapy is closely monitored and individualised. The INR is a well-validated and inexpensive surrogate marker for warfarin effects which is already in clinical practice. However, it is not helpful for predicting which dose of warfarin to use for starting anticoagulation.

Additional epidemiological studies are needed to assess the association between genotype and the absolute risk of adverse effects before a cost-effectiveness analysis can be completed.<sup>16</sup>

## Conclusion

The variability in warfarin dosage requirements is multifactorial, although genetic polymorphisms play a part. Current warfarin-dosing algorithms fail to take into account genetics and other individual patient factors. Theoretically, including these factors could help in predicting an individual's loading and maintenance doses for safer anticoagulation. However, linear regression analysis, taking into account genetic polymorphisms of CYP2C9 and VKORC1 (additive effect), body weight, body surface area and height, has so far been able to capture only approximately half of the large inter- and intra-patient variation in dose requirements. Vitamin K status and alcohol intake,

together with additional genetic factors, are likely to account for some of the remaining difference in warfarin requirements, but still need to be studied in a regression analysis. For now, incorporation of age, body surface area, CYP2C9 and VKORC1 genotype allow the best estimate of warfarin induction and maintenance dose.

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*Conflict of interest: none declared*

### Self-test questions

*The following statements are either true or false (answers on page 87)*

7. Increased dietary intake of vitamin K reduces a patient's warfarin requirements.
8. Most of the inter-individual variation in warfarin requirements can be explained by genetic variation in cytochrome P450 2C9.

## Dental notes

*Prepared by Michael McCullough, Chair, Therapeutics Committee, Australian Dental Association*

### Pharmacogenetics of warfarin

The international normalised ratio (INR) is a simple test commonly used by dentists to gauge the likelihood that a patient taking warfarin will have excessive haemorrhage following tooth extraction. There is a clearly defined range of INR values within which simple local post-extraction measures, such as suturing, pressure and tranexamic acid mouth rinses, are adequate to control bleeding. Patients within this range can continue warfarin.<sup>1</sup>

The large variation in INR values, related to genetic and dietary factors, particularly the intake of vitamin K, reinforces the need to have this test undertaken shortly before the dental procedure.

The metabolism of warfarin can be reduced by azole antifungals such as miconazole. Topical oral miconazole can profoundly increase the INR and thus the risk of bleeding due to over-anticoagulation.<sup>2,3</sup> Similarly metronidazole, which is commonly used in the management of oral infections, can greatly increase the INR. Dentists therefore need to review patients' current medication before prescribing any drugs, even those topically applied, for possible interactions with warfarin.

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## New drugs: transparency

Access to information about drugs is essential for the quality use of medicines. Since 2003 *Australian Prescriber* has therefore recorded details about the willingness of pharmaceutical companies to disclose the information that supported the Australian approval of their new products.<sup>1</sup> These details are published as the T(ransparency)-score at the end of each new drug comment in *Australian Prescriber*.

Table 1 shows the responses to requests for evaluation data between January 2007 and January 2009. The Editorial Executive Committee of *Australian Prescriber* is pleased to report that there has been an improvement since the previous

reports were published.<sup>1,2</sup> Most manufacturers now provide some information to assist in the preparation of the new drug comments. The Editorial Executive Committee hopes this trend to increased transparency continues.

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