Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin dosing: 2017 Update

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Abstract

This document is an update to the 2011 Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for *CYP2C9* and *VKORC1* genotypes and warfarin dosing. Evidence from the published literature is presented for *CYP2C9*, *VKORC1*, *CYP4F2*, and rs12777823 genotype-guided warfarin dosing to achieve a target international normalized ratio of 2-3 when clinical genotype results are available. In addition, this updated guideline incorporates recommendations for adult and pediatric patients that are specific to continental ancestry.

Keywords

warfarin; pharmacogenetics; VKORC1; CYP2C9; CYP4F2; CYP2C; clinical implementation; pharmacogenomics

Introduction

Warfarin is a widely used anticoagulant with a narrow therapeutic index and large inter-patient variability in the dose required to achieve target anticoagulation. Common genetic variants in *CYP2C9, VKORC1, CYP4F2* and the *CYP2C* cluster (e.g., rs12777823), plus known non-genetic factors, account for ~50% of warfarin dose variability. This document is an update to the 2011 Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for *CYP2C9* and *VKORC1* genotypes and warfarin dosing and aims to assist in the interpretation and use of *CYP2C9, VKORC1, CYP4F2,* and rs12777823 genotypes to estimate therapeutic warfarin dose among patients with a target international normalized ratio (INR) of 2-3, should clinical genotype results be available to the clinician. The Clinical Pharmacogenetics Implementation Consortium (CPIC) of the National Institute of Health's Pharmacogenomics Research Network develops peer-reviewed gene/drug guidelines that are published and updated periodically on https://cpicpgx.org/genes-drugs/ and http://www.pharmgkb.org based upon new developments in the field (1). These guidelines were written with a global audience in mind, although the majority of the data that underpin these guidelines arise from people of European ancestry, East Asia and African Americans.

Focused Literature Review

The Supplement includes a systematic literature review on *CYP2C9, VKORC1, CYP4F2* and other relevant genes/genotypes that have been associated with warfarin dosing. This

systematic review forms the basis for the recommendations contained in this guideline. Although some of these genes have also been associated with dose of other coumarin anticoagulants, the recommendations below are specific to warfarin.

Drug: Warfarin

Warfarin (Coumadin® and others) is the most commonly used oral anticoagulant worldwide, with annual prescriptions in the Western world typically equaling 0.5 to 1.5% of the population (2). It is prescribed for treatment and prevention of thromboembolic disorders (3). Although highly efficacious, warfarin dosing is notoriously challenging due to its narrow therapeutic index and wide inter-individual variability in dose requirements even among patients with the same target INR (4). Complications from inappropriate warfarin dosing are among the most frequently reported adverse events to the U.S. Food and Drug Administration (FDA) and one of the most common reasons for emergency room visits (5).

Warfarin is usually dosed empirically: an initial dose is prescribed, typically followed by at least weekly measurement of the INR and subsequent dose adjustment. The initial dose is often based on population averages (e.g., 4-5 mg/day), but in some settings, it is common to use loading doses during the first few days of anticoagulation. Irrespective of the method used to initiate warfarin, stable doses to achieve an INR of 2-3 range from 1 to 20 mg/day. The iterative process to define the appropriate dose can take weeks to months and during this period, patients are at increased risk of over- or under-anticoagulation, and thus risk of bleeding or thromboembolism.

Warfarin pharmacology and pharmacokinetics

Figure 1 highlights key elements of warfarin pharmacology and pharmacokinetics. Warfarin inhibits vitamin K epoxide reductase complex (6) and is administered as a racemic mixture, with S-warfarin being more potent than R-warfarin (3).

Genes: *CYP2C9*, *VKORC1* and *CYP4F2*

There is substantial candidate gene literature evaluating associations with warfarin dose requirements, as well as several reported genome-wide association studies (Supplemental Tables S1-S7). The genes with the strongest literature support, and for which we make recommendations for use in warfarin dosing, are *CYP2C9*, *VKORC1* and *CYP4F2*. Additionally, genome-wide association studies have identified an independently significant single nucleotide polymorphism (SNP) in the *CYP2C* cluster (7), which has also been incorporated into this updated recommendation.

CYP2C9 and warfarin

CYP2C9 is a hepatic drug-metabolizing enzyme in the cytochrome P450 (CYP450) superfamily (8), and is the primary metabolizing enzyme of S-warfarin (Figure 1). *CYP2C9* has over 60 known variant alleles (http://www.cypalleles.ki.se/cyp2c9.htm; **CYP2C9 allele definition table (9)**). Individuals homozygous for the reference *CYP2C9* allele *(CYP2C9*1)* have the "normal metabolizer" phenotype. Each named *CYP2C9* star (*) allele is defined by one or more specific SNPs and to date, and 18 alleles have been associated with decreased

enzyme activity (**CYP2C9 allele definition table-(9)**). The two most common decreased function alleles among individuals of European ancestry are *CYP2C9*2* (c.430C>T; p.Arg144Cys; rs1799853) and *CYP2C9*3* (c.1075A>C; p.Ile359Leu; rs1057910) (8). *CYP2C9* allele frequencies differ between racial/ethnic groups (8, 10).

In vitro and *in vivo* studies suggest *CYP2C9*2* and *3* impair metabolism of S-warfarin by ~30-40% and ~80-90%, respectively (8). Compared to patients homozygous for *CYP2C9*1*, individuals who inherit one or two copies of *CYP2C9*2* or *3* are at greater risk of bleeding during warfarin therapy (11, 12), require lower doses to achieve similar levels of anticoagulation, and require more time to achieve a stable INR (11) (Supplemental Table S1). Additional *CYP2C9* alleles (*CYP2C9*5, *6, *8,* and *11*) are associated with decreased function of the CYP2C9 enzyme and contribute to dose variability. These alleles are found with the highest frequency among those of African ancestry, and collectively are more common than *CYP2C9*2* and *3* in that population (**CYP2C9 frequency table; (9)**).

VKORC1 and warfarin

VKORC1 encodes the vitamin K epoxide reductase protein, the target enzyme of warfarin (6). VKORC1 catalyzes the conversion of vitamin K-epoxide to vitamin K, which is the rate-limiting step in vitamin K recycling (13).

A common variant upstream of *VKORC1* (c.-1639G>A, rs9923231) is significantly associated with warfarin sensitivity and patients with one or two -1639A require progressively lower warfarin doses than -1639G/G homozygotes (10, 14-18). The -1639G>A polymorphism is present on a haplotype that affects VKORC1 protein expression. (18).

Other common *VKORC1* SNPs or haplotypes do not further improve warfarin dose prediction (10, 16). The c.-1639G>A allele frequency varies among different ancestral populations (**VKORC1 frequency table; (19)**), and largely explains the differences in average dose requirements between whites, blacks and Asians (10, 17). Several rare non-synonymous *VKORC1* variants confer warfarin resistance (high dose requirements) and are detailed in Supplemental Table S2 (20).

CYP4F2 and warfarin

CYP4F2 is a primary liver vitamin K oxidase that catalyzes the metabolism of vitamin K to hydroxy-vitamin K1 and removes vitamin K from the vitamin K cycle (21) (Figure 1). It acts as an important counterpart to VKORC1 in limiting excessive accumulation of vitamin K. The non-synonymous variant *CYP4F2*3* (c.1297G>A; p.Val433Met; rs2108622) was first shown to affect enzyme activity and associated with warfarin dose in three independent white cohorts (22-24). Furthermore, including this *CYP4F2* variant in warfarin dosing models that included *CYP2C9, VKORC1* and clinical factors improved the accuracy of dose prediction (25). This correlation has been confirmed in subsequent studies with those of European and Asian ancestry, though not those of African ancestry (26, 27). Two large meta-analyses (one in Han Chinese that pulled in substantial Chinese literature) provide the best estimates for the influence data of *CYP4F2*3* on warfarin dose requirements (26, 27). They

suggest statistically significant but modest impacts of 8-11% higher warfarin doses in A allele carriers (Supplemental Table S3).

CYP2C rs12777823 and warfarin

rs12777823 is a SNP in the *CYP2C* cluster near the *CYP2C18* gene on chromosome 10 and is associated with a clinically relevant effect on warfarin dose through significant alterations in warfarin clearance, independent of *CYP2C9*2* and *3* (7). This association was first identified through a genome-wide association study in African Americans ($p=1.51\times10^{-8}$) and confirmed in a replication cohort ($p=5.04\times10^{-5}$); meta-analysis of the two cohorts together produced a p value of 4.5×10^{-12}. This study concluded that African Americans who are heterozygous or homozygous for the rs12777823 A allele require a dose reduction of ~ 7 or 9 mg/week, respectively (7). Regression analysis showed that addition of this SNP improves the dosing algorithm published by the International Warfarin Pharmacogenetics Consortium (IWPC) by 21%. Further studies have demonstrated the importance of this SNP in African Americans (28). Although this variant is common in other ethnic populations, an association with warfarin dose has only been detected among African Americans suggesting it is not the underlying cause but likely inherited with other variant(s) on a haplotype that influences warfarin dose in this population. Of note, an association was not observed in a cohort of Egyptians, thus it is not possible to make broad statements about this allele in people of continental African ancestry. Most African Americans are of West African ancestry; it is unknown whether similar associations are present in individuals from other parts of Africa.

Genetic Test Interpretation

CYP2C9. Clinical laboratories typically report *CYP2C9* genotype results using the star (*) allele nomenclature system and an interpretation that includes a predicted metabolizer phenotype. Most FDA-approved *CYP2C9* tests include only *2* and *3*, which is not as informative for African ancestry populations; however, some clinical laboratories may offer expanded *CYP2C9* panels validated as laboratory developed tests (LDTs) (for allele frequencies see: **CYP2C9 frequency table (9)**).

VKORC1—Clinical laboratories typically report *VKORC1* genotype results by c.-1639G>A (or the linked 1173C>T; rs9934438) genotype (e.g., G/A) and an interpretation on warfarin sensitivity. Most commercial genotyping platforms do not detect rare *VKORC1* variants that have been associated with warfarin resistance (**VKORC1 frequency table (19)**).

CYP4F2—Although not as commonly tested for as *CYP2C9* and *VKORC1*, some clinical laboratories may also test for *CYP4F2* using a targeted genotyping laboratory developed test to detect *CYP4F2*3* (c.1297G>A, p.Val433Met; rs2108622) variant. Results are typically reported by nucleotide (e.g., G/A), amino acid (e.g., Val/Met) or star (*) allele (*1/*3*) genotype and an interpretation related to warfarin dosing.

CYP2C rs12777823—Given the recent identification of the association between rs12777823 (g.96405502G>A) and warfarin dosing among African Americans, most clinical laboratories do not currently include this non-coding variant in their warfarin

pharmacogenetic genotyping panels. However, the increasing accessibility of clinical research genomics programs that return actionable results and the notable effect of this variant among African Americans suggests that some patients may have genotype results for this variant in the future. Results would likely be reported by genotype (e.g., G/A) and an interpretation related to warfarin dosing.

Genetic test options

Commercially available genetic testing options change over time. Additional information about pharmacogenetic testing can be found at the Genetic Testing Registry (http://www.ncbi.nlm.nih.gov/gtr/).

Incidental findings

No diseases have been linked to common *CYP2C9* variants independent of drug metabolism and response. Similarly, no diseases have been consistently linked to common *VKORC1* and *CYP4F2* variants that are interrogated in warfarin response tests. However, homozygosity for rare coding mutations in *VKORC1* are a known cause of combined deficiency of vitamin K-dependent clotting factors-2 (VKCFD2), which is a rare and potentially fatal bleeding disorder that can be reversed by oral administration of vitamin K (29).

Linking genetic variability to variability in drug-related phenotypes

Common variants in *CYP2C9*, *VKORC1*, and *CYP4F2* account for up to 18%, 30%, and 11% respectively, of the variance in stable warfarin dose among patients of European ancestry (10, 16, 17, 30, 31), but because of differing allele frequencies across populations, these variants explain less of the dose variability in patients of other ancestries. In particular, *CYP2C9*2* is virtually absent in Asians, and additional *CYP2C9* alleles (e.g. *5, *6, *8,* and *11* alleles) occur almost exclusively in persons of African ancestry and contribute to dose variability in this population. Other genes of potential importance are discussed in the Supplemental Material.

Published in 2013, the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) and Clarification of Optimal Anticoagulation through Genetics (COAG) trials examined the efficacy of genotype-guided warfarin dosing in randomized controlled trials (32, 33). In a homogenous European population, the EU-PACT trial showed shorter time to stable dose, improved percent time in therapeutic range, and reduced number of episodes with an INR>4 using a pharmacogenetic dosing algorithm compared to standard dosing (33). The COAG trial was conducted in an ethnically diverse cohort with 27% of participants of African ancestry (32). Overall, COAG did not show a difference in time to stable dose, percent time in therapeutic range, reduction in number of episodes with INR >4 or <2, or bleeding risk with a pharmacogenetic dosing algorithm compared to a clinical algorithm. In non-blacks, the pharmacogenetic dosing algorithm arm had more patients whose stable dose was within 1 mg per day of the algorithm-predicted dose (57 vs 39%, respectively). In contrast, the pharmacogenetic dosing algorithm was less accurate at predicting within 1 mg/day of the stable dose than the clinical algorithm in blacks (38 vs 48% respectively) (32). Blacks were more likely to have an INR above range with pharmacogenetic dosing, which could be due to the genotyping panel in the COAG trial being limited to *CYP2C9*2, *3* and *VKORC1*

c.-1639G>A. Other variants that influence warfarin dose and are more common in blacks (i.e., *CYP2C9*5*, **6*, **8*, and **11* and rs12777823) were not genotyped in the COAG trial and their absence likely led to significant overdosing in patients with these alleles (10, 34). Consequently, this updated CPIC guideline recommends against pharmacogenetic dosing of warfarin in blacks when only *CYP2C9*2* and **3* genotype results are available.

The Genetics-In Formatics Trial (GIFT) was a randomized controlled trial examining the effectiveness and safety of genotype-guided dosing versus clinical algorithm dosing in orthopedic patients with a composite outcome that included symptomatic and asymptomatic venous thromboembolism, major hemorrhage, INR 4, and death (35). It is the first warfarin pharmacogenetics trial powered for clinical outcomes. GIFT included genotyping for *CYP2C9*2* and **3*, *CYP4F2*3*, and *VKORC1-1639*, but did not include the African-specific *CYP2C9* alleles or rs12777823. The results of GIFT were presented in early 2017 and revealed a 27% reduction in the composite outcome with genotype-guided versus clinical algorithm dosing, documenting the clinical benefits of a genotype guided approach to warfarin dosing.(https://www.sciencedaily.com/releases/2017/03/170320091104.htm)

Therapeutic Recommendations: Adults

Recommendations for warfarin maintenance (chronic) dosage based on genetic information—We use the three-tiered rating system described previously (and in Supplemental Material) (1) in which ratings of strong, moderate, and optional are applied based on the evidence reviewed. The recommendations for dosing based on genotype contained herein include recommendations and are derived from numerous observational and prospective studies, and randomized trials that suggest the ability to more accurately identify stable therapeutic warfarin dose requirements through use of both genetic and clinical information. Data from prospective studies and randomized controlled trials are equivocal on whether the improvement in dosing prediction by pharmacogenetics dosing leads to improved clinical outcomes. The majority of the literature underpinning these guidelines arises from individuals of European ancestry, African Americans, and East Asians. However, the more limited literature in other populations generally suggests the guidelines are appropriate in them also.

Numerous studies have derived warfarin dosing algorithms that use both genetic and non-genetic factors to predict warfarin dose (16, 17, 36, 37). Two algorithms perform well in estimating stable warfarin dose (16, 17) and were created using more than 5000 subjects, though as noted above, more recent data suggest they do not perform acceptably in African Americans when used without modification for *CYP2C9* alleles frequently found in the African population (32). The Gage and IWPC algorithms or minor adjustments to them have also been the algorithms used in both randomized controlled trials and most of the prospective dosing studies. Dosing algorithms using genetic information outperform non-genetic clinical algorithms and fixed-dose approaches in dose prediction, except in African Americans when the algorithm only includes *CYP2C9*2* and **3* (16, 17, 32). Genetics-based algorithms also better predict warfarin dose than the FDA-approved warfarin label table (38).

Pharmacogenetic algorithm-based warfarin dosing—This guideline recommends that pharmacogenetic warfarin dosing be accomplished through the use of one of the pharmacogenetic dosing algorithms described above, as summarized in Figure 2. These algorithms, as originally published, are available in the Supplement and the dosing algorithm published by IWPC is also online at http://www.pharmgkb.org/do/serve? objId=PA162372936&objCls=Dataset#tabview=tab2. The two algorithms provide very similar dose recommendations. The clinical and genetic information used in one or both algorithms is shown in box 1. These algorithms compute the anticipated stable daily warfarin dose to one decimal and the clinician must then prescribe a regimen (e.g., an estimate of 4.3 mg/day might be given as 4 mg daily except 5 mg two days per week). An additional "dose revision" algorithm, that can be used on days 4-5 of therapy for dose refinement and uses genetic information, was tested in COAG and EU-PACT and can also be used (36) (Supplemental Table S5).

It is important to note that these algorithms do not include *CYP4F2, CYP2C9*5*, *6*, *8*, or *11* or rs12777823, and incorporation of these should be added when results are available, as described in Figure 2. The warfarindosing.org website contains both algorithms, the Gage algorithm (16) as the primary algorithm and the IWPC algorithm (17) as the secondary algorithm and can adjust for *CYP4F2, CYP2C9*5* and *6*. If utilizing warfarindosing.org, the user should be clear on whether the algorithm is or is not incorporating genotypes beyond *CYP2C9 *2* and *3* and *VKORC1,* which are the only three genotypes in the original version of both algorithms.

Pharmacogenetics-informed loading (or initiation) dose calculations—The use of a different initial warfarin dose (or "loading dose") is somewhat controversial and plays different roles in different regions of the world, based on experience and local standards. Recent data from a diverse U.S. based cohort suggest that failure to provide a loading dose in patients with zero or one variant alleles in *VKORC1* or *CYP2C9* may delay time to therapeutic INR and reduce time in therapeutic range in the initial month of therapy (39). A genetically-guided loading dose approach was developed by Avery *et al.* (37) and a slightly modified version was successfully implemented in the EU-PACT trial (33). In COAG *CYP2C9* variant alleles were not considered for the initial dose, providing a small loading dose on day 1. Whether differences in loading dose strategies between the EU-PACT and COAG trials contributed to differing results is not known. If loading doses are to be used, a genetically-informed approach to calculating the loading dose may be helpful. The majority of the experience with a genetically-informed loading regimen is in those of European ancestry. Determination of maintenance dose would be as described above.

Non-African ancestry recommendation—In patients who self-identify as non-African ancestry, the recommendation, as summarized in Figure 2, is to: 1) calculate warfarin dosing using a published pharmacogenetic algorithm (16, 17), including genotype information for *VKORC1-1639G>A* and *CYP2C9*2* and *3.* In individuals with genotypes associated with CYP2C9 poor metabolism (e.g., *CYP2C9 *2/*3, *3/*3*) or both increased sensitivity (*VKORC1-1639* A/A) and CYP2C9 poor metabolism, an alternative oral anticoagulant might be considered. The bulk of the literature informing these recommendations is in

European and Asian ancestry populations, but consistent data exist for other non-African populations. These recommendations are graded as *STRONG.* 2) If a loading dose is to be utilized, the EU-PACT loading dose algorithm that incorporates genetic information could be used (33). This recommendation is *OPTIONAL.* 3) While *CYP2C9*5, *6, *8,* or *11* variant alleles are commonly referred to as African specific alleles, they can occur among individuals who do not identify as, or know of their, African ancestry. If these variant alleles are detected, decrease calculated dose by 15-30% per variant allele or consider an alternative agent. Larger dose reductions might be needed in patients homozygous for variant alleles (i.e. 20-40%, e.g. *CYP2C9*2/*5*). This recommendation is graded as *OPTIONAL.* 4) If the *CYP4F2*3* (i.e., c.1297A, p.433Met) allele is also detected, increase the dose by 5-10%. This recommendation is also considered *OPTIONAL.* 5) The data do not suggest an association between rs12777823 genotype and warfarin dose in non-African Americans, thus rs12777823 should not be considered in these individuals (even if available).

African ancestry recommendation—In patients of African ancestry, *CYP2C9*5, *6, *8, *11* are important for warfarin dosing. If these genotypes are **not** available, warfarin should be dosed clinically without consideration for genotype. If *CYP2C9*5, *6, *8,* and *11* are known, then the recommendation, as shown in Figure 2, is to: 1) calculate warfarin dose using a validated pharmacogenetic algorithm, including genotype information for *VKORC1* c.-1639G>A and *CYP2C9*2 and *3 (16, 17); 2) if the individual carriers a *CYP2C9*5, *6, *8,* or *11* variant allele(s), decrease calculated dose by 15-30%. Larger dose reductions might be needed in patients who carry two variant alleles (e.g., *CYP2C9*5/*6*) (i.e. 20-40% dose reduction). 3) In addition, rs12777823 is associated with warfarin dosing in African Americans (mainly originating from West Africa). Thus, in African Americans a dose reductions of 10-25% in those with rs12777823 A/G or A/A genotype is recommended. These recommendations are considered *MODERATE.*

In individuals with genotypes that predict CYP2C9 poor metabolism or who have increased warfarin sensitivity (*VKORC1* c.-1639 A/A) and CYP2C9 poor metabolism, an alternative oral anticoagulant should be considered (see Supplemental material for definitions of strength of recommendations). As noted above, for non-African ancestry, if a loading dose is to be used, the EU-PACT algorithm (33) that incorporates genetic information could be used to calculate loading dose. This recommendation is *OPTIONAL.* The data do not support an impact on clinical phenotype for *CYP4F2* on warfarin dosing in those of African ancestry and so no recommendation is made for use of *CYP4F2* genotype data in blacks.

Recommendations for Pediatric Patients—As detailed in Supplemental Table S7, there is strong evidence for the use of *CYP2C9*2 and *3 and VKORC1-1639G>A* genotype to guide warfarin dosing in children of European ancestry. The studies in Japanese pediatric individuals are conflicting as *VKORC1* and *CYP2C9* could not be adequately evaluated due to the low numbers of *CYP2C9* variant carriers. For other ethnicities, there is no evidence documenting that *VKORC1* and *CYP2C9* are important. Furthermore, there are no data in children that included *CYP2C9*5, *6, *8,* or *11* genotyping. Based on the current evidence, in children of European ancestry and if *CYP2C9*2 and *3 and VKORC1-1639* genotype are available, calculate warfarin dosing based on a validated published pediatric

pharmacogenetic algorithm (Figure 3) (40, 41). A dosing tool that can be used in children of European ancestry is available at http://www.warfarindoserevision.com (42).

Other considerations

Given the effects of CYP2C9 on warfarin clearance, and given that the *CYP2C9* variant alleles are associated with reduced warfarin clearance, *CYP2C9* genotype may influence time to onset and offset of anticoagulation, as measured by INR (43). The Supplemental Material summarizes other considerations in the dosing of warfarin, including clinical factors and interacting drugs, some of which are included in the pharmacogenetic dosing algorithms (see Text Box). Other genes of potential importance are detailed in the Supplemental Material and Supplemental table S6, including *CALU* and *GGCX*. Most clinical genotyping platforms do not include these genes, nor do the dosing tables or published algorithms. The Supplemental Material also discusses incorporation of genetic information into the initial dose, and alternatives to warfarin.

Potential Benefits and Risks for the Patient

Incorporation of genetic information has the potential to shorten the time to stable INR, increase the time within the therapeutic INR range, and reduce under-dosing or over-dosing during the initial treatment period (33). If these benefits are achieved, they could result in a reduced risk of bleeding and thromboembolic events (12, 44). There are also potential risks. For example, using genetic information to guide dosing may lead to false security and inadequate INR monitoring. In particular, there are risks of using pharmacogenetic dosing in those of African ancestry if only *CYP2C9 *2* and *3* alleles are included. Genetic-guided dosing may increase the risk for over-dosing or under-dosing, especially in individuals who carry rare or untested variants and are assigned as "wild-type" by default (17, 32). The cost-benefit of genetic-guided therapy depends on the cost of genotyping and the reduction in adverse events (45), and most insurance plans do not currently pay for warfarin pharmacogenetic testing. Although there is substantial evidence associating *CYP2C9* and *VKORC1* variants with warfarin dosing, randomized clinical trials have demonstrated inconsistent results in terms of clinical outcomes (see *Linking genetic variability to variability in drug-related phenotypes*). Although genotyping is reliable when performed in qualified laboratories, an additional risk is an error in genotyping or reporting of genotype. Genotypes are life-long test results, so such error could have long-term adverse health implications.

Caveats: Appropriate Use and/or Potential Misuse of Genetic Tests

Many pharmacogenetic dosing algorithms are developed for a target INR of 2-3 (17) and so their utility for estimating therapeutic warfarin doses with other target INR ranges is uncertain; however, some algorithms accommodate the target INR explicitly (16, 42). Pharmacogenetic-guided warfarin dosing does not alter the requirements for regular INR monitoring. There are patients for whom genetic testing is likely to be of little or no benefit, including those who already have had long-term treatment with stable warfarin doses and those who are unable to achieve stable dosing due to variable adherence. The greatest potential benefit is early in the course of therapy (before therapy initiation or in the early

days of therapy) (36). It is likely that patients on therapy for many weeks to months, with careful INR monitoring, will derive little benefit from subsequent warfarin pharmacogenetics testing (46).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Text box

Patient characteristics utilized in the Gage (16), or IWPC (17) algorithms or both

- Age

- Sex

- Race

- Weight

- Height

- Smoking status

- Warfarin indication

- Target INR

- Interacting drugs

  - Inhibitors: Amiodarone, statins, sulfamethoxazole, azole antifungals

  - Inducers: Rifampin, phenytoin, carbamazepine

- Genetic variables

  - *CYP2C9* genotype

  - *VKORC1* genotype

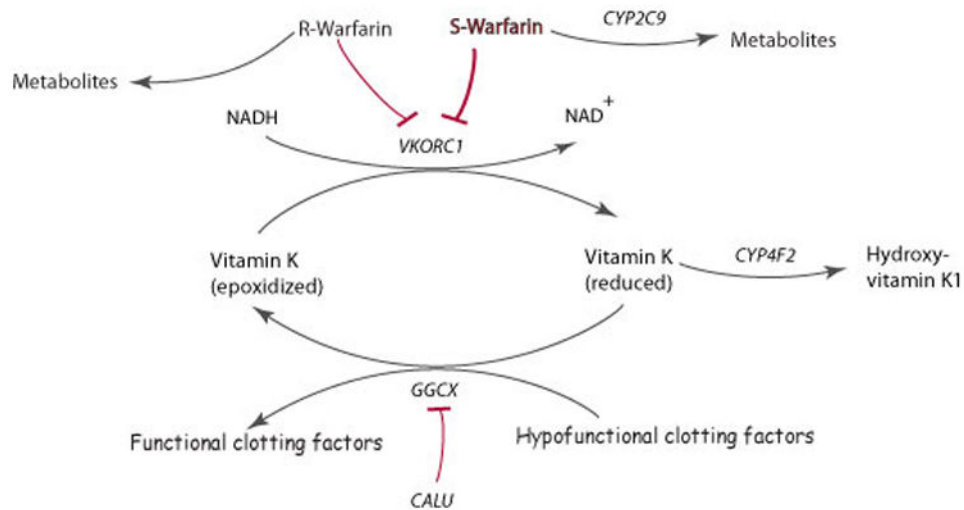 - Gage algorithm can also incorporate *CYP4F2* and *GGCX* genotypes

Figure 1.
Schematic representation of warfarin metabolism and its mechanism of action.
Warfarin is administered via a racemic mixture of the *R*- and *S*- stereoisomers. S-warfarin is
3-5 times more potent than R-warfarin and is metabolized predominantly to 7- and 6-
hydroxyl metabolites via CYP2C9. Warfarin exerts its anticoagulant effect through
inhibition of its molecular target VKORC1, which in turn limits availability of reduced
vitamin K, leading to decreased formation of functionally active clotting factors. These
clotting factors are glycoproteins that are post-translationally carboxylated by gamma-
glutamyl carboxylase (GGCX) to Gla-containing proteins. The endoplasmic reticulum
chaperone protein calumenin (CALU) can bind to and inhibit GGCX activity. The
metabolism of reduced vitamin K to hydroxyvitamin K1 is catalyzed by CYP4F2 which
removes vitamin K from the vitamin K cycle (adapted from warfarin pharmacokinetics (PK)
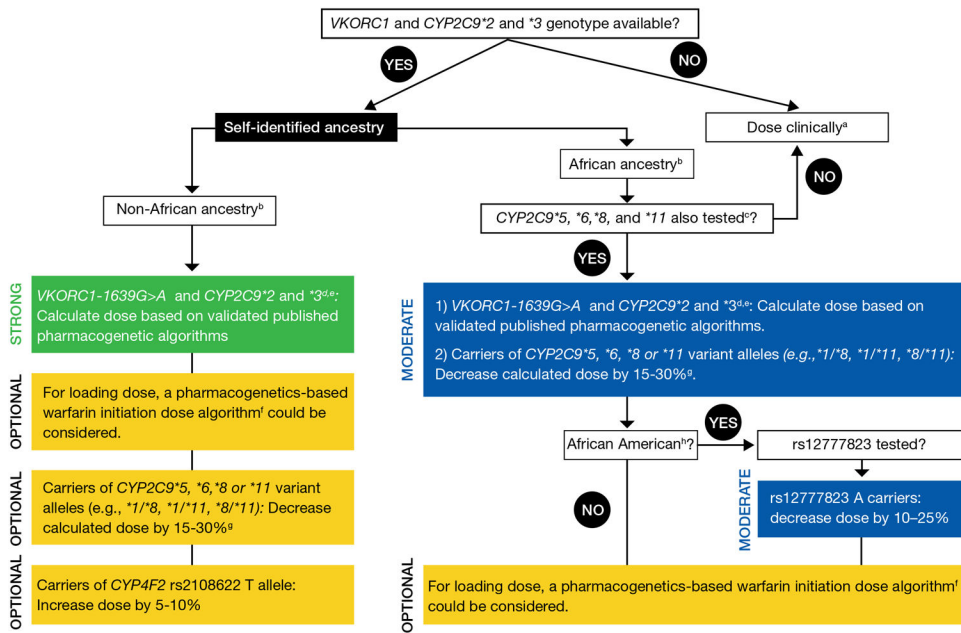and pharmacodynamics (PD) pathways at PharmGKB, http://www.pharmgkb.org/do/serve?
objId=PA451906&objCls=Drug#tabview=tab4).

Figure 2. Dosing recommendations for Warfarin dosing based on genotype for adult patients

[a]"Dose clinically" means to dose without genetic information, which may include use of a clinical dosing algorithm or standard dose approach

[b]Data strongest for European and East Asian ancestry populations and consistent in other populations.

[c]45-50% of individuals with self-reported African ancestry carry *CYP2C9*5,*6,*8,*11,* or rs12777823. IF *CYP2C9*5, *6, *8,* and *11* WERE NOT TESTED, DOSE WARFARIN CLINICALLY. Note: these data derive primarily from African Americans, who are largely from West Africa. It is unknown if the same associations are present for those from other parts of Africa.

[d]Most algorithms are developed for the target INR 2-3.

[e]Consider an alternative agent in individuals with genotypes associated with CYP2C9 poor metabolism (e.g., *CYP2C9*3/*3, *2/*3, *3/*3*) or both increased sensitivity (*VKORC1* A/G or A/A) and CYP2C9 poor metabolism.

[f]See the EU-PACT trial for pharmacogenetics-based warfarin initiation (loading) dose algorithm (33) with the caveat that the loading dose PG algorithm has not been specifically tested or validated in populations of African ancestry.

[g]Larger dose reduction might be needed in variant homozygotes (*i.e.* 20-40%).

[h]African American refers to individuals mainly originating from West Africa.
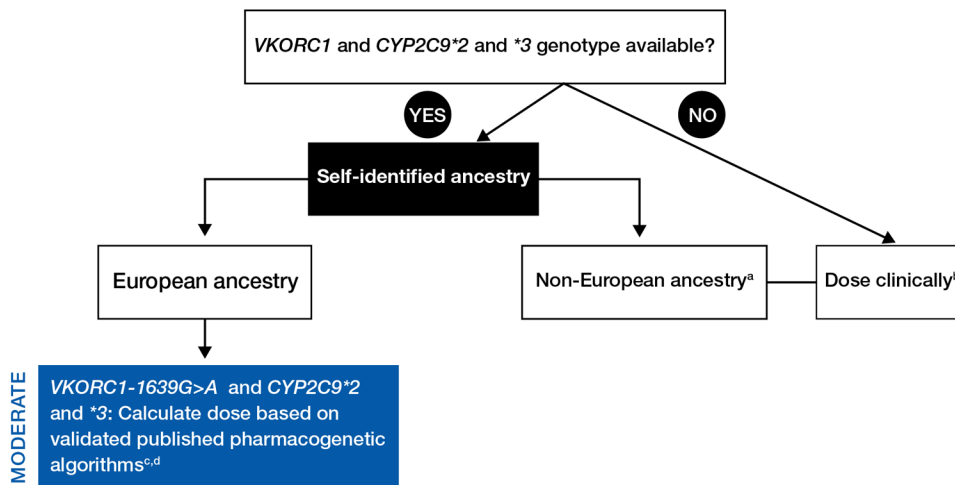
Figure 3. Dosing recommendations for Warfarin dosing based on genotype for pediatric patients

[a]Data strongest for European ancestry populations and consistent in most Japanese studies.

[b]"Dose clinically" means to dose without genetic information, which may include use of a clinical dosing algorithm or standard dose approach

[c]Validated published pediatric pharmacogenetic algorithms include Hamberg *et al.*(42) and Biss *et al.*(41)

[d]No studies in children included *CYP2C9*5, *6, *8,* or *11* genotyping.