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# *VKORC1-1639A* allele influences warfarin maintenance dosage among Blacks receiving warfarin anticoagulation: a retrospective cohort study

**Fatima Donia Mili**[*,1], **Tenecia Allen**[2], **Paula Weinstein Wadell**[1], **W Craig Hooper**[1], **Christine De Staercke**[1], **Christopher J Bean**[1], **Cathy Lally**[3], **Harland Austin**[3,†], **Nanette K Wenger**[2]

[1]Hemostasis Laboratory Branch, Division of Blood Disorders, Centers for Disease Control & Prevention, Atlanta, GA 30329, USA

[2]Emory Heart & Vascular Center, Emory University School of Medicine, Atlanta, GA 30322, USA

[3]Department of Epidemiology, Emory University Rollins School of Public Health, Atlanta, GA 30322, USA

## Abstract

**Aim:** The study objectives were to investigate the association between selected *CYP2C9* and *VKORC1* single nucleotide polymorphisms with serious bleeding or thrombotic risk, and to estimate mean daily maintenance dose of warfarin and international normalized ratio measurements among Blacks receiving warfarin anticoagulation.

**Methods:** We conducted a retrospective cohort study among 230 Black adults receiving warfarin for a minimum of three consecutive months with a confirmed date of first dosage.

**Results:** A lower mean daily maintenance dosage of warfarin was required to maintain an international normalized ratio measurement within the therapeutic range among Blacks with the *VKORC1*-1639G>A variant alleles ([G/A vs G/G, p = 0.02], [A/A vs G/A, p = 0.008] and [A/A vs G/G, p = 0.001]).

**Conclusion:** Data indicated that *VKORC1*-1639A variant allele influenced warfarin daily maintenance dosage among our small, likely admixed Black patient population.

[*]Author for correspondence: Tel.: +1 404 718 5743; fmili@cdc.gov.
[†]Deceased in 2013.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fca-2017-0025.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

**Keywords**

anticoagulation; Blacks; *CYP2C9*; pharmacogenetics thromboembolism; *VKORC1*; *VKORC1–1639G>A* warfarin

## Background

Since its approval for medical use in humans in 1954, warfarin – a vitamin K antagonist (VKA) – has been one of the most commonly prescribed drugs in history [1]. It is the most widely used oral anticoagulant worldwide to prevent and treat arterial and venous thromboembolism disorders. Main advantages of warfarin [2] are oral administration, high efficacy, low cost, monitoring of prothrombin time by international normalized ratio (INR) measurement and prevention of thromboembolism in valvular atrial fibrillation [3]. Warfarin, however, has considerable disadvantages, including a narrow therapeutic index, large inter-patient variability in dose–response relationship which requires frequent monitoring of prothrombin time by INR measurement, frequent dosage adjustments to avoid over-anticoagulation complicated by severe or life-threatening bleeding or under-anticoagulation complicated by thromboembolic events, and interactions with food and over-the-counter vitamins and herbs containing vitamin K – the natural antidote to warfarin [2,4–5]. Because warfarin anticoagulation is associated with a high rate and severity of complications, it has been listed among the 10 drugs with the largest number of adverse events requiring treatment in hospital emergency departments [6].

It is well established that the pharmacokinetics of warfarin are influenced by the cytochrome P450–2C9 complex (*CYP2C9*) [7–12]. Results from early studies revealed that single nucleotide polymorphisms (SNPs) in *CYP2C9* are associated with low warfarin dosage requirements *in vivo* [7] and in clinical studies [8]. Patients who were homozygous for *CYP2C9*1* allele metabolized warfarin normally, whereas patients with the *CYP2C9*2* and *CYP2C9*3* polymorphisms had a reduced enzymatic activity and, consequently, a reduced S-warfarin metabolism and inactive metabolites, over-anticoagulation and an increased risk of bleeding complications [8]. In a retrospective cohort study conducted among Whites [9], patients with *CYP2C9*2* and *CYP2C9*3* alleles experienced bleeding episodes more commonly, and required a lower maintenance dosage and a longer time to achieve stable dosing compared with homozygous patients for *CYP2C9*1*.

Subsequent to the identification of *VKORC1* [13,14], the gene coding for the enzyme targeted by coumarin derivatives, several polymorphisms in this gene – such as 1639G>A, 1173C>T, 1542G>C, 3730G>A, 6853G>C and 7566C>T – were shown to influence the pharmacodynamic effects of warfarin on INR and, consequently, to be associated with inter-individual variation in warfarin dosage requirements among White–American, African–American and Asian–American patients [15–17]. High dose (group A) and low-dose (group B) haplotypes (haplotype tag SNPs) – haplotypes H1, and H2 (group A) versus H7, H8 and H9 (group B) – have also been identified among patients receiving long-term warfarin maintenance therapy by screening 10 common noncoding *VKORC1* SNPs [17]. Findings from several investigations indicated that *VKORC1*-1639G>A is an important determinant

for the variability in warfarin dosage requirements among African Americans [11,18–20], as well as among Whites, Blacks (predominantly African–Americans) and Asians from 11 countries and four continents participating in the International Warfarin Pharmacogenetics Consortium (IWPC) [21]. In the IWPC study, *VKORC1*-1639G>A was associated with reduced warfarin dose requirements across the three ethnic groups studied. The inter-individual dose variation explained by *VKORC1*-1639G>A, however, was much lower in Blacks than in Whites, and was largely accounted for the lower allele frequency in Blacks compared with Whites.

One goal of warfarin dosing is to understand the role that pharmacogenetics plays in individualized therapy, particularly in dosage requirements and risk of complications [22–24]. Although numerous studies have been published describing the association between complications of warfarin therapy and *CYP2C9* or *VKORC1* polymorphisms among Whites, only a limited number of studies assessing these associations have been conducted among Blacks, populations characterized by a great genetic diversity [20] and by a high risk of thromboembolism and its complications [25]. There is evidence that genetic variations play a major role in the difference of warfarin dosage requirements not only between ethnic groups, but also within ethnic groups, such as the Black populations [20,26]. To investigate selected genetic factors that could influence the therapeutic maintenance dose of warfarin in Blacks, we conducted the Grady Coumadin Study, a small retrospective cohort study. We used a re-sequencing strategy to identify additional novel *VKORC1* SNPs in regions that might be implicated in the variability in warfarin dosage and that might explain in part the genetic variations among our Black population. Our objectives were to investigate the associations between selected *CYP2C9* and *VKORC1* polymorphisms and the risk of serious or life-threatening bleeding or thrombotic events among Black patients on warfarin therapy during a 1-year maintenance phase, and to estimate warfarin maintenance dose requirements and INR measurements among these patients within their 1 year of follow-up.

## Methods

### Study design & subject enrollment

The Grady Coumadin Study was a retrospective pilot cohort study carried out at the Coumadin Clinic of Grady Memorial Hospital, a large public hospital affiliated with Emory Healthcare, in Atlanta, GA, USA, between February 2005 and October 2006. The study protocol was approved by the Institutional Review Boards at the two participating institutions – Emory University School of Medicine and the Centers for Disease Control and Prevention (CDC). Eligibility criteria comprised: patients being on warfarin therapy for at least three consecutive months, having a confirmed date of first warfarin dosage, and patients being 18 years of age or older. These criteria encompassed patients with thromboembolic disease, myocardial infarction, atrial fibrillation and prosthetic heart valves. The exclusion criteria comprised: an inability to document the initial dose, an inability to collect a blood sample and documentation of repeated noncompliance with warfarin therapy by staff data collection. There was no restriction based on sex or race. Patients were enrolled in the study after informed consent was obtained; 245 patients were eligible to participate (n = 230 Blacks, n = 13 Whites and n = 2 of unknown race).

### Data collection & study end points

Data were collected by a clinic nurse and a cardiology fellow and consisted of a review of inpatient and outpatient medical records. Information on the INR measurements and warfarin dosing was obtained for each study participant. The target range for INR was 2.0–3.0 except for patients with prosthetic heart valves whose range was 2.5–3.5. Medical information abstracted from chart review was kept in a secured location in a locked file cabinet. A 5-ml sample of blood was drawn for DNA testing. Serious or life-threatening bleeding complications were defined as a drop in hematocrit (>35%) that was associated with clinical bleeding and the need for blood transfusion. Thrombotic complications were obtained by chart review and comprised embolic stroke, pulmonary embolism, deep vein thrombosis and other systemic embolism. Other variables collected from chart review that are known to influence warfarin dosage were race, sex, age at enrollment, indications for warfarin therapy (atrial fibrillation or flutter; congestive heart failure; cerebrovascular accident; deep vein thrombosis or pulmonary embolism, or both; left ventricular thrombus; and heart valve replacement or repair), co-morbid conditions (arrhythmia, congestive heart failure, diabetes mellitus, hypertension and protein C or S deficiency), vitamin or herbal supplement intake and current smoking status [2,4,27].

### Sample collection

Every attempt was made to collect the blood samples at the time of a routine blood draw, and samples were stored at 4°C until shipped via a courier to the CDC. DNA was extracted according to the manufacturer's protocol using the PureGene DNA extraction kit (MN, USA) and stored at the CDC. The DNA was tested only for variables related to bleeding and clotting disorders.

### Candidate SNP selection

For the *CYP2C9* and *VKORC1* genes, variants that result in a coding nonsynonymous amino acid substitution or have been previously reported to alter warfarin pharmacokinetics were selected for genotyping. Additionally, a re-sequencing strategy was employed to characterize additional genetic variation in this Black patient population. For SNP discovery, sequencing of the *VKORC1* exons and 5′ region were performed on a subset of study samples (n = 125). Primers for sequencing each of the *VKORC1* exons and 5′ region were designed in the laboratory and synthesized in the CDC core facility. DNA sequencing was performed on a 3730 DNA analyzer (Applied BioSystems, CA, USA), and results were analyzed using Sequencing Analysis 5.2 software (Applied Biosystems).

### Genotyping

Selected *CYP2C9* and *VKORC1* SNPs were genotyped with TaqMan ® Drug Metabolism genotyping assays according to manufacturer protocols (Applied Biosystems). Amplification was carried out in 25 μl in a GeneAmp PCR System 9700 (Applied BioSystems), and fluorescence signals from samples and no-template controls were analyzed on a 7900HT Fast Real-Time PCR System (Applied Biosystems). Genotypes were determined by manual review of each allelic discrimination plot with Sequence Detection System Version 2.3

software (Applied Biosystems). At least 10% of the samples were repeated and confirmed. All genotyping assay SNP results were validated by comparison with re-sequencing results.

We genotyped four *CYP2C9* polymorphisms – *CYP2C9*2* 430C>T (coding variant; R144C; rs1799853), *CYP2C9*3* 1075A>C (coding variant; I359L; rs1057910), *CYP2C9*5* 1080C>G (coding variant; D360E; rs28371686) and *CYP2C9*6* 187delA (coding variant; K273Rfs; rs9332131) [28] – and 16 *VKORC1* polymorphisms – *VKORC1*-1639G>A (upstream-variant-2KB; rs9923231), *VKORC1* 4633A>G (upstream-variant-2KB; rs184617062), *VKORC1* 4719T>C (intron-variant; rs17878259), *VKORC1* 4835G>A (upstream-variant-2KB; rs17883590), *VKORC1* 5373T>C (coding variant exon 1; novel), *VKORC1* 5387G>A (coding variant exon 1; novel), *VKORC1* 5396G>A (coding variant exon 1; Val29Leu; rs104894539), *VKORC1* 5417G>T(coding variant exon 1; Asp36Tyr; rs61742245), *VKORC1* 5445T>A (coding variant exon 1; Val45Ala; rs104894540), *VKORC1* 6642G>A (coding variant exon 2; Val66Met; rs72547529), *VKORC1* 6746C>T (intronic variant; novel), *VKORC1* 6763C>G (intronic variant; rs200039618), *VKORC1* 6853G>C (intronic variant; rs8050894), *VKORC1 6915C>T* (intronic variant; rs17886199), *VKORC1 8773C>T* (coding variant exon 3; Leu120Leu; rs7200749) and *VKORC1* 8798T>G (coding variant exon 3; Leu128Arg; rs104894542) [29]. Upstream-variant 2KB is defined as a sequence variant located within 2KB 5′ of a gene [28,29].

## Statistical methods

Primary end points were: serious or life-threatening bleeding complications, thrombotic complications, daily maintenance warfarin dosage (in mg) and INR at 6-month and 1-year follow-up. We calculated the minor allele frequency (MAF) – the frequency of the less common allele – for each of the *CYP2C9* and *VKORC1* polymorphisms, and grouped the MAFs into four categories: common alleles (MAF >5%), rare alleles (2% ≤ MAF ≤ 5%), very rare alleles (0% < MAF ≤ 1%) and alleles without variation (MAF = 0%). We = compared the distribution of selected demographic and clinical characteristics of each of the *CYP2C9* and the *VKORC1* variant alleles with their respective reference allele using chi-square test or Fisher's exact test, and calculated the risk ratios and 95% confidence intervals. Specifically, for the four *CYP2C9* genotypes, the reference alleles were, respectively, *CYP2C9*2* 430C/C, *CYP2C9*3* 1075A/A, *CYP2C9*5* 1080C/C, *CYP2C9*6* 817A/A. For the 16 *VKORC1* genotypes, the reference alleles were, respectively, *VKORC1*-1639G/G, *VKORC1* 4633A/A, *VKORC1* 4719T/T, *VKORC1* 4835G/G, *VKORC1* 5373T/T, *VKORC1* 5387G/G, *VKORC1* 5396G/G, *VKORC1* 5417G/G, *VKORC1* 5445T/T, *VKORC1* 6642G/G, *VKORC1* 6746C/C, *VKORC1* 6763C/C, *VKORC1* 6853G/G, *VKORC1 6915C/C*, *VKORC1 8773C/C*, *VKORC1* 8798T/T.

We compared the prescribed daily maintenance dosage of warfarin as well as the INR at 6-month and 1-year follow-up between the different alleles for each of the *CYP2C9* and *VKORC1* SNP within our group of patients using a nonparametric analysis based on the Wilcoxon-Mann–Whitney test (or Wilcoxon-rank-sum test), with a level of statistical significance at 0.05. We also analyzed variant allele data following the logarithmic transformation of the warfarin mean doses, and performed linear regression analysis to

assess the association between each genotype with the logarithmic transformed means of warfarin maintenance doses.

All statistical analyses were performed using SAS 9.3 (SAS Institute, Inc, NC, USA).

## Results

We restricted our analyses to Black participants (n = 230). Among the 230 Black participants, 29 patients (12.6%) were lost to follow-up during the 1-year follow-up period and, among those, six were lost before 6-month follow-up.

Allelic frequencies of the four *CYP2C9* polymorphisms and the 16 *VKORC1* polymorphisms among our Black population are summarized in Supplementary Table 1. We included in analyses the four polymorphisms with common alleles – *VKORC1*-1639G>A, *VKORC1* 6853G>C, *VKORC1* 6915C>T and *VKORC1* 8773C>T – and the two polymorphisms with rare alleles – *CYP2C9*2* and *VKORC1 4719T>C*. Because of the very small sample sizes, we excluded the six polymorphisms with very rare alleles – *CYP2C9*3*, *CYP2C9*5*, *CYP2C9*6*, *VKORC1* 4835G>A, *VKORC1* 6642G>A, *VKORC1* 6763C>G – as well as the eight polymorphisms showing little or no variation among our population – *VKORC1* 4633A>G, *VKORC1* 5373T>C *VKORC1* 5387G>A, *VKORC1* 5396G>A, *VKORC1* 5417G>T, *VKORC1* 5445T>A, *VKORC1* 6746C>T, *VKORC1* 8798T>G.

Table 1 presents the distribution of demographic and clinical characteristics by *CYP2C9*2*, *VKORC1* 4719T>C and *VKORC1*-1639 polymorphisms. The distribution of atrial fibrillation or atrial flutter was higher among Blacks with *VKORC1* 4719 variant alleles compared with Blacks with the reference allele (Fisher's exact test, p = 0.0009). We did not observe a significant difference in the occurrences of bleeding or thrombotic complications or in the distributions of demographic characteristics and co-morbid conditions among Blacks with *CYP2C9*2* or *VKORC1* 4719 variant alleles when compared with those with their respective reference allele (data not shown). None of the Black patients with *CYP2C9*2* or *VKORC1* 4719 genotype had antithrombin III deficiency, homocysteinemia or peripheral vascular disease.

Among Blacks with the variant alleles for *VKORC1*-1639 (Table 1), *VKORC1* 6853, *VKORC1* 6915 or *VKORC1* 8773 (data not shown), we did not observe a significant difference in the occurrences of bleeding or thrombotic complications, or a significant difference in the distributions of warfarin indications and co-morbid conditions compared with Blacks with the reference alleles within our group of patients (data not shown). None of the Black patients with *VKORC1*-1639, *VKORC1* 6853, *VKORC1* 6915 or *VKORC1* 8773 polymorphisms had antithrombin III deficiency, homocysteinemia or peripheral vascular disease.

As shown in Table 2, the mean daily maintenance dosage of warfarin required to maintain an INR measurement within the therapeutic range was not statistically different among Black study patients who were heterozygous for *CYP2C9*2* or *VKORC1* 4719T>C polymorphisms compared with those with their respective reference allele. A lower mean daily dosage of warfarin was required to maintain an INR measurement within the

therapeutic range among Blacks who were homozygotes or heterozygotes for *VKORC1*-1639 and *VKORC1* 6853 polymorphisms, and the difference in mean daily dosages of warfarin for these polymorphisms was statistically significant. When we considered the logarithm of warfarin mean daily maintenance dosages as the dependent variable in univariate linear regression models, we found a statistically significant association with *VKORC1*-1639 ($p = 0.001$) and with *VKORC1* 6853 ($p = 0.027$). For the *VKORC1*-1639 polymorphism, the warfarin maintenance genetic polymorphism for *VKORC1* 6853. Our data analysis indicated that *VKORC1–1639* and *VKORC1 6853* were correlated in a linear regression model, and were in linkage disequilibrium (LD; squared allele-frequency correlation [$r^2$] = 0.41). When *VKORC1*-1639 and *VKORC1 6853* polymorphisms were concomitantly included as independent variables for the logarithm of mean warfarin dosage in a multivariate linear regression model, only *VKORC1*-1639 remained statistically significant ($p = 0.036$).

## Discussion

This cohort study revealed that a lower mean daily maintenance dosage of warfarin was required to maintain an INR measurement within the therapeutic range among Blacks with *VKORC1*-1639G>A (rs9923231), and the dose effect of genetic polymorphism followed a negative linear trend with homozygous patients *VKORC1*-1639 A/A requiring the lowest dosage. We also found a statistically significant association between atrial fibrillation or atrial flutter and the rare SNP *VKORC1* 4719T>C (rs17878259). In our small, likely admixed Black patient population, many of the other polymorphisms were very rare or completely monomorphic showing no variation. We did not observe a significant difference in the occurrences of bleeding or thrombotic complications among Blacks with *VKORC1*-1639G>A (rs9923231), 6853G>C (rs8050894), 6915T>C (rs17886199), 8773C>T (rs7200749) polymorphisms (MAF >5%), and *VKORC1* 4719T>C (rs17878259) and *CYP2C9*2* (rs1799853) polymorphisms (2% ≤ MAF ≤ 5%), when compared with those with their respective reference alleles.

The genetic variability for the *CYP2C9* and *VKORC1* genotypes across ethnic groups varied among studies [21,30–32]. Our findings agreed with those of previous studies that *VKORC1*-1639G>A is a major predictor of warfarin maintenance dosage among Blacks, and patients with G/A or A/A variant alleles necessitated a lower warfarin dosage than those with G/G variant alleles [10–11,18–19,21]. Also in agreement with other investigations among African–Americans [33,34], the *VKORC1* 6853G>C polymorphism was not a predictor of warfarin dosage requirement, although Limdi *et al.*'s study [34] revealed that *VKORC1*-1639 and *VKORC1* 6853 were both predictive of warfarin dosage requirement among White–Americans. In contrast to other studies focusing on *VKORC1 8773* among White–Canadians [35], or on *VKORC1* 6915 among African–Americans [36], we did not find that *VKORC1* 8773 or *VKORC1* 6915 polymorphisms were associated with a lower warfarin dosage requirement in our Black population, likely because of the small sample size of the population. Furthermore, previous research investigations showed strong evidence that promoter and intronic variants *VKORC1*-1639G>A and *VKORC1* 6853G>C (as well as 1173C>T and 7566C>T) were in complete LD [16], and were both present in *VKORC1*2* haplotype (H2) [16,37]. Our data analysis indicated that *VKORC1*-1639 and *VKORC1* 6853

were in LD ([$r^2$] 0.41), confirming Geisen's finding [16]. Further adjustment for each genotype versus the other genotype led us to conclude that *VKORC1*-1639G>A was the important genetic factor associated with warfarin sensitivity among our Black study participants.

Because *CYP2C9*2*, *CYP2C9*3* and *VKORC1*-1639G>A variant alleles are less frequent in Blacks and can only partly explain their warfarin dose variability, further investigations were carried out to identify additional variant alleles that could alter the response to warfarin therapy in Black populations. For example, studies in multiethnic groups showed that *CYP2C9*5*, **6*, **8* and **11* variants were found in higher frequency in Blacks than in Whites. Specifically, these alleles influenced the variability in warfarin maintenance dosage among African–Americans and were associated with decreased function of the *CYP2C9* enzyme [10–12,38]. In indigenous African populations, a reduction of warfarin dose requirements was also observed with the *CYP2C9*8* polymorphisms in Black populations from South Africa [39], and with the *CYP2C9*5*, **6* and **11* polymorphisms in patients from Sudan [40]. In our study, however, *CYP2C9*5 and CYP2C9*6* variants were excluded from the analysis because of very low allelic frequencies, and *CYP2C9*8 and CYP2C9*11* variants were not studied.

Frequency distributions of minor alleles for *CYP2C9* and *VKORC1* polymorphisms vary across ethnically diverse populations [15–16,21,41]. In our study, genotype frequencies for *CYP2C9*2*, *CYP2C9*3*, *CYP2C9*5*, *CYP2C9*6, VKORC1*-1639G>A, *VKORC1* 6853G>C, *VKORC1* 6915C>T and *VKORC1* 8773C>T among our Black population were in line with those from several investigations in African–Americans [10–11,18–21,32–34,38,40–44] and various public resources [16,45–47]. Furthermore, frequencies of minor alleles for *CYP2C9*5*, *CYP2C9*6*, *VKORC1 6853G>C* and *VKORC1 8773G>A* polymorphisms in our Black population were similar to those among indigenous African populations, such as Black populations from South Africa [39], Benin [48], Mozambique or Angola [49]. Allelic frequencies for *CYP2C9**2, *CYP2C9**3 and *VKORC1*-1639G>A in our study of Black population, however, slightly differed from those reported in several studies conducted among Sub-Saharan African populations. For example, *CYP2C9**2 and *CYP2C9**3 variant alleles were not detected in any of genotyped study participants consistent with Black–Africans from Ghana [50,51], Benin [48,52], Mozambique [53] or South Africa [39,54], whereas, in our study, we detected 2 and 1% frequencies of minor alleles for *CYP2C9**2, *CYP2C9**3, respectively, among our Black participants. In addition, *VKORC1*-1639G>A allelic frequencies were lower in populations from Mozambique or Angola [49] (3.5 and 2.7%, respectively) compared with those in our Black population (13%).

Several novel genotypes shown to significantly improve warfarin dose prediction were identified in studies conducted among Black populations. The gene caluminium (*CALU*) – a variant in a *VKORC1* regulator – was shown to influence warfarin requirements [38,55]. In particular, *CALU* rs339097 A>G was associated with higher warfarin dose requirements in African–Americans [55]. The gamma-glutamyl carboxylase (*GGCX* [rs10654848]) gene was also identified in African–Americans and was significantly associated with higher warfarin stable dose requirements [36,38,43]. In the first genome-wide association study

focusing on genetic influence of warfarin dose requirements which included Blacks, investigators identified rs12777823 variant in the *CYP2C* cluster near the *CYP2C18* gene on chromosome 10 among their Black study participants [44]. They conducted an initial genome-wide association study followed by replication cohort studies, and found that Black participants (mainly originating from West Africa) with rs12777823 variant needed a warfarin dose reduction. Because this variant was not associated with warfarin dose requirements in Whites or in Asians, it was suggested that the rs12777823 variant was not causal but in LD with one or more rare causal variants in Blacks [56]. Nevertheless, the association between rs12777823 genotype and warfarin dose reduction was subsequently confirmed in further studies [57]. In a study on extreme warfarin doses with African–American participants, investigators found evidence that a population-specific regulatory variant in the folate homeostasis gene folylpolyglutamate synthase (*FPGS* [rs7856096]) might influence warfarin dose [58]. On the other hand, several other genes have been identified such as *EPHX1*, *APOE* or *CYP4F2*, and have been shown to influence warfarin dose requirements in Whites, but not, or inconsistently, in African-descent populations (findings from these studies are summarized in Suarez-Kurtz and Botton's comprehensive literature review [59]). In our study, we did not investigate the association between *CALU* rs339097, *GGCX* rs10654848, *CYP2C* rs12777823 or *FPGS* rs7856096 variants and warfarin dose variability among our Black population because our study was conducted before these SNPs were identified.

The difference in warfarin maintenance dosage requirement between ethnic groups has been attributed to genetic factors as well as to clinical and environmental factors. Numerous dosing algorithms for warfarin have been derived from multiple regression models to predict the stable therapeutic dosage of warfarin. For example, investigators from the IWPC derived dosing algorithms using data from geographically diverse patients, and included clinical factors (warfarin indication, target INR and interacting drugs), demographic variables (age, race, weight and height) and genetic variables (*CYP2C9* and *VKORC1* genotypes) [21,23]. They concluded that incorporating genotype information improved clinical outcomes, especially for patients who required much higher or lower warfarin dosages. A meta-analysis of randomized controlled trials comparing genotype-guided with standard-VKA (such as warfarin) dosing algorithms in adults initiating anticoagulation, however, did not find a decrease of a composite outcome of death, major bleeding and thromboembolic events [60]. They did observe an improvement in time in therapeutic range when they compared genotype-guided with fixed-VKA dosing algorithms, but not with clinical algorithms [60].

Since 2007, the US FDA mandated that information about *CYP2C9* and *VKORC1* variants be included in the labeling information of warfarin [22,61]. In 2010, the FDA updated the label to include dosing recommendations on *CYP2C9* and *VKORC1* genotypes [62]. *CYP2C9*2*, *CYP2C9*3* and *VKORC1*-1639G>A variants are included in the FDA table to guide warfarin dosing [63], as well as in the IWPC algorithm [23]. Although these polymorphisms accounted for more than 40% of the variability in warfarin dose in Whites, they explained only 20% of the variability in warfarin dose in Black populations [21–23]. Therefore, advances in pharmacogenetics-based warfarin therapy providing clinicians with a personalized approach to estimate the initial therapeutic dose of warfarin therapy when genotype information is available [63] benefit predominantly White populations. There is a

major limitation of current standard practice of genetic testing in clinical medical care associated with warfarin therapy since it is mainly applicable to the White patient populations from Europe or the USA. More research is needed in the Black populations to assist in the development of pharmacogenetics-based guidelines for warfarin dosing among populations of African ancestry.

Two large prospective clinical trials sponsored by the National Heart, Lung, and Blood Institute were conducted in the USA to examine warfarin pharmacogenetics – the Clarification of Optimal Anticoagulation Through Genetics (COAG) study and the Genetics Informatics Trial (GIFT) of Warfarin Therapy to Prevent Deep Venous Thrombosis study. Although the COAG clinical trial included an ethnically diverse population with 27% African Americans, it was limited to *CYP2C9*2*, *CYP2C9*3* and *VKORC1*-1639 genetic variants. The COAG trial found no difference in pharmacogenetic dosing of warfarin compared with a clinical algorithm [64,65]. Because genetic variants specific to African–Americans such as *CYP2C9 (*5, *6, *8* and **11*) variants, or rs12777823, were not included in the COAG trial, some authors suggested that the algorithm used in this trial over-estimated warfarin dose in African–Americans who had *CYP2C9 (*5, *6, *8* and **11*) variants [66,67]. The GIFT clinical trial, on the other hand, quantified the benefit of pharmacogenetic dosing of warfarin on clinical events in orthopedic patients and included *CYP2C9*2*, *CYP2C9*3*, *VKORC1*-1639 and *CYP4F2*3* genetic variants [68,69]. Results of the GIFT trial indicated that genotype-guided dosing was associated with a 27% reduction in the composite outcome of death, confirmed venous thromboembolism, warfarin overdose (INR ≥4) and major bleeding as compared with clinical algorithm dosing, and a statistically significant improvement in INR control among the study population. These results may be promising and could provide a good illustration of the importance of personalized medicine [69].

Our study had several strengths. First, it comprised a sample of Blacks, an ethnic group with wide, complex and heterogeneous genetic diversity for whom there is currently a knowledge gap related to warfarin pharmacogenetics. Second, we used a conservative case definition of a bleeding event and only patients who experienced major bleeding episodes were counted as bleeding events. By restricting bleeding events to those which were serious and life threatening, we created a more specific case definition with fewer false-positives. Because our case definition was not sensitive, we probably missed patients with mild or moderate bleeding episodes, and might have underestimated the risk of complications associated with *CYP2C9* and *VKORC1* variant alleles. Our study, however, has several limitations. First, bias from loss to follow-up or loss to competing risks might have occurred if censoring was related to the outcomes. Specifically, selection bias might have arisen if the 29 Black patients who withdrew from the cohort had different frequencies of bleeding or thrombotic events, or had different distributions of daily maintenance warfarin dosages according to *CYP2C9* and *VKORC1* variant alleles from those who remained in follow-up. Second, our study did not include information on the induction phase, and therefore, we were unable to estimate the time to stable dosing in relation to the genetic risk factors, in particular *VKORC1* polymorphisms. Third, we had small numbers making some data unreliable. Fourth, we did not have information on medications, such as amiodarone, statins, to assess drug interactions with warfarin. The majority of the results are related to dose and dose

would be directly affected by drug interactions. Finally, our Black population is likely admixed. However, admixture had to remain unresolved in our study because we could not test or control for admixture in association testing. There is large genomic diversity in Africa and tests which are developed fail to take this variation into account might fall short of offering universal efficacious testing.

## Conclusion

This pilot study consisting of a small, likely admixed patient population showed that *VKORC1*-1639G>A variant alleles were associated with a lower mean daily maintenance dosage of warfarin independently of the *VKORC1* 6853 polymorphism among Blacks receiving warfarin therapy. Because allele frequencies differ between ethnic groups, more studies are needed to detect novel genotypes among ethnically diverse populations receiving warfarin anticoagulation. Such studies might provide references for the development of dosing protocols designed to reduce the risk of complications associated with warfarin among different ethnically diverse populations.

Future research could include a cohort with a larger sample of Black participants from various ethnic groups of African ancestry to allow enrichment for rare alleles, as well as to adjust for censoring and potential confounding variables, and to detect gene–gene interactions and gene–environment interactions. Future research could also include clinical or epidemiologic studies designed to investigate whether *CYP2C9* and *VKORC1* polymorphisms and other genotypes are more important genetic determinants of warfarin dosage among patients with bleeding disorders, such as hemophilia, compared with those without bleeding disorders.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Financial & competing interest disclosure

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## Executive summary

- Although numerous published studies described the association between complications of warfarin anticoagulation and *CYP2C9* or *VKORC1* single nucleotide polymorphisms (SNPs) among Whites, few studies assessing these associations have been performed among Blacks.
- In this retrospective cohort study of a small, likely admixed Black patient population receiving warfarin anticoagulation, we investigated whether four genotyped *CYP2C9* SNPs and 16 *VKORC1* SNPs identified by re-sequencing were associated with serious bleeding or thrombotic risk. We also estimated mean daily maintenance dosage of warfarin and international normalized ratio in this population.
- We conducted the Grady Coumadin Study, a small retrospective cohort study among 230 Blacks 18 years or older (comprising 124 men and 106 women) receiving warfarin for a minimum of three consecutive months with a confirmed date of first dosage from February 2005 through October 2006.
- A lower mean daily maintenance dose of warfarin was required to maintain an international normalized ratio measurement within the therapeutic range among Blacks with the *VKORC1–1639G>A* variant alleles ([G/A vs G/G, p = 0.02], [A/A vs G/A, p = 0.008] and [A/A vs G/G, p = 0.001]) using Wilcoxon-Mann–Whitney test.
- The warfarin maintenance dose decreased with the increased number of variant alleles among Blacks with *VKORC1–1639G>A*, indicating a negative dose effect of genetic polymorphism (*P* trend test = 0.0008).
- Our data did not indicate a significant difference in the occurrences of bleeding or thrombotic complications among Blacks with *VKORC1–1639G>A*, *6853G>C*, *6915T>C*, *8773C>T* polymorphisms (MAF > 5%), and *VKORC1 4719T>C* and *CYP2C9*2* 430*C>T* polymorphisms (2% ≤ MAF ≤ 5%).
- Because allele frequencies differ between ethnic groups, more studies are needed to detect novel *CYP2C9* and *VKORC1* polymorphisms and other genotypes among ethnically diverse populations, especially of African ancestry, receiving warfarin anticoagulation.

**Table 1.**

Distribution of demographic and clinical characteristics by *CYP2C9*2 430C>T, VKORC1 4719T>C and VKORC1*-1639G>A single nucleotide polymorphisms among Blacks: The Grady Coumadin Study, Atlanta, Georgia, 2005–2006.

| Variables | *CYP2C9*2* | | *VKORC1 4719T>C* | | VKORC1–1639G>A | |
|---|---|---|---|---|---|---|
| | Variant allele C/T | Reference allele T/T | Variant allele T/C | Reference allele T/T | Variant alleles G/A or A/A combined | Reference allele G/G |
| **Demographics** | | | | | | |
| Subjects, no. (%) | 8 (4.2) | 184 (95.8) | 7 (3.2) | 213 (96.8) | 51 (24) | 165 (76) |
| Male, no. (%) | 4 (50.0) | 100 (54.4) | 4 (57.1) | 120 (56.3) | 31 (61) | 88 (53) |
| Age, mean (SD; years) | 57.4 (8.81) | 58.5 (12.2) | 61.4 (15.8) | 58.5 (12.0) | 59.2 (11.9) | 58.5 (12.3) |
| **6-month follow-up (days)** | | | | | | |
| Mean | 181 | 181 | 180 | 181 | 180.5 | 182 |
| Median | 182 | 183.5 | 188 | 183 | 182 | 185 |
| Range | 168–189 | 34–273 | 34–273 | 50–270 | 50–219 | 34–273 |
| **1-year follow-up (days)** | | | | | | |
| Mean | 369 | 358 | 347 | 360 | 357 | 361 |
| Median | 364 | 364 | 361 | 364 | 364 | 364 |
| Range | 361–392 | 258–406 | 266–385 | 258–406 | 262–384 | 258–406 |
| **Warfarin complications, no. (%)** | | | | | | |
| Serious bleeding | 2 (25.0) | 15(8.2) | 0 (0.0) | 18 (8.5) | 6(12) | 13(8) |
| Thrombosis | 0 (0.0) | 7 (3.8) | 0 (0.0) | 8 (3.8) | 1 (2) | 6(4) |
| **Indications for warfarin, no. (%)** | | | | | | |
| Atrial fibrillation/atrial flutter | 1 (12.5) | 73 (39.7) | 7 (100.0)[†] | 76 (35.7) | 21 (41) | 60 (36) |
| Coronary arterial disease | 0 (0.0) | 1 (0.54) | 0 (0.0) | 1 (0.47) | 0 (0.0) | 1 (0.6) |
| Dilated cardiomyopathy/ congestive heart failure | 0 (0.0) | 15(8.2) | 0 (0.0) | 17 (8.0) | 3 (6) | 14 (8) |
| Cerebrovascular accident | 2 (25.0) | 18 (9.8) | 0 (0.0) | 26 (12.2) | 9 (18) | 16(10) |
| DVT/PE | 4 (50.0) | 56 (30.4) | 0 (0.0) | 69 (32.4) | 12 (24) | 56 (34) |
| Left ventricular thrombus | 0 (0.0) | 6 (3.3) | 0 (0.0) | 6(2.8) | 2 (4) | 5(3) |
| Pulmonary hypertension | 0 (0.0) | 2(1.1) | 0 (0.0) | 2 (0.94) | 0 (0.0) | 2(1) |
| Peripheral vascular disease | 0 (0.0) | 1 (0.54) | 0 (0.0) | 1 (0.47) | 0 (0.0) | 1 (0.6) |
| Valve replacement/repair | 1 (12.5) | 31 (16.9) | 1 (14.3) | 34 (16.0) | 6(12) | 28 (17) |
| Other | 0 (0.0) | 1 (0.54) | 0 (0.0) | 2 (0.94) | 0 (0.0) | 2(1) |
| **Co-morbid conditions, no. (%)** | | | | | | |
| Atrial fibrillation/atrial flutter | 0 (0.0) | 1 (0.54) | 0 (0.0) | 2 (0.94) | 0 (0.0) | 2(1) |
| Arrhythmia | 1 (12.5) | 21 (11.4) | 0 (0.0) | 24 (11.3) | 7(14) | 16(10) |

| Variables | *CYP2C9*2* | | *VKORC1 4719T>C* | | VKORC1–1639G>A | |
|---|---|---|---|---|---|---|
| | **Variant allele C/T** | **Reference allele T/T** | **Variant allele T/C** | **Reference allele T/T** | **Variant alleles G/A or A/A combined** | **Reference allele G/G** |
| Cancer | 0 (0.0) | 14 (7.6) | 0 (0.0) | 17 (8.0) | 6(12) | 11 (7) |
| Congestive heart failure | 3(37.5) | 50 (27.2) | 3 (42.9) | 57 (26.8) | 11 (22) | 46 (28) |
| Cerebrovascular accident | 0 (0.0) | 8 (4.4) | 0 (0.0) | 8 (3.8) | 3 (6) | 5(3) |
| Diabetes mellitus | 1 (12.5) | 50 (27.2) | 2 (28.6) | 59 (27.7) | 13(25) | 47 (28) |
| Hypertension | 7(87.5) | 1 53 (83.2) | 6 (85.7) | 176 (82.6) | 43 (84) | 136 (82) |
| Valve replacement/repair | 0 (0.0) | 1 (0.54) | 0 (0.0) | 1 (0.47) | 1 (2) | 0 (0.0) |
| History of DVT/PE | 0 (0.0) | 4(2.2) | 0 (0.0) | 4 (1.9) | 1 (2) | 3(2) |
| Factor V Leiden | 0 (0.0) | 1 (0.54) | 0 (0.0) | 1 (0.47) | 0 (0.0) | 1 (0.6) |
| Protein C or S deficiency | 0 (0.0) | 3(1.6) | 0 (0.0) | 4 (1.9) | 2 (4) | 2(1) |
| **Current smoker, no. (%)** | 2 (25.0) | 59 (32.1) | 1 (14.3) | 68 (31.9) | 20 (39) | 49 (30) |
| **Vitamin or herb intake, no. (%)** | 3(37.5) | 46 (25.0) | 1 (14.3) | 54 (25.4) | 12 (24) | 40 (24) |

$^{\dagger}$Fisher exact test, p = 0.0009.

DVT: Deep vein thrombosis; PE: Pulmonary embolism; SD: Standard deviation; *VK*: *VKORC1* genotype.

**Table 2.**

Prescribed daily maintenance dose of warfarin and mean international normalized ratio in relation to *CYP2C9* and *VKORC1* single nucleotide polymorphisms with minor allele frequency ≥ 2% among Blacks: The Grady Coumadin Study, Atlanta, Georgia, 2005–2006.

| Single nucleotide polymorphism | No. | Daily maintenance warfarin dose (mg) | | International normalized ratio | | |
|---|---|---|---|---|---|---|
| | | Mean (SD) | Median (IQR) | Mean (SD) at enrollment | Mean (SD) at 6 months | Mean (SD) at 1 year |
| ***CYP2C9*2*** | | | | | | |
| *C/C* | 184 | 7.0 (4.5) | 6.1 (4.6) | 2.6 (1.1) | 2.7 (1.5) | 2.8 (1.5) |
| C/T | 8 | 4.8 (1.6) | 5.0 (2.0) | 2.3 (0.58) | 2.3 (0.61) | 2.6 (0.93) |
| T/T | 0 | - | - | - | - | - |
| ***VKORC1* 4719T>C** | | | | | | |
| T/T | 213 | 6.9 (4.5) | 6.1 (4.5) | 2.6 (1.1) | 2.6 (1.4) | 2.8 (1.4) |
| T/C | 7 | 5.1 (2.0) | 4.3 (3.9) | 2.7 (0.71) | 3.4 (1.7) | 2.4 (0.38) |
| C/C | 0 | - | - | - | - | - |
| ***VKORC1–1639G> A*** | | | | | | |
| *G/G* | 165 | 7.0 (3.8) | 6.4 (3.9) | 2.6 (1.1) | 2.6 (1.6) | 2.8 (1.5) |
| G/A | 45 | 6.9 (6.5)† | 5.0 (2.9) | 2.5 (0.96) | 2.7 (1.2) | 2.6 (1.3) |
| A/A | 6 | 2.9 (1.2)‡,§ | 2.5 (1.1) | 2.7 (1.0) | 2.3 (0.87) | 2.1 (0.49) |
| ***VKORC1* 6853G>C** | | | | | | |
| G/G | 116 | 7.2 (4.0) | 6.4 (3.9) | 2.5 (1.1) | 2.6 (1.3) | 2.7 (1.2) |
| G/C | 62 | 6.4 (4.5)¶ | 5.0 (3.9) | 2.7 (1.1) | 2.5 (0.93) | 2.8 (1.8) |
| C/C | 29 | 6.5 (6.2)# | 5.0 (4.4) | 2.4 (0.79) | 3.1 (2.3) | 2.5 (1.0) |
| ***VKORC1* 6915C>T** | | | | | | |
| C/C | 195 | 7.0 (4.7) | 6.1 (4.6) | 2.6 (1.1) | 2.6 (1.5) | 2.8 (1.4) |
| C/T | 23 | 6.4 (2.2) | 6.1 (2.6) | 2.7 (1.1) | 2.7 (1.2) | 2.5 (1.2) |
| T/T | 1 | 4.3 (0.0) | 4.3 (0.0) | 2.8 (0.0) | 2.5 (0.0) | 2.4 (0.0) |
| ***VKORC1* 8773C>T** | | | | | | |
| C/C | 138 | 6.8 (4.7) | 5.0 (3.6) | 2.6 (1.0) | 2.6 (1.6) | 2.8 (1.4) |
| C/T | 73 | 7.1 (4.2) | 6.4 (5.0) | 2.6 (1.1) | 2.6 (1.2) | 2.8 (1.6) |
| T/T | 9 | 5.9 (2.9) | 5.0 (4.1) | 2.6 (1.3) | 2.6 (0.97) | 2.3 (0.81) |

†Wilcoxon-Mann–Whitney test, p = 0.02 between G/A and G/G genotypes for *VKORC1–1639G>A*.

‡Wilcoxon-Mann–Whitney test, p = 0.001 between A/A and G/G genotypes for *VKORC1–1639G>A*.

§Wilcoxon-Mann–Whitney test, p = 0.008 between A/A and G/A genotypes for *VKORC1–1639G>A*.

¶Wilcoxon-Mann–Whitney test, p = 0.046 between G/C and G/G genotypes for *VKORC1 6853G>C*.

#Wilcoxon-Mann–Whitney test, p = 0.03 between C/C and G/G genotypes for *VKORC1 6853G>C*.

IQR: Interquartile range; SD: Standard deviation.