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Influence of *CYP2C9* and *VKORC1* on warfarin dose, anticoagulation attainment and maintenance among European American and African Americans

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Abstract

Aims—The influence of *CYP2C9* and *VKORC1* on warfarin dose, time to target INR, time to stabilization, and risk of over-anticoagulation (INR>4) was assessed after adjustment for clinical factors, intra-individual variation in environmental factors and unobserved heterogeneity.

Materials and Methods—Common *CYP2C9* and *VKORC1* polymorphisms were assessed in 302 European American and 273 African Americans on warfarin. Race-stratified multivariable analyses evaluated the influence of *CYP2C9* and *VKORC1* on warfarin response.

Results and Conclusion—*CYP2C9* and *VKORC1* accounted for up to 30% variability in warfarin dose among European Americans and 10% among African Americans. Neither *CYP2C9* nor *VKORC1* influenced time to target INR or stabilization among patients of either race or risk of over-anticoagulation among African Americans. The risk of over-anticoagulation was higher among European Americans with variant *VKORC11173C/T* ($p<0.01$) and marginally significant among those with variant *CYP2C9* ($p=0.08$) genotype.

Although *CYP2C9* and *VKORC1* genotyping can facilitate individualized initiation of warfarin dose in African and European Americans, the ability to predict risk of over-anticoagulation is inconsistent across race. Identification of other factors that can predict such risk consistently in a racially diverse group will facilitate individualized maintenance of warfarin therapy.

Keywords

Warfarin; Pharmacogenetics; CYP2C9; VKORC1; over-anticoagulation; cohort study; African Americans; European Americans

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Introduction

Although the efficacy of warfarin in the treatment and prevention of thromboembolic disorders (TEDs) is proven,[1,2] it is underutilized with difficulties in management and risk of complications being the main deterrents.[3–6] Recognition of genetic regulation of warfarin response has stimulated efforts aimed at quantifying this influence, but past efforts have focused on a limited number of polymorphisms in populations of mainly European descent. [7–17]Race appears to influence warfarin dose requirements with African Americans requiring larger and Asians requiring lower doses compared to Europeans.[18–23] This variation in dose requirement by race may at least partially be explained by genetic differences. Cytochrome P4502C9 (*CYP2C9)*2* and **3* variants are reported to have significant influence on warfarin dose among patients of European descent. [7–17] However among patients of African descent the *CYP2C9*-dose association has not been consistent.[24–26]African American populations contain known or putative poor-metabolizer alleles (*CYP2C9**5, *CYP2C9**6 and *CYP2C9**11) which are rarely found in European Americans.[27] However the influence of these variants has not been extensively documented. In contrast to the influence of *CYP2C9*, Vitamin K epoxide reductase (*VKORC1)1173C/T* variants have been shown to influence warfarin dose requirements among patients of both racial groups.[28]

*CYP2C9*2* and **3 VKORC11173C/T* variants have been associated with an increased risk of over-anticoagulation among European Americans[7,13,25,28–30] but not African Americans. [25,28] It is recognized that while genes may influence inter-individual variation in warfarin response, for the intra-individual variation environmental factors may be more important.[2, 17] For example in any given patient, the International Normalized Ratio (INR) deviates from target (2.5, range 2–3) over time in response to many independent perturbations. Such intra-individual variation in INR due to unobserved factors introduces heterogeneity. Difficulties in capturing and accounting for unobserved heterogeneity may underestimate the environmental contribution (e.g. medication changes) and overestimate the genetic contribution in explaining variance in warfarin response and limit the ability to assess gene-environment interactions. [17]

Herein we evaluate the influence of *CYP2C9* (*2 (rs1799853), *3 (rs1057910), *5 (rs28371686), *6 (rs9332131), and *11(rs28371685)) and four *VKORC1* single nucleotide polymorphisms [SNPs: *1173C/T* (rs9934438), *3730G/A* (rs7294), *2255C/T* (rs2359612), *1542G/C* (rs8050894)] on warfarin maintenance dose. We also assess the influence of *CYP2C9* and *VKORC1* polymorphisms on anticoagulation status and risk of over-anticoagulation after accounting for unobserved heterogeneity and clinical factors.

Patients & methods/Materials & methods

The Pharmacogenetic Optimization of Anticoagulation Therapy (POAT) is an ongoing prospective cohort study aimed at defining the influence of *CYP2C9* polymorphisms on warfarin response over a 2-year follow-up period.[26,31,32] The study is being conducted at the University of Alabama at Birmingham (UAB) enrolling patients from the anticoagulation clinic at The Kirklin Clinics (TKC-AC) and the Jefferson Clinic P.C., Jefferson County Health System (CGH-JC) under the approval of the respective Institutional Review Boards. Both clinics follow a standardized approach to manage anticoagulation therapy.[33]

Inclusion and Exclusion

Patients ≥20 years of age were identified at the initiation of warfarin therapy. Patients were considered eligible if the intended duration of anticoagulation therapy was at least 2 years, the target INR range was 2–3 and are managed at one of the two anticoagulation clinics.

Data Collection

A structured interview form was used at the time of enrollment to obtain a detailed medical lifestyle and concomitant medication history. Information on self-reported race, indication for therapy, demographics, height and weight, medications and co-morbid conditions was documented. Lifestyle and socioeconomic data included smoking, alcohol use, education, annual household income, medical insurance, physical activity, and dietary vitamin K intake. Medical history was then verified by medical records review.

All patients were followed at monthly intervals for up to two years from initiation of therapy. At each visit factors influencing warfarin response such as warfarin dose, INR, concurrent medications, dietary vitamin K (number of servings of foods rich in vitamin K consumed per week)[34] alcohol intake (number of alcoholic drinks per week), compliance and level of physical activity were documented.[35–37] Information on concurrent medications was updated at every clinic visit by self-report and verified by medical record review. Concomitant use of drugs such as non-steroidal anti-inflammatory drugs or antiplatelet agents, or drugs that alter warfarin pharmacokinetics, including CYP2C9 inhibitors (e.g., amiodarone), CYP2C9 inducers (e.g., rifampin), or CYP2C9 substrates (e.g., losartan) was recorded.[38,39]

DNA extraction and genotyping methodology for *CYP2C9* and *VKORC1* polymorphisms were detailed in recent manuscripts.[26,31] Blood (8ml) was collected in a Qaigen PAX gene tube and DNA was extracted using the PAXgene blood DNA extraction kits. Briefly *CYP2C9* genotyping was conducted using pyrosequencing methods and PCR-RFLP methodology. *VKORC1* genotyping (rs9934438, rs7294, rs2359612 and rs8050894) was conducted using the Sequenom iPLEX technology[40] at the Broad Institute.

Outcome definitions and Statistical Methods

Analysis of variance was used to assess group differences for continuous variables and χ^2 test of independence for categorical variables. The assumption of Hardy Weinberg Equilibrium (HWE) was tested using the χ^2 test of independence and exact statistics were obtained using a Markov Chain Monte Carlo algorithm.[41]

Dose was defined in two ways: the average maintenance dose required to maintain anticoagulation for the duration of therapy and stable dose defined as the first dose that leads to a stable INR over three consecutive visits following initiation of the drug. These INR measurements encompassing a period of at least 2 weeks, with a maximum difference between the mean daily dosages of 10%.[7] The distribution of dose was marginally skewed to the right therefore log transformation was done to attain normality. Race-stratified linear-regression analysis was conducted to assess the influence of *CYP2C9, VKORC1* genotype on log-transformed dose after adjustment for age, gender, BMI, clinic, income, education, health insurance, smoking status, level of physical activity, alcohol intake, vitamin K intake, comorbid conditions (e.g. CHF, renal failure and cancer) and drug interactions (e.g. amiodarone, statins, NSAIDs, antiplatelet agents). We evaluated dose genotype associations using models that assessed the additive and dominant effects of *VKORC1* and *CYP2C9*. Genotypes were included as covariates with 3-levels in the additive models and as covariates with 2 levels (wild-type versus variant) in the dominant models. The effect size associated with each predictor was calculated as the percentage of the variation in warfarin dose explained by the predictor, divided by the total variance in the regression model.

We also examined the time required to attain target INR (calculated as the difference in days from initiation of therapy to achieving the first INR in target range) and time to attain stable dose (calculated as the time from the initiation of therapy until attainment of stable dose) using the Cox Proportional Hazard (PH) models. To assess the risk of over-anticoagulation (INR>4)

the hazard ratio (HR) and 95% CI were obtained using the counting process format in the PH model.[42,43] This format allows individuals to contribute more than one event (INR>4). Valid confidence intervals were obtained by correction of dependence using robust variance estimation.[44]

Accounting for unobserved heterogeneity

In any given patient, INR deviates from target (2.5, range 2–3) over time in response to many independent perturbations. Such intra-individual variation in INR due to unobserved factors introduces heterogeneity, herein termed unobserved heterogeneity.

1. Although the fluctuation in INR control due to medication changes is accounted for in the counting process format analyses, inability to accurately quantify this interaction (e.g. plasma concentrations) limits our ability to account for all the heterogeneity introduced by such changes in drug regimen.
2. Changes in diet (e.g. vitamin K intake) are documented as change from the prior visit, not quantified using a full vitamin K inventory (or plasma concentrations) at each visit. This can potentially limit our ability in explaining the variance in INR due to more subtle changes in diet, thereby increasing heterogeneity.[45]
3. As the interval between INR measurements increases, the variation in INR increases.[46] Therefore by default patients who are evaluated more often (e.g. patients with higher comorbidity require more frequent consultations) may tend to have lower variation (heterogeneity) in the INR.[47]

We recognize that such unobserved heterogeneity may potentially influence the time to stabilization and over-anticoagulation. To capture its effect we computed a patient-specific variance growth rate (Vscore), a cumulative measure of time-weighted variance of the INR for each time interval (between visits) as proposed by Fihn et al,[48] with minor modification. This measure adjusts for the influence of the number of visits and the interval between visits on INR variation for each patient. The use of the Vscore from the preceding interval accounts for the patient-specific unobserved heterogeneity in the analyses of time to stabilization and time to over-anticoagulation.

$$\sigma^2 = \frac{1}{n-1}\sum_{i=1}^{n-1}\frac{(INR_i - 2.5)^2}{\tau_i} \qquad \text{Equation 1}$$

$\sigma 2$ = variance growth rate (Vscore)

n = number of visits (n-1 will compute the Vscore up to the preceding visit)

τ_i = duration in weeks since preceding clinic visit (INR measurement)

INR_i = International Normalized ratio at the i^{th} visit (target INR was 2–3 for all participants)

Modified from Fihn et al [48]

All multivariable analyses included *CYP2C9, VKORC1* genotype, age, race, gender, BMI, socio-demographic factors, clinic, indication and comorbid conditions. Changes in medications, Vscore, vitamin K and alcohol intake, and level of physical activity, warfarin dose and INRs were included as time-varying covariates. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC) at a non-directional alpha level of 0.05.

Results

Patients meeting eligibility criteria between August 2003 and April 2007 (n=621) were asked to participate in the study. Forty-three (6. 9%) patients declined participation. The cohort was comprised mainly of African Americans (47.2%) and European Americans (52.2%). As described in previous reports,[26,31,32] there were no significant differences in gender distribution, length of follow-up or BMI across race groups. European American patients were older, more physically active, and more likely to be light drinkers (1–7 drinks/week); enrolled from TKC-AC, have medical insurance, higher education and higher income (Table 1).

Stroke and venous thromboembolism were more common indications for therapy in African American patients, while atrial fibrillation and valvular heart disease were more common in European Americans. The prevalence of individual comorbid conditions differed across racial groups. More European American patients had undergone coronary artery bypass grafting (CABG) or percutaneous coronary angioplasty (PTCA), had hyperlipidemia and malignancies while the prevalence of end stage renal disease and renal insufficiency was higher among African Americans. Other than the higher use of antiplatelet agents at baseline, the use of non-steroidal analgesics, *CYP2C9* inhibitors or substrates was not significantly different across racial groups African American patients had a greater intra-individual variation in INR, captured by the variable VScore (p=0.026, Table 2).

Genotype distributions for *CYP2C9* and *VKORC1* were in HWE among European Americans (all p-values >0.5) and African Americans (all p-values >0.25). Of the variant *CYP2C9* alleles tested only *CYP2C9**5, CYP2C9*6, and *CYP2C9**11 were observed among African Americans while *CYP2C9**2, and *CYP2C9**3 were observed among European Americans and African Americans. European Americans had higher frequency of variant genotype for *CYP2C9, 1173C/T*, *2255C/T* and *1542G/C*, whereas African Americans had higher variant allele frequencies for *3730G/A* (Table 3). Due to significant racial differences in covariates (Tables 1 and 2) and frequencies of *CYP2C9* and *VKORC1* variants (Table 3) all further analyses were stratified by race.

Influence of *CYP2C9* and *VKORC1* Genotype on warfarin dose

In univariate analysis, among European Americans, variant *CYP2C9* and *VKORC1* (*1173C/T*, *2255C/T* and *1542G/C*) genotypes were associated with lower (all p-values <0.01) and *3730G/A* was associated with higher (p =0.008) warfarin dose. Among African Americans, variant *VKORC1* (*1173C/T*, *2255C/T*) genotypes were associated with lower dose (p <0.005) while variant *CYP2C9* (p =0.12) and *VKORC1* (*1542G/C*, p =0.15 and *3730G/A*, p =0.2) genotypes did not have a significant influence. Univariate associations of *CYP2C9* and *VKORC1* variants with dose demonstrate the higher dose requirements among African Americans (Figure 1).

Among European Americans, prior reports indicate strong linkage disequilibrium between the four *VKORC1* SNPs.[49,50] Therefore multivariable analyses included the most informative *VKORC1* SNP (*1173C/T*) along with *CYP2C9* and clinical covariates. Since the LD structure for *VKORC1* in African Americans is unknown, all four *VKORC1* SNPs were included in the initial model. However the final model retained only *VKORC11173C/T* as a significant predictor.

African Americans required higher warfarin maintenance doses compared to European Americans (Figure 2) after adjusting clinical and genetic covariates. The variability in dose explained by the *VKORC11173C/T* and *CYP2C9* was lower in African Americans compared to European Americans (p<0.0001). After adjusting for potential confounders, variant *CYP2C9* and *VKORC11173C/T* genotypes explained 30% variance in warfarin dose (20% in

the dominant model) among European Americans compared to 10% variance in does (7% in the dominant model) among African Americans (Table 4). The variance in dose accounted for by *CYP2C9* and *VKORC11173C/T* genotypes was similar for both dose definitions; average maintenance dose and first stable dose. The interaction between *CYP2C9* and *VKOR1173C/T* genotype was not significant for either race group. Age, gender, BMI, vitamin K intake, concomitant therapy with *CYP2C9* inhibitors also had significant influence on warfarin dose among patients of both race groups ($p<0.05$).

Influence of *CYP2C9* and *VKORC1173C/T* on anticoagulation control

We assessed the effects of *VKORC11173C/T* on time to target INR, stabilization or risk of over-anticoagulation after accounting for clinical covariates and unobserved heterogeneity (Table 5).

Neither *CYP2C9* nor *VKORC11173C/T* influenced the time to attain target INR or time to stabilization among patients of either race. European Americans patients with variant *VKORC11173C/T* genotype showed a 2 to 4 fold higher risk of over-anticoagulation (Figure 3a). Those with variant *CYP2C9* genotype were at a 2 to 3 fold higher risk; however, this finding was of marginal statistical significance (Table 5, Figure 3b). Neither *VKORC11173C/T* nor *CYP2C9* was significantly associated with risk of over anticoagulation among African Americans (Table 5, Figure 3c and 3d).

The interaction of *CYP2C9* and *VKOR1173C/T* genotypes was not significant for any endpoint. Time to stable dosing was not significantly influenced by Vscore prior to stabilization for patients of either race (p-values >0.1). Higher Vscore was associated with a significantly higher risk of over anticoagulation through out the duration of therapy ($p<0.0001$) among both racial groups. The risk was higher during the first 30 days of therapy ($p=0.06$ for European Americans, $p=0.03$ for African Americans) but not around the time when stabilization is first achieved ($p=0.56$ for European Americans, $p=0.81$ for African Americans).

Discussion

This prospective cohort study demonstrates the contribution of *CYP2C9* and *VKORC1* variants in determining warfarin dose in a racially diverse cohort. *VKORC11173C/T* genotype explains a higher proportion of variance in warfarin dose compared *CYP2C9* for both race groups.

By evaluating both additive and dominant effects of *VKORC1* and *CYP2C9* we demonstrate the variance in warfarin dose explained is higher when *VKORC1* and *CYP2C9* genotype covariates were modeled on an additive scale compared to dominant scale. This finding was consistent among both European Americans and African Americans. Among African Americans, the variance in warfarin dose explained by *CYP2C9* and *VKORC1* (dominant model) was consistent with prior reports.[25,26,28] However, contrary to prior reports and concordant with those of Momary et al,[24] the additive model explained a higher percent variance and revealed statistically significant effects of *CYP2C9* on warfarin dose in African Americans. Consistent with prior reports[24] and others age, gender, BMI, vitamin K intake, concomitant therapy with *CYP2C9* inhibitors also had significant influence on warfarin dose among patients of both race groups.[8,10–12,15,16,24,50–56]

By defining dose in two ways; dose at first stabilization and average maintenance dose we demonstrated consistent influence of *VKORC1* and *CYP2C9* genotype regardless of definition. *VKORC1* and *CYP2C9* along with clinical covariates explained up to 55% of the variance in dose in European Americans (up to 40% among African Americans) with *VKORC1* and *CYP2C9* accounting for up to 30% (10% among African Americans). Among European Americans these findings are consistent with the prior reports.[10,11,15,19–21,23,49,50,57,

58] However the latter estimates, derived from mainly retrospective studies in homogeneous populations, may not hold in racially diverse populations as demonstrated by Schellman et al. [28] In concordance with their findings, the variability in dose explained by the *VKORC11173C/T* and *CYP2C9* was lower in African Americans compared to European Americans.

Consistent with Schellman et al[28] and Kealy et al,[25] our findings demonstrate the lack of influence of *VKORC1* and *CYP2C9* on time to stabilization in patients of either race. However these findings are discordant with those reported by Schalekamp et al.[7,13,29,30] This discordance can potentially be explained by several factors including differences in the coumarin anticoagulant, dose initiation strategies, and important protocol differences such as exclusion of interacting drugs.

As reported by Schellman et al,[28] the *VKORC11173'T*' allele was significantly associated with an increased risk of over-anticoagulation among European Americans but not African Americans. The *CYP2C9* variant was marginally associated with an increased risk of over-anticoagulation among European Americans, which is consistent with previous studies[25]. Although we do not know the reason for the racial differences, we can speculate on the influence and interplay of various factors:

1. The contribution of unmeasured genetic/environmental factors to INR fluctuation may differ by race. This was supported by the significance of race-gene interactions in race adjusted analyses for time to stabilization (*VKORC1 1173C/T* x race, p=0.03) and risk of over anticoagulation (*VKORC1 1173C/T* x race, p=0.002; *CYP2C9* x race, p=0.09). Recognizing race specific differences we chose to conduct stratified analyses.
2. We had to combine the '*CT* and *TT*' genotypes for multivariable analyses because of the small number of patients with the '*TT*' genotype thereby diluting the effect of *VKORC1* polymorphism on risk of over-anticoagulation.
3. The association between the *VKORC1* polymorphisms studied and the causative polymorphism(s) that determines warfarin response is weaker in African Americans compared with European Americans because of different haplotype structures.
4. Genetic and environmental factors other then those studied influence the risk of over-anticoagulation in African Americans. This idea is supported by the higher intra-individual variation in INR among African Americans compared to European Americans.

Among European Americans, the risk ratios in our study were lower than those reported by Kealy et al and Schellamn et al.[25,28] This can perhaps be explained by the inclusion of a measure of unobserved heterogeneity in the analyses. Inability to account for such heterogeneity has been recognized as a limitation by several investigators.[9,17,28] Our results provide evidence that the higher risk of over-anticoagulation associated with variant *VKORC11173C/T* among European Americans is independent of such heterogeneity.

We recently reported a significantly increased risk of major hemorrhage conferred by variant *CYP2C9* genotype but not by variant *VKORC1 1173C/T* genotype after adjusting for the influence of INR at the time of the event and other clinical covariates. The risk was statistically significant among European Americans but not in African Americans. The latter finding may be due to the lower frequency of the variant genotypes in African Americans.[31] Along with *CYP2C9*, an elevated INR significantly increased the risk of major hemorrhage. For every unit increase in INR the risk of major hemorrhage increased by 75% (HR 1.74, 95%CI: 1.5, 2.1). In both African American and European American patients these gene-response associations highlight two things; the risk of hemorrhage is higher among patients who possess *CYP2C9*

variants and the risk of hemorrhage is higher among patients who have elevated INR. These effects are independent of each other.

To our knowledge, our cohort represents the largest population of African Americans genotyped for *CYP2C9* and *VKORC1*. Inclusion of the *5, *6 and *11 variants in the genotyping provides a robust estimate of the *CYP2C9* allele frequencies in this previously underrepresented racial group. We examined only four SNPs in the *VKORC1* gene (*1173C/T, 3730G/A, 2255C/T, 1542G/C*). We did not assess the -1639G/A polymorphism (rs9923231) as studies have demonstrated that the 1173C and -1639G allele are in linkage disequilibrium among both African Americans[28] and European Americans.[49] Three of these polymorphisms (*1173C/T, 2255C/T, 1542G/C*) are part of the haplotype that has been associated with a relatively low hepatic *VKORC1* mRNA expression in the liver of European Americans.[49] Furthermore, Rieder et al showed that the *1173C/T* polymorphism alone was as informative as *VKORC1* haplotypes for predicting warfarin dose in a Caucasian population.[49] The haplotype structure differs significantly between persons of European versus African descent[20,59] and may differ among African Americans across the US depending on the degree of racial admixture.[60–62] Therefore all four SNPs were included in the initial models for African Americans. Assessment of other *VKORC1* polymorphisms will help determine haplotype structure and may identify other influential *VKORC1* polymorphisms among African Americans.

We also recognize our sample-size was inadequate to detect significant *CYP2C9*-*VKORC1* interaction in either race group. After adjusting for statistically significant and clinically relevant covariates a post-hoc assessment of power demonstrates the adequacy of the cohort size to detect significant dose differences (between variant and wild-type genotype) for *VKORC1* among European Americans and African Americans and for *CYP2C9* among European Americans (power >80%). However among African Americans significant dose difference was not detected for *CYP2C9* (power ~40%) except when it was modeled on an additive scale for average dose. For most anticoagulation endpoints the risk ratios detected were consistent with the null for both European Americans and African Americans. Only risk ratios for INR >4 demonstrated an increased risk of over-anticoagulation among European Americans with variant VKORC1 genotype (power >80%) but not for variant CYP2C9 genotype (power ~70%). Documentation of vitamin K intake was based on patient report using vitamin K inventory and was not quantified by assay/measurements.[34] However, all measurements were used consistently; therefore, bias if any should be non-differential. We recognize that many factors including changes in vitamin K intake can contribute to INR fluctuation.[45,63] The inclusion of the Vscore potentially accounts for the changes in unmeasured/unobserved environmental influences. We assessed the influence of only two genes (*CYP2C9* and *VKORC1*) and recognize that other genes may influence warfarin response or modify the effect of these genes. ApoE has recently been shown to influence warfarin dose among African Americans. Other genes such as gamma-glutamyl carboxylase, calumenin, epoxide hydroxylase may influence warfarin dose in this race group. However, the extent to which variability in other genes in the warfarin pathways influences warfarin response is yet to be resolved.

Conclusion

In conclusion the *CYP2C9* and *VKORC1* variants are associated with lower warfarin dose requirements among both African Americans and European Americans. Although *CYP2C9* and *VKORC1* genotyping have the potential to facilitate the development of individually tailored warfarin dose in both African and European Americans, the ability to predict risk of over-anticoagulation is limited to European Americans. Perhaps future identification of other genetic/environmental factors that can predict such risk consistently in the racially diverse

group of patients encountered in clinical practice will facilitate individually tailored maintenance of warfarin therapy.

Executive Summary

- The limited representation of African Americans has hindered our understanding of genetic influences on warfarin response in this racial group.
- In this prospective cohort study we assessed the effect of *CYP2C9* *2, *3, *5, *6 and *11 and four *VKORC1* polymorphisms in African Americans and European Americans after adjustment for numerous mediators of warfarin response.
- We assessed the influence of *CYP2C9* and *VKORC1* polymorphisms on both stable dose and average maintenance dose. The evaluation of gene effects on both dominant and additive scale demonstrates the importance of both genes in determining warfarin dose in African Americans and European Americans.
- In assessing the risk of over-anticoagulation we accounted for unmeasured heterogeneity.
- *VKORC1* variants are associated with a higher risk of over-anticoagulation (with *CYP2C9* demonstrating a marginal significance) and in European Americans but not in African Americans.
- Although *CYP2C9* and *VKORC1* variants influence warfarin dose in both African and European Americans, further research is needed to identify genetic and environmental determinants of over-anticoagulation in African Americans.

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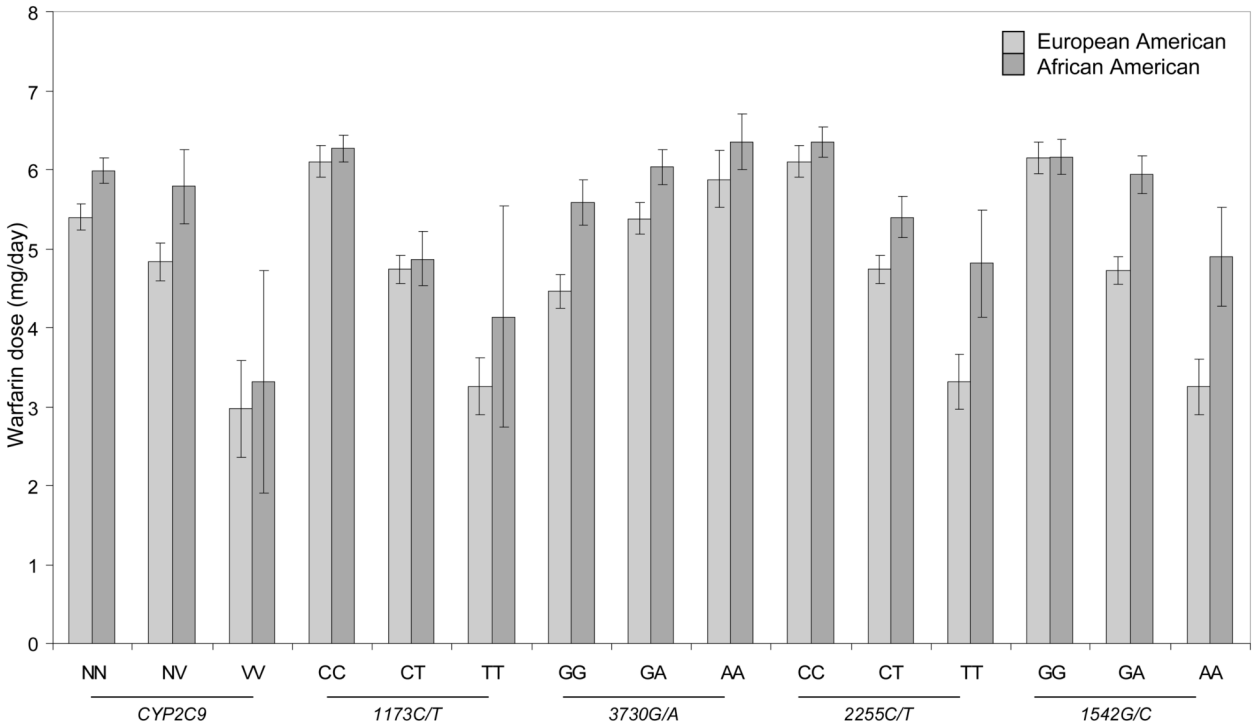

Figure 1.
Mean warfarin dose (mg/day) by *CYP2C9* and *VKORC1*, stratified by race.

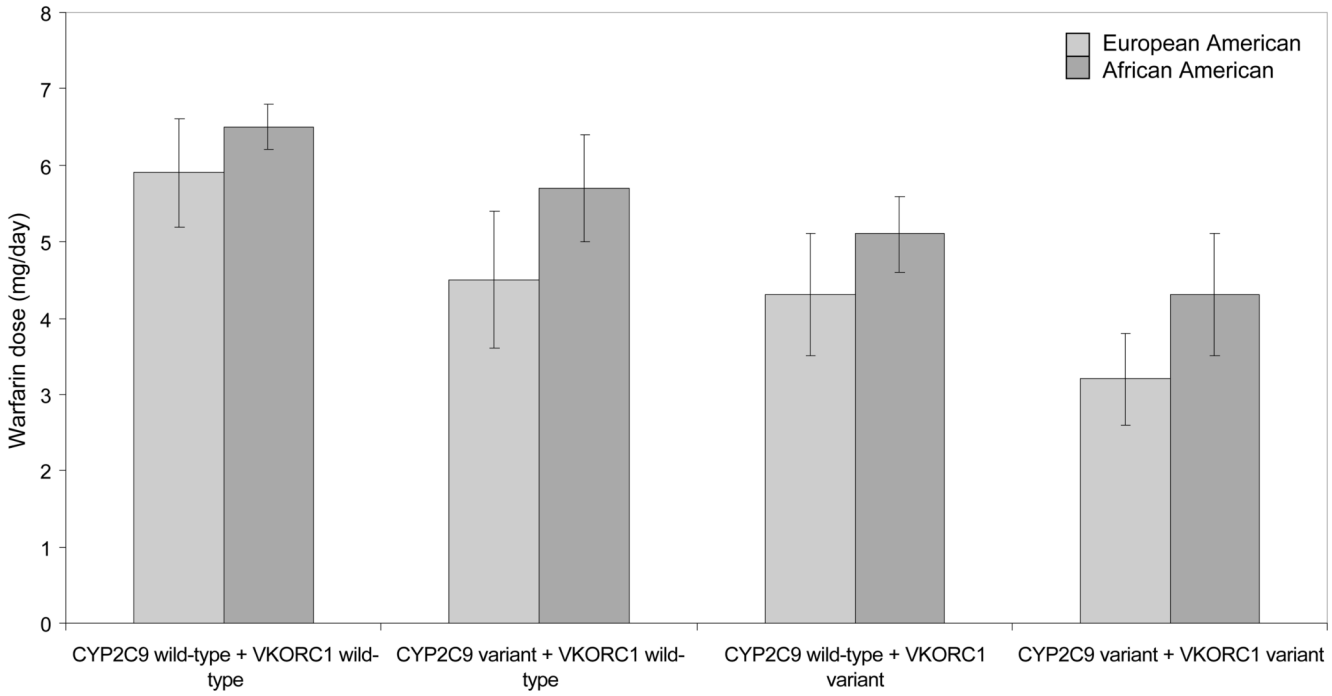

Figure 2.

Average warfarin dose (mg/day) by *CYP2C9* and *VKORC1*, stratified by race after adjusting for clinical variables. The referent patient is a 61 year old man with BMI =30, current non-smoker, non-drinker, with no comorbidities (e.g. CHF, cancer, renal failure) and no inhibitors of CYP2C9 (e.g. amiodarone).

2C9 wt indicates *CYP2C9* *1/*1 genotype; 2C9 v indicates one or more variant *CYP2C9* allele *(2, *3, *5, *6, *11)*. VKOR wt indicates *VKORC11173'CC'* genotype; VKOR v indicates *VKORC11173'CT'* or *'TT'* genotype.

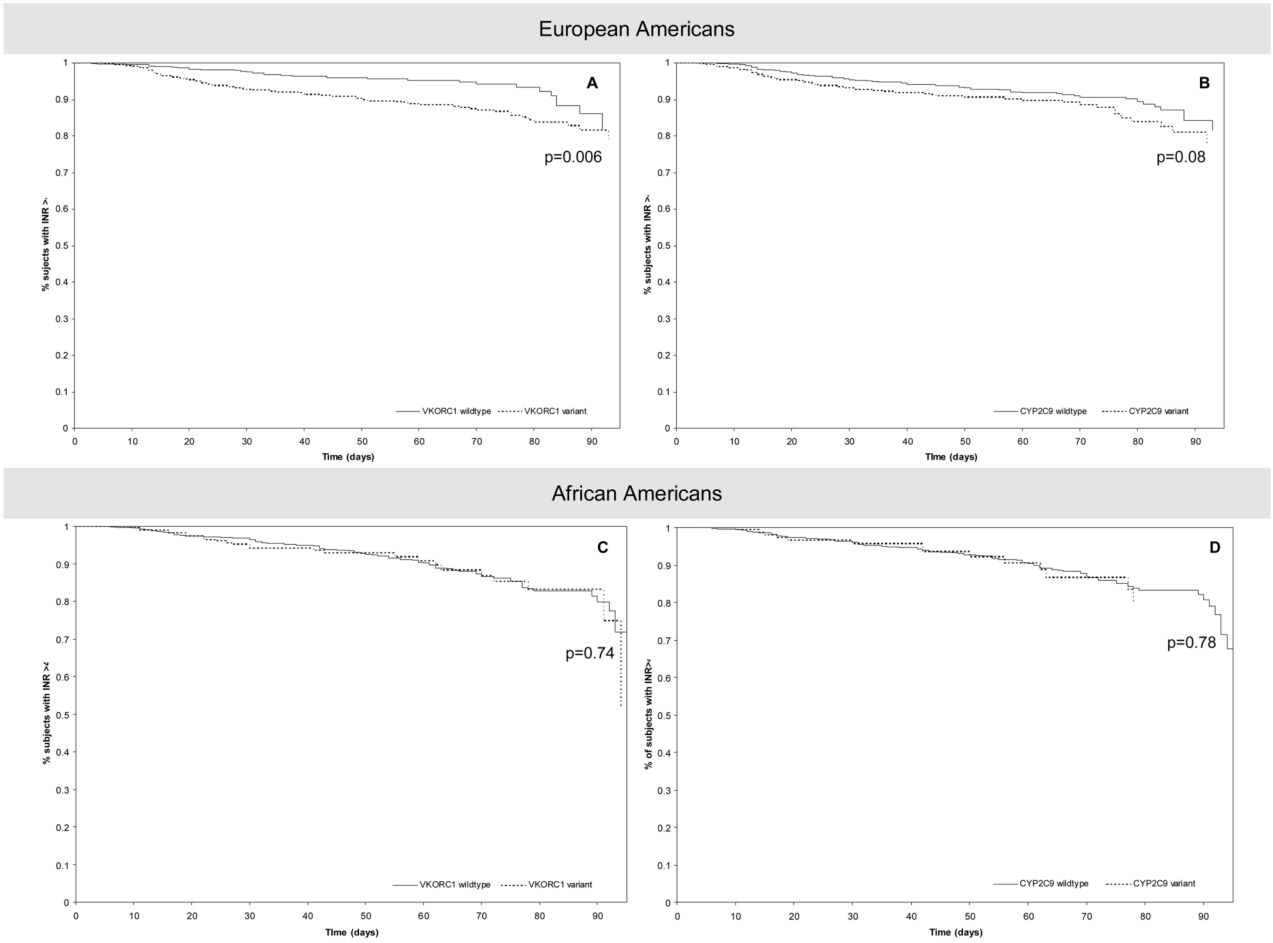

Figure 3.

Estimated survival curves from Cox PH model for European Americans (A and B) and African Americans (C and D). Dotted line indicates variant genotype; Full line indicates wild-type genotype.

A. Adjusted for *CYP2C9* genotype, age, sex, BMI, smoking, vitamin K intake, number of comorbid conditions, drug interactions, Vscore and socio-demographic factors

B. Adjusted for *VKORC1 1173C/T* genotype, age, sex, BMI, smoking, vitamin K intake, number of comorbid conditions, drug interactions, Vscore and socio-demographic factors.

C. Adjusted for *CYP2C9* genotype, age, sex, BMI, smoking, vitamin K intake, number of comorbid conditions, drug interactions, Vscore and socio-demographic factors.

D. Adjusted for *VKORC1 1173C/T* genotype, age, sex, BMI, smoking, vitamin K intake, number of comorbid conditions, drug interactions, Vscore and socio-demographic factors.

Table 1

Socio-demographic and lifestyle characteristics of the POAT study participants at baseline

Characteristic	African American (n=273)	European American (n=302)	p-value <0.0001
Age, mean (SD)	58.0 (±16.0)	63.9 (±14.7)	
Follow-up (months, Mean ± SD)	14.7 (±14.7)	15.1 (±10.8)	0.66
Body Mass Index, mean (SD)	30.1 (±7.3)	29.3 (±7.2)	0.18
	N (%)	**N(%)**	
Gender			
Female	144 (52.7%)	136 (45.0%)	0.065
Male	129 (47.3%)	166 (55.0%)	
Alcohol (drinks per week)[1]			
0	213 (78.0%)	186 (61.6%)	**<0.0001**
1–7	37 (13.6%)	100 (33.1%)	
>8	21 (7.7%)	16 (5.3%)	
Smoking Status[1]			
Current	51 (18.7%)	27 (8.9%)	**0.007**
Past	90 (33.0%)	119 (39.4%)	
Never	123 (45.0%)	147 (48.7%)	
Level of Physical Activity[1]			
Wheelchair bound	18 (6.6%)	10 (3.3%)	**<0.0001**
Uses Walker/Cane	46 (16.8%)	30 (9.9%)	
Ambulates without assistance	55 (20.1%)	54 (17.9%)	
Physically active	121 (44.3%)	196 (64.9%)	
Consistent/Intensive exercise	1 (0.4%)	12 (4.0%)	
Education			
< High School	84 (30.8%)	22 (7.3%)	**0.0007**
High School	123 (45.0%)	109 (36.1%)	
College	63 (23.0%)	132 (43.7%)	
Graduate School	3 (1.1%)	38 (12.6%)	
Annual Household Income[1]			
<15,000	98 (35.9%)	29 (9.6%)	**<0.0001**
15,000–25,000	78 (23.6%)	28 (9.3%)	
25,000–50,000	90 (33.0%)	107 (35.4%)	
50,000–100,000	5 (1.8%)	105 (34.8%)	
>100,000	1 (0.4%)	31 (10.3%)	

Characteristic	African American (n=273)	European American (n=302)	p-value <0.0001
Medical Insurance			
Medicare	85 (31.1%)	126 (41.7%)	**<0.0001**
Medicare Medicaid	5 (1.5%)	0 (0.0%)	
Medicaid	18 (6.6%)	10 (3.3%)	
Private	100 (36.6%)	142 (47.0%)	
None	64 (23.4%)	23 (7.6%)	
Site			
UAB Anticoagulation clinic	88 (32.2)	19 (6.3)	**<0.0001**
CGH-JC Anticoagulation clinic	185 (67.8)	283 (93.7)	

[*] 3 Hispanic patients excluded, all significant p-values **bolded**

Mean (SD) displayed for continuous variables and frequency counts (column percent) for categorical variables

[1] Information on missing for smoking (n=18), level of physical activity (n=2), alcohol (n=2), income (n=3), education (n=1) and Insurance (n=2). Private insurance includes various plans such as Blue Cross Clue Shield, Aetna, Travelers, etc.

Table 2

Clinical characteristics of the POAT study participants at enrollment.

Characteristic	African American (n=273)	European American (n=302)	p-value
Indication for warfarin therapy*			
Deep Vein Thrombosis	97 (35.5%)	62 (20.5%)	<0.0001
Pulmonary Thromboembolism	49 (17.9%)	27 (8.9%)	0.0014
Recurrent Venous Thromboembolism	22 (8.1%)	14 (4.6%)	0.091
Atrial Fibrillation	87 (31.9%)	174 (57.6%)	<0.0001
Valvular Heart Disease	32 (11.7%)	54 (17.9%)	0.04
Low Left Ventricular Ejection Fraction	42 (15.4%)	29 (9.6%)	0.03
Cardiac Thrombus	12 (4.4%)	15 (5.0%)	0.74
Myocardial Infarction	67 (24.5%)	95 (31.5%)	0.066
Transient Ischemic Attack	16 (5.9%)	20 (6.6%)	0.71
Stroke	57 (20.9%)	38 (12.6%)	0.007
Peripheral Vascular Disease	30 (11.0%)	39 (12.9%)	0.48
Site of thromboembolism**			
Arterial	109 (39.9%)	131 (43.4%)	0.4
Venous	138 (50.5%)	90 (29.8%)	<0.0001
Both	30 (11.0%)	21 (6.9%)	0.09
None	34 (12.4%)	44 (14.6%)	0.46
Comorbidity			
History of Myocardial Infarction	9 (3.3%)	30 (9.9%)	**0.0016**
History of CABG/ PTCA	30 (11.0%)	68 (22.5%)	**0.0002**
Cardiomyopathy	32 (11.7%)	43 (14.2%)	0.37
Coronary Artery Disease	82 (30.0%)	108 (35.8%)	0.14
Congestive Heart Failure	69 (25.3%)	59 (19.5%)	0.10
Hypertension	134 (49.1%)	132 (43.7%)	0.20
Hyperlipidemia	63 (23.1%)	118 (39.1%)	**< 0.0001**
Diabetes Mellitus	94 (34.4%)	87 (28.8%)	0.15
Malignancy	28 (10.3%)	56 (18.5%)	**0.005**
Prior Hemorrhage	24 (8.8%)	16 (5.3%)	0.1
Renal insufficiency	51 (18.7%)	36 (11.9%)	**0.024**
End Stage Renal Disease	36 (13.2%)	10 (3.3%)	**<0.0001**
Number of Comorbid Conditions			
Low (0 or 1)	88 (32.2%)	74 (24.5%)	**0.03**
Medium (2 to 4)	129 (47.2%)	141 (46.7%)	
High (5 or more)	56 (20.5%)	87 (28.8%)	
Concurrent Medications			
Antiplatelet agents	133 (49.3%)	189 (65.6%)	**0.001**
NSAIDS	37 (13.7%)	39 (12.9%)	0.78

Characteristic	African American (n=273)	European American (n=302)	p-value
CYP2C9 substrates	91 (33.7%)	96 (31.8%)	0.62
CYP2C9 inhibitors	50 (18.3%)	74 (24.4%)	0.072
VScore$^{\Psi}$	0.52 [0.27, 1.24]	0.50 [0.25, 1.13]	**0.026**

3 Hispanic patients excluded, significant p-values **bolded**

*All patients had a prescribed target INR range of 2–3. Patients with orthopedic surgery excluded due to short (3–6 month) treatment duration, patients with mechanical heart valve and hypercoagulable state excluded due to higher intensity of anticoagulation required

**Arterial thromboembolism includes patients with MI, Stroke & TIA. Venous thromboembolism includes patients with DVT & PE. Both include patients with venous and arterial events. None includes patients with no thromboembolic events (e.g. Atrial Fibrillation).

Patients can have more than one indication for therapy and comorbid conditions

CYp2C9 inhibitors included amiodarone, metronidazole, tamoxifen, propxyphene, co-trimoxazole, etc.. (Miners)

$^{\Psi}$Variability Score captures the INR variation after accounting for the influence of the number of visits and the interval between visits on INR variation for each individual patient (presented as Median and Intraqaurtile range, with Wilcoxon p-value)

Table 3

CYP2C9 and *VKORC1* genotype distribution among African Americans (AA) and European Americans (EA).

Characteristic	African American No. Positive (%)	European American No. Positive (%)	p
CYP2C9†	**(n=268)**	**(n=292)**	
*1/*1	237 (88.4)	193 (66.9)	**<0.0001**
*1/*2	7 (2.6)	58 (19.9)	
*1/*3	9 (3.3)	28 (9.6)	
*1/*5	3 (1.1)	0 (0.0)	
*1/*6	2 (0.74)	0 (0.0)	
*1/*11	7 (2.6)	0 (0.0)	
*2/*2	0 (0.0)	5 (1.7)	
*2/*3	0 (0.0)	7 (2.4)	
*3/*3	0 (0.0)	1 (0.3)	
*3/*6	1 (0.37)	0 (0.0)	
*5/*6	1 (0.37)	0 (0.0)	
*5/*11	1 (0.37)	0 (0.0)	
VKORC1†			
***1173C/T* (rs9934438)**	**(n=259)**	**(n=278)**	
CC	207 (79.9)	110 (39.6)	**<0.0001**
CT	49 (18.9)	113 (47.8)	
TT	3 (1.2)	35 (12.6)	
***1542G/C* (rs8050894)**	**(n=259)**	**(n=278)**	
GG	131 (50.6)	109 (39.2)	**<0.0001**
GA	112 (43.2)	134 (48.2)	
AA	16 (6.2)	35 (12.6)	
***2255C/T* (rs2359612)**	**(n=259)**	**(n=278)**	
CC	167 (64.5)	110 (39.6)	**<0.0001**
CT	79 (30.5)	132 (47.5)	
TT	13 (5.0)	36 (12.9)	
***3730G/A* (rs7294)**	**(n=261)**	**(n=275)**	
GG	72 (27.6)	105 (38.2)	**0.021**
GA	136 (52.1)	130 (47.3)	
AA	53 (20.3)	40 (14.5)	

†Excludes 3 Hispanic patients. Significant p-values bolded

Genotype data not available for 15 patients (*CYP2C9*) 38 patients for rs9934438 *(1173C/T)*, rs2359612 *(2255C/T)*, rs8050894 and 39 patients for rs7294 *(3730G/A)*. Missing genotype information did not differ by race (all p-values > 0.2).

Table 4

Multivariable analyses of the influence of *CYP2C9* and *VKORC1* polymorphisms on log warfarin dose

	Additive Model[1]			Dominant Model[2]		
	r^2model	r^2vkor	$r^2$2C9	r^2model	r^2vkor	$r^2$2C9
European Americans						
Average dose	53.8%	16.7%*	14.7%*	41.5%	9.6%*	9.5%*
Stable Dose	55.2%	18.5%*	11.8%*	45.6%	12.6%*	8.3%*
African Americans						
Average dose	37.9%	7.3%*	3.0%*	35.5%	6.6%*	1.0%
Stable Dose	43.8%	10.4%*	0.6%	40.8%	7.4%*	1.0%

[1] *VKORC1* and *CYP2C9* genotypes were included as covariates with 3-levels in the additive models.

[2] *VKORC1* and *CYP2C9* genotypes were included as covariates with 2 levels (wild-type versus variant) in the dominant models.

r^2_{model} denotes percent variation in warfarin dose explained by the model containing clinical and genetic covariates

r^2_{vkor} is the semi-partial r^2 denoting percent variation in warfarin dose explained by *VKORC1* SNP

r^2_{2C9} is the semi-partial r^2 denoting percent variation in warfarin dose explained by *CYP2C9* (Variant includes *2, *3, *5, *6 and *11 alleles) genotype

Average dose is dose required to maintain anticoagulation for the duration of therapy after attainment of target INR

Stable dose defined as the first dose that leads to a stable INR over three consecutive visits following initiation of the drug, with these INR measurements encompassing a period of at least 2 weeks, with a maximum difference between the mean daily dosages of 10%.

Adjusted for age, gender, BMI, Follow-up clinic, income, smoking status, education, health insurance, drug interactions (e.g. amiodarone), alcohol intake, vitamin K intake, comorbid conditions (CHF and cancer) and *CYP2C9* and *VKORC1*1173*C/T*.

* Genotype explains a statistically significant (at alpha level of 0.05) proportion of variance in warfarin dose

Table 5

Adjusted risk ratios regarding the influence of *CYP2C9* and *VKORC1* on time to attaining anticoagulation endpoints

Endpoint	CYP2C9 (variant vs. wild-type)		VKORC11173C/T (variant vs. wild-type)	
	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)
Time to first target INR				
European American	1.23 (0.96, 1.58)	1.09 (0.72, 1.65)	1.11 (0.87, 1.42)	1.09 (0.80, 1.49)
African American	1.27 (0.87, 1.87)	1.15 (0.73, 1.83)	1.11 (0.80, 1.52)	0.99 (0.69, 1.41)
Time to stable dosing*				
European American	1.11 (0.85, 1.45)	0.93 (0.59, 1.48)	0.96 (0.74, 1.25)	0.89 (0.63, 1.26)
African American	0.98 (0.63, 1.53)	0.85 (0.50, 1.43)	0.98 (0.66, 1.46)	1.41 (0.90, 2.22)
Time to INR >4 during first 30 days*$				
European American	1.23 (0.83, 1.81)	2.84 (0.91, 8.85)	2.67 (1.57, 4.57)	3.75 (1.52, 9.30)
African American	1.16 (0.56, 2.44)	0.77 (0.19, 3.09)	1.63 (0.93, 2.83)	1.26 (0.57, 2.76)
Time to INR >4 prior to stabilization$**				
European American	1.33 (0.98, 1.81)	2.15 (0.90, 5.13)	2.28 (1.55, 3.35)	2.69 (1.32, 5.47)
African American	1.00 (0.60, 1.69)	0.87 (0.34, 2.21)	1.24 (0.83, 1.86)	1.08 (0.69, 1.68)
Time to INR >4 during duration of follow-up*$				
European American	1.27 (1.03, 1.58)	1.82 (0.97, 3.43)	1.68 (1.33, 2.11)	1.91 (1.27, 2.89)
African American	1.11 (0.83, 1.49)	0.66 (0.34, 1.29)	0.93 (0.71, 1.23)	0.72 (0.43, 1.20)

CYP2C9 Variant genotype includes one or more (*2, *3, *5, *6 and *11) alleles.

Variant *VKORC11173C/T* includes '*TT* or *CT*'

All models adjusted for age, gender, BMI, *CYP2C9*, *VKOR1173C/T*, interaction between *CYP2C9*VKOR1173C/T*, number of comorbid conditions, vitamin K intake, drug interactions (e.g amiodarone), clinic site, education, income, indication, insurance and current smoking.

*Models adjusted for Vscore, the variance growth rate, a measure of INR variability up to the preceding visit was also included in analyses of time to stable dosing and time to INR >4.

$Models adjusted for correlation between repeat episodes of INR>4 within the patient

**$Median time to stabilization (95 days, Intraquartile range 42–175 days) was used to assess genotype related risk of over-anticoagulation. Analyses based on individual time to stabilization could not be conducted due to model non-convergence on stratification by race among African Americans.