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WARFARIN PHARMACOGENOMICS IN DIVERSE POPULATIONS

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Abstract

Genotype-guided warfarin dosing algorithms are a rational approach to optimize warfarin dosing and potentially reduce adverse drug events. Diverse populations, such as African Americans and Latinos, have greater variability in warfarin dose and higher risk for warfarin-related adverse events compared to individuals of European ancestry, suggesting that they may benefit more from improving warfarin dose estimation. However, the vast majority of literature on genotype-guided warfarin dosing is from populations of European ancestry, particularly the data from prospective randomized trials. Despite differing frequencies and effects of variants by race/ethnicity, most of evidence in diverse populations evaluates variants that are most common in populations of European ancestry. Algorithms that do not include variants important across race/ethnic groups are unlikely to provide a broad benefit to diverse populations. In some race/ethnic groups, development of race-specific or admixture-based algorithms may facilitate improved genotype-guided warfarin dosing algorithms above and beyond that seen in individuals of European ancestry. These observations should be considered in the interpretation of recent literature evaluating the clinical utility of genotype-guided warfarin dosing. Careful consideration of race/ethnicity and additional evidence focused on improving warfarin dosing algorithms across race/ethnic groups will be necessary for successful clinical implementation of warfarin pharmacogenomics. Finally, the evidence with warfarin pharmacogenomics has a broad significance for pharmacogenomic testing, emphasizing the consideration of race/ethnicity in

discovery of gene-drug pairs and development of clinical recommendations for pharmacogenetic testing.

INTRODUCTION

Warfarin is the most widely prescribed oral anticoagulant in the United States (US), accounting for approximately 34 million prescriptions.^{1, 2} Despite the approval of several direct oral anticoagulants (DOACs), warfarin remains a mainstay in the treatment and prevention of thromboembolic events. However, warfarin has a narrow therapeutic index and continues to be a leading cause of drug-related adverse events leading to hospitalization.³ Inappropriate dosing significantly increases the risk for thromboembolism, bleeding, hospitalization, and death.^{4, 5} This risk is highest during the initial months of warfarin treatment and efficient achievement of therapeutic anticoagulation may result in safer and more effective warfarin dosing.⁶

Warfarin doses needed to achieve therapeutic anticoagulation vary up to 20-fold among patients.⁷ Warfarin's narrow therapeutic index and dose variability has led to the development of dose prediction algorithms to facilitate attainment of optimal anticoagulation. Such dose prediction algorithms incorporate factors such as age, weight, race/ethnicity, drug interactions, and target International Normalized Ratio (INR). These algorithms are improved by the incorporation of genetic variants affecting warfarin dose, which led to refinement of these algorithms and updates in the FDA-approved warfarin labeling.^{8, 9} More than 30 genotype-guided warfarin dosing algorithms have been published for warfarin and other coumarin anticoagulants.¹⁰ However, uptake of these algorithms into clinical practice has been slow with few clinical guidelines supporting use of pharmacogenetic testing.¹¹

Average daily warfarin doses differ by race/ethnicity as daily dose needed to reach a stable therapeutic INR is estimated at 5.1mg in individuals of European ancestry, 5.7mg in individuals of African ancestry, 4.4mg in Latinos, 3.4mg in individuals of Asian ancestry, and 4.5mg in American Indian/Alaska Natives (AI/AN).^{12–14} Diverse populations such as African Americans (AAs) and Latinos are significantly underrepresented in studies that developed clinical and genotype-guided dosing algorithms. This underrepresentation and the results of recent studies have cast doubt on the utility of pharmacogenetic testing to estimate warfarin dose, especially in diverse populations.^{15–17} This is particularly concerning since these populations may have the most to gain from efforts to improve warfarin management. Warfarin dose variability appears to be greatest in populations without European ancestry.¹⁸ In addition, Latinos and AAs are at notably high risk for poor outcomes as a result of sub-optimal warfarin management, including an increased risk for warfarin-related intracranial hemorrhage compared to Europeans.^{19, 20}

In this manuscript, we review existing evidence for variants that affect warfarin dose requirements and the performance of genotype-guided dosing algorithms. We present detailed data on studies in diverse populations, focusing on major minority populations, in order to summarize the inclusion of race/ethnic groups, frequency of genetic variants, and performance of genotype-guided dosing algorithms by race/ethnicity. Finally, we offer

perspectives on the future directions of warfarin pharmacogenetic research and potential implications for clinical practice.

WARFARIN PHARMACOGENOMICS IN INDIVIDUALS OF EUROPEAN ANCESTRY

Single nucleotide polymorphisms (SNPs) in genes encoding proteins involved in warfarin pharmacokinetic and pharmacodynamics pathways have been associated with warfarin dose requirements. (Figure 1) Cytochrome P450 family 2 subfamily C member 9 (*CYP2C9*) is the enzyme primarily responsible for metabolizing the more potent S-enantiomer of warfarin to an inactive metabolite. Multiple SNPs in the gene *CYP2C9* alter the amino acid sequence and thus confer reduced enzyme function and decreased warfarin metabolism. Warfarin elicits its anticoagulant effect by inhibiting vitamin K epoxide reductase (*VKORC1*), the enzyme responsible in activating downstream clotting factors.²¹ A SNP in *CYP4F2*, which plays a role in catalyzing free vitamin K to hydroxyvitamin K, was observed to increase warfarin dose requirements.²² Other genes with variation affecting warfarin dose include calumenin (*CALU*),²³ which functions as a chaperone for γ -glutamyl carboxylation, γ -glutamyl carboxylase (*GGCX*),²⁴ which contributes to activating clotting factors, and NAD(P)H quinone dehydrogenase 1 (*NQO1*),²⁵ which helps reduce vitamin K to vitamin K hydroquinone, the activating co-factor for γ -glutamyl carboxylase.

In individuals of European ancestry, variants in *CYP2C9*, *VKORC1*, and *CYP4F2* explain an estimated 9, 25, and 2% of the variability in warfarin dose requirements, respectively.^{9, 18, 26} In response to the mounting evidence of *CYP2C9* and *VKORC1* SNPs impact on warfarin dose requirements, the United States Food & Drug Administration (FDA) updated the warfarin dosing label in 2010.⁸ This label includes a table of initial dosing recommendations based on *CYP2C9* and *VKORC1* genotypes. Although simple to implement clinically, the table ignores many of the other clinical factors affecting warfarin dose requirements. Among the most commonly used algorithms to predict stable warfarin dose are the Gage algorithm and the International Warfarin Pharmacogenetic Consortium (IWPC) algorithm.^{9, 18} These algorithms were developed in populations of primarily European ancestry and consider race/ethnicity as a variable within the algorithm. The vast majority of warfarin pharmacogenetic studies have been conducted in populations with European ancestry, especially studies that investigate the clinical utility of genotype-guided warfarin dosing.²⁷ (Figure 2 and Table S1 in Supplementary Materials)

Despite the FDA label change and the availability of these algorithms, uptake of genotype-guided warfarin dosing into the clinic has been slow. Proponents of warfarin pharmacogenetic testing support incorporation of testing recommendations into clinical guidelines, citing the wealth of evidence supporting improvement of initial warfarin dose estimation using genotype data. The Clinical Pharmacogenetics Implementation Consortium (CPIC) strongly advises using an individual's genotype to guide therapy based on the improved predictability to achieve a stable dose compared to standard care.¹¹ An important caveat is that CPIC guidelines assume genetic data is available upon warfarin initiation, and the guidelines do not provide recommendations of when to order pharmacogenetic testing.

Opponents of warfarin pharmacogenetic testing cite the lack of supporting data from randomized prospective trials as well as the cost of genotyping. The American College of Chest Physicians (ACCP) recommends against ordering pharmacogenetic testing to guide warfarin dosing due to a lack of data showing improvement in patient outcomes such as bleeding and thrombotic events.²⁸ However, ACCP's guideline did not address whether pharmacogenetic information should be used if available at the time of warfarin initiation.

The results of three marquis randomized control trials (RCTs) have differing results regarding the clinical utility of warfarin pharmacogenetic testing.^{15–17} The results of these three RCTs are summarized in Table S2 in the Supplementary Materials. The only one of these trials to observe a benefit used a fixed dose approach as a comparator. The European Pharmacogenetics of Anti-coagulant Therapy (EU-PACT) group recruited 454 patients and found a significant improvement in the percent of time patients were able to maintain a stable warfarin dose when initial dosing predictions were based on genotype-guided dosing versus fixed-dosing (67.4% vs 60.3% time in therapeutic range (TTR), $p<0.001$).¹⁶ Although a fixed dose approach may be more representative of clinical practice, it is unclear whether the pharmacogenetic data or the clinical data used to predict dose were driving the observed benefit. Conversely, the Clarification of Optimal Anticoagulation through Genetics (COAG) study in 955 patients and found no improvement in TTR when patients received predicted warfarin dosing based on genotype-guided methods compared to dosing based solely on clinical factors (45.2 versus 45.4% TTR, $p=0.91$).¹⁵ The results of COAG suggest that a clinically-guided algorithm is sufficient for improvement in dosing estimation.

A second EU-PACT study evaluated TTR for a genotype-guided algorithm and a clinical algorithm for dosing the warfarin derivatives acenocoumarol and phenprocoumon.¹⁷ This study observed no significant improvement in percent TTR in the genotype-guided group compared to patients receiving clinically-guided dosing (61.6% vs 60.2%, $p=0.52$). (Table S2 in Supplementary Materials) The interpretation and generalizability of these trials is complicated by discordant study designs, including differences in comparator groups, total number of subjects, follow-up time, warfarin loading dose, time of dose initiation, time of INR measurement, and time of genotype availability. In addition, these three trials used a surrogate marker, TTR, as a primary outcome rather than clinical outcomes such as bleeding or thromboembolic events, limiting interpretability in terms of clinical utility.

A possible explanation for the disparate results of these trials is the distribution of race/ethnicity. The EU-PACT and COAG trials used the IWPC and Gage algorithms, which were derived from individuals of primarily European ancestry and include the variants *CYP2C9*2*, *CYP2C9*3*, and *VCORC1-1639G>A*. While EU-PACT consisted of a patient cohort with 98.5% self-reported European ancestry, the COAG trial was composed of a more diverse cohort with 27% AA patients.^{15–17} In COAG, variability in warfarin dose explained by the genotype-guided algorithm was 52% in individuals of European ancestry and as low as 17% in AAs.²⁹ The AAs who were assigned to the genotype-guided arm, had significantly lower time spent in therapeutic range compared to the clinical-guided arm (35.2% vs. 43.5%, $p=0.0003$). (Table S2 in Supplementary Materials) These findings suggest that European-based genetic algorithms do not accurately predict warfarin dose in

AAs and the same concern exists for other underrepresented populations of non-European ancestry.

IDENTIFICATION OF WARFARIN PHARMACOGENOMIC VARIANTS IN DIVERSE POPULATIONS

Diverse populations encounter higher adverse health risks compared to European patients during warfarin therapy. Asians, Latinos, and AAs have higher risks of warfarin-related intracranial hemorrhage outcomes than individuals with European ancestry.²⁰ In addition, African Americans, Latinos, Asians, and Native Americans have significant reductions in TTR compared to individuals with European ancestry. An assessment of warfarin management showed individuals of African and Hispanic ancestry had lower median TTR than individuals of European ancestry (59% vs 62% vs 68%, $p < 0.0001$).¹⁹ A similar study found lower mean TTR in AAs, Asians, and Native Americans combined compared to European patients.^{19, 30} These observations suggest that diverse populations stand to benefit more from more accurate dose estimation provided by genotype-guided warfarin dosing.

Differences in the frequencies of warfarin pharmacogenetic variants partially explain the early clinical observations of different warfarin dose requirements by race/ethnicity. For instance, the *VKORC1* –1639G>A allele is less common in individuals with African ancestry, partially accounting for higher warfarin dose requirements in AAs. (Table 1) The *CYP2C9**2 and *3 alleles are more prevalent in individuals with European ancestry, while the *CYP2C9**8 and *11 alleles are more prevalent in AAs. These differences in minor allele frequency (MAF) are compounded by the existence of race-specific variants that contribute to warfarin dose variability. If important functional variants are not recognized or identified for a given race/ethnic group, the clinical utility of genotype-guided algorithms is limited. Literature identifying these variants and their frequencies is summarized below.

Individuals of African Ancestry

The majority of warfarin pharmacogenetic studies in individuals of African ancestry have been performed in AA populations from the US. *CYP2C9**2 and *CYP2C9**3 are not as useful in predicting warfarin dose requirements in AAs, partly because of their lower prevalence compared to individuals of European ancestry.³¹ Important warfarin pharmacogenetic variants in AAs include *CYP2C9**5, *6, *8, *11, and 18786T, rs12777823 in the *CYP2C* gene cluster, *VKORC1* –1639G>A, *GGCX*(CAA) 16/17, and *CALU*rs339097. (Table 1) Most of these variants have increased MAFs in AAs compared to individuals with European ancestry, with the exception of *VKORC1* –1639G>A. For example, the MAF of the *CALU*rs339097 is less than 1% in Europeans and 9% in AAs, and corresponds to an 11–15% higher warfarin dose in AAs.³² *CYP4F2**3 and *NQO1**2 have low MAFs compared to populations of European ancestry and do not account for significant warfarin dose variability in AAs.²⁵ The presence of the *GGCX*(CAA) 16/17 repeat polymorphism, which accounts for 2% of warfarin dose variability, is ten times more common in AAs than in populations of European ancestry.³³

In a targeted resequencing study of AAs, new variants affecting warfarin dose were found in both *VKORC1* and *CYP2C9*.³⁴ The presence of *VKORC1*-8191 and *CYP2C9* 18786T were significantly associated with higher warfarin doses and significantly improved warfarin dosing algorithm R^2 . A more recent genome-wide association study (GWAS) of warfarin dose variability in AAs also observed a significant association with rs12777823 G>A located near *CYP2C18*, a variant in a gene previously unknown to affect warfarin dose requirements.³⁵ Individuals heterozygous for the A allele required 6.92 mg less of warfarin per week, and homozygous individuals required 9.34 mg less of warfarin per week than those with the GG genotype. The rs12777823 SNP is also common in Europeans, but is not associated with warfarin dose in this population, suggesting that it is not functional, but rather inherited with a yet unidentified functional variant. The *CYP2C9**5, *6, *8, and *11 alleles occur almost exclusively in populations of African ancestry.³²

Latino Populations

The rich genetic diversity of the Latino population makes generalizations regarding the presence and frequency of variants difficult. The majority of published studies have been performed in either Mexican or Caribbean Latinos. In contrast to AAs, MAFs of variants affecting warfarin dose requirements in Latinos are similar to MAFs in populations with European ancestry.^{25, 32} (Table 1) The *CYP29**2, *CYP2C9**3, and *VKORC1*-1639A variants were shown to account for 56% of the variability observed in warfarin dose requirements in a population of primarily Mexican descent.³⁶ The variant *CYP4F2**3 has also been associated with an increase in warfarin dose requirements in both Mexican and Caribbean Latino populations.^{25, 37} In contrast to AAs and populations of European ancestry, *NQO1**2 has an increased MAF in Latinos and has been associated with an increase in warfarin dose requirements. The addition of *CYP4F2**3 and *NQO1**2 explained about 68% of warfarin dose variability in Latinos compared to 58% with algorithms that do not include these variants.²⁵

Individuals of Asian Ancestry

Aside from populations of European ancestry, Asian populations, particularly from China, are the best studied with respect to warfarin pharmacogenomics. However, these studies tend to focus on developing new algorithms based on clinical data and known European variants rather than discovery of new variants or clinical utility of genotype-guided algorithms, with some exceptions.^{12, 38, 39} In addition, few of these studies have been performed in Asian populations within the US. The variants identified that are known to significantly influence warfarin dose requirements include *CYP2C9**3, *VKORC1*-1639A, and *CYP4F2**3. Similar to populations of European ancestry, the total variability in warfarin dose explained by *CYP2C9**3, *VKORC1*-1639A, and *CYP4F2**3 is estimated at 40–63% in Asian populations.³⁹ In a Chinese subgroup, total warfarin dose variability explained by *CYP2C9**3 was comparable to populations of European ancestry at 11.2%. However, the effect of *CYP2C9**2 on warfarin dose is essentially non-existent in Asian patients at a population level as the MAF is negligible.(Table 1)

VKORC1-1639A is significantly associated with reduced warfarin dose requirements as AA, GA, and GG genotypes correspond to daily differences in warfarin dose of 3, 5, and 6 mg,

respectively.³² The frequency of *VKORC1*-1639 A allele in Asian populations is substantial (90%) compared to other race/ethnic groups, which partially explains the reduced warfarin dose requirements in Asian patients.¹² Variability in warfarin dose due to *VKORC1*-1639A is estimated at 29% in populations of Asian ancestry. The frequency of *CYP4F2**3 and the contribution of *CYP4F2**3 to warfarin dose variability in Asian populations is also comparable to populations of European ancestry.³⁹ Recently, variants in the cytochrome p450 oxidoreductase gene (*POR*) have also been observed to affect warfarin dose requirements in Chinese patients, similar to a study in individuals of European ancestry.⁴⁰

American Indian/Alaska Native (AI/AN)

American Indian/Alaska Native (AI/AN) populations are among the most underrepresented race/ethnic groups in warfarin pharmacogenetic studies. One study observed a correlation between AI ancestry and low warfarin dose requirements and a higher proportion of AI patients with low-dose requirements (<3 mg/day) compared to the rest of the population (i.e., 33% vs. 19%, $p < 0.01$).³⁷ However, this study was performed in Caribbean Latinos rather than an AI/AN population. Another study identified *CYP2C9**2, *3, *VKORC1*-1639A, and *CYP4F2**3 as contributors to warfarin dose variability in AI/AN patients.¹³ The AI/AN population displayed a high frequency of alleles conferring increased warfarin sensitivity, suggesting a reduction in warfarin dose requirements as a population. Two novel non-synonymous variants (*MIL* and *N218I*) were also identified in the *CYP2C9* gene.¹³ Although confirmation of the effects of these non-synonymous mutations is necessary, the presence of novel mutations suggests that additional research aimed at discovery of novel polymorphisms associated with warfarin dose variability are necessary.

GENOTYPE-GUIDED WARFARIN DOSING ALGORITHMS IN DIVERSE POPULATIONS

Populations with primarily European ancestry are by far the most well-studied race/ethnic group in terms of warfarin pharmacogenomic testing. Of twelve prospective randomized trials investigating genotype-guided warfarin dosing, individuals of European ancestry comprised 79.9% of all study populations combined, whereas Latinos comprised 2%, AAs 9.5%, and individuals of Asian ancestry 6.9%. (Table S1 in Supplementary Materials) The lack of exhaustive studies in non-European populations raises a fundamental question: Can algorithms developed in European populations be generalized to more diverse populations? Algorithms that were developed in populations of European ancestry may miss important variants in other race/ethnic groups. In addition, variants may have differing effects depending on the race/ethnic group, resulting in use of incorrect effect sizes and incorrect dose estimates.⁴¹ In the following section, we summarize literature evaluating the performance of European-derived warfarin pharmacogenetic algorithms and the development of race-specific algorithms in diverse populations.

Individuals of African Ancestry

Several studies have tested the performance of genotype-guided warfarin dosing algorithms in AAs. (Table S3 in Supplementary Materials) Studies that genotype polymorphisms from populations of European ancestry observe a relatively low amount of variance explained in

AA patients compared to individuals of European ancestry.⁴² This observation is consistent with the results of the COAG trial, which employed a European-derived algorithm and observed decreased time in therapeutic range as well as an increase in adverse outcomes in AAs.¹⁵ (Table S2 in Supplementary Materials) Subsequent studies developed AA-specific algorithms that incorporate variants with warfarin dose effects in AAs, such as *CYP2C9*5*, **6*, **8*, **11* and rs12777823. Such studies observe improvements in performance ($R^2=0.38$) versus traditional genotype-guided algorithms such as the IWPC ($R^2=0.26$).⁴³ (Table 2) In a retrospective analysis using electronic health records, clinical information alone explained 24% of variation in stable dose in AAs, the IWPC algorithm improved R^2 to 30%, and an expanded genetic algorithm, including *CYP2C9*6*, *CYP2C9*8*, *CYP4F2*3*, and *CALU* rs339097, improved the R^2 to 41%.⁴⁴ Similar results were observed by other studies, in which AA-specific algorithms explain more of the phenotypic variation ($R^2=0.27$) than IWPC ($R^2=0.15$) or clinical ($R^2=0.16$) algorithms.^{41, 45} Interestingly, a significant inverse correlation between predicted dose and percent West African ancestry was observed for the IWPC pharmacogenomics algorithm among patients requiring ≥ 60 mg per week ($\beta=-2.04$, $P=0.02$).⁴⁶

Taken together, these results support that incorporation of AA-specific variants facilitate improvements in prediction of warfarin dose variability in individuals of African ancestry. This constitutes strong evidence that algorithms derived from predominately European populations cannot be broadly applied to AAs. In fact, such algorithms may perform worse than clinical algorithms in AAs.^{15, 41} Available data in AAs supports a race-stratified approach if genotype-guided warfarin dosing algorithms are to be used. Recently, a race-stratified approach to genotype-guided dosing in AAs was recommended in updated warfarin CPIC guidelines.¹¹ However, despite the availability of new AA-derived algorithms, predicted variability in warfarin dose remains low relative to populations of European ancestry. This suggests that genotype-guided dosing will not have as much clinical utility in AAs as in individuals of European ancestry in the absence of identifying and incorporating additional variants influencing dose in AAs. In any case, the data highlight the limited amount of prospective randomized trials evaluating variants from AAs in genotype-guided warfarin dosing algorithms.

Latino Populations

The majority of the evidence evaluating genotype-guided warfarin dosing algorithms in Latinos has been performed in either Brazilian or Puerto Rican populations. Few studies have been performed in individuals with Mexican ancestry. Available evidence in Brazilian populations suggests that European-derived dosing algorithms including *CYP2C9*2*, *CYP2C9*3*, and *VKORC1* -1639G>A perform reasonably well ($R^2=40\text{--}51\%$).^{47, 48} (Table S4 in Supplementary Materials) A genotype-guided algorithm developed in Brazilian patients with a high percentage of European ancestry had a higher explanation of warfarin dose variability than the IWPC algorithm ($R^2=0.61$ vs $R^2=0.51$). (Table 2) In Puerto Rican populations, the amount of warfarin dose variability predicted using European-derived dosing algorithms is substantially higher ($R^2=67\text{--}77\%$).⁴⁹ (Table S4 in Supplementary Materials) A tailored algorithm for Puerto Rican patients also performed better than the IWPC algorithm ($R^2=67\%$ vs $R^2=36\%$).⁴⁹ (Table 2) Overall, these novel algorithms

developed for sub-populations of Latino populations predict warfarin dose more accurately than the IWPC algorithm. However, these algorithms are being tested in the same populations in which they are developed, which likely increases R^2 values, and these algorithms use different clinical variables than IWPC.

An admixture-based approach to genotype-guided warfarin dosing has also been developed.³⁷ Latino populations are characterized by a large amount of admixture and this approach is uniquely suited to their genetic diversity. In this study, an admixture index was calculated based on STRUCTURE-defined race (1 = European ancestry, 2 = Native American ancestry, 3 = African ancestry, 4 = Admixed individual). This admixture vector explained ~6% of the variance in effective warfarin dose requirements after taking into account the contribution of other covariates. In contrast to a race-specific algorithm, this approach accommodates for the fact that many race/ethnic groups will not fit neatly into an ancestral population. The approach may have broader implications for other race/ethnic groups moving forward and could bolster use of other estimates of admixture to be used, such as percent ancestry and principal components analysis.

Individuals of Asian ancestry

A relatively large number of studies have been published which evaluate the performance of genotype-guided warfarin dosing algorithms in populations of Asian ancestry. (Table S5 in Supplemental Materials). These studies are conducted primarily in individuals from China, Korea, and Japan, with virtually no studies from Asian populations within the US. Such studies have a heavy focus on variants associated with warfarin response from European populations, such as *CYP2C*3* (*CYP2C*2* has a negligible frequency in Asians populations) and *VKORC1* -1639A. Performance of genotype-guided dosing algorithms from these studies is comparable to the performance of the same algorithms in individuals of European ancestry, with R^2 ranging from 33% to 70%. (Table S5 in Supplemental Materials)

Multiple studies have evaluated the performance of algorithms developed in individuals of Asian ancestry versus traditional pharmacogenetic algorithms. (Table 2) Algorithms developed in populations of Asian ancestry perform comparably or slightly better than Gage and/or IWPC algorithms as indicated by R^2 . This result is consistent with population similarity in terms of allele frequencies to individuals of European ancestry. As with the literature relevant to Latinos, these algorithms are often tested in the same population in which they are developed, which likely increases algorithm performance, and these algorithms use different clinical variables than traditional dosing algorithms.

Although individuals with Asian ancestry are well-studied compared to other diverse populations, the body of evidence underscores the lack of consideration of variants outside traditional genotype-guided dosing algorithms. Individuals from China, Korea, and Japan are unlikely to capture genomic variability across Asian populations with greater African and European ancestry, which is likely to be present in the US. Therefore, it will be important to consider the diversity of other Asian populations when determining whether and what genotype-algorithms should be used clinically.

SUMMARY OBSERVATIONS AND LIMITATIONS IN THE EVIDENCE

Whereas variants tend to have similar effects on warfarin dose requirements across race/ethnic groups, differences in the frequency of these variants by race/ethnicity may drive differences in the contribution of these variants to warfarin dose variability. For some populations, such as populations of Asian ancestry, variants identified in populations of European ancestry may capture the majority of variance in warfarin dose. However, novel variants affecting warfarin dose have been identified in AAs, Latinos, and AI/AN groups, suggesting that variants identified in populations of European ancestry may not capture sufficient contributions to warfarin dose variability across diverse populations. This suggests that inclusion of these novel variants in genotype-guided algorithms may be important to improve the predictability of warfarin dose in diverse race/ethnic groups.

The majority of studies in diverse race/ethnic groups have sought to replicate associations of known variants rather than identify new variants. Moving forward, research aimed at discovery of novel polymorphisms affecting warfarin dose variability will likely be necessary if genotype-guided dosing will be implemented in these populations. Such studies would need to perform sequencing and/or GWAS rather than single SNP genotyping and thus incur larger costs to discover novel associations. Furthermore, the vast genomic diversity across the US and the globe will likely require repeated efforts for discovery of novel polymorphisms.⁵⁰ Because of smaller sample sizes in studies of diverse race/ethnic groups, variants with small but significant effects on warfarin response may also go undiscovered.

Multiple studies have been developed to improve the accuracy of genotype-guided warfarin dosing algorithms in minority populations. However, these studies typically evaluate clinical and genetic factors derived from populations of European ancestry rather than discovering new variants among diverse race/ethnic groups. While the evidence suggests that this approach may be reasonable in Asian populations and, to a lesser extent, Latino populations, this approach leads to inaccurate dosing estimation in AAs. Although variants have been discovered in AI/AN populations, no algorithms have been developed or tested.⁵⁸ Additional studies are needed to define important variants and refine genotype-guided algorithms for other minority groups. Definitions of race will be particularly important if these algorithms are to be implemented in more diverse populations. Although these studies provide useful examples, the populations studied are unlikely to capture the majority of world-wide genetic variability across race/ethnic groups. Many of these algorithms were developed in small populations and some have not been validated in independent populations. The algorithms are often inconsistent with respect to clinical variables used and how performance is measured (e.g. mean absolute error versus R^2).

FUTURE DIRECTIONS

Despite increasing use of DOACs, warfarin is likely to remain an important oral anticoagulant. Some socioeconomic populations may have access barriers to DOACs and a number of clinical concerns may limit DOAC use, such as FDA-approved indications, concerns about bleeding risk, contraindication in valvular heart disease, and availability/

expense of an antidote in the event of overdose. If warfarin remains heavily used in clinical practice, improving dosing accuracy should remain a priority considering the high contribution of the drug to adverse events.³ Whether improved dosing accuracy is accomplished using clinical algorithms, genotype-guided dosing algorithms or some other method, consideration of race/ethnicity will be critical considering the increased dose variability and adverse events rates in minority populations.^{18–20}

Selection of Genotype-Guided Warfarin Dosing Algorithms in Diverse Populations

Race and ethnicity are key considerations in any genetic study and pharmacogenomics is no exception. Previously, many studies have intentionally excluded diverse race/ethnic groups to eliminate the possibility for population stratification.²⁷ This highlights one potential reason for the limited evidence in non-European race/ethnic groups. A recent analysis on GWAS studies from 2009–2016 showed that European populations continue to account for a significant proportion of new studies (>80%) and other populations, including AAs, Latinos, and Native Americans, have remained relatively stagnant or decreased in proportion.⁵¹ Research in other race/ethnic groups may also be seen as unoriginal or “me too” studies. Other disadvantages in performing these studies is the expense associated with capturing genomic variability in many race/ethnic minorities, who may require the use of specially designed platforms and/or be highly admixed.

Genotype-guided warfarin dosing provides a case study that has broader significance across pharmacogenomics. Moving forward, any implementation of genotype-guided warfarin dosing algorithms is likely to require adequate consideration of race/ethnicity. As the evidence for many drug-gene pairs is primarily within individuals of European ancestry, more studies are needed to identify the effects of existing variants or new variants in diverse race/ethnic groups. It may be reasonable to use European-derived algorithms for some populations, but this approach is unlikely to be valid in other populations. To maximize the utility of pharmacogenomics, it is likely that separate algorithms will need to be developed for many race/ethnic groups. Recent data from population pharmacokinetic modelling support this notion.⁵²

The updated CPIC guidelines for genotype-guided warfarin dosing incorporates substantial changes based on African ancestry.¹¹ For patients with African ancestry, CPIC now recommends a 15–30% warfarin dose reduction if a patient carries *CYP2C9**5, *6, *8, or *11 alleles and recommends against using genotype-guided dosing if these alleles are not captured. If patients are African American, referring to individuals mainly originating from West Africa, the new guidelines recommend a 10–25% dose reduction in rs12777823 A carriers. All patients of non-African ancestry are treated using the European-derived algorithms from previous CPIC guidelines with optional reductions in dose for patients with *CYP2C9**5, *6, *8, or *11 alleles and for *CYP4F2* rs2108622 T carriers. The new CPIC guidelines thereby support the use of different algorithms based on African ancestry, where the evidence is strongest, but treat European, Asian, Hispanic, and AI/AN patients with the same algorithm. Interestingly, the guidelines support the exclusion of classic *CYP2C9* and *VKORC1* variant data in patients with African ancestry if *CYP2C9**5, *6, *8, or *11 alleles have not been genotyped.

Whether a new algorithm needs to be developed for subsets of ancestral populations is not clear. Admixture within an individual patient should be considered, especially in US populations. Admixture mapping may be required for selection of appropriate algorithms or for inclusion of an admixture variable. It is unlikely that an accurate algorithm could be developed for each nationality or individual race/ethnic group. The consideration of admixture will be particularly important for US populations and some progress has been made in developing these models.³⁷ Additional approaches such as machine learning and artificial neural networks have also been investigated.^{53, 54} Although this review focused on US populations, many quality studies have been published evaluating other race/ethnic groups across the globe. These studies can inform research in the US populations and ultimately provide guidance on the best process for accurately predicting warfarin dose.

Clinical Implementation of Genotype-Guided Warfarin Dosing Algorithms

A central consideration for the future direction of warfarin dosing algorithms is whether or not clinical implementation is justified by the present level of evidence. Recommendations are now available for clinical translation of genotypes and indeed the FDA has changed the labelling of warfarin to accommodate genotype data.^{8, 11} However, seminal clinical guidelines for cardiovascular care have yet to embrace warfarin pharmacogenomics, citing a lack of established efficacy in randomized controlled trials and a lack of proven effect on clinical outcomes.²⁸ Indeed, clinical guidelines have readily incorporated pharmacogenomic testing when supported by a randomized trial as with abacavir.⁵⁵ The limited clinical utility of pharmacogenetic testing observed in randomized controlled trials performed in individuals of European ancestry may not be generalizable to other race/ethnic groups. Based on the poor performance of genotype-guided algorithms in AAs, additional randomized trials may be needed in AAs and potentially other race/ethnic groups.

Thus a key issue in implementation is the threshold of evidence at which pharmacogenomics testing should be incorporated in the clinic. Some groups maintain that randomized controlled trials should not be required since a new drug entity is not being approved, simply an improvement in treatment with an existing drug. These groups cite the incorporation of renal dose adjustments in clinical care and a slew of other clinical adjustments that are not supported by a randomized controlled trial level of evidence. A randomized controlled trial for personalized medicine can be seen as a contradiction in terms, in that a randomized treatment structure is applied to personalized care.⁵⁶ Opponents of this view maintain the central requirement of randomized controlled trial level evidence in clinical decision making.

Pharmacoeconomic Considerations

One argument posits that a pharmacoeconomic threshold of evidence should be instituted. That is, since no new drug is being approved, financial justification for genetic testing should be the point at which the testing is incorporated into practice. This approach is indirectly supported by CPIC guidelines, which recommend employing pharmacogenomic data when it is available rather than order genetic testing each time a new warfarin prescription is ordered, essentially avoiding concerns regarding cost effectiveness.⁵⁷ This is a reasonable approach considering that no additional cost is incurred for testing, yet data are

still used to improve dosing recommendations. Although there will be relatively few patients with these data today, the number is increasing with increasing clinical and direct to consumer testing. This approach has the caveats that it introduces additional problems of consistency of reporting and interpretability of results.

Earlier cost-effective analysis studies performed from 2009–2010 suggest warfarin pharmacogenetic testing improved quality-adjusted life-years (QALY) gained but not at a cost-effective level.^{58–61} For a majority of economic studies, the accepted threshold deemed cost-effective is at \$50,000 per QALY.⁶² The calculated cost-effectiveness ratios of the studies ranged from \$50,000 to above \$170,000 per QALY with costs most influenced by the price of genetic testing, the clinical outcome, duration to receive genetic testing results, and the propensity of high risk patients to bleed or clot during warfarin therapy. Cost-effectiveness could be met if genotype-guided dosing methods improved the TTR by 5–9%, reduced the risk of major bleeding by 32%, improved warfarin management for high risk patients, improved genetic testing availability within 24 hours, and the cost of genetic testing fell under \$200.^{59, 60}

More recent pharmacoeconomic studies have suggested a growing improvement in cost-effectiveness. A meta-analysis of major randomized control trial studies comparing pharmacogenetic guided-dosing to standard dosing, showed an improvement of TTR by 6% and a reduction in the risk of major bleeding by 66%.⁶³ The European Pharmacogenetics of Anticoagulant Therapy, an active RCT, uses a bedside test that can provide results to guide therapy within 1.5 hours.⁶⁴ In addition, the cost of genetic testing continues to fall where prices for warfarin genotyping ranged from \$400 to \$550 in 2009 to \$25 to \$200 beginning in 2013.^{60, 65} Three recent simulation trials that incorporated these updated factors showed genetic testing before warfarin initiation therapy can be highly cost-effective and fall below the accepted \$50,000 per QALY gained threshold as compared to standard warfarin dosing.^{62, 66}

As technology moves forward, genotyping becomes less costly and more accurate, one would expect genomic data to be available on the majority of patients as standard of care. This would make genotyping for pharmacogenomics variants as well as ancestry markers unnecessary and potentially facilitate implementation into clinical care. Until genomic sequencing is commonplace within the clinic, pharmacogenomic implementation might be considered premature. Regardless, research into the influence of variants on drug response will inform clinical practice as this genomic data becomes available in the majority of patients.

CONCLUSIONS

The vast majority of published literature on genotype-guided warfarin dosing is derived from populations of European ancestry. In addition, the vast majority of evidence in more diverse populations evaluates genetic variation from populations of European ancestry. However, the frequency and effect of variants may differ based on race/ethnicity and additional studies in diverse populations are needed if pharmacogenetic testing is carried out in these groups. In some race/ethnic groups, development of race-specific or admixture-based algorithms may

facilitate improved safety and efficacy of warfarin above and beyond that seen in individuals of European ancestry. Finally, the evidence with warfarin pharmacogenomics may have a broader significance on pharmacogenomic testing, emphasizing the consideration of race/ethnicity in discovery of gene-drug pairs and development of guidelines. Ensuring broad benefit from genotype-guided warfarin dosing is contingent on accounting for genotypes that are important across populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:

Genes involved in warfarin metabolism and mechanism of action. *CALU* indicates calumenin; *CYP2C18*, cytochrome P450 family 2 subfamily C member 18; *CYP2C9* indicates cytochrome P450 family 2 subfamily C member 9; *CYP4F2*, cytochrome P450 family 4 subfamily F member 2; *GGCX*, gamma-glutamyl carboxylase; *NQO1*, NAD(P)H quinone dehydrogenase 1; *VKORC1*, vitamin K epoxide reductase.

Figure 2: Distribution of race/ethnicity in randomized prospective trials investigating genotype-guided warfarin dosing. Participant numbers are based on trials from Table S1 in Supplemental Materials. AA indicates African American.

Table 1:

Genetic Variants that contribute to warfarin dose requirements and their frequencies in race/ethnic groups

Allele	rs number	Warfarin Dose	Minor Allele Frequency (%) ^{1,2}				
			European	Latino	African	Asian	AI/AN ³
<i>CYP2C9*2</i>	rs1799853	↓	10	7	2	<1	5
<i>CYP2C9*3</i>	rs1057910	↓	7	4	<1	3	3
<i>CYP2C9*5</i>	rs28371686	↓	<1	<1	2	<1	-
<i>CYP2C9*6</i>	rs9332131	↓	<1	<1	1	<1	-
<i>CYP2C9*8</i>	rs7900194	↓	<1	<1	5	<1	<1
<i>CYP2C9*11</i>	rs28371685	↓	<1	<1	2	<1	<1
<i>CYP2C9 18786T</i>	rs7089580	↑	22	12	21	1	-
<i>CYP2C18G>A</i>	rs12777823	↓	15	11	25	31	-
<i>VKORC1</i> -1639G>A ⁴	rs9923231	↓	39	41	5	88	60
<i>VKORC1</i> -8191A>G	rs61162043	↑	61	57	46	12	-
<i>CYP4F2*3</i> (V433M)	rs2108622	↑	29	24	8	21	32
<i>GGCX</i> (CAA) 16,17	rs10654848	↑	<1	-	3	-	-
<i>NQO1*2</i>	rs1800566	↑	21	33	18	42	-
<i>CALUT>C</i>	rs339097	↑	<1	1	14	1	-

AA indicates African American; AI/AN, American Indian/Alaska Native; *CYP*, cytochrome P450; *VKORC1*, vitamin K epoxide reductase; *GGCX*, gamma-glutamyl carboxylase; *NQO1*, NAD(P)H dehydrogenase, quinone 1; *CALU*, calumenin.

¹⁾ Minor allele frequencies were obtained from NCBI dbSNP 1000 Genomes using super population codes for East Asian, European, African, and Admixed American and from Perera *et al.* 2011 and Limdi *et al.* 2008.

²⁾ “-” indicates that frequency is unknown or not available

³⁾ Frequency estimates for American Indian/Alaska Natives were obtained from Southcentral Foundation Health Corporation Cohort (Fohner *et al.* 2015)

⁴⁾ Frequency estimates include tag SNPs for *VKORC1* -1639G>A (1173T/6484T)

Table 2:

Performance of race/ethnicity-specific versus traditional genotype-guided warfarin dosing algorithms in diverse populations

Study algorithm ¹	Population	n ²	Genetic Factors ³	Clinical Factors ³	Genetic + Clinical algorithm (R ²)	Gage algorithm (R ²)	IWPC algorithm (R ²)
Individuals of African Ancestry							
Alzubiedi <i>et al.</i> 2016 ⁴³	African American	163	<i>CYP2C9</i> *2, *3, *5; <i>VKORC1</i> –1639G>A; <i>CYP4F2</i> *3; rs12777823	age, weight, CHF, CM	38%	NR	26%
Hernandez <i>et al.</i> 2014 ⁴⁶	African American	349	<i>CYP2C9</i> *2, *3, *5, *8, *11, 18786T; <i>VKORC1</i> –1639G>A, –8191A; rs12777823	age, weight, VTE	27%	NR	15%
Ramirez <i>et al.</i> 2012 ⁴⁴	African American	145	<i>CYP2C9</i> *2, *3, *6, *8; <i>VKORC1</i> –1639G>A; <i>CALU</i> rs339097; <i>CYP4F2</i> *3	age, BSA, sex, race, smoking, CM, VTE	41%	NR	29%
Latino Populations							
Ramos <i>et al.</i> 2012 ⁴⁹	Puerto Rican	163	<i>CYP2C9</i> *2, *3, *5; <i>VKORC1</i> –1639G>A	age, PE, CM, dose-adjusted INR	67%	NR	36%
Botton <i>et al.</i> 2011 ⁶⁷	Brazilian ⁴	279	<i>CYP2C9</i> *2, *3; <i>VKORC1</i> –1639G>A, rs7294; <i>CYP4F2</i> *3; F2 rs5896	age, weight, CM	63%	42%	46%
Individuals of Asian Ancestry							
Lin <i>et al.</i> 2016 ⁶⁸ (Miao <i>et al.</i> algorithm)	Chinese	208	<i>CYP2C9</i> *3; <i>VKORC1</i> –1639G>A	age, weight	34%	NR	27%
Cho <i>et al.</i> 2016 ⁶⁹	Korean	101	<i>CYP2C9</i> *3; <i>VKORC1</i> –1639G>A	age, weight	51%	32%	37%
Zhao <i>et al.</i> 2014 ⁷⁰ (Zhang <i>et al.</i> algorithm)	Chinese	122	<i>CYP2C9</i> *3; <i>VKORC1</i> –1639G>A	age, weight	67%	53%	31%
Lei <i>et al.</i> 2012 ⁷¹ (Wu <i>et al.</i> algorithm)	Chinese	368	<i>CYP2C9</i> *2, *3; <i>VKORC1</i> –1639G>A	age, sex, weight	55%	58%	52%
Cho <i>et al.</i> 2011 ⁷²	Korean	108	<i>CYP2C9</i> *3; <i>VKORC1</i> –1639G>A	age, BSA, CM	46%	4%	49%

BSA, body surface area; CALU, calumenin; CHF, congestive heart failure; CM, concomitant medication; *CYP*, Cytochrome P450; INR, international normalized ratio; IWPC, International Warfarin Pharmacogenetic Consortium; NR, not reported; PE, pulmonary embolism; *VKORC1*=Vitamin K epoxide reductase complex subunit 1; VTE, indication for venous thromboembolism.

¹) Algorithms studied were developed in the study cited unless otherwise specified. If multiple algorithms were compared in the reference study, the best performing algorithm is included in the table.

²) Indicates derivation cohort minus validation cohort

³) Genetic and clinical factors included in the final novel algorithm. *VKORC1* –1639A polymorphisms may be genotyped using tag SNPs such as *VKORC1* 1173T or 6484T.

⁴) Brazilian patients specified as having European ancestry