The Role of Pharmacogenomics in the Management of Traditional and Novel Oral Anticoagulants

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Abstract

Warfarin is the most commonly prescribed oral anticoagulant. However, it remains a difficult drug to manage mostly because of its narrow therapeutic index and wide inter-patient variability in anticoagulant effects. Over the past decade, there has been substantial progress in our understanding of genetic contributions to variable warfarin response, particularly in regard to warfarin dose requirements. The genes encoding for cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase complex 1 (VKORC1) are the major genetic determinants of warfarin pharmacokinetics and pharmacodynamics, respectively. Numerous studies have demonstrated significant contributions of these genes to warfarin dose requirements. The CYP2C9 gene has also been associated with bleeding risk with warfarin. The CYP4F2 gene influences vitamin K availability and makes minor contributions to warfarin dose requirements. Less is known about genes influencing warfarin response in African Americans compared to other racial groups, but this is the focus of on-going research. Several warfarin pharmacogenetic dosing algorithms and FDA-cleared genotyping tests are available for clinical use. Clinical trials are on-going to determine the clinical utility and cost effectiveness of genotype-guided warfarin dosing. Results from these trials will likely influence clinical uptake and third party payer reimbursement for genotype-guided warfarin therapy. Currently, there is a lack of pharmacogenetic data with the newly approved oral anticoagulant, dabigatran, and with other oral anticoagulants in the research and development pipeline.
Warfarin is the most commonly prescribed oral anticoagulant and is widely used for the prevention of thromboembolism or stroke in patients with prior thromboembolism, recent orthopedic surgery, atrial fibrillation, heart valve replacement, or other diseases that increase the risk for thrombosis. Dabigatran is the only warfarin competitor on the market in the U.S. However, dabigatran is currently approved only for the prevention of stroke in patients with atrial fibrillation. Little is known yet about the pharmacogenetics of dabigatran or other oral anticoagulants in the drug development pipeline. Thus, the majority of this review will focus on warfarin, with a brief discussion of potentially important genes related to dabigatran.

One of the primary challenges with warfarin therapy is determining the dose necessary to achieve therapeutic anticoagulation for an individual patient. Dose requirements vary as much as 20-fold among patients. Failure to achieve optimal anticoagulation significantly increases the risk for adverse sequelae. Clinical factors, including age, body size, diet and medications that interfere with warfarin metabolism, are well known to influence warfarin dose requirements. There is also recent evidence that decreased renal function reduces warfarin dose requirements and increases the risk for warfarin-related bleeding. Dose requirements also vary significantly by race, with higher mean maintenance doses in African Americans and lower mean doses in Asians compared to Caucasians. While clinical factors are obviously important considerations when dosing warfarin, factors such as age, body size, and interacting medications, account for only 15% to 20% of the overall variability in warfarin dose. It is now widely accepted that an individual’s genotype provides significant contribution to the warfarin dose he or she requires to attain optimal anticoagulation.

Current state of knowledge of genotype-guided anticoagulation therapy
Genes involved in warfarin pharmacokinetics and pharmacodynamics

Over the past decade, there has been substantial progress in our understanding of genetic contributions to warfarin response, particularly in regard to warfarin dose requirements. Genes encoding for proteins involved in warfarin metabolism and pharmacodynamics contribute to the inter-patient variability in warfarin response and largely explain racial differences in warfarin dose requirements. As shown in figure 1, the cytochrome P450 (CYP) 2C9 enzyme metabolizes the more potent S-enantiomer of warfarin primarily to the inactive 7-hydroxy-warfarin protein. Warfarin exerts its therapeutic effect by inhibiting the vitamin K epoxide reductase (VKOR). The genes encoding for CYP2C9 and VKOR (VKORC1) are the major genetic determinants of warfarin pharmacokinetics and pharmacodynamics, respectively. The CYP4F2 gene exerts lesser influences on warfarin pharmacodynamics through its effects on vitamin K availability.

CYP2C9 polymorphisms and warfarin clearance

The CYP2C9 gene is located on chromosome 10q24.1. Over 35 CYP2C9 alleles have been described. The CYP2C9*2 and *3 alleles are the most extensively studied and result from single nucleotide polymorphisms (SNPs) in the coding region of the gene leading to significant reductions in enzyme activity.9,10 There are racial differences in the prevalence of CYP2C9 alleles, as shown in Table 1.11-14 The *2 and *3 alleles occur in approximately one-third of Caucasians, but are much less prevalent among Asians and African Americans. The CYP2C9 *5, *6, *8, and *11 alleles predominate in African populations. The *8 allele is the most common variant in persons of African descent, occurring in up to one of every 9 African Americans.13,15 Decreased enzyme activity has been reported with the *5, *6, and *11 alleles, while data with the *8 allele are conflicting.16-18 Specifically, in-vitro data with the *8 allele
show greater activity toward tolbutamine.\textsuperscript{18} This is in contrast to the only clinical pharmacokinetic study published to date, which shows a reduction in phenytoin metabolism with the *8 allele.\textsuperscript{17} There are no pharmacokinetic data with the *8 allele using warfarin as a probe.

The CYP2C9*2 amino acid substitution occurs on the outer surface of the enzyme, while *3 substitution occurs internally.\textsuperscript{19} Neither appears to affect substrate binding. Rather, evidence suggests that they disrupt formation of intermediate compounds in the CYP2C9 catalytic cycle.\textsuperscript{20} The CYP2C9*2 and *3 alleles reduce S-warfarin clearance by 40\% and 75\%, respectively.\textsuperscript{21, 22} Accordingly, individuals with a *2 or *3 allele require significantly lower warfarin doses to achieve therapeutic anticoagulation. The *2 variant exerts lesser effects on dose compared to the *3 allele, as would be expected based on the pharmacokinetic data. Compared to the *1/*1 genotype, 20\% and 35\% reductions in warfarin dose are generally required with the CYP2C9*1/*2 and *1/*3 genotypes, respectively.\textsuperscript{23} Up to 80\% lower warfarin doses may be necessary for *3 homozygotes.\textsuperscript{23, 24}

Less is known about the effects of other CYP2C9 variants on warfarin clearance. However, the CYP2C9*5, *6, *8, and *11 alleles have been correlated with reduced clearance of other CYP2C9 substrates.\textsuperscript{17} Recent studies show lower warfarin dose requirements among African Americans with a *5, *6, *8, or *11 allele, suggesting that they might also reduce clearance of warfarin.\textsuperscript{13, 25}

\textit{VKORC1 genotype and warfarin pharmacodynamics}

The \textit{VKORC1} gene is located on chromosome 16p11.2 and was first described in the context of warfarin resistance, where exceptionally high doses of warfarin (e.g. >20 mg/day) are required to achieve therapeutic anticoagulation. Missense mutations in the coding region of the
VKORC1 gene contribute to warfarin resistance. While rare in the general population, approximately 8% of Ashkenazi Jewish individuals carry the VKORC1 Asp36Tyr mutation accounting for the higher prevalence of warfarin resistance in this population.

In 2005, investigators identified common VKORC1 variants occurring in the gene’s regulatory regions that explain the variability in warfarin dose in the general population. In a sentinel paper, Rieder et al described 10 common VKORC1 SNPs identified through a gene resequencing approach. In patients of European ancestry, these SNPs define two major haplotypes, or groups of SNPs inherited together more often than expected based on chance alone (i.e. in strong linkage disequilibrium). These haplotypes were designated as haplotypes A and B. Haplotype A was associated with lower mRNA expression and warfarin maintenance dose. The mean daily warfarin dose with the AA, AB, and BB haplotype combinations was 2.7 mg, 4.9 mg, and 6.2 mg, respectively.

Since then, Wang et al found that of the SNPs defining VKORC1 haplotype, only the -1639G>A (rs9923231) SNP in the gene promoter region and possibly the 1173C>T SNP in intron 1 (rs9934438) are functional. The -1639A and 1173T SNPs are in near complete linkage disequilibrium across populations (i.e. almost always inherited together). Studies have consistently demonstrated lower warfarin dose requirements with the -1639A (or 1173T) allele. On average, the -1639 AA, AG, and GG genotypes predict warfarin maintenance doses of 3 mg/day, 5 mg/day, and 6 mg/day, respectively. The -1639G>A and 1173C>T SNPs are similarly predictive of warfarin dose, and thus only one needs to be considered warfarin dosing decisions.

There are racial differences in the distribution of the -1639G>A genotype, as shown in Table 1. Approximately 50% of Caucasians have the AG (intermediate sensitivity) genotype. The AA (most sensitive, low dose) genotype predominates in Asians, whereas the GG (least
sensitive, high dose) genotype predominates in African Americans. The racial difference in *VKORC1* genotype distribution contributes to the higher mean warfarin maintenance dose in African Americans and lower mean dose in Asians, compared to Caucasians.\(^\text{12}\)

*CYP4F2 and warfarin pharmacodynamics*

The CYP4F2 enzyme catalyzes metabolism of vitamin K\(_1\) to hydroxyvitamin K\(_1\), which reduces the amount of vitamin K available for reduction to vitamin KH\(_2\), a necessary cofactor for clotting factor activation (Figure 1).\(^\text{31}\) The Val433Met (rs2108622) SNP in exon 2 leads to lower CYP4F2 protein concentration resulting in greater vitamin K availability. In a study of 3 independent Caucasian cohorts, the 433Met/Met genotype was correlated with approximately 1 mg/day higher warfarin dose requirements compared to the Val/Val genotype.\(^\text{32}\) Heterozygotes required intermediate doses. The association was confirmed in separate studies in Caucasian and Japanese patients.\(^\text{33-35}\) The 433Met allele occurs more commonly among Caucasians and Asians than in African Americans (Table 1). The low frequency in African Americans may explain why *CYP4F2* has not been associated with warfarin dose requirements in this racial group.\(^\text{13}\)

Genetic determinants of warfarin response

*Genome wide association studies for dose response associations*

Two genome wide association studies (GWAS) in Caucasians confirmed that the *CYP2C9* and *VKORC1* genes are the primary contributors to warfarin dose requirements in this population. Cooper et al\(^\text{36}\) assayed over 550,000 SNPs in an index population of 181 Caucasians, with replication in a cohort of 374 Caucasians. Takeuchi et al\(^\text{33}\) analyzed over 325,000 SNPs for association with warfarin dose in 1053 Swedish patients. Both studies showed that the *VKORC1*
-1639G>A variant was the most important genetic predictor of warfarin dose, explaining approximately 25% of the overall variability in dose requirements. The \textit{CYP2C9}*2 and \textit{CYP2C9}*3 variants provided moderate contributions to warfarin dose, predicting approximately 9% of the dose variance. The combination of \textit{VKORC1}, \textit{CYP2C9}, and clinical factors (age, sex, weight, amiodarone use, losartan use) explained 47% of total variance in warfarin maintenance dose.\textsuperscript{36} The \textit{CYP4F2} Val433Met SNP explained an additional 1% to 2% of the variability in maintenance dose among the Swedish patients.\textsuperscript{33} No other variant met genome-wide significance for association with warfarin maintenance dose in these studies. A third GWAS in Japanese showed similar results, with \textit{VKORC1} providing the greatest contribution to warfarin maintenance dose, and \textit{CYP2C9} and \textit{CYP4F2} providing lesser contribution.\textsuperscript{35} Whether the \textit{VKORC1} and \textit{CYP2C9} genes are the most important genetic determinants of warfarin dose in African Americans is not yet known, but is the subject of on-going investigation.

\textit{Genetic associations with warfarin bleeding risk}

The \textit{CYP2C9} variant alleles are associated with an increased risk of over-anticoagulation, especially during warfarin initiation.\textsuperscript{1,24,37-39} Some investigators have also reported an increased incidence of bleeding with the \textit{CYP2C9} variants.\textsuperscript{40,24,41,42} In a racially diverse cohort started on warfarin and followed prospectively for 2 years, the variant \textit{CYP2C9} genotype was associated with an increased risk for major, but not minor, bleeding.\textsuperscript{41} The risk for bleeding was similar among Caucasians and African Americans. Overall, \textit{CYP2C9} variants appear to increase the bleeding risk with warfarin approximately 2-fold.\textsuperscript{43} The \textit{VKORC1} -1639A allele is associated with higher INR values and more time spent with an INR above the therapeutic
range. However, in contrast to CYP2C9, the VKORC1 genotype does not appear to confer a clinically significant increase in bleeding risk.41

Genetic associations with time to achieve stable warfarin dosing

Some investigators have reported a delay in dose stabilization with the variant CYP2C9 genotype. However, the data are inconsistent.1,11,39 It is plausible that reduced warfarin metabolism secondary to CYP2C9 polymorphism prolongs the half-life of warfarin and time to achieve steady-state plasma concentrations. This pharmacokinetic effect may contribute to a slower rate of stabilization with a variant allele.

International Warfarin Pharmacogenetics Consortium

A number of investigators from around the world have been involved in elucidating genetic determinants of warfarin response. These groups have consistently shown that the CYP2C9 and VKORC1 variants explain much of the variability in warfarin dose requirements.1, 8, 25, 29, 45-48 However, data from individual groups are limited by smaller sample sizes and geographically confined populations. In order to ensure global clinical utility of pharmacogenetic data, investigators from the international community in collaboration with the Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB) formed the International Warfarin Pharmacogenetics Consortium (IWPC).49 The IWPC consists of 22 research groups from 4 continents and 11 countries. Investigators within the consortium pooled genotype and phenotype data for over 5,700 warfarin-treated patients to create a large, geographically, and ethnically diverse population from which to explore important pharmacogenetic questions. The first effort of the consortium was to create a dosing equation
containing both genetic data (CYP2C9 and VKORC1 genotypes) and nongenetic data (age, height, weight, amiodarone use, enzyme inducer use). The IWPC dosing algorithm explains 40% of the variability in warfarin dose among Caucasians, and approximately 25% among Asians and African Americans. In their most recent effort, IWPC investigators found that no other known VKORC1 SNP or haplotype contributed to warfarin dose requirements beyond that of the -1639G>A or 1173C>T variant.

Racial and ethnic considerations in warfarin pharmacogenetics

African Americans are largely underrepresented in candidate gene studies of warfarin response and were excluded from GWASs published to date. Only 14% of the patients included in the most recent IWPC effort were African American. Frequencies of the CYP2C9*2, CYP2C9*3 and VKORC1 -1639A variants are significantly lower among African Americans compared to Caucasians (Table 1). As a consequence, these SNPs explain significantly less of the variability in warfarin dose in African Americans compared to Caucasians (10% versus >30%, respectively).

_Lesser linkage disequilibrium among African Americans_

There is also lower linkage disequilibrium and greater variation in the African American genome. As a result, genetic variants that occur predominately in African Americans and contribute to warfarin dose response may go undetected in studies limited to persons of non-African ancestry. As mentioned previously, SNP discovery efforts in VKORC1, such as by Rieder et al., involved resequencing the entire VKORC1 gene in Caucasians only. All associations with warfarin dose were made with haplotypes found in Caucasians. Most
subsequent investigations have genotyped only one or two \textit{VKORC1} SNPs that differentiated between haplotype A and haplotype B in Caucasian patients. As mentioned above, only the -1639G>A and 1173C>T SNPs appear functional. Thus, unless the SNP under study is in complete linkage disequilibrium with one of the functional SNPs, the data may not accurately reflect the association between \textit{VKORC1} genotype and warfarin maintenance dose. For example, one of the earlier warfarin dose association studies in African Americans focused on the \textit{VKORC1} 1542G>C SNP. \textsuperscript{51} This SNP had been previously associated with warfarin dose requirements in Caucasians, in whom it is in near complete linkage disequilibrium with the -1639G>A and 1173C>T SNPs. \textsuperscript{12, 46} However, in African Americans, it is inherited much less frequently with the -1639G>A and 1173C>T SNPs. There was no association between the 1542G>C SNP and warfarin dose requirements in the African American population, which likely reflects the fact that the “wrong” SNP was tested.\textsuperscript{30} This example illustrates the importance of including the functional SNP, or at least a SNP in strong linkage disequilibrium with the functional SNP, in pharmacogenetic studies.

Table 2 compiles the frequencies of \textit{VKORC1} haplotypes from different studies.\textsuperscript{29, 52} From this, one can see that, in general, African Americans have a high percentage of haplotype group B (high dose haplotype), while Asians have the highest percentage of haplotype group A (low dose haplotype). This corresponds to a higher frequency of the -1639G allele in African Americans and higher frequency of the -1639A allele in Asians, compared to Caucasians. While the haplotype groups A and B capture 96% and 99% of the variation in Caucasians and Asians, respectively, they account for only 62-78% of the variation seen in African Americans.

\textit{Novel VKORC1 and CYP2C9 variants in African Americans}
Recently, investigators began searching for alternative and novel variants that predict warfarin dose response in African Americans. Through a targeted resequencing strategy of the \textit{CYP2C9} and \textit{VKORC1} genes (in which highly conserved coding, noncoding, and upstream gene regions as well as putative transcriptional binding sites were sequenced), Perera et al\textsuperscript{25} identified novel variation in the African American genome. Two SNPs, one in \textit{CYP2C9} (18786A>T) and one in \textit{VKORC1} (-8191A>G), were predictive of higher warfarin dose requirements in both discovery and validation cohorts, with a 5.2 mg/week increase for each \textit{VKORC1} -8191G allele and a 3.7 mg/week increases for each \textit{CYP2C9} 18786T allele. On regression analysis, the 2 novel SNPs, along with \textit{VKORC1} -1173C>T, known \textit{CYP2C9} alleles, and clinical factors, explained 40% of the overall variability in warfarin dose in the combined African American cohort. In contrast, the IWPC model explained only 26% of the variability in warfarin dose among African Americans.\textsuperscript{7} Thus, the new model containing the novel variants is significantly more predictive of warfarin dose requirements in African Americans than previously published models.

The \textit{VKORC1} -8191G allele is located upstream from the transcriptional start site and occurs in up to 72% of individuals of African ancestry. The \textit{CYP2C9} 18786T variant is located in intron 3 and occurs in approximately 40% of African Americans. Given their common occurrence, failure to account for these variants in warfarin dosing algorithms could result in under-dosing a significant portion of African Americans.

\textit{Calumenin genotype and warfarin dose requirements in African Americans}

Other studies have focused on alternative genes involved in activation of vitamin K dependent clotting factors. Calumenin serves as a chaperone for gamma glutamyl carboxylation
of clotting factors. Voora et al\textsuperscript{53} used a resequencing strategy to identify a calumenin SNP known by the National Center for Biotechnology Information (NCBI) reference SNP (refSNP) number rs339097. The minor G allele was overrepresented in African Americans requiring higher warfarin doses than predicted based on age, body size, and the \textit{CYP2C9} and \textit{VKORC1} genotypes. In a pooled analysis of African Americans, the G allele was associated with 11\% higher warfarin doses. The G allele occurs in about 25\% of African Americans, but less than 1\% of Caucasians, potentially contributing to the higher warfarin dose requirements among the former racial group.

\textit{Warfarin pharmacogenetics in Hispanics}

Patients of Hispanic ethnicity are also underrepresented in warfarin pharmacogenetic studies. Data from the National Health and Nutrition Examination Survey (NHANES) cohort show that \textit{VKORC1} allele frequencies in Hispanics are similar to those in non-Hispanic Caucasians.\textsuperscript{12} In a small study in U.S. Hispanics, the \textit{CYP2C9} and \textit{VKORC1} genotypes made similar contributions to the dose variability in Hispanics as previously reported in non-Hispanic Caucasians.\textsuperscript{54} Thus, until further data from Hispanics are available, Hispanic and non-Hispanic Caucasians can probably be considered similarly in terms of warfarin pharmacogenetics.

\textbf{Adoption and role in clinical practice of pharmacogenetic-guided warfarin therapy}

Regulatory stance on warfarin pharmacogenetics

In the past 5 years, warfarin labeling has undergone two important revisions. In August 2007, pharmacogenetic information was added. Then, in January 2010, a pharmacogenetic dosing table, “Range of expected therapeutic warfarin doses based on the \textit{CYP2C9} and \textit{VKORC1}
genotype” was added. The pharmacogenetic dosing table may help clinicians select an initial warfarin dose when the patient’s CYP2C9 and VKORC1 genotype information is available (Table 3). The table was derived from multiple clinical studies and generally accounts for other clinical factors (e.g. age, race, body weight, sex, concomitant medications, and comorbidities) influencing warfarin dose variability. The revised labeling recommends adjustment of subsequent dosages based on the results of prothrombin time/INR determination. Because the revised label does not require pharmacogenetic testing for warfarin initiation, the use of pharmacogenetic testing remains at the discretion of the clinician.

Warfarin pharmacogenetic dosing algorithms

Warfarin pharmacogenetic dosing algorithms are tools using non-genetic and genetic factors to estimate a therapeutic warfarin dose. The algorithms are linear regression models (Y = aX + b) with the dependent variable (i.e. the Y variable) being the stable warfarin dose, and the independent variables (i.e. the X variables) being non-genetic and genetic factors. For example;

\[
\text{Warfarin daily dose (mg/day)} = \exp(0.9751 - 0.3238 \times V\text{KOR} \text{-}1639\text{G}\text{A} + 0.4317 \times \text{body surface area} - 0.4008 \times C\text{YP2C9*3} - 0.00745 \times \text{age} - 0.2066 \times C\text{YP2C9*2} + 0.2029 \times \text{target INR} - 0.2538 \times \text{amiodarone} + 0.0922 \times \text{smokes} - 0.0901 \times \text{African-American race} + 0.0664 \times \text{deep vein thrombosis/pulmonary embolism})
\]

where the SNPs are coded 0 if absent, 1 if heterozygous, and 2 if homozygous; and race is coded as 1 if African American and 0 if otherwise.
To estimate a therapeutic warfarin dose, known values of the independent variables are added into the model. For example, for a 75-year old African American man who is 5’7” and 75 kg, carries \textit{VKORC1} -1639A/G and \textit{CYP2C9}*1/*1 genotypes, does not smoke, is not on amiodarone and is treated for atrial fibrillation with a target INR of 2.5, the model estimates a warfarin dose of 3.74 mg/day:

$$\exp[0.9751 – 0.3238 x 1 + 0.4317 x 1.88 – 0.4008 x 0 – 0.00745 x 75 – 0.2066 x 0 + 0.2029 x 2.5 – 0.2538 x 0 + 0.0922 x 0 – 0.0901 x 1 + 0.0664 x 0] = 3.74 \text{ mg/day}.$$  

Thus, the algorithms actually estimate a dose whereas the pharmacogenetic dosing table provides a dose range.

About 40 warfarin pharmacogenetic algorithms are currently available because many algorithms were derived from ethnically specific populations (e.g. Slovenians, Koreans, and Japanese etc).\textsuperscript{7, 8, 48, 57-59} All of the algorithms have the \textit{CYP2C9}*3 and the \textit{VKORC1} -1639G>A (or 1173C>T) alleles as independent variables. The algorithms derived from Asian populations do not include the \textit{CYP2C9}*2 allele because this allele is rare in this racial group. Some algorithms include additional SNPs in the \textit{CYP2C9} and \textit{VKORC1} genes (e.g. \textit{CYP2C9}*5, *6, *8 and *11, and \textit{VKORC1} 2255C>T etc) and/or other genes (e.g. \textit{CYP4F2}).\textsuperscript{13, 60, 61} The algorithms also contain various non-genetic factors. The majority include age, body size, amiodarone use and smoking status as independent variables. Some algorithms additionally have prosthetic valve replacement status, heart failure status, and amount of vitamin K intake.

The coefficient of determination ($R^2$) is a measure of how much a linear regression model (e.g. warfarin pharmacogenetic dosing algorithms) explains variability of the data. The higher an
$R^2$ value, the more the model explains the variability of the data. Warfarin pharmacogenetic
dosing algorithms usually have the $R^2$ values of 30-60%, with lower $R^2$ values in African
Americans. Pharmacogenetic dosing algorithms with additional SNPs and/or genes
tend to have slightly higher $R^2$ values than those with only $CYP2C9^*2$, *3 and $VKORC1$-
1639G/A. However, it remains to be determined whether the inclusion of these additional
genetic factors improves clinical outcomes of warfarin therapy.

The www.warfarindosing.org algorithm is freely available online. Whereas other dosing
algorithms require genetic test results prior to the first warfarin dose, www.warfarindosing.org
allows the results to be available before the sixth dose because it can account for previous
warfarin doses and INR values for warfarin dose estimation. As a result, it can be used to adjust
warfarin doses during warfarin initiation. Because it is often not feasible to have genotype
results ready before the first dose, www.warfarindosing.org may be clinically more applicable
than other dosing algorithms. In addition, it is easy to use and has an $R^2$ value about 60% on the
fifth day of warfarin therapy.

Comparisons of traditional and pharmacogenetic dosing methods

The accuracy of dose prediction has been compared among various warfarin dosing
methods. The warfarin dosing methods can be divided into pharmacogenetic and non-
pharmacogenetic methods. Pharmacogenetic methods include the dosing table in the warfarin
labeling and pharmacogenetic dosing algorithms. Non-pharmacogenetic dosing methods include
empiric dosing strategies (e.g. a fixed dose of 5 mg/day) and clinical dosing algorithms. A
clinical dosing algorithm is a linear regression model without a genetic variable. The coefficient
of determination, percent of patients whose estimated doses are within 20% of actual doses, and
mean absolute error (i.e. absolute difference between actual and estimated doses) are commonly used as measures of the accuracy of dosing prediction.

Data suggest that pharmacogenetic algorithms more accurately predict warfarin dose requirements than other dosing methods. In general, pharmacogenetic dosing algorithms have 15-40% higher $R^2$ values and are more likely to predict doses within 20% of the actual dose than other methods. In a direct comparison of various dosing methods, a pharmacogenetic dosing algorithm predicted more doses within 20% of the actual dose (52%) than a clinical dosing algorithm (39%), the dosing table in the warfarin labeling (43%), and an empiric dosing method (i.e. 5 mg/day; 37%). Pharmacogenetic algorithms particularly outperform more traditional dosing approaches for patients requiring warfarin doses $\leq$ 3 mg/day or $\geq$ 7 mg/day.

Because the $CYP2C9$ and $VKORC1$ polymorphisms account for 10-30% of warfarin dose variability, it is not surprising that genotype-based dosing is generally more accurate than non-genotype-based methods. However, it is interesting that the pharmacogenetic table in the warfarin labeling was less accurate than the pharmacogenetic algorithm at predicting warfarin dose. The lower accuracy of the table could be due to several factors. First, the table predicts doses within a range of 0.5 to 7 mg/day. In contrast, dosing algorithms can predict doses $>7$ mg/day. Secondly, the table may not adequately account for some of the clinical factors influencing warfarin dose variability. In a recent study, amiodarone use and female sex were identified as factors predicting lower accuracy of the dosing table. Interestingly, the table appears to be as effective as a pharmacogenetic dosing algorithm in African Americans, with 41% of doses predicted with the table and 42% predicted with the algorithm falling within 20% of the actual dose. In contrast to the IWPC algorithm, the table is more accurate at predicting doses within the intermediate range of 3-7 mg/day than predicting lower doses ($\leq$ 3 mg/day),
with 56% of doses in the intermediate range and 15% in the low range falling within 20% of the actual dose.\textsuperscript{64} Thus, the table may be considered for certain populations (e.g. African Americans), but in general, is less accurate than pharmacogenetic dosing algorithms, particularly for low doses.

\textit{Evaluation of various pharmacogenetic dosing algorithms}

The accuracy of various warfarin pharmacogenetic dosing algorithms has also been compared.\textsuperscript{60, 62, 65-68} The \texttt{www.warfarindosing.org} and IWPC dosing algorithms have been consistently identified as the most accurate of the algorithms.\textsuperscript{65-67} These two algorithms have mean absolute errors ranging 1.2-1.4 mg/day and predict 45-54% of doses within 20% of the actual dose. They also appear to perform similarly across race groups, with the percent of doses within 20% of the actual dose ranging from 37-53% in Asians, 39-52% in African Americans, and 47-56% in Caucasians. The algorithms perform better for doses \(>3\) and \(<7\) mg/day (54-60% within 20% of the actual dose) than for doses \(\leq 3\) mg/day (20-45% within 20% of the actual dose) or \(\geq 7\) mg/day (38-48% within 20% of the actual dose).\textsuperscript{67} The algorithms may be most accurate for the intermediate dose range because the majority of patients included in the derivation of the algorithm required doses \(>3\) and \(<7\) mg/day. Nonetheless, compared to non-genotype-based dosing methods, pharmacogenetic algorithms are significantly better at predicting doses \(<3\) and \(>7\) mg/day.\textsuperscript{7}

Warfarin pharmacogenetic algorithms have some limitations that should be noted. First, they do not include all of the known factors causing warfarin dose variability. Disease state (e.g., acute decompensated heart failure, thyroid dysfunction), vitamin K intake, and drugs interacting with warfarin (particularly CYP2C9 inducers) are excluded from most algorithms. Many
algorithms, including www.warfarindosing.org and the IWPC algorithm, do not contain genetic variables that are common in African Americans. This may contribute to the greater mean absolute error with the algorithms in African Americans. In addition, the algorithms have the lowest accuracy in the high warfarin dose range. They are especially unlikely to predict unusually high doses (e.g., 20 mg/day) because most do not include genetic variants associated with warfarin resistance. Finally, recent data suggest that pharmacogenetic algorithms may overestimate warfarin doses in elderly patients who require <2 mg/day. Thus, warfarin pharmacogenetic algorithms should be viewed as adjuncts to decrease uncertainty about initial warfarin doses; they do not replace close monitoring of INR and sound clinical judgment.

Warfarin pharmacogenetic tests

There are two mechanisms by which a warfarin pharmacogenetic test can be introduced for clinical use. The first involves a premarket clearance by the FDA as an in vitro diagnostic device or “test kit.” In this mechanism, manufacturers provide clinical laboratories with all the ingredients and instructions necessary to perform the test. The second mechanism involves an individual clinical laboratory developing and offering a test. In this mechanism, only component reagents used as part of laboratory-developed tests are regulated by the FDA. Of note, neither of these mechanisms requires clinical outcome data for clinical use.

As of May 1, 2011, four warfarin pharmacogenetic tests are available as in vitro diagnostic devices. As shown in Table 4, all of these tests genotype for three loci: CYP2C9*2, CYP2C9*3 and one VKORC1 SNP (VKORC1 -1639G/A or 1173C>T). All of the tests can be completed in 8 hours, including DNA extraction; the fastest ones provide genotype results in less than 2 hours.
Although the availability of FDA-cleared devices for warfarin pharmacogenetic testing makes genotype-guided warfarin initiation possible, several barriers to clinical adoption remain. First, many medical centers do not have warfarin pharmacogenetic testing available. In a recent survey, only 20% of hospitals in North America have testing available on site, suggesting the majority of the hospitals rely on outside commercial clinical laboratories. This outsourcing may make genotype-guided warfarin initiation impractical because of 3-7 days of turnaround time. Second, no other professional organization currently endorses warfarin pharmacogenetic testing in their guidelines because of the lack of the clinical utility data. Inclusion of testing recommendation in professional guidelines has been identified as a factor influencing reimbursement of new technology. As such, the Centers for Medicare and Medicaid Services (CMS) and many commercial insurance plans generally do not reimburse the cost of testing ($300-$500). There are two exceptions: 1) some plans may reimburse the cost if the need for testing can be justified (e.g., patients with high bleeding risk), 2) the CMS covers the cost to support development of evidence that may benefit Medicare beneficiaries if testing is performed for patients enrolled in a prospective, randomized, controlled clinical study measuring clinical outcomes (i.e., mortality, bleeding, or thromboembolism) of warfarin pharmacogenetic testing. Because of these barriers, warfarin pharmacogenetic testing is currently performed mainly for research purposes and for patients willing to pay for the cost.

Economic data

Since cost-effectiveness is an important factor influencing clinical adoption of new technology, studies have evaluated cost-effectiveness of warfarin pharmacogenetic testing and have produced mixed results. One early study indicated that genetic testing may save up to $1.1
billion per year. However, this study made several overly optimistic assumptions including the assumption that testing would have 100% efficacy and that those with a variant allele would have a 27% per year incidence of serious bleeding without genotype-guiding dosing.

Currently, the body of evidence suggests that warfarin pharmacogenetic testing may not be cost-effective at present. Such evidence includes studies showing that the marginal cost-effectiveness of testing exceeds $170,000 per quality-adjusted life-year (QALY) and that testing has only a 10% chance to be cost-effective (i.e., less than $50,000 per QALY). The incremental cost per unit outcome improved per QALY was greater than $50,000 for 62.1% of the time in one study. Similarly, a cost-effectiveness analysis based on results from a small randomized controlled clinical trial suggested that the incremental cost-effectiveness ratio was greater than $50,000 54% of the time.

Several factors may determine cost-effectiveness of warfarin pharmacogenetic testing. First, cost-effectiveness may vary by the population to be tested. Specifically, testing was shown to be cost-effective in elderly patients with atrial fibrillation. Secondly, the clinical utility of the testing will influence cost effectiveness. One study showed that testing is cost-effective if it increases the time spent within the therapeutic INR range by more than 5-9%. Other determinants are testing cost and turnaround time. Testing was shown to be cost-effective if it costs less than $200, is available within 24 hours and prevents greater than 32% of major bleeding in high risk patients. The cost and turnaround time are likely to be reduced with wide availability of testing and rapid advances in genotyping technology. Another consideration is that CYP2C9 genotype only needs to be determined once in a patient’s lifetime, and the results may have implications for other drugs that are CYP2C9 substrates (e.g. phenytoin, non-steroidal anti-inflammatory drugs, sulfonylureas). Ongoing, prospective, randomized trials are evaluating
the clinical utility of warfarin genetic testing and will provide important data to inform cost
effectiveness analyses. In particular, the Clinical and Economic Implications of Genetic Testing
for Warfarin Management study (ClinicalTrials.gov ID NCT00964353) is an ongoing trial with
the goal of assessing the clinical and cost effectiveness of existing pharmacogenetic algorithms
for the management of warfarin.

Future role/direction of pharmacogenetic guided anticoagulation therapy

Trials assessing the clinical utility of warfarin pharmacogenetics

The Centers for Disease Control and Prevention (CDC) define clinical utility of a genetic
test as evidence of improved measurable clinical outcomes, as well as its usefulness and added
value to patient management decision-making compared with current management without
genetic testing. Thus, clinical utility of a warfarin pharmacogenetic test may be the evidence
that testing decreases the risk of bleeding or thromboembolism in patients initiating warfarin
therapy. Because of the rarity of these events, most trials use markers for the risk of bleeding or
thrombosis, such as time in the therapeutic INR range, as their primary endpoint. This evidence
may be best obtained from a prospective, randomized trial comparing clinical outcomes between
patients newly initiating warfarin with and without pharmacogenetic testing.

As shown in Table 5, four small, prospective, randomized trials have evaluated the
clinical utility of warfarin pharmacogenetic testing to date with mixed results. For
example, Caraco et al reported that compared to warfarin dosing based on clinical factors
alone, dosing based on clinical factors plus CYP2C9 (but not VKORC1) genotype led to earlier
attainment of stable anticoagulation, more time spent within the therapeutic range, and a lower
incidence of minor bleeding. In contrast, a study of similar size showed no benefit of dosing based on both CYP2C9 and VKORC1 genotypes for the primary endpoint of the percentage of out-of-range INR values over the initial months of therapy. However, a subgroup analysis of this latter trial revealed a lower percentage of out-of-range INR values with genotype-guided versus standard dosing among patients who had either the CYP2C9*1/*1 and VKORC1 1173CC genotypes or multiple CYP2C9 and/or VKORC1 variant alleles.

All four of the trials completed to date had a small sample size (n ≤230), homogenous population, and excluded of bleeding and thromboembolism as primary outcomes. In addition, each study used a different pharmacogenetic dosing algorithm, and none used the IWPC or www.warfarindosing.org algorithms, which are believed to be the most accurate. For these reasons, the results of these studies should be largely viewed as inconclusive.

A comparative effectiveness study reported a significant clinical benefit with genotyping for CYP2C9 and VKORC1 variants at the initiation of warfarin therapy. Study patients were offered free genotyping, and the results were provided to the patient’s physician with an interpretative report. Controls consisted of patients who started warfarin the previous year and were not offered genotyping. Patients who underwent genotyping had fewer hospitalizations for any cause and fewer hospitalizations for bleeding or thromboembolism during the 6-month follow-up period compared to controls. These findings suggest that genotype-guided warfarin dosing is of benefit in a “real-world” setting. However, criticisms of the study include the use of historical controls and the non-randomized, non-blinded, and non-placebo controlled design.

At least four multicenter, randomized, controlled, clinical trials are underway in the U.S. and Europe to assess the clinical utility of warfarin pharmacogenetic testing: the Clarification of Optimal Anticoagulation through Genetics (COAG); the Genetics Informatics Trial (GIFT) of
Warfarin to Prevent Deep Venous Thrombosis; the Clinical, Economic Implication of Genetic Testing for Warfarin Management trial; and the European Pharmacogenetics of Anticoagulation Trial (EU-PACT). These trials are expected to be completed between March 2012 and August 2014. Perhaps the most anticipated of these trials is the National Heart, Lung, and Blood Institute-sponsored COAG trial (ClinicalTrials.gov ID NCT00839657), which began in September 2009. This is a prospective, double-blind trial with a planned enrollment of 1238 participants from at least 12 centers in the U.S. Participants are randomized to a genotype-guided or clinical warfarin dosing initiation strategy, with randomization stratified by race. Both strategies employ a dose initiation algorithm followed by a dose-revision algorithm for 5 days. A standard dose-titration algorithm is used by both groups for subsequent dose adjustment. The primary outcome measure is the percent of time spent within the therapeutic INR range during the initial 4 weeks of therapy. A secondary measure is the occurrence of an INR >4 or serious event during the initial 4 weeks of therapy. The study is expected to be completed in March 2012.

Guidelines for implementation for warfarin pharmacogenetics

One of the barriers to clinical uptake of warfarin pharmacogenetic testing is that many clinicians do not know how to interpret and apply the test results to adjust warfarin doses. To address this unmet need, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has been created “to provide clear, curated, peer-reviewed guidelines that translate pharmacogenomic test results into actionable prescribing decisions for specific drugs.” As such, the CPIC guidelines do not address which pharmacogenomic tests should be ordered to prescribe a drug. Instead, they provide guidance on how to interpret and apply pharmacogenetic test results to more appropriately prescribe a drug when the test results are available. The CPIC
consists of the Pharmacogenomics Research Network members, PharmGKB staff as well as experts in pharmacogenetics, pharmacogenomics and laboratory medicine. Their guidelines on the interpretation and application of warfarin pharmacogenetic test results are expected to be available in 2011. Although these guidelines may not change the current reimbursement policy of third party payers, they will be instrumental for clinicians to determine an appropriate warfarin dose for a patient with genotype results available.

Novel genotyping and bioinformatic methods in warfarin pharmacogenetics

As interest increases in evaluating the complexity of genetic association in disease and drug response with more comprehensive bioinformatics tools, so has interest in looking at such association in pharmacogenetic studies. The use of comparative genomics has recently been highlighted as a method to prioritize regions for sequencing and has led to the discovery of novel variants in both $CYP2C9^{25}$ and $CALU$, both of which affect warfarin dose. These studies incorporate the genomic sequence of several mammalian species (publicly available through http://uswest.ensembl.org/index.html), covering millions of years of evolution, to identify areas of deep sequence conservation, as these regions may contain elements responsible for gene expression and function.

An additional tool in uncovering regions that may play a role in gene regulation is through putative transcriptional site mapping. The idea behind this technique is that the identification of clusters of binding sites may signal the presence of a regulatory region as opposed to known transcriptional binding factor sequences that occur at random throughout the genome. Several $in silico$ methods exist to calculate the posterior probability of regulatory clusters with a DNA sequence. These methods have been used to identify regulatory regions in
CYP2C9 \(^{25}\) as well as other important drug metabolizing genes with important implications for drug response.\(^{90}\)

One last novel area of growing interest that is expected to be applied to warfarin pharmacogenetics is in the identification of expression quantitative trait loci (eQTLs). eQTLs are SNPs found throughout the genome that may directly (as in a regulatory element upstream of a gene) or indirectly (as in a SNP within a transcription binding factor that regulates a gene) affect the expression of a gene. Currently the publically available database, SCAN \(^{91}\) (http://scan.bsd.uchicago.edu/newinterface/about.html) can be queried to show SNPs throughout the genome found to be associated with gene expression for the lymphoblastoid cell lines (LCLs) of HapMap samples. The advantage to this method over other genome wide approaches is that the association made using eQTLs has a plausible biologic function as opposed to SNPs that may not be close to any known gene and have unknown function. The disadvantage is that genes that are not expressed in the tissue used to quantify gene expression (lymphoblastoid cell lines in the case of SCAN) cannot be evaluated. Use of these novel tools and techniques will likely accelerate our understanding of genetic variants contributing to anticoagulant drug response in the near future.

Pharmacogenetics of novel anticoagulants

Dabigatran is a reversible direct thrombin inhibitor that was approved in 2010 for the prevention of stroke in patients with atrial fibrillation. Rivaroxaban and apixaban are direct factor Xa inhibitors and are additional novel oral anticoagulation agents currently under study. There are no pharmacogenetic data with any of these agents to date. Candidate genes that might influence response to dabigatran, rivaroxaban, or apixaban include those encoding for proteins
involved in drug metabolism, drug transport, or drug target proteins. We will focus our
discussion of candidate genes on dabigatran, since it is the only agent currently approved.

Dabigatran etexilate is a prodrug that is rapidly hydrolyzed by nonspecific esterases to its
active form, dabigatran, following absorption. Based on both in-vitro studies and in-vivo studies
in healthy volunteers, the CYP450 enzymes do not appear to have a role in the conversion of the
prodrug to dabigatran or to dabigatran’s metabolism.92-94 Thus, unlike warfarin, CYP450
genotype is unlikely involved in dabigatran pharmacokinetics. On the other hand, the dabigatran
etexilate prodrug is a substrate for the polymorphic drug efflux transporter P-glycoprotein.
There is evidence that co-administration with the p-glycoprotein inhibitor amiodarone increases
AUC for dabigatran by 60%.95 P-glycoprotein is encoded by the ABCB1 gene, which is a
member of the ATP-binding cassette (ABC) transporter superfamily. A number of SNPs have
been identified in the ABCB1 promoter and exon (coding) regions and have been associated with
plasma concentrations of p-glycoprotein substrates.96 Thus, it is plausible that ABCB1 genotype
could influence dabigatran absorption and availability.

Dabigatran binds to the active site of thrombin to prevent conversion of fibrinogen to
fibrin.97 Thus, it is also possible that variants at the thrombin site could affect dabigatran
pharmacodynamics. However, variants affecting dabigatran pharmacokinetics or
pharmacodynamics have yet to be defined. Genetic samples were collected as part of the Phase
III clinical trials with dabigatran, and thus, pharmacogenetic studies are expected to emerge with
this newly approved agent.

Summary
In summary, there are substantial and convincing data supporting the clinical and analytical validity of warfarin pharmacogenetics. The *CYP2C9* and *VKORC1* genes are the primary determinants of warfarin dose requirements in Caucasian and Japanese patients. The genetic determinants of warfarin response in other racial groups are still being defined. Novel pharmacogenetic approaches may assist in identifying variants of importance in African Americans and other minority populations. There are several FDA-cleared tests for *CYP2C9* and *VKORC1* genotyping available. However, genotype-guided warfarin dosing has not yet become a reality in most medical centers despite the wealth of data supporting genetic influences of warfarin dose requirements. Many clinicians and third party payers are awaiting evidence of clinical utility and cost effectiveness before adopting genetic testing for anticoagulation management in the clinic setting. Results from on-going clinical trials are expected to address these issues and will likely determine the course of genotype-guided anticoagulant therapy. Whether pharmacogenetics will have a role in the treatment with newer anticoagulant agents has yet to be determined. However, pharmacogenetics with newer anticoagulants could be of great importance given the unavailability of routine monitoring parameters with these agents.
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diverse large cohort. Pharmacogenomics 2011;12:125-34.
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assessment of pharmacogenetically predictive warfarin dosing algorithms in patients of an


Acknowledgements

Table 1. Location and prevalence of variant *CYP2C9* and *VKORC1* alleles\textsuperscript{11-14}

<table>
<thead>
<tr>
<th>Allele</th>
<th>Location</th>
<th>Prevalence\textsuperscript{a}</th>
<th>Caucasians</th>
<th>Asians</th>
<th>African Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>CYP2C9</em>2 (144Cys)</td>
<td>Exon 3</td>
<td>20-24%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>3-4%</td>
</tr>
<tr>
<td><em>CYP2C9</em>3 (359Leu)</td>
<td>Exon 7</td>
<td>11-12%</td>
<td>8-9%</td>
<td>1-3%</td>
<td></td>
</tr>
<tr>
<td><em>CYP2C9</em>5 (360Glu)</td>
<td>Exon 7</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>1-2%</td>
</tr>
<tr>
<td><em>CYP2C9</em>6 (Null)</td>
<td>Exon 5</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>1-3%</td>
</tr>
<tr>
<td><em>CYP2C9</em>8 (150His)</td>
<td>Exon 3</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>12%</td>
</tr>
<tr>
<td><em>CYP2C9</em>11 (335Trp)</td>
<td>Exon 7</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>3-4%</td>
</tr>
<tr>
<td><em>VKORC1</em> -1639A</td>
<td>Promoter</td>
<td>60%</td>
<td>99%</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td><em>CYP4F2</em> 433Met</td>
<td>Exon 2</td>
<td>39%</td>
<td>37-45%</td>
<td>14%</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Percent of patients who carry a variant allele

Abbreviations: *CYP*, cytochrome P450; *VKORC1*, vitamin K epoxide reductase complex subunit
Table 2: *VKORC1* haplotype group (haplogroup) frequency across ethnicities.

<table>
<thead>
<tr>
<th>Population</th>
<th>Haplogroup A</th>
<th>Haplogroup B</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>European Americans</td>
<td>37%-42%</td>
<td>57%-58%</td>
<td>29, 52</td>
</tr>
<tr>
<td>African Americans</td>
<td>14%-21%</td>
<td>49%-58%</td>
<td>29, 52</td>
</tr>
<tr>
<td>Asians</td>
<td>85%-89%</td>
<td>10%-14%</td>
<td>29, 52</td>
</tr>
<tr>
<td>Peruvians</td>
<td>27%</td>
<td>71%</td>
<td>52</td>
</tr>
<tr>
<td>Mexicans</td>
<td>38%</td>
<td>57%</td>
<td>52</td>
</tr>
<tr>
<td>Africans</td>
<td>23%</td>
<td>49%</td>
<td>52</td>
</tr>
</tbody>
</table>
Table 3. Range of expected therapeutic warfarin doses based on *CYP2C9* and *VKORC1* genotypes (mg/day).\textsuperscript{55}

<table>
<thead>
<tr>
<th>VKORC1</th>
<th>CYP2C9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*1/*1</td>
</tr>
<tr>
<td>GG</td>
<td>5-7</td>
</tr>
<tr>
<td>GA</td>
<td>5-7</td>
</tr>
<tr>
<td>AA</td>
<td>3-4</td>
</tr>
</tbody>
</table>

Abbreviations: *CYP2C9*, cytochrome P450 2C9; *VKORC1*, vitamin K epoxide reductase complex subunit 1; G, guanine; A, adenine.
Table 4. Warfarin pharmacogenetic tests cleared by the US Food and Drug Administrationa

<table>
<thead>
<tr>
<th>Test name</th>
<th>Manufacturer</th>
<th>Alleles tested</th>
<th>Estimated time for assay completion (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Verigene® Warfarin Metabolism</td>
<td>Nanosphere Inc.</td>
<td><em>CYP2C9</em>2 and *3,</td>
<td>≤2</td>
</tr>
<tr>
<td>Nucleic Acid Test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiniti 2C9-VKORC1 Multiplex</td>
<td>AutoGenomics Inc.</td>
<td><em>CYP2C9</em>2 and *3,</td>
<td>6-8</td>
</tr>
<tr>
<td>Assay for warfarinb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eSensor® Warfarin Sensitivityc</td>
<td>Osmetech Molecular Diagnostics</td>
<td><em>CYP2C9</em>2 and *3,</td>
<td>3-4</td>
</tr>
<tr>
<td>eQ-PRC LC Warfarin Genotyping KIT</td>
<td>Trimgen Corporation</td>
<td><em>CYP2C9</em>2 and *3,</td>
<td>≤2</td>
</tr>
</tbody>
</table>

*aAs of May 1, 2011*


Abbreviations: CYP, cytochrome P450; VKORC1, vitamin K epoxide reductase complex subunit 1
Table 5. Summary of four prospective, randomized trials evaluating clinical utility of a warfarin pharmacogenetic test69, 81-83

<table>
<thead>
<tr>
<th>Study Population group</th>
<th>Study size</th>
<th>Study Standard</th>
<th>Primary outcome</th>
<th>Results</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caraco et al81 (2008) Caucasians Computerized</td>
<td>191</td>
<td>Caucasians Computerized</td>
<td>Time to reach warfarin first therapeutic dosing program INR range</td>
<td>Pharmacogenetics group: 14.1 ± 6.9 days</td>
<td>The pharmacogenetic algorithm was based on expected warfarin clearance by CYP2C9 genotype. <em>VKORC1</em> genotype was not used.</td>
</tr>
<tr>
<td>COUMAGEN69 206 Caucasians Published nomogram</td>
<td>206</td>
<td>Caucasians Published nomogram</td>
<td>Percent of out-of-range INRs in the first 90 days</td>
<td>Pharmacogenetics group: 30.7%</td>
<td>Investigators developed a pharmacogenetic dosing algorithm.</td>
</tr>
</tbody>
</table>

Standard group: 32.2 ± 21.1 days (P<0.001)
median 35 days ($P<0.001$) 3 days was capped at 3 mg/day in the pharmacogenetics group.

Burmester et al\textsuperscript{83} 230 Caucasian ancestry algorithm
Clinical dosing Absolute Pharmacogenetics group: Investigator
prediction error 0.80 mg/day
time to 1.32 mg/day
therapeutic dose. (P not reported)
Time in therapeutic range: Time in range: Pharmacogenetics group:
during the first 14 days 30.8 ± 28.4%
Standard group: 29.1 ± 15.5% ($P=0.56$)

Abbreviations: INR, International normalized ratio; CYP, cytochrome P450; VKORC1, vitamin K epoxide reductase subunit 1
Figure 1. Genes involved in the pharmacokinetics and pharmacodynamics of warfarin. The proteins shown in italics are encoded by polymorphic genes that provide significant contributions to warfarin disposition (CYP2C9), vitamin K disposition (CYP4F2), and clotting factor activation (VKOR and calumenin). CYP, cytochrome P450; VKOR, vitamin K epoxide reductase.