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The future of warfarin pharmacogenetics in under-represented minority groups

Larisa H Cavallari[1,*] and **Minoli A Perera**[2]

[1]Department of Pharmacy Practice, University of Illinois at Chicago, College of Pharmacy, 833 South Wood Street, Room 164, Chicago, IL 60612-7230, USA

[2]Department of Medicine, University of Chicago, Chicago, IL, USA

Abstract

Genotype-based dosing recommendations are provided in the US FDA-approved warfarin labeling. However, data that informed these recommendations were from predominately Caucasian populations. Studies show that variants contributing to warfarin dose requirements in Caucasians provide similar contributions to dose requirements in US Hispanics, but significantly lesser contributions in African–Americans. Further data demonstrate that variants occurring commonly in individuals of African ancestry, but rarely in other racial groups, significantly influence dose requirements in African–Americans. These data suggest that it is important to consider variants specific for African–Americans when implementing genotype-guided warfarin dosing in this population.

Keywords

African–American; CYP2C9; Hispanic; polymorphism; VKORC1; warfarin

Warfarin is widely prescribed to prevent thromboembolism and while it has been in use for over 60 years, it remains a challenging drug to manage. This is predominately because of its narrow therapeutic index and the significant interpatient variability in the dose that produces therapeutic anticoagulation. Specifically, warfarin is dosed to achieve an international normalized ratio (INR) of 2–3 for most indications, with sub- or supra-therapeutic dosing increasing the risk for thrombosis or bleeding, respectively [1,2]. The warfarin dose required to achieve therapeutic levels of anticoagulation (i.e., INR of 2–3) varies as much as 20-fold between individuals [3].

According to data from the National Electronic Injury Surveillance System, warfarin is the most common drug-related cause of hospitalization for adverse events among older adults in the USA, accounting for 33% of such hospitalizations [4]. An estimated 21,010 hospitalizations from 2007 to 2009 were for warfarin-related hemorrhages. The risk for hemorrhage is particularly elevated when the INR exceeds 4, as well as during the initial months of therapy [1]. Thus, it is imperative to efficiently achieve a safe and effective level of anticoagulation for patients starting warfarin.

*Author for correspondence: Tel.: +1 312 996 0886 Fax: +1 312 996 0379 humma@uic.edu.

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Current anticoagulation management guidelines recommend starting warfarin at a dose of 5 mg/day for most patients, with lower doses in the elderly or those taking medications that reduce CYP2C9-mediated warfarin metabolism [5,6]. However, the 5 mg/day dose will result in sub- or supra-therapeutic anticoagulation for approximately 60% of Caucasians and even more African–Americans [7]. The dose titration necessary in these patients prolongs the time to achieve therapeutic anticoagulation.

A number of factors influence warfarin dose requirements, including age, hepatic and renal function, concomitant use of drugs that interfere with warfarin metabolism (especially amiodarone) and vitamin K intake [5,8,9]. In addition, the genotypes for proteins involved in warfarin metabolism and pharmacodynamics provide significant contributions to warfarin dose requirements. Thus, rather than using an empiric approach to warfarin dosing, a number of investigators have proposed personalized warfarin dosing, with doses chosen based on genetic and clinical factors [7,10–13]. The goal of this individualized approach is to improve dosing accuracy and reduce the risk for sub- or supra-therapeutic anticoagulation during the initial months of therapy when the risks for adverse outcomes are highest.

African–Americans are significantly under-represented in pharmacogenetic studies with warfarin, and Hispanics are even less well represented. The performance of dosing algorithms derived from predominately Caucasian populations is questionable in under-represented patient groups. This article will discuss warfarin pharmacogenetics and its future potential, with a focus on African–American and Hispanic populations.

Genetic determinants of warfarin response

Numerous studies, predominately conducted in Caucasian and Asian populations, demonstrate that the *CYP2C9* and *VKORC1* genotypes contribute significantly to warfarin dose variability [3,7,14–18]. The CYP2C9 enzyme metabolizes the more active *S*-enantiomer of warfarin primary to an inactive 7-hydroxy metabolite, as shown in Figure 1 [5]. Warfarin inhibits VKOR to prevent generation of a reduced form of vitamin K that is necessary for γ-carboxylation and activation of clotting factors II, VII, IX and X. Thus, the *CYP2C9* gene affects warfarin pharmacokinetics, while the *VKORC1* gene (the gene encoding VKOR) impacts warfarin pharmacodynamics.

The most extensively studied *CYP2C9* variants are the *CYP2C9*2* (R144C) and *CYP2C9*3* (I359L) alleles, which lead to significant reductions in CYP2C9 activity. The location and frequencies of these alleles are shown in Table 1. Compared with the *CYP2C9*1/*1* genotype, the *CYP2C9 *1/*2, *1/*3* and *3/*3* genotypes reduce *S*-warfarin clearance by approximately 40, 60 and 90%, respectively [19,20]. As a result, significantly lower doses are usually needed in individuals with a *CYP2C9*2* or *CYP2C9*3* allele. For example, mean doses of 5.6, 3.9 and 2.9 mg/day were reported in Caucasians with the *CYP2C9*1/*1, *1/*2* and **1/*3* genotypes, respectively [19]. A dose of 1 mg/day or lower may be necessary in *CYP2C9*3* homozygotes. The *CYP2C9*2* and **3* alleles explain 9–12% of the total variability in warfarin dose requirements in Caucasians, but significantly less in African–Americans, likely because of their lower frequency in the latter population [15,17,21]. The *CYP2C9* genotype is also implicated in the risk for bleeding during warfarin therapy, especially during the warfarin initiation period [22].

The *VKORC1* genotype was originally recognized for causing warfarin resistance due to mutations in the gene-coding region [23]. More recently, common *VKORC1* single-nucleotide polymorphisms (SNPs) occurring in gene-regulatory regions and underlying usual warfarin dose variability were discovered [14,24]. Five common *VKORC1* SNPs are in strong linkage disequilibrium (i.e., almost always inherited together) in Caucasians and comprise two major haplotypes, designated as haplotypes A and B [14]. Haplotype A is

associated with a twofold lower *VKORC1* expression and significantly lower warfarin dose requirements [14]. Specifically, doses of 2.7, 4.9 and 6.2 mg/day were reported with the AA, AB and BB haplotype combinations, respectively.

Of the five variants comprising *VKORC1* haplotypes A and B, only the -1639G>A and possibly 1173C>T SNPs appear to be functional [25]. Thus, the majority of warfarin pharmacogenetic studies have focused on one of these two SNPs. The -1639G>A and 1173C>T are in strong linkage disequilibrium across populations and are similarly predictive of warfarin dose requirements, as shown in Figure 2 [26]. Thus, only one of these SNPs needs to be taken into account for pharmacogenetic dosing of warfarin. The *VKORC1* -1639G>A variant explains approximately 20–28% of the overall variability in dose requirements in Caucasians, but only 5–7% of the variability in African–Americans [15,17,21,26]. The reduced contribution of the -1639G>A genotype to dose variability in African–Americans is primarily due to the lower frequency of the -1639A (low dose) allele in this racial group (Table 1) [26]. Unlike *CYP2C9*, *VKORC1* genotype does not appear to affect bleeding risk with warfarin [22].

Data from two genome-wide association studies (GWAS) in Caucasians and a third in Asian individuals confirm that the *VKORC1* -1639G>A, *CYP2C9**2 and *CYP2C9**3 polymorphisms are the primary genetic determinants of warfarin dose requirements in these populations [15–17]. The combination of *VKORC1* -1639G>A, *CYP2C9* (**2* and **3*) and clinical factors (e.g., age, sex, weight and amiodarone use) explains approximately 55% of the total variance in warfarin maintenance dose in Caucasians, but only about 25% among African–Americans [3,26]. With the exception of the *CYP4F2* genotype discussed below, no other variant met genome-wide significance for association with warfarin dose requirements in Caucasian and Asian GWAS.

Warfarin pharmacogenetic dosing tools

In response to the wealth of data supporting the *CYP2C9* and *VKORC1* variants as determinants of warfarin response, the US FDA-approved warfarin labeling was revised in 2007 to include pharmacogenetic information. The label was again revised in 2010 to include a dosing table based on the *VKORC1* -1639G>A, *CYP2C9*2,* and *CYP2C9*3* genotypes [27]. In addition to this table, several pharmacogenetic dosing algorithms have been published [7,10–13,28]. The two most commonly cited algorithms are the International Warfarin Pharmacogenetic Consortium (IWPC) and Gage algorithms [7,11]. Both are based on data from large, predominately Caucasian populations, and both are freely available [101]. Data from over 4000 warfarin-treated patients (55% Caucasian and 30% Asian patients) were used to derive the IWPC algorithm [7]. The Gage algorithm was derived from a population of over 1000 patients, of whom 83% were Caucasian [11].

The Gage algorithm can account for previous warfarin doses and INR measurements to refine dose prediction [29]. Thus, the Gage algorithm may be most useful for patients who have already received one or more warfarin doses at the time of algorithm use. In this regard, there are data supporting the use of pharmacogenetic algorithms even after INR results are available. Specifically, Lenzini *et al.* found that, even after 4–5 days of warfarin therapy, a pharmacogenetic algorithm that incorporated previous warfarin doses and INR values more accurately predicted warfarin maintenance dose than clinical factors alone [29]. In a prospective cohort included in this study, the pharmacogenetic algorithm explained 42–58% of the variance in warfarin dose at 4–5 days, whereas a clinical algorithm explained 26–43% of the variance. A more recent study by the same investigator group supports the integration of genetic data into warfarin dosing decisions even after 9 days of therapy [30].

At this time point, a pharmacogenetic algorithm explained 74% of the variability in dose compared with 65% explained by clinical factors alone ($p < 0.01$).

Insight into *CYP2C9* genotype may also be useful with regard to decisions regarding warfarin dose titration. Specifically, reduced warfarin metabolism, secondary to *CYP2C9* variant genotype prolongs the half-life of warfarin and time to achieve steady-state plasma concentration and a stable INR [31,32]. Because of the prolonged rate of dose stabilization, patients with a variant *CYP2C9* allele may require slower than usual dose titration to avoid 'over-shooting' the target INR range.

Racial considerations in warfarin pharmacogenetics

African–American and Hispanic individuals are under-represented in warfarin pharmacogenetic studies. Specifically, only 9% of patients included in the IWPC cohort were African–American and even fewer were Hispanic [7]. Only 15% of patients included in the derivation of the Gage algorithm were African–American and 2% were Hispanic [11]. There are important differences in genetic structure and allele frequencies by race and ethnicity that may significantly impact the genotype–warfarin response relationship. Thus, it is important to confirm that associations in Caucasians extend to those of other racial and ethnic backgrounds.

Racial differences in warfarin dose requirements

Warfarin dose requirements vary significantly by race, as demonstrated in Figure 3, with higher median doses in African–Americans and lower doses in Asians compared with Caucasians [26,33]. Racial differences in genotype frequencies contribute to the observed racial differences in warfarin dose requirements [34–39,102]. In particular, the *VKORC1* -1639A, *CYP2C9*2* and *CYP2C9*3* alleles, all of which are associated with lower warfarin doses, are significantly less common in African–Americans compared with Caucasians and Asians (Table 1). Less is known about dose requirements in Hispanics. However, the limited data available suggest that Hispanic and non-Hispanic Caucasians require similar warfarin doses [33].

Racial differences in thrombotic risk

African–Americans are at greater risk for adverse outcomes as a result of subtherapeutic anticoagulation compared with Caucasians. Specifically, African–Americans have greater stroke-related disability and higher mortality rates from stroke and pulmonary embolism compared with Caucasians [40–42]. Thus, efficiently achieving therapeutic anticoagulation is especially important for African–Americans starting warfarin.

Compared with non-Hispanic Caucasians, Hispanics have a higher risk for stroke, suffer from stroke earlier in life and die at a younger age from stroke [43,44]. However, Hispanics have lower overall stroke-related mortality rates compared with non-Hispanic whites [45]. Hispanics also have a lower risk for first venous thromboembolism compared with non-Hispanic Caucasians [46]. However, Hispanic women have a higher rate of recurrent venous thromboembolism compared with non-Hispanic Caucasian women [42]. The incidence of recurrent thromboembolism is similar between Hispanic and non-Hispanic men. Thus, achieving and maintaining therapeutic anticoagulation in Hispanic women with a history of venous thromboembolism is especially important.

Racial differences in genetic 'structure'

It is widely recognized that populations of recent African ancestry generally have more genetic variation. Furthermore, the extent of linkage disequilibrium, or the association

between two SNPs on the same chromosome, can be much lower in populations of recent African ancestry than in populations of recent European or Asian ancestry [47]. In many cases, the linkage disequilibrium in the African genome extends for shorter distances and may be broken up with sections of the genome that have very low linkage disequilibrium between sections that have very high linkage disequilibrium (known as long range linkage disequilibrium) [47]. These patterns are typically absent in Caucasians.

Polymorphisms that tag for a particular haplotype, known as tag SNPs, may be associated with drug response without actually causing changes in gene function or expression. Rather, tag SNPs may be in high linkage disequilibrium with the causal variant that actually underlies the genotype–phenotype association. Until functional studies are performed, the actual causal SNP within a haplotype block is unknown. Thus, a tag SNP that is not in itself functional, but is in high linkage disequilibrium with the causal SNP, may be a good predictor of warfarin dose requirements in a European population. However, because of the lesser linkage disequilibrium in African–Americans, this SNP may not be inherited as often with the causal SNP in the African–American population. Thus, it will not be predictive of dose requirements in African–Americans. As an example, the *VKORC1* 1583G>C SNP was strongly associated with warfarin dose requirements in several Caucasian populations [14,48]. However, we previously observed no association between the 1583G>C SNP and dose requirements in African–Americans [49]. The 1583G>C SNP is not functional. However, in Caucasians, the 1583G>C SNP is in strong linkage disequilibrium with the functional -1639G>A SNP that decreases *VKORC1* expression [14,25]. In African–Americans, the 1583G>C and -1639G>A SNPs are in much weaker linkage disequilibrium, thus explaining the lack of association with the 1583G>C SNP in this racial group. This example demonstrates the importance of validating gene–drug response associations across populations, especially when there are no functional data available for the SNP in question.

As mentioned previously, several groups have constructed warfarin dosing algorithms that include the *VKORC1* -1639G>A, *CYP2C9*2* and *CYP2C9*3* SNPs. These algorithms explain 50–60% of variability in warfarin dose in Caucasians and Asians, but significantly less in African–Americans [3,7,26,28,50,51]. African–American heritage was found to be a predictor of warfarin doses greater than 5 mg/day, and this association was independent of dietary and vitamin K intake [8]. On the other hand, patients from the Far East (Chinese, Japanese and Malay patients), required the lowest mean doses of warfarin at 3.1 mg/day [52–54]. Dosing algorithms that include only variants predictive in Caucasians and Asians are much less predictive in African–Americans [7,51], which may be due to the large amount of genetic diversity and decreased linkage disequilibrium in the African–American genome [55].

Warfarin pharmacogenetics in African–American individuals

CYP2C9 genotype

While African–American individuals have a lower prevalence of the *CYP2C9*2* and **3* alleles compared with Caucasians, the *CYP2C9*5* (D360E), **6* (10601delA) and **8* (R150H) alleles occur almost exclusively in African populations, whereas the *CYP2C9*11* (R335W) allele occurs at a low frequency in both populations.The *CYP2C9*8* allele is one of the most common *CYP2C9* alleles in African–Americans (Table 1), occurring more often than the *CYP2C9*2* and **3* alleles combined. The *CYP2C9*5, *6* and **11* alleles have been associated with decreased enzyme activity, whereas functional data suggest that the effects of the *CYP2C9*8* allele on drug metabolism are substrate specific [56–58]. Specifically, the *CYP2C9*8* allele is reported to increase enzyme activity toward tolbutamide *in vitro,* decrease enzyme activity toward phenytoin *in vivo* and have no effect on losartan metabolism [57–59]. Consistent with the *in vivo* data with phenytoin, we recently found a

20% reduction in *S*-warfarin clearance in African–American individuals with the *CYP2C9*8* allele [60].

Data from our group and others show significantly lower warfarin dose requirements in African–American individuals with a *CYP2C9*5, *6, *8* or **11* allele compared with those with the *CYP2C9*1/*1* genotype [21,34,61]. Specifically, we observed an 18% lower median warfarin maintenance dose in African–Americans with a *CYP2C9*5, *6, *8* or **11* allele compared with those with the *CYP2C9*1/*1* genotype (median dose of 5.0 mg/day vs 6.1 mg/day, p = 0.004) [21]. When taking the *CYP2C9*2, *3, *5, *6, *8* and **11* alleles into account, the *CYP2C9* genotype explained approximately 8% of the variability in warfarin dose requirements among African–Americans, just slightly less than that explained by the *CYP2C9*2* and **3* alleles in Caucasians [15].

The *CYP2C9*8* allele by itself is associated with significantly lower warfarin dose requirements compared with the *CYP2C9 *1/*1* genotype, as shown in Figure 4 [21]. Furthermore, it predicts similar warfarin dose requirements as the other *CYP2C9* alleles combined in African–Americans. Since the *CYP2C9*8* allele occurs almost as often as other *CYP2C9* alleles combined in African–Americans and significantly impacts warfarin dose requirements, it is especially important to consider the *CYP2C9*8* allele in pharmacogenetic warfarin dosing for African–American patients [21].

VKORC1 genotype

Many of the warfarin pharmacogenetic studies to date have focused on *VKORC1* -1639G>A SNP, which distinguishes between haplotype groups A and B, as defined by Rieder *et al.* [14]. By compiling frequencies of haplotype groups found in several different studies (Table 2), we can see that, in general, African–American individuals have the highest prevalence of haplotype group B (high dose) and lowest prevalence of haplotype group A (low dose), while Asian individuals have the highest prevalence of haplotype group A [14,36]. While these haplotype groups capture 96 and 99% of the total *VKORC1* haplotypes in Caucasians and Asians, respectively, they account for only 62–78% of the total *VKORC1* haplotypes in African–Americans. This is due in part to the decrease in linkage disequilibrium and increase in the amount of variation found in populations of African ancestry. By focusing solely on the *VKORC1* -1639G>A variant, there is a significant portion of the genetic variation in African–Americans, in terms of *VKORC1* haplotype, that will not be captured.

Novel *CYP2C9* & *VKORC1* variants

Because African–Americans were excluded from initial efforts to elucidate SNPs associated with warfarin response, our laboratory sought to identify a novel variation that affects warfarin dose in African–Americans through a targeted gene resequencing approach. Our recent findings highlighted a novel *VKORC1* SNP in African–Americans that was associated with higher warfarin dose requirements in both a discovery (n = 122) and replication (n = 207) cohort [34]. This SNP (*VKORC1*-8191, rs61162043) was found approximately 8 kb upstream of the *VKORC1* start site and was only identified via resequencing of the region in African–Americans. Furthermore, this SNP is not on the current 1 million or 2.5 million SNP arrays nor is it in strong linkage dis equilibrium with SNPs on these arrays. Therefore, conventional GWAS would not have identified this variant as associated with warfarin dose.

In addition to the *VKORC1* variant, the authors also identified a novel *CYP2C9* variant (rs7089580, intron 3) that was associated with higher dose requirements of warfarin [34]. The inclusion of these two SNPs in a regression model allowed us to explain 40% of the variability in warfarin dose in the combined (discovery plus replication) cohort of over 300

African–Americans. With the addition of sequence-level data, the authors will be closer to identifying novel ethnicity-specific variants GWAS and candidate gene investigations are underpowered or unable to capture.

Alternative genes associated with warfarin response

CYP4F2—The CYP4F2 enzyme is involved in the metabolism of vitamin K_1. Increased vitamin K_1 metabolism leads to reduced quantity of vitamin KH, available for clotting factor activation. The V433M SNP occurs in exon 2 of the *CYP4F2* gene, with the 433M allele leading to lower CYP4F2 concentration and a reduced ability to metabolize vitamin K_1 [62]. Consequently, individuals with the 433 M/M genotype will likely have greater vitamin K availability. The *CYP4F2* V433M genotype has been associated with warfarin dose requirements in both Caucasian and Asian populations, with increased requirements with the 433 M allele [16,17,39]. The *CYP4F2* genotype explains approximately 1–2% of the variability in warfarin dose requirements in these populations [17]. However, the *CYP4F2* genotype was not associated with warfarin maintenance doses in African–Americans, possibly because of the low frequency of the 433M allele in this population (Table 1) [21].

CALU—Calumenin regulates γ-glutamyl carboxylation of vitamin K-dependent-clotting factors by inhibiting γ-carboxylase activity (Figure 1) [63]. As such, the *CALU* gene is a potential candidate for influencing warfarin pharmaco dynamics. With this in mind, Voora *et al.* resequenced the *CALU* gene and identified the rs339097 SNP, which was significantly more common in African–Americans compared with Caucasians (25% vs <1%) and was associated with warfarin dose requirements in the former population [37]. In particular, the minor G allele at this position was associated with 11% higher warfarin doses in African–Americans. A subsequent study in Egyptian individuals confirmed that the *CALU* variant increases warfarin dose requirements [64].

GGCX—Another potential candidate for influencing warfarin pharmacodynamics is the gene encoding γ-glutamyl carboxylase (GGCX) (Figure 1) The GGCX enzyme catalyzes the biosynthesis of vitamin K-dependent clotting factors by carboxy lating protein-bound glutamate residues. Rare *GGCX* mutations cause deficiencies in vitamin K-dependent clotting factors [65]. More common *GGCX* variants have been reported to influence warfarin dose variability; however, there are limited data with regards to this gene in African–Americans [66–68].

Our group recently found that the rs10654848 (CAA) microsatellite in intron 6 of the *GGCX* gene is associated with higher warfarin dose requirements in African–Americans [38]. Specifically, we identified 8–17 repeats of the CAA sequence among 338 warfarin-treated African–Americans. We found that 5% of African–Americans carried the (CAA)16 or 17 repeat, which was correlated with fourfold greater odds of needing a warfarin dose >7.5 mg/day. The *GGCX* (CAA)n genotype was also associated with warfarin dose variability, explaining 2% of the total variability in maintenance dose among African–Americans. In a comparative cohort of 183 Swedish warfarin-treated patients, only one patient (0.5%) had a (CAA)16 repeat, and none carried the 17 repeat. Interestingly, the Caucasian (CAA)16 repeat carrier also had the *CYP2C9*1/*2* genotype and was taking amiodarone, both of which are expect to lower dose requirements. Yet, this patient required a warfarin maintenance dose of 11 mg/day. The higher prevalence of the (CAA)16 or 17 allele among African–Americans may contribute to the higher dose requirements observed in the African–American population. The mechanism underlying higher doses with the (CAA)16/17 repeat is unclear. However, one possibility is that a higher number of repeats enhances the activity of the enzyme, thus leading to greater clotting factor carboxylation and higher warfarin dose requirements.

Warfarin pharmacogenetics in Hispanic individuals

The Hispanic population is the largest and most rapidly growing minority population in the USA [103]. According to data from the US Census Bureau, there are approximately 43 million Hispanic individuals living in the USA, and this population is expected to increase more than twofold in the next 3–4 decades [103]. Despite this, most warfarin pharmacogenetic studies either excluded or enrolled a marginal percentage (<15%) of Hispanic individuals [7,11,14,69,70]. Data from the NHANES cohort demonstrate that *VKORC1* alleles occur at similar frequencies in US Hispanic and non-Hispanic Caucasians [26]. However, whether *VKORC1* predicts warfarin dose requirements in Hispanic individuals is not well studied.

To address this issue, our group assessed warfarin pharmacogenetics in a small inner-city US Hispanic population, mostly consisting of Mexican–Americans [33]. In addition to determining the combination of clinical and genetic factors that influence warfarin dose requirements, we tested the performance of published warfarin dosing algorithms that were derived from predominately European Caucasians, in this population. The *VKORC1* -1639A, *CYP2C9*2* and *CYP2C9*3* alleles occurred at frequencies similar to those reported in Caucasian individuals. Compared with the *VKORC1* -1639GG genotype, the median warfarin dose requirements were 30% lower with the AG genotype and 62% lower with AA genotype. Median dose requirements were 42% lower with the *CYP2C9*2* or **3* allele compared with the *CYP2C9*1/*1* genotype. Together with clinical factors, *VKORC1* and *CYP2C9* genotypes explained 56% of the interpatient variability in warfarin dose requirements in our Hispanic cohort, which is consistent with the contribution of these variables to dose requirements in non-Hispanic Caucasians [7,11,51]. Both the IWPC and Gage algorithms were predictive of warfarin dose requirements in Hispanic individuals, each explaining approximately 45% of the variance in dose. This is similar to the performance of these algorithms in non-Hispanic Caucasians [11,51,71]. These data suggest that similar factors affect warfarin dose requirements in Hispanic and non-Hispanic Caucasians and that dosing algorithms derived from non-Hispanic Caucasian cohorts are applicable to Hispanics of Mexican descent living in the USA.

Future directions in pharmacogenetic research

Novel approaches to examining gene–drug response associations

New methodology has been proposed to examine SNP association in the context of genome-wide approaches. One method that has been used in both disease association and chemotherapy response association is expression quantitative trait loci (eQTL) analysis. Such analysis involves both genome-wide SNP genotyping and gene-expression array data in the same set of individuals. This approach allows the investigator to identify a genetic variant associated with a phenotype of interest (through genome-wide SNP genotyping) as well as the gene(s) whose expression is affected by the variant (through gene-expression array data). By understanding which genes are affected by a particular variant, the investigator gains insight into the mechanism underlying the association between the variant and the outcome of interest. eQTL studies have led to novel SNP associations in chemotherapy sensitivity, which subsequently led to translatable clinical outcomes in ovarian cancer [72], as well as a publically available database to search for genotype/gene expression associations [73].

The primary drawback to these eQTL studies, particularly for applying eQTL methods to warfarin pharmacogenetic research, is that many of the studies were conducted in lympho blastic cell lines that are less than ideal for pharmacokinetic phenotypes. In particular, lymphoblastic cell lines do not express CYP450 drug metabolizing enzymes and thus, could

not be used to interrogate the pharmaco genetics of warfarin metabolism. A recent publication highlighted findings in liver eQTL, which could have important implications in drug metabolism genetics [74]. There are no studies utilizing eQTL strategies for warfarin pharmacogenetics to date. Therefore, use of such would be a novel approach to examining the genetics of warfarin pharmacokinetics. However, since most of the liver eQTL studies have been conducted in predominantly Caucasian populations, the translation of these findings into other ethnicities has similar limitations as those seen in GWAS and genetic association studies discussed previously.

GWAS in African–Americans

The IWPC recently completed a GWAS in a relatively large cohort of African–Americans. Preliminary results were presented at the 2011 American Heart Association Scientific Sessions and are published in abstract form [75]. This study found novel variation in the *CYP2C* locus associated with warfarin dose requirements. Interestingly, this novel signal is not related to the known *CYP2C9*2* and *CYP2C9*3* variants that have been previously implicated as contributors to warfarin dose requirements.

Studies such as the GWAS in African–American individuals show that by studying non-Caucasian populations, one can reveal novel variation that cannot be found otherwise. However, the use of GWAS in African–American individuals also has its limitations. A recent publication by our laboratory interrogated the coverage of approximately 250 pharmacogenes (i.e., genes important in pharmacogenomics) on several different high-throughput genotyping platforms [76]. We also investigated the coverage of these pharmacogenes in the HapMap Project, which is commonly used as the reference set for SNP imputation in GWAS, compared with the 1000 Genomes Project data, which were generated through deeper sequencing and provide greater SNP coverage. The authors found that the HapMap data provided high overall coverage of pharmacogenes, with 84% of SNPs in 1000 Genomes found in the HapMap database. However, none of the genotyping platforms studied (which included the Illumina Omni 2.5 million array and the Axiom™ Genomic Database [Affymetrix, CA, USA], which contains 11 million SNPs) showed more than 85% coverage of SNPs in pharmacogenes when using 1000 Genomes data as the reference. In addition, many of the platforms studied did not reach maximum coverage of approximately 70–80% for SNPs with minor allele frequencies <20%.

These findings illustrate one of the limitations of genome-wide technologies. The implication of these findings is that SNPs with minor allele frequencies below 20% are not well represented on available platforms for genome wide analysis. When focusing on the *CYP2C9* gene specifically, we found that the Axiom database showed the highest overall coverage of the platforms studied at 68%. These coverage estimates take into account linkage disequilibrium within and near the *CYP2C9* gene and highlight that as much as 32% of the variation in this gene, which is important in warfarin dosing, would never be captured in a GWAS. Furthermore, the novel *VKORC1* variant associated with higher warfarin dose requirements that we identified through resequencing in African–Americans (described previously) was not captured by any of the genotyping platforms evaluated, nor is it in HapMap [34]. Thus, without a sequenced-based methodology, the authors would never have uncovered this SNP.

Findings from the authors' analysis of genotyping platforms and HapMap coverage also call into question the assumption that only very low-frequency alleles are absent in GWAS genotyping methods as they relate to pharmacogenes. While these studies were performed in a Caucasian population, we assume the coverage would be even less for populations such as African–Americans that carry greater amounts of genetic variation. Therefore, a

comprehensive strategy, employing both GWAS and resequencing, may be required to fully evaluate GWAS, particularly for African–Americans.

Implementing warfarin pharmacogenetics

Genetic data are seldom used in most institutions to make warfarin dosing decisions. This is despite the substantial data supporting genetic determinants of warfarin dose requirements and the dosing tools available to assist with applying genetic data to dose prediction. Furthermore, at least five FDA-cleared platforms are available for genotype-guided dosing [77]. Guidelines from the American College of Chest Physicians and the American College of Medical Genetics actually recommend against routine use of genetic information to guide warfarin dosing [78,79]. In addition, few third party payers reimburse for genetic testing for warfarin management at this time; although the Centers for Medicare and Medicaid Services may provide coverage if testing is performed in the context of a controlled clinical study. A major reason that genotype-guided therapy is not better accepted is that many clinicians and policy makers require further evidence of the clinical utility of such testing [5,79].

Findings from two small trials and a comparative effectiveness study provide some evidence of the clinical utility of genotype-guided warfarin dosing [10,80,81]. The two trials demonstrated reduced time to reach stable warfarin dosing with a pharmacogenetic dosing approach [10,80]. In the comparative effectiveness study, patients who accepted free genotyping for the *CYP2C9*2*, *CYP2C9*3* and *VKORC1* -1639G>A variants, with results provided to their physician, had 30% fewer hospitalizations for any cause and for bleeding or thromboembolism during the 6 months following warfarin initiation compared with historical controls [81]. By contrast to these positive data, two other small trials found no benefit with pharmacogenetic dosing compared to clinical-based dosing in terms of percentage of out-of-range INR values [12,13]. However, an exploratory analysis of one trial demonstrated a significant benefit with pharmacogenetic dosing for patients with either multiple variant alleles or with no variant alleles compared with carriers of a single gene variant [12]. African–Americans were either excluded or only marginally represented in these trials. Overall, while studies provide some support with regards to the clinical utility of warfarin pharmacogenetics, the data are inconsistent and the utility of genotype-guided dosing in African–Americans in particular is unclear.

In order to more definitively address the clinical utility of pharmacogenetic warfarin dosing, several large clinical trials, including the National Heart, Lung and Blood Institute sponsored COAG trial, are addressing the issue. The COAG trial is a prospective, multicenter, double-blind trial, in which patients are randomized to a pharmacogenetic or clinical dosing strategy, with the outcome measures of percentage of time spent within the therapeutic INR range (primary outcome) and the occurrence of an INR >4 or serious event (secondary outcome) during the initial month of therapy [82] . Unlike previous trials, the COAG trial is powered to account for the potential lack of benefit with genotype-guided therapy in patients with a single variant. In addition, the COAG trial aims to enroll a significant proportion of minority patients in order to examine the utility of pharmacogenetic dosing across populations. The COAG trial is expected to be completed in March 2013.

An additional barrier to pharmacogenetic dosing for African–Americans is that African-specific variants, such as *CYP2C9*8, CALU* rs339097, the *GGCX* rs10654848 (CAA) microsatellite and the newly discovered *CYP2C9* rs7089580 and *VKORC1* rs61162043 alleles, are not included on FDA-cleared warfarin geno typing platforms. In addition, most pharmacogenetic algorithms do not include African-specific SNPs. An exception is the Gage algorithm, which is revised periodically as data become available. At the time of writing, the

Gage algorithm includes the *CYP2C9*5* and *CYP2C9*6* alleles, but not the *CYP2C9*8* allele.

Conclusion

African–Americans and Hispanics are significantly under-represented in pharmacogenetic studies with warfarin. There are important differences in genetic structure and genotype frequencies by race that may contribute to racial differences in warfarin pharmacogenetics. For US Hispanics, the limited data available suggest that warfarin pharmacogenetics in this population resembles that in non-Hispanic Caucasians, at least for Hispanics of Mexican descent. However, for African–Americans, the major variants influencing warfarin dose requirements in Caucasians provide lesser contribution to dose variability in African–Americans. In addition, there are variants occurring almost exclusively in persons of African descent that significantly impact warfarin dose requirements.

The most commonly used pharmacogenetic dosing algorithms were derived from predominately Caucasian populations, and while these appear to perform well in US Hispanics, their ability to predict warfarin maintenance dose is reduced in African–Americans [11,33,51]. African-specific variants that are predictive of warfarin dose requirements are not included in most dosing algorithms, the FDA-approved dosing table or on FDA-cleared genotyping platforms. Neglecting to account for these variants will likely result in lower accuracy of pharmacogenetic dosing algorithms in African–Americans. A recent model that incorporated two novel African-specific variants was able to explain 40% of the dose variability in African–Americans [34]. Refinement of this model to account for additional African-specific SNPs, such as the SNP recently identified in a GWAS, will likely further improve the utility of pharmacogenetic dosing for African–Americans [75]. Trials addressing the clinical utility of warfarin pharmacogenetics are ongoing. However, questions related to the utility of warfarin pharmacogenetics in African–Americans may remain after trial completion, given that many African-specific SNPs will not be accounted for. For pharmacogenetics to reach their full potential for persons of African ancestry, the data suggest that the African-specific variants should be accounted in dosing algorithms and genotyping efforts.

Future perspective

The Clinical Pharmacogenetics Implementation Consortium recently published guidelines on how to interpret and apply genetic test results to adjust warfarin doses. These guidelines do not address when to order a genetic tests but rather, how to dose warfarin when genetic test results are available. The guidelines strongly support the use of genetic information to guide warfarin dosing when genotype is known and recommend using either the IWPC or Gage algorithm to do so [83].

From a practical standpoint, when using genotype-guided therapy, an empiric dose of 5 mg (or lower if the patient is older or taking a CYP2C9 inhibitor) may initially be administered if genotyping results cannot be obtained prior to the first warfarin dose. Then, the Gage algorithm, which allows for integration of previous warfarin doses and INR values to refine subsequent dosing, may be used once genotype results are available. The Gage algorithm may be particularly useful for warfarin dosing in African–Americans since it is periodically updated to include variants associated with warfarin dose in various populations. The dosing table in the warfarin labeling is an alternative to the IWPC or Gage algorithm in the event that access to these algorithms is unavailable. However, data suggest that the dosing table may be less accurate at dose prediction than pharmacogenetic algorithms [84].

The ability to accurately predict dose requirements could improve time to stable warfarin dosing and potentially reduce the risks associated with sub- and supra-therapeutic anticoagulation early in the course of therapy. This would have particular implications for African–American individuals and Hispanics who are greater risk for adverse sequelae from suboptimal warfarin dosing than those of other racial or ethnic backgrounds [40–43].

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Executive summary

Genetic determinants of warfarin response

- Substantial data show that the *VKORC1* -1639G>A (or 1173C>T), *CYP2C9*2* and *CYP2C9*3* SNPs are the major genetic contributors to warfarin dose requirements in Caucasian and Asian individuals.
- The combination of *VKORC1* -1639G>A, *CYP2C9*2, CYP2C9*3* and clinical factors (e.g., age, sex, weight and amiodarone use) explains 50–60% of the total variance in warfarin dose requirements in Caucasians, but only an estimated 25% in African–Americans.

Warfarin pharmacogenetic dosing tools

- A US FDA-approved table and several algorithms are available to assist with genotype-guided warfarin dosing.
- However, these dosing tools were derived largely from Caucasian populations and are less accurate in African–Americans.

Racial considerations in warfarin pharmacogenetics

- African–American and Hispanic individuals are significantly under-represented in pharmacogenetic studies with warfarin.
- There are important differences in genetic structure and genotype frequencies by race that may contribute to racial differences in warfarin pharmacogenetics.

Warfarin pharmacogenetics in African–American individuals

- Variants in the *CYP2C9*, *VKORC1, CALU* and *GGCX* genes that occur almost exclusively in African–Americans have been significantly associated with warfarin dose requirements.

Warfarin pharmacogenetics in Hispanic individuals

- Limited data in US Hispanics suggest that warfarin pharmacogenetics in this population resembles that in non-Hispanic Caucasians.
- Pharmacogenetic dosing algorithms derived from predominantly Caucasian populations perform well in US Hispanics.

Future directions in pharmacogenetic research

- The first genome-wide association study in African–Americans was recently completed and preliminary data show a novel association with warfarin response at the *CYP2C* locus.
- New methodology to examine single-nucleotide polymorphisms associations with drug response in the context of genome-wide approaches, such as use of expression quantitative trait loci, could lead to novel insight into the mechanism underlying gene–warfarin response associations.

Implementing warfarin pharmacogenetics

- The Clinical Pharmacogenetics Implementation Consortium recently provided guidelines for using genetic information to guide warfarin dosing.
- However, barriers to clinical implementation of warfarin pharmacogenetics remain and include uncertainty as to its clinical utility.

■ An additional barrier for African–Americans is that US FDA-cleared genotyping platforms and most pharmacogenetic dosing algorithms do not include African-specific variants.

■ An ongoing clinical trial, which aims to enroll a significant number of ethnic minorities, will provide data on the clinical utility of genotype-guided warfarin dosing.

Conclusion

■ For pharmacogenetics to reach its full potential for persons of African ancestry, the data suggest that genotype-guided warfarin dosing should account for African-specific variants.

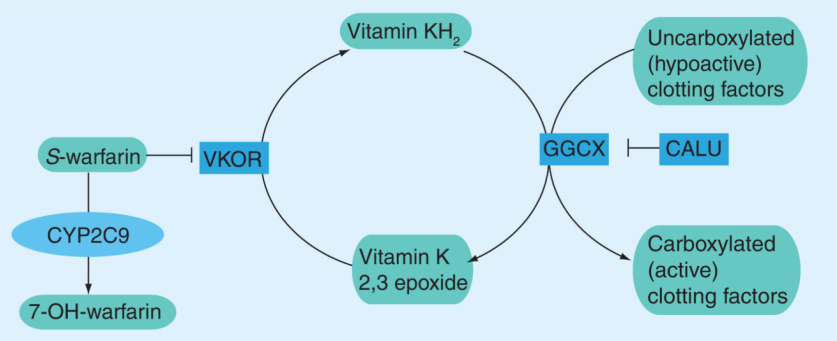

Figure 1. Genes affecting warfarin

Pharmacokinetics (shown in oval boxes) and pharmacodynamics (shown in rectangular boxes).

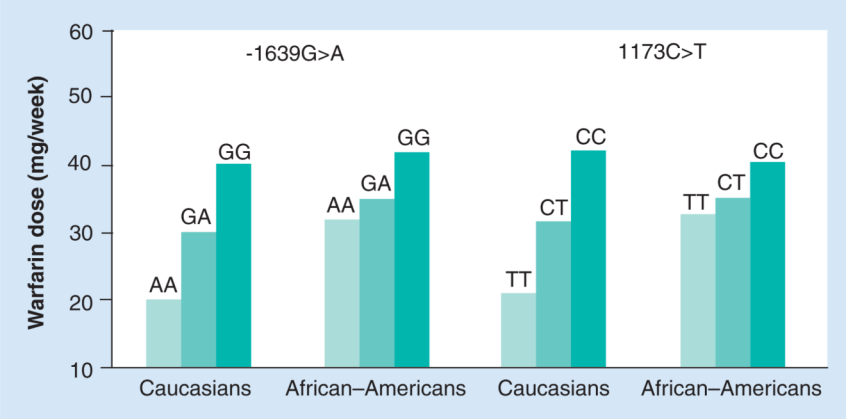

Figure 2. Median warfarin dose requirements by *VKORC1* -1639G>A and 1173C>T genotypes in Caucasians and African–Americans, according to data from the International Warfarin Pharmacogenetics Consortium

Data taken from [26].

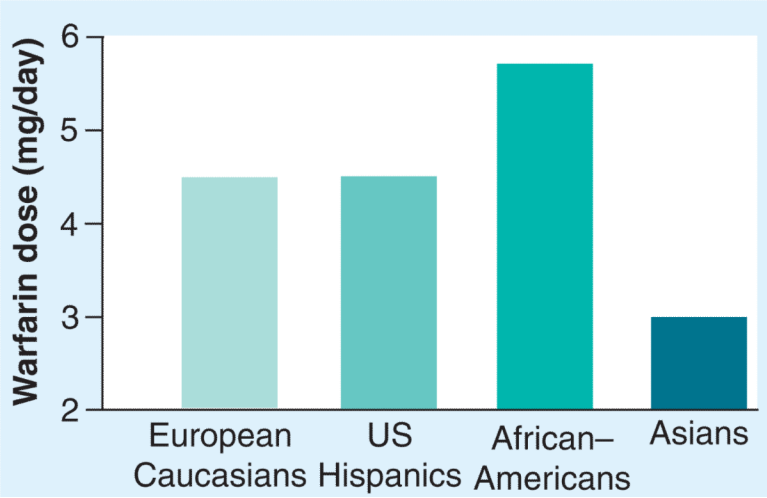

Figure 3. Median warfarin dose requirements by race and ethnicity
Data taken from [26,33].

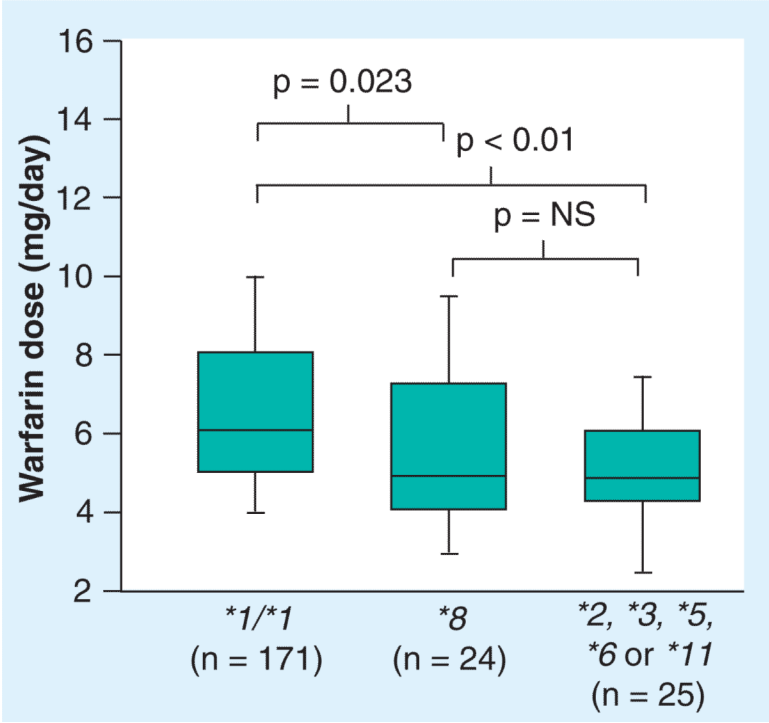

Figure 4. Median (range) warfarin dose requirements in African–Americans with the *CYP2C9*1/*1* genotype compared with *CYP2C9*8* allele carriers and carriers of a *CYP2C9*2, *3, *5, *6* or *11* allele

Lines within boxes represent medians. Lower and upper borders of the boxes represent 25th and 75th percentiles, respectively. Whiskers below and above boxes represent 10th and 90th percentiles, respectively.

NS: Not significant.

Data taken from [21].

Table 1

Location and frequency of gene alleles contributing to warfarin response.

Allele	Location	Frequency		
		European Caucasians	*US Hispanics*	*African–Americans*
*CYP2C9*2*	Exon 3	0.10	0.07	0.02
*CYP2C9*3*	Exon 7	0.06	0.05	0.01
*CYP2C9*5*	Exon 7	<0.01	<0.01	0.01
*CYP2C9*6*	Exon 5	<0.01	<0.01	0.01
*CYP2C9*8*	Exon 3	<0.01	<0.01	0.06
*CYP2C9*11*	Exon 7	<0.01	<0.01	0.04
CYP2C9 rs7089580	Intronic	0.24	0.11	0.23
VKORC1 -1639A	5-UTR	0.40	0.46	0.11
VKORC1 rs61162043	5-UTR	Unknown	Unknown	0.47
CALU rs339097	Intronic	<0.01	Unknown	0.11–0.14
CYP4F2 433M	Exon 2	0.23	0.22	0.09
GGCX (CAA)16, 17	Intronic	<0.01	Unknown	0.03

Data taken from [34–39,102].

Table 2

Frequency of *VKORC1* haplotype groups in African–American individuals compared with Caucasian and Asian individuals.

Haplotype group	African–Americans (%)	Non-Hispanic Caucasians (%)	Asians (%)
A	14–21	37–42	85–89
B	49–58	57–58	10–14

Data taken from [14,36].