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Genes Affecting Warfarin Response—Interactive or Additive?

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Abstract

Genotypes for cytochrome P450 (CYP) 2C9 and vitamin K epoxide reductase complex 1 (VKORC1) contribute significantly to the inter-patient variability in warfarin dose requirements. These genotypes in addition to clinical factors explain approximately 50% of the dose variability in Europeans, but less in other populations. Thus, a large portion of the variability remains unexplained and has been the focus of on-going research. Trials evaluating the clinical utility of genotype-guided warfarin dosing have shown a benefit in Europeans, but not in an ethnically diverse cohort. Identifying and accounting for variants important in non-European populations will likely be necessary before a benefit with genotype-guided dosing will be realized in these populations.

Keywords

warfarin; genotype; CYP2C9; VKORC1

Warfarin is the most commonly prescribed oral anticoagulant, accounting for 35 million prescriptions in the United States in 2011.¹ Even with approval of newer agents that do not require routine laboratory monitoring, warfarin continues to be the mainstay of therapy, especially for low socioeconomic populations who have access barriers to newer, more expensive medications.^{2,3} Warfarin has been in use for over 60 years, providing extensive experience with the drug. Yet, it remains one of the most challenging drugs to manage secondary to its narrow therapeutic index and unpredictable dose requirements for optimal anti-coagulation. Numerous investigators have sought to identify predictors of warfarin dose requirements and refine methods for optimizing warfarin dosing. Much of this effort has focused on genetic factors and the *CYP2C9* and *VKORC1* genotypes in particular.

Cytochrome P450 (CYP) 2C9 is the primary enzyme that metabolizes the more potent *S*-enantiomer of warfarin. Although over 50 *CYP2C9* alleles have been identified, most studies have focused solely on the *2 and *3 alleles. These are the most common reduced function alleles in Europeans but are significantly less common in other populations. For example, in this issue, Li et al reported that over 35% of Whites had a *2 or *3 allele compared to only

8% of Blacks. Six to 8% of Asians had a *3 allele, whereas none carried the *2 allele. Vitamin K epoxide reductase complex 1 (VKORC1) encodes for the target protein of warfarin. The *VKORC1* -1639G > A polymorphism affects gene and protein expressions and, consequently, the amount of warfarin needed for optimal anticoagulation.^{4,5} Notably, the *VKORC1* AA genotype, conferring reduced protein expression, is the predominate genotype in Asian populations, contributing to the lower dose requirements generally observed among Asians compared to European and African populations.

Genome wide association studies have confirmed that the *CYP2C9* and *VKORC1* genotypes are the primary genetic determinants of warfarin dose requirements in Europeans and Asians.⁶⁻⁸ Pharmacogenetic dosing algorithms that include genotypes and clinical factors, such as age, body size, and medications that affect *CYP2C9* activity, have been developed to assist with warfarin dosing. Algorithms by Gage et al⁹ and the International Warfarin Pharmacogenetics Consortium (IWPC)¹⁰ are the most widely accepted. However, both algorithms better predict dose for Europeans compared to Asians and African Americans, which is not surprising since Europeans comprised the majority of the derivation and validation cohorts for each. In addition, the *VKORC1* -1639G>A, *CYP2C9**2, and *CYP2C9**3 genotypes combined with clinical factors included in the algorithms explain approximately 40%–50% of the interpatient variability in dose requirements in Europeans, but only about 30% of the variability in African Americans and Asians.^{11,12} Thus, a significant amount of the variability remains unexplained, and investigators continue to search for genetic factors that might improve warfarin dose prediction, especially for non-European patient populations.

In this issue, Li et al investigate the predictive value of the gene-gene interaction between *CYP2C9* and *VKORC1* on estimating a patient's stable warfarin dose. The authors note that in previous warfarin dose prediction algorithms, *CYP2C9* and *VKORC1* genotypes were treated as independent predictors, and the interaction between the 2 factors has not yet been included in dosing algorithms. Their novel approach was to incorporate a *CYP2C9*-*VKORC1* interaction term into the IWPC warfarin dose prediction algorithm¹⁰ in the hope of improving its predictive ability.

This analysis included a study population of 1,297 Chinese patients who underwent cardiac valve replacement, along with 4,101 patients of European, African American, or Asian descent from the IWPC. The primary outcome measure was the actual stable warfarin dose subjects required (in milligrams per week). A linear regression model was created, and interaction terms were tested in the model using various *CYP2C9* genotype groupings with the *2 and *3 alleles. In the African American and Asian populations, the effect of *VKORC1* and *CYP2C9* genotypes appeared to be additive, as the interaction between them was not found to be statistically significant ($P > .05$). However, in the Chinese and European populations, the effects of *VKORC1* and *CYP2C9* genotypes on stable warfarin dose appeared to be nonadditive ($P < .05$).

To estimate the effect of this interaction term on dose prediction, a new algorithm including the *VKORC1*-*CYP2C9* interaction term was created using 80% of the European and Chinese populations, and then tested in a validation cohort consisting of the remaining 20%

of patients. This random sampling was repeated 100 times. The mean differences from these samplings showed that even in these populations, the interaction terms appeared to have a negligible effect on dose prediction (improvement in $R^2 = 0.01$). In addition, when comparing the ratios of patients within 20% of their actual stable warfarin dose, the interaction term showed no appreciable improvement in percentage or mean absolute error.

Based on the distinct mechanisms by which variations in *VKORC1* and *CYP2C9* are thought to influence warfarin response, these findings are not entirely surprising. The authors postulate that *S*- and *R*-warfarin could have different binding affinities for *VKORC1* encoded by the variant –1639 A allele. In fact, previous reports indicate that variation in *VKORC1* may affect warfarin binding as well as the *R/S*-potency ratio.^{13,14} The –1639 A variant exists in the *VKORC1* promoter region, so the fact that it has not been reported as one of the variants involved in warfarin binding is of no surprise. However, a recent article reported that homozygotes for the *VKORC1* 1173 C > T polymorphism were more sensitive to *R*-warfarin than those with the CC genotype.¹³ These findings are particularly relevant because the 1173 C> T polymorphism is in near complete linkage disequilibrium with the –1639 G> A polymorphism, making it an excellent proxy for –1639 G> A genotype.¹¹

The interaction analyses only included the *CYP2C9**2, *3, and *VKORC1* –1639G > A variants. In recent years, investigators have discovered a number of genotypes with contributions to warfarin dose requirements beyond that of the *CYP2C9**2, *3, and *VKORC1* genotypes. In the African American population, the rs12777823 and *FPGS* genotypes in addition to the *CYP2C9**5, *6, *8, and *11 alleles have emerged as important influences of dose requirements.^{15–17} In both European and Asian populations, the *CYP4F2* genotype contributes to dose requirements, though it explains only a small percentage of the overall dose variability.^{7,18} The *CYP4F2* enzyme is involved in the metabolism of vitamin K, and the Val433Met polymorphism decreases vitamin K metabolism necessitating higher warfarin doses for optimal anticoagulation.¹⁹ There are also a number of putative defective *CYP2C9* alleles in Asians, including the *14, *16, *19, *23, *27, *29, *33, and *34 alleles recently discovered in a Chinese population.^{20,21} Including these variants may have altered the results found by Li et al in the African American and Asian populations. The authors acknowledge this limitation, stating that insufficient genetic data were available to include these variants.

The anticipated culmination of this line of research is the use of genetic data in clinical practice to better inform warfarin dosing and potentially minimize the risks for adverse warfarin-related events. However, prior to endorsing genotype-guided dosing many have called for data demonstrating the clinical utility of this approach. Two trials examining the clinical utility of genotype-guided warfarin dosing were published in late 2013. The EU-PACT trial was conducted in a homogenous European population, whereas the COAG trial was conducted in an ethnically diverse US cohort, with a large portion of African Americans.^{22,23} Asians were minimally represented in each. Both trials used a pharmacogenetic algorithm that included the *CYP2C9* and *VKORC1* genotypes plus clinical factors (eg, age, body size, smoking status, amiodarone use, etc). The COAG trial used the algorithm by Gage et al, and the EU-PACT trial used a modified version of the IWPC algorithm. Of the known *CYP2C9* variant alleles, only *2 and *3 were included. The EU-

PACT trial showed that genotype-guided dosing resulted in better anticoagulation control compared to standard dosing. The COAG trial compared genotype-guided dosing to clinically based dosing and reported no difference in anticoagulation control over the initial weeks of therapy with the 2 approaches. However, in the African American subgroup, pharmacogenetic dosing led to a lower percent time in therapeutic range compared to clinically based dosing.

Several groups have questioned whether results from the COAG trial, particularly in African Americans, were attributable to failure to account for genetic variants important in African Americans.^{24–27} Specifically, African Americans with a *CYP2C9**5, *6, *8, *11, rs12777823, or *FPGS* variant, all of which are associated with lower warfarin dose requirements, would not have been identified as needing a lower dose and thus may have been overdosed in the COAG trial. This might explain why genotype-guided dosing led to more supratherapeutic INR values compared to clinically based dosing in African Americans. Ultimately, a warfarin pharmacogenetic dosing algorithm containing genotypes important in various ethnic groups may allow better prediction of warfarin dose requirements on a global scale. Until then, dose prediction with existing models will likely be of the most benefit for European populations.

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