BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2007.03053.x

Influence of *APOE* genotypes and *VKORC1* haplotypes on warfarin dose requirements in Asian patients

Suman Lal,[1] Edwin Sandanaraj,[1] Srinivasa Rao Jada,[1] Ming-Chai Kong,[2] Lai-Heng Lee,[2] Boon-Cher Goh,[3] Soo-Chin Lee[3] & Balram Chowbay[1]

[1]Laboratory of Clinical Pharmacology, Division of Medical Sciences, Humphrey Oei Institute of Cancer Research, National Cancer Centre, Singapore, [2]Department of Haematology, Singapore General Hospital, Singapore and [3]Department of Haematology-Oncology, National University Hospital, Singapore

Correspondence
Dr Balram Chowbay PhD, Principal Clinical Pharmacologist Division of Medical Sciences, 11 Hospital Drive, Humphrey Oei Institute of Cancer Research, National Cancer Centre, Singapore 169610.
Tel.: 65 6436 8321;
Fax: 65 6372 0161;
E-mail: ctebal@nccs.com.sg

Keywords
apolipoproteinE, Asian, *VKORC1* haplotypes, warfarin dose

Received
15 March 2007

Accepted
14 September 2007

Published *OnlineEarly*
8 November 2007

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Recent studies on pharmacogenetics of warfarin have implicated apolipoproteinE (*APOE*) polymorphisms to influence the vitamin K dependent coagulation cascade and hence the efficacy of warfarin.
- Studies among Caucasian and African Americans showed a significant but conflicting role of apolipoproteinE (APOE) isoforms in warfarin pharmacogenetics.
- The contribution of APOE isoforms in influencing variations in warfarin requirements in Asian subjects remains to be investigated.

WHAT THIS STUDY ADDS

- This is the first report of a population study in Asians exploring the role of isoforms encoded by three *APOE* alleles (*ε2*, *ε3*, *ε4*) in influencing warfarin dose requirements.
- The present study showed that the APOE *ε3*/*ε3* isoform is the predominant genotype in the Asian population.
- The study also showed that APOE isoforms may not be important in affecting warfarin pharmacodynamics in Asian patients. It also suggested that the impact of different APOE isoforms depended on the frequency of *APOE* genotypes in the population, in particular the *ε4* allele containing genotypes.

AIMS

To investigate the influence of *APOE* genotypes and *VKORC1* haplotypes on warfarin dose requirements in Asian patients.

METHODS

A total of 174 Asian patients (Chinese, $n = 96$; Malays, $n = 50$; Indians, $n = 28$) who had stable daily warfarin doses for at least 1 month were recruited. Following genomic DNA extraction from venous blood, pharmacogenetic analysis of *APOE* and *VKORC1* genes was done by DNA sequencing.

RESULTS

The majority of the Asian patients (78%) harboured the *APOE ε3/ε3* genotype. Different *APOE* genotypes were found not to have any significant influence on mean daily warfarin dose requirements. Warfarin dose requirements in the pooled Asian patients homozygous for the *VKORC1* H1 haplotype were significantly lower compared with patients homozygous for the H7 haplotype (H1-H1 *vs.* H7-H7: 2.79 ± 1.06 mg day^{-1} *vs.* 5.45 ± 2.3 mg day^{-1}, $P < 0.001$).

CONCLUSIONS

The present study suggests that *APOE* variants have minimal impact on warfarin dose requirements in Asian patients, probably due to the low frequency of *ε4* allele containing genotypes.

Introduction

Warfarin is a commonly prescribed oral anticoagulant with wide interindividual variations in therapeutic response. Risk of haemorrhage frequently complicates warfarin therapy and occurs at a rate of 7.6–16.5 per 100 patients per year [1]. Warfarin is administered as a racemate of R- and S-enantiomers with S-warfarin being 3–5 fold more active than R-warfarin and metabolized mainly by the cytochrome P450 2C9 (CYP2C9) enzyme [2]. The *CYP2C9* gene is polymorphic and the reference *CYP2C9*1* allele yields a more active enzyme than the *CYP2C9*2* and **3* alleles. Warfarin exerts its pharmacodynamic effects by inhibiting VKORC1 (vitamin K epoxide reductase complex 1), a multicomponent lipid-protein enzyme system [3]. Major warfarin dose related haplotypes [H1(CCATTG), H7(TCGTCA) and H9(TAGTCG)] have been assigned based on the rs7196161, rs17880887, rs9923231, rs2884737, rs9934438 and rs17880624 polymorphisms in the *VKORC1* gene [4].

Recent pharmacogenetic studies of warfarin have implicated apolipoproteinE (*APOE*) polymorphisms to influence the vitamin K dependent coagulation cascade and hence the efficacy of warfarin [5, 6]. Plasma vitamin K concentrations are dependent on the APOE receptor mediated clearance of chylomicron particles and their remnants where it serves as a ligand mediating the uptake of vitamin K into hepatocytes [7]. APOE is a glycoprotein consisting of 299 amino acids encoded by the human *APOE* gene on chromosome 19q13.2 which is 3.7 kb and consists of 4 exons. Two nucleotide variations on exon 4 in the *APOE* gene give rise to three isoforms encoded by three *APOE* alleles, namely, *ε2* (Cys112/Cys158), *ε3* (Cys112/Arg158) and *ε4* (Arg112/Arg158) [8]. The receptor affinities of the proteins encoded by the three allelic isoforms of *APOE* towards chylomicron remnants varies in the order *ε2* < *ε3* < *ε4* [9]. Further investigations to elucidate the relationships between the different *APOE* genotypes, vitamin K concnetrations and warfarin dose requirements showed that patients homozygous for the *APOE ε4* allele required higher warfarin doses and that the allele accounted for 6% of the variance in warfarin dose requirements among the CYP2C9 extensive metabolizers. However, Visser *et al.* [10] reported that patients homozygous for the *APOE ε2* allele required higher doses of acenocoumarol than their counterparts harbouring the *APOE ε3/ε4 or ε4/ε4*, implying that rapid uptake of vitamin K among subjects with an *APOE ε2* allele could increase its hepatic availability, raising warfarin requirements.

The objective of the present study was to determine the influence of APOE *ε2, ε3 and ε4* allelic isoforms on warfarin dose requirements in Asian patients of known *VKORC1* genotypes. To the best of our knowledge, this is the first study exploring the relation of *APOE* pharmacogenetics to warfarin dose requirements in Asians.

Methods

Study subjects and genotyping

A total of 174 Asians comprising of 96 Chinese, 50 Malays and 28 Indians were enrolled from the Anticoagulation Clinic at the Singapore General Hospital and the National University Hospital, Singapore. Malay and Indian subjects were also enrolled in the study by Lee *et al.* [11]. Ethnicity was assigned by verification against National Registry Identification Cards (NRIC). All patients were required to have their daily warfarin doses stable for at least 1 month and stabilized INR values (range 2–3) within the desired therapeutic range over the same duration. The indications for anticoagulant therapy were mainly for prevention or treatment of thromboembolic diseases such as atrial fibrillation, deep vein thrombosis or prosthetic valve replacement. Patients with congestive heart failure (NYHA class 3 or greater), liver cirrhosis or thyroid disease and patients on medications known to interact with warfarin were excluded from the study. All patients gave written informed consent to participate and the protocol was approved by the institution ethics committee.

Genomic DNA was extracted from venous blood using the phenol-chloroform method and *APOE* genotypes were determined by PCR (F: 5′-TCAAGGTTGCAGTGAACCATGTTC AGGC-3′, R: 5′-AGAGCTAGGGAAGGACAGA GACAGAGC-3′) and fragments analyzed by direct DNA sequencing (Beckman Coulter Inc., CA, USA) with 5′-TGATGGA CGAGA CCATGAAGGAGTTG-3′ as the sequencing primer. Genotyping for the *VKORC1* polymorphisms was also performed in all the subjects using the technique previously described [11]. *VKORC1* haplotypes were assigned according to Rieder *et al.* [4].

Statistical analysis

The chi-squared and Fisher's exact test was used to analyze allele and genotype frequencies. Individual haplotypes were derived using the expectation maximization algorithm. The nonparametric Kruskal–Wallis test was used to measure the variability in warfarin dose requirements in relation to *APOE* genotype and *VKORC1* haplotype pairs. For the analysis of covariance, covariates included *APOE* and *VKORC1* genotype status, age, weight, height, gender, ethnic group status and the INR. Bonferroni correction was applied for multiple testings. All statistical analyses including estimation of confidence intervals were done using Stata (STATA statistical software release 7.0, USA).

Results

The median age of the patients was 58 years (range 29–87 years). Univariate and multivariate analysis showed that age ($P < 0.001$), ethnicity (Chinese, $P = 0.017$; Malays,

Table 1

APOE genotype and allele frequencies and *VKORC1* haplotypes in Asian patients on warfarin therapy

Populations	n	*APOE* genotype frequencies, *n* (%)					*APOE* allele frequencies (95% CI)		
		ε2/ε2	*ε2/ε3*	*ε3/ε3*	*ε3/ε4*	*ε4/ε4*	*ε2*	*ε3*	*ε4*
Chinese	96	0	16 (16.7)	67 (69.8)	12 (12.5)	1 (1.1)	0.08 (0.04, 0.12)	0.84 (0.79, 0.89)	0.07 (0.03, 0.11)
Malays	50	0	4 (8.0)	42 (84.0)	4 (8.0)	0	0.04 (0.001, 0.08)	0.92 (0.87, 0.97)	0.04 (0.001, 0.08)
Indians	28	0	1 (3.6)	26 (92.9)	1 (3.6)	0	0.02 (–0.02, 0.06)	0.96 (0.91, 1.01)	0.02 (–0.02, 0.06)
Pooled Asians	174	0	21 (12.1)	135 (78.2)	17 (9.8)	1 (0.6)	0.06 (0.04, 0.08)	0.89 (0.86, 0.92)	0.04 (0.02, 0.06)

		VKORC1 haplotype frequencies (%)			*VKORC1* haplotype pairs, *n*			
	n	H1 (CCATTG)	H7 (TCGTCA)	H9 (TAGTCG)	H1-H1	H1-H9	H7-H7	H7-H9
Chinese	96	86.4	12.5	1.1	82	1	13	–
Malays	50	80.0	20.0	0	40	–	10	–
Indians	28	7.1	85.7	7.2	2	–	24	2
Pooled Asians	174	71.8	26.4	1.8	124	1	47	2

n, number of subjects.

Table 2

APOE genotypes and *VKORC1* haplotype pairs in relation to daily warfarin dose requirements (mean ± SD) in Asian patients

Gene	Genotype/ Haplotype pairs	Warfarin dose (mg day^{-1}; mean ± SD)							
		n	Pooled Asian	*n*	Chinese	*n*	Malays	*n*	Indians
APOE	*ε2/ε2*	0	NA	0	NA	0	NA	0	NA
	ε2/ε3	21	3.26 ± 1.21	16	3.29 ± 1.40	4	2.82 ± 1.08	1	5
	ε3/ε3	135	4.06 ± 2.15	67	3.71 ± 1.68	42	3.34 ± 1.44	26	6.13 ± 2.9
	ε3/ε4	17	3.91 ± 1.88	12	4.02 ± 2.10	4	3.06 ± 1.19	1	6
	ε4/ε4	1	3.75	1	3.75	0	NA	0	NA
VKORC1	H1-H1	124	2.79 ± 1.06	82	2.82 ± 1.05	40	2.92 ± 1.11	2	1.75 ± 0.50
	H1-H9	1	7.5	1	7.5	–	–	–	–
	H7-H7	47	5.45 ± 2.3	13	5.71 ± 1.40	10	4.71 ± 1.39	24	6.41 ± 2.53
	H7-H9	1	3	–	–	–	–	1	3
		1	9	–	–	–	–	1	9

n, number of subjects; NA, not appropriate.

$P < 0.001$; Indians, $P < 0.001$) and *VKORC1* haplotype (H1, $P < 0.001$; H7, $P < 0.001$) correlated significantly with daily warfarin dose requirements. Patients older than 60 years of age required significantly lower daily doses of warfarin ($n = 82$; 3.08 ± 1.49 mg day^{-1}) compared with younger patients ($n = 92$; 4.71 ± 2.18 mg day^{-1}; $P < 0.0001$). The daily warfarin doses were significantly different among the three ethnic groups ($P = 0.0001$) and were significantly lower among Chinese (3.68 ± 1.68 mg day^{-1}) and Malays (3.28 ± 1.39 mg day^{-1}) than in Indians (6.21 ± 2.94 mg day^{-1}, $P < 0.001$ in each case).

The *APOE* genotype frequencies were found to be in Hardy–Weinberg equilibrium (Table 1) and the allele frequencies were comparable with previous reports [12]. There were no patients carrying the *ε2/ε2* or the *ε2/ε4* genotype whereas the *ε4/ε4* genotype was observed only in the Chinese subjects (1%). The daily warfarin doses in patients harbouring different *APOE* genotypes were in the similar range and pairwise analysis showed no significant difference ($P > 0.05$ in each case) (Table 2). Analysis of patients based on ethnicity as well as stratification by warfarin doses (greater than or less than 5 mg day^{-1}) did not reveal any significant influence of the *APOE* genotypes on warfarin dose requirements.

The majority of the patients carried the low-dose (H1; 71.8%) *VKORC1* haplotype, followed by the high-dose (H7; 26.4% and H9; 1.8%) haplotypes (Table 1). The warfarin dose requirements in the pooled Asian patients homozygous for the H1 haplotype were significantly lower compared with patients homozygous for the H7 haplotype (H1-H1 *vs.* H7-H7: 2.79 ± 1.06 mg day^{-1}*vs.* 5.45 ± 2.3 mg day^{-1}, $P < 0.001$) (Table 2). The warfarin dose requirements were also significantly lower among subjects homozygous for the H1 haplotype compared with patients homozygous for the H7 haplotype when examined in individual ethnic groups ($P < 0.02$ in each case).

Discussion

The majority of the Asian patients in the present study carried the *ε3/ε3* genotype (78.2%), which is associated with intermediate vitamin K clearance. The *ε4/ε4* genotype which is associated with faster clearance of vitamin K-containing chylomicrons was detected in only one patient, suggesting minimal phenotypic impact of this rare isoform on warfarin dosage requirement in Asians. Similar to the study by Visser *et al.* [10], a recent study by Sconce *et al.* [13] showed a small (2%) but significant impact of the *ε4* allele on low dose warfarin requirement in Caucasians. This genotypic-phenotypic effect, however, was absent in the Italian population, probably due to the low frequency of the *ε4* allele in this population [14]. In another report, the presence of the *ε4* allele associated significantly with higher warfarin dose requirements among African-Americans but not among Caucasians, who incidentally had a lower frequency of the *ε4* allele [15]. Taken collectively, these studies suggest that the impact of different APOE isoforms depend on the frequency of *APOE* genotypes in the population, in particular the *ε4* allele containing genotypes which range from 37.8% in African Americans to 3.6% among Indians in the present study [15]. The influence of *APOE* genotypes may also vary with different coumarin anticoagulants such as acenocoumarol and phenprocoumon which have different pharmacokinetic profiles compared with warfarin [16].

Recent studies suggest that the *VKORC1* haplotypes are a major determinant of warfarin dose requirements in patients of different ethnic background [4]. Similar to previous reports among Asians [17], this study showed that subjects harbouring the H7 haplotypes tend to require higher doses of warfarin in comparison with the H1 haplotype, independent of individual ethnic status. The contributions of *CYP2C9* genetic polymorphisms have been reported to have minimal impact on warfarin dosing in Asian patients as the frequency of the low-activity *CYP2C9*2* and **3* alleles is low in Asian ethnic groups [18].

In conclusion, the present study suggests that the reported influence of *APOE* variants on warfarin dose requirements may not be important in affecting warfarin pharmacodynamics in Asian patients, probably due to the low frequency of the ε4 allele containing genotypes.

The authors would like to acknowledge the Singapore General Hospital Research Fund (SRF #96/03) for funding this study.

REFERENCES

1 Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirements and risk of bleeding complications. Lancet 1999; 353: 717–9.

2 Kaminsky L, Zhang Z. Human P450 metabolism of warfarin. Pharmacol Ther 1997; 73: 67–74.

3 Li T, Chang CY, Jin DY, Lin PJ, Khvorova A, Stafford DW. Identification of the gene for vitamin K epoxide reductase. Nature 2004; 427: 541–4.

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

5 Kohnke H, Sorlin K, Granath G, Wadelius M. Warfarin dose related to apolipoprotein E (*APOE*) genotype. Eur J Clin Pharmacol 2005; 61: 381–8.

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

7 Saupe J, Shearer MJ, Kohlmeier M. Phylloquinone transport and its influence on gamma-carboxyglutamate residues of osteocalcin in patients on maintenance hemodialysis. Am J Clin Nutr 1993; 58: 204–8.

8 Rocchi A, Pellegrini S, Siciliano G, Murri L. Causative and susceptibility genes for Alzheimer's disease: a review. Brain Res Bull 2003; 61: 1–24.

9 Weintraub MS, Eisenberg S, Breslow JL. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. J Clin Invest 1987; 80: 1571–7.

10 Visser LE, Trienekens PH, De Smet PA, Vulto AG, Hofman A, van Duijn CM, Stricker BH. Patients with an ApoE epsilon4 allele require lower doses of coumarin anticoagulants. Pharmacogenet Genomics 2005; 15: 69–74.

11 Lee SC, Ng SS, Oldenburg J, Chong PY, Rost S, Guo JY, Yap HL, Rankin SC, Khor HB, Yeo TC, Ng KS, Soong R, Goh BC. Interethnic variability of warfarin maintenance requirement is explained by *VKORC1* genotype in an Asian population. Clin Pharmacol Ther 2006; 79: 197–205.

12 Seet WT, Mary Anne TJ, Yen TS. Apolipoprotein E genotyping in the Malay, Chinese and Indian ethnic groups in Malaysia: a study on the distribution of the different apoE alleles and genotypes. Clin Chim Acta 2004; 340: 201–5.

13 Sconce EA, Daly AK, Khan TI, Wynne HA, Kamali F. *APOE* genotype makes a small contribution to warfarin dose requirements. Pharmacogenet Genomics 2006; 16: 609–11.

14 Kohnke H, Scordo MG, Pengo V, Padrini R, Wadelius M. Apolipoprotein E (APOE) and warfarin dosing in an Italian population. Eur J Clin Pharmacol 2005; 61: 781–3.

15 Kimmel SE, Christie J, Kealey C, Chen Z, Price M, Thorn CF, Brensinger CM, Newcomb CW, Whitehead AS. Apolipoprotein E genotype and warfarin dosing among

Caucasians and African Americans. Pharmacogenomics J 2007;[Epub ahead of print].

16 Yacobi A, Levy G. Pharmacokinetics of warfarin enantiomers in rats. J Pharmacokinet Biopharm 1974; 2: 239–55.

17 Tham LS, Goh BC, Nafziger A, Guo JY, Wang LZ, Soong R, Lee SC. A warfarin-dosing model in Asians that uses single-nucleotide polymorphisms in vitamin K epoxide reductase complex and cytochrome P450 2C9. Clin Pharmacol Ther 2006; 80: 346–55.

18 Zhao F, Loke C, Rankin SC, Guo JY, Lee HS, Wu TS, Tan T, Liu TC, Lu WL, Lim YT, Zhang Q, Goh BC, Lee SC. Novel CYP2C9 genetic variants in Asian subjects and their influence on maintenance warfarin dose. Clin Pharmacol Ther 2004; 76: 210–9.