BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2010.03709.x

# New genetic variant that might improve warfarin dose prediction in African Americans

**Hedi Schelleman,[1] Colleen M. Brensinger,[1] Jinbo Chen,[1] Brian S. Finkelman,[1] Mark J. Rieder[3] & Stephen E. Kimmel[1,2]**

*[1]Center for Clinical Epidemiology and Biostatistics, Department of Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, and [2]Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA and [3]Department of Genome Sciences, University of Washington, Seattle, WA, USA*

**Correspondence**
Associate Professor Stephen E. Kimmel MD MSCE, University of Pennsylvania School of Medicine, 717 Blockley Hall, 423 Guardian Drive, Philadelphia, PA 19104-6021, USA.
Tel.: +1 215 898 1740
Fax: +1 215 573 3106
E-mail: stevek@mail.med.upenn.edu

**Keywords**
ALB, CYP2C9, EPHX1, GGCX, warfarin, VKORC1

**Received**
8 February 2010

**Accepted**
12 May 2010

## WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

Variants in the *CYP2C9* (i.e. *2 and *3) and *VKORC1* (i.e. 1173C/T or –1639G/A) genes have been shown to influence warfarin dose requirements. However, these factors seem to explain less of the dose variability in African Americans who have a lower prevalence of the *CYP2C9* *2 and *3 and *VKORC1* 1173T alleles.

## WHAT THIS STUDY ADDS

In African Americans, the VKORC1 rs17886199 variant was statistically significantly associated with log-transformed warfarin maintenance dose, independent of the influence of *VKORC1* 1173C>T and *CYP2C9* *2 and *3. However, replication of our finding is needed to confirm the association of rs1786199 SNP in African Americans, since Limdi *et al.* [3] did not examine the effect of this SNP because the prevalence of the rs1786199 A-allele was too low.

## AIMS

To raise hypotheses with regards to whether genetic variants in the *VKORC1*, *CYP2C9*, *EPHX1*, *GGCX* and *ALB* genes might influence warfarin dose in African Americans and Caucasians, independent of the effects of the *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variants.

## METHODS

From a prospective cohort study, we obtained additional DNA on 36 Caucasian and 22 African American warfarin users who reached maintenance dose and genotyped them for tagSNPs ($r^2 < 0.8$) in *VKORC1*, *EPHX1*, *GGCX* and *ALB* genes, and one exonic *CYP2C9* SNP. Linear regression models were fitted to estimate the relationship (*P* value) between log-transformed maintenance dose and each SNP and the amount of the warfarin dose variability accounted for by each SNP (partial $R^2$).

## RESULTS

In African Americans, the *VKORC1* rs17886199 A-allele was associated with a lower dose (GG = 46.3 mg and GA = 25.6 mg; $P = 0.002$), independent of the *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variants. Even after applying Bonferroni correction, the *P* value would still be considered statistically significant. The *VKORC1* rs17886199 variant was not found in Caucasians. In Caucasians, the *EPHX1* rs1051741 T-allele was associated with a lower dose (CC = 41.3 mg and CT = 30.0 mg; $P = 0.04$). The latter was no longer statistically significant after applying Bonferroni correction.

## CONCLUSIONS

Our pilot study suggests that the *VKORC1* rs17886199 variant could influence warfarin maintenance dose among African Americans, even after accounting for the influence of the *VKORC1* 1173C>T variant. Future studies with a larger sample size will be needed to confirm our findings.

## Introduction

Warfarin is highly efficacious at reducing the risk of thromboembolism, and it is one of the top 20 medications prescribed in the US. A complicating factor in the management of warfarin therapy is its narrow therapeutic index and the large inter-individual variability in the warfarin maintenance dose requirements (e.g. 0.5 to 11.0 mg $day^{-1}$ [1]). In general, most patients are started on an empiric dose (for example, 5 mg $day^{-1}$) and, based on the observed international normalized ratio (INR), the dose can be titrated upwards or downwards in order to reach a stable INR within the target range. Warfarin dosing algorithms might predict a better starting dose for a patient, and potentially shorten the period required to achieve a stable warfarin dose, which might reduce the bleeding risk (from over-coagulation) or thromboembolism risk (under-coagulation).

In the last decade, the *CYP2C9* *2 and *3 and *VKORC1* 1173C>T or −1639G>A variants have been shown to account for a large amount ($R^2$ > 10%) of the variability in warfarin dose requirements in Caucasians, but these factors seem to explain less of the dose variability in African Americans who have a lower prevalence of the *CYP2C9* *2 and *3 and *VKORC1* 1173T alleles [2]. This suggests that there may be other genetic variants within the *VKORC1* and *CYP2C9* and/or other genes that have a stronger influence on warfarin maintenance dosing in African Americans compared with Caucasians.

Limdi and colleagues found that of the *VKORC1* SNPs they evaluated the *VKORC1* 1173C>T and −1639G>A SNPs were the best predictors of warfarin dose in African Americans [3]. The *CYP2C9* *5, *6, and *11 variants might contribute beyond the effect of the *CYP2C9* *2 and *3 variants in African Americans [4]. There are several other genes [5] that might influence warfarin dosing that have not been tested in African Americans. Of these genes, genetic variants within epoxide hydrolase 1 (*EPHX1*) and gamma-glutamyl carboxylase (*GGCX*) might have a small influence on warfarin dose in Caucasians or Asians [5–8]. No study has evaluated whether another potential candidate gene, namely albumin (*ALB*), influences warfarin dosing.

The purpose of this study was to raise hypotheses with regards to whether genetic variants in the *VKORC1*, *CYP2C9*, *EPHX1*, *GGCX* and *ALB* genes might influence warfarin dose in African Americans and Caucasians, independent of the effects of the *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variants.

## Methods

### Study population and data collection

From April 2002 to December 2005, subjects were prospectively recruited at two anticoagulation clinics: the Hospital of the University of Pennsylvania (HUP) and the Philadelphia Veterans Affairs Medical Center (PVAMC) in Philadelphia, PA. All subjects 21 years and older and initiating warfarin therapy once daily with a target INR of 2.0 to 3.0 who presented to one of the clinics were considered eligible for the study. Subjects with abnormal INRs prior to initiating warfarin and those with anti-phospholipid antibody in whom the INR measurement may not be valid were excluded [9]. From this cohort, we obtained additional DNA on 58 patients who reached maintenance dose. Maintenance dose was defined as the dose that leads to a stable INR over three consecutive visits that were at least 1 day apart following initiation of the drug [10]. The study was approved by the Institutional Review Boards of the participating hospitals, and all subjects provided informed, written consent.

Data on patient demographics, medical history, medication use, warfarin dose and diet were obtained prospectively by trained study interviewers using standardized questionnaires, and INR was measured during each visit. Genomic DNA was obtained from Oragene saliva kits (DNA Genotek Inc.), which were mailed to each participant. DNA was extracted and analyzed by collaborative investigators blinded to patient characteristics or outcomes.

### SNP selection and genotyping

Tagging SNPs (tagSNPs) within the *VKORC1* gene were selected from the University of Washington sequence database (http://gvs.gs.washington.edu/GVS/index.jsp), which was derived from sequencing 24 African Americans and 24 Caucasians. TagSNPs within the *EPHX1*, *GGCX* and *ALB* gene were selected from HAPMAP database. All tagSNPs were selected from the African Americans/Yorubans (West-Africa) and Caucasians to derive the optimal panel of tagSNPs for our African American sample [11]. Using LDselect we chose all tagSNPs within the candidate genes (including 2 kb upstream or downstream from the candidate genes) that had a minor allele frequency (MAF) ≥5% and an $r^2$ < 0.8 in either African Americans/Yorubans or Caucasians. In total, 14 *VKORC1*, 16 *EPHX1*, 8 *GGCX1* and 4 *ALB* taqSNPs were selected. However, for two *VKORC1* SNPs, two *EPHX1* SNPs and one *ALB* SNP, primers or probes could not be designed because of the proximity of neighbouring genetic variants. All remaining SNPs were genotyped using TaqMan assay (Applied Biosystems). For the *CYP2C9* gene, we selected the exonic SNP with the highest reported MAF in African Americans (data about PCR primers, probes and PCR conditions are available upon request from the authors). All SNPs were analyzed using Taqman 5′ Nuclease Real-Time PCR assays on an ABI prism 7900 HT instrument (Applied Biosystems).

The *VKORC1* 1173C/T (rs9934438) and −1639G/A (rs9923231) variants and *CYP2C9* *2 and (rs1799853) and *3 (rs1057910) variants were genotyped in the original cohort [12, 13].

### Outcomes

The primary outcome for the study was the maintenance dose of warfarin, defined as the dose that leads to a stable INR over three consecutive visits that were at least 1 day apart following initiation of the drug, as previously described [10].

### Statistical analysis

Each SNP was categorized in three levels based on genotype data. Hardy–Weinberg equilibrium test was performed on each SNP to assess whether genotype frequencies were in conformity with predictions based on random union of the two alleles within each race group separately. *A priori*, we decided to exclude all SNPs that were either not in Hardy–Weinberg equilibrium (defined as $P < 0.0001$ [14]), had a genotyping error rate of >5% in our duplicate samples (5% of our cohort), had a genotyping error in one of two CEPH samples, had fewer than 70% of the patients genotyped, or had an MAF less than 5% in African Americans and Caucasians.

The distribution of the warfarin maintenance dose was right-skewed and was, therefore, log-transformed in an effort to achieve constant variance and normality. Linear regression models were fitted to estimate the relationship (*P* value) between log-transformed maintenance dose and each SNP (additive model) and the amount of the warfarin dose variability accounted for by each SNP (partial $R^2$), while adjusting for *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variant. All analyses were stratified by race and were performed using SAS version 9.1 (SAS Institute, Cary, NC).

## Results

The study included 22 African Americans and 36 Caucasians. The minimum number of days between visits used to calculate maintenance dose was 14.0. The unadjusted median weekly maintenance dose was 45.0 mg (minimum = 10.0 mg and maximum = 80.0 mg) in 21 African Americans with the *VKORC1* 1173CC genotype and 30.0 mg in one African American with the CT genotype (30.00 mg). As expected, Caucasians with the CC had a higher weekly maintenance dose (45.0 mg, minimum = 22.0 mg and maximum = 95.0 mg) than Caucasians with the CT (30.0 mg, minimum = 17.5 mg and maximum = 55.0 mg) and TT genotypes (23.0 mg, minimum = 20.0 mg and maximum = 26.0 mg). The *VKORC1* 1173C>T variant explained slightly more of the warfarin dose variability (i.e. 2% in African Americans and 35% in Caucasians), due to its higher minor allele frequency, compared with the −1639G>A variant (i.e. 0.2% in African Americans and 28% in Caucasians). Therefore, we have only shown the results adjusting for the 1173C>T variant. The *CYP2C9* *2 and *3 variants accounted for an additional 0% in African Americans and 2% in Caucasians of the variability in log-transformed warfarin maintenance dose.

### Effect of VKORC1 SNPs on maintenance dose of warfarin

In total, we excluded three *VKORC1* SNPs from the analysis because the MAF was less than 5% in African Americans and Caucasians. Prior to adjusting for the *VKORC1* 1173C>T variant and *CYP2C9* *2 and *3 variants, the rs17886199 [GG = 46.25 mg and GA = 25.63 mg; difference in log-transformed maintenance dose = −0.68 (95% CI −0.29, −1.07); $P = 0.003$] and rs7199949 [GG = 50.00 mg, GC = 45.00 mg, and CC = 32.50 mg; difference in log-transformed maintenance dose assuming a dominant model = −0.24 (95% CI −0.02, −0.47); $P = 0.047$] variants were statistically significantly associated with log-transformed warfarin maintenance dose requirements in African Americans. After adjusting for the *VKORC1* 1173C>T variant and *CYP2C9* *2 and *3 variants, only the rs17886199 [difference in log-transformed maintenance dose = −0.74 (95% CI −0.34, −1.15); $P = 0.002$] remained statistically significantly associated with log-transformed warfarin maintenance dose, and it accounted for 42% of the variability in log-transformed warfarin maintenance dose in African Americans (Table 1). There was no strong linkage disequilibrium between the rs17886199 and 1173C>T variants ($r^2 = 0.01$) and moderate linkage disequilibrium between the rs17886199 and rs7199949 SNPs ($r^2 = 0.32$).

The *VKORC1* rs17886199 variant was not found in Caucasians. Among all *VKORC1* SNPs, except for the rs17708472 variant ($P = 0.69$), there was an unadjusted, statistically significantly association with log-transformed warfarin maintenance dose requirements ($P < 0.05$, data not shown). However, after adjusting for the *VKORC1* 1173C>T variant and *CYP2C9* *2 and *3 variant, none of the VKORC1 SNPs remained statistically significantly associated (Table 1). This is most likely explained by the high linkage disequilibrium (for almost all SNPs the $r^2 > 0.5$) between the *VKORC1* SNPs and the 1173C>T variant in Caucasians.

### Effect of EPHX1 SNPs on maintenance dose of warfarin

Prior and after adjusting for the *VKORC1* 1173C>T variant and *CYP2C9* *2 and *3 variant, none of the *EPHX1* SNPs were statistically significantly associated with log-transformed warfarin maintenance dose requirements in African Americans (Table 2). Nonetheless, three of the *EPHX1* variants seem to explain more than 10% of the dose variability ($R^2 \geq 10\%$ = rs2740170: CC = 45.00 mg and CT = 35.00 mg; difference in log-transformed maintenance dose = −0.35 [95% CI 0.12, −0.82], $P = 0.16$; rs2740171: CC = 47.50 mg, CA = 36.25 mg, and AA = 26.25 mg; difference in log-transformed maintenance dose = −0.30 [95% CI 0.05, −0.65], $P = 0.12$; and rs1051741: CC = 36.25 mg and CT = 52.50 mg; difference in log-transformed maintenance dose

## Table 1

Maintenance dose of warfarin by *VKORC1* SNPs, stratified by race

| SNP | Major allele (A) | Minor allele (B) | AA (*n*) | AB (*n*) | BB (*n*) | AA Median dose (min–max) | AB Median dose (min–max) | BB Median dose (min–max) | *P* value* | Partial $R^2$* |
|---|---|---|---|---|---|---|---|---|---|---|
| **African American** | | | | | | | | | | |
| **rs17878544** | A | G | 9 | 11 | 2 | 37.50 (10.00–62.50) | 47.50 (25.00–80.00) | 38.75 (30.00–47.50) | 0.53 | 0.02 |
| **rs2884737** | A | C | 19 | 2 | 0 | 45.00 (10.00–80.00) | 32.50 (30.00–35.00) | – | † | † |
| **rs17708472** | G | A | 19 | 3 | 0 | 45.00 (10.00–80.00) | 35.00 (32.50–50.00) | – | 0.91 | <0.001 |
| **rs17886199** | G | A | 18 | 4 | 0 | 46.25 (27.50–80.00) | 25.63 (10.00–37.50) | – | 0.002 | 0.42 |
| **rs8050894** | C | G | 13 | 9 | 0 | 37.50 (10.00–67.50) | 45.00 (30.00–80.00) | – | 0.27 | 0.07 |
| **rs2359612** | G | A | 15 | 7 | 0 | 47.50 (10.00–80.00) | 35.00 (26.25–62.50) | – | 0.92 | <0.001 |
| **rs7200749** | G | A | 14 | 8 | 0 | 41.25 (10.00–80.00) | 40.00 (27.50–67.50) | – | 0.73 | 0.01 |
| **rs7294** | T | C | 7 | 9 | 5 | 52.50 (27.50–67.50) | 45.00 (25.00–62.50) | 37.50 (10.00–80.00) | 0.28 | 0.07 |
| **rs7199949** | G | C | 8 | 9 | 5 | 50.00 (27.50–67.50) | 45.00 (25.00–80.00) | 32.50 (10.00–37.50) | 0.07 | 0.18 |
| **Caucasian** | | | | | | | | | | |
| **rs17878544** | A | G | 35 | 1 | 0 | 37.50 (17.50–95.00) | 42.50 | – | – | – |
| **rs2884737** | A | C | 22 | 10 | 3 | 43.75 (22.00–80.00) | 30.00 (17.50–95.00) | 22.50 (20.00–23.00) | 0.36 | 0.02 |
| **rs17708472** | G | A | 28 | 8 | 0 | 36.75 (17.50–95.00) | 42.25 (22.00–56.25) | – | 0.69 | 0.003 |
| **rs17886199** | G | A | 34 | 0 | 0 | 36.75 (17.50–95.00) | – | – | – | – |
| **rs8050894** | C | G | 16 | 16 | 4 | 52.50 (25.00–80.00) | 30.00 (17.50–95.00) | 22.75 (20.00–26.00) | 0.10 | 0.05 |
| **rs2359612** | G | A | 16 | 14 | 6 | 47.50 (25.00–80.00) | 28.75 (17.50–95.00) | 24.50 (20.00–55.00) | 0.46 | 0.01 |
| **rs7200749** | G | A | 34 | 1 | 0 | 36.75 (17.50–95.00) | 42.50 | – | – | – |
| **rs7294** | T | C | 12 | 13 | 6 | 47.50 (27.50–80.00) | 35.00 (17.50–95.00) | 28.00 (22.00–52.50) | 0.20 | 0.03 |
| **rs7199949** | G | C | 11 | 15 | 10 | 45.00 (25.00–80.00) | 35.00 (17.50–95.00) | 28.00 (20.00–55.00) | 0.85 | <0.001 |

*The relationship between each SNP and log-transformed maintenance dose was adjusted for the *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variants. †The *VKORC1* 1173C>T and rs2884737A>C were in complete linkage disequilibrium. Therefore, we could not run the statistical analyses.

= 0.41 [95% CI –0.16, 0.97], $P = 0.18$) and potentially could influence warfarin dosing if these SNPs are retested in a study with larger sample sizes. Nonetheless, since the *P* values are between 0.10 and 0.20, these results could be due to chance.

In Caucasians, the rs1051741 variant was statistically significantly associated with warfarin dose requirements (CC = 41.25 mg and CT = 30.00 mg; difference in log-transformed maintenance dose = –0.32 [95% CI –0.03, –0.61]; $P = 0.04$) after adjusting for the variants known to be influence warfarin maintenance dose (i.e. *VKORC1* 1173C>T variant and *CYP2C9* *2 and *3 variants). This variant explained an additional 8% of variability in log-transformed warfarin maintenance dose requirements in Caucasians (Table 2).

### *Effect of GGCX, ALB, and CYP2C9 SNPs on maintenance dose of warfarin*

Prior to and after adjusting for the *VKORC1* 1173C>T variant and *CYP2C9* *2 and *3 variant, none of the *GGCX*, *ALB*, or *CYP2C9* SNPs was statistically significantly associated with log-transformed warfarin maintenance dose requirements in African Americans or Caucasians (Tables 3–5). However, the *CYP2C9* *9 variant appeared to account for more than 10% of the variability in log-transformed warfarin maintenance dose requirements in African Americans (no *9 = 41.25 mg and one *9 = 52.50 mg; difference in log-transformed maintenance dose = 0.34 [95% CI –0.17, 0.86]; $P = 0.22$), a finding that could be due to chance.

## Discussion

This study demonstrated that the *VKORC1* rs17886199 A-allele was statistically significantly associated with lower log-transformed warfarin maintenance dose in African Americans, independently of the *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variants. Even if we conservatively account for the nine tagSNPs we evaluated within the *VKORC1* gene, the *P* value would still be considered statistically significant. The *VKORC1* rs17886199 variant was not found in Caucasians. None of the other *VKORC1* SNPs remained statistically significantly associated with warfarin dose after adjusting for the *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variants in African Americans and Caucasians, which makes rs17886199 the most likely *VKORC1* candidate SNP to influence warfarin dose in African-Americans. Furthermore, none of the *EPHX*, *GGCX*, *ALB* or *CYP2C9* SNPs was statistically significantly associated with log-transformed warfarin maintenance dose requirements in African Americans. Nonetheless, there were three *EPHX1* and one *CYP2C9* variants that might have a meaningful influence on dose variability in African Americans; this requires retesting in a larger population of warfarin users. Of the *EPHX*, *GGCX*, *ALB* or *CYP2C9* SNPs tested in Caucasians, only the *EPHX1* rs1051741 was statistically significantly associated with log-transformed warfarin maintenance dose requirements. However, this SNP was no longer statistically significantly associated with warfarin dosing after accounting for the number of tagSNPS we evaluated within the *EPHX1* gene.

## Table 2

Maintenance dose of warfarin by *EPHX1* SNPs, stratified by race

| SNP | Major allele (A) | Minor allele (B) | AA (*n*) | AB (*n*) | BB (*n*) | AA Median dose (min–max) | AB Median dose (min–max) | BB Median dose (min–max) | *P* value* | Partial $R^2$* |
|---|---|---|---|---|---|---|---|---|---|---|
| **African American** | | | | | | | | | | |
| **rs1877724** | C | T | 18 | 4 | 0 | 46.25 (10.00–80.00) | 35.00 (27.50–45.00) | – | 0.66 | 0.01 |
| **rs3766934** | G | T | 16 | 5 | 1 | 36.25 (25.00–80.00) | 45.00 (10.00–55.00) | 67.50 | 0.99 | <0.001 |
| **rs2671272** | G | A | 8 | 10 | 4 | 45.00 (32.50–67.50) | 35.00 (10.00–80.00) | 38.13 (25.00–52.50) | 0.35 | 0.05 |
| **rs2234697** | C | T | 13 | 5 | 0 | 45.00 (10.00–80.00) | 35.00 (25.00–52.50) | – | 0.71 | 0.01 |
| **rs1051740** | T | C | 13 | 5 | 1 | 47.50 (10.00–80.00) | 35.00 (25.00–52.50) | 45.00 | 0.95 | <0.001 |
| **rs2292566** | G | A | 20 | 1 | 1 | 41.25 (10.00–80.00) | 47.50 | 26.25 | 0.53 | 0.02 |
| **rs2260863** | C | G | 10 | 8 | 4 | 33.75 (25.00–80.00) | 50.00 (32.50–62.50) | 31.88 (10.00–50.00) | 0.25 | 0.07 |
| **rs2740168** | G | A | 10 | 7 | 5 | 33.75 (10.00–67.50) | 47.50 (30.00–80.00) | 35.00 (25.00–47.50) | 0.53 | 0.02 |
| **rs10915884** | C | T | 17 | 5 | 0 | 37.50 (10.00–67.50) | 45.00 (35.00–80.00) | – | 0.27 | 0.07 |
| **rs2740170** | C | T | 17 | 5 | 0 | 45.00 (25.00–80.00) | 35.00 (10.00–55.00) | | 0.16 | 0.11 |
| **rs2740171** | C | A | 9 | 12 | 1 | 47.50 (27.50–80.00) | 36.25 (10.00–67.50) | 26.25 | 0.12 | 0.14 |
| **rs2234922** | A | G | 10 | 8 | 3 | 41.25 (25.00–67.50) | 41.25 (10.00–80.00) | 32.50 (26.25–52.50) | 0.62 | 0.02 |
| **rs1051741** | C | T | 18 | 4 | 0 | 36.25 (10.00–80.00) | 52.50 (35.00–62.50) | – | 0.18 | 0.10 |
| **rs4149229** | G | A | 18 | 4 | 0 | 41.25 (10.00–62.50) | 47.50 (25.00–80.00) | – | 0.64 | 0.01 |
| **Caucasian** | | | | | | | | | | |
| **rs1877724** | C | T | 20 | 12 | 3 | 41.88 (17.50–95.00) | 33.75 (17.50–80.00) | 36.00 (26.00–40.00) | 0.25 | 0.02 |
| **rs3766934** | G | T | 29 | 7 | 0 | 37.50 (17.50–95.00) | 42.00 (23.00–63.75) | – | 0.87 | <0.001 |
| **rs2671272** | G | A | 21 | 12 | 3 | 40.00 (17.50–95.00) | 28.75 (17.50–56.25) | 45.00 (35.00–50.00) | 0.45 | 0.01 |
| **rs2234697** | C | T | 23 | 4 | 1 | 30.00 (17.50–95.00) | 65.00 (40.00–80.00) | 42.50 | 0.40 | 0.02 |
| **rs1051740** | T | C | 12 | 15 | 3 | 35.50 (17.50–63.75) | 52.50 (20.00–95.00) | 30.00 (25.00–42.00) | 0.12 | 0.05 |
| **rs2292566** | G | A | 27 | 9 | 0 | 37.50 (17.50–95.00) | 55.00 (17.50–80.00) | – | 0.82 | 0.001 |
| **rs2260863** | C | G | 21 | 5 | 9 | 40.00 (17.50–95.00) | 27.50 (17.50–56.25) | 42.50 (23.00–63.75) | † | † |
| **rs2740168** | G | A | 15 | 15 | 5 | 41.25 (17.50–63.75) | 30.00 (17.50–95.00) | 35.00 (20.00–80.00) | 0.64 | 0.005 |
| **rs10915884** | C | T | 22 | 13 | 1 | 38.75 (17.50–95.00) | 30.00 (20.00–75.00) | 80.00 | 0.92 | <0.001 |
| **rs2740170** | C | T | 22 | 9 | 4 | 38.75 (17.50–95.00) | 27.50 (17.50–56.25) | 47.50 (25.50–63.75) | 0.97 | <0.001 |
| **rs2740171** | C | A | 21 | 11 | 4 | 37.50 (17.50–95.00) | 30.00 (17.50–56.25) | 47.50 (25.50–63.75) | 0.61 | 0.01 |
| **rs2234922** | A | G | 21 | 12 | 2 | 40.00 (17.50–80.00) | 36.25 (17.50–95.00) | 30.00 (25.00–35.00) | 0.62 | 0.01 |
| **rs1051741** | C | T | 29 | 7 | 0 | 41.25 (17.50–95.00) | 30.00 (17.50–42.50) | – | 0.04 | 0.08 |
| **rs4149229** | G | A | 35 | 1 | 0 | 37.50 (17.50–95.00) | 42.50 | – | – | – |

*The relationship between each SNP and log-transformed maintenance dose was adjusted for the *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variants. †SNP was not in Hardy–Weinberg equilibrium ($P < 0.0001$).

## Table 3

Maintenance dose of warfarin by GGCX SNPs, stratified by race

| SNP | Major allele (A) | Minor allele (B) | AA (*n*) | AB (*n*) | BB (*n*) | AA Median dose (min–max) | AB Median dose (min–max) | BB Median dose (min–max) | *P* value* | Partial $R^2$* |
|---|---|---|---|---|---|---|---|---|---|---|
| **African American** | | | | | | | | | | |
| **rs7568458** | A | T | 10 | 11 | 1 | 41.25 (10.00–67.50) | 45.00 (25.00–80.00) | 35.00 | 0.58 | 0.02 |
| **rs6751560** | G | A | 18 | 4 | 0 | 40.00 (10.00–80.00) | 43.75 (26.25–52.50) | – | 0.96 | <0.001 |
| **rs17026452** | G | C | 18 | 3 | 1 | 40.00 (10.00–80.00) | 37.50 (26.25–50.00) | 52.50 | 0.78 | 0.005 |
| **rs699664** | C | T | 2 | 10 | 10 | 48.75 (35.00–62.50) | 40.00 (25.00–80.00) | 41.25 (10.00–67.50) | 0.44 | 0.04 |
| **rs2592551** | G | A | 5 | 12 | 5 | 35.00 (26.25–62.50) | 45.00 (25.00–80.00) | 32.50 (10.00–67.50) | 0.55 | 0.02 |
| **rs10179904** | G | A | 20 | 2 | 0 | 41.25 (10.00–80.00) | 36.25 (27.50–45.00) | – | 0.71 | 0.01 |
| **rs11676382** | C | G | 22 | 0 | 0 | 41.25 (10.00–80.00) | – | – | – | – |
| **rs17026447** | A | C | 20 | 1 | 0 | 41.25 (10.00–80.00) | 52.50 | – | – | – |
| **Caucasian** | | | | | | | | | | |
| **rs7568458** | A | T | 6 | 18 | 12 | 32.75 (20.00–55.00) | 36.75 (17.50–80.00) | 40.63 (17.50–95.00) | 0.93 | <0.001 |
| **rs6751560** | G | A | 33 | 3 | 0 | 37.50 (17.50–95.00) | 42.50 (23.00–50.00) | – | – | – |
| **rs17026452** | G | C | 30 | 4 | 1 | 38.75 (17.50–95.00) | 28.75 (23.00–42.50) | 50.00 | 0.38 | 0.02 |
| **rs699664** | C | T | 12 | 22 | 2 | 40.63 (17.50–95.00) | 36.75 (17.50–80.00) | 36.25 (22.50–50.00) | 0.84 | <0.001 |
| **rs2592551** | G | A | 15 | 20 | 1 | 40.00 (17.50–95.00) | 38.75 (17.50–80.00) | 22.50 | 0.64 | 0.005 |
| **rs10179904** | G | A | 33 | 3 | 0 | 37.50 (17.50–95.00) | 42.50 (20.00–55.00) | – | – | – |
| **rs11676382** | C | G | 32 | 4 | 0 | 36.75 (17.50–95.00) | 41.63 (27.50–42.50) | – | 0.84 | <0.001 |
| **rs17026447** | A | C | 31 | 5 | 0 | 37.50 (17.50–95.00) | 42.50 (27.50–55.00) | – | 0.31 | 0.02 |

*The relationship between each SNP and log-transformed maintenance dose was adjusted for the *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variants.

## Table 4

Maintenance dose of warfarin by *ALB* SNPs, stratified by race

| SNP | Major allele (A) | Minor allele (B) | AA (*n*) | AB (*n*) | BB (*n*) | AA Median dose (min–max) | AB Median dose (min–max) | BB Median dose (min–max) | *P* value* | Partial $R^2$* |
|---|---|---|---|---|---|---|---|---|---|---|
| **African American** | | | | | | | | | | |
| **rs3775486** | C | A | 8 | 10 | 4 | 48.75 (26.25–67.50) | 40.00 (10.00–80.00) | 33.75 (27.50–47.50) | 0.37 | 0.05 |
| **rs3926327** | C | T | 19 | 1 | 1 | 45.00 (10.00–80.00) | 30.00 | 35.00 | 0.55 | 0.02 |
| **rs962004** | C | T | 6 | 13 | 3 | 33.75 (25.00–52.50) | 45.00 (10.00–80.00) | 35.00 (26.25–67.50) | 0.66 | 0.01 |
| **Caucasian** | | | | | | | | | | |
| **rs3775486** | C | A | 11 | 18 | 6 | 40.00 (23.00–63.00) | 39.38 (17.50–95.00) | 35.00 (22.50–45.00) | 0.19 | 0.03 |
| **rs3926327** | C | T | 19 | 16 | 1 | 40.00 (20.00–95.00) | 38.75 (17.50–80.00) | 23.00 | 0.24 | 0.03 |
| **rs962004** | C | T | 8 | 16 | 11 | 40.63 (22.50–95.00) | 40.00 (17.50–80.00) | 36.00 (23.00–63.00) | 0.39 | 0.01 |

*The relationship between each SNP and log-transformed maintenance dose was adjusted for the *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variants.

## Table 5

Maintenance dose of warfarin by *CYP2C9* SNP, stratified by race

| SNP | Major allele (A) | Minor allele (B) | AA (N) | AB (N) | BB (N) | AA Median dose (min–max) | AB Median dose (min–max) | BB Median dose (min–max) | *P* value* | Partial $R^2$* |
|---|---|---|---|---|---|---|---|---|---|---|
| **African American** | | | | | | | | | | |
| **rs2256871 (*9)** | A | G | 10 | 7 | 0 | 41.25 (10.00–67.50) | 52.50 (30.00–80.00) | – | 0.22 | 0.11 |
| **Caucasian** | | | | | | | | | | |
| **rs2256871 (*9)** | A | G | 26 | 2 | 0 | 35.50 (17.50–95.00) | 48.75 (42.50–55.00) | – | – | – |

*The relationship between each SNP and log-transformed maintenance dose was adjusted for the *VKORC1* 1173C>T and *CYP2C9* *2 and *3 variants.

Limdi and colleagues also evaluated whether other genetic variants within the *VKORC1* gene might explain more of the warfarin dose variability in African Americans [3]. However, because the MAF of the rs17886199 variant was below 5% and in strong linkage disequilibrium with the 1173C>T polymorphism in their study, it was not analyzed in their population. A possible explanation for the contrasting results is that our population of African Americans might originate from a different area within Africa and/or have less European ancestry which might have resulted in a higher MAF and lower linkage disequilibrium. In the University of Washington sequence data of African Americans, the MAF was 8% and there was no strong linkage disequilibrium with the 1173C>T polymorphism ($r^2 = 0.02$).

There are several limitations to our study. The main limitation is that this is a small pilot study which was only powered to detect very large warfarin dose difference between genotype groups. Even for a SNP with a MAF of 0.5, the difference in log-maintenance weekly dose needed to be 0.40 (which corresponds to an untransformed weekly dose of 11.66 mg) in African-Americans and 0.33 (which corresponds to an untransformed weekly dose of 9.86 mg) in Caucasians to have 80% power to detect an association. Given the small size of this study, the proportion of warfarin dose variability explained by the rs17886199 variant could not be precisely quantified. Further, because of the small sample size, we were unable to adjust for other potential confounding factors such as age, gender, smoking or concomitant medications that influence warfarin dosing, and therefore we likely overestimated the amount of dose variability explained by each SNP. Further, we did not have sufficient power to evaluate haplotypes. Another limitation is that we cannot exclude the possibility of type I error (false positives) due to the number of comparisons we made. Nonetheless, after applying Bonferroni correction for the nine tagSNPs we evaluated within the *VKORC1* gene, the rs17886199 variant was still associated with warfarin dosing in African Americans.

Despite these limitations, this study suggests that the *VKORC1* rs17886199 variant might influence warfarin dose in African Americans, even after correction for multiple testing. Further, it is possible that genetic variants in the *CYP2C9* and *EPHX1* gene might have a meaningful influence on warfarin dosing in African Americans and Caucasians beyond the effect of the known genetic variants. Nonetheless, future studies with larger sample size will be needed to confirm our findings before definitive conclusions can be made.

## Competing interests

Dr Schelleman has had travel to scientific conferences paid for by pharmacoepidemiology training funds contributed

by pharmaceutical companies. Ms Brensinger, has served as a statistical consultant for Pfizer unrelated to warfarin. Dr Chen and Mr. Finkelman have no competing interests to declare. Dr Rieder has applied for a patent related to genotyping for warfarin dosing. Dr Kimmel has received an honorarium from Ortho McNeil for a talk on warfarin. He has also done consulting work for GlaxoSmithKline, Novartis, Centocor, and Pfizer, all unrelated to warfarin. He has received grant funding from the Aetna Foundation for warfarin adherence research, from Pfizer for adherence research, and from the NIH for warfarin adherence and pharmacogenetics research.

*This study was funded by NIH grant R01HL066176 and P20RR020741. The funding agencies had no role in the design and conduct of the study, collection, management, analysis and interpretation of the data or preparation, review or approval of the manuscript. The authors thank Jane Jaskowiak at CCEB for her dedication to our field work. Further, we would like to acknowledge the Molecular Diagnosis and Genotyping Facility at the University of Pennsylvania where the genotyping was performed.*

## REFERENCES

**1** Wadelius M, Sorlin K, Wallerman O, Karlsson J, Yue QY, Magnusson PK, Wadelius C, Melhus H. Warfarin sensitivity related to CYP2C9, CYP3A5, ABCB1 (MDR1) and other factors. Pharmacogenomics J 2004; 4: 40–8.

**2** Schelleman H, Limdi NA, Kimmel SE. Ethnic differences in warfarin maintenance dose requirement and its relationship with genetics. Pharmacogenomics 2008; 9: 1331–46.

**3** Limdi NA, Beasley TM, Crowley MR, Goldstein JA, Rieder MJ, Flockhart DA, Arnett DK, Acton RT, Liu N. VKORC1 polymorphisms, haplotypes and haplotype groups on warfarin dose among African-Americans and European-Americans. Pharmacogenomics 2008; 9: 1445–58.

**4** Limdi NA, Goldstein JA, Blaisdell JA, Beasley TM, Rivers CA, Acton RT. Influence of CYP2C9 genotype on warfarin dose among African-Americans and European-Americans. Personalized Medicine 2007; 4: 157–69.

**5** Wadelius M, Chen LY, Eriksson N, Bumpstead S, Ghori J, Wadelius C, Bentley D, McGinnis R, Deloukas P. Association of warfarin dose with genes involved in its action and metabolism. Hum Genet 2007; 121: 23–34.

**6** Rieder MJ, Reiner AP, Rettie AE. Gamma-glutamyl carboxylase (GGCX) tagSNPs have limited utility for predicting warfarin maintenance dose. J Thromb Haemost 2007; 5: 2227–34.

**7** Wang TL, Li HL, Tjong WY, Chen QS, Wu GS, Zhu HT, Hou ZS, Xu S, Ma SJ, Wu M, Tai S. Genetic factors contribute to patient-specific warfarin dose for Han Chinese. Clin Chim Acta 2008; 396: 76–9.

**8** Pautas E, Moreau C, Gouin-Thibault I, Golmard JL, Mahe I, Legendre C, Taillandier-Heriche E, Durand-Gasselin B, Houllier AM, Verrier P, Beaune P, Loriot MA, Siguret V. Genetic factors (VKORC1, CYP2C9, EPHX1, and CYP4F2) are predictor variables for warfarin response in very elderly, frail inpatients. Clin Pharmacol Ther 2010; 87: 57–64.

**9** Moll S, Ortel TL. Monitoring warfarin therapy in patients with lupus anticoagulants. Ann Intern Med 1997; 127: 177–85.

**10** de Bakker PI, Burtt NP, Graham RR, Guiducci C, Yelensky R, Drake JA, Bersaglieri T, Penney KL, Butler J, Young S, Onofrio RC, Lyon HN, Stram DO, Haiman CA, Freedman ML, Zhu X, Cooper R, Groop L, Kolonel LN, Henderson BE, Daly MJ, Hirschhorn JN, Altshuler D. Transferability of tag SNPs in genetic association studies in multiple populations. Nat Genet 2006; 38: 1298–303.

**11** Kealey C, Chen Z, Christie J, Thorn CF, Whitehead AS, Price M, Samaha FF, Kimmel SE. Warfarin and cytochrome P450 2C9 genotype: possible ethnic variation in warfarin sensitivity. Pharmacogenomics 2007; 8: 217–25.

**12** Schelleman H, Chen Z, Kealey C, Whitehead AS, Christie J, Price M, Brensinger CM, Newcomb CW, Thorn CF, Samaha FF, Kimmel SE. Warfarin response and vitamin K epoxide reductase complex 1 in African Americans and Caucasians. Clin Pharmacol Ther 2007; 81: 742–7.

**13** Rieder MJ, Reiner AP, Gage BF, Nickerson DA, Eby CS, McLeod HL, Blough DK, Thummel KE, Veenstra DL, Rettie AE. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. N Engl J Med 2005; 352: 2285–93.

**14** Balding DJ. A tutorial on statistical methods for population association studies. Nat Rev Genet 2006; 7: 781–91.