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## Race-specific influence of *CYP4F2* on dose and risk of hemorrhage among warfarin users

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### Summary

**Objective**—The p.V433M in cytochrome P450 4F2 (rs2108622, *CYP4F2*\*3) is associated with higher warfarin dose and lower risk of hemorrhage among European Americans. Herein, we evaluate the influence of *CYP4F2*\*3 on warfarin dose, time to target INR and stable dose, proportion of time spent in target range (PTTR), as well as the risk of overanticoagulation and hemorrhage among European and African Americans.

**Design**—*CYP4F2*\*3 was genotyped in 1238 patients initiated on warfarin in a prospective inception cohort. Multivariable linear regression was used to assess warfarin dose and PTTR; proportional hazards analysis was performed to evaluate time to target INR and stable dose, overanticoagulation, and hemorrhage.

**Setting**—Two outpatient anticoagulation clinics

**Participants**—1238 anticoagulated patients

**Outcomes**—Warfarin dose (mg/day), time to target INR and stable dose, PTTR, over anticoagulation (INR>4), and major hemorrhage.

**Results**—Minor allele frequency for the *CYP4F2*\*3 variant was 30.3% among European Americans and 8.4% among African Americans. *CYP4F2*\*3 was associated with higher dose among European Americans but not African Americans. Compared to *CYP4F2*\*1/\*1, \*1/\*3 was associated with a statistically nonsignificant increase in dose (4.5%, p=0.22) and \*3/\*3 was

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associated with a statistically significant increase in dose (13.2% ,  $p=0.02$ ). *CYP4F2* genotype did not influence time to target INR, time to stable dose, or PTTR in either race group. *CYP4F2*\*3/\*3 was associated with a 31% lower risk of over anticoagulation ( $p=0.06$ ). Incidence of hemorrhage was lower among participants with *CYP4F2* \*3/\*3 compared to \*1/\*3 or \*1/\*1 (IRR=0.45; 95% CI: 0.14 – 1.11;  $p=0.09$ ). After controlling for covariates, the *CYP4F2* \*3/\*3 was associated with a 52% lower risk of hemorrhage although this was not statistically significant ( $p=0.24$ ).

**Conclusion**—Possession of *CYP4F2*\*3 variant influences warfarin dose among European Americans but not African Americans. The *CYP4F2*-dose, *CYP4F2*-overanticoagulation, and *CYP4F2*-hemorrhage association follows a recessive pattern with possession of *CYP4F2*\*3/\*3 genotype likely demonstrating a protective effect. These findings need further confirmation.

### Keywords

*CYP4F2*; race; warfarin dose; PTTR; over anticoagulation; hemorrhage

### Introduction

The management of warfarin therapy is complicated by a wide variation in dose and response across patients. Investigations in diverse racial populations have identified the contribution of clinical factors such as age, body mass index (BMI), vitamin K intake, comorbidities, and concurrent medications to variability in warfarin dosing.<sup>1, 2</sup> Evaluation of genetic underpinnings have revealed the importance of single nucleotide polymorphism (SNPs) within the cytochrome P450 2C9 (*CYP2C9*) and vitamin K oxidoreductase complex subunit 1 (*VKORC1*) genes in influencing warfarin dose. These genes explain significantly more variability in warfarin dose requirements among European Americans than in African Americans.<sup>3-12</sup>

Investigators have also identified the influence of *CYP4F2* on warfarin dose.<sup>13</sup> *CYP4F2* is a  $\omega$ -hydroxylase responsible for the hydroxylation of leukotriene B<sub>4</sub>, and is expressed in the liver, kidneys, lungs, and white blood cells.<sup>14</sup> It is also a vitamin K1 mono-oxidase (VK1), catalyzing the hydroxylation of VK1.<sup>15</sup> Patients possessing p.V433M (rs2108622; *CYP4F2*\*3) have a reduced capacity to metabolize VK1 in the liver, and the corresponding increase in VK1 levels necessitates a higher warfarin dose to elicit the therapeutic anticoagulant response.<sup>15</sup> The *CYP4F2*\*3 variant is associated with higher warfarin dose requirements in European Americans,<sup>13, 15-22</sup> Asians<sup>23-26</sup> and Hispanics<sup>27</sup> but not among Brazilians<sup>18</sup> or African Americans<sup>8, 27</sup>. Among European Americans, studies support the association of *CYP4F2*\*3 with dose variability, attainment of therapeutic INR, and attainment of stable dose.<sup>13, 15-22, 28, 29</sup> Recently, *CYP4F2* has been associated with a lower hemorrhage risk,<sup>30</sup> and increased risk of stent thrombosis<sup>31</sup> among patients of European descent.

The *CYP4F2*\*3 polymorphism has been incorporated in the warfarin dosing algorithm offered at [warfarindosing.org](http://warfarindosing.org), although it is not yet a part of the guidelines provided by the Clinical Pharmacogenetics Implementation Consortium (CPIC).<sup>32</sup> Recent clinical trials such as Clarification of Optimal Anticoagulation through Genetics (COAG) and European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) do not include *CYP4F2*\*3 in their

dosing algorithms.<sup>33, 34</sup> Although, there is enough evidence of the influence of *CYP4F2*\*3 on warfarin dose, its influence on warfarin response, especially among African Americans has not been extensively evaluated. Herein, we evaluate the influence of *CYP4F2* on warfarin dose, time to attain therapeutic INR and stable dose, anticoagulation control (assessed as proportion of time spent in target range; PTTR), over anticoagulation (INR>4), and major hemorrhage among European and African American warfarin users.

## Methods

The prospective Warfarin Pharmacogenetics Cohort (WPC) recruited patients 20 years of age initiating warfarin therapy and managed at an anticoagulation clinic under the approval of the Institutional Review Board at the University of Alabama at Birmingham and at Emory University. Warfarin therapy requiring a target international normalized ratio (INR) range of 2-3 was initiated in patients with venous thromboembolism, stroke/transient ischemic attacks, atrial fibrillation, myocardial infarction, peripheral arterial disease, and others (i.e., cardiac thrombus). Patients requiring a higher (INR 2.5 to 3.5; e.g. mechanical heart valves) or lower (INR 1.5-2.5; e.g. pulmonary arterial hypertension) intensity of anticoagulation were excluded. The goals of the study were to identify the influence of clinical and genetic factors on warfarin dose, anticoagulation control, and risk of hemorrhage.

Patients were followed monthly from initiation of warfarin therapy for up to 2 years. A detailed history was obtained that including self-reported race, education, income, medical insurance, height and weight, blood urea nitrogen, serum creatinine, hemoglobin and hematocrit, indication for warfarin therapy, co-morbid conditions, medications, smoking status, alcohol use, and vitamin K intake as detailed in previous publications.<sup>3, 4, 35, 36</sup> At each clinic visit, the INR, warfarin dose, concurrent medications, level of physical activity, dietary vitamin K intake, alcohol intake, and medication compliance were recorded. Changes in concomitant medications that influence warfarin pharmacodynamics (antiplatelet agents) or pharmacokinetics (including *CYP2C9* inhibitors [e.g. amiodarone and statins] and *CYP2C9* inducers [e.g. rifampin]) were documented at each visit and verified through medical record review and pharmacy refill records.

In addition to *VKORC1* (rs9923231) and *CYP2C9* (\*2 [rs1799853], \*3 [rs1057910]), we assessed *CYP4F2*\*3 (rs2108622), and the African American specific *CYP2C9* SNPs (\*5 [rs28371686], \*6 [rs9332131], \*11 [rs28371685]), and the *CYP2C* SNP (rs12777823).<sup>3, 4, 35, 36</sup> The assumption of Hardy Weinberg Equilibrium was met for all SNPs ( $p>0.20$ ).

## Outcome definitions

Warfarin dose (mg/day; log transformed to attain normality of residuals) was defined as the average dose required for maintenance of therapeutic anticoagulation (INR 2-3).

Time to attaining a therapeutic INR was calculated as number of days from warfarin initiation to the first INR of >2. Time required to attain stable warfarin dose was defined based on attainment of three consecutive target INRs in target INR range (2-3) with the INRs assessed at least 2 weeks apart.

Proportion of time spent in target range (PTTR) was calculated for each patient using the Rosendaal linear interpolation method.<sup>37</sup> This method assumes that a linear relationship exists between two consecutively measured INR values and allows one to allocate a specific INR value to each day for each patient. Time in target range (TTR) for each patient was assessed by the percentage of interpolated INR values within the target range of 2.0–3.0 after attainment of first INR in target range.

Overanticoagulation was defined as INR>4. Major hemorrhages included serious, life threatening and fatal bleeding episodes as per Schulman et al.<sup>38</sup> For all major hemorrhagic complications during the 2-year follow-up, the complication site (e.g. gastrointestinal tract), gravity of the event (e.g. requiring transfusion, surgical intervention, etc.), and laboratory findings (INR, hemoglobin/hematocrit, etc.) at the time of the event were objectively documented. Isolated subtherapeutic or suprathreshold INRs in the absence of bleeding were not classified as events.

During follow-up, all major hemorrhagic complications were captured and verified through review of admissions and emergency department visits. Only medically documented events were included in the analyses. The Alabama Center for Health Statistics was queried to verify cause of death for all deceased to ensure inclusion of deaths due to hemorrhagic complications. All complications were documented by the study nurse, confirmed by the principal investigator, and finally reviewed independently by the Medical Director (TMB) of the Anticoagulation Clinic.

### Statistical methods

Analysis of variance was used to assess group differences for continuous variables and chi-square ( $\chi^2$ ) test of independence for categorical variables. The Hardy-Weinberg equilibrium assumption was tested using the  $\chi^2$  test. Multivariable linear regression analysis was performed to evaluate the association between *CYP4F2*\*3 on warfarin dose and PTTR after accounting for sociodemographic factors (age, race, gender, body surface area [BSA]), clinical factors (e.g. kidney impairment, based on estimated glomerular filtration rate eGFR >60, 30-59, <30ml/min/1.73 m<sup>2</sup> calculated using the Modification of Diet in Renal Disease Study equation),<sup>39</sup> concurrent medications (e.g. amiodarone, statins), and variants in *CYP2C9*, *VKORC1* and *rs12777823*.

The influence of *CYP4F2*\*3 on time to target INR (2-3), time to stable dose, the risk of over anticoagulation (INR>4) and major hemorrhage was assessed using the counting process format in the proportional hazard (PH) model. Robust variance estimation was used to correct for the dependence among multiple events per individual and provide 95% confidence intervals (CIs) for the hazard ratios (HRs) of interest. Departures from the PH assumption were assessed by evaluating interactions of the predictors and a function of survival time. All analyses were performed using SAS version 9.3 at a non-directional alpha level of 0.05 accounting for demographic, clinical, and genetic factors. As the current evidence suggests that the influence of *CYP4F2*\*3 on warfarin response may be dependent on race, we evaluated the *CYP4F2*-dose, anticoagulation control, and hemorrhage risk after stratifying by race. We evaluated the influence of *CYP4F2*\*3 using additive (\*1/\*1 versus

\*1/\*3 versus \*3/\*3), dominant (\*1/\*1 versus \*1/\*3 or \*3/\*3), and recessive (\*1/\*1 or \*1/\*3 versus \*3/\*3) models.

### Role of the funding source

The study was funded by the NIH and the funders did not have any role in the study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

A total of 1238 patients were genotyped for the *CYP4F2*\*3 variant (Table 1). The minor allele frequency (MAF) for the *CYP4F2*\*3 variant was 30.3% among European Americans and 8.4% among African Americans. The prevalence of *CYP2C9*\*2 and *VKORC1* was higher among patients with the *CYP4F2*\*3 variant compared to the wild-type allele. However, the prevalence of *CYP2C9*\*3, \*5, \*6, and \*11, and rs12777823 did not differ by *CYP4F2* genotype. Patients with the *CYP4F2*\*3 variant were older and more likely to be on warfarin for atrial fibrillation, whereas patients with *CYP4F2* wild-type genotype were more likely to be on warfarin for venous thromboembolism and to have chronic kidney disease. The prevalence of comorbid conditions such as hypertension, hyperlipidemia, and diabetes mellitus did not differ by *CYP4F2* genotype nor did the use of statins, amiodarone or antiplatelet therapy.

Patients with the *CYP4F2*\*3 variant required higher warfarin dose compared to the wild-type genotype (Table 2) after adjusting for race, clinical and genetic factors. Among European Americans, compared to *CYP4F2*\*1/\*1, possession of \*1/\*3 genotype was associated with a statistically nonsignificant increase in dose by 4.5% and possession of \*3/\*3 genotype was associated with statistically significant increase in dose by 13.2% (Figure 1). Although African Americans possessing \*3/\*3 genotype required 19.9% higher warfarin dose, this finding was not statistically significant, likely due to the limited (n=2) prevalence of this genotype among African Americans (Figure 1). Gender (p=0.76), smoking status (p=0.54), and antiplatelet therapy (p=0.55) were not statistically significant in the model and were therefore removed from further analysis. Age, BSA, *CYP2C9*\*3, and *VKORC1* were significantly associated with dose among both European and African Americans (p<0.001, all). *CYP2C9*\*2 was significantly associated only with dose among European Americans (p<0.001). Similarly, rs12777823 was significantly associated only with dose among African Americans (p<0.001).

Time to target INR (>2) did not differ by *CYP4F2* genotype among European Americans (p=0.10) or African Americans (p=0.47). These associations remained unchanged after adjusting for clinical and genetic factors. Similarly, time to stable warfarin dose did not significantly differ by *CYP4F2* genotype among European Americans (p = 0.98) and African Americans (p = 0.08).

Patients possessing *CYP4F2*\*3 variant had higher PTTR compared to those with the wild-type genotype. However this association was not statistically significant after accounting for clinical and genetic factors (Table 3). As the *CYP4F2*- PTTR association did not differ significantly by race, the final model adjusted for race. As previously shown, age (p<0.001),

African-American race ( $p<0.001$ ), BSA ( $p=0.002$ ) and chronic kidney disease ( $p<0.001$ ) were significantly associated with PTTR whereas gender ( $p=0.62$ ), amiodarone use ( $p=0.76$ ), statin use ( $p=0.13$ ), antiplatelet use ( $p=0.16$ ), *CYP2C9* ( $p=0.97$ ), *VKORC1* ( $p=0.94$ ), and rs12777823 ( $p=0.34$ ) had no significant association with anticoagulation control.

Compared to patients possessing the *\*1/\*1* genotype, the risk of over anticoagulation ( $\text{INR}>4$ ) was not significantly different among those with *\*1/\*3* genotype (HR: 0.90, 95% CI: 0.76 – 1.07;  $p=0.22$ ) and was lower among those with *\*3/\*3* genotype (HR: 0.69, 95% CI: 0.47 – 1.01,  $p=0.06$ ; Figure 2), although this was not statistically significantly different. As the *CYP4F2*-overanticoagulation association did not differ by race, the final model adjusted for race. African-American race ( $p<0.001$ ), BSA ( $p=0.002$ ), chronic kidney disease ( $p<0.001$ ), antiplatelet use ( $p=0.008$ ), *CYP2C9* variants ( $p=0.006$ ) and age ( $p=0.05$ ) had a significant influence on over anticoagulation. Gender ( $p=0.81$ ), amiodarone use ( $p=0.49$ ), statin use ( $p=0.23$ ), and rs12777823 ( $p=0.35$ ) and *VKORC1* ( $p=0.07$ ) did not influence the risk of over anticoagulation.

Over the accrued 1762 person-years of follow up, 140 major hemorrhagic events were observed. Major hemorrhages by site included gastrointestinal ( $n=84$ ), genitourinary ( $n=18$ ), retroperitoneal ( $n=6$ ), intracranial bleeds ( $n=12$ ), hemoptysis ( $n=4$ ), and hematomas ( $n=16$ ). On average, patients with major hemorrhage had significantly lower hematocrit values compared to those without major hemorrhages ( $35.5 \pm 6.9$  vs.  $37.3 \pm 8.7$ ,  $p=0.007$ ). The overall incidence of major hemorrhage was 7.9/100 person-years (95% CI: 6.7 – 9.3 per 100 p-yrs). The overall incidence of hemorrhage was 55% lower among patients with *CYP4F2* *\*3/\*3* genotype compared to *\*1/\*3* or *\*1/\*1* genotype, however this finding was not statistically significantly different (Table 4). European Americans with *CYP4F2* *\*3/\*3* had a 49% lower risk of hemorrhage compared to those with the *\*1/\*3* or *\*1/\*1* genotype;— although this finding was not statistically significant (Table 4). Due to the limited ( $n=2$ ) prevalence of the *\*3/\*3* genotype, we could not evaluate the gene-hemorrhage association among African Americans.

As the *CYP4F2* *\*3/\*3* genotype appears to be protective for major hemorrhage, we assessed its influence further by accounting for other clinical and genetic factors, categorizing the *CYP4F2* *\*1/\*3* and *\*1/\*1* as the referent genotype (recessive model). While possession of *CYP4F2* *\*3/\*3* genotype was associated with a 52% lower risk of major hemorrhage, this finding was not statistically significant (HR: 0.48, 95% CI: 0.14 – 1.66,  $p=0.24$ ).

We categorized *\*1/\*3* or *\*1/\*1* genotype as the referent genotype based on observed incidence of hemorrhage in our cohort. However, a recent report<sup>30</sup> evaluated the *CYP4F2* *\*3*-hemorrhage association using a dominant model categorizing *\*1/\*1* as the referent genotype and *\*1/\*3* and *\*3/\*3* as the variant genotype. Using this categorization, *CYP4F2* *\*3* was not significantly associated with risk of hemorrhage (Table 4). This finding remained unchanged after adjusting for other clinical and genetic factors (HR: 0.88; 95% CI: 0.59-1.33;  $p=0.54$ ).

## Discussion

To our knowledge, this is the first paper to comprehensively evaluate the association of *CYP4F2*\*3 with warfarin dose, PTTR, risk of overanticoagulation, and risk of major hemorrhage among chronic warfarin users. We also assessed whether possession of the *CYP4F2*\*3 variant has a consistent effect across race groups.

The MAF for the *CYP4F2*\*3 variant in our cohort was 30.3% among European Americans and 8.4% among African Americans, which is similar to previous reports for both European Americans and African Americans.<sup>8, 13, 17, 18, 27</sup> Given that the bulk of the evidence regarding the influence of *CYP4F2* among warfarin users is from patients of European descent, we compared our findings with previous reports in this race group. And given the limited reports in the literature among African Americans, we presented our findings acknowledging that our ability to interpret *CYP4F2*\*3-outcome associations among African Americans was limited because of the lower MAF of the variant in this race group. Moreover, as only two patients possessed the \*3/\*3 genotype, we could not assess the *CYP4F2*\*3-response association using the recessive model.

Possession of *CYP4F2*\*3 variant was associated with higher warfarin dose requirements. Inclusion of *CYP4F2*\*3 in the dose prediction algorithm improved the proportion of variability explained by 0.6% which is concordant with previous reports.<sup>13, 17, 40</sup> Consistent with previous reports, the influence of *CYP4F2* on warfarin dose varied by race in our study. Among European Americans, possession of *CYP4F2*\*3 variant was associated with higher dose requirements with the *CYP4F2*\*3/\*3 genotype associated with significantly higher dose requirements (recessive effect).

The limited African-American participation has previously prevented the evaluation of the *CYP4F2*-dose association separately in this race group,<sup>41, 42</sup> and has been unable to test the association after accounting for other clinical and genetic factors.<sup>8, 18</sup> Our findings are consistent with those reported by others<sup>27</sup> who demonstrated the lack of influence of *CYP4F2*\*3 variant on warfarin dose requirement among 260 African Americans using an additive model. Moreover, as only 2 out of the 531 African Americans in our cohort possessed the *CYP4F2*\*3/\*3 genotype, we could not evaluate whether the *CYP4F2*\*3 variant exerted a recessive effect on dose in this race group.

Concordant with previous reports among patients of European descent,<sup>17, 20, 28</sup> *CYP4F2*\*3 did not influence time to attainment of first target INR 2 or time to stable dose among European Americans. Researchers evaluating the influence of 59 *CYP4F2* SNPs on time to target INR and stable dose reported that the *CYP4F2*\*3-time to therapeutic INR association ( $p=0.03$ ) did not remain significant after correction for multiple testing.<sup>28</sup> Possession of the *CYP4F2*\*3 variant did not influence PTTR after controlling for clinical and genetic factors in either race group in our study. The findings among European Americans are consistent with results of a previous study reporting that *CYP4F2*\*3 was not significantly associated with PTTR (\*1/\*1 vs. \*3/\*3,  $p=0.39$ ; \*1/\*3 vs. \*3/\*3,  $p=0.14$ ).<sup>40</sup> We are the first to report the lack of effect of the *CYP4F2*\*3 variant on time to target INR, time to stable dose, and PTTR among African Americans.

Previous evaluations have not shown a significant association between *CYP4F2* and overanticoagulation among patients of European descent.<sup>17, 20, 28, 40</sup> We found no effect of *CYP4F2* on risk of overanticoagulation among European or African Americans. Our results suggest a recessive effect of the \*3 variant, with the *CYP4F2*\*3/\*3 genotype associated with a lower risk of over anticoagulation.

To our knowledge, this is the first report on the *CYP4F2*-hemorrhage association among African Americans. There are limited data on the *CYP4F2*-hemorrhage association among European Americans.<sup>17, 43</sup> In a recent study of community-dwelling warfarin users of mainly European descent, possession of the \*3 variant was associated with a 38% lower odds of major hemorrhage.<sup>30</sup> The authors assessed the *CYP4F2*-hemorrhage association using a dominant model categorizing *CYP4F2*\*1/\*3 and \*3/\*3 versus \*1/\*1. However, our results do not support these findings. We recognize that categorization of genotypes as variant (\*1/\*3 and \*3/\*3) versus wild-type (\*1/\*1) is accepted. Therefore, we report the *CYP4F2*-hemorrhage association using both categorizations. However, our data regarding the incidence of hemorrhage demonstrates that the protective effect (if any) associated with *CYP4F2* is related to possession of the \*3/\*3 genotype. Possession of the \*3/\*3 genotype is protective as supported by the absolute risk of hemorrhage observed among European Americans in our cohort. However, we could not evaluate the protective association of the *CYP4F2*\*3 variant among African Americans given the lower minor allele frequency of the *CYP4F2*\*3 in this group and the absence of major hemorrhage among the two African Americans with the *CYP4F2*\*3/\*3 genotype.

While we assessed the differential effect of *CYP4F2*\*3 among African and European Americans, we recognize that these results may not be generalizable to Hispanics and Asians.<sup>23, 24, 27, 44, 45</sup> Additional studies are needed to identify predictors that may differentially influence warfarin response in these race groups. As we assessed only the *CYP4F2*\*3 polymorphism, we could not investigate for haplotype block structures in the two race groups. Assessment of additional SNPs in the *CYP4F2* gene and investigating their association with warfarin response may reveal as yet undiscovered causal variants among African Americans. Moreover, it is likely that incorporation of other genetic (e.g. *GGCX*, *CALU*, *CYP2C9*\*8)<sup>27, 46, 47</sup> and clinical factors (including drug-drug interactions) not assessed in this study may further improve dose prediction and alter the effect-sizes of the predictors evaluated herein. We recognize this limitation.

## Conclusion

Our findings confirm that possession of *CYP4F2*\*3 variant influences warfarin dose among European Americans but not African Americans. The influence of *CYP4F2*\*3 on warfarin dose variability among European Americans is smaller than that accounted for by *CYP2C9* and *VKORC1*. The *CYP4F2*-dose association follows a recessive model. Similarly, *CYP4F2*-overanticoagulation and *CYP4F2*-hemorrhage association follow a recessive pattern with possession of *CYP4F2*\*3/\*3 genotype likely demonstrating a protective effect. These findings need to be confirmed in larger studies that can evaluate the consistency of the effects in different race groups.



## Acknowledgements

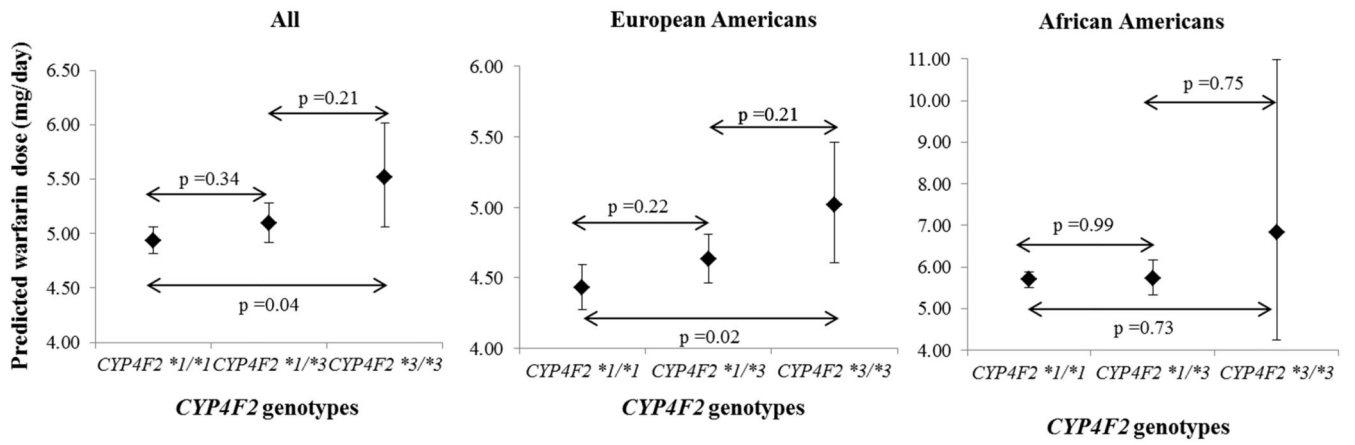
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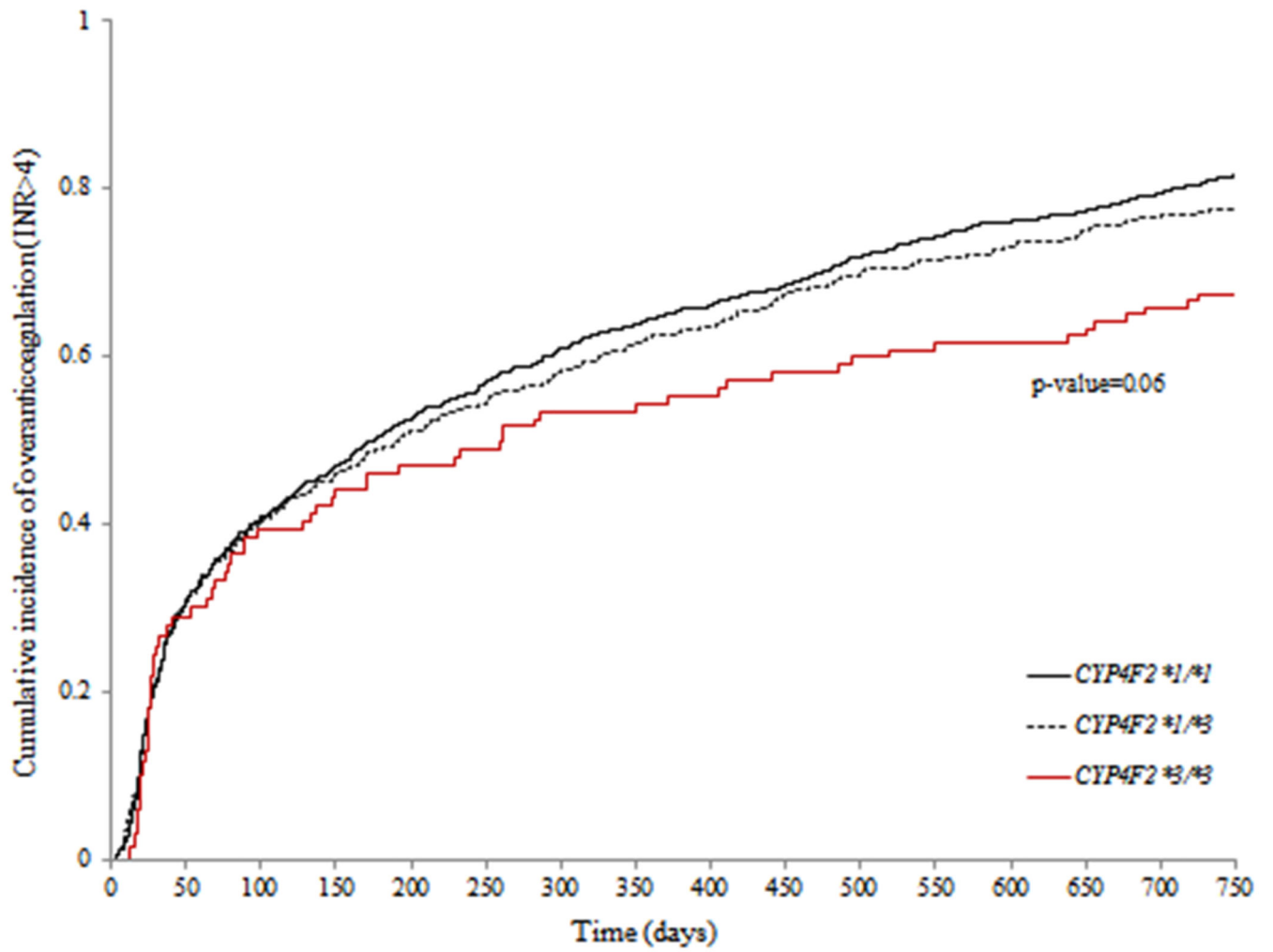
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**Figure 1.** Warfarin dose by *CYP4F2* genotypes for all, European Americans and African Americans patients.



**Figure 2.**  
Cumulative incidence of overanticoagulation (INR > 4) by *CYP4F2* genotype

**Table 1**  
**Clinical, genetic and socio-demographic characteristics of patients by *CYP4F2* genotypes**

Characteristics	<i>CYP4F2</i> *1/*1 n=784	<i>CYP4F2</i> *1/*3 n=390	<i>CYP4F2</i> *3/*3 n=64	P Value
	Mean ± SD	Mean ± SD	Mean ± SD	
Follow-up, years	1.5 ± 0.9	1.3 ± 0.9	1.3 ± 0.9	0.06
Age, years	60.1 ± 15.6	63.0 ± 15.7	63.5 ± 15.6	0.005
BSA, m <sup>2</sup>	2.0 ± 0.3	2.0 ± 0.3	2.0 ± 0.3	0.24
	N (%)	N (%)	N (%)	
Female	390 (49.7)	176 (45.1)	24 (37.5)	0.08
Race <sup>a</sup>				<0.001
European Americans	340 (43.4)	305 (78.2)	62 (96.9)	
African Americans	444 (56.6)	85 (21.8)	2 (3.1)	
Indication for Warfarin therapy				
Venous thromboembolism	359 (45.6)	142 (36.4)	23 (35.9)	0.005
Stroke /TIA	50 (6.4)	15 (3.9)	3 (4.7)	0.19
Atrial Fibrillation	300 (38.3)	193 (49.5)	32 (50.0)	<0.001
Myocardial infarction	12 (1.5)	10 (2.6)	0 (0.0)	0.24
Peripheral arterial disease	9 (1.2)	3 (0.8)	1 (1.6)	0.77
Other	53 (6.8)	27 (6.9)	5 (7.8)	0.95
Comorbid conditions				
Hypertension	528 (68.0)	244 (63.9)	41 (64.1)	0.34
Hyperlipidemia	359 (46.3)	196 (51.3)	31 (48.4)	0.27
Diabetes mellitus	253 (32.6)	117 (30.6)	16 (25.0)	0.40
Chronic Kidney disease <sup>b</sup>				0.03
eGFR > 60ml/min/1.73m <sup>2</sup>	492 (62.8)	240 (61.7)	36 (56.3)	
eGFR >30-59ml/min/1.73m <sup>2</sup>	209 (26.7)	123 (31.6)	25 (39.1)	
eGFR < 30 ml/min/1.73m <sup>2</sup>	82 (10.5)	26 (6.7)	3 (4.7)	
Concurrent medications				
Statins <sup>c</sup>	411 (52.8)	222 (56.9)	33 (51.7)	0.38
Anti-platelets <sup>d</sup>	443 (56.9)	240 (61.5)	40 (62.5)	0.26
Amiodarone	75 (9.6)	45 (11.5)	10 (15.6)	0.24
Percent patients possessing > 1 minor allele				
<i>CYP2C9</i> *2	109 (14.0)	81 (20.8)	16 (25.0)	0.003
<i>CYP2C9</i> *3	58 (7.5)	37 (9.5)	5 (7.8)	0.47
<i>CYP2C9</i> *5, *6, *11	13 (1.7)	6 (1.5)	0 (0.0)	0.58
<i>VKORC1</i>	283 (36.3)	203 (52.5)	39 (61.9)	<0.001
rs12777823	292 (37.3)	130 (33.3)	17 (26.6)	0.12

All patients enrolled were prescribed warfarin with a target INR range of 2-3.

Patients requiring a higher (INR 2.5 to 3.5; e.g. mechanical heart valves) or lower (INR 1.5-2.5; e.g. pulmonary arterial hypertension) intensity of anticoagulation were excluded.

SD: Standard Deviation, BSA: body surface area, TIA: transient ischemic attack, eGFR: estimated glomerular filtration rate

<sup>a</sup> Asians (n=4; 0.3%) and Hispanics (n=4; 0.3%) were combined with the European Americans.

<sup>b</sup> All eGFR are based on National Kidney Foundation staging using the Modification of Diet in Renal Disease study equation and categorized into 3 categories: eGFR > 60; eGFR >30-59 and eGFR < 30 ml/min/1.73m<sup>2</sup>

<sup>c</sup> Statins included any of the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors

<sup>d</sup> Antiplatelet agents included aspirin, clopidogrel, and, dipyridamole as mono or dual therapy

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**Table 2**  
**Predicted warfarin dose in mg/day and 95% confidence intervals (95%CI) based on**  
***CYP4F2* genotypes**

	<i>Total</i>	<i>CYP4F2</i> *1/*1		<i>CYP4F2</i> *1/*3		<i>CYP4F2</i> *1/*3	
		<i>N</i>	<b>Dose (95%CI)</b>	<i>N</i>	<b>Dose (95%CI)</b>	<i>N</i>	<b>Dose (95%CI)</b>
All	1238	784	4.9 (4.8 – 5.1)	390	5.1 (4.9 – 5.3)	64	5.5 (5.1 – 6.0)
European Americans	707	340	4.4 (4.3 – 4.6)	305	4.6 (4.5 – 4.8)	62	5.0 (4.6 – 5.5)
African Americans	531	444	5.7 (5.5 – 5.9)	85	5.7 (5.3 – 6.2)	2	6.8 (4.3 – 10.9)

<sup>a</sup> Adjusted for age (centered at 40 years); body surface area (BSA) centered at 2.01, chronic kidney disease (CKD), amiodarone therapy, *CYP2C9*\*2, *CYP2C9*\*3, *CYP2C9*\*5, \*6 and \*11 together, *VKORC1*, and rs12777823 variants. CKD was based on the Modification of Diet in Renal Disease Study equation. Patients were categorized into 3 categories: eGFR ≥ 60 (no CKD or mild CKD stage 1 and 2), eGFR=30-59 (moderate CKD; stage 3) and eGFR < 30 (severe CKD; stage 4 and 5). *CYP2C9*\*2, *CYP2C9*\*3, and *VKORC1* were included as additive; 0 if no variants; 1 if heterozygous and 2 if homozygous for the variant allele. *CYP2C9*\*5, \*6 and \*11 together, and rs12777823 were categorized as 0 if no variants and 1 if heterozygous or homozygous for the variant allele.

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**Table 3**  
**Anticoagulation control as assessed by percent time in target range (PTTR; INR 2-3) by**  
***CYP4F2* genotype**

Parameter	<i>CYP4F2</i> *1/*1	<i>CYP4F2</i> *1/*3	<i>CYP4F2</i> *3/*3	P Value
Number of patients	784	390	64	
Number of visits	21008	10420	1864	
Follow-up (months)	13193	6643	1289	
Follow-up months/patient	16.8 ± 10.6	17.0 ± 10.8	20.1 ± 10.4	0.06
<b>Distribution of treatment time spent below range, in range and above range</b>				
PTBR	29.6	26.2	26.0	0.09
PTTR	52.5	57.1	56.4	0.002
PTAR	17.9	16.7	17.6	0.57
<b>Distribution of treatment time spent below range, in range and above range after controlling for confounders<sup>a</sup></b>				
PTBR	28.6	27.5	29.4	0.77
PTTR	53.5	55.5	53.8	0.34
PTAR	17.9	17.0	16.8	0.73

INR: International Normalized Ratio

PTBR denotes percent time spent below range (INR 2-3); PTTR denotes percent time spent in range (INR 2-3); PTAR denotes percent time spent above range (INR 2-3)

<sup>a</sup> Adjusted analysis includes age, race, BSA, current smoking, chronic kidney disease, concurrent statin and antiplatelet use, *CYP2C9*, and *VKORC1* variants

**Table 4**  
**Incidence (per 100 person-years) of Major Hemorrhage by *CYP4F2* genotype**

<i>CYP4F2</i> genotype	N	Events	FU years	Incidence	Incidence rate ratio	P Value
All						
*1/*1	784	92	1099.5	8.37 (6.75 -10.26)	Reference	—
*1/*3	390	44	554.6	7.93 (5.76-10.65)	0.95 (0.66 -13.53)	0.85
*3/*3	64	4	107.5	3.72 (1.00-9.53)	0.44 (0.14-1.10)	0.09
European Americans						
*1/*1	340	36	504.0	7.14 (5.00-9.89)	Reference	—
*1/*3	305	35	438.2	7.99 (5.56-11.11)	1.12 (0.70-1.79)	0.64
*3/*3	62	4	103.8	3.85 (1.04-9.87)	0.54 (0.16-1.40)	0.24
African Americans						
*1/*1	444	56	595.5	9.40 (7.10-12.21)	Reference	—
*1/*3	85	9	116.4	7.73 (3.53-14.68)	0.82 (0.38-1.61)	0.61
*3/*3	2	0	3.7	—	—	—
<b><i>CYP4F2</i> genotype (*1/*1 or *1/*3 versus *3/*3)</b>						
All						
*1/*1 or *1/*3	1174	136	1654.1	8.22 (6.89 -9.73)	Reference	—
*3/*3	64	4	107.5	3.72 (1.00-9.53)	0.45 (0.14-1.11)	0.09
European Americans						
*1/*1 or *1/*3	645	71	942.2	7.54 (5.89-9.51)	Reference	—
*3/*3	62	4	103.8	3.85 (1.04-9.87)	0.51 (0.16-1.28)	0.22
African Americans						
*1/*1 or *1/*3	529	65	711.9	9.13 (7.10-11.56)	—	—
*3/*3	2	0	3.7	—	—	—
<b><i>CYP4F2</i> genotype (*1/*1 versus *1/*3 or *3/*3)</b>						
All						
*1/*1	784	92	1099.5	8.37 (6.75 -10.26)	Reference	—
*1/*3 or *3/*3	454	48	662.1	7.25 (5.35-9.61)	0.87 (0.61-1.22)	0.42
European Americans						
*1/*1	340	36	504.0	7.14 (5.00-9.89)	Reference	—
*1/*3 or *3/*3	367	39	542.0	7.20 (5.12-9.84)	1.01 (0.64-1.59)	0.98
African Americans						
*1/*1	444	56	595.5	9.40 (7.10-12.21)	Reference	—
*1/*3 or *3/*3	87	9	120.1	7.49 (3.42-14.23)	0.80 (0.37-1.56)	0.55

FU years: Follow-up years