

## Editorial

# Pharmacogenetics of Warfarin

**Pantep Angchaisuksiri**

*Division of Hematology, Department of Medicine Ramathibodi Hospital, Bangkok, Thailand.*

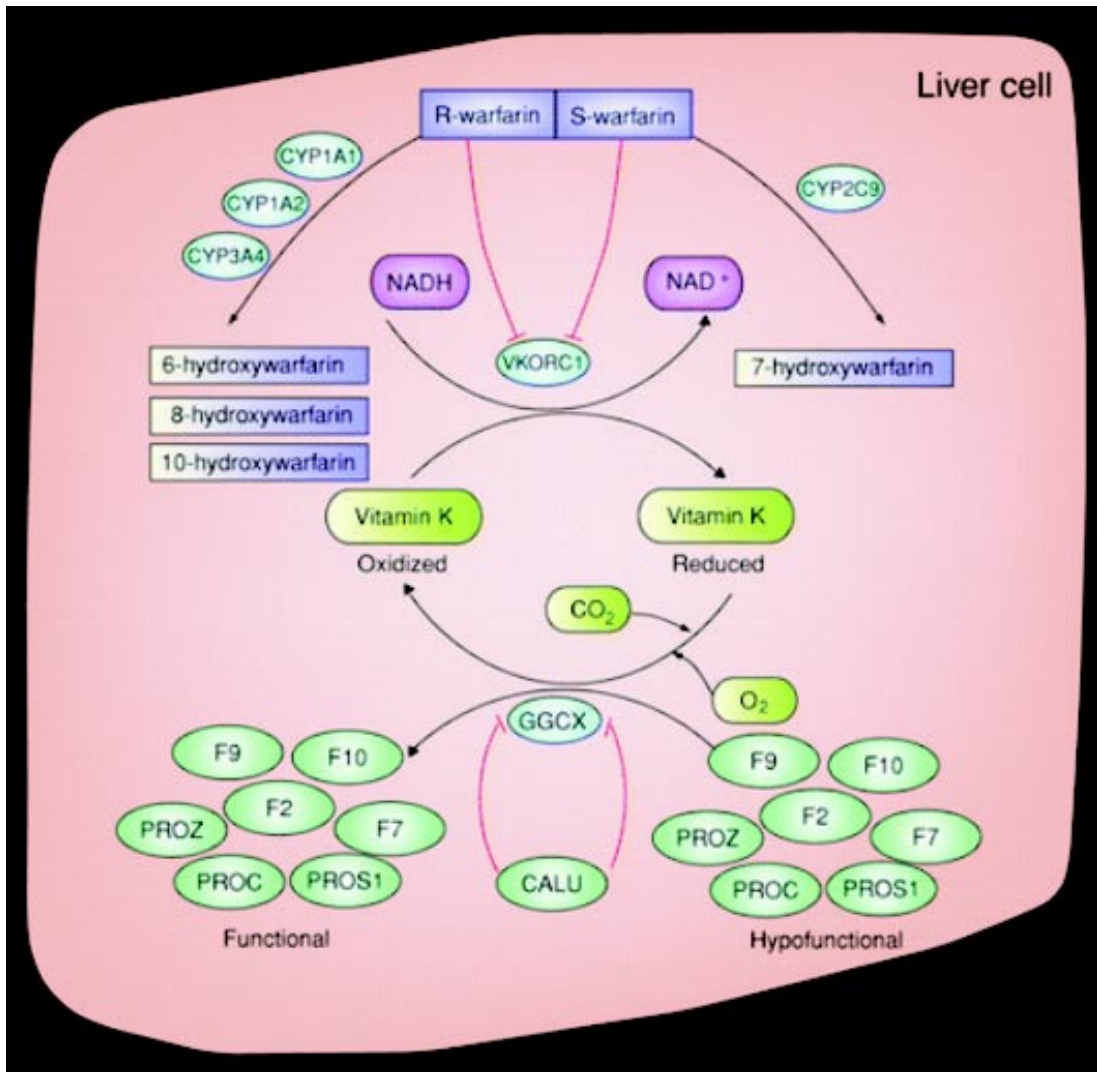
Warfarin is the mainstay of anticoagulation therapy. It is used for the prevention and treatment of venous thromboembolism, myocardial infarction, and strokes.<sup>1</sup> Its clinical use, however, is complicated by the fact that it has a narrow therapeutic index with associated adverse effects that are potentially serious, i.e., bleeding, and the dosage requirement to produce a required degree of anticoagulation varies widely between patients. The reason for the latter is multifactorial and includes determinants such as drugs,<sup>1</sup> age,<sup>24</sup> diet,<sup>5</sup> race and genetic factors.<sup>6,11</sup> There has been, to date, no reliable means of predicting an individual's response to warfarin prior to initiating therapy.

The discovery of common polymorphisms related to warfarin pharmacokinetics and pharmacodynamics over the last 10 years provides a unique opportunity to develop a pharmacogenetics-based approach to oral anticoagulant therapy. By using a pharmacogenetics-based approach to predict the patient's maintenance dose *a priori*, initiation of warfarin therapy may become safer and more efficient.

## Pharmacology of warfarin

Warfarin produces its anticoagulant effect through the inhibition of vitamin K epoxide reductase (VKOR) (Figure 1). VKOR is a multi-component lipid-protein enzyme system in the endoplasmic reticulum, which regenerates reduced vitamin K from its 2,3-epoxide.<sup>12</sup> Reduced vitamin K or vitamin K hydroquinone is an essential cofactor for the post-translational  $\gamma$ -carboxylation of glutamic acid residues on coagulation factors II, VII, IX, and X, as well as the anticoagulant proteins C, S, and Z by  $\gamma$ -glutamyl carboxylase (GGC).<sup>1</sup> Vitamin K epoxide reductase complex subunit 1 (VKORC1) is a recently identified gene that confers *in vitro* VKOR activity and is inhibited by warfarin.<sup>13,14</sup> Warfarin depletes the pool of reduced vitamin K causing the liver to synthesize nonfunctional coagulation factors, and producing an anticoagulated state.

Warfarin is manufactured as racemic mixtures of the S- and R-enantiomers, and after oral ingestion they are metabolized by the cytochrome P450 (CYP) complex (Figure 1). This cytochrome P450 is largely responsible for the metabolism



**Figure 1. Pathway showing metabolism and site of action of warfarin.**

The liver takes up free warfarin where it is biologically active and metabolized by the CYP2C9 complex. Commercially-available warfarin is a racemic mixture, with each of the 2 enantiomers having its own route of metabolism. The S-enantiomer, metabolized by CYP2C9, more strongly blocks vitamin K epoxide reductase (VKOR), thereby preventing regeneration of reduced vitamin K. Reduced vitamin K is needed for  $\gamma$ -carboxylation of glutamic acid residues on coagulation factors II (prothrombin), VII, IX, and X.

CALU: Calumenin;  $\text{CO}_2$ : Carbon dioxide; CYP: Cytochrome P450; GGCX:  $\gamma$ -glutamylcarboxylase; NAD: Nicotine adenine dinucleotide;  $\text{O}_2$ : Oxygen; PRO: Protein; VKOR: Vitamin K epoxide reductase.

of S-warfarin, which is the enantiomer predominantly responsible for the drug's anticoagulant activity.<sup>15</sup> Once in the liver, the two isomers of warfarin have separate metabolic pathways. CYP2C9 is largely responsible for the hydroxylation of S-warfarin into two inactive metabolites, 6- and 7-hydroxy S-warfarin, in approximately a 1:3 ratio.<sup>16</sup> R-warfarin is hydroxylated by CYP1A1, CYP1A2 and CYP3A4 into inactive metabolites that are excreted in the urine.<sup>17</sup> S-warfarin is 3-5 times more effective at inhibiting VKOR compared with R-warfarin.<sup>18</sup> The difference in potencies accounts for why S-warfarin is responsible for 70% of the anticoagulant effect of warfarin.<sup>18</sup> Genetic factors determining the activity of CYP2C9 have been recently demonstrated to be important.

### CYP2C9 polymorphisms

Over the years, several polymorphisms have been identified in the CYP2C9 gene. The most

common allele (CYP2C9\*1) is considered the wild-type allele. Across populations, the distribution of CYP2C9 polymorphisms varies significantly (Table 1). In Caucasian populations, almost 80% of all alleles are wild type, with the remainder being composed primarily of the CYP2C9\*2 or CYP2C9\*3 single nucleotide polymorphisms (SNPs). In African-American, Chinese, Korean, Taiwanese, Japanese, and African populations, nearly 95% of all alleles are wild type. CYP2C9\*2 and CYP2C9\*3 have greatly reduced catalytic activity compared to the wild-type enzyme CYP2C9\*1,<sup>10,19</sup> and retrospective studies have shown associations between the various genotypes and warfarin dose requirement and adverse effects.<sup>2-4,10,19-21</sup> Heterozygotes, homozygotes and compound heterozygotes of CYP2C9\*2 and CYP2C9\*3 require reduced warfarin maintenance doses compared with wild types<sup>19-29</sup> (Table 2). In addition, those with a CYP2C9\*2 or CYP2C9\*3 genotype required longer time to

**Table 1.** CYP2C9 allele frequency among various ethnic groups.

| Ethnicity | CYP2C9*1 | CYP2C9*2 | CYP2C9*3 | CYP2C9*4 | CYP2C9*5 |
|-----------|----------|----------|----------|----------|----------|
| Caucasian | 0.808    | 0.127    | 0.070    | 0        | 0.001    |
| Black     | 0.942    | 0.034    | 0.015    | 0        | 0.018    |
| Asian     | 0.982    | 0        | 0.018    | 0        | 0        |

CYP: Cytochrome P450.

**Table 2.** CYP2C9 genotype and warfarin dose reductions.

| Genotype    | Reduction in Warfarin Dose (%) |
|-------------|--------------------------------|
| CYP2C9*1/*2 | 13-30                          |
| CYP2C9*1/*3 | 21-47                          |
| CYP2C9*2/*3 | 51-73                          |
| CYP2C9*2/*2 | 22-47                          |
| CYP2C9*3/*3 | 71-87                          |
| CYP2C9*1/*5 | 8                              |

CYP: Cytochrome P450.

achieve stable dosing.<sup>21</sup> Unless warfarin is initiated with a pharmacogenetics-based dose, this delay is presumably because the trial-and-error process of finding the therapeutic dose takes longer when using traditional dosing in CYP2C9 variants. The effect of the other variant alleles on the maintenance warfarin dose has yet to be quantified.

It is apparent, however, that other factors, also possibly genetic, are important because, even when matched according to CYP2C9 genotype, the dosing requirements for a similar degree of anticoagulation varies across populations and appear to be related to racial ancestry. For example, patients of Asian descent (Chinese,<sup>6,9,11</sup> Japanese,<sup>10</sup> and Malay<sup>9,11</sup>) require a lower maintenance dose of warfarin than Caucasians and Indians; by contrast, a higher dose is needed in African-Americans.<sup>7,8</sup> Therefore variants discovered in CYP2C9 can only partially explain some of the inter-individual differences in warfarin dosage but cannot account for the inter-ethnic differences.

### **VKORC1 polymorphisms**

Warfarin's anticoagulant activity results from inhibition of hepatic VKOR that affects the synthesis of various coagulation factors. Recently, variants of the VKORC1 gene have been described to have potentially functional consequences.<sup>30-36</sup> For instance, Rieder and colleagues identified informative SNPs in VKORC1 that correlate with warfarin dose requirements.<sup>36</sup> They performed direct resequencing of polymerase

chain reaction amplicons encompassing 5 kilobases in the upstream promoter region, 4 kilobases of intragenic (intron and exon) sequence, and 2 kilobases of the 3' downstream region of the VKORC1 locus. Overall they identified 28 SNPs, of which ten (all non-coding) SNPs had a minor allele frequency greater than 5%. By binning SNPs in linkage disequilibrium together, they determined a set of 5 tagSNP bins in linkage disequilibrium and associated it with warfarin maintenance dose in the derivation cohort. They recommended a minimal SNP set composed of 4 sites (C861A, T5808G, G6853C, and G9041A) to identify five major haplotypes (H1, H2, H7, H8 and H9). Those having either H1 or H2 haplotypes required significantly lower dose of warfarin than those having H7, H8 or H9 (Table 3). In addition, these VKORC1 haplotypes were correlated with the level of expression of mRNA of VKORC1 in human liver.

Evidence from various clinical and population studies suggests that persons of Asian, European, and African ancestry tend to require, on average, lower, intermediate, and higher doses of warfarin (approximately 3.0, 5.0, and 6.5 mg per day, respectively).<sup>6,8,23,37</sup> Because group A haplotypes (H1, H2) predicted the low-warfarin-dose phenotype and were relatively common in the Asian-American population, it is likely that the association between ancestral origin and dose is, in part, an effect of the VKORC1 haplotype. Conversely, the prevalence of group B haplotypes (H7, H8, H9) was relatively high in the African-American population, potentially giving rise to

**Table 3.** VKORC1 haplotype and its effect on warfarin dose.\*

| Haplotype<br>Identification Code | Haplotype<br>Sequence <sup>†</sup> | Mean Maintenance                                                  | P Value |
|----------------------------------|------------------------------------|-------------------------------------------------------------------|---------|
|                                  |                                    | Dose among<br>Homozygous Patients<br>(95% CI) <sup>‡</sup> mg/day |         |
| H1                               | CCGATCTCTG                         | 2.9 (2.2-3.7)                                                     | <0.0001 |
| H2                               | CCGATCTCTG                         | 3.0 (2.5-3.6)                                                     | <0.001  |
| H3                               | CCGGTCCCCG                         | NA                                                                | NA      |
| H4                               | CCGGTCCGTG                         | NA                                                                | NA      |
| H5                               | TCGAGCTCTG                         | NA                                                                | NA      |
| H6                               | TCGGTCCGCG                         | NA                                                                | NA      |
| H7                               | TCGGTCCGCA                         | 6.0 (5.2-6.9)                                                     | <0.001  |
| H8                               | TAGGTCCGCA                         | 4.8 (3.4-6.7)                                                     | 0.76    |
| H9                               | TACGTTGCGG                         | 5.5 (4.5-6.7)                                                     | 0.05    |

\*CI denotes confidence interval, and NA not analyzed.

<sup>†</sup>For each haplotype sequence, the single-nucleotide polymorphisms are listed in sequential order along the VKORC1 gene, at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566, and 9041.

<sup>‡</sup>Analyses were adjusted for age, sex, use or nonuse of aminodarone, use or nonuse of losartan, and CYP2C9 genotype. The mean warfarin dose among all the patients was 5.15 mg per day (95 percent confidence interval, 4.78 to 5.51).

the increased dose requirement in this population (Table 4).

G6853C is also in tight linkage disequilibrium with several other VKORC1 SNPs, including C6484T (+1173) and G3673A (-1639).<sup>36</sup> These latter two SNPs have also been commonly used for studying an association between VKORC1 genotype and the warfarin dose.<sup>38-40</sup> Patients with the VKORC1 1173TT required less warfarin compared with those with the VKORC1 1173CT genotype or the VKORC1 1173CC genotype.<sup>38</sup> Patients with the VKORC1 1639AA genotype required less warfarin compared with those with

the VKORC1 1639GA genotype or VKORC1 1639GG genotype.<sup>39,40</sup>

The VKORC1 genotype appears to be the most important genetic factor determining variability in warfarin dose. Changes in the VKORC1 gene could alter warfarin dosage requirements both inter-individually and inter-ethnically.<sup>39</sup> Its effect was approximately three times that of the CYP2C9 genotype. Approximately 25% of the variance in warfarin dose was explained by the VKORC1 haplotype alone, whereas CYP2C9 explained 6% to 10% of the variability.<sup>36</sup>

**Table 4.** Distribution of VKORC1 haplotype in European-American, African-American, and Asian-American Populations.

| Haplotype Identification<br>Code or Group | Frequency of Haplotype in American Populations* |                             |                            |
|-------------------------------------------|-------------------------------------------------|-----------------------------|----------------------------|
|                                           | European (N=119)                                | African (N=96) <sup>‡</sup> | Asian (N=120) <sup>‡</sup> |
|                                           | <i>proportion (number of haplotypes)</i>        |                             |                            |
| Haplotype distribution                    |                                                 |                             |                            |
| H1                                        | 0.12 (28)                                       | 0.07 (14)                   | 0.89 (213)                 |
| H2                                        | 0.26 (61)                                       | 0.06 (12)                   | 0                          |
| H7                                        | 0.21 (49)                                       | 0.42 (80)                   | 0.10 (25)                  |
| H8                                        | 0.14 (34)                                       | 0.01 (2)                    | 0                          |
| H9                                        | 0.24 (56)                                       | 0.06 (11)                   | 0                          |
| Other haplotypes                          | 0.04 (10)                                       | 0.38 (73) <sup>‡</sup>      | 0.01 (2)                   |
| Group distribution                        |                                                 |                             |                            |
| Group A (H1, H2)                          | 0.37 (89)                                       | 0.14 (26)                   | 0.89 (213)                 |
| Group B (H7, H8, H9)                      | 0.58 (139)                                      | 0.49 (93)                   | 0.10 (25)                  |
| Total of groups A and B                   | 0.96 (228)                                      | 0.62 (119)                  | 0.99 (238)                 |

\* The total number of haplotypes analyzed is twice the number of persons assessed.

<sup>‡</sup>There were significant differences between the African-American and European-American populations and between the Asian-American and European-American populations, both in terms of haplotype ( $P < 0.001$ ) and in terms of group distribution (A or B) ( $P < 0.001$ ).

<sup>‡</sup>The haplotypes consisted largely of H3 and H6.

#### Other potential targets

GGC confers functionality to vitamin K-dependent clotting factors (factors II, VI, IX, and X) through post-translational carboxylation. Since the prevalence of CYP2C9 variants is low in Asian populations, Shikata and colleagues studied the effect of mutations in GGC and factor II and VII on warfarin sensitivity in 45 Japanese patients. The mean warfarin maintenance dose increased with the number of CAA repeats in intron 6 of GGC, with no association with warfarin clearance. In addition, the G402A mutation in factor

VII, or T494C mutation in factor II, was associated with higher (11% and 28%, respectively) maintenance warfarin doses, compared with wild types. The combination of these three mutations with CYP2C9\*3 accounted for nearly 50% of the variability in this population.<sup>41</sup> In 147 Italian patients on warfarin, those with the factor II Thr/Thr 165 genotype, required more (4.2 mg) warfarin than those with a Met 165 allele (2.9 mg), and those with the factor VII GG-401 genotype required more (4.1 mg) compared with those with the T-401 allele (3.1 mg).<sup>42</sup> Additionally, an

Ala-10Thr mutation in the propeptide segment of factor IX, which reduces the affinity of factor IX for GGC, conferred warfarin sensitivity in one patient.<sup>43</sup>

### **Nongenetic determinants of anticoagulant dose**

In addition to the genetic factors, other factors also influence anticoagulant dose. Increased age is associated with decreased requirements and correlates with an 8% decrease in warfarin dose per decade.<sup>2,22-24,44</sup> The mechanism behind the dose reductions in the elderly relates to enhanced responsiveness (i.e., pharmacodynamic) and/or reduced clearance (i.e., pharmacokinetic) alterations, both of which may be related to reduced liver volume with advancing age.<sup>2,4,45-47</sup>

Warfarin dose requirements are also positively correlated with weight and body surface area (BSA) with a 14% change in warfarin dose per standard deviation change in BSA.<sup>2,23</sup> Body mass index does not correlate with coumarin dose requirements.<sup>7,48</sup> BSA correlates with liver size and, therefore, hepatic clearance of warfarin, and may explain the increased dose requirements.<sup>46</sup> On average, men require larger warfarin doses than do women.<sup>8,49</sup> However, this effect is of borderline significance when controlling for BSA.<sup>23,48</sup>

While vitamin K levels are associated with the INR and dose requirements, in multivariate analyses including age and CYP2C9 genotype, vitamin K concentrations did not significantly affect dose requirements, suggesting that age may correlate with vitamin K levels.<sup>4,50</sup> Dietary vitamin K intake was not associated with war-

farin maintenance dose in two studies,<sup>8,23</sup> but was in another,<sup>2</sup> suggesting that this requires further investigation.

Many drugs have the potential to interact with warfarin or to prolong the prothrombin time when taken in combination with warfarin.<sup>1</sup> It would be prudent to take special care prescribing any new drug for patients who are being treated with warfarin and to monitor the prothrombin time more frequently during the initial stages of combined drug therapy.

### **Putting it all together**

Given the diverse array of genetic and nongenetic determinants of the variation in the dose response to oral anticoagulants, how do they perform in a single model? Several groups have modeled the maintenance warfarin dose based on a combination of genetic and nongenetic factors. Gage and colleagues studied 369 patients on stable warfarin therapy and found the following to be independent predictors of warfarin dose: age in years, BSA, number of CYP2C9\*2 and CYP2C9\*3 alleles, target INR, use of amiodarone, and use of 5-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors ('statins'). This model explained 39% of the inter-individual variability in warfarin maintenance dose.<sup>23</sup> Two other models, though enrolling fewer patients, resulted in similar findings and predictive ability,<sup>2,22</sup> but neither of these models have included the new polymorphisms in VKORC1, GGC, or clotting factors II or VII. Sconce and colleagues reported that 50-55% of the variability in the thera-

peutic warfarin dose can be predicted based on CYP2C9\*2, CYP2C9\*3, VKORC1 and clinical factors.<sup>40</sup> In a cohort of Italian patients homozygous carriers of an 1173C→T polymorphism in intron 1 of VKORC1 had significantly lower warfarin maintenance doses compared with wild types (3.5 versus 6.2 mg) and heterozygotes (3.5 versus 4.8 mg), after considering the effects of age and CYP2C9 genotype. In this model, the 1173 polymorphism explained 14% of the individual variation in warfarin dose.<sup>31</sup>

#### Future directions

As the highest risk of excessive anticoagulation and bleeding is during the initiation of therapy, pharmacogenetics-based warfarin therapy has the potential to reduce the risk of bleeding, shorten the time to stabilization of therapy, and improve the efficiency of initiating warfarin therapy by accurately estimating the warfarin maintenance dose a priori.<sup>51</sup> Alternatively, prospective identification of high-risk individuals with one or more polymorphisms may benefit from more frequent monitoring during initiation, substitution of alternative anticoagulants, or every-other-day warfarin dosing. Pharmacogenetics-based warfarin therapy will not obviate the need for INR monitoring or dose adjustments. Instead, pharmacogenetics-based therapy may reduce the effort and expense of dose titration during the induction period. Before pharmacogenetics-based warfarin dosing is widely used, additional variants (i.e., VKORC1, factor II, factor VII, and GGC polymorphisms)

that independently affect the warfarin dose need to be incorporated into the dosing algorithm. Next, the benefits of pharmacogenetics-based dosing should be assessed in a randomized controlled trial against traditional trial and error dosing. Finally, if the trial is successful, the cost-effectiveness of pharmacogenetics-based therapy needs to be assessed.

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