The future prospects of pharmacogenetics in oral anticoagulation therapy

Farhad Kamali & Munir Pirmohamed¹

Wolfson Unit of Clinical Pharmacology, School of Clinical and Laboratory Sciences, University of Newcastle, Newcastle upon Tyne, and

¹Department of Pharmacology, The University of Liverpool, Liverpool, UK

Correspondence

Dr Farhad Kamali, Wolfson Unit of Clinical Pharmacology, Claremont Place, University of Newcastle upon Tyne, Newcastle upon Tyne, NE2 4HH, UK.

Tel: + 44 19 1222 8043

Fax: + 44 19 1222 5827

E-mail: farhad.kamali@ncl.ac.uk

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Coumarins are the mainstay of oral anticoagulation for the treatment and prophylaxis of thromboembolic disorders. They have a narrow therapeutic index and regular monitoring is therefore required to avoid serious adverse effects. There is wide interindividual variability in dosage requirements, which makes anticoagulation response unpredictable. Current dosing titrations are haphazard and inconvenient and poor initial control leads to morbidity, and occasional mortality, because of bleeding and further thromboembolism. Recent discoveries have helped to characterize the factors that contribute to the interindividual variability in responses to coumarins. Patient and environmental factors that affect anticoagulation response to coumarins include age, body size, dietary vitamin K status, concurrent disease and drug interactions. More recently, single nucleotide polymorphisms in the 2C9 isoform of cytochrome P450 (CYP2C9) and vitamin K epoxide reductase (VKOR) have been shown to make significant contributions to the variability in coumarin dosage requirements. Polymorphisms in other genes that mediate the actions of coumarins may also contribute to this variability. Racial and cultural differences influence dosage requirements, which can be explained, at least in part, by genetic and dietary factors. Incorporation of genetic and environmental factors could help in the prediction of more individualized loading and maintenance doses for safer anticoagulation therapy.

Coumarins, typified by warfarin, have been the mainstay of anticoagulation therapy for thromboembolic disorders for over 50 years. Recent randomized trials have shown that anticoagulant therapy can reduce the risk of ischaemic stroke attributable to atrial fibrillation [1] and this is now the main clinical indication for warfarin.

Poor control of anticoagulation with warfarin can lead to significant morbidity and even death, with associated effects on the patient's quality of life, health service utilization and costs. About 50% of patients who take warfarin fail to stabilize within the target anticoagulation range. The risk of bleeding related to warfarin is most strongly related to the intensity of anticoagulation [2]. Bleeding increases markedly when the International Normalized Ratio (INR) exceeds 3.0, the annual risk rising from 1.6% in

non-anticoagulated older subjects to 5% (relative risk 3.0) at an INR of 2.5 and 50% (relative risk 30) at an INR of 4.0 [3, 4]. Furthermore, over-anticoagulation occurs mainly in the early stages of treatment, when the optimal dose is being established. The risk of bleeding during the first month of treatment is 10 times the risk at 12 months [5]. Underdosing is associated with an increased risk of thromboembolism – a recent survey showed that almost one in five patients consistently have an INR <2.0 [6]. These problems are due principally to interindividual variation in the response to warfarin, which makes prediction and achievement of an accurate maintenance dose difficult [7, 8]. Frequent anticoagulation monitoring, particularly in the early stages of therapy, is therefore required to avoid adverse effects [9].

Environmental factors that affect warfarin dosage requirements

Factors that affect warfarin dosage requirements include:

- hepatocellular damage, which results in increased sensitivity to warfarin owing to reduced clotting factor synthesis and reduced metabolism of warfarin;
- cardiac disease, because of hepatic congestion, which causes abnormal liver function and reduced clotting factor synthesis;
- pyrexia, which increases warfarin sensitivity by enhancing the rate of degradation of vitamin K-dependent clotting factors;
- drug interactions, which can increase or reduce dosage requirements depending on the drug. They can be pharmacodynamic (having no effect on plasma concentrations of warfarin, but affecting the INR), pharmacokinetic (altering plasma concentrations of warfarin), or both;
- age; warfarin dosage requirements fall with increasing age [10] and warfarin dose correlates with liver size, which falls with increasing age [11];
- dietary vitamin K intake (discussed in more detail below).

Genetic factors that affect warfarin dosage requirements

Genetic polymorphism in warfarin metabolism affects warfarin dosage requirements. Warfarin is a racemic mixture of R and S enantiomers and displays stereoselective metabolism. The S enantiomer is about three times as potent as the R enantiomer. Two common allelic variants in the CYP2C9 gene, CYP2C9*2 and CYP2C9*3, encode enzymes that are, respectively, about 12% and 5% as efficient as the wild-type enzyme that hydroxylates S warfarin [12–14]. These single nucleotide polymorphisms (SNPs) are relatively common in Caucasians. For instance, about 1% of the population are homozygous carriers and about 22% are heterozygous carriers of the CYP2C9*2 allele, while the corresponding figures for CYP2C9*3 are 0.4% and about 15%; another 1.4% are compound heterozygotes (CYP2C9*2*3) [15]. Carriage of variant alleles is associated with low warfarin dosage requirements and an increased risk of bleeding, particularly at the start of warfarin therapy [16], and patients who are homozygous for CYP2C9*3*3 require the lowest doses. There also seems to be a gene–dose effect, in that heterozygotes require intermediate dosages.

Recently, the gene that encodes vitamin K epoxide reductase (termed VKORC1), the target enzyme for warfarin, has been cloned [17, 18] and nonsynonymous

mutations have been found in warfarin-resistant patients. Further studies have resulted in the identification of a number of common polymorphisms in noncoding sequences. One of these is located in intron 1 (C1173T) and is associated with lower warfarin dosage requirements [19]. This polymorphism forms part of two common haplotypes, which also include an upstream polymorphism at position 1639 [20]. Heterozygosity or homozygosity for either of these haplotypes is associated with lower hepatic expression of VKORC1 [20] and lower anticoagulant dosage requirements compared with those who are homozygous for combinations of the other haplotypes.

Three studies have now shown that the CYP2C9 and VKORC1 genotypes, together with environmental factors such as age and body size, explain nearly two-thirds of the interindividual variability in warfarin dosage requirements [20–22]. However, over one-third of the variability is still unaccounted for. The possibility that other genes besides CYP2C9 and VKORC1 might contribute has been investigated. There appears to be little or no contribution from the genes that encode gamma-glutamyl carboxylase, various clotting factors, microsomal epoxide hydrolase, P450 isoforms other than CYP2C9 and certain isoforms of glutathione S-transferase [22–24]. However, more than 30 genes are involved in the mechanism of action of warfarin and all of them are being investigated in a large prospective study in the UK.

The impact of ethnicity on sensitivity to warfarin

Racial and cultural differences also influence warfarin dosage requirements. Hong Kong Chinese patients reportedly required only half the warfarin maintenance dosage required by Caucasian patients [25], while resident Asian patients in southern California required a nearly 40% lower average warfarin dose than Caucasian or Hispanic patients (3.1 mg day^{-1} vs. 5.1 mg day^{-1}) [26]. Similarly, the adjusted mean weekly warfarin dosage requirement to maintain an INR between 2.0 and 3.0 was lowest in Asian Americans (24 mg) and highest in African-Americans (43 mg), compared with Hispanics (31 mg) and Caucasians (36 mg) [27]. African-American ancestry has been identified as a predictor of high warfarin dosage requirements [28], while patients from the Indian subcontinent require more warfarin than their Chinese and Malay counterparts, even after correction for body weight [29].

Some of the interethnic differences in warfarin dosage requirements can be attributed to genetic differences. The CYP2C9*2 variant appears to be absent in Chinese and Inuits, but is present in Native Canadian

Indians and Caucasians at frequencies of 0.03 and 0.08–0.15, respectively [30]. The CYP2C9*3 variant is not detected in Inuits, but occurs in Native Canadian Indians at a frequency similar to that in other ethnic groups. The CYP2C9*2 variant is absent, and the frequency of the CYP2C9*3 variant is low, in Japanese compared to the frequencies found in Jewish Israelis [31]. While the CYP2C9*2 and CYP2C9*3 allele frequencies in Indian patients are similar to those found in Caucasians [29, 32], the CYP2C9*2 allele is very rare or absent in East Asian populations, including Chinese, Malays, Japanese and Koreans [33–35]. The CYP2C9*5 variant is found only in African-Americans, of whom about 3% carry the variant allele [36]. However, this variant has 8–18% of wild-type CYP2C9 enzyme activity [36] and therefore does not explain the greater warfarin dosage requirements in African-Americans [28]. The CYP2C9*3 allele has been associated with lower warfarin dosage requirements in Chinese and Indians [37–39]. However, because this variant is uncommon in Chinese and Malays, it cannot account for their lower warfarin dosage requirements [29]. Chinese CYP2C9*3 carriers require a lower warfarin maintenance dosage than Indian CYP2C9*3 carriers, while in Malays the presence of the CYP2C9*3 allele is not associated with lower warfarin dosage requirements. Altogether, these findings strongly suggest that other factors are involved in the lower warfarin dosage requirements in Chinese and Malays [29], which is consistent with other studies that suggest that CYP2C9 variants cannot wholly explain these interethnic differences [40–42]. Many other CYP2C9 variants have been identified in other ethnic groups, and some seem to alter the function of CYP2C9 [43]. However, whether these have effects on warfarin dosage requirements is not yet known.

Some evidence suggests that VKORC1 also plays an important role in ethnic differences in warfarin sensitivity. In a recent study the frequencies of VKORC1 AA, AG and GG genotypes in Chinese patients taking warfarin (80, 18 and 2.7%) were significantly different from those in Caucasians (14, 47 and 39%), which is concordant with the clinical observation that the Chinese require lower dosages of warfarin than their Caucasian counterparts to achieve the same degree of anticoagulation [42]. Recently described polymorphisms associated with low warfarin dosage requirements (dbSNP: rs9934438; dbSNP: rs17878363) are in complete linkage disequilibrium with the VKORC1*2 haplotype. Analysis of database-derived VKORC1 genotypes in African-Americans and Chinese has shown that haplotype frequencies in these populations differ significantly from those in Europeans (for VKORC1*2: Europeans

42%, Chinese 95%, African-Americans 14%). These observations suggest that VKORC1 could be the principal genetic modulator of ethnic differences in warfarin response [44]. However, novel variants in CYP2C9 and VKORC1 still need further investigation, as do other genes in the warfarin pathway, as determinants of warfarin dosage requirements in non-Caucasian populations.

The effect of dietary vitamin K on warfarin dosage requirements

A significant proportion of the variability in warfarin dosage requirements cannot be explained by genetic factors alone. Vitamin K is an essential cofactor for the normal production of clotting factors II, VII, IX and X. Warfarin causes anticoagulation by reducing the regeneration of vitamin K and thereby inhibiting the activation of vitamin K-dependent blood clotting factors. Dietary vitamin K intake in excess of 250 µg day⁻¹ is associated with reduced warfarin sensitivity [45]. In 73 patients with stable control of warfarin anticoagulation INR values were significantly negatively correlated with plasma vitamin K concentrations [46]. This is consistent with the finding in patients taking long-term warfarin that the INR fell by 0.2 for every 100-µg increase in vitamin K intake over the previous 4 days [47]. It is possible that vitamin K status at the start of warfarin therapy affects the pharmacodynamic response to the drug [48].

Future prospects of genetic screening for anticoagulation therapy

Since the risk of bleeding due to over-anticoagulation occurs mainly at the start of therapy, knowledge of a patient's genotype and other environmental factors could be beneficial in planning an induction regimen that is likely to avoid this. The induction regimens in current use are only partly successful in achieving target values for anticoagulation. The most widely recommended is that reported by Fennerty *et al.* [49]. This was initially described in patients with a mean age of 52 years, but has been poorly validated in older age groups [49–53], who account for most of the anticoagulated population. The Fennerty regimen is associated with over-anticoagulation and is poorly predictive of maintenance warfarin dosage requirements [51]. In the north-east of England 62% and 12% of patients aged ≥70 years taking long-term warfarin had values of INR of ≥4.0 and ≥6.0, respectively, during the induction phase of the Fennerty regimen, while the corresponding values in those under 70 years of age were 29% and 4%, respectively [52]. Similar figures have been reported by

others [53]. Using the dosing algorithm based on a regression model proposed by Sconce *et al.* [21], it can be estimated that the daily warfarin dosage requirement for a 170 cm tall 90-year-old patient 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 [1.16, 95% confidence interval (CI) 0.91, 1.44 vs. 7.39, 95% CI 6.71, 8.11]. This clearly highlights the inadequacy of the current warfarin dosing regimens and the need for a more individualized approach to warfarin therapy.

Although CYP2C9 and VKORC1 genetic variants may affect warfarin dosage requirements, they cannot wholly explain the observed interindividual and inter-ethnic differences in warfarin sensitivity, which suggests that other factors, which are yet to be discovered, are involved. These could include other genetic and environmental factors that are common and unique to the culture of the various ethnic groups.

Conclusions

The variability in warfarin dosage requirements is multifactorial. Uncertainty in response makes current loading regimens inaccurate, as they fail to take into account individual patient factors that have a major influence on anticoagulation responses. Incorporation of genetic and environmental factors could help in the prediction of more individualized loading and maintenance warfarin doses for safer anticoagulation therapy.

Alternative oral anticoagulants, in particular oral direct thrombin inhibitors, are currently under development. However, they are unlikely to come into general use in the near future because of the lack of appropriate information from high-quality randomized controlled trials, lack of effective monitoring and antidotes, and concerns over toxicity; for example, ximelagatran has been associated with a 7% frequency of liver function abnormalities [54], which may themselves require monitoring. Warfarin will therefore remain the oral anticoagulant of choice for the foreseeable future. Research is needed to improve the safety of warfarin anticoagulation during the initial and maintenance stages of therapy. Poor initial control leads to morbidity and occasional mortality because of bleeding and further thromboembolism, with current dosing titrations being haphazard and inconvenient.

Until now, most studies on the effects of CYP2C9 and VKORC1 genotypes on responses to warfarin have been performed in Caucasians. The effects in other ethnic groups, in whom other polymorphisms and haplotypes may be common, are less clear and require additional large studies.

In the UK, the environmental and genetic factors that may be involved in determining warfarin dosage requirements are currently being prospectively analysed. Variations in 30 genes are being assessed, together with clinical measurements, pharmacokinetic measurements (warfarin concentrations) and pharmacodynamic measurements (vitamin K, vitamin K epoxide and clotting factor concentrations), as possible predictors of anticoagulation responses to warfarin. Further prospective clinical studies to assess dosing algorithms, incorporating the contribution of genetic and environmental factors to allow individualization of warfarin dosage, are also likely to be required, during both the initiation and maintenance stages of therapy. If such studies show an improvement in the outcome of therapy, successful implementation of the new algorithms into clinical practice will depend on the use of methods that allow rapid and easy genotyping in clinical settings, and the demonstration that genotype-guided prescription is cost-effective.

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