Role of warfarin pharmacogenetic testing in clinical practice

For over 60 years, chronic oral anticoagulation with warfarin has been the standard therapy for patients with venous thromboembolism, and it is used in the prevention of systemic embolism in patients with atrial fibrillation and prosthetic heart valves [1,2]. Warfarin is the most commonly prescribed vitamin K antagonists worldwide and is administered to more than 1 million patients in the USA annually [3].

Chronic anticoagulation with warfarin is limited by its narrow therapeutic range, risk of bleeding, numerous drug and dietary interactions and the inconvenience of regular international normalized ratio (INR) monitoring. These problems result in discontinuation of warfarin therapy in many patients who are at high risk of thromboembolism.

Major bleeding is an important concern with anticoagulation therapy, and warfarin is one of the leading causes of attendance to the emergency department and hospitalization owing to adverse drug events [4]. Analysis of 3791 warfarin-treated patients from the National Registry of Atrial Fibrillation revealed that the rate of admissions for bleeding was 5.2 per 100 patient years. Of these, 67.3% were gastrointestinal and 15.4% were intracranial hemorrhage; the overall 30-day mortality of patients admitted with major hemorrhage was 21.6% [5].

Recently, variations in two genes, vitamin K epoxide reductase (VKOR) complex 1 (VKORC1) and cytochrome P450, subfamily IIC, polypeptide 9 (CYP2C9) have been associated with variations in warfarin metabolism among individuals. Patients with CYP2C9*2 and *3 variants have longer times to dose stabilization and are at higher risk of serious and life-threatening bleeding. VKORC1 polymorphisms significantly influence time to first therapeutic warfarin range, and variants in this gene determine low-, intermediate- and high-warfarin dose requirements. The prevalence of CYP2C9 and VKORC1 polymorphisms vary among different ethnic groups, and can account for over 30% of variance in warfarin dose. Recent studies suggest that the pharmacogenomics-guided dosing algorithm can accurately predict warfarin dosage and might reduce adverse events. We aim to review the pharmacogenetics of warfarin metabolism and the clinical role of genetic testing for warfarin therapy.

KEYWORDS: anticoagulation therapy  CYP2C9  pharmacogenetics  polymorphism  VKORC1  warfarin

Chronic oral anticoagulation with warfarin is difficult to maintain within the therapeutic range and requires frequent monitoring and dose adjustments. Variations in two genes, VKORC1 and CYP2C9, have been associated with variation in warfarin metabolism among individuals. Patients with CYP2C9*2 and *3 variants have longer times to dose stabilization and are at higher risk of serious and life-threatening bleeding. VKORC1 polymorphisms significantly influence time to first therapeutic warfarin range, and variants in this gene determine low-, intermediate- and high-warfarin dose requirements. The prevalence of CYP2C9 and VKORC1 polymorphisms vary among different ethnic groups, and can account for over 30% of variance in warfarin dose. Recent studies suggest that the pharmacogenomics-guided dosing algorithm can accurately predict warfarin dosage and might reduce adverse events. We aim to review the pharmacogenetics of warfarin metabolism and the clinical role of genetic testing for warfarin therapy.

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Search method & strategy

References for this review were identified through searches of MEDLINE, EMBASE and Cochrane Library with the search terms...
‘anticoagulation therapy OR warfarin OR oral anticoagulants’ AND ‘polymorphism’, ‘pharmacogenetics’, ‘CYP2C9’, ‘VKORC1’, ‘cost–effectiveness’. The search included papers that were published up until December 2009. Only papers published in English were reviewed.

**Pharmacology of warfarin**

Warfarin is a water-soluble, strongly protein-bound drug with high bioavailability and a biological half-life of 36–42 h. It is manufactured as a mixture of R- and S-enantiomers, with the more potent S-form of the drug being metabolized primarily by the CYP2C9 enzyme of the cytochrome P450 system [1,6].

Clotting factors II, VII, IX and X are synthesized in the liver as inactive proteins and require reduced vitamin K as a cofactor for the γ-glutamyl carboxylation they require to become functional. Reduced vitamin K is regenerated by VKOR by converting oxidized vitamin K to its reduced form (Figure 1) [1]. The anticoagulant effect of warfarin is mediated through inhibition of VKOR enzyme complex, specifically the VKORC1 subunit [7], resulting in hepatic synthesis of partially carboxylated or decarboxylated proteins with reduced coagulant activity. Warfarin also inhibits carboxylation of regulatory anticoagulant proteins C and S, which has the potential to cause thrombogenic risk during initial administration. Peak anticoagulant effect occurs 36–72 h after drug administration when clotting factors, especially prothrombin, are cleared from the circulation [8].

**Monitoring of warfarin therapy**

Warfarin has a narrow therapeutic range and over- or underdosing of warfarin can lead to catastrophic hemorrhagic or thrombotic
complications. In order to achieve the optimal dose, each patient’s prothrombin time INR – a measure of anticoagulation status – is monitored regularly and maintained within a narrow range by warfarin dosage adjustment. Maintaining INR within the narrow therapeutic window is challenging because intra-individual variability can be as much as tenfold owing to factors such as adjustment of concomitant drugs, diet and disease-state interactions. The risk of iatrogenic adverse events is highest during warfarin initiation partly because the ideal therapeutic warfarin dose is unknown for different individuals as a result of interindividual variability in factors such as genetics, age, sex and body surface area [9–12]. Existing dosage algorithms are empirical and rely on trial-and-error dosing, rather than being tailored to individual clinical and genetic factors [13].

Pharmacogenomics of warfarin

More than 30 genes have been discovered so far that are involved in warfarin metabolism and action. The CYP2C9 gene is one of the most important genes in the pharmacokinetics of warfarin, and the VKORC1 gene also plays a key role in pharmacodynamics of warfarin [14].

CYP2C9

CYP2C9 is the key enzyme in metabolism of the more potent S-warfarin. Since 1999, genetic polymorphisms in CYP2C9 have been studied as a cause of interindividual variation of warfarin response [11,15]. The CYP2C9 gene is located on chromosome 10q:24.2, is approximately 55-kb long and contains 9 exons and encodes for a 60-kDa microsomal protein [16,17]. The frequency of the most common allele, designated CYP2C9*1, varies from 81 to 96% in different ethnic groups, and is considered the wild-type genotype. A total of 13 polymorphisms of the CYP2C9 gene have been identified. The commonest, CYP2C9*2 (C430T, rs1799852) and CYP2C9*3 (A1075C, rs1057910), occur at a frequency of 6–13% and 1–9%, respectively [13,18]. A meta-analysis of 7907 subjects found that 72.7% were wild-type homozygous, 15.4% were CYP2C9*1/*2, 9.6% were CYP2C9*1/*3, 1% were CYP2C9*2/*2, 1% were CYP2C9*2/*3 and 0.3% were CYP2C9*3/*3 [14].

The maximum rate of metabolism (V_{max}) of CYP2C9*2 (Arg144Cys) is 50% less than that of the wild-type, resulting in a 30–50% lower turnover of S-warfarin. CYP2C9*3 (Ile359Leu) has markedly higher K_{m} and lower intrinsic clearance, leading to approximately 90% decrease in S-warfarin 7-hydroxylation [9]. Possession of either of these genes, therefore, is associated with slower metabolism of warfarin and higher susceptibility to warfarin overdose.

In a prospective study of patients commencing on warfarin therapy, Schwarz et al. found that the CYP2C9 genotype was a significant predictor of time to the first INR being greater than four days and a significant predictor of the average dose of warfarin required after 28 days to achieve therapeutic INR, after adjustment for variables such as age, race, sex and use of amiodarone. The dose was highest in those with *1/*1 genotype (5.18 mg/day), intermediate in those with the *1/*2 or *1/*3 genotype (4.25 mg/day) and lowest in those with the *2/*2, *3/*3 or *2/*3 genotypes (3.36 mg/day) [12].

In another retrospective study evaluating the association between variant CYP2C9 alleles and clinical outcomes, it was found that during warfarin initiation, patients who had at least one variant allele, compared with those with the wild-type genotype, required a lower maintenance dose, as well as a significantly longer time (median difference of 95 days) to reach a stable dose. These patients were also more likely to be over-anticoagulated (INR > 4.0) and had a higher risk of a serious or life-threatening bleeding event (hazard ratio: 2.39; 95% CI: 1.18–4.86) [11].

The CYP2C9*3 variant, in particular, was found to be strongly associated with supratherapeutic anticoagulation in the Warfarin Genetics study [9]. In this study of 1496 patients, homozygosity for *3 substantially increased the risk of over-anticoagulation (HR: 21.84; 95% CI: 9.46–50.42) during the first 5 weeks of treatment. Moreover, 12.5% of CYP2C9*3/*3 patients experienced severe bleeding during the first month compared with 0.27% of patients with other genotypes (p = 0.07).

The influence of CYP2C9 polymorphism on warfarin dose requirements was evaluated in a recent meta-analysis of 39 studies. Warfarin dose reductions associated with the five most common variant CYP2C9 genotypes were calculated, and it was reported that, compared with the CYP2C9*1/*1 genotype, patients with *1/*2, *1/*3, *2/*2, *2/*3 and *3/*3 required warfarin doses that were 19.6% (95% CI: 17.4–21.9), 33.7% (29.4–38.1), 36.0% (29.9–42.0), 56.7% (49.1–64.3) and 78.1% (72.0–84.3) lower, respectively [14]. Using multiple linear regression models, it was determined that 5–18% of variation in warfarin dosage can be accounted for by polymorphisms of CYP2C9 [4,19,20–24].
VKORC1
As mentioned earlier, VKOR is the target enzyme for warfarin to exert its anticoagulation effect. This enzyme is inhibited by warfarin to regenerate the reduced vitamin K from its epoxide form. Reduced vitamin K is an essential cofactor for γ-glutamyl carboxylase (GGCX), the enzyme catalyzing the post-translational carboxylation of vitamin K-dependent clotting factors II, VII, IX and X, resulting in reduced coagulation [25].

The enzyme VKOR was first identified in 1974 [26], but the gene coding for VKORC1, was only identified in 2004 by Li et al. as being located on chromosome 16p11.2, and is approximately 4-kb long [7]. Ten common SNPs have been identified in the European–American population, at positions 381, 861, 2653, 3673, 5808, 6009, 6484, 6853, 7566 and 9041. Among these, five SNPs at positions 381, 3673, 6484, 6853 and 7566 were strongly predictive of the approximately 25% variance in warfarin dose. Five common haplotypes (H1, H2, H7, H8 and H9) were identified as having more than 5% frequency in the European–American population, and these haplotypes were grouped into two according to their association with warfarin dose requirement (Table 2). Group A, comprising H1 and H2, is associated with low warfarin dosage, and group B, comprising H7, H8 and H9, is associated with high dosage, respectively. The haplotypes group A/A requires 2.7 ± 0.2 mg of warfarin per day, group A/B 4.9 ± 0.2 mg per day and B/B 6.2 ± 0.3 mg per day [27,28]. This relationship was confirmed by Schwarz et al. in their study to determine the influence of genetic variant during the initiation of anticoagulation therapy [12]. In this study, the authors also demonstrated that patients with group A haplotype were more sensitive to warfarin in that the therapeutic range of INR, as well as first out-of-range INR, were reached in a shorter period of time.

CYP4F2 & other genetic factors
In addition to VKORC1 and CYP2C9, there are several other genes that may influence variation in warfarin metabolism. The DNA variant (rs2108622; V433M) in CYP4F2 was recently demonstrated to be associated with warfarin dose variation in a cohort study [29], where patients with TT alleles required approximately 1 mg/day more warfarin than patients with CC alleles. This correlation was confirmed by Borgiani et al. using multiple linear regression analyses [30].

With the completion of the Human Genome Project, geneticists have recently begun assaying genetic markers covering the entire genome to systematically search for genes that cause disease. This genome-wide association study (GWAS) has been extended to identify genes that alter response to warfarin. In 2009, Takeuchi et al. tested approximately 326,000 GWAS SNPs in 1053 Swedish patients for association with warfarin dose. By univariate regression, the strongest statistical signals (p < 10⁻⁸) were found at SNPs clustering near VKORC1 and the second lowest p-values (p < 10⁻³) from CYP2C9. In addition, by applying multivariate regression and adjusting for known genetic and demonstrated genetic predictors for dose, genome-wide significance (p = 8.3 × 10⁻³) at CYP4F2 was also detected, which accounted for approximately 1.5% of dose variance [31].

Other genes that might have influential effects on warfarin, include those coding for clotting factors (such as factors II, VII, IX and X), as well as apolipoprotein E (ApoE), calumenin, microsomal epoxide hydrolase (mEH) and multidrug resistance 1 (ABCB1), have been studied but have not been consistently demonstrated to have a significant association with warfarin dose requirements [4].

Interethnic genetic variation
Ethnicity is an important factor influencing warfarin dosage requirement. Chinese patients are known to be more sensitive to warfarin and require 40–50% lower maintenance dose of warfarin when compared with Europeans [32], while the African–American population requires a higher maintenance dose. These differences can be partly explained by the difference in frequencies of the CYP2C9 and VKORC variants (Table 3).

The frequency of distribution of CYP2C9*1, *2 and *3 in Caucasians are 80, 13 and 7%, respectively. The frequency of CYP2C9*1 alleles is 96.2%, and of CYP2C9*3 is 2–4%, in the Chinese population, while CYP2C9*2 is virtually absent in the Asian population [33]. There is a novel CYP2C9 allele found in Chinese subjects, the C-65 allele, with a frequency of 5% [34]. As previously mentioned, variant *3 is associated
with significantly lower warfarin dosage requirement. The novel C-65 allele is also associated with a relatively lower daily warfarin dosage requirement. Among the African–American population, the frequency of CYP2C9*1 alleles is 94.7%, CYP2C9*2 alleles 1.1% and CYP2C9*3 alleles 1.8% [35]. A recent study by Scott et al. found a new variant CYP2C9 allele, CYP2C9*8, and this was the most prevalent allele among the studied African–American population (~one in 11 individuals) [36].

Different frequencies of VKORC1 allele distribution among ethnic groups further contributes to interethnic variability. The prevalence of the AA haplotype group, which is more sensitive to warfarin, is only 35–37% in Caucasians, but is much higher, approximately 83–89%, in Asian subjects. The haplotype group B, which requires a larger warfarin dose, has a higher prevalence in the Caucasian population, approximately 58–64%, but is only found in 10–13% of Asian subjects [28,37]. Among the African–American population, the prevalence of low-dose group A is lower [38].

### Clinical application of the pharmacogenomics-guided dosing algorithm

The most delicate period of warfarin therapy is at the initiation phase, as mentioned earlier. Traditional clinical practice often employs a fixed loading dose regimen (e.g., 5–10 mg for the first 2–3 days) [39], and adjustment of dosage by trial and error according to the INR. Alternatively, dosing algorithms have been developed that incorporate clinical factors, such as age, body weight, sex, concurrent medication and indication of warfarin regimen [40,41]. However, these algorithms are complicated, and their application in clinical practice is limited. Computer programs that incorporate these clinical variables have also been developed, and studies have demonstrated that their usage helps to maintain a more stable INR and reduce complication rates in both the induction and maintenance phase [42,20]. An example can be found on the Warfarin Dosing website, which generates an initiation dose, as well as maintenance dose, based on clinical information entered [101].

Since the discovery of CYP2C9 and VKORC1 genes, their association with warfarin dosing requirement has been examined in numerous studies. Results from selected studies that examined the association of both CYP2C9 and VKORC1 with warfarin dosage are summarized in Table 4. The largest among them was the study by the International Warfarin Pharmacogenetics Consortium (IWPC), who examined the association of pharmacogenetics factors and clinical variables with warfarin dosage in a cohort of more than 4000 patients, subsequently deriving a pharmacogenetics model incorporating these variables and validating the model in a separate group of 1000 patients. In this study, the group derived two models from the ‘derivation cohort’, one incorporating both the genetic information and clinical variables, and the other with only clinical variables. These two models were then

### Table 3. Frequency of genetic variant distribution in Caucasian and Asian.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Caucasian (%)</th>
<th>Asian (%)</th>
<th>African–American (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9 *1</td>
<td>80</td>
<td>96</td>
<td>94.7†</td>
</tr>
<tr>
<td>*2</td>
<td>13</td>
<td>0</td>
<td>1.1</td>
</tr>
<tr>
<td>*3</td>
<td>7</td>
<td>4</td>
<td>1.8</td>
</tr>
<tr>
<td>VKORC1 (group A)</td>
<td>35–37</td>
<td>83–89</td>
<td>10–23</td>
</tr>
<tr>
<td>VKORC1 (group B)</td>
<td>58–64</td>
<td>10–13</td>
<td>49–80</td>
</tr>
</tbody>
</table>

†Inferred frequency. Adapted from [33,35,37,38].

### Table 4. Contribution of genetic variants and clinical variables† to warfarin dosage.

<table>
<thead>
<tr>
<th>Study</th>
<th>VKORC1 (%)</th>
<th>CYP2C9 (%)</th>
<th>Gene + clinical† (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquilante et al.</td>
<td>28.8</td>
<td>11.4</td>
<td>51.4</td>
<td>[21]</td>
</tr>
<tr>
<td>Carlquist et al.</td>
<td>15.1</td>
<td>18.3</td>
<td>44.6</td>
<td>[22]</td>
</tr>
<tr>
<td>Bodin et al.</td>
<td>37</td>
<td>14</td>
<td>54</td>
<td>[23]</td>
</tr>
<tr>
<td>Sconce et al.</td>
<td>15</td>
<td>17</td>
<td>54.2</td>
<td>[24]</td>
</tr>
<tr>
<td>IWPC†</td>
<td>27.7</td>
<td>5.5</td>
<td>31.4</td>
<td>[43]</td>
</tr>
<tr>
<td>Wadelius et al.</td>
<td>29.3</td>
<td>11.8</td>
<td>58.7</td>
<td>[19]</td>
</tr>
</tbody>
</table>

†Clinical variables include age, sex, drug interaction, race, BMI and so on. percentage of genetic contribution in univariate analysis. IWPC: International Warfarin Pharmacogenetics Consortium.
validated in a second cohort, and were compared against a fixed-dose regimen of 5 mg of warfarin daily by calculating the percentage of patients whose predicted dose of warfarin was within 20% of the actual stable therapeutic dose. The results demonstrated that the addition of genetic information provided a dosage prediction that was significantly closer to the actual dosage required than estimates derived from a clinical algorithm or the fixed-dose approach (8.5 vs 9.9 vs 13.0%). The accuracy of the pharmacogenetic model in dose prediction was more obvious in patients requiring extreme dosages (i.e., <21 mg/week and >49 mg/week groups), who comprised of 46% of the study population. It also demonstrated that the pharmacogenetic model can reduce the chance of a poor dose estimate (i.e., >20% deviation from actual dosage) by 7.57% when compared with the clinical model. In this study, the number of patients needed to be genotyped in order to obtain an improvement in dosage prediction was 13.2 for the comparison with the clinical algorithm and 6.0 for the comparison with the fixed-dose regimen [43].

Another prospective multicenter study by the Swedish Warfarin Genetics (WARG) also demonstrated that by using a multiregression model including variants of VKORC1 (in this study, the group only examine variants of rs9923231), CYP2C9*2 and *3, and clinical information derived from a group of more than 1000 patients, over 50% of the variance in warfarin dose can be explained. This group also validated their model in a separate group of 181 genotyped individuals, and the dosage association was similar to that of the derivation cohort [44]. The WARG group also illustrated several interesting findings. First, carriers of VKORC1 variant alleles reached first therapeutic INR more rapidly and spent more time in the therapeutic range (INR 2–3). Second, homozygous CYP2C9*3 was associated with unstable anticoagulation, with the least time in the therapeutic range, and was strongly associated with supratherapeutic INR. CYP2C9*3 was also associated with increased bleeding risk, although cases not reaching statistical significance when the whole observation period was taken into account.

However, these data were observational. Whether applying these pharmacogenetics dosing algorithms to clinical practice translates into better clinical outcomes, such as more rapid attainment of therapeutic INR or a reduction in percentage of out-of-range INR, still needs to be clarified. There are only handfuls of randomized trials published that address such issues, and their results remain inconclusive.

The study by Caraco et al. used only CYP2C9 variants as the genetic variants in their dosing algorithm. This study was able to demonstrate that patients in the genotype-guided group reached therapeutic INR in a shorter period of time, spent more time in the desired INR range, and reached pharmacodynamic steady state earlier than the control group. However, the dosing algorithm used in the control group of this study was not derived from a population study, but was based on deduction from previous clinical data. This study also demonstrated that the incidence of minor bleeding was lower in the genotype-guided group, although no difference was detected in terms overanticoagulation-associated major bleeding incidence [44].

The trial by Anderson et al. comprised 200 patients randomized to pharmacogenetics-guided or standard dosing. The authors demonstrated that a pharmacogenetics-guided regimen offers significantly more accurate dosage prediction, and that the improved accuracy was more apparent in predicting the dose requirement in the extreme groups that is, those who require higher or lower than average doses of warfarin, echoing the findings of the IWPC. Pharmacogenomics-guided regimens also significantly decreased the number of required dose adjustments. However, in this study, the authors were not able to demonstrate any significant difference in the percentage in out-of-range INR between the two arms. Although a significant reduction of the percentage of out-of-range INR was demonstrated in the subgroup with multiple variant alleles and wild-type alleles, this did not translate into significant reduction of clinical adverse events. In fact, the serious clinical events in this study were unrelated to out-of-range INR [45].

Another prospective randomized trial by Hillman et al. compared a dosing model based on CYP2C9 genotype with a fixed dose of 5 mg in a small group of 38 patients [46]. Although the primary end point was to measure the feasibility of genotype-guided warfarin dosing instead of comparing the clinical outcomes of different dosing regimens, this study reiterates the idea that dose prediction was more accurate with genotype testing. This study was also able to demonstrate that the adverse event occurrence rate was lower in the genotype-guided group. However, the study was underpowered to draw any conclusions from the results. Nevertheless, in this study, the authors were able to demonstrate that a genotype-guided dosing model was feasible in clinical practice.
Cost–effectiveness of pharmacogenetic testing

One limitation on the widespread usage of genetic testing in clinical practice is its cost. Commercially available genetic testing kits for both CYP2C9 and VKORC1 cost approximately US$400 [47,102], with a turnaround time of approximately 1–6 h [45,103]. However, this incremental up-front cost may be potentially off-set by downstream benefits, such as reduction in adverse events and hospitalization, and survival benefits.

Several cost–effectiveness analyses of genetic testing have been performed using the decision-tree model. In the study by Eckman et al., genetic testing was not a cost-effective strategy in reducing adverse events [47]. By setting the risk reduction at 32% for pharmacogenetics-guided dosing and estimating the cost for genetic testing at US$400, the cost per quality-adjusted life year (QALY) gained by genetic testing on a male aged 69 years with atrial fibrillation and a congestive heart failure, hypertension, age (≥75 years), diabetes, prior stroke (CHADS2) score of two and HEMORR:HAGES score of zero was US$172,000 (which is well over the generally accepted societal willingness-to-pay of US$50,000 per QALY gained). In this model, the utility of routine genetic testing is unlikely to be cost effective unless a more than 90% reduction in adverse events can be achieved, or if the marginal cost is less than US$140. The author also commented that if the model is conducted on an individual with high risk of bleeding (HEMORR:HAGE score of 1–2), the marginal cost-effective ratio might decrease to below the acceptable US$50,000 per QALY.

Another cost–effectiveness analysis published recently showed even higher costs per QALY gained (US$347,059) [48]. However, in this model, the author used a lower risk reduction (relative percentage reduction in out-of-range INRs) of 7%. If a risk reduction of more than 30% is achieved, genetic testing will cost less than US$50,000 per QALY gained, assuming that genetic testing is the only additional cost.

In both of these analyses, the cost–effectiveness would be improved if genetic testing were cheaper or if the genotype-guided dosing algorithm was used selectively in patients in whom problem in INR control is anticipated.

Future of warfarin genetic testing

In August 2007, the FDA updated the label of warfarin to include the statement that “lower initiation doses should be considered for patients with certain genetic variations in CYP2C9 and VKORC1 enzymes” [104], and the FDA has subsequently approved the first commercial genetic test for CYP2C9 and VKORC1 [105]. These initiatives have highlighted the importance of genetic influence on warfarin dosage, and have encouraged future research on utilizing genetic information on warfarin dosing. However, the FDA stopped short of recommending universal genetic testing for all patients on warfarin therapy. Meanwhile, the recent guidelines from the American College of Chest Physicians (IL, USA) published in June 2008 were against the use of pharmacogenetics-based initial dosing to individualize warfarin dosing [1].

The main factor holding back a general recommendation of genetic screening for patients on warfarin therapy is the lack of strong clinical evidence of its efficacy. Despite the strong correlation and the good predictive power of genetic information with regard to warfarin dosage, definitive evidence concerning the usefulness of incorporating this information into dosing algorithms in clinical practice has not been established in recently published randomized trials. It can be postulated that the lack of clinical efficacy in applying genetic information to warfarin dosing is owing to intra-individual variability, such as diet, concomitant drugs and comorbidity during the warfarin-maintenance phase. Genetic polymorphism being a constant variable might not manifest such strong influence beyond the initiation of the therapy. To further elucidate this issue, a large multicenter Classification of Optimal Anticoagulation through Genetics (COAG) study, as well as a single-blinded and randomized controlled trial, the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) study, are underway to determine if knowledge of genetic information will improve the efficacy and safety of warfarin therapy.

Another concern of applying genetic testing is its cost. From the results of current cost–effectiveness studies, unless the risk reduction of using genetic-guided dosing algorithm is more than 60%, genetic-guided dosing may not be a cost-effective strategy. With the results from the existing pilot clinical trials, the benefit of genetic-guided dosing is far smaller than 50%. Nevertheless, it can be speculated that if genetic testing is performed on individuals with a high risk of bleeding, such as those with a high HEMORR:HAGE index, or on populations with a high prevalence of susceptible polymorphisms (such as Asians), the cost–effectiveness model may tilt in favor of genetic testing.
Conclusion
Better understanding of the individual genetic polymorphisms contributing to the variations in pharmaceutical response of warfarin has enabled us to see beyond the mere biochemical aspects of warfarin dosing by trial and error. The discovery of genes coding for CYP2C9 and VKORC1 offers us the opportunity to realize the hopes of personalized medicine in which an individual can be prescribed with a specific regimen matching their particular genetic makeup so that the benefit of treatment can be maximized while minimizing complications. Warfarin, being one of the most widely prescribed drugs and having a narrow therapeutic window, seems to be an ideal candidate for the application of the concept of genetic-guided medicine. However, warfarin titration is a tedious process, and the availability of pharmacogenetic testing is not intended to loosen the vigilance of the physician. Rather, it serves to heighten our awareness of the multitudes of factors that affect warfarin dosing, some of which still elude our knowledge, and to alert us of the importance of conscientious clinical monitoring.

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Executive summary

CYP2C9 genotype is a significant predictor of the average dose of warfarin and the time needed to reach the first out-of-range international normalized ratio
- Variant CYP2C9 alleles require a lower maintenance dose, and longer time to reach the therapeutic international normalized ratio range and increase the risk of overanticoagulation.

VKORC1 significantly influences warfarin dosage requirement
- VKORC1 haplotype group A is associated with a lower maintenance dose, and haplotype group B is associated with a higher maintenance dose.

Inter-ethnic variation of warfarin dose can be explained by genetic difference
- Compared with Caucasians, Chinese populations have a higher prevalence of CYP2C9*3 and VKORC1 group A haplotypes, and so are more sensitive to warfarin.

Pharmacogenomics-guided dosing algorithms provide accurate dosage prediction, but their application in randomized controlled trials yielded inconclusive results
- The pharmacogenetics model was more accurate in dose prediction than using clinical variables alone in a multivariate analysis.
- The pharmacogenetics model, however, was only tested in a handful of small-scaled randomized controlled trials, and the evidence of benefit yielded is not strong.

Large-scale randomized controlled trials are underway to address this issue.

Genetic testing may not be cost effective
- Current cost-effectiveness studies do not support routine pharmacogenetic testing for warfarin dosing.

Bibliography
Papers of special note have been highlighted as:
* of interest
** of considerable interest

11 Discusses the fact that warfarin is superior to clopidogrel and suggests aspirin for prevention of stroke in patients with atrial fibrillation.
Demonstrates that CYP2C9 polymorphism is associated with increased risk of bleeding.


Demonstrates the impact of CYP2C9 and VKORC1 polymorphism on warfarin dosage.


Shows the impact of genetic variation and clinical characteristics on warfarin dosage.


A quantitative dosing algorithm incorporating genotypes for 2C9 and VKORC1 could substantially improve initial warfarin dose selection and reduce related complications.


Shows that genetic polymorphisms predict high risk of overdose before initiation of anticoagulation.


Proposes and validates genotype-guided warfarin dosing.


Demonstrates the association of VKORCI with warfarin dosage.


Population study of VKORCI prevalence in a Chinese population.


**Available latest study on the impact of genetic variation and clinical variables on warfarin dosage.

Caraco Y, Bleton S, Muszkat M: CYP2C9 genotype-guided warfarin prescribing enhances the efficacy and safety


** Largest randomized trial on genotype-guided warfarin dosing. **


Cost–effectiveness study of genotype-guided warfarin dosing.


## Websites

101 Warfarin Dosing www.warfarindosing.org


103 Trimgen eQ-PCRTM LC Warfarin Genotyping Kit www.trimgen.com/product_warfarin.asp


105 Table of Valid Genomic Biomarkers in the Context of Approved Drug Labels www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm