BRIEF REPORT

Implications of Polymorphisms in the BCKDK and GATA-4 Gene Regions on Stable Warfarin Dose in African Americans

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VKORC1 and CYP2C9 genotypes explain less variability in warfarin dose requirements in African Americans compared with Europeans. Variants in BCKDK and GATA-4 gene regions, purported to regulate VKORC1 and CYP2C9 expression, have been shown to play an important role in warfarin dose requirements in Europeans and Asians, respectively. We sought to determine whether rs56314408 near BCKDK or GATA-4 rs2645400 influence warfarin dose requirements in 200 African Americans. Unlike the strong linkage disequilibrium (LD) between rs56314408 and VKORC1 rs9923231 in Europeans, they were not in LD in African Americans. No associations were found on univariate analysis. On multivariable analysis, rs56314408 was associated (P = 0.027) with dose in a regression model excluding VKORC1 rs9923231, and GATA-4 rs2645400 was associated (P = 0.032) with dose in a model excluding CYP2C (CYP2C9*2, *3, *5, *6, *8, and *11, CYP2C rs12777823) variants. Neither variant contributed to dose in the model that included both VKORC1 rs9923231 and CYP2C variants. Our results do not support contributions of the studied variants to warfarin dose requirements in African Americans. However, they illustrate the value of studies in African descent populations, who have low LD in their genome, in teasing out genetic variation underlying drug response associations. They also emphasize the importance of confirming associations in persons of African ancestry.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ The literature is limited in warfarin pharmacogenomic studies in African Americans. VKORC1 and CYP2C9 genotypes explain less variability in warfarin dose requirements in African Americans compared with those of European ancestry. Variants in BCKDK and GATA-4 gene regions, purported to regulate VKORC1 and CYP2C9 expression, have been studied in Europeans and Asians, respectively, but not in African Americans.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ We sought to determine whether rs56314408 near BCKDK or GATA-4 rs2645400 influence warfarin dose requirements in African Americans.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
✔ Our results do not support major contributions of studied variants to warfarin dose requirements in African Americans. However, our findings illustrate the value of studies in African descent populations, who have low linkage disequilibrium in their genome, in teasing out genetic variation underlying drug response associations. They also highlight the importance of confirming associations in persons of African ancestry.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
✔ Results do not support the inclusion of rs56314408 near BCKDK or GATA-4 rs2645400 in pharmacogenomic dosing algorithms for African Americans.

Warfarin remains commonly prescribed despite the availability of direct-acting oral anticoagulants.1 It is, however, a challenging drug to dose because of its narrow therapeutic index and pronounced interpatient variability in dose requirements. Warfarin dose requirements are influenced by VKORC1 and CYP2C9 genotypes and clinical factors.2 VKORC1-1639G>A (rs9923231) and CYP2C9*2 and *3 are the variants most commonly included in pharmacogenetic dosing algorithms and explain more than 30% of dose variability in Europeans, but only about 10% of the variability in African Americans.3–6 The CYP2C9*5, *6, *8, and *11 and the rs12777823 variant in the CYP2C cluster explain an additional 11% of the dose variability in African Americans.4,7,8 Pharmacogenomic studies on warfarin are mostly done in Europeans and Asians. There is, therefore, a gap in the literature on warfarin pharmacogenomic studies in minorities.

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like African Americans who show differences in minor allele frequencies (MAF) and have a lower linkage disequilibrium (LD) pattern.  

A recent study in a European population suggested that the rs56314408 C>T single nucleotide polymorphism (SNP), located on chromosome 16 in an enhancer upstream of the branched chain ketoacid dehydrogenase kinase (BCKDK) gene, could be a functional variant regulating VKORC1 gene expression. However, rs56314408 is in high LD with VKORC1 rs9923231 in Europeans, and thus, whether this SNP influences warfarin dose independent of the VKORC1 rs9923231 variant could not be determined in Europeans. Therefore, Cavalli et al. mentioned that it is warranted to genotype rs56314408 in warfarin-treated African Americans to see whether it improves warfarin dose predictions. In another study, the GATA binding protein 4 (GATA-4) rs2645400 T>G variant was significantly associated with stable warfarin dose in Asians with prosthetic cardiac valves having the homozygous wild-type (i.e., *1/*1) CYP2C9 genotype. On multivariable analysis, rs2645400/rs4841588 combination increased contribution to the overall warfarin dose variability. This study, therefore, suggested that GATA-4 plays a role in the regulation of CYP2C9 gene expression and can be predictive of stable warfarin dose. 

Neither rs56314408 nor rs2645400 has been studied in African Americans. Therefore, this study aimed to determine whether the rs56314408 variant near BCKDK, which is in low LD with VKORC1 rs9923231 in African Americans, and/or the GATA-4 rs2645400 variant are associated with warfarin dose requirements in African Americans and explain additional variability in dose beyond that of the VKORC1 and CYP2C (CYP2C9*2, *3, *5, *6, *8, and *11 and CYP2C rs12777823) polymorphisms. In our study, we are using these two SNPs as cases to illustrate the importance of carrying out pharmacogenomic studies in African Americans.

METHODS

Study population

This study included samples and data from a previously described cohort of warfarin-treated African Americans enrolled from the University of Illinois at Chicago who had reached a stable warfarin dose, defined as the dose that produced a therapeutic international normalized ratio for three consecutive clinic visits. Only those for whom sufficient DNA was available to genotyping were included. As we have previously reported and consistent with previous studies of genetic associations with warfarin response, exclusion criteria included history of liver dysfunction or serum transaminase levels greater than 3 times the upper limit of normal, or liver cirrhosis; advanced malignancy; hospitalization within 4 weeks before the index visit, which is the third clinic visit where informed consent was obtained and data were collected; or febrile/ diarrheal illness within 2 weeks of the index visit. In addition, patients with samples that did not genotype for at least one SNP were excluded, as well as patients requiring ≥ 90 mg/week, as doses ≥ 90 mg/week are often associated with warfarin resistance variants (e.g., VKORC1 variants) that were not genotyped as part of this study. The study was approved by the institutional review boards at the University of Florida and the University of Illinois at Chicago, and all patients provided written informed consent for the use of their samples and data. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2008.

Data collection

As previously described, either a buccal cell or venous blood sample was collected from each patient for genotyping. Clinical data were collected from the electronic medical record or through patient interview. Genotypes for VKORC1 rs9923231 and CYP2C variants were available from previous efforts.

Genotyping

Genomic DNA from clinical samples was isolated using the PureGene kit (Qiagen, Valencia, CA). Genotyping for rs56314408 and rs2645400 was carried out by polymerase chain reaction using HotStarTaq Plus Master Mix and followed by pyrosequencing on PSQ HS 96 system (Qiagen). The polymerase chain reaction and pyrosequencing primers are shown in Table S1.

Statistical analyses

The Hardy–Weinberg equilibrium (HWE) assumption was tested for each polymorphism of interest using the exact test on PLINK version 1.07. The $r^2$ and $D'$ values between rs56314408 and VKORC1 rs9923231 were determined using the Haploview. Weekly warfarin dose was tested for normality using the Shapiro–Wilks test.

Univariate analysis using nonparametric Kruskal–Wallis and Mann–Whitney $U$ tests was performed to determine genotype associations with weekly warfarin dose requirements. The Kruskal–Wallis test was performed for rs56314408 because an additive model was used for this polymorphism, and Mann–Whitney $U$ test was used for GATA-4 rs2645400 because only three patients were homozygous variant for this polymorphism, making a dominant model preferable. Multiple linear regression analysis was then conducted to test the association among each polymorphism and warfarin dose requirements, while adjusting for VKORC1 rs9923231 and CYP2C variants, separately and in different combinations. Each regression model included clinical factors (age, body surface area, history of stroke or transient ischemic attack, and current smoking status) previously associated with warfarin dose requirements in African Americans. The variability of warfarin dose explained, or $R^2$, was reported for each model. A $P$ value of 0.05 was considered statistically significant. Given a previous association between the GATA-4 rs2645400 SNP and warfarin dose specific to those with the CYP2C9*1/*1 genotype, subgroup analyses (univariate and multivariable) were also performed in this subset. Including at least 180 patients and using MAF of 0.09 provided 80% power to detect a difference in weekly warfarin dose by SNP of ≥ 8.7 mg with an alpha of 0.05. All analyses were conducted using SAS software version 9.4 Copyright 2013 (SAS Institute, Cary, NC).

RESULTS

A total of 200 patients were genotyped for rs56314408 and rs2645400, with at least 180 included in each...
analysis. The majority (78%) was women, with a mean age of 55 ± 16 years, mean body surface area of 2.0 ± 0.3 m², and median weekly warfarin dose of 42.5 mg (interquartile range 34.5–56.1 mg). Twenty-six percent of patients had history of stroke or transient ischemic attack, and 19% were current smokers. The MAFs of rs56314408 and rs2645400 were 0.46 and 0.10, respectively, which is consistent with that reported in the 1000 genomes African ancestry in Southwest USA (ASW) population (0.45 and 0.11, respectively).\(^{20}\) Both variants were in Hardy–Weinberg equilibrium. Rs56314408 and VKORC1 rs9923231 were not in LD in our African American population (\(r^2 = 0.07, D' = 0.77\)). Weekly warfarin dose was not normally distributed. Therefore, values were natural log-transformed prior to regression analysis.

Neither variant was associated with warfarin dose requirements on univariate analysis (Figure 1). On multiple linear regression, including clinical factors and CYP2C variants, rs56314408 was associated with warfarin dose requirements (\(P = 0.027, R^2 = 33\%\); Table 1, model 1). However, this association was no longer evident when VKORC1 rs9923231 was added to the model. Similarly, GATA-4 rs2645400 was associated with dose in a model, including clinical factors and VKORC1 rs9923231 (\(P = 0.032, R^2 = 31\%\); Table 1, model 2), but not after inclusion of the CYP2C variants into the model. A model including clinical factors plus VKORC1 rs9923231 and CYP2C variants explained 38% of the variability in warfarin dose requirements in the study population but showed no association between rs56314408 or rs2645400 and dose (Table 1, model 3).

When limiting the analysis to those with CYP2C9*1/*1 genotype, there was no statistically significant association between the GATA-4 rs2645400 SNP and warfarin dose on univariate analysis (\(P = 0.100\)). It was marginally associated in a multiple linear regression model with clinical factors and VKORC1 rs9923231 (\(P = 0.045, R^2 = 34\%\); Table S2, model 2a), but the association was no longer significant after adjusting for the CYP2C rs12777823 variant (\(P = 0.177, R^2 = 35\%\); Table S2, model 2b).

**DISCUSSION**

In our African American population, we found no association between rs56314408 near BCKDK or GATA-4 rs2645400 and warfarin dose requirements. These data, therefore, do not support that either of these polymorphisms provides a major contribution to warfarin dose variability in African Americans.

Our results with rs56314408 are inconsistent with those by Cavalli et al., who proposed that rs56314408, which is an allele-specific SNP in high LD with VKORC1 rs9923231 in Europeans, could be a functional variant regulating VKORC1 expression based on in vitro assays.\(^{10}\) Given the strong LD between rs56314408 and VKORC1 rs9923231 in Europeans, it is difficult to discern if the effects of rs56314408 on gene transcription result in important effects on warfarin response that are independent of the VKORC1 variant in this population. However, our data from African Americans, in whom there is no LD between rs56314408 and VKORC1 rs9923231, suggest that any effect of rs56314408 on transcription does not translate into clinically significant differences in warfarin dose requirements, at least in African Americans. Specifically, the loss of association between rs56314408 and warfarin dose when VKORC1 rs9923231 was added to the model suggests that rs56314408 does not influence warfarin dose requirements independent of the VKORC1 rs9923231 variant.

In a separate study of 201 Asian patients with prosthetic heart valves, none of 11 GATA-4 variants tested, including the rs2645400 variant, was associated with warfarin dose in the population overall.\(^{11}\) However, when limiting univariate analysis to those with the CYP2C9 *1/*1 genotype, the rs2645400 G allele was associated with lower stable warfarin dose. On multivariable analysis, the rs2645400/rs4841588 combination accounted for 1.2% of the overall interindividual variability in warfarin dose requirements.\(^{11}\) GATA-4 is a liver-specific transcription factor recently shown to play an important role in regulating CYP2C9 gene expression.\(^{21}\) Although we observed a marginally significant association between the GATA-4 rs2645400 SNP and warfarin dose in the subpopulation of CYP2C9*1 allele homozygotes on multiple linear regression, it was no longer associated after adjusting for CYP2C rs12777823, a SNP associated with warfarin clearance and dose requirements in African ancestry patients.\(^{8}\) It is possible that the lower frequency of the SNP in African Americans vs. Asians contribute to the disparate findings between studies. Of note, we did not genotype for the rs4841588 SNP examined in the Jeong et al. study,

![Figure 1](image-url)  
**Figure 1** Warfarin dose requirements by (a) rs56314408 near the BCKDK gene and (b) GATA-4 rs2645400. In the boxplots shown, horizontal lines within boxes represent medians. The lower and upper boundaries represent the 25th and 75th percentiles, respectively. The lower and upper whiskers represent the minimum and maximum warfarin doses that are not outliers, respectively.
and, thus, cannot comment on its association in African Americans.

Our study with the rs56314408 variant further demonstrates the value of studies in African Americans in pinpointing the responsive variant underlying drug response associations within a haplotype block. This has been similarly demonstrated with polymorphisms within the VKORC1 gene. Specifically, Rieder et al. identified five polymorphisms (−4931T>C, −1639G>A, 1173C>T, 1542G>C, and 2255C>T) in strong LD in a European ancestry population defining a VKORC1 haplotype that conferred significant effects on warfarin dose requirements.22 However, the specific polymorphism underlying the dose association was unknown and could not be determined from dose association studies in European ancestry populations. In subsequent studies in African Americans, in whom there is significantly lower LD in the VKORC1 gene, we and others found that −1639G>A, but not 1542G>C, was responsible for observed associations between VKORC1 genotype and warfarin dose requirements.23,24

Our findings with the GATA-4 rs2645400 genotype emphasize that an association observed in one ethnic population should not be generalized to other ethnic populations, particularly those of African ancestry. This has been previously demonstrated with the CYP4F2 genotype, which is associated with higher warfarin dose requirements in European and Asian ancestry populations, but not in African Americans.13,25,26 As a result, recent guidelines by the Clinical Pharmacogenetics Implementation Consortium (CPIC) make no recommendations regarding the CYP4F2 genotype for persons of African ancestry, but recommend higher doses with the CYP4F2 variant allele in non-African ancestry populations.27

In conclusion, our results do not support a major contribution of either the BCKDK rs56314408 or GATA-4 rs2645400 variant to warfarin dose requirements in African Americans. However, we cannot rule out that these variants may provide smaller contributions to warfarin dose requirements that we were unable to detect because of our limited sample size. Importantly, our findings illustrate the value of studies in African descent populations in teasing out genetic variation underlying drug response associations. They also highlight the importance of confirming associations in persons of African ancestry.

Supporting Information. Additional supporting information may be found in the online version of this article at the publisher’s website:

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1. Kirley, K., Qato, D.M., Kornfield, R., Stafford, R.S. & Alexander, G.C. National trends in oral anticoagulant use in the United States, 2007 to 2011. Circ. Cardiovasc. Qual. Outcomes 5, 615–621 (2012).
2. Wadelius, M. & Pirmohamed, M. Pharmacogenomics of warfarin: current status and future challenges. Pharmacogenomics J. 7, 99–111 (2007).
3. Limdi, N.A. et al. VKORC1 polymorphisms, haplotypes and haplotype groups on warfarin dose among African-Americans and European-Americans. Pharmacogenomics 9, 1445–1458 (2008).
4. Cavalli, L.H. et al. Genetic and clinical predictors of warfarin dose requirements in African Americans. Clin. Pharmacol. Ther. 87, 459–464 (2010).
5. Gage, B.F. et al. Use of pharmacogenetic and clinical factors to predict the therapeutic dose of warfarin. Clin. Pharmacol. Ther. 84, 326–331 (2008).
6. Caldwell, M.D. et al. Evaluation of genetic factors for warfarin dose prediction. Clin. Med. Res. 5, 8–16 (2007).
7. Liu, Y. et al. Decreased warfarin clearance associated with the CYP2C9 R150H (∗8) polymorphism. Clin. Pharmacol. Ther. 91, 660–665 (2012).
8. Perera, M.A. et al. Genetic variants associated with warfarin dose in African-American individuals: a genome-wide association study. Lancet 382, 790–796 (2013).
9. Perera, M.A., Cavallari, L.H. & Johnson, J.A. Warfarin pharmacogenetics: an illustration of the importance of studies in minority populations. Clin. Pharmacol. Ther. 95, 242–244 (2014).
10. Cavalli, M. et al. Novel regulatory variant detected on the VKORC1 haplotype that is associated with warfarin dose. Pharmacogenomics 17, 1305–1314 (2016).
11. Jeong, E., Lee, K.E., Jeong, H., Chang, B.C. & Gwak, H.S. Impact of GATA4 variants on stable warfarin doses in patients with prosthetic heart valves. Pharmacogenomics J. 15, 33–37 (2015).
12. Aquilante, C.L. et al. Influence of coagulation factor, vitamin K epoxide reductase complex subunit 1, and cytochrome P450 2C9 gene polymorphisms on warfarin dose requirements. Clin. Pharmacol. Ther. 79, 291–302 (2006).
13. Caldwell, M.D. et al. CYP4F2 genetic variant alters required warfarin dose. Blood 111, 4106–4112 (2008).
14. Rost, S. et al. Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2. Nature 427, 537–541 (2004).
15. Andrisin, T.E., Humma, L.M. & Johnson, J.A. Collection of genomic DNA by the non-invasive mouthwash method for use in pharmacogenetic studies. Pharmacotherapy 22, 954–960 (2002).
16. Purcell, S. et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. 81, 559–575 (2007).
17. Shaun Purcell. PLINK Version 1.07 (2009). September 19, 2020.
18. Barrett, J.C., Fry, B., Maller, J. & Daly, M.J. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265 (2005).
19. Hernandez, W. et al. Ethnicity-specific pharmacogenetics: the case of warfarin in African Americans. Pharmacogenomics J. 14, 223–228 (2014).
20. Genomes Project C. et al. A global reference for human genetic variation. Nature 526, 68–74 (2015).
21. Mwinyi, J. et al. New insights into the regulation of CYP2C9 gene expression: the role of the transcription factor GATA-4. Drug Metab. Dispos. 38, 415–421 (2010).
22. Rieder, M.J. et al. Effect of VKORC1 haplotypes on transcriptional regulation and warfarin dose. N. Engl. J. Med. 352, 2285–2293 (2005).
23. Momary, K.M. et al. Factors influencing warfarin dose requirements in African-Americans. Pharmacogenomics 8, 1535–1544 (2007).
24. Wang, D. et al. Regulatory polymorphism in vitamin K epoxide reductase complex subunit 1 (VKORC1) affects gene expression and warfarin dose requirement. Blood 112, 1013–1021 (2008).
25. Chai, P.C. et al. Genome-wide association study identifies genetic determinants of warfarin responsiveness for Japanese. Hum. Mol. Genet. 19, 4735–4744 (2010).
26. Bress, A. et al. Effect of N001 and CYP4F2 genotypes on warfarin dose requirements in Hispanic-Americans and African-Americans. Pharmacogenomics 13, 1925–1935 (2012).
27. Johnson, J.A. et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for pharmacogenetics-guided warfarin dosing: 2017 update. Clin. Pharmacol. Ther. 102, 397–404 (2017).

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