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ORIGINAL ARTICLE

Apolipoprotein E genotype and warfarin dosing among Caucasians and African Americans

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Warfarin sodium is a vitamin K antagonist that is plagued by large variability in patient response, including higher dose requirements among African Americans. Polymorphisms in the gene encoding apolipoprotein E (*APOE*) may partly explain this variability by altering transport of vitamin K to the liver. In a prospective cohort study of 232 individuals (52.2% Caucasian and 47.8% African American) initiating warfarin therapy, the weekly maintenance dose was significantly higher for African Americans than for Caucasians (mean 42.9 versus mean 36.9 mg, $P=0.018$), and the ε4 allele was more common among African Americans (37.8 versus 26.4% for Caucasians). In multivariable analyses, the presence of the ε4 allele was associated with a statistically significantly higher warfarin dose among African Americans (median 45.0 mg in ε4 carriers versus 35.0 mg in non-ε4 carriers, $P=0.014$) but not Caucasians (38.1 versus 35.0 mg, $P=0.60$). In addition, warfarin maintenance dose increased among African Americans according to genotype previously associated with differential hepatic chylomicron clearance (ε2/ε2 or ε2/ε3: 30.0 mg; ε3/ε3: 35.0 mg; ε3/ε4 or ε4/ε4: 45.0 mg; $P=0.012$), although the ε4/ε4 genotype was rare and not clearly associated with higher doses. The association of *APOE* with warfarin dosing was independent of *CYP2C9* and *VKORC1* polymorphisms. *APOE* polymorphisms thus may be important determinants of warfarin maintenance dose and could explain at least some of the observed racial differences in dose requirements.
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Keywords: apolipoprotein E; warfarin; genetics; prospective cohort

Introduction

Warfarin sodium is the mainstay of oral anticoagulation therapy in the US and many other countries. When dosed properly it is a highly efficacious drug for maintaining sufficient, but not excessive, levels of anticoagulation. However, there is large interindividual variability in warfarin dose requirements.[1] In addition, African Americans have been noted to require higher doses of warfarin than Caucasians.[2,3]

The above observations have led to investigations aimed at identifying functional genetic variants that alter the response to warfarin. Two such sets of variants within the cytochrome P-450 CYP2C9 enzyme and the vitamin K epoxide reductase complex 1 (VKORC1) have been clearly associated with a lower warfarin maintenance dose requirement.[4,5] The genotypes that are associated with these lower dose requirements are relatively common in Caucasians but rare in African Americans.[2,3] Thus, one possible explanation for higher dose requirements in African Americans is the relative rarity of these variants. Another possible explanation is the presence of other functional genetic variants in African Americans.

One such possible source of variability in individual and race-specific warfarin responses is the lipoprotein, apolipoprotein E (APOE). Because warfarin affects coagulation by inhibiting the regeneration of reduced vitamin K from oxidized vitamin K in the liver, the availability of hepatic vitamin K is likely to alter the dose requirement of warfarin.[6] The main circulating form of vitamin K, phylloquinone, is bound to chylomicrons and chylomicron remnants within the plasma, and APOE influences chylomicron remnant uptake by the liver.[7] There are three common variants of the *APOE* gene (designated ε2, ε3 and ε4) that encode the three common isoforms of the APOE molecule; their prevalence varies by race, with ε4 being more common in African Americans than in Caucasians.[8] Each isoform varies in its ability to facilitate clearance of vitamin-K-rich lipoproteins from plasma, with ε4 having the greatest clearance.[9–12] Plasma vitamin K levels also vary by *APOE* genotype,

Table 1 Clinical characteristics by APOE genotype[a]

Variable	ε2/ε2, ε2/ε3 (n = 34)	ε3/ε3 (n = 124)	Any ε4 (n = 64)	P-value[a]	Non-ε4 (n = 158)	Any ε4 (n = 74)	P-value[b]
Age (years)							
Median (25–75%)	59.0 (53.0–72.0)	60.0 (50.0–71.5)	58.0 (44.5–67.0)	0.254	60.0 (51.0–72.0)	59.0 (48.0–67.0)	0.2027
Race (African American)	18 (52.9%)	51 (41.1%)	35 (54.7%)	0.1560	69 (43.7%)	42 (56.8%)	0.0629
Gender (female)	15 (44.1%)	36 (29.0%)	20 (31.3%)	0.2449	51 (32.3%)	23 (31.1%)	0.8553
Body mass index							
<25 kg/m²	4 (11.8%)	45 (36.6%)	18 (28.6%)	0.0764	49 (31.2%)	20 (27.4%)	0.7928
25–30 kg/m²	15 (44.1%)	37 (30.1%)	19 (30.2%)	—	52 (33.1%)	24 (32.9%)	—
>30 kg/m²	15 (44.1%)	41 (33.3%)	26 (41.3%)	—	56 (35.7%)	29 (39.7%)	—
Most drinks on one occasion							
Median (25–75%)	1.0 (0.0–3.0)	2.0 (0.0–4.0)	1.0 (0.0–3.0)	0.4973	2.0 (0.0–4.0)	1.0 (0.0–3.0)	0.2204
Number of medications that potentiate warfarin at baseline							
0	6 (17.6%)	24 (19.4%)	14 (21.9%)	0.0165	30 (19.0%)	18 (24.3%)	0.0144
1	19 (55.9%)	46 (37.1%)	14 (21.9%)	—	65 (41.1%)	16 (21.6%)	—
2+	9 (26.5%)	54 (43.5%)	36 (56.3%)	—	63 (39.9%)	40 (54.1%)	—
Use of medications that inhibit warfarin at baseline	11 (32.4%)	52 (41.9%)	23 (35.9%)	0.5143	63 (39.9%)	25 (33.8%)	0.3730
Indication for warfarin							
Atrial fibrillation/flutter	14 (41.2%)	64 (51.6%)	26 (40.6%)	0.4341	78 (49.4%)	30 (40.5%)	0.3711
DVT/PE	13 (38.2%)	30 (24.2%)	27 (42.2%)	—	43 (27.2%)	30 (40.5%)	—
Dilated cardiomyopathy	3 (8.8%)	11 (8.9%)	5 (7.8%)	—	14 (8.9%)	6 (8.1%)	—
Stroke/TIA	2 (5.9%)	6 (4.8%)	2 (3.1%)	—	8 (5.1%)	3 (4.1%)	—
Other	2 (5.9%)	13 (10.5%)	4 (6.3%)	—	15 (9.5%)	5 (6.8%)	—
Smoking status							
Never smoked	12 (35.3%)	49 (39.5%)	22 (34.4%)	0.8125	61 (38.6%)	25 (33.8%)	0.7774
Past smoker	19 (55.9%)	58 (46.8%)	32 (50.0%)	—	77 (48.7%)	39 (52.7%)	—
Current smoker	3 (8.8%)	17 (13.7%)	10 (15.6%)	—	20 (12.7%)	10 (13.5%)	—
History of prior warfarin	6 (17.6%)	31 (25.2%)	13 (20.3%)	0.5644	37 (23.6%)	15 (20.3%)	0.5756
Vitamin K intake median (25–75%)	60.6 (21.1–110.2)	44.1 (20.2–88.5)	33.6 (15.7–112.8)	0.5904	45.0 (20.4–94.0)	32.6 (15.5–112.8)	0.5573
Time to maintenance (days) median (25–75%)	53.0 (38.0–78.0)	49.0 (27.5–115.5)	68.0 (38.5–127.0)	0.2226	49.0 (28.0–98.0)	64.5 (36.0–120.0)	0.1563
CYP2C9 variant	7 (21.9%)	35 (28.7%)	12 (18.8%)	0.3021	42 (27.3%)	14 (18.9%)	0.1700
VKORC1: any T	13 (38.2%)	48 (39.3%)	22 (34.9%)	0.8406	61 (39.1%)	25 (34.2%)	0.4795

Abbreviations: DVT = deep vein thrombosis, PE = pulmonary embolism, TIA = transient ischemic attack.Numbers differ between the three-level *APOE* variable and the Any ε4 variable because 10 patients with ε2/ε4 were excluded *a priori* from the three-level variable (see text).
[a]*P* values comparing clinical characteristics by *APOE* genotype. For these comparisons among the three-level APOE variable, we *a priori* excluded patients with the ε4/ε2 genotype in the primary analyses, as described under Materials and methods. These 10 patients are therefore also excluded from the analysis in this table.
[b]*P* values comparing clinical characteristics by *APOE* genotype.

with the highest levels among ε2 allele carriers and the lowest in ε4 carriers.[10–12] Accordingly, some investigators have proposed that the lower plasma levels of vitamin K in ε4 carriers lead to lower anticoagulant requirements,[13] whereas others have suggested that the greater uptake of vitamin K into the liver leads to increased availability of vitamin K for coagulation proteins and thus a higher anticoagulant drug requirement to achieve adequate anticoagulation in ε4 carriers.[14] To date, observational studies of the effect of *APOE* genotype on anticoagulant requirements have produced contrasting results.[13–16]

The INR Adherence and Genetics (IN-RANGE) study is a prospective cohort study with a primary aim of examining the relationship between *APOE* genotype and warfarin response. The study also sought to determine the effect of *APOE* polymorphisms in African Americans, a group not yet studied, independent of the effects of *CYP2C9* and *VKORC1* variants.

Table 2 Distribution of APOE genotype by race

Race	*APOE genotype*	*Percentage (N)*	*Median dose (Q1–Q3)*
Caucasian	ε2/ε3	13.22 (16)	32.5 (23.8–41.9)
	ε3/ε3	60.33 (73)	35.0 (22.5–43.8)
	ε2/ε4	2.48 (3)	35.0 (35.0–75.0)
	ε3/ε4	23.14 (28)	40.6 (26.5–52.5)
	ε4/ε4	0.83 (1)	25.0 (25.0–25.0)
African-American	ε2/ε2	2.70 (3)	22.0 (17.5–30.0)
	ε2/ε3	13.51 (15)	32.5 (26.3–52.5)
	ε3/ε3	45.95 (51)	35.0 (26.3–52.5)
	ε2/ε4	6.31 (7)	35.0 (30.0–55.0)
	ε3/ε4	27.03 (30)	46.3 (38.8–55.0)
	ε4/ε4	4.50 (5)	35.0 (35.0–37.5)

Abbreviation: APOE, apolipoprotein E.

Results

Patient population and distribution of genotypes
The study cohort comprised 232 patients (mean age 58.8 ± 14.9 years) of whom 121 (52.2%) were self-reported Caucasian and 111 (47.8%) were self-reported African American. The clinical characteristics of the cohort by *APOE* genotype are shown in Table 1 and the distribution of *APOE* genotypes within the Caucasian and African American subgroups is shown in Table 2. The allele frequencies for ε2, ε3 and ε4 were, respectively, 7.9, 78.5 and 13.6% in Caucasians and 12.6, 66.2 and 21.1% in African Americans. As expected, the *APOE* ε4 variant was more common in African Americans. The allele distribution was in Hardy–Weinberg equilibrium for both groups ($P > 0.25$). Patients with the *APOE* ε4 variant also were more likely to use more medications that potentiate the effect of warfarin (Table 1). The prevalence of *APOE* variants was the same among those who reached maintenance dose (*APOE* ε2, ε3 and ε4 variants: 10, 73 and 17%) versus those who did not (*APOE* ε2, ε3 and ε4 variants: 10, 72 and 18%).

Effect of APOE *genotype on maintenance dose of warfarin*
In the entire cohort, ε4 carriers had a significantly higher median weekly maintenance dose (44.4 mg) than non-carriers (35.0 mg; $P = 0.0043$). Those with the ε2/ε2 and ε2/ε3 genotypes had the lowest median maintenance dose (31.2 mg), ε3/ε3 homozygotes had an intermediate maintenance dose (35.0 mg) and those with the ε4/ε3 or ε4/ε4 genotypes had the highest maintenance dose (45.0 mg); these differences were statistically significant ($P = 0.0118$). As explained under Materials and methods, the 10 ε2/ε4 patients were excluded *a priori* from the analysis of the three-level variable. These 10 patients had a median weekly maintenance dose of 35.0 mg, the same as that of the ε3/ε3 homozygotes.

Table 3 Maintenance dose of warfarin by APOE genotype, stratified by race[a]

APOE genotype	Caucasians			African Americans		
	N	*Median dose (Q1–Q3)*	P-*value*[b]	N	*Median dose (Q1–Q3)*	P-*value*[b]
Any ε4			0.60			0.014
Non-ε4	89	35.0 (22.5–43.8)		69	35.0 (26.3–50.0)	
Any ε4	32	38.1 (26.5–52.5)		42	45.0 (35.0–55.0)	
Three-level APOE			0.81			0.012
ε2/ε2, ε2/ε3	16	32.5 (23.8–41.9)		18	30.0 (25.0–50.0)	
ε3/ε3	73	35.0 (22.5–43.8)		51	35.0 (26.3–52.5)	
ε3/ε4, ε4/ε4	29	40.0 (25.5–52.5)		35	45.0 (35.0–55.0)	

Abbreviation: APOE, apolipoprotein E.
[a]Q1 = quartile 1, Q3 = quartile 3.
[b]Adjusted for age; sex; race; insurance status; study site; *CYP2C9* genotype; *VKORC1* genotype, history of prior warfarin use; medications that interact with warfarin; employment; marital status; body mass index; indication for warfarin; smoking; maximum number of drinks of alcohol consumed on any one occasion; and history of arrhythmias, myocardial infarction, other heart disease, stroke, pulmonary embolism, deep vein thrombosis, hyperthyroidism, cancer, peptic ulcer disease and clotting disorder.

Table 4 Multivariable models for any ε4 and for APOE three-level variable using square root transformed warfarin dose

Parameter	*Beta coefficients*	
	Any E4 versus. No E4[a]	*Three-level APOE Variable*[a]
Intercept	8.912	8.935
VKORC1 (any T versus no T)	−1.097[b]	−1.112[b]
CYP2C9 (any variant versus wild type)	−0.497[c]	−0.487[c]
Age (per 1 year increase)	−0.023[c]	−0.021[c]
Number of medications that potentiate warfarin	−0.180[a]	−0.180[c]
Employment status (disabled versus working)	−0.820[d]	−0.872[d]
Employment status (retired versus working)	−0.301	−0.407
Employment status (unemployed versus working)	−0.857[c]	−0.970[c]
BMI (<25 versus >30)	−0.687[d]	−0.685[d]
BMI (25–30 versus >30)	−0.689[d]	−0.652[d]
APOE		
Any ε4		
Any ε 4[a]race interaction term	0.517 ($P=0.20$)	—
Any ε 4 versus no E4, among Caucasian	0.152	—
Any ε 4 versus no E4, among African American	0.670[c]	—
APOE three-level		
APOE three-level [a]race interaction term	—	0.567 ($P=0.056$)
APOE three-level among Caucasian	—	−0.051
APOE three-level among African American	—	0.516[c]

Abbreviations: BMI, body mass index.
[a]Both models also adjusted for site, race (interaction term), gender, indication for warfarin, self-reported insurance, marital status, smoking status, most alcoholic drinks on one occasion, use of medications that inhibit warfarin at baseline and history of prior warfarin use, abnormal heart rhythm, MI, other heart condition, stroke, PE, DVT, hyperthyroidism, cancer, peptic ulcer disease and abnormal clotting.
Univariate R^2 for Any ε4 versus no ε4: Caucasian, 2.1%, African American 3.9%.
Full model R^2: Caucasian 57.3%, African American 54.6%.
Univariate R^2 for APOE three-level variable: Caucasian 1.4%, African American 4.2%.
Full model R^2: Caucasian 57.1%, African American 54.1%.
[b]$P \leqslant 0.0001$.
[c]$0.01 < P \leqslant 0.05$.
[d]$0.001 < P \leqslant 0.01$.

When stratified by race, the warfarin weekly maintenance dose was higher among African Americans (median 37.5 mg, mean 42.9 mg) than Caucasians (median 35.0 mg, mean 36.9 mg), a difference that was statistically significant ($P=0.018$). The effects of *APOE* genotype on warfarin maintenance dose, stratified by race, are shown in Table 3. In the multivariable model adjusting for confounders, selected as described under Materials and methods, being an ε4 carrier was associated with a statistically significant higher dose in African Americans (multivariable $P=0.014$) but not Caucasians (multivariable $P=0.60$, multivariable P value for interaction of genotype by race = 0.20, Tables 3 and 4). The three-level variable for *APOE* genotype showed the highest dose among those with ε3/ε4 or ε4/ε4 genotypes, the next highest among ε3/ε3 homozygotes and the lowest among those with ε2/ε3 or ε2/ε2 genotypes in both African Americans and Caucasians (Tables 3 and 4 and Figure 1). However, the association was statistically significant only within the African American subgroup (multivariable $P=0.012$), not the Caucasian subgroup (multivariable $P=0.81$; multivariable P value for interaction = 0.056). There were only six patients with the ε4/ε4 genotype and they did not clearly require lower doses. However, the one Caucasian patient with ε4/ε4 was a VKORC1 1173C/T heterozygote, a genotype that is associated with low maintenance doses. In addition, one of the highest dose requirements was among one of the five African Americans with the ε4/ε4 genotype, 105 mg/week. The median dose among those with ε2/ε4 was 35.00 mg in both the few African Americans ($n=7$) and the few Caucasians ($n=3$) with this genotype, again consistent with the ε3/ε3 group.

APOE genotype was associated with higher maintenance doses among both African Americans and Caucasians who were homozygous wild type for *CYP2C9*, but this difference was only statistically significant among African Americans (Table 5). Among subjects with a *CYP2C9* variant, there were no statistically significant effects of APOE within either race, but there were very few subjects in these subgroups (Table 5).

There was no apparent effect of *APOE* genotype on time to establishing a maintenance dose in the Caucasian or African American subgroups, when either ε4 carrier status ($P=0.84$

for Caucasians, $P=0.46$ for African Americans) or the three-level *APOE* genotype variable ($P=0.27$ for Caucasians, $P=0.63$ for African Americans) was considered.

Discussion

This study demonstrates that the *APOE* ε4 allele is associated with a significantly higher warfarin maintenance dose requirement than other *APOE* alleles and that the relationship between different *APOE* genotype and dose requirement may follow the same pattern as the published relationships between *APOE* genotype and hepatic chylomicron clearance.[7,9–12] These findings were independent of *CYP2C9* polymorphisms, the *VKORC1* 1173C/T variant, and numerous other factors that can alter warfarin dose requirements. The results were statistically significant among African Americans but not Caucasians; however, the ε4 allele was less common among Caucasians, which could have limited our ability to detect an effect in this group.

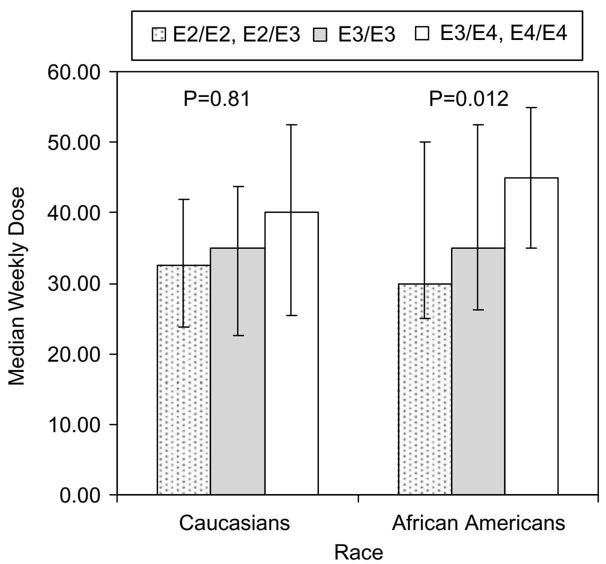

Figure 1 Median warfarin maintenance dose by *APOE* genotype. The median warfarin maintenance dose by the three-level *APOE* variable among Caucasians and African Americans is shown. The bars represent the 25th and 75th percentiles.

Our findings could, at least in part, explain the higher warfarin dose requirement among African Americans that has previously been noted by others.[2] The ε4 allele was associated with a higher maintenance dose of warfarin in African Americans, even after adjusting for the different frequencies of *CYP2C9* and *VKORC1* classes. Although the *APOE* genotype may have an effect on maintenance dose in Caucasians, the effect was less pronounced and not statistically significant. Regardless of any potential differences in magnitude of effect between African Americans and Caucasians, the fact that the *APOE* ε4 allele is more common and the *CYP2C9* and *VKORC1* variants are much less common in African Americans than Caucasians suggests that different maintenance dose requirements could be driven by different prevalences of functionally relevant genetic polymorphisms between these two groups.

Hepatic clearance of chylomicrons, and therefore the vitamin K carried by chylomicrons, is at least partly dependent on *APOE* genotype, with ε4 carriers having the most rapid clearance.[10] Thus, one hypothesis is that the APOE ε4 variant facilitates a relatively high uptake of vitamin K by the liver and thus enhances the availability of vitamin K for carboxylation of clotting factors.[14] However, a contrasting hypothesis is that the greater hepatic clearance of vitamin K mandated by the APOE ε4 variant does not translate into greater availability of vitamin K for

Table 5 **Maintenance dose of warfarin by APOE genotype by race, stratified by CYP2C9**

	Caucasians						*African Americans*					
	CYP2C9 Homozygous wild type			*Any CYP2C9 *2 or *3 variant*			*CYP2C9 Homozygous wild type*			*Any CYP2C9 *2 or *3 variant*		
ApoE genotype	N	*Median dose (Q1–Q3)*	P-*value*[a]	N	*Median dose (Q1–Q3)*	P-*value*[a]	N	*Median dose (Q1–Q3)*	P-*value*[a]	N	*Median dose (Q1–Q3)*	P-*value*[a]
Any E4			0.44			0.91			0.023			0.30
Non-E4	52	36.75		35	27.50		60	33.75		7	45.00	
Any E4	20	46.25		12	28.75		40	45.00		2	42.50	
Three-level APOE			0.96			0.73			0.018			0.43
E2/E2, E2/E3	10	37.50		6	26.25		15	30.00		1	52.50	
E3/E3	42	36.75		29	30.00		45	35.00		6	40.00	
E3/E4, E4/E4	19	45.00		10	26.50		33	45.00		2	42.50	

Abbreviations: APOE, apolipoprotein E, Q1 = quartile 1, Q3 = quartile 3.
[a]Adjusted for age; sex; race; insurance status; study site; *CYP2C9* genotype; *VKORC1* genotype, history of prior warfarin use; medications that interact with warfarin; employment; marital status; body mass index; indication for warfarin; smoking; maximum number of drinks of alcohol consumed on any one occasion; and history of arrhythmias, myocardial infarction, other heart disease, stroke, pulmonary embolism, deep vein thrombosis, hyperthyroidism, cancer, peptic ulcer disease and clotting disorder.

the formation of functional coagulation factors and may, in fact, be associated with reduced availability because of greater catabolism of vitamin K.[13,17] Our results support the former hypothesis.

Three prior studies have examined the effects of *APOE* genotype on warfarin dose requirement. Kohnke *et al.*[14] studied 183 Swedish patients (33.5% with one or two ε4 alleles) and found that, among *CYP2C9* wild-type homozygotes, those with the ε4/ε4 genotype required significantly higher warfarin maintenance doses than those with one or no ε4 alleles. Although our study had too few ε4/ε4 patients to draw meaningful conclusions about this genotype class, our results are consistent with a similar effect of *APOE* genotype on maintenance dose, extending this finding to African Americans. A follow-up study by Kohnke *et al.*[15] failed to confirm the effect of *APOE* genotype on warfarin dose requirement in Italian patients; however, that cohort had only 20 (14%) ε4 carriers and no ε4/ε4 homozygotes. Sconce *et al.*[16] found that the ε4 allele was associated with lower doses in a Caucasian population, but, interestingly, ε4 carriers had the highest plasma vitamin K concentrations, not the lowest as might be expected.[10–12] Clearly, the relationship between APOE genotype and warfarin dose requirements is complex; further study is needed to better understand the interactions between APOE and race, other genotypes, diet and vitamin K levels. In a retrospective study of different anticoagulants, acenocoumarol and phenprocoumon, Dutch Caucasian ε4 carriers and homozygotes required a significantly lower mean dose of acenocoumarol, but not of phenprocoumon, relative to ε3/ε3 homozygotes.[13] Comparisons between our findings and those of this last study are necessarily limited, however, because of the use of different medications and a different definition of maintenance dose that may not represent steady-state requirements. Furthermore, the significant findings were limited to patients taking acenocoumarol and thus could represent the play of chance. With respect to warfarin specifically, our study is consistent with the prior Swedish study.[14]

There are several strengths and potential limitations that must be considered in interpreting our results. Our study was prospective and specifically designed to test the hypothesis that *APOE* genotype would have an effect on warfarin maintenance dose. The prospective cohort design should prevent selection bias, a common concern in retrospective case–control studies. We also were able to control for numerous other factors that are known to alter warfarin response. However, we were only able to adjust for a single polymorphism in VKORC1. Although this polymorphism has been associated with lower warfarin dose in multiple studies and is just as informative as inferred haplotypes in Caucasians,[5,18,19] further assessment of other VKORC1 polymorphisms is needed. Due to our sample size, we also were limited in our ability to test for interactions by race, and interpretation of our findings must be tempered by the sample size and the possibility of erroneous findings. Our findings among ε4/ε4 patients is also not consistent with prior study, but we had very few patients with this genotype. Finally, our results may not generalize to other populations.

In conclusion, our study demonstrates that *APOE* genotype may affect warfarin maintenance dose requirements, particularly among African Americans, with higher doses needed by ε4 allele carriers and lower doses needed by those with the ε2/ε2 and ε2/ε3 genotypes. These findings could at least partly explain the higher dose requirements noted previously in African Americans. Although the ε4/ε4 genotype was rare and not clearly associated with lower doses in this study, our study suggests that *APOE* genotype may play some role, along with other factors, in determining warfarin response and may explain some of the racial differences in warfarin dose requirements.

Materials and methods

Study population and data collection

From April 2002 to December 2005, patients were prospectively recruited at three anticoagulation clinics: the Hospital of the University of Pennsylvania (HUP) and the Philadelphia Veterans Affairs Medical Center (PVAMC) in Philadelphia, PA, and the Penn State Milton S Hershey Medical Center (HMC) in Hershey, PA. Patients 21 years and older initiating warfarin therapy once daily with a target INR of 2.0–3.0 who presented to one of the clinics were considered eligible for the study. We excluded patients with abnormal INRs before initiating warfarin and those with anti-phospholipid antibody in whom the INR measurement may not be valid.[20] This study also excluded nine patients who were not self-reported Caucasian/white or African American/black.

Information on patient demographics, medical history, medication use, warfarin dose, diet and INR was obtained prospectively by trained study interviewers using standardized questionnaires. Genomic DNA was obtained from buccal swabs and was analyzed by facility staff blinded to patient characteristics or outcomes.

Genotyping

DNA was extracted from buccal swab preparations using a method adapted from Richards *et al.*[21] Two sets of swabs were taken from each participant and used for validation of methods and quality control.

APOE genotypes (based on single nucleotide polymorphisms (SNPs) rs429358 and rs7412) were determined by the molecular genotyping and diagnostics facility at HUP using a standard method.[22] Briefly, polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assays were performed in 50-μl reactions in PCR buffer that contained 5 μl of genomic DNA, 200 nM of the forward ((MDX 216) 5′-GCACGGCTGTCCAAGGAGCTGCAGGC-3′) and reverse ((MDX 217) 5′-GGCGCTCGCGGATGGCGCT GAG-3′)) primers, 200 μM dNTPs, 15 mM $MgCl_2$, 5.0 μl 10% dimethylsulfoxide, and 1.25 U Amplitaq gold. Thermocycling conditions consisted of 1 min at 95°C, followed by 45 cycles of 95°C for 30 s, 65°C for 30 s and 72°C for 30 s, with a final extension step of 72°C for 10 min. The 270-bp product was digested with 1.5U *Hha*I (New England Biolabs, Ipswich, MA, USA) at 37°C for 2 h. The products were separated on

4% agarose/Nusieve gels and visualized by ethidium bromide staining and UV illumination. The ε2 allele yielded bands of 91 and 83 bp; the ε3 allele yielded bands of 91, 48 and 35 bp; and the ε4 allele yielded bands of 72, 48 and 35 bp.

The region containing the CYP2C9*2 SNP (rs1799853) was PCR amplified in a 25-μl volume that contained 5 μl of genomic DNA, 200 nM of the forward (5′-GTATTTTGGC CTGAAACCCATA-3′) and reverse (5′-GGCCTTGGTTTTT CTCAACTC-3′) primers, 200 μM dNTPs, NW3 buffer (16 mM $(NH_4)_2SO_4$, 67.5 mM Tris, 0.01% Tween 20), 2 mM $MgCl_2$, and 1.5 U Amplitaq (Roche, Indianapolis, IN, USA). The 452-bp product was digested with 1.5 U *Ava*II (New England Biolabs) at 37°C for 6 h. The products were separated on 3% agarose gels and visualized by ethidium bromide staining and UV illumination. For CYP2C9*3 (rs1057910), PCR amplifications were performed using 200 μM dNTPs, NW3 buffer, 2 mM $MgCl_2$ and 1.5 U Hotstar Taq Polymerase (Qiagen, Valencia, CA, USA) in a final volume of 25 μl. Genomic DNA (5 μl) and 0.2 pg of heteroduplex generator were coamplified in the presence of 200 nM of the forward primer (5′-CAGGAAGAGATTGAACGTGTG-3′) and the reverse primer (5′-ACAAACTTACCTTGGGAATGAGA-3′). The heteroduplexes and homoduplexes were separated on 12% polyacrylamide (2.6% crosslinking) minigels run at 150 V for 2 h and visualized using ethidium bromide staining and UV illumination. The CYP2C9*3 heteroduplex generator was validated against the Nsi I RFLP and gave the same results.

VKORC1 1173C/T (rs9934438) polymorphism was chosen because it has been demonstrated in several studies to be associated with lower dose requirements and is just as informative as inferred haplotypes in Caucasians.[5,18,19] The region containing the VKORC1 1173C/T (based on SNP rs9934438) variant was PCR amplified in 25-μl reactions in PCR buffer that contained 5 μl of genomic DNA, 200 nM forward (5′-AAGATGAAAAGCAGGGCCTAC-3′) and reverse (5′-CCGAGAAAGGTGATTTCCAA-3′) primers, 200 μM each dNTP, 1X PCR buffer (Qiagen), and 1 U HotStar Taq polymerase (Qiagen). Thermocycling conditions consisted of 95°C for 5 min, followed by 45 cycles of 95°C for 40 s, 59°C for 50 s and 72°C for 40 s, with a final extension step of 75°C for 5 min. The 195-bp product was digested with 2 U *Sty*I (New England Biolabs) at 37°C for 6 h; the 1173T allele is cleaved to yield fragments of 125 and 70 bp. The products were separated on 12% polyacrylamide gels and visualized by ethidium bromide staining and UV illumination.

Outcomes

The primary outcome for the study was the maintenance dose of warfarin, determined using the standard definition as the dose that leads to a stable INR over three consecutive visits following initiation of the drug.[5] In addition, we examined the time required to reach the maintenance dose.

Statistical analysis

The demographic and clinical characteristics of subjects were compared by *APOE* genotype using χ^2 or exact tests for categorical variables and Wilcoxon rank sum tests for continuous variables. The Hardy–Weinberg equilibrium test was used to assess whether genotype frequencies were in conformity with predictions based on allele frequencies. *APOE* genotype was categorized in two ways, first based on the presence or absence of any *APOE* ε4 allele (e.g., ε4/ε4, ε4/ε3 and ε4/ε2 versus non-ε4), the 'ε4 carrier' group. This was based on prior studies demonstrating that people with an ε4 allele clear vitamin-K-rich lipoproteins from the circulation more efficiently and have lower plasma vitamin K concentrations than those who do not carry an *APOE* ε4 allele[10,11] and a prior study suggesting that the presence of the ε4 allele is associated with higher warfarin dose requirements.[14] Second, we categorized *APOE* genotypes into three groups (the 'three-level APOE' variable), based on data suggesting that plasma vitamin K levels vary by these groups, with levels being highest among those with the ε2/ε2 or ε2/ε3 genotype, intermediate with ε3/ε3, and lowest with ε4/ε4 or ε4/ε3.[11] The ε2/ε4 genotype is rare[23] and vitamin K levels have not been studied in this class; therefore, the 10 ε2/ε4 patients were excluded *a priori* from analysis of the three-level variable.[10,12]

The distribution for the warfarin maintenance dose was right-skewed, so transformations were performed to achieve approximate normality using the family of Box–Cox transformation[24] using a maximum likelihood criterion,[25] resulting in a square-root transformation on the maintenance dose. Linear regression analyses based on this transformation were then performed to test for differences in maintenance dose by *APOE* genotype and other potential confounders. Covariates that were associated with maintenance dose with a bivariate P value <0.2 were included in a multivariable linear regression model. Cox proportional hazards models were used to examine differences in the time to maintenance therapy by *APOE* genotype. For these analyses, patients who did not reach maintenance therapy were censored at their last follow-up visits.

All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA) and Stata version 9 (StataCorp, College Station, TX, USA). The study was approved by the Institutional Review Boards at all participating hospitals, and all patients provided informed, written consent.

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