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

Dr. Larisa H. Cavallari, Pharm.D., Dr. Jaekyu Shin, Pharm.D., M.S., and Dr. Minoli A. Perera, Pharm.D., Ph.D.

Department of Pharmacy Practice, University of Illinois at Chicago, Chicago, Illinois (Dr. Cavallari); the Department of Clinical Pharmacy, University of California–San Francisco, San Francisco, California (Dr. Shin); and the Department of Medicine, University of Chicago, Chicago, Illinois (Dr. Perera)

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 interpatient variability in anticoagulant effects. Over the past decade, there has been substantial progress in our understanding of genetic contributions to variable warfarin response, particularly with regard to warfarin dose requirements. The genes encoding for cytochrome P450 (CYP) 2C9 (*CYP2C9*) and vitamin K epoxide reductase complex subunit 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-American patients compared with other racial groups, but this is the focus of ongoing research. Several warfarin pharmacogenetic dosing algorithms and United States Food and Drug Administration–cleared genotyping tests are available for clinical use. Clinical trials are ongoing 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. There is still a lack of pharmacogenetic data for the newly approved oral anticoagulants, dabigatran and rivaroxaban, and with other oral anticoagulants in the research and development pipeline. These data, once known, could be of great importance as routine monitoring parameters for these agents are not available.

Keywords

warfarin; polymorphism; dabigatran; genotype; pharmacogenetics; cytochrome P450; *CYP2C9* gene; vitamin K epoxide reductase complex subunit 1; *VKORC1* gene

Warfarin is the most commonly prescribed oral anticoagulant and is widely used for the prevention of thromboembolism or stroke in patients with previous thromboembolism, recent orthopedic surgery, atrial fibrillation, heart valve replacement, or other diseases that increase the risk for thrombosis. Dabigatran and rivaroxaban are the only warfarin competitors on the market in the United States. However, dabigatran is approved only for

the prevention of stroke in patients with atrial fibrillation. Rivaroxaban is approved for prophylaxis of venous thromboembolism after orthopedic surgery. Little is known about the pharmacogenetics of dabigatran, rivaroxaban, or other oral anticoagulants in the drug development pipeline. Thus, most of this review focuses on warfarin, with a brief discussion of potentially important genes related to dabigatran.

One of the primary challenges with warfarin therapy is determining the dosing regimen necessary to achieve therapeutic anticoagulation for an individual patient. Dose requirements vary as much as 20-fold among patients.¹ Failure to achieve optimal anticoagulation significantly increases the risk for adverse sequelae.^{2, 3} Clinical factors, including age, body size, diet, and drugs that interfere with warfarin metabolism, are well known to influence warfarin dose requirements.⁴ There is also recent evidence that decreased renal function reduces warfarin dose requirements and increases the risk for warfarin-related bleeding.^{5, 6} Dose requirements also vary significantly by race, with higher mean maintenance doses in African-Americans and lower mean doses in Asians compared with doses in Caucasians. Whereas clinical factors are obviously important considerations when dosing warfarin, factors such as age, body size, and interacting drugs account for only 15–20% of the overall variability in warfarin dose.^{1, 7, 8} It is now widely accepted that an individual's genotype significantly influences the warfarin dose required 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 with regard to warfarin dose requirements. Genes encoding for proteins involved in warfarin metabolism and pharmacodynamics contribute to the interpatient 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-hydroxywarfarin protein. Warfarin exerts its therapeutic effect by inhibiting vitamin K epoxide reductase (VKOR). The genes encoding for CYP2C9 (*CYP2C9*) and VKOR (*VKORC1*) are the major genetic determinants of warfarin pharmacokinetics and pharmacodynamics, respectively. The *CYP4F2* gene exerts lesser influence 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 *CYP2C9**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 *CYP2C9**8 allele is one of the most common variant in persons of African descent, occurring in up to one of every nine African-Americans.^{13, 15} Decreased enzyme activity has been reported with the *CYP2C9**5, *6, and *11 alleles, whereas data with the *CYP2C9**8 allele are conflicting.^{16–18} Specifically, in vitro data with the *CYP2C9**8 allele show greater activity toward tolbutamide.¹⁸ This is in contrast to a clinical pharmacokinetic study, which shows a reduction in phenytoin metabolism in patients with the *CYP2C9**8 allele.¹⁷ We know of no pharmacokinetic data with the *CYP2C9**8 allele using warfarin as a probe.

The *CYP2C9**2 amino acid substitution occurs on the outer surface of the enzyme, whereas the *CYP2C9**3 substitution occurs internally.¹⁹ Neither appears to affect substrate binding. Rather, evidence suggests that they disrupt formation of intermediate compounds in the *CYP2C9* catalytic cycle.²⁰ The *CYP2C9**2 and *3 alleles reduce S-warfarin clearance by 40% and 75%, respectively.^{21, 22} In accordance, individuals with a *CYP2C9**2 or *3 allele require significantly lower warfarin doses to achieve therapeutic anticoagulation. The *CYP2C9**2 variant exerts lesser effects on dose compared with the *CYP2C9**3 allele, as would be expected based on the pharmacokinetic data. Compared with the *CYP2C9**1/*1 genotype, 20% and 35% reductions in warfarin dose are generally required with the *CYP2C9**1/*2 and *1/*3 genotypes, respectively.²³ Up to 80% lower warfarin doses may be necessary for *CYP2C9**3 homozygotes.^{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.¹⁷ Recent studies show lower warfarin dose requirements among African-Americans with a *CYP2C9**5, *6, *8, or *11 allele, suggesting that these alleles might also reduce clearance of warfarin.^{13, 25}

VKORC1 Genotype and Warfarin Pharmacodynamics—The *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.²⁶ Although 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.²⁷

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.^{28, 29} In a sentinel article, the authors described 10 common *VKORC1* SNPs identified through a gene resequencing approach.²⁹ 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 messenger RNA expression and warfarin maintenance dose. The mean daily warfarin doses with the AA, AB, and BB haplotype combinations were 2.7, 4.9, and 6.2 mg, respectively.

Since then, another group of investigators 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 (rs9934438) in intron 1 are functional.³⁰ The -1639A and 1173T SNPs are in near complete linkage disequilibrium across populations (i.e., almost always inherited together).¹² 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, 5, 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 in 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 requirements in African-Americans and lower mean dose in Asians, compared with Caucasians.¹²

CYP4F2 Gene and Warfarin Pharmacodynamics—The CYP4F2 enzyme catalyzes the metabolism of vitamin K₁ to hydroxyvitamin K₁, which reduces the amount of vitamin K available for reduction to vitamin K_{H2}, a necessary cofactor for clotting factor activation (Figure 1).³¹ The Val433Met (rs2108622) SNP in exon 2 leads to lower CYP4F2 protein concentration, resulting in greater vitamin K availability. In a study of three independent Caucasian cohorts, the 433Met/Met genotype was correlated with approximately 1-mg/day higher warfarin dose requirements compared with the Val/Val genotype.³² Heterozygotes required intermediate doses. The association was confirmed in separate studies in Caucasian and Japanese patients.^{33–35} The 433Met allele occurs more commonly in Caucasians and Asians than in African-Americans (Table 1). The low prevalence in African-Americans may explain why *CYP4F2* has not been associated with warfarin dose requirements in this racial group.¹³

Genetic Determinants of Warfarin Response

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

Genetic Associations with Warfarin Bleeding Risk—The *CYP2C9* variant alleles are associated with an increased risk of overanticoagulation, especially during warfarin initiation.^{1, 24, 37–39} Some investigators have reported an increased occurrence of bleeding with the *CYP2C9* variants.^{24, 40–42} In a racially diverse cohort that was started on warfarin and was followed prospectively for 2 years, the variant *CYP2C9* genotype was associated with an increased risk for major, but not minor, bleeding.⁴¹ The risk for bleeding was similar among Caucasians and African-Americans. Overall, *CYP2C9* variants appear to increase the bleeding risk with warfarin approximately 2-fold.⁴³ The *VKORC1* –1639A allele is associated with higher international normalized ratio (INR) values and more time spent with an INR above the upper limit of the therapeutic range.^{1, 33, 37, 44} 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 small sample sizes and geographically confined populations. 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 four continents and 11 countries. Investigators within the consortium pooled genotype and phenotype data for more than 5700 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).⁷ The IWPC dosing algorithm explains 40% of the variability in warfarin dose among Caucasians, and approximately 25% among Asians and African-Americans.¹² 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.¹²

Racial and Ethnic Considerations in Warfarin Pharmacogenetics

African-Americans are largely underrepresented in candidate gene studies of warfarin response and were excluded from the published genomewide association studies. Only 14% of the patients included in the most recent IWPC effort were African-American.¹² Frequencies of the *CYP2C9**2, *CYP2C9**3, and *VKORC1* –1639A variants are significantly lower among African-Americans compared with Caucasians (Table 1). As a consequence, these SNPs explain significantly less of the variability in warfarin dose in African-Americans compared with Caucasians (10% vs > 30%).^{12, 45, 50}

Lower Linkage Disequilibrium Among African-Americans—There is also lower linkage disequilibrium and greater variation in the African-American genome compared with non-Africans.⁵¹ As a result, genetic variants that occur predominantly 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 in the sentinel article,²⁹ involved resequencing the *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 *VKORC1* SNPs that differentiated between haplotype A and haplotype B in Caucasian patients. As mentioned earlier, 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 *VKORC1* genotype and warfarin maintenance dose. For example, one of the earlier warfarin dose association studies in African-Americans focused on the *VKORC1* 1542G>C SNP.⁵² 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.^{12, 46} In African-Americans, however, 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.³⁰ This example illustrates the importance of including the functional SNP, or at least an SNP in strong linkage disequilibrium with the functional SNP, in pharmacogenetic studies.

Table 2 compiles the frequencies of *VKORC1* haplotypes from different studies.^{29, 53} From this, one can see that, in general, African-Americans have a high percentage of haplotype group B (high-dose haplotype), whereas 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 with Caucasians. Although 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.

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 *CYP2C9* and *VKORC1* genes (in which highly conserved coding, noncoding, and upstream gene regions as well as putative transcriptional binding sites were sequenced), one group of investigators identified novel variation in the African-American genome.²⁵ Two SNPs, one in *CYP2C9* (18786A>T) and one in *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 *VKORC1* –8191G allele and 3.7-mg/week increases for each *CYP2C9* 18786T allele. On regression analysis, the two novel SNPs, along with *VKORC1* –1173C>T, known *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 explains only 26% of the variability in warfarin dose among African-Americans.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 *VKORC1* –8191G allele is located upstream from the transcriptional start site and occurs in up to 72% of individuals of African ancestry. The *CYP2C9* 18786T variant is located in intron 3 and occurs in approximately 40% of African-Americans. Given the common occurrence of these variants, failure to account for them in warfarin dosing algorithms could result in underdosing a significant portion of African-Americans.

Calumenin Genotype and Warfarin Dose Requirements in African-Americans

—Other studies have focused on other genes involved in activation of vitamin K–dependent clotting factors. Calumenin serves as a chaperone for γ -glutamyl carboxylation of clotting factors. One group of investigators used a resequencing strategy to identify a calumenin SNP, known by the National Center for Biotechnology Information reference SNP number rs339097.⁵⁴ The minor G allele was overrepresented in African-Americans requiring higher warfarin doses than predicted based on age, body size, and the *CYP2C9* and *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 in less than 1% of Caucasians, potentially contributing to the higher warfarin dose requirements among the former racial group.

Warfarin Pharmacogenetics in Hispanics—Patients of Hispanic ethnicity are also under-represented in warfarin pharmacogenetic studies. Data from the National Health and Nutrition Examination Survey cohort showed that *VKORC1* allele frequencies in U.S. Hispanics are similar to those in non-Hispanic Caucasians.¹² In a small study in U.S. Hispanics, the *CYP2C9* and *VKORC1* genotypes made similar contributions to the dose

variability in Hispanics as previously reported in non-Hispanic Caucasians.⁵⁵ Thus, until further data from Hispanics are available, Hispanic and non-Hispanic Caucasians can probably be considered similarly in terms of warfarin pharmacogenetics.

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. In January 2010, a pharmacogenetic dosing table was added (Table 3).⁵⁶ The pharmacogenetic dosing table may help clinicians select an initial warfarin dose when the patient's *CYP2C9* and *VKORC1* genotype information is available. The table was derived from multiple clinical studies and generally accounts for other clinical factors (e.g., age, race, body weight, sex, concomitant drugs, and comorbidities) influencing warfarin dose variability. The revised labeling recommends adjustment of subsequent dosages based on the results of prothrombin time or 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 that use nongenetic and genetic factors to estimate a therapeutic warfarin dose. The algorithms are linear regression models ($y = ax + b$), with the dependent variable (the y variable) being the stable warfarin dose, and the independent variables (the x variables) being nongenetic and genetic factors. The following is an example:

$$\begin{aligned} \text{Warfarin daily dose (mg/day)} = & \exp [0.9751 - 0.3238 \times VKORC1-1639G > A + \\ & 0.4317 \times \text{body surface area} - 0.4008 \times \\ & CYP2C9 * 3 - 0.00745 \times \text{age} - 0.2066 \times \\ & CYP2C9 * 2 + 0.2029 \times \text{target INR} - 0.2538 \times \\ & \text{amiodarone} + 0.0922 \times \text{smokes} - 0.0901 \times \\ & \text{African-American race} + 0.0664 \times \text{deep vein} \\ & \text{thrombosis or pulmonary embolism}] \end{aligned}$$

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.⁸

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 *VKORC1* -1639A/G and *CYP2C9**1/*1 genotypes, does not smoke, is not receiving 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:

$$\begin{aligned} \exp [0.9751 - 0.3238 \times 1 + 0.4317 \times 1.88 - \\ 0.4008 \times 0 - 0.00745 \times 75 - 0.2066 \times 0 + \\ 0.2029 \times 2.5 - 0.2538 \times 0 + 0.0922 \times 0 - \\ 0.0901 \times 1 + 0.0664 \times 0] = 3.74 \text{ mg/day} \end{aligned}$$

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

About 40 warfarin pharmacogenetic algorithms are available because many algorithms were derived from ethnically specific populations (e.g., Slovenians, Koreans, and Japanese).^{7, 8, 48, 57–59} All of the algorithms have the *CYP2C9**3 and the *VKORC1* –1639G>A (or 1173C>T) alleles as independent variables. The algorithms derived from Asian populations do not include the *CYP2C9**2 allele because this allele is rare in this racial group. Some algorithms include additional SNPs in the *CYP2C9* and *VKORC1* genes (e.g., *CYP2C9**5, *6, *8, and *11, and *VKORC1* 2255C>T) and/or other genes (e.g., *CYP4F2*).^{13, 60, 61} The algorithms also contain various nongenetic 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 the R^2 value, the more the model explains the variability of the data. Warfarin pharmacogenetic dosing algorithms usually have R^2 values of 30–60%, with lower R^2 values in African-Americans.^{7, 8, 48, 50, 57–59} Pharmacogenetic dosing algorithms with additional SNPs and/or genes tend to have slightly higher R^2 values than those with only *CYP2C9**2, *CYP2C9**3, and *VKORC1* –1639G/A.^{61, 62} However, it remains to be determined whether the inclusion of these additional genetic factors improves clinical outcomes of warfarin therapy.

Whereas most warfarin dosing algorithms require genetic test results before the first warfarin dose, one online algorithm, available at no cost at 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.⁵⁹ 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, the www.WarfarinDosing.org algorithm may be clinically more applicable than other dosing algorithms. In addition, it is easy to use and has an R^2 value of approximately 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 nonpharmacogenetic methods. Pharmacogenetic methods include the dosing table in the warfarin labeling and pharmacogenetic dosing algorithms. Nonpharmacogenetic 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 R^2 value, percentage 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 do 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 are other methods.^{7, 8} 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%]).⁶³ Pharmacogenetic algorithms

particularly outperform more traditional dosing approaches for patients requiring warfarin doses of 3 mg/day or lower, or 7 mg/day or higher.⁷

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 0.5–7 mg/day.⁵⁶ In contrast, dosing algorithms can predict doses greater than 7 mg/day. Second, 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.⁶⁴ Of interest, 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.⁶³ 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 (< 3 mg/day), with 56% of doses in the intermediate range and 15% in the low range falling within 20% of the actual dose.⁶⁴ Thus, the table may be considered for certain populations (African-Americans) but in general is less accurate than pharmacogenetic dosing algorithms, particularly for low doses.

Evaluation of Various Pharmacogenetic Dosing Algorithms—The accuracy of various warfarin pharmacogenetic dosing algorithms has also been compared.^{60, 62, 65–68} The www.WarfarinDosing.org and IWPC dosing algorithms have been consistently identified as the most accurate of the algorithms.^{65–67} These two algorithms have mean absolute errors of 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 percentage 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 higher than 3 and lower than 7 mg/day (54–60% within 20% of the actual dose) than for doses of 3 mg/day or lower (20–45% within 20% of the actual dose) or 7 mg/day or higher (38–48% within 20% of the actual dose).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 higher than 3 and lower than 7 mg/day. Nonetheless, compared with non-genotype-based dosing methods, pharmacogenetic algorithms are significantly better at predicting doses lower than 3 and higher than 7 mg/day.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 the www.WarfarinDosing.org and the IWPC algorithms, 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.⁶⁷ 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.⁶⁹ Finally, recent data suggest that pharmacogenetic algorithms may overestimate warfarin doses in elderly patients who require less than 2 mg/day.⁷⁰ 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 United States Food and Drug Administration (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 situation, 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.⁷¹ This outsourcing may make genotype-guided warfarin initiation impractical because of 3–7 days of turnaround time. Second, no professional organization endorses warfarin pharmacogenetic testing in its guidelines because of the lack of the clinical utility data. Inclusion of a testing recommendation in professional guidelines has been identified as a factor influencing reimbursement of new technology.⁷² 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: some plans may reimburse the cost if the need for testing can be justified (e.g., patients with high bleeding risk), and 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.⁷³ Because of these barriers, warfarin pharmacogenetic testing is performed mainly for research purposes and for patients willing to pay 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/year.⁷⁴ 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%/year incidence of serious bleeding without genotype-guided dosing.

The body of evidence suggests that warfarin pharmacogenetic testing may not currently be cost-effective. Such evidence includes studies showing that the marginal cost-effectiveness of testing exceeds \$170,000/quality-adjusted life-year (QALY) and that testing has only a 10% chance to be cost-effective (i.e., < \$50,000/QALY).⁷⁵ The incremental cost per unit outcome improved per QALY was greater than \$50,000 for 62.1% of the time in one study.⁷⁶ 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.⁷⁷

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.⁷⁸ Second, 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%.⁷⁹ 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.⁷⁵ 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, nonsteroidal antiinflammatory 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 and Direction of Pharmacogenetic-Guided Anticoagulation Therapy

Trials Assessing the Clinical Utility of Warfarin Pharmacogenetics

The Centers for Disease Control and Prevention 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.⁸⁰ Thus, clinical utility of a warfarin pharmacogenetic test may be the evidence that testing decreases the risk of bleeding or thromboembolism in patients starting 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 end point. This evidence may be best obtained from a prospective, randomized trial comparing clinical outcomes between those patients newly starting warfarin who receive pharmacogenetic testing versus those who do not.

To date, four small, prospective, randomized trials have evaluated the clinical utility of warfarin pharmacogenetic testing, with mixed results (Table 5).^{69, 81–83} For example, one study reported that compared with 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 occurrence of minor bleeding.⁸¹ In contrast, a study of similar size showed no benefit of dosing based on both *CYP2C9* and *VKORC1* genotypes for the primary end point of the percentage of out-of-range INR values over the initial months of therapy.⁶⁹ 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 had small sample sizes (< 230 patients) and homogenous populations and excluded 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.^{65–67} 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 start of warfarin therapy.⁸⁴ Study patients were offered free genotyping, and the results were provided to the patients' physicians 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 with 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 its nonrandomized, nonblinded, and non-placebo-controlled design.

At least four multicenter, randomized, controlled, clinical trials are under way in the United States and Europe to assess the clinical utility of warfarin pharmacogenetic testing: the Clarification of Optimal Anticoagulation Through Genetics (COAG) trial⁸⁵; the Genetics Informatics Trial (GIFT) of Warfarin to Prevent Deep Venous Thrombosis trial⁸⁶; the Clinical and Economic Implications of Genetic Testing for Warfarin Management trial⁸⁷; and the European Pharmacogenetics of Anticoagulation Therapy—Warfarin (EU-PACT) trial.⁸⁸ 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, which began in September 2009.⁸⁵ This is a prospective, double-blind trial with a planned enrollment of 1238 participants from at least 12 centers in the United States.⁸⁹ Participants are randomly assigned to a genotype-guided or clinical warfarin dosing initiation strategy, with randomization stratified by race. Both strategies use 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 dosage adjustment. The primary outcome measure is the percentage of time spent within the therapeutic INR range during the initial 4 weeks of therapy. A secondary measure is the occurrence of an INR greater than 4 or serious event during the initial 4 weeks of therapy. The study is expected to be completed in March 2012.

Guidelines for Implementation of Warfarin Pharmacogenetics

Another barrier to clinician acceptance of warfarin pharmacogenetic testing is that many clinicians do not know how to interpret and apply the test results to adjust warfarin doses.⁷¹ 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.”⁹⁰ 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 Pharmacogenomics Research Network members (a group of scientists focused on understanding how a person's genes affect his/her response to drug therapy), PharmGKB staff, and other experts in pharmacogenetics, pharmacogenomics, and laboratory medicine. Their guidelines on the interpretation and application of warfarin pharmacogenetic test results are expected to be available within the year. 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 has increased 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* and calumenin, both of which affect warfarin dose.^{25, 54} 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^{91, 92} exist to calculate the posterior probability of regulatory clusters with a DNA sequence. These methods have been used to identify regulatory regions in *CYP2C9* as well as other important drug metabolizing genes with important implications for drug response.^{25, 93}

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 (eQTL). The eQTL are SNPs found throughout the genome that may directly (as in a regulatory element upstream of a gene) or indirectly (as in an SNP within a transcription binding factor that regulates a gene) affect the expression of a gene. The publically available database SCAN⁹⁴ (available from <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 of population samples taken from the International HapMap Project. The advantage to this method over other genomewide approaches is that the association made using eQTL 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. Rivaroxaban was approved in 2011 for prophylaxis of deep vein thrombosis after knee or hip replacement. To our knowledge, no pharmacogenetic data have been published on any of these agents. 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. Our discussion of candidate genes focuses on dabigatran and rivaroxaban since these are the only agents currently approved.

Dabigatran etexilate is a prodrug that is rapidly hydrolyzed by nonspecific esterases to its active form, dabigatran, after absorption. Based on both in vitro and in vivo studies in healthy volunteers, the CYP enzymes do not appear to have a role in the conversion of the prodrug to dabigatran or to dabigatran's metabolism.⁹⁵⁻⁹⁷ Thus, unlike warfarin, the *CYP* genotype is unlikely involved in dabigatran pharmacokinetics. However, the dabigatran etexilate prodrug is a substrate for the polymorphic drug efflux transporter P-glyco-protein.

There is evidence that coadministration with the P-glycoprotein inhibitor amiodarone increases the area under the concentration-time curve for dabigatran by 60%.⁹⁸ P-glycoprotein is encoded by the *ABCB1* gene, which is a member of the adenosine triphosphate-binding cassette transporter superfamily. Several SNPs have been identified in the *ABCB1* promoter and exon (coding) regions and have been associated with plasma concentrations of P-glycoprotein substrates.⁹⁹ 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.¹⁰⁰ 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; thus, pharmacogenetic studies are expected to emerge with this newly approved agent.

The CYP enzymes, specifically CYP3A4/5 and CYP2J2, are involved in the metabolism of rivaroxaban and serve as a potential source of genetic variability in the drug's pharmacokinetics.¹⁰¹ Similar to dabigatran, rivaroxaban is a P-glycoprotein substrate.¹⁰¹ Thus, *ABCB1* genotype may influence the drug's disposition. Polymorphisms at the drug's target site are potential sources of variability in rivaroxaban's pharmacodynamics.

Conclusion

There are substantial and convincing data supporting the clinical and analytic 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 available for *CYP2C9* and *VKORC1* genotyping. 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 ongoing 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, the pharmacogenetics with these anticoagulants could be of great importance given the unavailability of routine monitoring parameters with these agents.

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Figure 1.

Genes involved in the pharmacokinetics and pharmacodynamics of warfarin. The proteins CYP2C9, CYP4F2, VKOR, and calumenin 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.

Table 1Location and Prevalence of Variant *CYP2C9* and *VKORC1* Alleles^{11–14}

Allele	Location	Prevalence (%) ^a		
		Caucasians	Asians	African-Americans
<i>CYP2C9</i> *2 (144Cys)	Exon 3	20–24	< 1	3–4
<i>CYP2C9</i> *3 (359Leu)	Exon 7	11–12	8–9	1–3
<i>CYP2C9</i> *5 (360Glu)	Exon 7	< 1	< 1	1–2
<i>CYP2C9</i> *6 (Null)	Exon 5	< 1	< 1	1–3
<i>CYP2C9</i> *8 (150His)	Exon 3	< 1	< 1	12
<i>CYP2C9</i> *11 (335Trp)	Exon 7	< 1	< 1	3–4
<i>VKORC1</i> –1639A	Promoter	60	99	20
<i>CYP4F2</i> 433Met	Exon 2	39	37–45	14

CYP = cytochrome P450; *VKORC1* = vitamin K epoxide reductase complex subunit 1 gene.

^aPercentage of patients who carry a variant allele.

Table 2Frequency of *VKORC1* Haplotype Group by Race-Ethnicity

Population	Haplogroup A	Haplogroup B
European-Americans ^{29, 53}	37–42%	57–58%
African-Americans ^{29, 53}	14–21%	49–58%
Asians ^{29, 53}	85–89%	10–14%
Peruvians ⁵³	27%	71%
Mexicans ⁵³	38%	57%
Africans ⁵³	23%	49%

VKORC1 = vitamin K epoxide reductase complex subunit 1 gene.

Table 3

Ranges of Expected Therapeutic Warfarin Daily Doses Based on *CYP2C9* and *VKORC1* Genotypes⁵⁶

<i>CYP2C9</i>					
<i>VKORC1</i> -1639	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3
GG	5-7 mg	5-7 mg	3-4 mg	3-4 mg	3-4 mg
GA	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg
AA	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg

CYP2C9 = cytochrome P450 2C9 gene; *VKORC1* = vitamin K epoxide reductase complex subunit 1 gene; G = guanine; A = adenine.

Table 4

Warfarin Pharmacogenetic Tests Cleared by the U.S. Food and Drug Administration as of May 1, 2011

Name of Test (manufacturer)	Alleles Tested	Estimated Time for Assay Completion (hrs)
Verigene Warfarin Metabolism Nucleic Acid Test (Nanosphere, Inc., Northbrook, IL)	<i>CYP2C9</i> *2 and *3, <i>VKORC1</i> 1173C/T	2
Infiniti 2C9-VKORC1 Multiplex Assay for Warfarin (AutoGenomics, Inc., Vista, CA) ^a	<i>CYP2C9</i> *2 and *3, <i>VKORC1</i> –1639G/A	6–8
eSensor Warfarin Sensitivity (GenMark Dx, Carlsbad, CA) ^b	<i>CYP2C9</i> *2 and *3, <i>VKORC1</i> –1639G/A	3–4
eQ-PRC LC Warfarin Genotyping Kit (Trimgen Corp., Sparks, MD)	<i>CYP2C9</i> *2 and *3, <i>VKORC1</i> –1639G/A	2

CYP = cytochrome P450; *VKORC1* = vitamin K epoxide reductase complex subunit 1 gene.

^a The expanded Infiniti CYP450 2C9-VKORC1 Assay offers testing for *CYP2C9**2, *3, *4, *5, *6, and *11, *VKORC1* –1639G>A, and six additional *VKORC1* variants.

^b eSensor Warfarin Plus Test offers testing for *CYP2C9**2, *3, *5, *6, *11, *14, *15, and *16, *VKORC1* –1639G>A, and *CYP4F2* Valine433Methionine.

Table 5

Summary of Four Prospective Randomized Trials Evaluating Clinical Utility of a Warfarin Pharmacogenetic Test

Study Population	Standard Dosing	Primary Outcome	Results	Comments
Caucasians (Israelis) (n=191) ⁸¹	Computerized warfarin dosing program	Time to reach first INR within therapeutic range	Pharmacogenetic dosing group: 14.1 ± 6.9 days (mean ± SD) Standard dosing group: 32.2 ± 21.1 days (mean ± SD) (p<0.001)	Pharmacogenetic algorithm was based on expected warfarin clearance by <i>CYP2C9</i> genotype <i>VKORC1</i> genotype was not used
Caucasians (n=206) ⁶⁹	Published nomogram	Percentage of out-of-range INRs in first 90 days	Pharmacogenetic dosing group: 30.7% Standard dosing group: 33.1% (p=0.47)	Investigators developed their own pharmacogenetic dosing algorithm
Asians (Chinese) (n=122) ⁸²	Fixed starting dose of 2.5 mg/day	Time to a stable warfarin dose	Pharmacogenetic dosing group: 24 days (median) Standard dosing group: 35 days (median) (p<0.001)	All patients had heart valve replacements Warfarin dose for first 3 days was capped at 3 mg/day in the pharmacogenetics group
Caucasian ancestry (n=230) ⁸³	Clinical dosing algorithm	Absolute prediction error relative to therapeutic dose Percentage of time in therapeutic range during first 14 days	Mean absolute error: Pharmacogenetic dosing group: 0.80 mg/day Standard dosing group: 1.32 mg/day (p value NR) Time in therapeutic range: Pharmacogenetic dosing group: 30.8 ± 28.4% (mean ± SD) Standard dosing group: 29.1 ± 15.5% (mean ± SD) (p=0.56)	Investigators developed and validated pharmacogenetic dosing algorithm

INR = international normalized ratio; *CYP2C9* = cytochrome P450 2C9 gene; *VKORC1* = vitamin K epoxide reductase subunit 1 gene; NR = not reported.