Trends in Cardiovascular Medicine 25 (2015) 33–41

Available online at www.sciencedirect.com

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Warfarin pharmacogenetics

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ABSTRACT

The cytochrome P450 (CYP) 2C9 and vitamin K epoxide reductase complex 1 (VKORC1) genotypes have been strongly and consistently associated with warfarin dose requirements, and dosing algorithms incorporating genetic and clinical information have been shown to be predictive of stable warfarin dose. However, clinical trials evaluating genotype-guided warfarin dosing produced mixed results, calling into question the utility of this approach. Recent trials used surrogate markers as endpoints rather than clinical endpoints, further complicating translation of the data to clinical practice. The present data do not support genetic testing to guide warfarin dosing, but in the setting where genotype data are available, use of such data in those of European ancestry is reasonable. Outcomes data are expected from an on-going trial, observational studies continue, and more work is needed to define dosing algorithms that incorporate appropriate variants in minority populations; all these will further shape guidelines and recommendations on the clinical utility of genotype-guided warfarin dosing.

Introduction

Since its approval in 1954, warfarin has been widely prescribed for the prophylaxis and treatment of venous thromboembolism and complications associated with atrial fibrillation and cardiac valve replacement. Even with the availability of newer agents shown to be noninferior to warfarin, warfarin remains the most commonly prescribed oral anticoagulant [1–4]. Owing to its narrow therapeutic index, warfarin is also a leading cause of serious adverse drug events and is implicated in 33% of hospitalizations secondary to adverse drug events among older adults in the U.S. [5]. Therapy with warfarin is further complicated by marked inter-patient variability in the dose necessary for optimal anticoagulation, defined as an international normalized ratio (INR) of 2–3 for most indications. Genotype is a major determinant of warfarin dose requirements and also impacts risk for over-anticoagulation and hemorrhage, especially in the initial months of therapy [6]. Guidelines and dosing algorithms are available to assist with application of genotype data to dose warfarin. However, clinical trials evaluating the effect of genotype-guided warfarin dosing on time in therapeutic range (TTR) produced variable results. Other trials are on-going, including one powered to detect clinical endpoints. Herein, we review the evidence for genetic associations with warfarin response, evaluate the design and results from clinical trials, and discuss evidence needed to further define the role for genotyping to guide warfarin dosing.

Overview of genetic associations with warfarin dose requirements

The primary genes contributing to warfarin dose requirements are vitamin K epoxide reductase complex 1 (VKORC1) and cytochrome P450 2C9 (CYP2C9). The CYP2C9 enzyme metabolizes the more potent S-enantiomer of warfarin, while

The authors have indicated there are no conflicts of interest.

Supported in part by NIH, USA Grant nos. U01 GM074492 to J.A.J. and U01 HG007269 to J.A.J. and L.H.C.

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http://dx.doi.org/10.1016/j.tcm.2014.09.001
1050-1738/

VKORC1 is the target protein for warfarin. Specifically, warfarin inhibits VKORC1, thus preventing reduction of vitamin K to vitamin KH_2, a necessary co-factor for carboxylation and activation of clotting factors. The gene for CYP4F2, which metabolizes vitamin K, provides a minor contribution to dose requirements, particularly in European and Asian populations.

A single polymorphism in the *VKORC1* regulatory region, c.-1639G>A (rs9923231), predicts dose requirements across ethnic groups [7]. The minor A allele at this position is associated with approximately twofold lower gene expression and significantly lower warfarin dose requirements compared to the G allele [8]. The frequency of the A allele varies by ethnicity, as shown in Table 1, with the highest frequency in Asians, intermediate frequency in Europeans, and the lowest frequency in African Americans. Differences in allele frequency account for the lower dose requirements generally observed in Asian populations and higher requirements in Africans compared to Europeans.

The majority of *CYP2C9* variants impacting warfarin dosing are nonsynonymous single nucleotide polymorphisms (SNPs) that occur in the exonic regions of the gene and lead to reduced enzyme activity against S-warfarin and consequently lower warfarin dose requirements [9,10]. An exception is the *6 allele (rs9332131), which results from a single nucleotide deletion, shift in the reading frame, and loss of function. The *2 (R144C, rs1799853) and *3 (I359L, rs1057910) alleles are the primary dysfunctional alleles in Europeans but are less common in African Americans; the *2 allele rarely occurs in Asians (Table 1). Additional functional polymorphisms occurring primarily in African populations include *5 (D360E, rs28371686), *6, *8 (R150H, rs7900194), and *11 (R335W, rs28371685). The number of individuals expected to carry a *CYP2C9* variant allele, based on allele frequency data, are shown in Table 2.

The *CYP4F2* V433M (rs2108622) SNP was first identified as a contributor to warfarin dose requirements in Europeans, with variant allele homozygotes requiring higher doses [11]. The association was subsequently replicated in Asians but not in African Americans, in whom the variant allele is much less common (Table 1) [12,13]. In functional studies, the 433M allele led to reduced hepatic concentrations of CYP4F2 and decreased vitamin K metabolism [14]. As a consequence, variant allele carriers are expected to have higher vitamin K concentrations and require higher warfarin doses to inhibit vitamin K-dependent clotting factor activation.

Table 1 – Minor allele frequencies by ethnicity [12,13,15,18,24,33].

Variant	Europeans	African Americans	Asians
VKORC1-1639A	0.37	0.10	0.86
*CYP2C9*2*	0.13	0.02	ND
*CYP2C9*3*	0.06	0.01	0.02
CYP2C9*5	**ND**	**0.01**	**ND**
CYP2C9*6	**ND**	**0.01**	**ND**
CYP2C9*8	**ND**	**0.06**	**ND**
CYP2C9*11	**ND**	**0.02**	**ND**
CYP4F2 433Met	**0.29**	**0.07**	**0.29**
rs12777823	**0.14**	**0.25**	**0.32**

ND = not detected or rare. Variants shown in bold text were not included in either the COAG or EU-PACT study.

Table 2 – Expected prevalence of carriers of *CYP2C9* variants by ethnicity.

CYP2C9 allele	Ethnicity		
	Europeans	African Americans	Asians
*2	0.24	0.04	ND
*3	0.12	0.02	0.04
*5	ND	0.02	ND
*6	ND	0.02	ND
*8	ND	0.12	ND
*11	ND	0.04	ND

Carriers of variant alleles calculated from allele frequencies in Table 1. Carriers are defined as those with one (e.g., *1/*2 or *2/*3) or two (i.e., *2/*2) copies of the variant allele.

A genome-wide association study (GWAS) in African Americans revealed an additional SNP, rs12777823G>A, associated with variability in warfarin dose requirements in this population [15]. This SNP occurs in chromosome 10 near the *CYP2C18* gene, with the minor A allele correlated with reduced S-warfarin clearance and dose requirements. Interestingly, while the variant is common across populations (Table 1), its association with warfarin dose is only evident in African Americans, which suggests that it is likely not the causative allele but rather inherited with one or more alleles influencing warfarin response in West African populations. Further research is required to elucidate the mechanism underlying the association between rs12777823 and dose requirements in African Americans.

Unequivocal data that genotype predicts warfarin dose requirements

Multiple candidate gene studies have consistently demonstrated that the *VKORC1* and *CYP2C9* genotypes influence the inter-patient variability in warfarin dose requirements, together explaining 10–45% of the overall variance, depending on the population studied and genotypes detected [16–18]. This has been confirmed by several GWAS [12,19,20]. The *CYP4F2* SNP explains approximately 1% of the overall variability in warfarin dose requirements [12,20]. Specific effects of these and other variants on dose are shown in Table 3. The *CYP2C9* genotype has also been correlated with risk for over-anticoagulation and hemorrhage during warfarin therapy. The risk for bleeding is highest in the initial 3–6 months following warfarin initiation but continues with chronic therapy [6,21]. In a meta-analysis of 22 studies including over 6000 patients, the *CYP2C9*2*, *3, and *VKORC1*-1639A alleles were associated with an increased risk for over-anticoagulation, defined as INR > 4 [22]. Interestingly, the association between *VKORC1*-1639G>A genotype and over-anticoagulation was evident only in the initial month of therapy, whereas the risk conferred by *CYP2C9* extended beyond this period, suggesting that the effects of the *VKORC1* genotype are realized early on while the influence of *CYP2C9* genotype